nutrients

Vitamins C and D

Global and Population Health Perspectives

Edited by

Anitra Carr and Adrian Gombart

Printed Edition of the Special Issue Published in *Nutrients*

www.mdpi.com/journal/nutrients

MDPI

Vitamins C and D: Global and Population Health Perspectives

Vitamins C and D: Global and Population Health Perspectives

Editors

Anitra Carr
Adrian Gombart

MDPI • Basel • Beijing • Wuhan • Barcelona • Belgrade • Manchester • Tokyo • Cluj • Tianjin

Editors

Anitra Carr
University of Otgao
New Zealand

Adrian Gombart
Oregon State University
USA

Editorial Office
MDPI
St. Alban-Anlage 66
4052 Basel, Switzerland

This is a reprint of articles from the Special Issue published online in the open access journal *Nutrients* (ISSN 2072-6643) (available at: https://www.mdpi.com/journal/nutrients/special_issues/Vitamin_C_D_Global_Health).

For citation purposes, cite each article independently as indicated on the article page online and as indicated below:

LastName, A.A.; LastName, B.B.; LastName, C.C. Article Title. *Journal Name* **Year**, *Volume Number*, Page Range.

ISBN 978-3-0365-6117-2 (Hbk)
ISBN 978-3-0365-6118-9 (PDF)

Contents

About the Editors

Anitra Carr

Anitra Carr is Director of the Nutrition in Medicine Research Group at the University of Otago, Christchurch, NZ. Dr. Carr is recognised as an international expert and opinion leader on the role of vitamin C in human health and disease. Dr. Carr was awarded an American Heart Association Fellowship to research vitamin C in cardiovascular disease at the Linus Pauling Institute, Oregon State University, USA. Whilst there she produced a number of high impact publications that have helped inform US and EU recommendations around vitamin C dietary intakes. Dr. Carr was subsequently awarded a Health Research Council of NZ Fellowship to investigate the role of vitamin C in severe infection and is also carrying out translational research into the role of vitamin C in the prevention and treatment of chronic diseases such as cancer and diabetes. Dr. Carr has >80 publications on vitamin C alone and was recently awarded a University of Otago Gold Medal for sustained research excellence.

Adrian Gombart

Adrian Gombart is currently a principal investigator in the Linus Pauling Institute and professor in the Department of Biochemistry and Biophysics at Oregon State University. He is recognized as an international expert and opinion leader on the role of vitamin D in the innate immune response against infection. His laboratory studies the regulation of antimicrobial peptides by vitamin D and other nutritional compounds. The organizers of the 2005 Vitamin D Workshop recognized his contributions to the field of vitamin D and immunity with a Young Investigator Award. In addition, his group studies the interaction between plant polyphenols, the host microbiota and immune system with a goal of understanding their role in treating obesity and metabolic syndrome. Dr. Gombart received his PhD in Microbiology from the University of Washington. He was a research assistant and associate professor at Cedars-Sinai Medical Center and the David Geffen School of Medicine at UCLA in the Department of Biomedical Sciences and Division of Hematology and Oncology before joining the Linus Pauling Institute.

nutrients

MDPI

Editorial

Multi-Level Immune Support by Vitamins C and D during the SARS-CoV-2 Pandemic

Anitra C. Carr [1,*] and Adrian F. Gombart [2,3]

[1] Nutrition in Medicine Research Group, Department of Pathology and Biomedical Science, University of Otago, Christchurch 8011, New Zealand
[2] Linus Pauling Institute, Oregon State University, Corvallis, OR 97331, USA; adrian.gombart@oregonstate.edu
[3] Department of Biochemistry and Biophysics, Oregon State University, Corvallis, OR 97331, USA
* Correspondence: anitra.carr@otago.ac.nz; Tel.: +643-364-0649

Abstract: Vitamins C and D have well-known immune supportive roles, with deficiencies in both vitamins predisposing to increased risk and severity of respiratory infections. Numerous studies have indicated that administration of these vitamins, particularly to people who are deficient, can decrease the risk and severity of respiratory infections. This has stimulated an interest in the potential efficacy of these vitamins in people with novel coronavirus (SARS-CoV-2) infection and its more severe disease (COVID-19). In this overview, we highlight the current research evidence around the multiple levels of immune support provided by vitamins C and D in the context of general respiratory infections and with a focus on the current SARS-CoV-2 pandemic. These include: prevention of infection; attenuating infection symptoms and severity; adjunctive therapy for severe disease; attenuating ongoing sequelae (long COVID); and immunisation support. Although some of these topics have not yet been investigated in great depth concerning SARS-CoV-2 and COVID-19, extensive research into the role of these vitamins in general respiratory infections has highlighted directions for future research in the current pandemic.

Keywords: SARS-CoV-2; COVID-19; vitamin C; vitamin D; pneumonia; sepsis; long COVID; immunisation; immune support

Citation: Carr, A.C.; Gombart, A.F. Multi-Level Immune Support by Vitamins C and D during the SARS-CoV-2 Pandemic. *Nutrients* **2022**, *14*, 689. https://doi.org/10.3390/nu14030689

Received: 20 January 2022
Accepted: 31 January 2022
Published: 6 February 2022

1. Introduction

Since the novel coronavirus (SARS-CoV-2) and the associated disease (COVID-19) were declared a global pandemic in early 2020, there has been a worldwide effort to establish therapies to prevent and treat the respiratory infection and more severe disease. Although vaccination status is now high in most high-income countries, vaccination rates are still relatively low in many low-middle income countries. Furthermore, despite high vaccination rates in much of the developed world, SARS-CoV-2 infection cases and COVID-19 morbidity and mortality remain high. As such, additional means of supporting optimal immune function are crucial.

Both the innate and adaptive immune systems are absolutely reliant on appropriate nutritional support for optimal functioning [1]. Of the various immune-supportive micronutrients, vitamins C and D are two of the most well-established [2,3]. Deficiencies of these vitamins are common in many regions of the world and within specific subpopulations [4,5]. Deficiencies of in these vitamins are known to impair the immune system, resulting in severe respiratory infections [6–8]. In the case of vitamin C, pneumonia is a common complication of chronic vitamin C deficiency (scurvy) and is one of the most common causes of mortality in people with scurvy [6]. In addition, infection itself can cause further depletion of micronutrients, particularly vitamin C, thus necessitating higher intakes to restore optimal micronutrient status [9].

In this overview, we highlight the current evidence around the multiple levels of immune support provided by vitamins C and D in the context of general respiratory infec-

tions and with a focus on the current SARS-CoV-2 pandemic. These include: prevention of infection; attenuating infection symptoms and severity; adjunctive therapy for severe disease; attenuating ongoing sequelae (long COVID); and immunisation support. Although some of these topics have not yet been investigated in great depth concerning SARS-CoV-2 and COVID-19, extensive research into the role of these vitamins in general respiratory infections has highlighted directions for future research in the current pandemic.

2. Prevention of Respiratory Infection

2.1. Vitamin C

Coronaviruses are one of the many types of viruses that can cause the common cold [10]. Meta-analysis of 24 trials investigating vitamin C for the prevention of the common cold has indicated that prophylactic supplementation with doses $\geq$200 mg/day did not decrease the incidence of the common cold in the general population [11]. In the case of SARS-CoV2 infection, one case-control study has attempted to estimate the effect of regular vitamin C supplementation on the incidence of SARS-CoV-2 infection [12]. Cases and controls were health-care workers who tested positive and negative, respectively, for SARS-CoV-2 infection. Of the 372 participants, 67 participants took vitamin C supplements (500 mg) once or twice daily. There was, however, no significant association with SARS-CoV-2 infection compared with the control group.

In contrast, meta-analysis of a handful of trials in people under enhanced physical stress who were regularly supplementing with vitamin C indicated a >50% decrease in the incidence of colds [11]. This may be particularly relevant in cases of severe infection where vitamin C appears to act as part of the body's stress response [13]. Psychological stress is also known to negatively affect the immune system [14], and could potentially enhance requirements for vitamin C [15]. Thus, future research around the role of vitamin C in the prevention of SARS-CoV-2 infection should specifically focus on subgroups who are under enhanced physical or psychological stress and who are at risk of vitamin C deficiency [16].

2.2. Vitamin D

It is clear that an inverse association exists between vitamin D status and the risk of acute respiratory tract infections [17]. Similarly, meta-analysis of 54 studies and 1,403,715 patients has indicated that those with low vitamin D levels had a higher susceptibility to SARS-CoV-2 infection and associated hospitalisation [18]. However, due to inherent study limitations caution should be exercised in interpreting the results as another meta-analysis with 11 cohort studies and 536,105 patients, did not show a link between vitamin D deficiency or insufficiency and susceptibility to SARS-CoV-2 infection [19].

It should be noted that meta-analyses of trials investigating vitamin D supplementation and risk of acute respiratory tract infection have indicated that those with a low vitamin D status at the start of the trial tend to achieve better results following supplementation [20,21]. Furthermore, recent meta-analyses show supplementation protects against acute respiratory infections when given as daily, but not as an intermittent bolus dose [21,22]. These aspects should be taken into consideration during the design of future SARS-CoV-2 trials.

3. Attenuating Infection Symptoms and Severity

3.1. Vitamin C

In the absence of specific comorbidities that increase the risk of developing severe COVID-19 [23], infection with SARS-CoV-2 can be relatively mild and even asymptomatic [24]. To date, one published trial has investigated the effects of vitamin C supplementation on SARS-CoV-2 symptoms [25]. This trial was underpowered as it was halted early, but nevertheless showed a non-significant 1.2-day decrease in duration to reach 50% reduction in symptoms in the participants who received supplemental vitamin C (8 g/day). Furthermore, independent statistical analysis of the results showed a significant 70% increase in the rate of recovery in the vitamin C group compared to standard care [26]. Similarly, in the case of the common cold, meta-analysis of 31 trials indicated that prophylactic vitamin

C supplementation in the general population can decrease both the duration and severity of the common cold, with up to an 18% decreased duration in children who received ≥1 g/day [11]. Supplementation with gram doses of vitamin C following initiation of cold symptoms also provided a dose-dependent decrease in the duration of the common cold [11].

Pneumonia is a common complication of severe respiratory infections, including severe SARS-CoV-2 infection [27]. Epidemiological evidence has suggested that people in the highest quartile of vitamin C status had a lower incidence of pneumonia than those in the lowest quartile [28]. Furthermore, meta-analysis has indicated that prophylactic supplementation with vitamin C can decrease the risk of developing pneumonia [29]. As such, regular vitamin C supplementation may attenuate progression of mild SARS-CoV-2 infection to the more severe complication of pneumonia observed in COVID-19.

3.2. Vitamin D

Meta-analysis of eight observational studies has suggested an association between vitamin D deficiency and risk of developing community-acquired pneumonia [30]. Early epidemiological research showed inverse associations between mean vitamin D status of 20 European countries and COVID-19 cases in those countries [31]. In a retrospective cohort study of 4,599 veterans with a positive SARS-CoV-2 test, after adjusting for all covariates, an inverse dose-response relationship between increasing vitamin D concentrations and decreasing probability of hospitalisation from COVID-19 was observed [32]. A number of meta-analyses supported a link between vitamin D deficiency and the severity of SARS-CoV-2 infection, with the largest indicating that those with low vitamin D levels were at an increased risk of ICU admission due to acute respiratory distress syndrome [18].

There is currently debate as to whether low serum vitamin D is caused by infection or if deficiency negatively affects immune defense. A meta-analysis of one population study and seven clinical studies that reported serum vitamin D levels pre-infection or on the day of hospital admission indicated that low serum levels are a predictor rather than a side effect of SARS-CoV-2 infection [33]. Furthermore, regression suggested a point of zero mortality at serum vitamin D levels ≥50 ng/mL [33].

Low-to-moderate evidence suggests possible benefits from vitamin D supplementation in adults and children with upper respiratory tract infections and influenza [34]. Similarly, there is mixed evidence for a beneficial role for vitamin D supplementation on need for ICU admission in patients with SARS-CoV-2 infection, with only two RCTs currently published [19,35]. Additional well-designed RCTs are needed to evaluate the efficacy of vitamin D supplementation in affecting SARS-CoV-2 infection outcomes.

4. Adjunctive Therapy for Severe Disease

4.1. Vitamin C

Severe COVID-19 is characterised by the complications of pneumonia, acute respiratory distress syndrome (ARDS) and sepsis, typically requiring hospitalisation and intensive care for respiratory support [36]. Patients with pneumonia, ARDS and sepsis generally have severely depleted vitamin C levels [9]; comparable findings have been reported with COVID-19 patients [37]. Vitamin C supplementation of hospitalised patients with pneumonia has indicated decreased respiratory symptoms in the most severely ill and a dose-dependent decrease in the duration of hospital stay [29]. Patients with sepsis and ARDS in intensive care require gram doses of vitamin C to restore optimal vitamin C status; this is typically administered parenterally [9]. A recent RCT in septic patients with ARDS indicated decreased mortality and increased ICU and hospital free days in the group who received intravenous vitamin C (200 mg/kg/day) [38].

The World Health Organisation in 2020 highlighted intravenous vitamin C as a potential adjunctive therapy for patients with critical COVID-19 [39]. To date, five intervention trials have been published. The first trial to be carried out in patients with COVID-19-related pneumonia indicated a trend towards decreased 28-day mortality in the most severely ill

patients who received intravenous vitamin C (24 g/day) [40]. Unfortunately, this trial was terminated early due to decreasing numbers of patients. Another trial that administered high-dose vitamin C (~28 g/d) to COVID-19 patients, who were receiving hydroxychloroquine, azithromycin, zinc, and vitamin D3, showed quicker recovery (symptom free and discharged from hospital) [41]. Three other trials in patients with severe COVID-19 who were administered lower doses of intravenous vitamin C (50 mg/kg/day and 6–8 g/day) showed no effects on mortality, but did find patients became symptom free earlier and spent fewer days in hospital [42], and had lower body temperature and improvements in oxygen saturation and respiratory rate [43,44]. Further trials are currently underway.

4.2. Vitamin D

Adjunct vitamin D supplementation in children with pneumonia did not reveal any significant reduction in the duration of hospitalisation, resolution of fever or acute illness, or mortality rate [45]. However, in adults with community-acquired pneumonia, vitamin D supplementation may benefit those with deficiency [46]. In the case of COVID-19, meta-analysis of two RCTs and a quasi-experimental study failed to show significant differences with vitamin D supplementation on various patient outcomes, including mechanical ventilation and mortality [19,47].

The potential for vitamin D to reduce inflammatory responses suggests it could function as an adjunctive therapy for recovery from COVID-19. A two-week 5000 IU oral vitamin D supplementation reduced the time to recovery for cough and loss-of-taste among patients with sub-optimal vitamin D status [48]. A study using 60,000 IU vitamin D for 8-10 days in individuals with low vitamin D levels showed a significant reduction in inflammatory markers as compared to standard care [49]. On the other hand, in a multicenter double-blind RCT, hospitalised patients (n = 120) receiving a single oral dose of 200,000 IU vitamin D did not show a significant reduction in hospital length of stay as compared to placebo [50]. Oral administration with calcifediol (25(OH)D) rather than vitamin D, raises serum 25(OH)D levels much more rapidly and may improve immune function and patient outcomes [51–54]. Further well designed RCTs are needed to determine the potential to use vitamin D in adjunctive therapy for COVID-19.

5. Attenuating Ongoing Sequelae

5.1. Vitamin C

The post-acute sequelae of COVID-19 (or 'long COVID') is characterised by persistent physical, cognitive and psychological symptoms which can significantly impair quality of life [55]. These include fatigue, muscle and joint pain, shortness of breath, chest pain, cough, and headache, as well as cognitive impairment, memory loss, anxiety and sleep disorders. Fatigue is a common symptom of viral infections in general and is one of the most frequently reported symptoms in long COVID [56]. Of note, fatigue, lethargy and low mood are early, preclinical symptoms of the vitamin C deficiency disease scurvy, whilst muscle and joint pain are common symptoms of clinical scurvy [57,58]. The vitamin C status of people with long COVID has not yet been assessed. Nevertheless, vitamin C intervention has been shown to improve symptoms of fatigue in people with various acute and chronic conditions, including herpes zoster infection [59], and has been proposed as a feasible therapy for the post viral fatigue of long COVID [60]. Furthermore, vitamin C supplementation has been reported to ameliorate pain in various acute and chronic conditions, including pain associated with viral infections [61].

Although not yet published, some current vitamin C and COVID trials (such as LOVIT-COVID) are assessing the longer-term health-related quality of life outcomes of short-term intravenous vitamin C administration to patients with COVID-19. However, it is unlikely that four days of intravenous vitamin C administration during critical COVID will have significant longer-term quality of life effects as cessation of vitamin C administration can result in a return to low baseline levels of vitamin C in many patients [38,62]. Thus, in order

to see potential prevention of, or recovery from, long COVID ongoing daily vitamin C administration in appropriate dosages would be required. This remains to be established.

5.2. Vitamin D

Investigators are beginning to publish studies examining the relationship between vitamin D and long-term effects following SARS-CoV-2 infection and COVID-19. A recent study investigated the potential link between serum vitamin D levels and fatigue and reduced exercise tolerance in 149 patients at a median of 79 days after COVID-19 illness [63]. No relationship between vitamin D levels and ongoing ill-health was found after multivariable regression analysis. This relationship requires additional research and some vitamin D supplementation studies (e.g., VitD-COVID19) are examining physical activity as a secondary outcome.

6. Immunisation Support

6.1. Vitamin C

Vaccination against SARS-CoV-2 and the resultant activation of the immune system is likely to result in enhanced utilisation of immune supportive micronutrients, such as vitamin C. In cases of severe vitamin C deficiency, infants have been observed to go into shock following routine childhood vaccinations; rapid vitamin C administration was able to rescue many cases [64,65]. Animal studies have indicated that administration of vitamin C during vaccination or antigen challenge can stimulate an earlier and higher antibody response [66–68].

In 2019, the World Health Organization highlighted vaccine hesitancy as one of the top 10 threats to global health [69]. In the case of COVID-19 vaccination, a survey conducted in the USA found that 70% of vaccine-hesitant adults were worried about potential side effects of COVID-19 vaccines [70]. As discussed above, vitamin C can potentially attenuate symptoms of fatigue and pain [60,61]. Furthermore, decreased anaphylaxis and mortality from shock have been observed in vitamin C supplemented animals following challenge of passively sensitised animals [68]. As such, studies in people at risk of vitamin C deficiency who are receiving SARS-CoV-2 vaccines appear warranted.

6.2. Vitamin D

Vitamin D activates transcription of numerous genes involved in immune system support [3]. Research has suggested that low vitamin D status was associated with poorer hepatitis B vaccine response [71]. Meta-analysis of vitamin D deficiency and immunogenic response to influenza vaccine indicated lower seroprotection rates in response to vaccination against specific strains of influenza A and B virus in vitamin D deficient patients [72]. However, calcitriol co-administered intramuscularly with a commercially available influenza vaccine in 175 human volunteers provided no significant differences in hemagglutination titers at one or three months postvaccination [73]. In addition, initial research into vitamin D status and antibody response to COVID-19 mRNA vaccination in healthy adults did not show any significant association [74]. However, vitamin D may be able to support immune functions other than antibody response [75].

7. Summary and Conclusions

Immune support by micronutrients can be provided at various stages along the disease spectrum, from prevention of infection to adjunctive therapy for mild and severe disease, and its long-term sequelae, as well as support during immunisation. Table 1 summarises the evidence for the role of vitamins C and D in these different phases of immune support for SARS-CoV-2 and COVID-19, or general respiratory infections. Of note, there is significant overlap in the risk factors for vitamins C and D deficiency [16,76] and those for severe COVID-19 morbidity and mortality [23]. Therefore, restoring optimal vitamin C and D status in people with risk factors for both COVID-19 and vitamin deficiency may help to attenuate not only the risk of infection, but also the severity of complications.

Table 1. Summary of multi-level immune support by vitamins C and D for SARS-CoV-2 infection and COVID-19, or general respiratory infections.

	Vitamin C		Vitamin D	
	SARS-CoV-2 and COVID-19*	**General Respiratory Infections**	**SARS-CoV-2 and COVID-19**	**General Respiratory Infections**
Prevention of infection	X risk reduction in case-control study [12]	X common cold risk in general population [11] ↓ common cold risk in people under stress [11]	↑ risk observed if low status [18]	↑ risk of acute RTI observed if low status [17] ↓ risk in people with low status if given vitD daily [20–22]
Attenuating infection symptoms and severity	? some evidence of decreased duration [25] ↑ rate of recovery [26]	↓ duration and severity of common cold [11] ↓ development of pneumonia [29]	↑ hospitalisation and ARDS observed if low status [18,32] X ICU admission (2 RCTs) [19]	↑ risk of pneumonia observed with deficiency [30] ? limited benefit in upper RTI and influenza [34]
Adjunctive therapy for severe disease	? some evidence of decreased mortality [40] ↑ rate of recovery [41,42] ↑ oxygenation [43,44]	↓ hospital stay in pneumonia [29] ↓ mortality, ICU and hospital stay in ARDS [38]	X mechanical ventilation or mortality (2 RCTs) [47]	X resolution or mortality in childhood pneumonia [43] ? may benefit those with deficiency [46]
Attenuating ongoing sequelae	? as yet unknown effects	↓ fatigue and pain in viral infections [60,61]	X fatigue, exercise tolerance [63]	? not assessed
Immunisation support	? as yet unknown effects	↑ antibody response in animals [66–68] ↓ anaphylaxis, shock mortality in animals [68] ↓ post immunisation shock in deficient infants [64,65]	X antibody response to mRNA vaccine in healthy adults [74]	↓ immunogenic response to some influenza vaccines if deficient [72] X hemagglutination titres following influenza vaccine [75]

* X no effect, ? uncertain effect, ↓ decreased, ↑ increased. Individual studies are cited where meta-analyses are not available. Some studies are observational only. ARDS; acute respiratory distress syndrome, RCTs; randomised controlled trials, RTI; respiratory tract infections.

Vitamin C has numerous well-known immune supportive roles; however, its efficacy depends on appropriate timing, doses and rout of administration. Figure 1 summarises the different requirements for vitamin C along the spectrum of illness. To date, studies have indicated minimal effect on risk of SARS-CoV-2 and other upper respiratory tract infections with oral vitamin C, unless the person is under enhanced stress. However, there is some evidence that vitamin C can attenuate the duration and severity of respiratory and SARS-CoV-2 infections, thus potentially preventing progression to more severe conditions such as pneumonia, ARDS, sepsis and COVID-19. Intravenous vitamin C is typically administered to patients as adjunctive therapy once they enter into intensive care and preliminary studies indicate potential improvements in patients with ARDS and COVID-19. Larger trials are currently underway.

Vitamin D is implicated in numerous biological activities of the innate and adaptive immune system and observational studies suggest an inverse relationship between serum vitamin D concentrations and risk or severity of COVID-19. Some RCTs show vitamin D supplementation could reduce SARS-CoV-2 positivity, but data on ICU admission or all-cause mortality in those patients with moderate to severe disease is mixed. While further well-designed studies are needed, the evidence from prior work with respiratory tract infections and recent COVID-19 studies suggest taking a vitamin D supplement to reach serum vitamin D levels greater than 30 ng/mL is safe and could reduce risks associated with COVID-19.

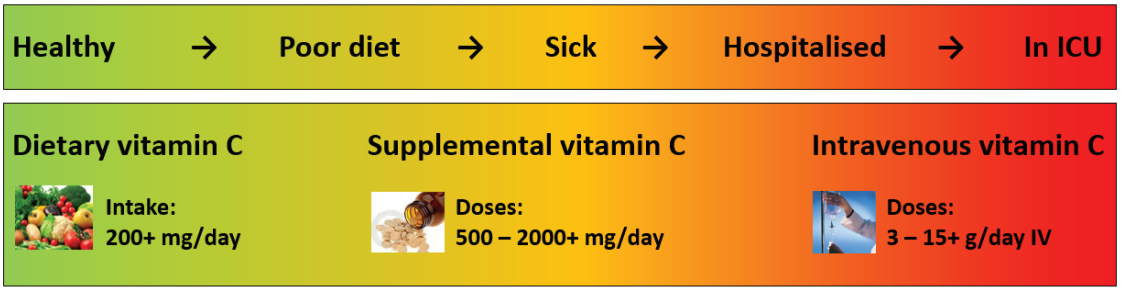


Figure 1. Vitamin C requirements along the spectrum of illness. Routs of administration and doses are those recommended or typically used. ICU, intensive care unit; IV, intravenous.

To date, minimal research has been carried out around the potential effects of micronutrients on mitigating the debilitating symptoms of long COVID. Therefore, additional good-quality studies are needed in this area. Future studies investigating micronutrient support during immunisation should focus on people with comorbidities and other risk factors for both COVID-19 and vitamin deficiency. Furthermore, these studies should not focus exclusively on the antibody response, but also consider the other multiple levels of immune support offered by these vitamins, including potentially attenuating vaccine side effects. More research in these areas may provide evidence that could help with mitigating vaccine hesitancy.

Author Contributions: Conceptualisation, A.C.C.; writing—original draft preparation, A.C.C. and A.F.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest in relation to this article.

References

1. Calder, P.C.; Carr, A.C.; Gombart, A.F.; Eggersdorfer, M. Optimal nutritional status for a well-functioning immune system is an important factor to protect against viral infections. *Nutrients* **2020**, *12*, 1181. [CrossRef]
2. Carr, A.C.; Maggini, S. Vitamin C and immune function. *Nutrients* **2017**, *9*, 1211. [CrossRef]
3. Gombart, A.F.; Pierre, A.; Maggini, S. A review of micronutrients and the immune system-working in harmony to reduce the risk of infection. *Nutrients* **2020**, *12*, 236. [CrossRef]
4. Rowe, S.; Carr, A.C. Global vitamin C status and prevalence of deficiency: A cause for concern? *Nutrients* **2020**, *12*, 2008. [CrossRef] [PubMed]
5. Hilger, J.; Friedel, A.; Herr, R.; Rausch, T.; Roos, F.; Wahl, D.A.; Pierroz, D.D.; Weber, P.; Hoffmann, K. A systematic review of vitamin D status in populations worldwide. *Br. J. Nutr.* **2014**, *111*, 23–45. [CrossRef] [PubMed]
6. Hemilä, H. Vitamin C and infections. *Nutrients* **2017**, *9*, 339. [CrossRef] [PubMed]
7. Jat, K.R. Vitamin D deficiency and lower respiratory tract infections in children: A systematic review and meta-analysis of observational studies. *Trop. Doct.* **2017**, *47*, 77–84. [CrossRef]
8. Jolliffe, D.A.; Griffiths, C.J.; Martineau, A.R. Vitamin D in the prevention of acute respiratory infection: Systematic review of clinical studies. *J. Steroid Biochem. Mol. Biol.* **2013**, *136*, 321–329. [CrossRef]
9. Carr, A.C. Vitamin C in pneumonia and sepsis. In *Vitamin C: New Biochemical and Functional Insights*; Chen, Q., Vissers, M., Eds.; CRC Press/Taylor & Francis: Boca Raton, FL, USA, 2020; pp. 115–135.
10. Holford, P.; Carr, A.C.; Jovic, T.H.; Ali, S.R.; Whitaker, I.S.; Marik, P.E.; Smith, A.D. Vitamin C—An adjunctive therapy for respiratory infection, sepsis and COVID-19. *Nutrients* **2020**, *12*, 3760. [CrossRef]
11. Hemilä, H.; Chalker, E. Vitamin C for preventing and treating the common cold. *Cochrane Database Syst. Rev.* **2013**, *1*, CD000980. [CrossRef]
12. Behera, P.; Patro, B.K.; Singh, A.K.; Chandanshive, P.D.; S, R.R.; Pradhan, S.K.; Pentapati, S.S.K.; Batmanabane, G.; Mohapatra, P.R.; Padhy, B.M.; et al. Role of ivermectin in the prevention of SARS-CoV-2 infection among healthcare workers in India: A matched case-control study. *PLoS ONE* **2021**, *16*, e0247163. [CrossRef]
13. Marik, P.E. Vitamin C: An essential "stress hormone" during sepsis. *J. Thorac. Dis.* **2020**, *12*, S84–S88. [CrossRef] [PubMed]
14. Segerstrom, S.C.; Miller, G.E. Psychological stress and the human immune system: A meta-analytic study of 30 years of inquiry. *Psychol. Bull.* **2004**, *130*, 601–630. [CrossRef] [PubMed]

15. Moritz, B.; Schmitz, A.E.; Rodrigues, A.L.S.; Dafre, A.L.; Cunha, M.P. The role of vitamin C in stress-related disorders. *J. Nutr. Biochem.* **2020**, *85*, 108459. [CrossRef]
16. Carr, A.C.; Rowe, S. Factors affecting vitamin C status and prevalence of deficiency: A global health perspective. *Nutrients* **2020**, *12*, 1963. [CrossRef] [PubMed]
17. Pham, H.; Rahman, A.; Majidi, A.; Waterhouse, M.; Neale, R.E. Acute respiratory tract infection and 25-hydroxyvitamin D concentration: A systematic review and meta-analysis. *Int. J. Environ. Res. Public Health* **2019**, *16*, 3020. [CrossRef]
18. Chiodini, I.; Gatti, D.; Soranna, D.; Merlotti, D.; Mingiano, C.; Fassio, A.; Adami, G.; Falchetti, A.; Eller-Vainicher, C.; Rossini, M.; et al. Vitamin D status and SARS-CoV-2 infection and COVID-19 clinical outcomes. *Front Public Health* **2021**, *9*, 736665. [CrossRef] [PubMed]
19. Chen, J.; Mei, K.; Xie, L.; Yuan, P.; Ma, J.; Yu, P.; Zhu, W.; Zheng, C.; Liu, X. Low vitamin D levels do not aggravate COVID-19 risk or death, and vitamin D supplementation does not improve outcomes in hospitalized patients with COVID-19: A meta-analysis and GRADE assessment of cohort studies and RCTs. *Nutr. J.* **2021**, *20*, 89. [CrossRef] [PubMed]
20. Autier, P.; Mullie, P.; Macacu, A.; Dragomir, M.; Boniol, M.; Coppens, K.; Pizot, C.; Boniol, M. Effect of vitamin D supplementation on non-skeletal disorders: A systematic review of meta-analyses and randomised trials. *Lancet. Diabetes Endocrinol.* **2017**, *5*, 986–1004. [CrossRef]
21. Martineau, A.R.; Jolliffe, D.A.; Hooper, R.L.; Greenberg, L.; Aloia, J.F.; Bergman, P.; Dubnov-Raz, G.; Esposito, S.; Ganmaa, D.; Ginde, A.A.; et al. Vitamin D supplementation to prevent acute respiratory tract infections: Systematic review and meta-analysis of individual participant data. *BMJ* **2017**, *356*, i6583. [CrossRef]
22. Jolliffe, D.A.; Camargo, C.A., Jr.; Sluyter, J.D.; Aglipay, M.; Aloia, J.F.; Ganmaa, D.; Bergman, P.; Bischoff-Ferrari, H.A.; Borzutzky, A.; Damsgaard, C.T.; et al. Vitamin D supplementation to prevent acute respiratory infections: A systematic review and meta-analysis of aggregate data from randomised controlled trials. *Lancet Diabetes Endocrinol.* **2021**, *9*, 276–292. [CrossRef]
23. Centers for Disease Control and Prevention. COVID-19: People with Certain Medical Conditions. Available online: https://www.cdc.gov/coronavirus/2019-ncov/need-extra-precautions/people-with-medical-conditions.html (accessed on 12 December 2021).
24. Chen, X.; Huang, Z.; Wang, J.; Zhao, S.; Wong, M.C.; Chong, K.C.; He, D.; Li, J. Ratio of asymptomatic COVID-19 cases among ascertained SARS-CoV-2 infections in different regions and population groups in 2020: A systematic review and meta-analysis including 130 123 infections from 241 studies. *BMJ Open* **2021**, *11*, e049752. [CrossRef] [PubMed]
25. Thomas, S.; Patel, D.; Bittel, B.; Wolski, K.; Wang, Q.; Kumar, A.; Il'Giovine, Z.J.; Mehra, R.; McWilliams, C.; Nissen, S.E.; et al. Effect of high-dose zinc and ascorbic acid supplementation vs usual care on symptom length and reduction among ambulatory patients with SARS-CoV-2 infection: The COVID A to Z randomized clinical trial. *JAMA Netw. Open* **2021**, *4*, e210369. [CrossRef] [PubMed]
26. Hemilä, H.; Carr, A.; Chalker, E. Vitamin C may increase the recovery rate of outpatient cases of SARS-CoV-2 infection by 70%: Reanalysis of the COVID A to Z randomized clinical trial. *Front. Immunol.* **2021**, *12*, 674681. [CrossRef]
27. Budinger, G.R.S.; Misharin, A.V.; Ridge, K.M.; Singer, B.D.; Wunderink, R.G. Distinctive features of severe SARS-CoV-2 pneumonia. *J. Clin. Invest.* **2021**, *131*. [CrossRef] [PubMed]
28. Myint, P.K.; Wilson, A.M.; Clark, A.B.; Luben, R.N.; Wareham, N.J.; Khaw, K.T. Plasma vitamin C concentrations and risk of incident respiratory diseases and mortality in the European Prospective Investigation into Cancer-Norfolk population-based cohort study. *Eur. J. Clin. Nutr.* **2019**, *73*, 1492–1500. [CrossRef]
29. Hemilä, H.; Louhiala, P. Vitamin C for preventing and treating pneumonia. *Cochrane Database Syst. Rev.* **2013**, *8*, Cd005532. [CrossRef]
30. Zhou, Y.F.; Luo, B.A.; Qin, L.L. The association between vitamin D deficiency and community-acquired pneumonia: A meta-analysis of observational studies. *Medicine* **2019**, *98*, e17252. [CrossRef]
31. Ilie, P.C.; Stefanescu, S.; Smith, L. The role of vitamin D in the prevention of coronavirus disease 2019 infection and mortality. *Aging Clin. Exp. Res.* **2020**, *32*, 1195–1198. [CrossRef]
32. Seal, K.H.; Bertenthal, D.; Carey, E.; Grunfeld, C.; Bikle, D.D.; Lu, C.M. Association of vitamin D status and COVID-19-related hospitalization and mortality. *J. Gen. Intern. Med.* **2022**, 1–9. [CrossRef] [PubMed]
33. Borsche, L.; Glauner, B.; von Mendel, J. COVID-19 mortality risk correlates inversely with vitamin D3 status, and a mortality rate close to zero could theoretically be achieved at 50 ng/mL 25(OH)D3: Results of a systematic review and meta-analysis. *Nutrients* **2021**, *13*, 3596. [CrossRef] [PubMed]
34. Yamshchikov, A.V.; Desai, N.S.; Blumberg, H.M.; Ziegler, T.R.; Tangpricha, V. Vitamin D for treatment and prevention of infectious diseases: A systematic review of randomized controlled trials. *Endocr. Pract.* **2009**, *15*, 438–449. [CrossRef] [PubMed]
35. Tentolouris, N.; Samakidou, G.; Eleftheriadou, I.; Tentolouris, A.; Jude, E.B. The effect of vitamin D supplementation on mortality and intensive care unit admission of COVID-19 patients. A systematic review, meta-analysis and meta-regression. *Diabetes Metab. Res. Rev.* **2021**, e3517. [CrossRef] [PubMed]
36. Fan, E.; Beitler, J.R.; Brochard, L.; Calfee, C.S.; Ferguson, N.D.; Slutsky, A.S.; Brodie, D. COVID-19-associated acute respiratory distress syndrome: Is a different approach to management warranted? *Lancet Respir. Med.* **2020**, *8*, 816–821. [CrossRef]
37. Holford, P.; Carr, A.C.; Zawari, M.; Vizcaychipi, M.P. Vitamin C intervention for critical COVID-19: A pragmatic review of the current level of evidence. *Life* **2021**, *11*, 1166. [CrossRef]

38. Fowler, A.A., 3rd; Truwit, J.D.; Hite, R.D.; Morris, P.E.; DeWilde, C.; Priday, A.; Fisher, B.; Thacker, L.R., 2nd; Natarajan, R.; Brophy, D.F.; et al. Effect of vitamin C infusion on organ failure and biomarkers of inflammation and vascular injury in patients with sepsis and severe acute respiratory failure: The CITRIS-ALI randomized clinical trial. *JAMA* **2019**, *322*, 1261–1270. [CrossRef]
39. World Health Organization. *A Coordinated Global Research Roadmap: 2019 Novel Coronavirus*; WHO: Geneva, Switzerland, 2020; p. 67.
40. Zhang, J.; Rao, X.; Li, Y.; Zhu, Y.; Liu, F.; Guo, G.; Luo, G.; Meng, Z.; De Backer, D.; Xiang, H.; et al. Pilot trial of high-dose vitamin C in critically ill COVID-19 patients. *Ann. Intensive Care* **2021**, *11*, 5. [CrossRef]
41. Ried, K.; BinJemain, T.; Sali, A. Therapies to prevent progression of COVID-19, including hydroxychloroquine, azithromycin, zinc, and vitamin D3 with or without intravenous vitamin C: An international, multicenter, randomized trial. *Cureus* **2021**, *13*, e19902. [CrossRef] [PubMed]
42. Kumari, P.; Dembra, S.; Dembra, P.; Bhawna, F.; Gul, A.; Ali, B.; Sohail, H.; Kumar, B.; Memon, M.K.; Rizwan, A. The role of vitamin C as adjuvant therapy in COVID-19. *Cureus J. Med. Sci.* **2020**, *12*, e11779. [CrossRef]
43. JamaliMoghadamSiahkali, S.; Zarezade, B.; Koolaji, S.; Seyed Alinaghi, S.; Zendehdel, A.; Tabarestani, M.; Sekhavati Moghadam, E.; Abbasian, L.; Dehghan Manshadi, S.A.; Salehi, M.; et al. Safety and effectiveness of high-dose vitamin C in patients with COVID-19: A randomized open-label clinical trial. *Eur. J. Med. Res.* **2021**, *26*, 20. [CrossRef]
44. Tehrani, S.; Yadegarynia, D.; Abrishami, A.; Moradi, H.; Gharaei, B.; Rauofi, M.; Maghsoudi Nejad, F.; Sali, S.; Khabiri, N.; Abolghasemi, S. An investigation into the effects of intravenous vitamin C on pulmonary CT findings and clinical outcomes of patients with COVID 19 pneumonia A randomized clinical trial. *Urol. J.* **2021**, *18*, 6863.
45. Das, R.R.; Singh, M.; Naik, S.S. Vitamin D as an adjunct to antibiotics for the treatment of acute childhood pneumonia. *Cochrane Database Syst. Rev.* **2018**, *7*, Cd011597. [CrossRef] [PubMed]
46. Slow, S.; Epton, M.; Storer, M.; Thiessen, R.; Lim, S.; Wong, J.; Chin, P.; Tovaranonte, P.; Pearson, J.; Chambers, S.T.; et al. Effect of adjunctive single high-dose vitamin D3 on outcome of community-acquired pneumonia in hospitalised adults: The VIDCAPS randomised controlled trial. *Sci. Rep.* **2018**, *8*, 13829. [CrossRef] [PubMed]
47. Rawat, D.; Roy, A.; Maitra, S.; Shankar, V.; Khanna, P.; Baidya, D.K. Vitamin D supplementation and COVID-19 treatment: A systematic review and meta-analysis. *Diabetes Metab. Syndr.* **2021**, *15*, 102189. [CrossRef] [PubMed]
48. Sabico, S.; Enani, M.A.; Sheshah, E.; Aljohani, N.J.; Aldisi, D.A.; Alotaibi, N.H.; Alshingetti, N.; Alomar, S.Y.; Alnaami, A.M.; Amer, O.E.; et al. Effects of a 2-week 5000 IU versus 1000 IU vitamin D3 supplementation on recovery of symptoms in patients with mild to moderate Covid-19: A randomized clinical trial. *Nutrients* **2021**, *13*, 2170. [CrossRef]
49. Lakkireddy, M.; Gadiga, S.G.; Malathi, R.D.; Karra, M.L.; Raju, I.; Ragini; Chinapaka, S.; Baba, K.; Kandakatla, M. Impact of daily high dose oral vitamin D therapy on the inflammatory markers in patients with COVID 19 disease. *Sci. Rep.* **2021**, *11*, 10641. [CrossRef] [PubMed]
50. Murai, I.H.; Fernandes, A.L.; Sales, L.P.; Pinto, A.J.; Goessler, K.F.; Duran, C.S.C.; Silva, C.B.R.; Franco, A.S.; Macedo, M.B.; Dalmolin, H.H.H.; et al. Effect of a single high dose of vitamin D3 on hospital length of stay in patients with moderate to severe COVID-19: A randomized clinical trial. *JAMA* **2021**, *325*, 1053–1060. [CrossRef]
51. Entrenas Castillo, M.; Entrenas Costa, L.M.; Vaquero Barrios, J.M.; Alcalá Díaz, J.F.; López Miranda, J.; Bouillon, R.; Quesada Gomez, J.M. Effect of calcifediol treatment and best available therapy versus best available therapy on intensive care unit admission and mortality among patients hospitalized for COVID-19: A pilot randomized clinical study. *J. Steroid Biochem. Mol. Biol.* **2020**, *203*, 105751. [CrossRef]
52. Nogues, X.; Ovejero, D.; Pineda-Moncusí, M.; Bouillon, R.; Arenas, D.; Pascual, J.; Ribes, A.; Guerri-Fernandez, R.; Villar-Garcia, J.; Rial, A.; et al. Calcifediol treatment and COVID-19-related outcomes. *J. Clin. Endocrinol. Metab.* **2021**, *106*, e4017–e4027. [CrossRef]
53. Maghbooli, Z.; Sahraian, M.A.; Jamalimoghadamsiahkali, S.; Asadi, A.; Zarei, A.; Zendehdel, A.; Varzandi, T.; Mohammadnabi, S.; Alijani, N.; Karimi, M.; et al. Treatment with 25-hydroxyvitamin D(3) (calcifediol) is associated with a reduction in the blood neutrophil-to-lymphocyte ratio marker of disease severity in hospitalized patients with COVID-19: A pilot multicenter, randomized, placebo-controlled, double-blinded clinical trial. *Endocr. Pract.* **2021**, *27*, 1242–1251. [CrossRef]
54. Loucera, C.; Peña-Chilet, M.; Esteban-Medina, M.; Muñoyerro-Muñiz, D.; Villegas, R.; Lopez-Miranda, J.; Rodriguez-Baño, J.; Túnez, I.; Bouillon, R.; Dopazo, J.; et al. Real world evidence of calcifediol or vitamin D prescription and mortality rate of COVID-19 in a retrospective cohort of hospitalized Andalusian patients. *Sci. Rep.* **2021**, *11*, 23380. [CrossRef]
55. Aiyegbusi, O.L.; Hughes, S.E.; Turner, G.; Rivera, S.C.; McMullan, C.; Chandan, J.S.; Haroon, S.; Price, G.; Davies, E.H.; Nirantharakumar, K.; et al. Symptoms, complications and management of long COVID: A review. *J. R. Soc. Med.* **2021**, *114*, 428–442. [CrossRef]
56. Sandler, C.X.; Wyller, V.B.B.; Moss-Morris, R.; Buchwald, D.; Crawley, E.; Hautvast, J.; Katz, B.Z.; Knoop, H.; Little, P.; Taylor, R.; et al. Long COVID and post-infective fatigue syndrome: A review. *Open Forum Infect. Dis.* **2021**, *8*, ofab440. [CrossRef] [PubMed]
57. Levine, M.; Conry-Cantilena, C.; Wang, Y.; Welch, R.W.; Washko, P.W.; Dhariwal, K.R.; Park, J.B.; Lazarev, A.; Graumlich, J.F.; King, J.; et al. Vitamin C pharmacokinetics in healthy volunteers: Evidence for a recommended dietary allowance. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 3704–3709. [CrossRef] [PubMed]
58. Hodges, R.E.; Hood, J.; Canham, J.E.; Sauberlich, H.E.; Baker, E.M. Clinical manifestations of ascorbic acid deficiency in man. *Am. J. Clin. Nutr.* **1971**, *24*, 432–443. [CrossRef]
59. Schencking, M.; Vollbracht, C.; Weiss, G.; Lebert, J.; Biller, A.; Goyvaerts, B.; Kraft, K. Intravenous vitamin C in the treatment of shingles: Results of a multicenter prospective cohort study. *Med. Sci. Monit.* **2012**, *18*, CR215–CR224. [CrossRef] [PubMed]

60. Vollbracht, C.; Kraft, K. Feasibility of vitamin C in the treatment of post viral fatigue with focus on long COVID, based on a systematic review of IV vitamin C on fatigue. *Nutrients* **2021**, *13*, 1154. [CrossRef]
61. Carr, A.C.; McCall, C. The role of vitamin C in the treatment of pain: New insights. *J. Transl. Med.* **2017**, *15*, 77. [CrossRef]
62. de Grooth, H.J.; Manubulu-Choo, W.P.; Zandvliet, A.S.; Spoelstra-de Man, A.M.E.; Girbes, A.R.; Swart, E.L.; Oudemans-van Straaten, H.M. Vitamin-C pharmacokinetics in critically ill patients: A randomized trial of four intravenous regimens. *Chest* **2018**, *153*, 1368–1377. [CrossRef]
63. Townsend, L.; Dyer, A.H.; McCluskey, P.; O'Brien, K.; Dowds, J.; Laird, E.; Bannan, C.; Bourke, N.M.; C, N.C.; Byrne, D.G.; et al. Investigating the relationship between vitamin D and persistent symptoms following SARS-CoV-2 infection. *Nutrients* **2021**, *13*, 2430. [CrossRef]
64. Kalokerinos, A. *Every Second Child*; Keats Pub: Wilkes-Barre, PA, USA, 1981; p. 174.
65. Kalokerinos, A.; Dettman, G. Sudden death in infancy syndrome in Western Australia. *Med. J. Aust.* **1976**, *2*, 31–32. [CrossRef]
66. Otomaru, K.; Oishi, S.; Fujimura, Y.; Iwamoto, Y.; Nagai, K.; Ijiri, M. Effects of vitamin C supplementation on the blood oxidative stress and antibody titre against Histophilus somni vaccination in calves. *J. Vet. Res.* **2021**, *65*, 73–78. [CrossRef] [PubMed]
67. Prinz, W.; Bloch, J.; Gilich, G.; Mitchell, G. A systematic study of the effect of vitamin C supplementation on the humoral immune response in ascorbate-dependent mammals. I. The antibody response to sheep red blood cells (a T-dependent antigen) in guinea pigs. *Int. J. Vitam. Nutr. Res.* **1980**, *50*, 294–300. [PubMed]
68. Feigen, G.A.; Smith, B.H.; Dix, C.E.; Flynn, C.J.; Peterson, N.S.; Rosenberg, L.T.; Pavlovic, S.; Leibovitz, B. Enhancement of antibody production and protection against systemic anaphylaxis by large doses of vitamin C. *Res. Commun. Chem. Pathol. Pharmacol.* **1982**, *38*, 313–333. [CrossRef]
69. World Health Organization. Ten threats to global health in 2019. Available online: http://www.who.int/emergencies/ten-threats-to-global-health-in-2019 (accessed on 29 July 2019).
70. The Delphi Group at Carnegie Mellon University. *COVID-19 Symptom Survey: Topline Report on COVID-19 Vaccination in the United States*; Carnegie Mellon University: Pittsburgh, PA, USA, 2021; p. 83.
71. Kashi, D.S.; Oliver, S.J.; Wentz, L.M.; Roberts, R.; Carswell, A.T.; Tang, J.C.Y.; Jackson, S.; Izard, R.M.; Allan, D.; Rhodes, L.E.; et al. Vitamin D and the hepatitis B vaccine response: A prospective cohort study and a randomized, placebo-controlled oral vitamin D(3) and simulated sunlight supplementation trial in healthy adults. *Eur. J. Nutr.* **2021**, *60*, 475–491. [CrossRef] [PubMed]
72. Lee, M.D.; Lin, C.H.; Lei, W.T.; Chang, H.Y.; Lee, H.C.; Yeung, C.Y.; Chiu, N.C.; Chi, H.; Liu, J.M.; Hsu, R.J.; et al. Does vitamin D deficiency affect the immunogenic responses to influenza vaccination? A systematic review and meta-analysis. *Nutrients* **2018**, *10*, 409. [CrossRef]
73. Kriesel, J.D.; Spruance, J. Calcitriol (1,25-dihydroxy-vitamin D3) coadministered with influenza vaccine does not enhance humoral immunity in human volunteers. *Vaccine* **1999**, *17*, 1883–1888. [CrossRef]
74. Chillon, T.S.; Demircan, K.; Heller, R.A.; Hirschbil-Bremer, I.M.; Diegmann, J.; Bachmann, M.; Moghaddam, A.; Schomburg, L. Relationship between vitamin D status and antibody response to COVID-19 mRNA vaccination in healthy adults. *Biomedicines* **2021**, *9*, 1714. [CrossRef]
75. Goncalves-Mendes, N.; Talvas, J.; Dualé, C.; Guttmann, A.; Corbin, V.; Marceau, G.; Sapin, V.; Brachet, P.; Evrard, B.; Laurichesse, H.; et al. Impact of vitamin D supplementation on influenza vaccine response and immune functions in deficient elderly persons: A randomized placebo-controlled trial. *Front. Immunol.* **2019**, *10*, 65. [CrossRef]
76. Amrein, K.; Scherkl, M.; Hoffmann, M.; Neuwersch-Sommeregger, S.; Köstenberger, M.; Tmava Berisha, A.; Martucci, G.; Pilz, S.; Malle, O. Vitamin D deficiency 2.0: An update on the current status worldwide. *Eur. J. Clin. Nutr.* **2020**, 1–16. [CrossRef]

nutrients

MDPI

Review

Optimal Nutritional Status for a Well-Functioning Immune System Is an Important Factor to Protect against Viral Infections

Philip C. Calder [1], Anitra C. Carr [2], Adrian F. Gombart [3] and Manfred Eggersdorfer [4,*]

[1] Faculty of Medicine, University of Southampton and NIHR Southampton Biomedical Research Centre, University Hospital Southampton NHS Foundation Trust, Tremona Road, Southampton SO16-6YD, UK; P.C.Calder@soton.ac.uk

[2] Nutrition in Medicine Research Group, Department of Pathology & Biomedical Science, University of Otago, Christchurch, P.O. Box 4345, Christchurch 8140, New Zealand; anitra.carr@otago.ac.nz

[3] Linus Pauling Institute, Department of Biochemistry and Biophysics, Oregon State University, 307 Linus Pauling Science Center, Corvallis, OR 97331, USA; adrian.gombart@oregonstate.edu

[4] Department of Internal Medicine, University Medical Center Groningen, 9713 GZ Groningen, The Netherlands

* Correspondence: m.eggersdorfer@bluewin.ch

Received: 10 March 2020; Accepted: 18 April 2020; Published: 23 April 2020

Abstract: Public health practices including handwashing and vaccinations help reduce the spread and impact of infections. Nevertheless, the global burden of infection is high, and additional measures are necessary. Acute respiratory tract infections, for example, were responsible for approximately 2.38 million deaths worldwide in 2016. The role nutrition plays in supporting the immune system is well-established. A wealth of mechanistic and clinical data show that vitamins, including vitamins A, B_6, B_{12}, C, D, E, and folate; trace elements, including zinc, iron, selenium, magnesium, and copper; and the omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid play important and complementary roles in supporting the immune system. Inadequate intake and status of these nutrients are widespread, leading to a decrease in resistance to infections and as a consequence an increase in disease burden. Against this background the following conclusions are made: (1) supplementation with the above micronutrients and omega-3 fatty acids is a safe, effective, and low-cost strategy to help support optimal immune function; (2) supplementation above the Recommended Dietary Allowance (RDA), but within recommended upper safety limits, for specific nutrients such as vitamins C and D is warranted; and (3) public health officials are encouraged to include nutritional strategies in their recommendations to improve public health.

Keywords: immune system; viral infection; influenza; COVID-19; micronutrients; vitamins; omega-3 fatty acids; minerals; vitamin C; vitamin D

1. Introduction

Acute respiratory tract infections are a major cause of morbidity and mortality across the globe, as illustrated by both seasonal influenza epidemics, and the recent outbreak of the coronavirus disease, COVID-19, caused by SARS-CoV-2 infection. The World Health Organization (WHO) estimates that worldwide, seasonal influenza alone results in 3–5 million cases of severe illness that require hospitalization, and 290,000–650,000 deaths annually [1]. In aggregate, acute respiratory tract illnesses were estimated to be responsible for approximately 2.38 million deaths worldwide in 2016 [2,3]. Indeed, severe lower respiratory tract infections were the most common cause of sepsis-related death globally from 1990–2017 [4].

A number of standard public health practices have been developed to help limit the spread and impact of respiratory viruses, such as regular hand washing, avoiding those showing symptoms of infection, and covering coughs [5]. For certain viruses, such as influenza, annual vaccination campaigns designed to prime the immune response in case of exposure exist in many countries. Influenza is caused by a single-stranded RNA virus, and as such exhibits high mutation rates and rapid evolution, which may allow these viruses to escape from pre-existing neutralizing antibodies in the host [6]. Vaccination programs therefore must make predictions each year as to which strains to vaccinate against, with varying degrees of success. In the US, the Centers for Disease Control and Prevention estimate the current year influenza vaccine to be 45% effective for preventing medically attended, laboratory-confirmed influenza virus. This is consistent with estimates from the previous years when the influenza vaccines were antigenically matched to the circulating viruses [7]. Since the 2011–2012 season, vaccine efficacy has ranged from 19%–54% [8].

The immune system is comprised of both the innate (fast, non-antigen specific) and adaptive (slower, antigen-specific) responses. The innate immune system is comprised of physical barriers that help prevent pathogen entry (e.g., skin, gut epithelium), antimicrobial peptides, the complement system, and a variety of phagocytic and other cells (e.g., neutrophils, macrophages, natural killer cells), that recognize the presence of pathogens via the expression of nonspecific pattern-recognition receptors [9]. The innate system moves quickly to recognize and destroy "non-self" threats, typically via inflammatory processes, and then resolve the inflammation and repair the damage caused by these events [9]. However, innate immunity does not increase efficacy or speed of response with repeated exposure to a pathogen. Subsequent to the innate response, the adaptive response is engaged. The adaptive response includes antigen-specific cells, e.g., T lymphocytes, subsets of which coordinate the overall adaptive response or kill virally-infected cells, and B lymphocytes, which can be activated to secrete antibodies specific to the infecting pathogen [9]. While slower to respond than the innate system, the adaptive system is responsible for generating immunological "memory", whereby a repeated infection with the same pathogen will generate a vigorous, fast antigen-specific response [9]. The induction of immunological memory is the mechanism by which vaccines can provide protection against subsequent pathogen exposure.

Undoubtedly, public hygiene practices and, when available, vaccinations can be effective mechanisms to provide protection against infectious disease. However, vaccines can take years to create, are not available against all viruses (including the current coronavirus SARS-CoV-2), and provide varying levels of protection. The morbidity and mortality numbers cited above highlight the need for additional strategies to support the immune system, in order to reduce the impact of respiratory and other infections.

2. Nutritional Impact on Immunity

Often missing in public health discussions around immunity and infection are nutritional strategies to support optimal function of the immune system. This is surprising, given that the importance that nutrition plays in immune function is well established. Several vitamins, including vitamins A, B_6, B_{12}, C, D, E, and folate; and trace elements, including zinc, iron, selenium, magnesium, and copper, play important and complementary roles in supporting both the innate and adaptive immune systems. Deficiencies or suboptimal status in micronutrients negatively affect immune function and can decrease resistance to infections [10–12]. Indeed, with the exceptions of vitamin E and magnesium, each of these micronutrients has been granted health claims in the European Union for contributing to the normal function of the immune system [13]. Other nutrients such as omega-3 fatty acids also support an effective immune system, specifically by helping to resolve the inflammatory response [14].

The mechanistic roles that micronutrients play to optimize immune function have been well-described recently [10,12]. Most micronutrients exhibit pleiotropic roles in supporting immune function. With respect to innate immunity, the vitamins and minerals listed above collectively function to support the development and maintenance of physical barriers; production and activity of antimicrobial

proteins; growth, differentiation and motility/chemotaxis of innate cells; phagocytic and killing (e.g., oxidative burst) activities of neutrophils and macrophages; and promotion of and recovery from inflammation (e.g., cytokine production and antioxidant activity). They also support adaptive immunity, via lymphocyte differentiation, proliferation and homing; cytokine production; antibody production; and the generation of memory cells. The roles that vitamins C and D play in immunity are particularly well elucidated. Vitamin C affects several aspects of immunity, including supporting epithelial barrier function, growth and function of both innate and adaptive immune cells, white blood cell migration to sites of infection, phagocytosis and microbial killing, and antibody production [10]. Many immune cells have vitamin D receptors that affect their function after ligand binding, and as such vitamin D profoundly influences immunity. For example, it promotes differentiation of monocytes to macrophages and increases their killing capacity; modulates the production of inflammatory cytokines; and supports antigen presentation. Furthermore, vitamin D metabolites appear to regulate production of specific antimicrobial proteins that directly kill pathogens, and thus are likely to help reduce infection including in the lungs [15,16].

As mentioned above, inflammation is a key component of the immune response. This response is caused by a variety of pro-inflammatory mediators, produced by several different types of cells, resulting in the influx of fluid, immune cells, and other mediators that function to eliminate the infection. Inflammation typically resolves quickly at the end of the immune response, due to activation of specific negative-feedback mechanisms. Among these, the omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) present at the site of inflammation are enzymatically converted to specialized pro-resolving mediators (SPMs) known as resolvins, protectins, and maresins. These molecules, along with others, function together to orchestrate the resolution of inflammation and to support healing, including in the respiratory tract [14,17]. Notably, nutritional deficiencies in these essential fatty acids can result in delayed or suboptimal resolution of inflammation [17]. This could be very important in the context of severe COVID-19 which manifests as uncontrolled inflammation, the so-called cytokine storm [18,19], linked with acute respiratory distress syndrome (ARDS). A number of the SPMs formed from EPA and DHA have been shown in animal models to both protect against and resolve acute lung injury and ARDS [20–24]. Nutritional formulas containing antioxidants and rich in EPA and DHA have been used in several human trials of patients with ARDS. A recent Cochrane review of these trials identified a significant improvement in blood oxygenation and significant reductions in ventilation requirement, new organ failures, length of stay in the intensive care unit and mortality at 28 days [25]. Taken together, these findings suggest an important role for EPA and DHA in ameliorating inflammation and lung injury, perhaps acting via conversion to SPMs.

It is not surprising, then, that deficiencies and even suboptimal status of these nutrients can impair immune functions. Depending on the deficient nutrient or nutrients, there can be decreases in the numbers of lymphocytes, impairment of phagocytosis and microbial killing by innate immune cells, altered production of cytokines, reduced antibody responses, and even impairments in wound healing [12]. These functional impairments are, presumably, what lead to the clinical immune-related manifestations of deficiency. Indeed, people deficient in vitamin C are susceptible to severe respiratory infections such as pneumonia [10,26]. A recent meta-analysis reported a significant reduction in the risk of pneumonia with vitamin C supplementation, particularly in individuals with low dietary intakes [27]. In older patients, disease severity and risk of death were reduced with supplementation, particularly in the case where initial plasma levels of vitamin C were low [27]. Vitamin C supplementation has also been shown to decrease the duration and severity of upper respiratory tract infections, such as the common cold, and significantly decrease the risk of infection when given prophylactically in people under enhanced physical stress [26,28].

Likewise, vitamin D deficiency increases the risk for respiratory infection. Observational studies report an association between low blood concentrations of 25-hydroxyvitamin D (the major vitamin D metabolite) and susceptibility to acute respiratory tract infections [29,30]. Consistent with these findings, several recent meta-analyses have concluded that vitamin D supplementation can reduce the risk of

respiratory tract infections in both children and adults [11,31–35]. In 2017, Martineau and colleagues performed a systematic review and meta-analysis of individual participant data (n = 10,933) from 25 randomized, double blind, placebo controlled trials of vitamin D supplementation with a specified outcome of acute respiratory tract infection (ARI). They found a 12% reduction for experiencing at least one ARI irrespective of dosing schedule [11]. They found a 19% reduction in individuals taking a daily or weekly dose without bolus doses and no benefit with bolus dosing. Among those receiving a daily or weekly dose, they observed a 25% reduction for those with baseline 25(OH)D levels ≥25 nmol/L (12 ng/mL) and a 70% reduction for those with baseline levels <25 nmol/L [23]. They concluded that daily or weekly vitamin D supplementation protected against ARI overall and that it was safe.

Clinical outcomes also demonstrate a role for vitamin E in respiratory tract infections. In a randomized controlled trial of 617 nursing home residents, daily supplementation for one year with 200 IU vitamin E reduced the risk of upper respiratory tract infections, but not lower respiratory tract infections [36]. Vitamin E enhances T cell-mediated immune function in the face of age-related decline [37]. In one study, supplementation of older adults with vitamin E improved natural killer cell activity, neutrophil chemotaxis and phagocytosis, and mitogen-induced lymphocyte proliferation [38]. In a second study, vitamin E supplementation improved T cell-mediated immunity as measured by increased production of antibodies to hepatitis B virus and tetanus vaccines [39].

Finally, marginal zinc deficiency can also impact immunity. Zinc is important for maintenance and development of cells in both the innate and adaptive immune systems. Zinc deficiency results in impaired formation, activation and maturation of lymphocytes, disturbs the intercellular communication via cytokines, and weakens the innate host defense [40,41]. Those deficient in zinc, particularly children, are prone to increased diarrheal and respiratory morbidity [42,43].

Furthermore, data from animal models and epidemiological studies in people indicate that deficiency in specific nutrients, particularly selenium and vitamin E, can lead to reproducible genetic mutations and increased virulence of certain viruses, including coxsackievirus, poliovirus, and murine influenza [44,45]. In a double-blind placebo controlled study, an increase of selenium intake by otherwise healthy subjects with relatively low levels of plasma selenium concentrations improved cellular immunity. Subjects receiving selenium cleared an oral live attenuated poliomyelitis vaccine more rapidly and sequence analysis of the viral genome showed lower numbers of mutations as compared to those receiving the placebo. These data suggest that suboptimal nutrient status in the host population could lead to the emergence of more pathogenic strains of viral diseases, thereby increasing the risks and burdens associated with these illnesses. Given the current situation, it may be beneficial to further pursue this line of investigation.

Optimal intake of all these nutrients ideally would be achieved through the consumption of a well-balanced and diverse diet, but this can be difficult to accomplish for the general population. Indeed, it is generally accepted that nutrient inadequacies and deficiencies are widespread [46–50] (and references therein). Biochemical markers of nutrient status are particularly useful in assessing inadequacy or deficiency, and lead to the conclusion that intakes often are not sufficient. For example, extensive data have been published, using blood 25-hydroxyvitamin D levels to assess vitamin D status. A systematic review involving 195 studies in forty-four countries reported that 37.3% of the studies found mean values lower than 50 nmol/L [51]. The US Institute of Medicine (IOM) committee that reviewed Dietary Reference Intakes (DRI) for vitamin D has suggested that those with concentrations less than this level are at risk for inadequacy, while those with concentrations between 50–75 nmol/L are considered sufficient [52,53]. Interestingly, while the highest vitamin D levels were reported in North America, data from the United States still indicate that 8% of the non-infant population was at risk for vitamin D deficiency, and 17% exhibited concentrations below the 25(OH)D level that is associated with desirable intake [53]. Other studies based on 25(OH)D levels indicate that vitamin D inadequacy or deficiency are also prevalent in Europe and China [54–56]. Similarly, a recent systematic review involving 132 studies of serum alpha-tocopherol status indicated that 13% of the values were below the threshold of deficiency (12 mmol/L). Deficiency was noted in the Americas, Asia Pacific, Europe,

the Middle East and Africa [57]. The situation with vitamin C is similar. Currently, the most commonly used vitamin C cutoff levels are approximately ≤23–28 μmol/L for hypovitaminosis C and ≤11 μmol/L for deficiency [58]. The evidence indicates that vitamin C insufficiency or deficiency is common in low and middle-income countries (e.g., Mexico, Brazil, India), and not uncommon in high income countries (e.g., US, Singapore, New Zealand), particularly in at-risk subpopulations [53,59–67]. Furthermore, the WHO and the Food and Agriculture Organization (FAO) of the United Nations have described that, based on blood markers, vitamin A and iron deficiencies are widespread and of significant global concern [46,49,50]. Status data in the general population or specific subpopulations also reveal inadequacies or deficiencies in various countries, including in developed nations, for vitamins B6, B12, and folate, as well as zinc and selenium [53,59,60,68–73]. Finally, a global survey of EPA + DHA status in the blood, from 298 studies, found "low" or "very low" status (i.e., levels associated with increased risk of cardiovascular related mortality) of EPA + DHA in most of the countries assessed [74]. Collectively, the totality of these data strongly suggest that micronutrient and omega-3 inadequacies or deficiencies are prevalent around the globe.

It should also be noted that optimal nutritional support for the immune system can require intakes above the RDA for some micronutrients, while at the same time infections and other stressors can reduce micronutrient status in the body. Vitamin C levels, in particular, decrease during times of infection and higher intakes are required to restore normal blood levels [10,75]. These higher intakes and blood levels are associated with improved clinical outcomes. For example, supplementation of pneumonia patients with ≥200 mg/d vitamin C restored depleted plasma and cellular vitamin C levels, and resulted in decreased respiratory symptom scores and a dose-dependent decrease in hospital length of stay [76,77].

3. Recommendations and Conclusions

Thus, a set of clear nutritional recommendations is needed (Table 1). First, supplementation with micronutrients and omega-3 fatty acids is a safe, effective, and low-cost way to help eliminate nutritional gaps and support optimal immune function, and therefore reduce the risk and consequences of infections [10,12]. Intakes should follow recommended upper safety limits set by expert authorities, such as the European Food Safety Authority and, in the United States, the IOM. Thus, a multivitamin and mineral supplement that supplies the basic micronutrient requirements (e.g., RDA) for vitamins and minerals is recommended in addition to the consumption of a well-balanced diet.

Table 1. Recommended intakes of selected nutrients to support optimal immune function.

Nutrient	Rationale	Recommendation
Vitamins and trace elements	These micronutrients play important roles in supporting the cells and tissues of the immune system. Deficiencies or suboptimal status in these micronutrients negatively affect immune function and can decrease resistance to infections.	A multivitamin and trace element supplement that supplies the nutrient requirements (e.g., 100% US RDA for age and gender) [78] for vitamins and trace elements including vitamins A, B_6, B_{12}, C, D, E, and folate, and trace elements including zinc, iron, selenium, magnesium and copper. This is in addition to the consumption of a well-balanced diet.
Vitamin C	Doses of ≥200 mg/day provide saturating levels in the blood, and support reduction in the risk, severity and duration of upper and lower respiratory tract infections. Requirements for vitamin C increase during infection.	Daily intake of at least 200 mg/day for healthy individuals. In individuals who are sick, 1–2 g/day is recommended.
Vitamin D	Daily supplementation of vitamin D reduces the risk of acute respiratory tract infections.	Daily intake of 2000 IU/day (50 μg/day).
Zinc	Marginal zinc deficiency can impact immunity. Those deficient in zinc, particularly children, are prone to increased diarrheal and respiratory morbidity.	Daily intake in the range of 8–11 mg/day.
Omega-3 fatty acids (EPA + DHA)	Omega-3 fatty acids support an effective immune system, including by helping to resolve inflammation.	Daily intake of 250 mg/day of EPA + DHA.

Second, we recommend supplementation above the RDA for vitamins C and D. As noted above, recent meta-analyses concluded significant reductions in the risk and impact of both upper and lower respiratory tract infections such as the common cold and pneumonia, including disease severity and risk of death in older patients, with vitamin C supplementation [27,28,79]. Based on this evidence, a daily intake of at least 200 mg/day for healthy individuals is recommended. This level is above the US RDA of 75 and 90 mg/day for female and male adults, respectively [80]. It should be noted that vitamin C requirements depend on health status, and 1–2 g/day are recommended to restore normal blood levels in individuals who are sick, beginning at the onset of symptoms. These levels are within the US tolerable upper limit (TUL) for adults of 2 g/day (note that the upper limit for children aged 1–3 years is 400 mg/day) [80].

Several recent meta-analyses have concluded that vitamin D supplementation reduces the risk of respiratory tract infections in both children and adults [11,31–35]. Protective effects were seen with those receiving daily or weekly vitamin D, but not with less frequent bolus doses [11,32]. A daily intake of 2000 IU (50 μg) is recommended. This is above the US RDA of 400–800 IU (depending on age), but below the TUL for those over 1 year of age (2500–4000 IU) [52].

A third recommendation involves the omega-3 fatty acids EPA and DHA. An adequate intake supports the resolution of inflammation via the production of anti-inflammatory metabolites of these fatty acids, including in the respiratory tract [14,17]. An intake of 250 mg EPA + DHA per day is recommended, consistent with global, regional and national expert recommendations [81–83].

Public health practices, such as vaccinations and hygiene measures, are important measures that help limit the spread and impact of infections, including against acute respiratory viruses. However, the present situation with SARS-CoV-2 infection and severe outcomes of COVID-19 and the annual morbidity and mortality figures for respiratory infections overall make it clear that these practices alone are not sufficient. New strains of influenza continuously emerge, necessitating development of new vaccines with varying efficacy, and outbreaks of novel viruses can be enormously difficult to contain. As such, additional safe and cost-effective strategies are needed to support the immune system, and further protect individuals and populations from harm. One compelling strategy is to provide sufficient nutritional support for the immune system. As described above, optimal nutrient intake, including supplementing above the RDA for certain immune-supporting vitamins, promotes optimal immune function, helps to control the impact of infections, and could help limit the emergence of novel, more virulent strains of pathogenic viruses. We, therefore, strongly encourage public health officials to also include nutritional strategies in their arsenal to improve public health and to limit the impact of seasonal and emerging viral infections.

Author Contributions: The outline of the publication was developed with input from all authors based on an expert webinar meeting they held and the conclusions they reached concerning the role of the immune system to reduce risk for infections including viral infections; All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: P.C.C. has research funding from BASF AS and Bayer Consumer Care; acts as an advisor/consultant to BASF AS, DSM, Cargill, Smartfish, Nutrileads, Bayer Consumer Care, and Pfizer (now GSK) Consumer Healthcare; has received reimbursement for travel and/or speaking from Danone, Fresenius Kabi, Baxter, Pfizer (now GSK) Consumer Healthcare, Abbott, Smartfish, Biogredia and the California Walnut Commission; and is President and member of the Board of Directors of the European Branch of the International Life Sciences Institute. A.C.C. has received research funding from Bayer Consumer Care and travel reimbursement from DSM. M.E. acts as an advisor for DSM and received travel reimbursement from DSM. He is member of the Scientific Board of PM International and President of the Gesellschaft für angewandte Vitaminforschung. A.F.G. has received research funding from Bayer Consumer Care and has acted as an advisor/consultant for and has received reimbursement for travel and/or speaking from Bayer Consumer Care.

References

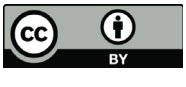
1. World Health Organization Influenza (Seasonal). Available online: https://www.who.int/news-room/fact-sheets/detail/influenza-(seasonal) (accessed on 2 March 2020).

2. Naghavi, M.; Abajobir, A.A.; Abbafati, C.; Abbas, K.M.; Abd-Allah, F.; Abera, S.F.; Aboyans, V.; Adetokunboh, O.; Afshin, A.; Agrawal, A.; et al. Global, regional, and national age-sex specific mortality for 264 causes of death, 1980–2016: A systematic analysis for the Global Burden of Disease Study 2016. *Lancet* **2017**, *390*, 1151–1210. [CrossRef]
3. Troeger, C.; Blacker, B.; Khalil, I.A.; Rao, P.C.; Cao, J.; Zimsen, S.R.M.; Albertson, S.B.; Deshpande, A.; Farag, T.; Abebe, Z.; et al. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory infections in 195 countries, 1990–2016: A systematic analysis for the Global Burden of Disease Study 2016. *Lancet Infect. Dis.* **2018**, *18*, 1191–1210. [CrossRef]
4. Rudd, K.E.; Johnson, S.C.; Agesa, K.M.; Shackelford, K.A.; Tsoi, D.; Kievlan, D.R.; Colombara, D.V.; Ikuta, K.S.; Kissoon, N.; Finfer, S.; et al. Global, regional, and national sepsis incidence and mortality, 1990–2017: Analysis for the Global Burden of Disease Study. *Lancet* **2020**, *395*, 200–211. [CrossRef]
5. U.S. Centers for Disease Control Take 3 Actions to Fight Flu. Available online: https://www.cdc.gov/flu/prevent/preventing.htm (accessed on 2 March 2020).
6. Visher, E.; Whitefield, S.E.; McCrone, J.T.; Fitzsimmons, W.; Lauring, A.S. The mutational robustness of Influenza A virus. *PLoS Pathog.* **2016**, *12*, e1005856. [CrossRef] [PubMed]
7. Dawood, F.S.; Chung, J.R.; Kim, S.S.; Zimmerman, R.K.; Nowalk, M.P.; Jackson, M.L.; Jackson, L.A.; Monto, A.S.; Martin, E.T.; Belongia, E.A.; et al. Interim estimates of 2019–20 seasonal influenza vaccine effectiveness—United States, February 2020. *Morb. Mortal. Wkly. Rep.* **2020**, *69*, 177–182. [CrossRef] [PubMed]
8. U.S. Centers for Disease Control Seasonal Influenza Vaccine Effectiveness, 2018–2019. Available online: https://www.cdc.gov/flu/vaccines-work/2018-2019.html (accessed on 2 March 2020).
9. Murphy, K.; Weaver, C. *Janeway's Immunobiology*, 9th ed.; Taylor & Francis: Philadelphia, PA, USA, 2017; pp. 1–35.
10. Carr, A.C.; Maggini, S. Vitamin C and immune function. *Nutrients* **2017**, *9*, 1211. [CrossRef]
11. Martineau, A.R.; Jolliffe, D.A.; Hooper, R.L.; Greenberg, L.; Aloia, J.F.; Bergman, P.; Dubnov-Raz, G.; Esposito, S.; Ganmaa, D.; Ginde, A.A.; et al. Vitamin D supplementation to prevent acute respiratory tract infections: Systematic review and meta-analysis of individual participant data. *BMJ* **2017**, *356*, i6583. [CrossRef]
12. Gombart, A.F.; Pierre, A.; Maggini, S. A review of micronutrients and the immune system–working in harmony to reduce the risk of infection. *Nutrients* **2020**, *12*, 236. [CrossRef]
13. EU Register on Nutrition and Health Claims. Available online: https://ec.europa.eu/food/safety/labelling_nutrition/claims/register/public/?event=search (accessed on 5 March 2020).
14. Calder, P.C. Omega-3 polyunsaturated fatty acids and inflammatory processes: Nutrition or pharmacology?: Omega-3 fatty acids and inflammation. *Br. J. Clin. Pharmacol.* **2012**, *75*, 645–662. [CrossRef]
15. Gombart, A.F. The vitamin D–antimicrobial peptide pathway and its role in protection against infection. *Future Microbiol.* **2009**, *4*, 1151. [CrossRef]
16. Greiller, C.; Martineau, A. Modulation of the immune response to respiratory viruses by vitamin D. *Nutrients* **2015**, *7*, 4240–4270. [CrossRef] [PubMed]
17. Basil, M.C.; Levy, B.D. Specialized pro-resolving mediators: Endogenous regulators of infection and inflammation. *Nat. Rev. Immunol.* **2016**, *16*, 51–67. [CrossRef] [PubMed]
18. Mehta, P.; McAuley, D.F.; Brown, M.; Sanchez, E.; Tattersall, R.S.; Manson, J.J. COVID-19: Consider cytokine storm syndromes and immunosuppression. *Lancet* **2020**, *395*, 1033–1034. [CrossRef]
19. Pedersen, S.F.; Ho, Y.-C. SARS-CoV-2: A Storm is Raging. *J. Clin. Investig.* **2020**. [CrossRef]
20. Gao, Y.; Zhang, H.; Luo, L.; Lin, J.; Li, D.; Zheng, S.; Huang, H.; Yan, S.; Yang, J.; Hao, Y.; et al. Resolvin D1 improves the resolution of inflammation via activating NF-κB p50/p50–mediated cyclooxygenase-2 expression in acute respiratory distress syndrome. *J. Immunol.* **2017**, *199*, 2043–2054. [CrossRef]
21. Wang, Q.; Yan, S.-F.; Hao, Y.; Jin, S.-W. Specialized pro-resolving mediators regulate alveolar fluid clearance during acute respiratory distress syndrome. *Chin. Med. J.* **2018**, *131*, 982–989. [CrossRef]
22. Sham, H.P.; Walker, K.H.; Abdulnour, R.-E.E.; Krishnamoorthy, N.; Douda, D.N.; Norris, P.C.; Barkas, I.; Benito-Figueroa, S.; Colby, J.K.; Serhan, C.N.; et al. 15-epi-Lipoxin A_4, Resolvin D2, and Resolvin D3 induce NF-κB regulators in bacterial pneumonia. *J. Immunol.* **2018**, *200*, 2757–2766. [CrossRef]

23. Sekheri, M.; El Kebir, D.; Edner, N.; Filep, J.G. 15-Epi-LXA$_4$ and 17-epi-RvD1 restore TLR9-mediated impaired neutrophil phagocytosis and accelerate resolution of lung inflammation. *Proc. Natl. Acad. Sci. USA* **2020**. [CrossRef]
24. Zhang, H.-W.; Wang, Q.; Mei, H.-X.; Zheng, S.-X.; Ali, A.M.; Wu, Q.-X.; Ye, Y.; Xu, H.-R.; Xiang, S.-Y.; Jin, S.-W. RvD1 ameliorates LPS-induced acute lung injury via the suppression of neutrophil infiltration by reducing CXCL2 expression and release from resident alveolar macrophages. *Int. Immunopharmacol.* **2019**, *76*, 105877. [CrossRef]
25. Dushianthan, A.; Cusack, R.; Burgess, V.A.; Grocott, M.P.; Calder, P.C. Immunonutrition for acute respiratory distress syndrome (ARDS) in adults. *Cochrane Database Syst. Rev.* **2019**. [CrossRef]
26. Hemilä, H. Vitamin C and infections. *Nutrients* **2017**, *9*, 339. [CrossRef]
27. Hemilä, H.; Louhiala, P. Vitamin C for preventing and treating pneumonia. *Cochrane Database Syst. Rev.* **2013**. [CrossRef] [PubMed]
28. Hemilä, H.; Chalker, E. Vitamin C for preventing and treating the common cold. *Cochrane Database Syst. Rev.* **2013**. [CrossRef] [PubMed]
29. Cannell, J.J.; Vieth, R.; Umhau, J.C.; Holick, M.F.; Grant, W.B.; Madronich, S.; Garland, C.F.; Giovannucci, E. Epidemic influenza and vitamin D. *Epidemiol. Infect.* **2006**, *134*, 1129–1140. [CrossRef]
30. Jolliffe, D.A.; Griffiths, C.J.; Martineau, A.R. Vitamin D in the prevention of acute respiratory infection: Systematic review of clinical studies. *J. Steroid Biochem. Mol. Biol.* **2013**, *136*, 321–329. [CrossRef]
31. Autier, P.; Mullie, P.; Macacu, A.; Dragomir, M.; Boniol, M.; Coppens, K.; Pizot, C.; Boniol, M. Effect of vitamin D supplementation on non-skeletal disorders: A systematic review of meta-analyses and randomised trials. *Lancet Diabetes Endocrinol.* **2017**, *5*, 986–1004. [CrossRef]
32. Martineau, A.R.; Jolliffe, D.A.; Greenberg, L.; Aloia, J.F.; Bergman, P.; Dubnov-Raz, G.; Esposito, S.; Ganmaa, D.; Ginde, A.A.; Goodall, E.C.; et al. Vitamin D supplementation to prevent acute respiratory infections: Individual participant data meta-analysis. *Health Technol. Assess* **2019**, *23*, 1–44. [CrossRef]
33. Rejnmark, L.; Bislev, L.S.; Cashman, K.D.; Eiríksdottir, G.; Gaksch, M.; Grübler, M.; Grimnes, G.; Gudnason, V.; Lips, P.; Pilz, S.; et al. Non-skeletal health effects of vitamin D supplementation: A systematic review on findings from meta-analyses summarizing trial data. *PLoS ONE* **2017**, *12*, e0180512. [CrossRef]
34. Bergman, P.; Lindh, Å.U.; Björkhem-Bergman, L.; Lindh, J.D. Vitamin D and respiratory tract infections: A systematic review and meta-analysis of randomized controlled trials. *PLoS ONE* **2013**, *8*, e65835. [CrossRef]
35. Charan, J.; Goyal, J.P.; Saxena, D.; Yadav, P. Vitamin D for prevention of respiratory tract infections: A systematic review and meta-analysis. *J. Pharmacol. Pharmacother.* **2012**, *3*, 300. [CrossRef]
36. Meydani, S.N.; Leka, L.S.; Fine, B.C.; Dallal, G.E.; Keusch, G.T.; Singh, M.F.; Hamer, D.H. Vitamin E and respiratory tract infections in elderly nursing home residents: A randomized controlled trial. *JAMA* **2004**, *292*, 828–836. [CrossRef] [PubMed]
37. Wu, D.; Meydani, S. Age-associated changes in immune function: Impact of vitamin E intervention and the underlying mechanisms. *Endocr. Metab. Immune Disord. Drug Targets* **2014**, *14*, 283–289. [CrossRef] [PubMed]
38. De la Fuente, M.; Hernanz, A.; Guayerbas, N.; Manuel Victor, V.; Arnalich, F. Vitamin E ingestion improves several immune functions in elderly men and women. *Free Radic. Res.* **2008**, *42*, 272–280. [CrossRef] [PubMed]
39. Meydani, S.N. Vitamin E supplementation and in vivo immune response in healthy elderly subjects. A randomized controlled trial. *JAMA* **1997**, *277*, 1380–1386. [CrossRef]
40. Gammoh, N.Z.; Rink, L. Zinc in infection and inflammation. *Nutrients* **2017**, *9*, 624. [CrossRef]
41. Maares, M.; Haase, H. Zinc and immunity: An essential interrelation. *Arch. Biochem. Biophys.* **2016**, *611*, 58–65. [CrossRef]
42. Aggarwal, R.; Sentz, J.; Miller, M.A. Role of zinc administration in prevention of childhood diarrhea and respiratory illnesses: A meta-analysis. *Pediatrics* **2007**, *119*, 1120–1130. [CrossRef]
43. Roth, D.E.; Richard, S.A.; Black, R.E. Zinc supplementation for the prevention of acute lower respiratory infection in children in developing countries: Meta-analysis and meta-regression of randomized trials. *Int. J. Epidemiol.* **2010**, *39*, 795–808. [CrossRef]
44. Beck, M.A.; Levander, O.A.; Handy, J. Selenium deficiency and viral infection. *J. Nutr.* **2003**, *133*, 1463S–1467S. [CrossRef]
45. Beck, M.; Handy, J.; Levander, O. Host nutritional status: The neglected virulence factor. *Trends Microbiol.* **2004**, *12*, 417–423. [CrossRef]

46. Food and Agriculture Organization of the United Nations. *Europe and Central Asia Regional Overview of Food Insecurity 2016: The Food Insecurity Transition*; FAO: Budapest, Hungary, 2017; pp. 1–44.
47. Maggini, S.; Pierre, A.; Calder, P. Immune function and micronutrient requirements change over the life course. *Nutrients* **2018**, *10*, 1531. [CrossRef]
48. Bailey, R.L.; West, K.P., Jr.; Black, R.E. The epidemiology of global micronutrient deficiencies. *Ann. Nutr. Metab.* **2015**, *66*, 22–33. [CrossRef] [PubMed]
49. World Health Organization; U.S. Centers for Disease Control and Prevention. *Worldwide Prevalence of Anaemia 1993–2005: WHO Global Database of Anaemia*; WHO: Geneva, Switzerland, 2008; pp. 1–41.
50. World Health Organization. *The World Health Report 2002: Reducing Risks, Promoting Healthy Life*; WHO: Geneva, Switzerland, 2002; pp. 1–248.
51. Hilger, J.; Friedel, A.; Herr, R.; Rausch, T.; Roos, F.; Wahl, D.A.; Pierroz, D.D.; Weber, P.; Hoffmann, K. A systematic review of vitamin D status in populations worldwide. *Br. J. Nutr.* **2014**, *111*, 23–45. [CrossRef] [PubMed]
52. U.S. Institute of Medicine. *Dietary Reference Intakes for Calcium and Vitamin D*; National Academies Press: Washington, DC, USA, 2011.
53. US Centers for Disease Control and Prevention. *Second National Report on Biochemical Indicators of Diet and Nutrition in the U.S. Population*; CDC: Atlanta, GA, USA, 2012; pp. 1–484.
54. Cashman, K.D.; Dowling, K.G.; Skrabakova, Z.; Gonzalez-Gross, M.; Valtuena, J.; De Henauw, S.; Moreno, L.; Damsgaard, C.T.; Michaelsen, K.F.; Molgaard, C.; et al. Vitamin D deficiency in Europe: Pandemic? *Am. J. Clin. Nutr.* **2016**, *103*, 1033–1044. [CrossRef] [PubMed]
55. Hu, Y.; Chen, J.; Wang, R.; Li, M.; Yun, C.; Li, W.; Yang, Y.; Piao, J.; Yang, X.; Yang, L. Vitamin D nutritional status and its related factors for Chinese children and adolescents in 2010–2012. *Nutrients* **2017**, *9*, 1024. [CrossRef] [PubMed]
56. Yun, C.; Chen, J.; He, Y.; Mao, D.; Wang, R.; Zhang, Y.; Yang, C.; Piao, J.; Yang, X. Vitamin D deficiency prevalence and risk factors among pregnant Chinese women. *Public Health Nutr.* **2017**, *20*, 1746–1754. [CrossRef]
57. Peter, S.; Friedel, A.; Roos, F.F.; Wyss, A.; Eggersdorfer, M.; Hoffmann, K.; Weber, P. A systematic review of global alpha-tocopherol status as assessed by nutritional intake levels and blood serum concentrations. *Int. J. Vitam. Nutr. Res.* **2016**, *14*, 261–281. [CrossRef]
58. Lykkesfeldt, J.; Poulsen, H.E. Is vitamin C supplementation beneficial? Lessons learned from randomised controlled trials. *Br. J. Nutr.* **2010**, *103*, 1251–1259. [CrossRef]
59. García, O.; Ronquillo, D.; del Caamaño, M.; Camacho, M.; Long, K.; Rosado, J.L. Zinc, vitamin A, and vitamin C status are associated with leptin concentrations and obesity in Mexican women: Results from a cross-sectional study. *Nutr. Metab.* **2012**, *9*, 59. [CrossRef]
60. Villalpando, S.; Montalvo-Velarde, I.; Zambrano, N.; Carcia-Guerra, A.; Ramirez-Silva, C.I.; Shamah-Levy, T.; Rivera, J.A. Vitamin A, and C and folate status in Mexican children under 12 years and women 12–49 years: A probabilistic national survey. *Salud Publica Mex.* **2003**, *45*, S508–S519. [CrossRef]
61. García, O.; Ronquillo, D.; del Carmen Caamaño, M.; Martínez, G.; Camacho, M.; López, V.; Rosado, J. Zinc, iron and vitamins A, C and E are associated with obesity, inflammation, lipid profile and insulin resistance in Mexican school-aged children. *Nutrients* **2013**, *5*, 5012–5030. [CrossRef] [PubMed]
62. Madruga de Oliveira, A.; Rondó, P.H.C.; Mastroeni, S.S.; Oliveira, J.M. Plasma concentrations of ascorbic acid in parturients from a hospital in Southeast Brazil. *Clin. Nutr.* **2008**, *27*, 228–232. [CrossRef] [PubMed]
63. Ravindran, R.D.; Vashist, P.; Gupta, S.K.; Young, I.S.; Maraini, G.; Camparini, M.; Jayanthi, R.; John, N.; Fitzpatrick, K.E.; Chakravarthy, U.; et al. Prevalence and risk factors for vitamin C deficiency in north and south India: A two centre population based study in people aged 60 years and over. *PLoS ONE* **2011**, *6*, e28588. [CrossRef] [PubMed]
64. Schleicher, R.L.; Carroll, M.D.; Ford, E.S.; Lacher, D.A. Serum vitamin C and the prevalence of vitamin C deficiency in the United States: 2003-2004 National Health and Nutrition Examination Survey (NHANES). *Am. J. Clin. Nutr.* **2009**, *90*, 1252–1263. [CrossRef] [PubMed]
65. Hughes, K.; New, A.L.; Lee, B.L.; Ong, C.N. Plasma vitamins A, C and E in the general population of Singapore, 1993 to 1995. *Ann. Acad. Med. Singapore* **1998**, *27*, 149–153. [PubMed]

66. Hughes, K.; Ong, C.N. Vitamins, selenium, iron, and coronary heart disease risk in Indians, Malays, and Chinese in Singapore. *J. Epidemiol. Community Health* **1998**, *52*, 181–185. [CrossRef]
67. Pearson, J.; Pullar, J.; Wilson, R.; Spittlehouse, J.; Vissers, M.; Skidmore, P.; Willis, J.; Cameron, V.; Carr, A. Vitamin C status correlates with markers of metabolic and cognitive health in 50-year-olds: Findings of the CHALICE cohort study. *Nutrients* **2017**, *9*, 831. [CrossRef]
68. Bird, J.; Murphy, R.; Ciappio, E.; McBurney, M. Risk of deficiency in multiple concurrent micronutrients in children and adults in the United States. *Nutrients* **2017**, *9*, 655. [CrossRef]
69. Bruins, M.J.; Bird, J.K.; Aebischer, C.P.; Eggersdorfer, M. Considerations for secondary prevention of nutritional deficiencies in high-risk groups in high-income countries. *Nutrients* **2018**, *10*, 47. [CrossRef]
70. Gibson, R.S.; Heath, A.-L.M.; Limbaga, M.L.S.; Prosser, N.; Skeaff, C.M. Are changes in food consumption patterns associated with lower biochemical zinc status among women from Dunedin, New Zealand? *Br. J. Nutr.* **2001**, *86*, 71–80. [CrossRef]
71. Baqui, A.H.; Black, R.E.; Fischer Walker, C.L.; Arifeen, S.; Zaman, K.; Yunus, M.; Wahed, M.A.; Caulfield, L.E. Zinc supplementation and serum zinc during diarrhea. *Indian J. Pediatr.* **2006**, *73*, 493–497. [CrossRef] [PubMed]
72. Combs, G.F., Jr. Biomarkers of selenium status. *Nutrients* **2015**, *7*, 2209–2236. [CrossRef] [PubMed]
73. Stoffaneller, R.; Morse, N. A review of dietary selenium intake and selenium status in Europe and the Middle East. *Nutrients* **2015**, *7*, 1494–1537. [CrossRef]
74. Stark, K.D.; Van Elswyk, M.E.; Higgins, M.R.; Weatherford, C.A.; Salem, N. Global survey of the omega-3 fatty acids, docosahexaenoic acid and eicosapentaenoic acid in the blood stream of healthy adults. *Prog. Lipid Res.* **2016**, *63*, 132–152. [CrossRef] [PubMed]
75. Carr, A.C. Vitamin C in pneumonia and sepsis. In *Vitamin C: New Biochemical and Functional Insights*; Chen, Q., Vissers, M.C.M., Eds.; CRC Press: Boca Raton, FL, USA, 2020; pp. 115–135.
76. Hunt, C.; Chakravorty, N.K.; Annan, G.; Habibzadeh, N.; Schorah, C.J. The clinical effects of vitamin C supplementation in elderly hospitalised patients with acute respiratory infections. *Int. J. Vit. Nutr. Res.* **1994**, *64*, 212–219.
77. Mochalkin, N.I. Ascorbic acid in the complex treatment of patients with acute pneumonia. *Voen. Meditsinskii Zhurnal.* **1970**, *9*, 17–21.
78. Institute of Medicine. *Dietary Reference Intakes: The Essential Guide to Nutrient Requirements*; The National Academies Press: Washington, DC, USA, 2006.
79. Ran, L.; Zhao, W.; Wang, J.; Wang, H.; Zhao, Y.; Tseng, Y.; Bu, H. Extra dose of vitamin C based on a daily supplementation shortens the common cold: A meta-analysis of 9 randomized controlled trials. *BioMed Res. Int.* **2018**. [CrossRef]
80. Institute of Medicine. *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids*; The National Academies Press: Washington, DC, USA, 2000.
81. EFSA Panel on Dietetic Products. Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. *EFSA J.* **2010**, *8*, 1461.
82. Food and Agriculture Organization of the United Nations. Chapter 2: Summary of conclusions and dietary recommendations on total fat and fatty acids. In *Fats and Fatty Acids in Human Nutrition: Report of An Expert Consultation: 10–14 November 2008, Geneva*; Food and Agriculture Organization of the United Nations: Rome, Italy, 2010; pp. 9–20.
83. Chinese Nutrition Society. *Chinese Dietary Reference Intakes Summary (2013)*; People's Medical Publishing House: Beijing, China, 2013; p. 16.



nutrients

MDPI

Review

Vitamin C—An Adjunctive Therapy for Respiratory Infection, Sepsis and COVID-19

Patrick Holford [1,*], Anitra C. Carr [2], Thomas H. Jovic [3,4], Stephen R. Ali [3,4], Iain S. Whitaker [3,4], Paul E. Marik [5] and A. David Smith [6]

1 Institute for Optimum Nutrition, Ambassador House, Richmond TW9 1SQ, UK
2 Nutrition in Medicine Research Group, Department of Pathology & Biomedical Science, University of Otago, Christchurch 8140, New Zealand; anitra.carr@otago.ac.nz
3 Reconstructive Surgery & Regenerative Medicine Research Group, Institute of Life Sciences, Swansea University Medical School, Swansea University, Swansea SA2 8PY, UK; Thomas.Jovic@wales.nhs.uk (T.H.J.); Stephen.Ali@wales.nhs.uk (S.R.A.); Iain.Whitaker@wales.nhs.uk (I.S.W.)
4 Welsh Centre for Burns & Plastic Surgery, Morriston Hospital, Swansea SA6 6NL, UK
5 Division of Pulmonary and Critical Care Medicine, Eastern Virginia Medical School, Norfolk, VA 23507, USA; marikpe@evms.edu
6 Department of Pharmacology, University of Oxford, Oxford OX1 3QT, UK; david.smith@pharm.ox.ac.uk
* Correspondence: pat@patrickholford.com; Tel.: +44-(0)-7944-689108

Received: 19 October 2020; Accepted: 3 December 2020; Published: 7 December 2020

Abstract: There are limited proven therapies for COVID-19. Vitamin C's antioxidant, anti-inflammatory and immunomodulating effects make it a potential therapeutic candidate, both for the prevention and amelioration of COVID-19 infection, and as an adjunctive therapy in the critical care of COVID-19. This literature review focuses on vitamin C deficiency in respiratory infections, including COVID-19, and the mechanisms of action in infectious disease, including support of the stress response, its role in preventing and treating colds and pneumonia, and its role in treating sepsis and COVID-19. The evidence to date indicates that oral vitamin C (2–8 g/day) may reduce the incidence and duration of respiratory infections and intravenous vitamin C (6–24 g/day) has been shown to reduce mortality, intensive care unit (ICU) and hospital stays, and time on mechanical ventilation for severe respiratory infections. Further trials are urgently warranted. Given the favourable safety profile and low cost of vitamin C, and the frequency of vitamin C deficiency in respiratory infections, it may be worthwhile testing patients' vitamin C status and treating them accordingly with intravenous administration within ICUs and oral administration in hospitalised persons with COVID-19.

Keywords: COVID-19; SARS-CoV-2; coronavirus; vitamin C; ascorbate; colds; pneumonia; sepsis; immunonutrition; supplementation

1. Introduction

Vitamin C, ascorbic acid, is an essential water-soluble nutrient. It is synthesised in plants from fructose and in almost all animals from glucose. It is not synthesised by primates, most bats, guinea pigs, and a small number of birds and fish since the final enzyme, gulonolactone oxidase (GULO), required for ascorbic acid synthesis is missing due to gene mutations that occurred prior to the evolution of Homo sapiens [1]. All these species are therefore dependent on vitamin C in their food. Primates are dependent on an adequate supply provided by fruits and vegetation intake ranging from 4.5 g/day for gorillas [2] to 600 mg/day for smaller monkeys (7.5 kg—a tenth of human size) [3].

The EU Average Requirement of 90 mg/day for men and 80 mg/day for women is to maintain a normal plasma level of 50 μmol/L [4], which is the mean plasma level in UK adults [5]. This is sufficient

to prevent scurvy but may be inadequate when a person is under viral exposure and physiological stress. An expert panel in cooperation with the Swiss Society of Nutrition recommended that everyone supplement with 200 mg "to fill the nutrient gap for the general population and especially for the adults age 65 and older. This supplement is targeted to strengthen the immune system" [6]. The Linus Pauling Institute recommends 400 mg for older adults (>50 years old) [7].

Pharmacokinetic studies in healthy volunteers support a 200 mg daily dose to produce a plasma level of circa 70 to 90 μmol/L [8,9]. Complete plasma saturation occurs between 1 g daily and 3 g every four hours, being the highest tolerated oral dose, giving a predicted peak plasma concentration of circa 220 μmol/L [10]. The same dose given intravenously raises plasma vitamin C levels approximately ten-fold. Higher intakes of vitamin C are likely to be needed during viral infections with 2–3 g/day required to maintain normal plasma levels between 60 and 80 μmol/L [11,12]. Whether higher plasma levels have additional benefit is yet to be determined, but would be consistent with the results of the clinical trials discussed in this review.

2. Vitamin C Deficiency in Pneumonia, Sepsis and COVID-19

Human plasma vitamin C levels decline rapidly under conditions of physiological stress including infection, trauma, and surgery, not uncommonly resulting in overt vitamin C deficiency in hospitalised patients, defined as a plasma level of vitamin C ≤ 11 μmol/L [13–18]. Two studies in hospitals in Paris reported that 17 to 44% of patients had vitamin C plasma levels less than ≤ 11 μmol/L [14,15]. In a Canadian university hospital, it was found that 19% of patients had vitamin C plasma levels ≤ 11 μmol/L [16]. In a study of surgical patients in Australia, it was found that 21% had vitamin C plasma levels ≤ 11 μmol/L [17]. A survey of elderly Scottish patients hospitalised as a consequence of acute respiratory infections reported that 35% of patients had vitamin C plasma levels ≤ 11 μmol/L [18]. The UK's National Diet and Nutrition Survey, based on a cross section of the UK population, reports that 4% of 65+ year olds and 40% of those institutionalised in care homes have vitamin C levels ≤ 11 μmol/L [5,19], indicating the way in which older people with low vitamin C status may be especially susceptible to critical infection.

The vitamin C-deficiency disease scurvy has long been associated with pneumonia which led to the view that vitamin C may influence susceptibility to respiratory infections [20]. In other words, people deficient in vitamin C may be more susceptible to severe respiratory infections such as pneumonia. A prospective study of 19,357 men and women followed over 20 years found that people in the top quartiles of baseline plasma vitamin C concentrations had a 30% lower risk of pneumonia [21]. Furthermore, meta-analysis has indicated a reduction in the risk of pneumonia with oral vitamin C supplementation, particularly in individuals with low dietary intakes [22].

Post-mortem investigations of severe COVID-19 have demonstrated a secondary organising pneumonia phenomenon [23]; therefore, studies investigating vitamin C in relation to pneumonia may be relevant [18,24–27] (Table 1). The most recent study, from New Zealand, reported that patients with pneumonia had depleted vitamin C levels compared with healthy controls (23 μmol/L vs. 56 μmol/L, $p < 0.001$). The pneumonia cohort comprised 62% with hypovitaminosis C and 22% with vitamin C ≤ 11 μmol/L, compared with 8% hypovitaminosis C and no cases with ≤11μmol/L in the healthy controls [24]. The more severely ill patients in the ICU had mean vitamin C levels of 11 μmol/L. Similar findings have been reported in other studies of critically ill septic patients [28–33] (Table 1). A New Zealand study of patients with sepsis found that 40% had vitamin C ≤ 11 μmol/L and the majority of the patients had hypovitaminosis C (serum level < 23 μmol/L), despite receiving recommended enteral and parenteral intakes of the vitamin [29].

Table 1. Vitamin C status of patients with pneumonia, sepsis and severe COVID-19.

Study Type	Cohort	Vitamin C (μmol/L) (% Deficient, % Hypovitaminosis C)	Refs.
Pneumonia			
Case control	Healthy volunteers (n = 50)	56 ± 2 [a] (0% [b], 8% [c])	[24]
	Community-acquired pneumonia (n = 50)	23 ± 3 (22%, 62%)	
Case control	Healthy volunteers (n = 20)	66 ± 3	[25]
	Pneumonia cases (n = 11)	31 ± 9	
Case control	Healthy participants (n = 28)	49 ± 1	[26]
	Lobular pneumonia (n = 35):		
	Acute—did not survive (n = 7)	17 ± 1	
	Acute—survived (n = 15)	24 ± 1	
	Convalescent cases (n = 13)	34 ± 1	
Intervention (placebo group)	Pneumonia/bronchitis (n = 29):		[18]
	Week 0	24 ± 5 (40%) [b]	
	Week 2	19 ± 3 (37%)	
	Week 4	24 ± 6 (25%)	
Intervention (control group)	Pneumonia cases (n = 70):		[27]
	Day 0	41	
	Day 5–10	23–24	
	Day 15–20	32–35	
	Day 30	39	
Sepsis			
Intervention (baseline)	Sepsis with ARDS (n = 83):		[28]
	Day 0	22 (11–37) [d]	
	Day 2	23 (9–37)	
	Day 4	26 (9–41)	
	Day 7	29 (12–39)	
Observational	Septic shock patients (n = 24)	15 ± 2 (38% [b], 88% [c])	[29]
Intervention (baseline)	Severe sepsis patients (n = 24)	18 ± 2	[30]
Case control	Healthy controls (n = 6)	48 ± 6	[31]
	Severe sepsis (n = 19)	14 ± 3	
	Septic shock (n = 37)	14 ± 3	
Case control	Healthy controls (n = 14)	76 ± 6	[32]
	Septic encephalopathy (n = 11)	19 ± 11	
Case control	Healthy controls (n = 34)	62 (55–72) [d]	[33]
	ICU (injury, surgery, sepsis) (n = 62)	11 (8–22)	
Severe COVID-19			
Observational	Critically ill COVID-19 (n = 21)	22 ± 4 (45%[b], 70% [c]) [e]	[34]
	Survivors (n = 11)	29 ± 7 (40%, 50%)	
	Non-survivors (n = 10)	15 ± 2 (50%, 90%)	
Observational	COVID-associated ARDS (n = 18)	17 with <9 μmol/L	[35]
		1 with 14 μmol/L	

[a]—Data represent mean and SEM; [d]—median (and interquartile range); [b]—Percentage of patients with vitamin C deficiency (<11 μmol/L); [c]—Percentage of patients with hypovitaminosis C (<23 μmol/L); [e]—Personal communication (Cristian Arvinte, North Suburban Medical Center, Thornton, CO, USA). COVID—coronavirus disease; ICU—intensive care unit; ARDS—acute respiratory distress syndrome. A part of this table has been reproduced from [36].

As yet, there have been few studies reporting the vitamin C status of patients with COVID-19 (Table 1). A study of 21 critically ill COVID-19 patients admitted to ICU in the US found a mean level of 22 μmol/L, thus a majority had hypovitaminosis C. The mean level for 11 survivors was 29 μmol/L compared to 15 μmol/L for the 10 non-survivors; of these five (50%) had ≤11 μmol/L [34]. A study in an ICU in Barcelona of 18 COVID-19 patients meeting acute respiratory distress syndrome (ARDS) criteria found that 17 had undetectable levels of vitamin C (i.e., <9 μmol/L) and one patient had a low vitamin C (14 μmol/L) [35]. Thus, low vitamin C levels are common in critically ill hospitalised patients with respiratory infections, pneumonia, sepsis and COVID-19, the most likely explanation being increased metabolic consumption [37].

3. Mechanisms of Action of Vitamin C in Infections, Sepsis and COVID-19

Vitamin C has important anti-inflammatory, immunomodulating, antioxidant, antithrombotic and antiviral properties [38–40]. The vitamin demonstrates direct virucidal activity and has effector mechanisms in both the innate and adaptive immune systems [41–44]. The effects of vitamin C on immunity during infection are many and include the development and maturation of T-lymphocytes and the functions of phagocytosis and chemotaxis of leucocytes [45]. It also has a vital role as an antioxidant whereby phagocytes import oxidised vitamin C (dehydroascorbic acid) and regenerate it to reduced vitamin C (ascorbic acid) [46,47].

Importantly, and with specific reference to the critical phase of COVID-19, vitamin C contributes to the downregulation of cytokines, protecting the endothelium from oxidant injury and has an essential role in tissue repair [48,49]. The interaction between oxidative stress and the induction of genes integral to the inflammatory response, including TNFα, IL-1, IL-8, and ICAM-1 has been shown to be mediated through activation of NF-κB [50]. Vitamin C lessens reactive oxidative species (ROS) and inflammation via attenuation of NF-κB activation [51]. Vitamin C significantly increases superoxide dismutase, catalase and glutathione and decreases serum TNFα and IL-1β levels in a rat ARDS model [52]. These effects of vitamin C may be due to its epigenetic regulation of various genes, i.e., upregulation of antioxidant proteins and downregulation of proinflammatory cytokines, rather than its direct scavenging of oxidants.

While SARS-CoV-2 downregulates the expression of type-1 interferons (the host's primary anti-viral defence mechanism) [53], vitamin C upregulates these key host defence proteins [40]. In GULO knockout mice, vitamin C shows in vivo anti-viral immune responses and a reduction in viral titres in the lung in the early stage of infection, especially against influenza virus, through increased production of interferon [54]. Animal studies show that vitamin C reduces the incidence and severity of bacterial and viral infections [55], including increased resistance of chick embryo tracheal organ cultures to coronavirus infection and protection of broiler chicks against avian coronavirus [56,57].

Based on the identification of ACE2 as the receptor for SARS-CoV-2 entry, there is a hypothesis that the increased risk of severe COVID-19 is a function of upregulated ACE2, as is found in the co-morbidities of diabetes, cardiovascular disease and hypertension [58]. The SARS-CoV-2 spike glycoprotein is able to bind to ACE2 [59]. It is noteworthy that, in human arterial endothelial cells, vitamin C abolished ACE2 upregulation induced by IL-7 [60].

Although there are many potential targets for vitamin C in the process of infection, viral replication and pathology in COVID-19, it is noteworthy that a key protease in the virus, Mpro, whose function is to activate several viral non-structural proteins, has been proposed as a target. In a modelling study using the crystal structure of Mpro, the active site of this enzyme was found to bind magnesium ascorbate, which had the strongest binding out of 106 nutraceuticals. The authors suggested that ascorbate might, therefore, be a powerful inhibitor of the enzyme [61].

The critical and often fatal phase of COVID-19, primarily triggered by the host's reaction to dead virus particles, occurs with increased production of potent proinflammatory cytokines and chemokines, resulting in the development of multi-organ failure [62]. This may result in neutrophil migration and accumulation in the lung interstitium and bronchoalveolar space and is considered a

key determinant of progression of ARDS [63]. Neutrophil extracellular trap formation (NETosis) is a cell death pathway different from apoptosis and necrosis that traps and inactivates pathogens [64]. This is a maladaptive response that may contribute to tissue and organ damage leading to organ failure. Vitamin C deficiency in GULO-knockout mice showed enhanced NETosis in the lungs of septic animals and increased circulating cell-free DNA suggesting that vitamin C is a novel regulator of NETosis [65]. Furthermore, vitamin C enhances lung epithelial barrier function in an animal model of sepsis by promoting epigenetic and transcriptional expression of protein-channels at the alveolar capillary membrane that regulate alveolar fluid clearance which include cystic fibrosis transmembrane conductance regulator, aquaporin-5, the Na^+/K^+-ATPase pump and epithelial sodium channel [66].

There is also increasing evidence that vitamin C, which is a pleiotropic stress hormone, may be playing a critical role in mediating the adrenocortical stress response, particularly in sepsis [38]. Vitamin C concentrations are three to ten times higher in the adrenal glands than in any other organ [67]. It is released from the adrenal cortex under conditions of physiological stress (ACTH stimulation), including viral exposure, raising plasma levels fivefold [68]. Vitamin C enhances cortisol production and potentiates the anti-inflammatory and endothelial cytoprotective effects of glucocorticoids [69,70]. Exogenous glucocorticoid steroids are the only proven disease-modifying treatment for COVID-19 [71]. The postulated mechanisms for vitamin C's amelioration of COVID-19 pathology are shown in Figure 1.

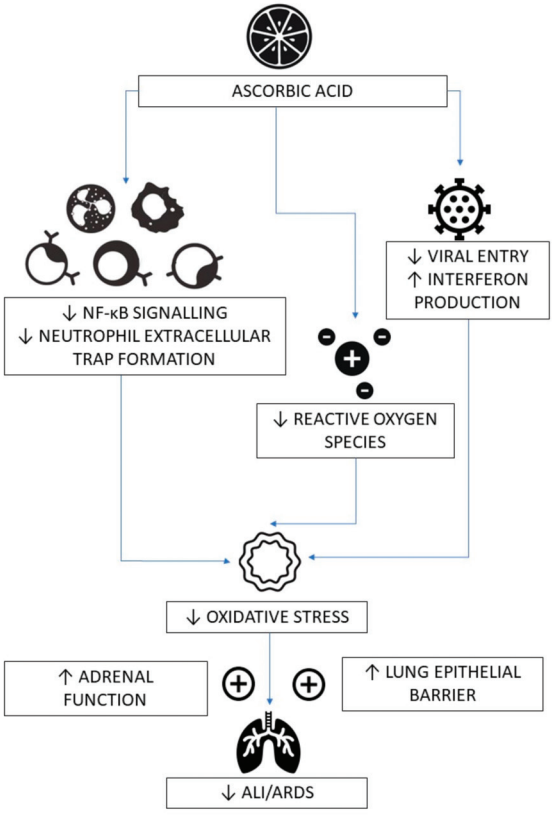


Figure 1. Postulated mechanisms for vitamin C's amelioration of COVID-19 pathology. ↓—decreased; ↑—increased; ALI—acute lung injury; ARDS—acute respiratory distress syndrome; NF-κB—nuclear factor kappa B.

4. Clinical Evidence for the Role of Vitamin C in Colds

Nobel laureate Linus Pauling concluded from randomised controlled trials (RCTs) that vitamin C prevented and alleviated colds thus popularising its use in the 1970s [72,73]. A Cochrane Review of placebo-controlled trials giving oral vitamin C for preventing and treating colds found that supplementation above 200 mg did not reduce the incidence in the general population [74]. However, in five trials involving a total of 598 marathon runners, skiers and soldiers on subarctic exercises vitamin C reduced the incidence of colds by 52% ($p < 0.0001$) [74]. Based on these findings, vitamin C appears to influence resistance to viral infections in special conditions, such as during brief periods of severe physical exercise.

Whereas trials where vitamin C has been administered only after the onset of symptoms have not shown consistent benefits, trials which regularly administered vitamin C reduced the duration of infections in adults by 8% and in children by 14%, with an apparent dose-dependency up to 6–8 g/day [55,74]. In children, 1 to 2 g/day vitamin C reduced cold duration by 18%, with the severity of colds being reduced by regular administration [74].

The latest UK placebo-controlled trial illustrates the meaningful clinical difference between the number of colds, cold duration and severity [75]. This trial comprised 168 volunteers who were randomised to receive a placebo or vitamin C (2 × 500 mg daily) over a 60-day winter period. The vitamin C group had fewer colds (37 vs. 50, $p = 0.05$), and even fewer virally challenged 'cold' days (85 vs. 178, $p = 0.03$) and a shorter duration of severe symptom days (1.8 vs. 3.1 days, $p = 0.03$). The number of participants who had two colds during the trial was significantly reduced (2/84 on vitamin C vs. 16/84 in the placebo group; $p = 0.04$) [75].

In summary, cold symptoms have been shown to be less severe and resolve more quickly with oral vitamin C with a dose-dependent effect. Colds, caused by over 100 different virus strains, some of which are coronaviruses, are defined by a group of symptoms similar to the majority of those who get SARS-CoV-2 infection and do not convert into the acute illness phase. This similarity of symptoms and the disease-modifying effect of vitamin C across a wide range of cold-related viruses is further rationale for considering that vitamin C's effects in reducing severity and duration of infection is not virus-specific and could thus also potentially alleviate SARS-CoV-2 related symptoms. Each of these effects—reduced duration, severity and number of colds—could reasonably be hypothesised, in the context of SARS-CoV-2, to reduce conversion from mild infection to the critical phase of COVID-19. Given the consistent effect of regular vitamin C intake on the duration and severity of colds, and the low cost and safety, it would be appropriate for patients with respiratory virus infections to have the benefits of therapeutic vitamin C assessed.

Since the disease caused by the novel coronavirus can be more severe than ordinary viral infections, the above estimates may justify a regular increased daily intake of vitamin C for the period when the prevalence of the virus is high, when a patient suffers from a virus infection with active cold symptoms, in those testing PCR positive to SARS-CoV-2 and in COVID-19 hospitalised patients; an oral dose of up to 6–8 g/day might be considered. Pauling's recommendation of 1 g every hour of oral ascorbic acid during active infection has yet to be studied in an RCT, therefore, the most effective dose has yet to be determined.

5. Clinical Evidence for the Role of Vitamin C in Pneumonia

In 1951, Klenner investigated the effects of high doses of vitamin C, given intravenously, against viral diseases including pneumonia [76]. A Cochrane review on pneumonia and vitamin C identified three prophylactic RCTs reporting the number of pneumonia cases in participants who were administered oral vitamin C [22]. Each of these found a ≥80% lower incidence of pneumonia for the vitamin C group [77–79]. One was an RCT giving 2 g/day versus placebo to US Marine recruits during a two-month recruit training period and reported 1/331 cases of pneumonia in the vitamin C group versus 7/343 cases in the placebo group ($p = 0.044$) [77].

Two therapeutic trials were identified (Table 2). One was an RCT with elderly people in the UK (mean age 81 years), hospitalised with acute bronchitis or pneumonia. The study found that the plasma vitamin C level at baseline was 23 µmol/L (hypovitaminosis C) and one third of the patients had a vitamin C level of ≤11 µmol/L [18]. Vitamin C (0.2 g/day) reduced the respiratory symptom score in the more ill patients but not the less ill. There were six deaths during the study, all among the more ill patients: five in the placebo group, but only one in the vitamin C group. The other RCT, in the former Soviet Union, administered two different doses, a variable high or low dose relating to the dosage of antibiotics given [27]. The duration of hospital stay in the control group was 23.7 days. In the low dose vitamin C group (0.25–0.8 g/day) hospital stay was 19% shorter and in the high-dose group (0.5–1.6 g/day) it was 36% shorter. A benefit was also reported in relation to erythrocyte sedimentation rate and the normalisation of chest X-ray and temperature.

Table 2. Vitamin C trials in patients with pneumonia, sepsis and severe COVID-19.

Patients	Intervention Dose (Duration)	Patient Outcomes	Refs.
Pneumonia			
Pneumonia/bronchitis (n = 57):	Oral vitamin C (28 day):	↓ respiratory symptom score in most severely ill	[18]
• Placebo (n = 29)	0 g/day	17% mortality in placebo group	
• Treatment (n = 28)	0.2 g/day	4% mortality in treatment group	
Pneumonia (n = 140):	Oral vitamin C (10 day):	↓ hospital length of stay:	[27]
• Control (n = 70)	0 g/day	24 days in control group	
• Low dose (n = 39)	0.25–0.8 g/day	19 days in low dose group	
• High dose (n = 31)	0.5–1.6 g/day	15 days in high dose group	
Sepsis			
Sepsis and ARDS (n = 167):	IV vitamin C (4 day):	X systemic organ failure score X C-reactive protein, thrombomodulin X ventilator-free days ↓ 28 day mortality ↑ ICU-free days ↑ hospital-free days	[28]
• Placebo (n = 83)	0 mg/kg bw/day		
• Treatment (n = 84)	200 mg/kg/day		
Septic shock (n = 100):	IV vitamin C (until ICU discharge)	↓ vasopressor duration ↓ ICU length of stay X length of mechanical ventilation X renal replacement therapy X ICU mortality	[80]
• Placebo (n = 50)	0 g/day		
• Treatment (n = 50)	6 g/day		
Septic shock (n = 28):	IV vitamin C (3 day):	↓ norepinephrine dose and duration ↓ 28 day mortality X ICU length of stay	[81]
• Placebo (n = 14)	0 mg/kg bw/day		
• Treatment (n = 14)	100 mg/kg bw/day		
Severe sepsis (n = 24)	IV vitamin C (4 day):	↓ systemic organ failure score ↓ C-reactive protein, procalcitonin, thrombomodulin	[30]
• Placebo (n = 8)	0 mg/kg bw/day		
• Low dose (n = 8)	50 mg/kg bw/day		
• High dose (n = 8)	200 mg/kg bw/day		
Severe COVID-19			
Critical COVID-19 (n = 54)	IV vitamin C (7 day):	X ventilation-free days ↑ PaO_2/FiO_2 ↓ Interleukin-6 ↓ 28 day mortality in patients with SOFA scores ≥ 3	[82]
• Placebo (n = 28)	0 g/day		
• Treatment (n = 26)	24 g/day		

ARDS—acute respiratory distress syndrome; COVID—coronavirus disease; FiO_2—fraction of inspired oxygen; IV—intravenous; PaO_2—partial pressure of oxygen; SOFA—sequential organ failure assessment; ↓—decrease; X—no change. A part of this table has been reproduced from [36].

6. Clinical Evidence for the Role of Vitamin C in Critically Ill Septic Patients

The major cause for concern regarding COVID-19 is the high frequency of ICU treatment that is needed. Meta-analyses of intravenous vitamin C supplementation in critically ill (burns, sepsis and

septic shock) patients indicated that it can lead to vasopressor sparing effects, reduced duration of ICU stay and a reduced need for mechanical ventilation [83]. In six trials, orally administered vitamin C in doses of 1–3 g/day reduced the length of ICU stay by 8.6% ($p = 0.003$) [84]. In five trials including 471 patients requiring ventilation for over 10 h, a dosage of 1–6 g/day of vitamin C reduced ventilation time by 25% ($p < 0.0001$) [85].

There is clear evidence that vitamin C levels decline precipitously in critically ill patients and in those with sepsis (Table 1) [36]. Although 0.1 g/day of vitamin C can maintain a normal plasma level in a healthy person, much higher doses (2–3 g/day) are needed to keep plasma vitamin C levels of critically ill patients within the normal range [11,86]. Being water-soluble, and thus excreted within hours, frequency of dose is important to maintain sufficient blood levels during active infection. Limitations in bioavailability in conditions of rapid vitamin C depletion in critically unwell patients have generated the hypothesis that the required therapeutic plasma levels to optimally reduce oxidative stress and exert an anti-inflammatory effect are more effectively achieved with intravenous administration than with oral administration alone [29,87].

Clinicians using intravenous vitamin C in severely ill COVID-19 patients have reported clinical effects upon administration of 3 g every 6 h together with steroids and anti-coagulants [88]. However, clear evidence for the most effective dose and frequency has not yet been determined. A four-group randomised pharmacokinetic trial testing 2 or 10 g/day, either delivered as a twice-daily bolus infusion or continuous infusion, found that the 2 g/day dose was associated with normal plasma concentrations, and the 10 g/day dose was associated with supranormal plasma concentrations, increased oxalate excretion, and metabolic alkalosis. The study's authors also concluded that sustained therapy is needed to prevent hypovitaminosis C [11].

Vitamin C has been reported to reduce mortality in septic patients requiring vasopressor treatment randomly assigned to be given 25 mg/kg body weight/day intravenous vitamin C every 6 h versus placebo (Table 2). Mortality at 28 days was significantly lower in the ascorbic acid than the placebo group (14% vs. 64%, respectively; $p = 0.009$) [81].

In the largest trial of intravenous vitamin C in sepsis-associated ARDS, the CITRIS-ALI trial, patients were given placebo or vitamin C at a dose of 50 mg/kg every 6 h for 4 days, thus providing 15 g/day for a 75 kg person (Table 2). Patients in the vitamin C group did not have significantly improved markers of inflammation, vascular injury or organ dysfunction which were the primary outcomes [28]. However, there were statistically significant benefits in three of the four clinically relevant outcomes, i.e., mortality ($p = 0.03$), duration of ICU-free days ($p = 0.03$) and hospital-free days ($p = 0.04$). Reanalysis of the data indicated that, during the 4-day vitamin C administration, mortality was 81% lower, but after the cessation of vitamin C administration, there was no difference between the two trial groups [89]. By the end of the 4-day vitamin C administration, the mortality rate was 23% (19/83) in the placebo group and 5% (4/84) in the vitamin C group ($p = 0.0007$). This difference of 18% corresponds to the number needed to treat of 5.5. Furthermore, the study authors, in recognition of the exclusion of sequential organ failure assessment (SOFA) scores in deceased patients, reported in a post hoc analysis assigning deceased patients a SOFA score of 20 and discharged patients a SOFA score of zero, that there was a 60% probability that any random patient from the placebo group had a higher SOFA score than any random patient from the vitamin C group ($p = 0.03$) at 96 h [90].

Another trial randomised 216 patients to low-dose intravenous vitamin C (1.5 g every 6 h thus providing 7.5 g/day), thiamine, and hydrocortisone for up to 10 days or until septic shock resolved, with a mean of 3.4 days, versus hydroxycortisone alone, and found no effect on the primary outcome of vasopressor-free time to 7 days or on 90-day mortality [91]. Two limitations of this study are the delay in giving vitamin C [92], and the absence of a vitamin C only arm [93]; hence, this study only shows that the addition of vitamin C, possibly too late in the disease process and for too short a time, to hydroxycortisone treatment added no treatment advantage.

7. Clinical Evidence for the Role of Vitamin C in COVID-19

Given the potential benefit of vitamin C, in oral and intravenous doses of 2–8 g/day, to reduce duration and severity of the common cold, pneumonia, sepsis and ARDS, this warrants investigation in relation to whether early oral supplementation could be beneficial in preventing conversion from mild infection to more critical COVID-19 infection and, if given intravenously to those with critical COVID-19 symptoms, in reducing mortality and ICU stay, thus speeding up recovery.

Interestingly, many of the risk factors for COVID-19 overlap with those for vitamin C deficiency [94]. Certain sub-groups (male, African American, older, those suffering with co-morbidities of diabetes, hypertension, COPD), all at higher risk of severe COVID-19, have also been shown to have lower serum vitamin C levels [95]. Average plasma vitamin C levels are generally lower in men than women, even with comparative intakes of vitamin C, which has been attributed to their higher body weight [94]. A hypothesis of altered sodium-dependent vitamin C transporter (SVCT1 and 2) expression in these sub-groups has also been proposed [95]. In old versus young rat hepatocytes, the vitamin C level declines by 66%, which is largely attributed to reduced absorption due to a 45% decline in SVCT1 with age [96]. It is noteworthy that inflammatory cytokines, also present in co-morbidities, downregulate SVCT2, resulting in the depletion of intracellular vitamin C [97,98].

There are currently 45 trials registered on Clinicaltrials.gov investigating vitamin C with or without other treatments for COVID-19. In the first RCT to test the value of vitamin C in critically ill COVID-19 patients, 54 ventilated patients in Wuhan, China, were treated with a placebo (sterile water) or intravenous vitamin C at a dose of 24 g/day for 7 days [82] (Table 2). After 7 days of treatment, the ratio of PaO_2/FiO_2 in the vitamin C group was 229 mmHg versus 151 mmHg in the control group ($p = 0.01$), and this also improved over time in the vitamin C group, but fell in the control group. On day 7, the IL-6 level was lower in the vitamin C group than in the placebo group: 19 pg/mL versus 158 pg/mL ($p = 0.04$). The more severely ill patients with SOFA scores ≥ 3 in the vitamin C group exhibited a reduction in 28-day mortality: 18% versus 50% ($p = 0.05$) in univariate survival analysis (Figure 2). No study-related adverse events were reported. The effects of treatment on the ratio PaO_2/FiO_2 and on IL-6 are clinically important, but further studies are needed to determine if the trend in lower mortality can be confirmed. The trial was originally designed for 140 subjects and was thus underpowered, with only 54 patients due to a lack of new admissions.

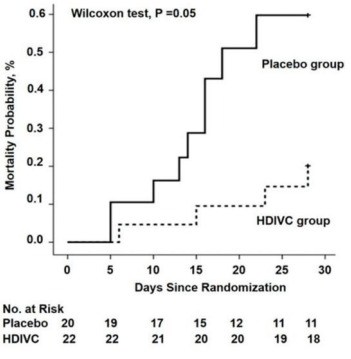


Figure 2. The 28-day mortality from randomization (day 1) to day 28 in a trial of high-dose intravenous vitamin C (HDIVC) in patients with COVID-19. Kaplan–Meier analysis was used to estimate the 28-day mortality and survival curves were compared with the Wilcoxon test ($p = 0.05$) among severe COVID-19 patients (baseline SOFA score ≥ 3). Cox regression was used as multiple comparisons (HR, 0.32 (95%CI, 0.10–1.06); $p = 0.06$). HDIVC—high-dose intravenous vitamin C. Reproduced with permission from Zhang J. et al. [82].

The largest registered trial is the Lessening Organ Dysfunction with Vitamin C-COVID (LOVIT-COVID) trial in Canada, which is recruiting 800 patients who are randomly assigned to vitamin C (intravenous, 50 mg/kg every 6 h) or a placebo for 96 h, i.e., equivalent to 15 g/day for a 75 kg person (NCT04401150). This protocol has also been added as a vitamin C arm in the Randomized, Embedded, Multifactorial Adaptive Platform Trial for Community-Acquired Pneumonia (REMAP-CAP; NCT02735707). The study design provides further rationale for the use of vitamin C in COVID-19 patients [99]. There is also a high-dose (10 g/day) vitamin C intervention study in 500 adults is in progress in Palermo, Italy (NCT04323514).

There is concern, however, that these study designs limit the use of vitamin C to a maximum of four days, which may be inadvisable in acutely ill patients due to the potential return of symptoms if the inflammation is not resolved. This issue was illustrated by the CITRIS-ALI trial, which showed a maximum reduction in mortality compared to placebo on day 4, the final day of vitamin C administration, but a decreased difference between the groups after 28 days [87,89].

In the UK, the Chelsea and Westminster hospital ICU, where adult ICU patients were administered 1 g of intravenous vitamin C every 12 h together with anticoagulants [100], has reported 29% mortality [101], compared to the average 41% reported by the Intensive Care National Audit and Research Centre (ICNARC) for all UK ICUs [102]. While the authors have stated that the addition of an antioxidant in the form of vitamin C could have contributed to the lower mortality rate, it should be noted that other clinical factors and procedures could also account for the improved mortality and that the Chelsea and Westminster ICU serves a more affluent sector of the population with less deprivation on the basis of the Index of Multiple Deprivation (IMD). Deprivation, while a risk factor for COVID-19 mortality, is also a predictor of low vitamin C status. In the UK, an estimated 25% of men and 16% of women in the low-income/materially deprived population are deficient in vitamin C > 11 μmol/L [103].

The Frontline COVID-19 Critical Care Expert Group (FLCCC), a group of emergency medicine experts, have reported that, with the combined use of 6 g/day intravenous vitamin C (1.5 g every 6 h), plus steroids and anticoagulants, mortality was 5% in two ICUs in the US (United Memorial Hospital in Houston, Texas, and Norfolk General Hospital in Norfolk, Virginia), the lowest mortality rates in their respective counties [88].

A case report of 17 COVID-19 patients who were given 1 g of intravenous vitamin C every 8 h for 3 days reported a mortality rate of 12% with 18% rates of intubation and mechanical ventilation and a significant decrease in inflammatory markers, including ferritin and D-dimer, and a trend towards decreasing FiO_2 requirements [104]. Another case of unexpected recovery following high-dose intravenous vitamin C has also been reported [105]. While these case reports are subject to confounding and are not prima facie evidence of effects, they do illustrate the feasibility of using vitamin C for COVID-19 with no adverse effects reported.

8. Safety of Oral and Intravenous Vitamin C

The US DRI, having thoroughly considered the wide literature on vitamin C and many kinds of speculated harms, stated that the safe range is up to 2 g/day [106]. The European Food Safety Authority stated that the lowest observable adverse effect level is 3–4 g/day (in relation to gastrointestinal effects) [107]. Injectable vitamin C phials state "there are no contraindications to the administration of ascorbic acid. As much as 6 g has been administered parenterally to normal adults without evidence of toxicity" [108].

Three concerns have been raised regarding high doses of vitamin C: diarrhoea from high oral ingestion, kidney stones, particularly due to kidney dysfunction in the case of intravenous vitamin C (i.e., if high doses cannot be cleared), and unsuitability for those with specific genetically inherited metabolic issues that affect vitamin C utilisation. The latter relates to those with glucose-6-phosphate deficiency (G6PD) and also haemochromatosis and thalassaemia due to enhanced iron absorption with vitamin C. G6PD deficiency is not considered an exclusion criterion in the use of up to 6 g/day oral or intravenous vitamin C [109]. The FLCCC report that 3 g every 6 h appears to be safe in patients with

G6PD. It may be wise for those with haemochromatosis or thalassaemia to avoid high-dose vitamin C taken with iron-rich foods or supplements and short-term high-dose vitamin C to be medically monitored [110].

Looser bowel movements and diarrhoea rarely occur below 3 g/day and tolerance is increased considerably when fighting a viral infection [111]. Diarrhoea has not been reported as a complication in hospital-based oral treatment and does not occur with intravenous vitamin C administration. A survey of 9328 patients given an average intravenous dose of 24 g of vitamin C every 4 days, primarily for cancer, infection or fatigue, reported that 101 (1%) had side effects, mostly minor, including lethargy/fatigue, a change in mental status and vein irritation/phlebitis [112].

Regarding kidney stone formation, the Kidney Stone Research Laboratory of the University of Cape Town conducted a controlled trial in which ten volunteer subjects were required to ingest 4 g of vitamin C per day for five days. Unlike the earlier studies, they put a preservative in the urine collection bottles to prevent the conversion of ascorbate to oxalic acid. The samples were analysed for numerous physicochemical risk factors of kidney stone formation. These risk factors were not significantly altered and the authors concluded that ingestion of large doses of vitamin C does not increase the risk of forming kidney stones and earlier trials had faulty study designs involving unpreserved urine samples [113]. A prospective cohort study of 85,557 women with no history of kidney stones, with 1078 incidences of kidney stones over 14 years of follow-up, reported that vitamin C was not associated with a risk of kidney stone development [114]. A systematic review of studies giving vitamin C found a correlation between ascorbic acid supplementation and the incidence of kidney stones in men, but not women [115]. A study administering intravenous ascorbic acid in doses ranging from 0.2 to 1.5 g/kg body weight measured urinary oxalic excretion during and over 6 h post infusion. The authors conclude that less than 0.5% of a very large intravenous dose of ascorbic acid was recovered as urinary oxalic acid in people with normal renal function [116]. A cautious position would be to exclude those with a history of kidney stones or kidney dysfunction from high-dose oral or intravenous vitamin C unless medically supervised. Short-term high-dose vitamin C in the region of 2–8 g/day is unlikely to be of significant concern in people with normal kidney function.

9. Conclusions

Vitamin C's potential benefits, low cost, safety profile and multiple disease-modifying actions, including antioxidant, anti-inflammatory and immunomodulating effects, make it an attractive therapeutic candidate in reducing viral load with oral supplementation in the range of 2–8 g/day to help attenuate the conversion to the critical phase of COVID-19. Likewise, vitamin C has potential benefits in treating acute respiratory infections and mitigating inflammation in critical COVID-19 patients with intravenous vitamin C infusion in the range of 6–24 g/day, for correcting disease-induced deficiency, reducing inflammation, enhancing interferon production and supporting the anti-inflammatory actions of glucocorticosteroids, especially given the high level of fatality for patients with severe COVID-19.

Given the remarkable safety of vitamin C, frequent deficiency among patients with COVID-19 and extensive evidence of potential benefits, the current treatment is justified on compassionate grounds pending more COVID-19 clinical trial data becoming available, not only for intravenous use within ICUs, but also orally with doses between 2 and 8 g/day in hospitalised patients due to increased need when fighting a viral infection, as concluded in recent reviews [36,117,118]. The clinical choice of oral versus intravenous vitamin C may be guided by similar criteria for administering oral versus intravenous antibiotics, considering both the severity of the illness and whether the patient is able to swallow oral medication at least four times a day.

People in high-risk groups for COVID-19 mortality, and at risk of vitamin C deficiency, should be encouraged to supplement with vitamin C daily to ensure vitamin C adequacy at all times, and to increase the dose when virally infected to up to 6–8 g/day [119]. Whether or not this will prevent conversion to the critical phase of COVID-19 has yet to be determined.

Author Contributions: Conceptualisation, P.H.; writing—original draft preparation, P.H., T.H.J., S.R.A, I.S.W., A.C.C.; writing—review and editing, P.E.M., A.D.S.; visualisation, T.H.J., S.R.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: The authors would like to thank Harri Hemila from the Department of Public Health, University of Helsinki, for his helpful feedback, as well as Gordon Brydon and Sheri Friedman for their help with referencing and secretarial support when preparing the paper for publication. A.C.C. is supported by a Health Research Council of New Zealand Sir Charles Hercus Health Research Fellowship.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Drouin, G.; Godin, J.R.; Page, B. The genetics of vitamin C loss in vertebrates. *Curr. Genomics* **2011**, *12*, 371–378. [CrossRef]
2. Milton, K. Micronutrient intakes of wild primates: Are humans different? *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **2003**, *136*, 47–59. [CrossRef]
3. Milton, K. Nutritional characteristics of wild primate foods: Do the diets of our closest living relatives have lessons for us? *Nutrition* **1999**, *15*, 488–498. [CrossRef]
4. European Food Safety Authority Panel on Dietetic Products, Nutrition and Allergies. Scientific opinion on dietary reference values for vitamin C. *EFSA J.* **2013**, *11*, 3418. [CrossRef]
5. Bates, B.; Collins, D.; Cox, L.; Nicholson, S.; Page, P.; Roberts, C.; Steer, T.; Swan, G. *National Diet and Nutrition Survey Years 1 to 9 of the Rolling Programme (2008/2009–2016/2017): Time Trend and Income Analyses*; Public Health England: London, UK, 2019.
6. Berger, M.M.; Bischoff-Ferrari, H.A.; Zimmermann, M.; Herter, I.; Spieldenner, J.; Eggersdorfer, M. *White Paper on Nutritional Status in Supporting a Well-Functioning Immune System for Optimal Health with a Recommendation for Switzerland*; SGE: Bern, Switzerland, 2020.
7. Linus Pauling Institute; Micronutrient Information Center. *Micronutrients for Older Adults Oregon State University*. 2020. Available online: https://lpi.oregonstate.edu/mic/life-stages/older-adults (accessed on 20 October 2020).
8. Levine, M.; Conry-Cantilena, C.; Wang, Y.; Welch, R.W.; Washko, P.W.; Dhariwal, K.R.; Park, J.B.; Lazarev, A.; Graumlich, J.F.; King, J.; et al. Vitamin C pharmacokinetics in healthy volunteers: Evidence for a recommended dietary allowance. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 3704–3709. [CrossRef] [PubMed]
9. Levine, M.; Wang, Y.; Padayatty, S.J.; Morrow, J. A new recommended dietary allowance of vitamin C for healthy young women. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 9842–9846. [CrossRef]
10. Padayatty, S.J.; Sun, H.; Wang, Y.; Riordan, H.D.; Hewitt, S.M.; Katz, A.; Wesley, R.A.; Levine, M. Vitamin C pharmacokinetics: Implications for oral and intravenous use. *Ann. Intern. Med.* **2004**, *140*, 533–537. [CrossRef]
11. De Grooth, H.J.; Manubulu-Choo, W.P.; Zandvliet, A.S.; Spoelstra-de Man, A.M.E.; Girbes, A.R.; Swart, E.L.; Oudemans-van Straaten, H.M. Vitamin-C pharmacokinetics in critically ill patients: A randomized trial of four intravenous regimens. *Chest* **2018**, *153*, 1368–1377. [CrossRef]
12. Hume, R.; Weyers, E. Changes in leucocyte ascorbic acid during the common cold. *Scott. Med. J.* **1973**, *18*, 3–7. [CrossRef]
13. Evans-Olders, R.; Eintracht, S.; Hoffer, L.J. Metabolic origin of hypovitaminosis C in acutely hospitalized patients. *Nutrition* **2010**, *26*, 1070–1074. [CrossRef]
14. Teixeira, A.; Carrie, A.S.; Genereau, T.; Herson, S.; Cherin, P. Vitamin C deficiency in elderly hospitalized patients. *Am. J. Med.* **2001**, *111*, 502. [CrossRef]
15. Fain, O.; Paries, J.; Jacquart, B.; Le Moel, G.; Kettaneh, A.; Stirnemann, J.; Heron, C.; Sitbon, M.; Taleb, C.; Letellier, E.; et al. Hypovitaminosis C in hospitalized patients. *Eur. J. Intern. Med.* **2003**, *14*, 419–425. [CrossRef] [PubMed]
16. Gan, R.; Eintracht, S.; Hoffer, L.J. Vitamin C deficiency in a university teaching hospital. *J. Am. Coll. Nutr.* **2008**, *27*, 428–433. [CrossRef] [PubMed]
17. Ravindran, P.; Wiltshire, S.; Das, K.; Wilson, R.B. Vitamin C deficiency in an Australian cohort of metropolitan surgical patients. *Pathology* **2018**, *50*, 654–658. [CrossRef]

18. Hunt, C.; Chakravorty, N.K.; Annan, G.; Habibzadeh, N.; Schorah, C.J. The clinical effects of vitamin C supplementation in elderly hospitalised patients with acute respiratory infections. *Int. J. Vitam. Nutr. Res.* **1994**, *64*, 212–219. [PubMed]
19. Bates, C.J.; Prentice, A.; Cole, T.J.; van der Pols, J.C.; Doyle, W.; Finch, S.; Smithers, G.; Clarke, P.C. Micronutrients: Highlights and research challenges from the 1994-5 National Diet and Nutrition Survey of people aged 65 years and over. *Br. J. Nutr.* **1999**, *82*, 7–15. [CrossRef] [PubMed]
20. Hemilä, H.; Louhiala, P. Vitamin C may affect lung infections. *J. R. Soc. Med.* **2007**, *100*, 495–498. [CrossRef] [PubMed]
21. Myint, P.K.; Wilson, A.M.; Clark, A.B.; Luben, R.N.; Wareham, N.J.; Khaw, K.T. Plasma vitamin C concentrations and risk of incident respiratory diseases and mortality in the European Prospective Investigation into Cancer-Norfolk population-based cohort study. *Eur. J. Clin. Nutr.* **2019**, *73*, 1492–1500. [CrossRef] [PubMed]
22. Hemilä, H.; Louhiala, P. Vitamin C for preventing and treating pneumonia. *Cochrane Database Syst. Rev.* **2013**. [CrossRef]
23. Kory, P.; Kanne, J.P. SARS-CoV-2 organising pneumonia: 'Has there been a widespread failure to identify and treat this prevalent condition in COVID-19?'. *BMJ Open Respir. Res.* **2020**, *7*, e000724. [CrossRef]
24. Carr, A.C.; Spencer, E.; Dixon, L.; Chambers, S.T. Patients with community acquired pneumonia exhibit depleted vitamin C status and elevated oxidative stress. *Nutrients* **2020**, *12*, 1318. [CrossRef]
25. Bakaev, V.V.; Duntau, A.P. Ascorbic acid in blood serum of patients with pulmonary tuberculosis and pneumonia. *Int. J. Tuberc. Lung. Dis.* **2004**, *8*, 263–266.
26. Chakrabarti, B.; Banerjee, S. Dehydroascorbic acid level in blood of patients suffering from various infectious diseases. *Proc. Soc. Exp. Biol. Med.* **1955**, *88*, 581–583. [CrossRef] [PubMed]
27. Mochalkin, N.I. Ascorbic acid in the complex therapy of acute pneumonia. *Voen. Med. Zhurnal* **1970**, *9*, 17–21.
28. Fowler, A.A., III; Truwit, J.D.; Hite, R.D.; Morris, P.E.; DeWilde, C.; Priday, A.; Fisher, B.; Thacker, L.R., II; Natarajan, R.; Brophy, D.F.; et al. Effect of vitamin C infusion on organ failure and biomarkers of inflammation and vascular injury in patients with sepsis and severe acute respiratory failure: The CITRIS-ALI randomized clinical trial. *JAMA* **2019**, *322*, 1261–1270. [CrossRef]
29. Carr, A.C.; Rosengrave, P.C.; Bayer, S.; Chambers, S.; Mehrtens, J.; Shaw, G.M. Hypovitaminosis C and vitamin C deficiency in critically ill patients despite recommended enteral and parenteral intakes. *Crit. Care* **2017**, *21*, 300. [CrossRef] [PubMed]
30. Fowler, A.A.; Syed, A.A.; Knowlson, S.; Sculthorpe, R.; Farthing, D.; DeWilde, C.; Farthing, C.A.; Larus, T.L.; Martin, E.; Brophy, D.F.; et al. Phase I safety trial of intravenous ascorbic acid in patients with severe sepsis. *J. Transl. Med.* **2014**, *12*, 32. [CrossRef] [PubMed]
31. Doise, J.M.; Aho, L.S.; Quenot, J.P.; Guilland, J.C.; Zeller, M.; Vergely, C.; Aube, H.; Blettery, B.; Rochette, L. Plasma antioxidant status in septic critically ill patients: A decrease over time. *Fundam. Clin. Pharmacol.* **2008**, *22*, 203–209. [CrossRef] [PubMed]
32. Voigt, K.; Kontush, A.; Stuerenburg, H.-J.; Muench-Harrach, D.; Hansen, H.C.; Kunze, K. Decreased plasma and cerebrospinal fluid ascorbate levels in patients with septic encephalopathy. *Free Radic. Res.* **2002**, *36*, 735–739. [CrossRef] [PubMed]
33. Schorah, C.J.; Downing, C.; Piripitsi, A.; Gallivan, L.; Al-Hazaa, A.H.; Sanderson, M.J.; Bodenham, A. Total vitamin C, ascorbic acid, and dehydroascorbic acid concentrations in plasma of critically ill patients. *Am. J. Clin. Nutr.* **1996**, *63*, 760–765. [CrossRef]
34. Arvinte, C.; Singh, M.; Marik, P.E. Serum levels of vitamin C and vitamin D in a cohort of critically ill COVID-19 patients of a north American community hospital intensive care unit in May 2020: A pilot study. *Med. Drug Discov.* **2020**. [CrossRef] [PubMed]
35. Chiscano-Camón, L.; Ruiz-Rodriguez, J.C.; Ruiz-Sanmartin, A.; Roca, O.; Ferrer, R. Vitamin C levels in patients with SARS-CoV-2-associated acute respiratory distress syndrome. *Crit. Care* **2020**, *24*, 522. [CrossRef] [PubMed]
36. Carr, A.C. Vitamin C in pneumonia and sepsis. In *Vitamin C: New Biochemical and Functional Insights*; Chen, Q., Vissers, M., Eds.; CRC Press/Taylor & Francis: Boca Raton, FL, USA, 2020; pp. 115–135.
37. Marik, P.E.; Hooper, M.H. Doctor-your septic patients have scurvy! *Crit. Care* **2018**, *22*, 23. [CrossRef] [PubMed]

38. Marik, P.E. Vitamin C: An essential "stress hormone" during sepsis. *J. Thorac. Dis.* **2020**, *12*, S84–S88. [CrossRef]
39. Marik, P.E. Vitamin C for the treatment of sepsis: The scientific rationale. *Pharmacol. Ther.* **2018**, *189*, 63–70. [CrossRef]
40. Colunga Biancatelli, R.M.L.; Berrill, M.; Marik, P.E. The antiviral properties of vitamin C. *Expert Rev. Anti Infect. Ther.* **2020**, *18*, 99–101. [CrossRef]
41. Thomas, W.R.; Holt, P.G. Vitamin C and immunity: An assessment of the evidence. *Clin. Exp. Immunol.* **1978**, *32*, 370–379.
42. Dahl, H.; Degre, M. The effect of ascorbic acid on production of human interferon and the antiviral activity in vitro. *Acta Pathol. Microbiol. Scand. B* **1976**, *84*, 280–284. [CrossRef]
43. Webb, A.L.; Villamor, E. Update: Effects of antioxidant and non-antioxidant vitamin supplementation on immune function. *Nutr. Rev.* **2007**, *65*, 181–217. [CrossRef]
44. Hemila, H. Vitamin C and infectious diseases. In *Vitamin C*; Paoletti, R., Sies, H., Bug, J., Grossi, E., Poli, A., Eds.; Springer: Milan, Italy, 1998; pp. 73–85.
45. Carr, A.C.; Maggini, S. Vitamin C and immune function. *Nutrients* **2017**, *9*, 1211. [CrossRef]
46. Wang, Y.; Russo, T.A.; Kwon, O.; Chanock, S.; Rumsey, S.C.; Levine, M. Ascorbate recycling in human neutrophils: Induction by bacteria. *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 13816–13819. [CrossRef] [PubMed]
47. Nualart, F.J.; Rivas, C.I.; Montecinos, V.P.; Godoy, A.S.; Guaiquil, V.H.; Golde, D.W.; Vera, J.C. Recycling of vitamin C by a bystander effect. *J. Biol. Chem.* **2003**, *278*, 10128–10133. [CrossRef] [PubMed]
48. May, J.M.; Qu, Z.C. Ascorbic acid prevents oxidant-induced increases in endothelial permeability. *Biofactors* **2011**, *37*, 46–50. [CrossRef] [PubMed]
49. May, J.M.; Harrison, F.E. Role of vitamin C in the function of the vascular endothelium. *Antioxid. Redox Signal* **2013**, *19*, 2068–2083. [CrossRef]
50. Sen, C.K.; Packer, L. Antioxidant and redox regulation of gene transcription. *FASEB J.* **1996**, *10*, 709–720. [CrossRef]
51. Chen, Y.; Luo, G.; Yuan, J.; Wang, Y.; Yang, X.; Wang, X.; Li, G.; Liu, Z.; Zhong, N. Vitamin C mitigates oxidative stress and tumor necrosis factor-alpha in severe community-acquired pneumonia and LPS-induced macrophages. *Mediators Inflamm.* **2014**, *2014*, 426740. [CrossRef]
52. Erol, N.; Saglam, L.; Saglam, Y.S.; Erol, H.S.; Altun, S.; Aktas, M.S.; Halici, M.B. The protection potential of antioxidant vitamins against acute respiratory distress syndrome: A rat trial. *Inflammation* **2019**, *42*, 1585–1594. [CrossRef]
53. Blanco-Melo, D.; Nilsson-Payant, B.E.; Liu, W.C.; Uhl, S.; Hoagland, D.; Møller, R.; Jordan, T.X.; Oishi, K.; Panis, M.; Sachs, D.; et al. Imbalanced host response to SARS-CoV-2 drives development of COVID-19. *Cell* **2020**, *181*, 1036–1045. [CrossRef] [PubMed]
54. Kim, Y.; Kim, H.; Bae, S.; Choi, J.; Lim, S.Y.; Lee, N.; Kong, J.M.; Hwang, Y.I.; Kang, J.S.; Lee, W.J. Vitamin C is an essential factor on the anti-viral immune responses through the production of interferon-a/b at the initial stage of influenza A virus (H3N2) infection. *Immune Netw.* **2013**, *13*, 70–74. [CrossRef]
55. Hemilä, H. Vitamin C and infections. *Nutrients* **2017**, *9*, 339. [CrossRef]
56. Atherton, J.G.; Kratzing, C.C.; Fisher, A. The effect of ascorbic acid on infection chick-embryo ciliated tracheal organ cultures by coronavirus. *Arch. Virol.* **1978**, *56*, 195–199. [CrossRef] [PubMed]
57. Davelaar, F.G.; Bos, J. Ascorbic acid and infectious bronchitis infections in broilers. *Avian Pathol.* **1992**, *21*, 581–589. [CrossRef] [PubMed]
58. Gan, R.; Rosoman, N.P.; Henshaw, D.J.E.; Noble, E.P.; Georgius, P.; Sommerfeld, N. COVID-19 as a viral functional ACE2 deficiency disorder with ACE2 related multi-organ disease. *Med. Hypotheses* **2020**, *144*, 110024. [CrossRef] [PubMed]
59. Ni, W.; Yang, X.; Yang, D.; Bao, J.; Li, R.; Xiao, Y.; Hou, C.; Wang, H.; Liu, J.; Yang, D.; et al. Role of angiotensin-converting enzyme 2 (ACE2) in COVID-19. *Crit. Care* **2020**, *24*.
60. Ma, S.; Sun, S.; Li, J.; Fan, Y.; Qu, J.; Sun, L.; Wang, S.; Zhang, Y.; Yang, S.; Liu, Z.; et al. Single-cell transcriptomic atlas of primate cardiopulmonary aging. *Cell Res.* **2020**. [CrossRef] [PubMed]
61. Kumar, V.; Jena, M. In silico virtual screening-based study of nutraceuticals predicts the therapeutic potentials of folic acid and its derivatives against COVID-19. *Res. Square* **2020**. [CrossRef]
62. Bosmann, M.; Ward, P.A. The inflammatory response in sepsis. *Trends Immunol.* **2013**, *34*, 129–136. [CrossRef]

63. Grommes, J.; Soehnlein, O. Contribution of neutrophils to acute lung injury. *Mol. Med.* **2011**, *17*, 293–307. [CrossRef]
64. Brinkmann, V.; Reichard, U.; Goosmann, C.; Fauler, B.; Uhlemann, Y.; Weiss, D.S.; Weinrauch, Y.; Zychlinsky, A. Neutrophil extracellular traps kill bacteria. *Science* **2004**, *303*, 1532–1535. [CrossRef]
65. Mohammed, B.M.; Fisher, B.J.; Kraskauskas, D.; Farkas, D.; Brophy, D.F.; Fowler, A.A., III; Natarajan, R. Vitamin C: A novel regulator of neutrophil extracellular trap formation. *Nutrients* **2013**, *5*, 3131–3151. [CrossRef]
66. Fisher, B.J.; Kraskauskas, D.; Martin, E.J.; Farkas, D.; Wegelin, J.A.; Brophy, D.; Ward, K.R.; Voelkel, N.F.; Fowler, A.A., III; Natarajan, R. Mechanisms of attenuation of abdominal sepsis induced acute lung injury by ascorbic acid. *Am. J. Physiol. Lung Cell Mol. Physiol.* **2012**, *303*, L20–L32. [CrossRef] [PubMed]
67. Hornig, D. Distribution of ascorbic acid, metabolites and analogues in man and animals. *Ann. N. Y. Acad. Sci.* **1975**, *258*, 103–118. [CrossRef] [PubMed]
68. Padayatty, S.J.; Doppman, J.L.; Chang, R.; Wang, Y.; Gill, J.; Papanicolaou, D.A.; Levine, M. Human adrenal glands secrete vitamin C in response to adrenocorticotrophic hormone. *Am. J. Clin. Nutr.* **2007**, *86*, 145–149. [CrossRef]
69. Kodama, M.; Kodama, T.; Murakami, M.; Kodama, M. Vitamin C infusion treatment enhances cortisol production of the adrenal via the pituitary ACTH route. *In Vivo* **1994**, *8*, 1079–1085. [PubMed]
70. Barabutis, N.; Khangoora, V.; Marik, P.E.; Catravas, J.D. Hydrocortisone and ascorbic acid synergistically prevent and repair lipopolysaccharide-induced pulmonary endothelial barrier dysfunction. *Chest* **2017**, *152*, 954–962. [CrossRef] [PubMed]
71. Recovery Collaborative Group; Horby, P.; Lim, W.S.; Emberson, J.R.; Mafham, M.; Bell, J.L.; Linsell, L.; Staplin, N.; Brightling, C.; Ustianowski, A.; et al. Dexamethasone in hospitalized patients with Covid-19-preliminary report. *N. Engl. J. Med.* **2020**. [CrossRef] [PubMed]
72. Pauling, L. The significance of the evidence about ascorbic acid and the common cold. *Proc. Natl. Acad. Sci. USA* **1971**, *68*, 2678–2681. [CrossRef]
73. Pauling, L. *Vitamin C the Common Cold and Flu*; Freeman: San Francisco, CA, USA, 1970.
74. Hemilä, H.; Chalker, E. Vitamin C for preventing and treating the common cold. *Cochrane Database Syst. Rev.* **2013**. [CrossRef] [PubMed]
75. Van Straten, M.; Josling, P. Preventing the common cold with a vitamin C supplement: A double-blind, placebo-controlled survey. *Adv. Ther.* **2002**, *19*, 151–159. [CrossRef]
76. Klenner, F.R. Massive doses of vitamin C and the virus diseases. *South Med. Surg.* **1951**, *113*, 101–107.
77. Pitt, H.A.; Costrini, A.M. Vitamin C prophylaxis in marine recruits. *JAMA* **1979**, *241*, 908–911. [CrossRef] [PubMed]
78. Kimbarowski, J.A.; Mokrow, N.J. Colored precipitation reaction of the urine according to Kimbarowski (FARK) as an index of the effect of ascorbic acid during treatment of viral influenza. *Dtsch Gesundheitsw.* **1967**, *22*, 2413–2418. [PubMed]
79. Glazebrook, A.J.; Thomson, S. The administration of vitamin C in a large institution and its effect on general health and resistance to infection. *J. Hyg.* **1942**, *42*, 1–19. [CrossRef]
80. Nabil Habib, T.; Ahmed, I. Early adjuvant intravenous vitamin C treatment in septic shock may resolve the vasopressor dependence. *Int. J. Microbiol. Adv. Immunol.* **2017**, *5*, 77–81.
81. Zabet, M.H.; Mohammadi, M.; Ramezani, M.; Khalili, H. Effect of high-dose ascorbic acid on vasopressor's requirement in septic shock. *J. Res. Pharm. Pract.* **2016**, *5*, 94–100.
82. Zhang, J.; Rao, X.; Li, Y.; Zhu, Y.; Liu, F.; Guo, G.; Luo, G.; Meng, Z.; De Backer, D.; Xiang, H.; et al. High-dose vitamin C infusion for the treatment of critically ill COVID-19. *Res. Square* **2020**. [CrossRef]
83. Zhang, M.; Jativa, D.F. Vitamin C supplementation in the critically ill: A systematic review and meta-analysis. *SAGE Open Med.* **2018**, *6*. [CrossRef]
84. Hemila, H.; Chalker, E. Vitamin C can shorten the length of stay in the ICU: A meta-analysis. *Nutrients* **2019**, *11*, 708. [CrossRef]
85. Hemila, H.; Chalker, E. Vitamin C may reduce the duration of mechanical ventilation in critically ill patients: A meta-regression analysis. *J. Intensive Care* **2020**, *8*, 15. [CrossRef]
86. Long, C.L.; Maull, K.I.; Krishnan, R.S.; Laws, H.L.; Geiger, J.W.; Borghesi, L.; Franks, W.; Lawson, T.C.; Sauberlich, H.E. Ascorbic acid dynamics in the seriously ill and injured. *J. Surg. Res.* **2003**, *109*, 144–148. [CrossRef]

87. Kashiouris, M.G.; L'Heureux, M.; Cable, C.A.; Fisher, B.J.; Leichtle, S.W.; Fowler, A.A. The emerging role of vitamin C as a treatment for sepsis. *Nutrients* **2020**, *12*, 292. [CrossRef] [PubMed]
88. Marik, P.E.; Kory, P.; Varon, J.; Iglesias, J.; Meduri, G.U. MATH+ protocol for the treatment of SARS-CoV-2 infection: The scientific rationale. *Expert Rev. Anti Infect. Ther.* **2020**, 1–7. [CrossRef] [PubMed]
89. Hemilä, H.; Chalker, E. Reanalysis of the effect of vitamin C on mortality in the CITRIS-ALI trial: Important findings dismissed in the trial report. *Front. Med.* **2020**. [CrossRef] [PubMed]
90. Fowler, A.A., III; Fisher, B.J.; Kashiouris, M.G. Vitamin C for sepsis and acute respiratory failure—Reply. *JAMA* **2020**, *323*, 792–793. [CrossRef]
91. Fujii, T.; Luethi, N.; Young, P.J.; Frei, D.R.; Eastwood, G.M.; French, C.J.; Deane, A.M.; Shehabi, Y.; Hajjar, L.A.; Oliveira, G.; et al. Effect of Vitamin C, Hydrocortisone, and Thiamine vs Hydrocortisone Alone on Time Alive and Free of Vasopressor Support Among Patients with Septic Shock: The VITAMINS Randomized Clinical Trial. *JAMA* **2020**, *323*, 423–431. [CrossRef]
92. Long, M.T.; Kory, P.; Marik, P. Vitamin C, hydrocortisone, and thiamine for septic shock. *JAMA* **2020**, *323*, 2203–2204. [CrossRef]
93. Carr, A.C. Is the VITAMINS RCT indicating potential redundancy between corticosteroids and vitamin C? *Crit. Care* **2020**, *24*, 129. [CrossRef]
94. Carr, A.C.; Rowe, S. Factors affecting vitamin C status and prevalence of deficiency: A global health perspective. *Nutrients* **2020**, *12*, 1963. [CrossRef]
95. Patterson, G.; Isales, C.M.; Fulzele, S. Low level of vitamin C and dysregulation of vitamin C transporter might be involved in the severity of COVID-19 Infection. *Aging Dis.* **2020**, *12*.
96. Michels, A.J.; Joisher, N.; Hagen, T.M. Age-related decline of sodium-dependent ascorbic acid transport in isolated rat hepatocytes. *Arch. Biochem. Biophys.* **2003**, *410*, 112–120. [CrossRef]
97. Subramanian, V.S.; Sabui, S.; Subramenium, G.A.; Marchant, J.S.; Said, H.M. Tumor Necrosis Factor alpha (TNF-alpha) reduces intestinal vitamin C uptake: A role for NF-kB-mediated signaling. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2018**, *315*, G241–G248. [CrossRef]
98. Subramanian, V.S.; Sabui, S.; Moradi, H.; Marchant, J.S.; Said, H.M. Inhibition of intestinal ascorbic acid uptake by lipopolysaccharide is mediated via transcriptional mechanisms. *Biochim. Biophys. Acta Biomembr.* **2018**, *1860*, 556–565. [CrossRef] [PubMed]
99. Domain-Specific Appendix: VITAMIN, C. REMAP-CAP: Randomized, Embedded, Multifactorial Adaptive Platform Trial for Community-Acquired Pneumonia 2020. Available online: https://static1.squarespace.com/static/5cde3c7d9a69340001d79ffe/t/5f1bba732cda7f10310643fe/1595652735252/REMAP-CAP+Vitamin+C+Domain+Specific+Appendix+V2+-+08+June+2020_WM.pdf (accessed on 26 September 2020).
100. Vizcaychipi, M.P.; Shovlin, C.L.; McCarthy, A.; Howard, A.; Brown, A.; Hayes, M.; Singh, S.; Christie, L.; Sisson, A.; Davies, R.; et al. Development and implementation of a COVID-19 near real-time traffic light system in an acute hospital setting. *Emerg. Med. J.* **2020**, *37*, 630–636. [CrossRef] [PubMed]
101. ICNARC Report on COVID-19 in Critical Care: Chelsea and Westminster Hospital Intensive Care Unit. London. 2020. Available online: https://www.patrickholford.com/uploads/2020/chelwesticnarcreportjune.pdf (accessed on 12 June 2020).
102. ICNARC Report on COVID-19 in Critical Care. London. 2020. Available online: https://www.patrickholford.com/uploads/2020/nationwideicnarcreportjune.pdf (accessed on 26 June 2020).
103. Mosdol, A.; Erens, B.; Brunner, E.J. Estimated prevalence and predictors of vitamin C deficiency within UK's low-income population. *J. Public Health* **2008**, *30*, 456–460. [CrossRef] [PubMed]
104. Hiedra, R.; Lo, K.B.; Elbashabsheh, M.; Gul, F.; Wright, R.M.; Albano, J.; Azmaiparashvili, Z.; Patarroyo Aponte, G. The use of IV vitamin C for patients with COVID-19: A case series. *Expert Rev. Anti Infect. Ther.* **2020**, *18*, 1259–1261. [CrossRef] [PubMed]
105. Waqas Khan, H.M.; Parikh, N.; Megala, S.M.; Predeteanu, G.S. Unusual early recovery of a critical COVID-19 patient after administration of intravenous vitamin C. *Am. J. Case Rep.* **2020**, *21*, e925521. [PubMed]
106. Vitamin C: Fact Sheet for Health Professionals USA: National Institutes of Health. 2020. Available online: https://ods.od.nih.gov/factsheets/VitaminC-HealthProfessional/ (accessed on 10 October 2020).
107. Scientific Committee on Food Scientific Panel on Dietetic Products Nutrition and Allergies. *Tolerable Upper Intake Levels for Vitamins and Minerals*; EFSA: Parma, Italy, 2006.

108. Phoenix Labs. Ascorbic Acid Injection 500mg/5ml Clonee, Ireland 2014. Available online: https://www.medicines.org.uk/emc/product/1520/smpc#gref (accessed on 23 November 2020).
109. Marik, P.E. Is intravenous vitamin C contraindicated in patients with G6PD deficiency? *Crit. Care* **2019**, *23*, 109. [CrossRef] [PubMed]
110. Gerster, H. High-dose vitamin C: A risk for persons with high iron stores? *Int. J. Vitam. Nutr. Res.* **1999**, *69*, 67–82. [CrossRef] [PubMed]
111. Cathcart, R.F. Vitamin C, titrating to bowel tolerance, anascorbemia, and acute induced scurvy. *Med. Hypotheses* **1981**, *7*, 1359–1376. [CrossRef]
112. Padayatty, S.J.; Sun, A.Y.; Chen, Q.; Espey, M.G.; Drisko, J.; Levine, M. Vitamin C: Intravenous use by complementary and alternative medicine practitioners and adverse effects. *PLoS ONE* **2010**, *5*, e11414. [CrossRef]
113. Auer, B.L.; Auer, D.; Rodgers, A.L. The effect of ascorbic acid ingestion on the biochemical and physicochemical risk factors associated with calcium oxalate kidney stone formation. *Clin. Chem. Lab. Med.* **1998**, *36*, 143–147. [CrossRef] [PubMed]
114. Curhan, G.C.; Willett, W.C.; Speizer, F.E.; Stampfer, M.J. Intake of vitamins B6 and C and the risk of kidney stones in women. *J. Am. Soc. Nephrol.* **1999**, *10*, 840–845.
115. Jiang, K.; Tang, K.; Liu, H.; Xu, H.; Ye, Z.; Chen, Z. Ascorbic acid supplements and kidney stones incidence among men and women: A systematic review and meta-analysis. *Urol. J.* **2018**, *16*, 115–120.
116. Robitaille, L.; Mamer, O.A.; Miller, W.H., Jr.; Levine, M.; Assouline, S.; Melnychuk, D.; Rousseau, C.; Hoffer, L.J. Oxalic acid excretion after intravenous ascorbic acid administration. *Metabolism* **2009**, *58*, 263–269. [CrossRef] [PubMed]
117. Calder, P.C. Nutrition, immunity and COVID-19. *BMJ Nutr. Prev. Health* **2020**, *3*, e000085. [CrossRef] [PubMed]
118. Jovic, T.H.; Ali, S.R.; Ibrahim, N.; Jessop, Z.M.; Tarassoli, S.P.; Dobbs, T.D.; Holford, P.; Thornton, C.A.; Whitaker, I.S. Could vitamins help in the fight against COVID-19? *Nutrients* **2020**, *12*, 2550. [CrossRef] [PubMed]
119. Calder, P.C.; Carr, A.C.; Gombart, A.F.; Eggersdorfer, M. Optimal nutritional status for a well-functioning immune system is an important factor to protect against viral infections. *Nutrients* **2020**, *12*, 1181. [CrossRef]

nutrients

MDPI

Review

Feasibility of Vitamin C in the Treatment of Post Viral Fatigue with Focus on Long COVID, Based on a Systematic Review of IV Vitamin C on Fatigue

Claudia Vollbracht [1,2,*] and Karin Kraft [2]

[1] Medical Science Department, Pascoe Pharmazeutische Präparate GmbH, 35383 Giessen, Germany
[2] Department of Internal Medicine, University Medicine Rostock, 18057 Rostock, Germany; karin.kraft@med.uni-rostock.de
* Correspondence: claudia.vollbracht@pascoe.de

Abstract: Fatigue is common not only in cancer patients but also after viral and other infections. Effective treatment options are still very rare. Therefore, the present knowledge on the pathophysiology of fatigue and the potential positive impact of treatment with vitamin C is illustrated. Additionally, the effectiveness of high-dose IV vitamin C in fatigue resulting from various diseases was assessed by a systematic literature review in order to assess the feasibility of vitamin C in post-viral, especially in long COVID, fatigue. Nine clinical studies with 720 participants were identified. Three of the four controlled trials observed a significant decrease in fatigue scores in the vitamin C group compared to the control group. Four of the five observational or before-and-after studies observed a significant reduction in pre–post levels of fatigue. Attendant symptoms of fatigue such as sleep disturbances, lack of concentration, depression, and pain were also frequently alleviated. Oxidative stress, inflammation, and circulatory disorders, which are important contributors to fatigue, are also discussed in long COVID fatigue. Thus, the antioxidant, anti-inflammatory, endothelial-restoring, and immunomodulatory effects of high-dose IV vitamin C might be a suitable treatment option.

Keywords: ascorbic acid; post-viral fatigue; lack of concentration; sleep disturbances; depression

Citation: Vollbracht, C.; Kraft, K. Feasibility of Vitamin C in the Treatment of Post Viral Fatigue with Focus on Long COVID, Based on a Systematic Review of IV Vitamin C on Fatigue. *Nutrients* **2021**, *13*, 1154. https://doi.org/10.3390/nu13041154

Academic Editor: Carol S. Johnston

Received: 2 March 2021
Accepted: 27 March 2021
Published: 31 March 2021

1. Introduction

Fatigue often occurs as a symptom of severe diseases, such as cancer or autoimmune diseases. Chronic fatigue syndrome (CFS) is defined as a separate clinical entity, although the symptoms are very similar: besides intense fatigue, most patients with CFS report attendant symptoms such as pain, cognitive dysfunction, and unrefreshing sleep [1,2]. Since fatigue is still difficult to treat, there is an urgent need for effective treatment options.

Fatigue is also currently coming into focus as a major symptom of long COVID. Patient data from all over the world show that COVID-19 not only attacks people's health during the acute infection but also often results in post-infection problems, which are summarized under the term long COVID [3]. SARS-CoV-2 positive persons can be grouped into asymptomatic infection (no symptoms that are consistent with COVID-19), mild or moderate (symptoms but $SpO_2 \geq 94\%$), severe ($SpO_2 < 94\%$, $PaO_2/FiO_2 < 300$ mm Hg, respiratory frequency > 30 breaths/min, or lung infiltrates > 50%), or critical illness (respiratory failure, septic shock, and/or multiple organ dysfunction) [4]. Symptoms of long COVID can overlap with the post–intensive care syndrome that has been described in patients without COVID-19, but symptoms after COVID-19 have also been reported in patients with milder illness, including outpatients. Obviously, COVID-19 is a multi-system disease characterized by organ and vessel dysfunctions mainly caused by cytokine storm and microembolism. Presumably, this also applies to the post-acute recovery phase. The frequency, nature, and causes of long COVID are currently being investigated intensely.

Until the end of 2020, post-viral fatigue syndrome was listed under the WHO indication code G93.3 (CFS). As of January 2021, the WHO has defined new ICD-10 code numbers

as part of the attention to COVID-19: U08.9 Personal history of COVID-19, unspecified; and U09.9 Post-COVID-19 condition.

Post-viral fatigue is associated with various infectious diseases (SARS coronavirus, Epstein–Barr virus, Ross River virus, enteroviruses, human herpesvirus-6, Ebola virus, West Nile virus, Dengue virus, and parvovirus; bacteria such as *Borrelia burgdorferi*, *Coxiella burnetii*, and *Mycoplasma pneumoniae*; and even parasites, such as *Giardia lamblia*), which often show very different symptoms during the acute stage [1]. Post-viral fatigue syndrome is rather similar to CFS. In this context, it is interesting to note that CFS often begins with an infection during a period of increased physical activity or stress. This corresponds to the current situation in which patients with long COVID are or were affected not only by the infection but likely also by psychological and/or somatic stress during the lockdown.

In a recently published cohort study from Wuhan, which investigated 1733 patients after hospitalization for COVID-19 (half of them were younger than 57 years), even 6 months after the acute infection, 63% of those who had recovered suffered from fatigue or muscle weakness, 26% suffered from sleep disturbances, and 23% suffered from anxiety or depression. Patients who had been more severely ill during their hospital stay had more severely impaired pulmonary diffusion capacities and abnormal chest imaging manifestations [5]. The US National Institute for Health Research started a dynamic review on persistent COVID-19 symptoms in October 2020 and pointed out that not only hospitalized patients but also those with milder courses can be affected [3].

A recent systematic review and meta-analysis identified more than 50 long-term effects of COVID-19, with fatigue, anosmia, pulmonary dysfunction, abnormal chest X-ray/CT, and neurological disorders being the most common. It was estimated that 80% of individuals with a confirmed COVID-19 diagnosis continued to suffer from at least one problem beyond two weeks following acute infection. Most of the symptoms were similar to the symptomatology developed during the acute phase of COVID-19. The five most common symptoms were fatigue (58%), headache (44%), attention deficit disorder (27%), hair loss (25%), and dyspnea (24%) [6].

Although studies are still rather heterogeneous, it is already clear that post-viral fatigue accompanied by sleep disturbances and cognitive deficits is one of the most common complaints of long COVID.

The pathophysiology of COVID-19 is characterized by inflammation and oxidative stress leading to vascular and organ damage, as well as to the suppression of adaptive immune responses [7]. It can be assumed that the post-acute recovery phase is also accompanied by oxidative stress, inflammation, and thus a deficiency of antioxidants such as vitamin C. To date, post-infectious vitamin C plasma levels have not been evaluated. However, a deficiency is most likely since infections are known to be associated with high consumption of vitamin C, and deficiencies in acute infections are frequent [8], especially for patients with pneumonia and COVID-19 [9–13].

A clinically relevant vitamin C deficiency is a disease-eliciting condition, as the water-soluble vitamin is one of the body's most important antioxidants and is involved as a co-factor in more than 150 metabolic functions [14]. The term "vitamin C" encompasses the terms ascorbic acid and ascorbate. The latter is the biologically active form that is oxidized to dehydroascorbate when reactive oxygen species are neutralized. As an enzymatic co-factor, it is particularly important for the synthesis of collagen and carnitine, the bioavailability of tetrahydrobiopterin, and thus the formation of serotonin, dopamine, and nitric oxide, the synthesis of noradrenaline, the biosynthesis of amidated peptides, the degradation of the transcription factor HIF-1 α, and the hypomethylation of DNA [8,15]. Fatigue, pain, cognitive disorders, and depression-like symptoms are known symptoms of a vitamin C deficiency [16].

It is therefore clinically plausible that vitamin C administration could alleviate fatigue (1) by treating vitamin C deficiency symptoms, including fatigue, and (2) by neuroprotective and vasoprotective effects due to its antioxidant and anti-inflammatory properties.

The aim of this publication is to provide a feasibility analysis of whether the use of intravenous (IV) vitamin C in post-viral fatigue, particularly after COVID-19, should be further investigated. For this purpose, the pathophysiological factors underlying fatigue were investigated through a narrative review, and possible approaches for a therapeutic benefit of vitamin C in this condition were elicited. In addition, a systematic review was conducted to evaluate the study evidence on IV vitamin C in fatigue. The review focuses on high-dose IV vitamin C because, in contrast to oral application, only the IV route results in pharmacological plasma levels (>220 μM) [17,18]. Moreover, the high plasma levels reached after IV application offer the advantage of rapid bioavailability in the tissues [19]. Studies with oral vitamin C administration are often of low quality because vitamin C blood levels were rarely determined, and therefore, bioavailability and compliance could not be verified. This bias can be avoided with IV administration, which has the advantage of 100% bioavailability and compliance and additionally facilitates the circumvention of genetically determined resorption differences, which are described for the vitamin C transporter in patients with COVID-19 [20].

2. Materials and Methods

For the narrative feasibility analysis, the search terms "fatigue" and "review" as well as "fatigue" and "oxidative stress" were used in the Medline database.

For the systematic review, the Medline and Cochrane Central databases were searched: Medline with the mesh terms "fatigue" and "ascorbic acid" and Cochrane Central (PubMed, Embase, ICTRP, CT.gov (accessed on 25 February 2021)) with "fatigue" and "vitamin C". The results were screened by the authors for clinical studies with IV vitamin C. Eligibility criteria were the evaluation of fatigue by a score and the therapeutic use of IV vitamin C > 1g. Studies detected via secondary literature were supplemented. As fatigue was investigated in the context of the EORTC-Q30 in studies on quality of life in oncological patients treated with high-dose vitamin C, these studies were added to the search result.

3. Results

The search in PubMed for the search terms "fatigue" and "ascorbic acid" resulted in 43 publications, the search in Cochrane Central resulted in 62 publications, 6 were identified via publications that used EORTC Q30 questionnaires, and 8 were duplicates. Ninety-three publications were excluded because they were not clinical studies (n = 31), were case reports (n = 2), or they did not meet the eligibility criteria (because they used oral vitamin C, often combined with several substances (n = 37), did not use vitamin C (n = 20), or were study registrations without published results (n = 3)). From the 10 full-text publications, one was discarded because there was no information as to how intensity of fatigue was measured. (Figure 1).

From the nine identified clinical studies with 720 participants, three were randomized and controlled studies, one was a retrospective controlled cohort study, one was a phase I study, one was a before-and-after study, and the remaining ones were prospective observational studies.

For the evaluation of fatigue, four studies used EORTC QLQ-C30, three used a Likert scale and, two used a numeric rating scale. The IV vitamin C doses administered ranged from appr. 3.5 g to >75 g/day (three studies with >50 g, two studies with 10 g, three studies with 7.5 g, and one with approximately 3.5 g).

Three of the four controlled trials observed a significant decrease in fatigue in the vitamin C group compared to the control group ($p < 0.005$). In all observational before-and-after studies, a reduction in fatigue was reported. In the four studies that performed a statistical comparison of the pre–post values, the differences were significant ($p < 0.01$) (Table 1).

Table 1. Clinical studies investigating intravenous vitamin C in conditions with fatigue. * p-value for pre vs. post; ** p-value for verum vs. control; bw: body weight; NRS: numeric rating scale.

Reference	Study Type; Number of Patients (n); Underlying Disease	IV Vitamin C Dose	Additional Interventions	Estimation of Fatigue	Impact on Fatigue and Related Parameters
			Oncology		
[21]	Single-center, phase II, randomized clinical trial; $n = 97$; extensively pretreated patients with advanced, refractory non-small-cell lung cancer	1 g/kg bw, 3 times/week, 25 treatments in total	Vitamin C group received concurrently modulated electro-hyperthermia; both groups received best supportive care	EORTC QLQ-C30	Fatigue (mean ± SD) Verum group: pre: 46.48 ± 17.52, post: 20.63 ± 18.14 (* $p < 0.0001$) Control group: pre: 39.93 ± 20.59, post: 61.34 ± 25.32 (* $p < 0.0001$) (** $p < 0.0001$) Physical function ↑ (** $p < 0.0001$) Cognitive function (** $p = 0.1026$) Dyspnea ↓ (** $p < 0.0001$) Insomnia (** $p = 0.0772$ Pain ↓(p** $p < 0.0001$)
[22]	Single-center phase I clinical trial; $n = 17$; patients with refractory, advanced solid tumors (stage III-IV; colon, pancreas, breast, etc.)	0.8–3 g/kg bw, 4 times/week for 4 weeks	None	EORTC QLQ-C30	Fatigue ↓ (pre: 49/ post 11) Physical function ↑ (pre 69/post 87) Cognitive function ↑ (pre 75/post 83) Dyspnea ↓ (pre 24/post 0) Insomnia ↓ (pre 31/post 17) Pain ↓ (pre 36/ post 0)
[23]	Multi-center, prospective observational trial; $n = 60$; patients with advanced tumors (lung, breast, stomach, colonm etc.)	Increasing dosages up to 50 g and more to achieve plasma levels of 350–400 mg/dL 2 times/week for 4 weeks	+/− chemotherapy	EORTC QLQ-C30	Fatigue (mean ± SD) Pre: 42.4 ± 28.7 post: 28.4 25.7 (* $p < 0.01$) Physical function ↑ (* $p < 0.05$) Cognitive function ↑ (* $p < 0.01$) Dyspnea (not significant) Insomnia ↓ (* $p < 0.01$) Pain ↓ (* $p < 0.05$)
[24]	Single-center, prospective before-and-after study; $n = 39$, terminal cancer patients (stomach, colon, lungs, breast, gall bladder, etc.)	10 g 2 times/week for one week	None	EORTC QLQ-C30	Fatigue (mean ± SD) Pre: 52 ± 24, post: 40 ± 19 (* $p = 0.001$) Physical function ↑ (* $p = 0.037$) Cognitive function ↑ (* $p = 0.002$) Dyspnea ($p = 0.051$) Insomnia ↓ (* $p = 0.029$) Pain ↓ (* $p = 0.013$)

Table 1. *Cont.*

Reference	Study Type; Number of Patients (n); Underlying Disease	IV Vitamin C Dose	Additional Interventions	Estimation of Fatigue	Impact on Fatigue and Related Parameters
[25]	Multi-center, retrospective, cohort study; n = 125, patients with breast cancer UICC IIa-IIIb	≥7.5 g at least 1 time/week for at least 4 weeks	+/− chemotherapy, radiation	3-point Likert scale	Fatigue (mean ± SD) During adjuvant therapy (first 6 months after operation): Verum: pre: 1.53 ± 1.11, post: 0.71 ± 0.89 Control: pre 1.68 ± 1.004, post: 1.24 ± 0.936 (** p = 0.004) During after care (6–12 month after operation): Verum: 0.34 ± 0.58 Control: 0.64 ± 0.718 (** p = 0.023) Sleep disorders ↓ (** p = 0.005) Depression ↓ (** p = 0.01)
			Infection, allergies		
[26]	Multi-center, prospective observational trial; n = 67; patients with herpes zoster infection	7.5 or 15 g; on average 8 infusions within 2–3 weeks	55.8% received anti-infective drug	4-point Likert scale	Fatigue improved in 78.2% of the patients; Impaired concentration improved in 81.8% of the patients
[27]	Multi-center, prospective observational trial; n = 71; patients with respiratory and cutaneous allergies	7.5 g; 2–3 times/week for 2–3 weeks in acute and 11–12 weeks in chronic states	35 % received anti-allergic drugs	4-point Likert scale	Sum score (0–12) of the 4 symptoms: fatigue, sleep disorders, depression, and lack of mental concentration decreased from 5.93 to 1.09 (* $p < 0.0001$) Fatigue improved in 93.5% of patients Sleep disorders improved in 92.5%, depression in 95.5%, and impaired concentration in 91.7%
			Others		
[28]	Single-center, randomized, double-blind, controlled clinical trial; n = 97; patients under-going laparoscopic colectomy	50 mg/ kg bw; Single application after induction of anesthesia	Analgesics	NRS (0–10)	No significant differences in fatigue score 2, 6, and 24 h post operation Pain ↓ (** $p < 0.05$)
[29]	Multi-center, randomized, double-blind, controlled clinical trial; n = 147; apparently healthy full-time worker	10 g, single application	None	NRS (0–10)	Fatigue (mean ± SD) Verum: Pre: 5.64 ± 2.02, after 2 h: 5.10 ± 2.04, after 24 h: 4.97 ± 2.33 Control: Pre: 5.54 ± 2.07, after 2 h: 5.31 ± 2.00, after 24 h: 5.66 ± 2.16 (** p = 0.004) Plasma vitamin C increased after 2 h, marker for oxidative stress decreased in the verum group (** $p < 0.001$)

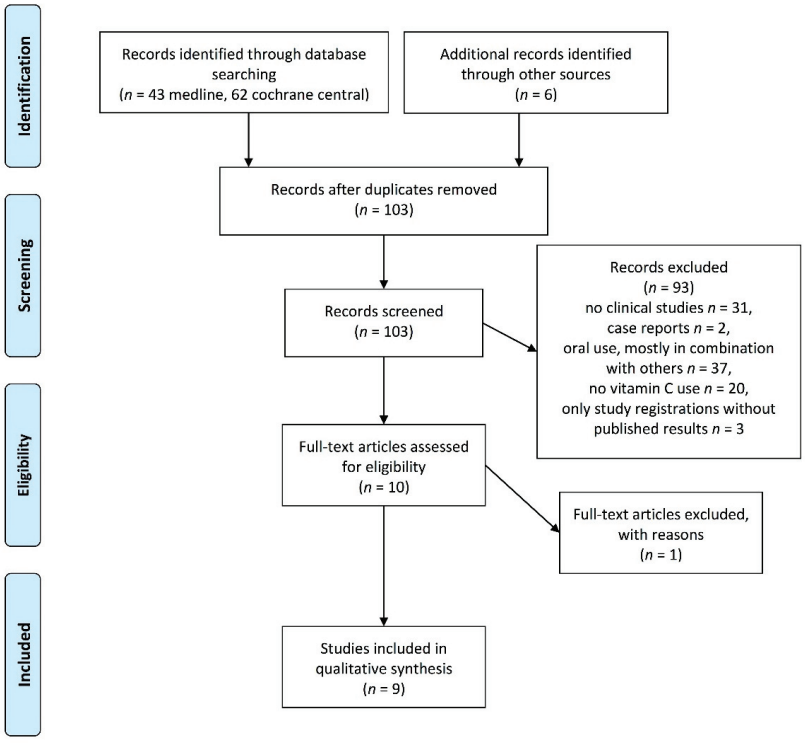


Figure 1. Documentation of study selection for the systematic review according to PRISMA guidelines.

4. Discussion

Altogether, nine clinical studies with 720 participants were identified. In three of the four controlled trials, a significant decrease in fatigue was detected in the high-dose vitamin C group compared to the control group. Vitamin C had no effect on acute post-operative fatigue. Four of the five observational or before-and-after studies performed a statistical comparison of pre–post values and observed a significant reduction in fatigue. To date, the effect of IV vitamin C on fatigue has been studied mainly in cancer patients. Additionally, there is one study in herpes zoster [26], one in allergies [27], one post-operative [28], and one in apparently healthy full-time employees [29].

Despite the different underlying diseases, high-dose vitamin C showed a significant reduction in fatigue in almost all studies. The most recent study in patients with advanced lung cancer [21] is particularly compelling: while fatigue continued to increase in the control group despite the best supportive therapy, it decreased significantly in the group with vitamin C plus hyperthermia. The oncology studies mostly used the EORCT QOL-C30, which also examines physical and cognitive dysfunction, dyspnea, insomnia, and pain. These complaints were also frequently alleviated by vitamin C. In cancer, very high doses of vitamin C are tested because of its chemotherapeutic potential. Three of the five oncology studies used doses >50 g [21–23]. In two studies, the dose was calculated based on body weight (bw) and ranged between 0.8 and 3 g vitamin C per kg bw [22]. For a 75 kg person, this means between 60 and 225 g of vitamin C per infusion. The two remaining studies used much lower (by a factor of 10) doses per application, yet fatigue was significantly reduced [24,25]. This means that very high doses do not seem to be necessary for improving

quality of life such as reducing fatigue. In their review of vitamin C in cancer-associated fatigue, Carr et al. [30] discussed the underlying mechanisms of action and concluded that the rapid correction of deficiency states, the effect as a co-factor of enzymatic reactions, and the anti-oxidative and anti-inflammatory effects are particularly important. All these effects do not require extremely high doses of vitamin C. The only study that investigated the effects of IV vitamin C in a viral disease (herpes zoster) also used a smaller amount (7.5 g) but with a high frequency (every second or third day) [26]. Fatigue improved in 78.2%, and impaired concentration improved in 81.8% of the patients. The same dose was used for the treatment of allergies, where fatigue is also a problem that affects the quality of life [27].

While the change in fatigue was only evaluated after 3 or more weeks in most studies, the study in apparently healthy full-time workers [29] reported an acute reduction in fatigue. One of the oncological studies [24] evaluated fatigue after one week and detected significant relief after this short treatment period.

The narrative feasibility analysis revealed that fatigue is most common in autoimmune diseases, intestinal bowel diseases, neurological diseases, and cancer [31–34]. Shared features of these diseases are inflammation and oxidative stress, which reinforce each other. Oxidative stress seems to be not only a convincing contributor but also a promising biomarker of the treatment of fatigue [35–39]. In cancer patients, an exercise intervention upon cessation of radiation or chemotherapy resulted in a reduction in fatigue [35]. The improvement was accompanied by a significant decrease in markers of oxidative stress. Changes in total and affective fatigue exhibited significant correlations with changes in plasma 8-hydroxy-deoxyguanosine over time, while behavioral and sensory fatigue changes were significantly correlated with protein carbonyls. Increases in antioxidant capacity were significantly correlated with reductions in affective, sensory, and cognitive fatigue [35]. Fatigue in patients with systemic lupus erythematosus with low disease activity is associated with increased markers of oxidative stress (F(2)-isoprostane). In a multivariate model, F(2)-isoprostane was a significant predictor of fatigue severity after adjustment for age, body mass index, pain, and depression [39]. Oxidative stress, impaired sleep homeostasis, mitochondrial dysfunction, immune activation, and (neuro-)inflammation can aggravate each other in a vicious pathophysiological loop in CFS [37]. Even in the pathophysiology of idiopathic CFS, oxidative stress seems to be a key contributor [36]. Compared to healthy controls, patients with idiopathic CFS have significantly elevated markers of oxidative stress (including reactive oxygen species, malondialdehyde, and F2-isoprostane) and reduced levels of antioxidant parameters, which include total antioxidant activity and catalase, superoxide dismutase, SOD, and GSH activity [36].

Fatigue is also very well known in cancer: not only does it accompany chemotherapy and radiation, which contribute to oxidative stress, but it can also persist long after completion of oncological treatment [34,40].

Inflammation and oxidative stress interfere with neurotransmitter metabolism, resulting in increased glutamatergic and decreased monoaminergic neurotransmission (serotonin, noradrenaline, and dopamine) via differing routes. These negatively affect neurotransmitter functioning in various cerebral areas that are involved in fatigue [41]. In this context, it is important to consider that oxidative stress not only reduces the bioavailability of neurotransmitters due to increased degradation, decreased formation, and distribution but also results in a decrease in antioxidants. Vitamin C is one of the most important endogenous antioxidants and is reduced in many chronic inflammatory diseases such as rheumatoid arthritis, inflammatory bowel diseases, and cancer [42–45]. Furthermore, together with vitamin C, vitamin B6, B12, and folic acid are important enzymatic cofactors of the synthesis of serotonin, dopamine, and noradrenaline.

Oxidative stress is also a major influencing factor for endothelial dysfunction and circulatory disorders. High-dose IV vitamin C combats overwhelming oxidative stress and restores endothelial and organ function [46]. In the case of COVID-19, oxidative stress not only triggers organ damage but also causes immune thrombosis via the formation

of neutrophil extracellular traps (NETs). This results in embolisms and dysfunction of microcirculation [7,47]. The situation is aggravated by the fact that SARS-CoV-2 also penetrates endothelial cells via ACE receptors and triggers a chain reaction of endothelial damage, infiltration of neutrophils, and resulting NETs [48]. As for vitamin C, it is essential for the phagocytosis of consumed neutrophils by macrophages. If this clearance does not take place, necrosis of the neutrophils occurs, leading to NETs and thus to circulatory disorders [8,47]. Therefore, an early application of high-dose vitamin C is proposed to possibly prevent the development of severe COVID-19 courses [7,47]. Indeed, in a first pilot study in COVID-19 patients requiring intensive care, high-dose IV vitamin C significantly improved oxygenation, reduced organ-damaging cytokine storm (IL-6), and showed a trend towards reduced mortality in severely ill patients [49]. A significant reduction in mortality and improvement of oxygen status by high-dose vitamin C was observed in a recent retrospective cohort study [50]. From these findings, it can be hypothesized that vitamin C administration may also be associated with a therapeutic post-viral benefit in the case of persistent symptoms. Randomized controlled trials, such as LOVIT-COVID (NCT04401150) or EVICT-CORONA-ALI (NCT04344184), are still ongoing.

A recent review of long COVID described abnormal chest X-rays/CT in 34% of the patients 6 months after infection. Markers reported to be elevated were D-dimer, NT-proBNP, C-reactive protein, serum ferritin, procalcitonin, and IL-6 [6], which implies involvement in circulatory disorders, cardiac insufficiency, and inflammatory reactions.

Another cause of persistent symptoms could be the induction of immune responses to self-epitopes during acute severe COVID-19. First observations point to IgG autoantibodies that are widely associated with myopathies, vasculitis, and antiphospholipid syndromes in SARS-CoV-2 infected subjects [51]. The observation of autoimmune antibodies is interesting, as fatigue is a known major problem in autoimmune diseases such as multiple sclerosis [37,52], rheumatoid arthritis [33,53], diabetes mellitus type 1 [54], systemic lupus erythematosus [39], and inflammatory bowel diseases [55].

Inflammation results in an overlap of fatigue, disturbed sleep, cognitive deficits, pain, and depression-like symptoms [41], the very pattern of symptoms observed in long COVID. These factors, which accompany and probably promote fatigue in long COVID, were alleviated in the clinical studies on IV vitamin C.

Therefore, the effects of IV vitamin C on post-viral COVID-19 fatigue should be investigated in clinical trials.

5. Conclusions

Oxidative stress and inflammation can cause and maintain fatigue, cognitive impairment, depression, and sleep disturbances. They disrupt the formation and functioning of important neurotransmitters and of blood circulation. Vitamin C is one of the most effective physiological antioxidants, showing anti-inflammatory effects, especially if applied intravenously in pharmacological doses. It restores endothelial function, and it is an enzymatic co-factor in the synthesis of various neurotransmitters.

High-dose IV vitamin C has been investigated in four controlled and five observational or before-and-after studies in patients with cancer, allergies, and herpes zoster infections. The results show a reduction in fatigue and attendant symptoms such as sleep disturbances, depressive symptoms, pain, and cognitive disorders.

COVID-19 is a multisystem disease in which oxidative stress is partly responsible for excessive inflammation and circulatory disorders such as immune thrombosis. Vitamin C deficiency has been demonstrated in COVID-19 and other acute severe infections and should also be investigated in long COVID. Furthermore, the effects of high-dose IV vitamin C on long COVID-associated fatigue should be investigated in clinical trials.

Author Contributions: Conceptualization, C.V. and K.K.; methodology, C.V.; validation, C.V., and K.K.; formal analysis, C.V.; investigation, C.V.; data curation, C.V.; writing—original draft preparation, C.V.; writing—review and editing, C.V. and K.K.; visualization, C.V.; supervision, K.K.; project administration, C.V.; funding acquisition, C.V. All authors have read and agreed to the published version of the manuscript.

Funding: Charge for the open-access journal was sponsored by Pascoe Pharmazeutische Praeparate GmbH, Germany.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Not applicable.

Conflicts of Interest: K.K. declare that she has no competing interests. C.V. is employed part time at Pascoe Pharmazeutische Praeparate GmbH (Giessen, Germany).

References

1. Komaroff, A.L.; Bateman, L. Will COVID-19 Lead to Myalgic Encephalomyelitis/Chronic Fatigue Syndrome? *Front. Med. (Lausanne)* **2020**, *7*, 606824. [CrossRef]
2. Bleijenberg, G.; van der Meer, J.W.M. Chapter 442: Chronic Fatigue Syndrome. In *Harrison's Principles of Internal Medicine, 20e*; McGraw-Hill Education: New York, NY, USA, 2018.
3. NIHR. Living with Covid19. Available online: https://evidence.nihr.ac.uk/themedreview/living-with-covid19/ (accessed on 25 February 2021).
4. NIH. COVID-19 Treatment Guidlines: Clinical Spectrum of SARS-CoV-2 Infection. Available online: https://www.covid19treatmentguidelines.nih.gov/overview/clinical-spectrum/ (accessed on 15 March 2021).
5. Huang, C.; Huang, L.; Wang, Y.; Li, X.; Ren, L.; Gu, X.; Kang, L.; Guo, L.; Liu, M.; Zhou, X.; et al. 6-month consequences of COVID-19 in patients discharged from hospital: A cohort study. *Lancet* **2021**, *397*, 220–232. [CrossRef]
6. Lopez-Leon, S.; Wegman-Ostrosky, T.; Perelman, C.; Sepulveda, R.; Rebolledo, P.A.; Cuapio, A.; Villapol, S. More than 50 Long-term effects of COVID-19: A systematic review and meta-analysis. *medRxiv* **2021**. [CrossRef]
7. Schonrich, G.; Raftery, M.J.; Samstag, Y. Devilishly radical NETwork in COVID-19: Oxidative stress, neutrophil extracellular traps (NETs), and T cell suppression. *Adv. Biol. Regul.* **2020**, *77*, 100741. [CrossRef]
8. Carr, A.C.; Maggini, S. Vitamin C and Immune Function. *Nutrients* **2017**, *9*, 1211. [CrossRef]
9. Chiscano-Camon, L.; Ruiz-Rodriguez, J.C.; Ruiz-Sanmartin, A.; Roca, O.; Ferrer, R. Vitamin C levels in patients with SARS-CoV-2-associated acute respiratory distress syndrome. *Crit. Care* **2020**, *24*, 522. [CrossRef]
10. Carr, A.C.; Spencer, E.; Dixon, L.; Chambers, S.T. Patients with Community Acquired Pneumonia Exhibit Depleted Vitamin C Status and Elevated Oxidative Stress. *Nutrients* **2020**, *12*, 1318. [CrossRef]
11. Xing, Y.; Zhao, B.; Yin, L.; Guo, M.; Shi, H.; Zhu, Z.; Zhang, L.; He, J.; Ling, Y.; Gao, M.; et al. Vitamin C supplementation is necessary for patients with coronavirus disease: An ultra-high-performance liquid chromatography-tandem mass spectrometry finding. *J. Pharm Biomed. Anal.* **2021**, *196*, 113927. [CrossRef]
12. Pincemail, J.; Cavalier, E.; Charlier, C.; Cheramy-Bien, J.P.; Brevers, E.; Courtois, A.; Fadeur, M.; Meziane, S.; Goff, C.L.; Misset, B.; et al. Oxidative Stress Status in COVID-19 Patients Hospitalized in Intensive Care Unit for Severe Pneumonia. *Pilot Study Antioxid.* **2021**, *10*, 257. [CrossRef]
13. Arvinte, C.; Singh, M.; Marik, P.E. Serum Levels of Vitamin C and Vitamin D in a Cohort of Critically Ill COVID-19 Patients of a North American Community Hospital Intensive Care Unit in May 2020: A Pilot Study. *Med. Drug Discov.* **2020**, *8*, 100064. [CrossRef]
14. Blaszczak, W.; Barczak, W.; Masternak, J.; Kopczynski, P.; Zhitkovich, A.; Rubis, B. Vitamin C as a Modulator of the Response to Cancer Therapy. *Molecules* **2019**, *24*, 453. [CrossRef] [PubMed]
15. Dattola, A.; Silvestri, M.; Bennardo, L.; Passante, M.; Scali, E.; Patruno, C.; Nistico, S.P. Role of Vitamins in Skin Health: A Systematic Review. *Curr. Nutr. Rep.* **2020**, *9*, 226–235. [CrossRef] [PubMed]
16. Institute of Medicine (US). Panel on Dietary Antioxidants and Related Compounds. In *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Carotenoids*; National Academies Press: Washington, DC, USA, 2000.
17. Levine, M.; Padayatty, S.J.; Espey, M.G. Vitamin C: Concentration-function approach yields pharmacology and therapeutic discoveries. *Adv. Nutr.* **2011**, *2*, 78–88. [CrossRef]
18. Padayatty, S.J.; Sun, H.; Wang, Y.; Riordan, H.D.; Hewitt, S.M.; Katz, A.; Wesley, R.A.; Levine, M. Vitamin C pharmacokinetics: Implications for oral and intravenous use. *Ann. Intern. Med.* **2004**, *140*, 533–537. [CrossRef]
19. Kuiper, C.; Vissers, M.C.; Hicks, K.O. Pharmacokinetic modeling of ascorbate diffusion through normal and tumor tissue. *Free Radic. Biol. Med.* **2014**, *77*, 340–352. [CrossRef]

20. Patterson, T.; Isales, C.M.; Fulzele, S. Low level of Vitamin C and dysregulation of Vitamin C transporter might be involved in the severity of COVID-19 Infection. *Aging Dis.* **2021**, *12*, 14–26. [CrossRef]
21. Ou, J.; Zhu, X.; Chen, P.; Du, Y.; Lu, Y.; Peng, X.; Bao, S.; Wang, J.; Zhang, X.; Zhang, T.; et al. A randomized phase II trial of best supportive care with or without hyperthermia and vitamin C for heavily pretreated, advanced, refractory non-small-cell lung cancer. *J. Adv. Res.* **2020**, *24*, 175–182. [CrossRef]
22. Stephenson, C.M.; Levin, R.D.; Spector, T.; Lis, C.G. Phase I clinical trial to evaluate the safety, tolerability, and pharmacokinetics of high-dose intravenous ascorbic acid in patients with advanced cancer. *Cancer Chemother. Pharm.* **2013**, *72*, 139–146. [CrossRef]
23. Takahashi, H.; Mizuno, H.; Yanagisawa, A. High-dose intravenous vitamin C improves quality of life in cancer patients. *Pers. Med. Universe* **2012**, *1*, 49–53. [CrossRef]
24. Yeom, C.H.; Jung, G.C.; Song, K.J. Changes of terminal cancer patients' health-related quality of life after high dose vitamin C administration. *J. Korean Med. Sci.* **2007**, *22*, 7–11. [CrossRef] [PubMed]
25. Vollbracht, C.; Schneider, B.; Leendert, V.; Weiss, G.; Auerbach, L.; Beuth, J. Intravenous vitamin C administration improves quality of life in breast cancer patients during chemo-/radiotherapy and aftercare: Results of a retrospective, multicentre, epidemiological cohort study in Germany. *In Vivo* **2011**, *25*, 983–990.
26. Schencking, M.; Vollbracht, C.; Weiss, G.; Lebert, J.; Biller, A.; Goyvaerts, B.; Kraft, K. Intravenous vitamin C in the treatment of shingles: Results of a multicenter prospective cohort study. *Med. Sci. Monit.* **2012**, *18*, CR215. [CrossRef] [PubMed]
27. Vollbracht, C.; Raithel, M.; Krick, B.; Kraft, K.; Hagel, A.F. Intravenous vitamin C in the treatment of allergies: An interim subgroup analysis of a long-term observational study. *J. Int. Med. Res.* **2018**, *46*, 3640–3655. [CrossRef]
28. Jeon, Y.; Park, J.S.; Moon, S.; Yeo, J. Effect of Intravenous High Dose Vitamin C on Postoperative Pain and Morphine Use after Laparoscopic Colectomy: A Randomized Controlled Trial. *Pain Res. Manag.* **2016**, *2016*, 9147279. [CrossRef]
29. Suh, S.Y.; Bae, W.K.; Ahn, H.Y.; Choi, S.E.; Jung, G.C.; Yeom, C.H. Intravenous Vitamin C Administration Reduces Fatigue in Office Workers: A Double-blind Randomized Controlled Trial. *Nutr. J.* **2012**, *11*, 7. [CrossRef] [PubMed]
30. Carr, A.C.; Vissers, M.C.; Cook, J.S. The effect of intravenous vitamin C on cancer- and chemotherapy-related fatigue and quality of life. *Front. Oncol.* **2014**, *4*, 283. [CrossRef] [PubMed]
31. Finsterer, J.; Mahjoub, S.Z. Fatigue in healthy and diseased individuals. *Am. J. Hosp. Palliat Care* **2014**, *31*, 562–575. [CrossRef]
32. Borren, N.Z.; van der Woude, C.J.; Ananthakrishnan, A.N. Fatigue in IBD: Epidemiology, pathophysiology and management. *Nat. Rev. Gastroenterol. Hepatol.* **2019**, *16*, 247–259. [CrossRef]
33. Elera-Fitzcarrald, C.; Rocha, J.; Burgos, P.I.; Ugarte-Gil, M.F.; Petri, M.; Alarcon, G.S. Measures of Fatigue in Patients With Rheumatic Diseases: A Critical Review. *Arthritis Care Res.* **2020**, *72* (Suppl. 10), 369–409. [CrossRef]
34. Mohandas, H.; Jaganathan, S.K.; Mani, M.P.; Ayyar, M.; Rohini Thevi, G.V. Cancer-related fatigue treatment: An overview. *J. Cancer Res. Ther.* **2017**, *13*, 916–929. [CrossRef]
35. Repka, C.P.; Hayward, R. Effects of an Exercise Intervention on Cancer-Related Fatigue and Its Relationship to Markers of Oxidative Stress. *Integr. Cancer Ther.* **2018**, *17*, 503–510. [CrossRef]
36. Lee, J.S.; Kim, H.G.; Lee, D.S.; Son, C.G. Oxidative Stress is a Convincing Contributor to Idiopathic Chronic Fatigue. *Sci. Rep.* **2018**, *8*, 12890. [CrossRef]
37. Morris, G.; Stubbs, B.; Kohler, C.A.; Walder, K.; Slyepchenko, A.; Berk, M.; Carvalho, A.F. The putative role of oxidative stress and inflammation in the pathophysiology of sleep dysfunction across neuropsychiatric disorders: Focus on chronic fatigue syndrome, bipolar disorder and multiple sclerosis. *Sleep Med. Rev.* **2018**, *41*, 255–265. [CrossRef]
38. Fukuda, S.; Nojima, J.; Motoki, Y.; Yamaguti, K.; Nakatomi, Y.; Okawa, N.; Fujiwara, K.; Watanabe, Y.; Kuratsune, H. A potential biomarker for fatigue: Oxidative stress and anti-oxidative activity. *Biol. Psychol.* **2016**, *118*, 88–93. [CrossRef] [PubMed]
39. Segal, B.M.; Thomas, W.; Zhu, X.; Diebes, A.; McElvain, G.; Baechler, E.; Gross, M. Oxidative stress and fatigue in systemic lupus erythematosus. *Lupus* **2012**, *21*, 984–992. [CrossRef]
40. Pearson, E.J.M.; Morris, M.E.; di Stefano, M.; McKinstry, C.E. Interventions for cancer-related fatigue: A scoping review. *Eur. J. Cancer Care* **2018**, *27*. [CrossRef]
41. Korte, S.M.; Straub, R.H. Fatigue in inflammatory rheumatic disorders: Pathophysiological mechanisms. *Rheumatology* **2019**, *58*, v35–v50. [CrossRef]
42. Lunec, J.; Blake, D.R. The determination of dehydroascorbic acid and ascorbic acid in the serum and synovial fluid of patients with rheumatoid arthritis (RA). *Free Radic. Res. Commun.* **1985**, *1*, 31–39. [CrossRef] [PubMed]
43. Jaswal, S.; Mehta, H.C.; Sood, A.K.; Kaur, J. Antioxidant status in rheumatoid arthritis and role of antioxidant therapy. *Clin. Chim. Acta* **2003**, *338*, 123–129. [CrossRef]
44. Carr, A.C.; Cook, J. Intravenous Vitamin C for Cancer Therapy-Identifying the Current Gaps in Our Knowledge. *Front. Physiol.* **2018**, *9*, 1182. [CrossRef]
45. Subramanian, V.S.; Sabui, S.; Subramenium, G.A.; Marchant, J.S.; Said, H.M. Tumor necrosis factor alpha reduces intestinal vitamin C uptake: A role for NF-kappaB-mediated signaling. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2018**, *315*, G241–G248. [CrossRef]
46. Spoelstra-de Man, A.M.E.; Elbers, P.W.G.; Oudemans-Van Straaten, H.M. Vitamin C: Should we supplement? *Curr. Opin Crit. Care* **2018**, *24*, 248–255. [CrossRef]
47. Holford, P.; Carr, A.C.; Jovic, T.H.; Ali, S.R.; Whitaker, I.S.; Marik, P.E.; Smith, A.D. Vitamin C-An Adjunctive Therapy for Respiratory Infection, Sepsis and COVID-19. *Nutrients* **2020**, *12*, 3760. [CrossRef]

48. Leppkes, M.; Knopf, J.; Naschberger, E.; Lindemann, A.; Singh, J.; Herrmann, I.; Sturzl, M.; Staats, L.; Mahajan, A.; Schauer, C.; et al. Vascular occlusion by neutrophil extracellular traps in COVID-19. *EBioMedicine* **2020**, *58*, 102925. [CrossRef]
49. Zhang, J.; Rao, X.; Li, Y.; Zhu, Y.; Liu, F.; Guo, G.; Luo, G.; Meng, Z.; De Backer, D.; Xiang, H.; et al. Pilot trial of high-dose vitamin C in critically ill COVID-19 patients. *Ann. Intensive Care* **2021**, *11*, 5. [CrossRef]
50. Gao, D.; Xu, M.; Wang, G.; Lv, J.; Ma, X.; Guo, Y.; Zhang, D.; Yang, H.; Jiang, W.; Deng, F.; et al. The efficiency and safety of high-dose vitamin C in patients with COVID-19: A retrospective cohort study. *Aging* **2021**, *13*. [CrossRef]
51. Bhadelia, N.; Belkina, A.C.; Olson, A.; Winters, T.; Urick, P.; Lin, N.; Rifkin, I.; Kataria, Y.; Yuen, R.R.; Sagar, M.; et al. Distinct Autoimmune Antibody Signatures Between Hospitalized Acute COVID-19 Patients, SARS-CoV-2 Convalescent Individuals, and Unexposed Pre-Pandemic Controls. *medRxiv* **2021**. [CrossRef]
52. Rottoli, M.; La Gioia, S.; Frigeni, B.; Barcella, V. Pathophysiology, assessment and management of multiple sclerosis fatigue: An update. *Expert Rev. Neurother.* **2017**, *17*, 373–379. [CrossRef]
53. Cramp, F. The role of non-pharmacological interventions in the management of rheumatoid-arthritis-related fatigue. *Rheumatology* **2019**, *58*, v22–v28. [CrossRef]
54. Griggs, S.; Morris, N.S. Fatigue Among Adults With Type 1 Diabetes Mellitus and Implications for Self-Management: An Integrative Review. *Diabetes Educ.* **2018**, *44*, 325–339. [CrossRef] [PubMed]
55. Nocerino, A.; Nguyen, A.; Agrawal, M.; Mone, A.; Lakhani, K.; Swaminath, A. Fatigue in Inflammatory Bowel Diseases: Etiologies and Management. *Adv. Ther.* **2020**, *37*, 97–112. [CrossRef] [PubMed]

nutrients

MDPI

Review

Global Vitamin C Status and Prevalence of Deficiency: A Cause for Concern?

Sam Rowe [1] and Anitra C. Carr [2,*]

[1] Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool L35QA, UK; sam.rowe@lstmed.ac.uk

[2] Nutrition in Medicine Research Group, Department of Pathology & Biomedical Science, University of Otago, Christchurch 8011, New Zealand

* Correspondence: anitra.carr@otago.ac.nz; Tel.: +64-3364-0649

Received: 24 May 2020; Accepted: 3 July 2020; Published: 6 July 2020

Abstract: Vitamin C is an essential nutrient that must be obtained through the diet in adequate amounts to prevent hypovitaminosis C, deficiency and its consequences—including the potentially fatal deficiency disease scurvy. Global vitamin C status and prevalence of deficiency has not previously been reported, despite vitamin C's pleiotropic roles in both non-communicable and communicable disease. This review highlights the global literature on vitamin C status and the prevalence of hypovitaminosis C and deficiency. Related dietary intake is reported if assessed in the studies. Overall, the review illustrates the shortage of high quality epidemiological studies of vitamin C status in many countries, particularly low- and middle-income countries. The available evidence indicates that vitamin C hypovitaminosis and deficiency is common in low- and middle-income countries and not uncommon in high income settings. Further epidemiological studies are required to confirm these findings, to fully assess the extent of global vitamin C insufficiency, and to understand associations with a range of disease processes. Our findings suggest a need for interventions to prevent deficiency in a range of at risk groups and regions of the world.

Keywords: vitamin C status; hypovitaminosis C; vitamin C deficiency; low and middle income; LMIC; dietary intake; supplement; non-communicable disease; communicable disease; infection

1. Introduction

Vitamin C (ascorbic acid) is an essential nutrient that must be consumed on a regular basis to prevent deficiency [1]. Maintenance of the body pool of vitamin C is dependent on dietary intake, efficient absorption, recycling and renal reuptake of the vitamin [2]. Vitamin C concentrations in blood and tissues are tightly controlled via specialized sodium-dependent vitamin C transporters (SVCTs) [3]. Different tissues and organs have variable requirements for vitamin C, as reflected by their vitamin C concentrations [4]. Tissues with the highest concentrations of the vitamin include the brain, adrenals and pituitary gland. This reflects one of the major functions of vitamin C, which is to act as a cofactor for a family of biosynthetic and regulatory metalloenzymes, including those involved in the synthesis of catecholamine and peptide hormones [5,6]. Recent research has also indicated a role for vitamin C in genetic and epigenetic regulation via enzymes that regulate gene transcription and the methylation of DNA and histones [7,8]. As a result, vitamin C has the potential to regulate thousands of genes in the body and thus play pleiotropic roles in human health and disease.

Historically, recommended intakes of micronutrients have been based on daily intakes required to prevent disease secondary to deficiency. Previous reviews of micronutrients have indicated high rates of global deficiency [9]. Due to a growing body of evidence that increased vitamin C intake has a beneficial effect on long-term health outcomes [1], many regulatory authorities have increased recommended intakes of vitamin C in their respective countries [10]. Vitamin C intakes of 100–200 mg/day will maintain

blood concentrations at adequate to saturating status i.e., 50–75 μmol/L [11]. When blood concentrations fall to the hypovitaminosis C range (i.e., <23 μmol/L), symptoms of vitamin C insufficiency may become apparent, such as fatigue, lethargy and mood changes, e.g., irritability and depression [11]. People with hypovitaminosis C are at high risk of developing vitamin C deficiency (defined as <11 μmol/L), putting them at risk of developing clinical scurvy, which is fatal if left untreated [12].

Global vitamin C status and prevalence of insufficiency has never been fully reported, despite increasing concern over micronutrient malnutrition in low-middle income countries (LMICs) resulting in poor health and higher rates of morbidity and mortality [13,14]. Here we provide an overview of global vitamin C status and prevalence of insufficiency and the associated potential public health impact of deficiency.

2. Selection and Assessment of Vitamin C Status Publications

For the illustrative purposes of this review, we describe key papers reporting the status of predominately healthy or randomly selected groups globally. A thorough literature search was conducted using the PubMed database using the keywords: vitamin C, ascorbic acid, ascorbate, blood, plasma, serum, concentration, level, status. No restrictions were placed on the publication date, study location, sex or age of participants. Further literature was found within the reference lists of published papers. Original English language publications containing plasma or serum vitamin C concentrations of healthy adults, pregnant women, children and adolescents are described. The vitamin C status of specific patient groups is not described. Countries were categorized as high, upper middle, lower middle or low income based on the World Bank income classifications [15]. Due to the scarcity of research in many LMICs smaller studies from LMICs with a minimum of 90 individuals are described. Where multiple large studies were available, e.g., USA, the most recent and largest studies are described. Where available, mean vitamin C concentrations (μmol/L) and mean dietary intakes (mg/day), rates of hypovitaminosis C and frank deficiency (%), are described. Currently, there are no internationally accepted cut off values to define hypovitaminosis C and vitamin C deficiency. Therefore, the most commonly used values have been used, e.g., ≤23–28 μmol/L for hypovitaminosis C and ≤11 μmol/L for deficiency. All plasma and serum concentrations are provided as mean and SD (or IQR) in μmol/L (converted from mg/dL or μg/mL as required) and missing data were calculated using weighted means.

3. Global Vitamin C Status and Prevalence of Insufficiency

3.1. High Income Countries

Several large epidemiological studies assessing vitamin C status and/or prevalence of deficiency in adults have been carried out in Europe and North America (Table 1). The largest study to have measured vitamin C status globally is the European EPIC-Norfolk study carried out in England (1993–1997) [16,17]. This study assessed >22,400 participants (aged 40–79 years), revealing a weighted mean of 54 μmol/L vitamin C (48 and 59 μmol/L for men and women, respectively), and a prevalence of deficiency of 1.4% (2.2% for men and 0.8% for women). Dietary intakes of vitamin C, determined using 7-day food diaries, were 85 mg/day for the cohort (83 and 87 mg/day for men and women, respectively). The smaller National Diet and Nutrition Survey carried out in 1994/1995 in the UK (England and Scotland) in >1300 elderly participants (aged ≥65 years) showed a lower vitamin C status (44 ± 25 μmol/L), and a higher prevalence of deficiency (14%) [18], more comparable to the French POLA study of the same age group (see below). The third MONICA study, carried out in Glasgow, Scotland in 1992 in >1200 adults, indicated an even higher prevalence of deficiency of 20% for the cohort (26% for men and 14% for women) [19].

Table 1. Vitamin C status and prevalence of deficiency and hypovitaminosis C in adults from high-income countries.

Country (Region) Sampling Dates	Population (Age Range)	Vitamin C Status (μmol/L) [a]	Deficiency (% <11 μmol/L)	Hypovitaminosis C (% <23 or 28 μmol/L)	Dietary Intake (mg/day) [a]	References
Europe						
England (Norfolk) 1993–1997	22,474 total (40–79 years)	54 [b]	1.4	12	85 [b]	[16,17]
	10,267 males	48 [b]	2.2	17	83 [b]	
	12,207 females	59 [b]	0.8	8	87 [b]	
UK (England and Scotland) 1994–1995	1310 elderly (≥65 years)	44 (25)	14	–	–	[18]
Scotland (Glasgow) 1992	1267 total (25–74 years)	–	20	44	–	[19]
	632 males	–	26	52	–	
	635 females	–	14	36	–	
France (all regions) 1994–1995	12,741 total (35–60 years)	56 [b]	1.0	–	100 [b]	[20,21]
	5028 males	50 (23)	1.8	–	103 (48)	
	7713 females	60 (31)	0.4	–	98 (44)	
France (South; Sète) 1995–1997	1987 total (>60 years)	36 [b]	9 [b]	–	–	[22]
	874 elderly males	32	13 [b]	–	–	
	1113 elderly females	40	6 [b]	–	–	
France (Paris) <1991	837 total (≥18 years)	48 [b]	–	–	–	[23]
	361 males	41	–	–	–	
	476 females	53	–	–	–	
France (Nancy)	459 males (20–60 years)	39 [b]	–	–	80 [b]	[24]
Finland (North Karelia) 1992–2002	1616 total (25–64 years)	40 [b]	–	–	–	[25,26]
	974 males	37	2.2	4.4	–	
	642 females	44	–	–	–	
Finland (Eastern) 1984–1989	1605 males (42–60 years)	48 (23)	5.7	–	–	[27]
Austria (Graz) 1991–1994	786 total (45–86 years)	58 (21)	–	–	–	[28]
	330 males	50 (20)	–	–	–	
	456 females	64 (19)	–	–	–	
Spain (Alicante province) 2000–2001	545 total (>65 years)	45 [b]	–	–	131 [b]	[29]
	252 elderly males	38 (20)	–	–	125 (64)	
	293 elderly females	51 (18)	–	–	136 (70)	

Table 1. *Cont.*

Country (Region) Sampling Dates	Population (Age Range)	Vitamin C Status (μmol/L) [a]	Deficiency (% <11 μmol/L)	Hypovitaminosis C (% <23 or 28 μmol/L)	Dietary Intake (mg/day) [a]	References
Europe (France, Ireland, Spain Netherlands)	349 total (25–45 years)	59 [b]	–	–	–	[30]
	175 males	54 (13–103) [d]	–	–	–	
	174 females	64 (6–117) [d]	–	–	–	
Germany (Giessen) 1994–2004	279 total (62–92 years)	71 [b]	–	–	90 [b]	[31]
	98 elderly males	62 (55–74) [c]	–	–	84 (61–116) [c]	
	181 elderly females	76 (62–89) [c]	–	–	93 (70–132) [c]	
North America						
United States 2003–2004	4438 total (≥20 years)	49 (32–71) [c]	8.4	–	–	[32]
	2153 males	45 (27–66) [c]	10.0	–	–	
	2285 females	53 (38–76) [c]	6.9	–	–	
Canada (16 sites countrywide) 2012–2013	1615 total (20–79 years)	53	<3	–	–	[33]
	804 males	47	–	–	–	
	811 females	59	–	–	–	
Canada (Toronto) 2004–2008	979 total (20–29 years)	31	14	47	140/242 [b,e]	[34]
	287 males	29	16	37	228	
	692 females	33	13	45	248	
Asia-Pacific						
Japan (Shibata, Niigata Prefecture) 1977	2185 total (40–89 years)	51 [b]	–	–	–	[35]
	919 males	43 (19)	–	–	–	
	1266 females	57 (17)	–	–	–	
Japan (Itabashi, Tokyo) 2006	655 elderly females (70–84 years)	51 (9)	–	–	–	[36]
Singapore 1993–1995	941 total (30–69 years)	37 [b]	12 [b]	–	–	[37,38]
	468 males	32 [b]	17 [b]	–	–	
	473 females	41 [b]	6.0 [b]	–	–	
New Zealand (Canterbury region) 2010–2013	369 total (50 years)	44	2.4	13	110	[39]
	174 males	41	4.0	15	113	
	195 females	47	1.0	11	107	

[a] Data represent mean (SD), if provided; [b] For missing data, weighted means were calculated; [c] Mean or median and interquartile range; [d] Mean and range; [e] Intake without/with supplement use.

The SU.VI.MAX study carried out in France (1994/1995) assessed factors influencing blood concentrations of antioxidant vitamins in >12,700 French participants aged 35–60 years [20,21]. This study showed comparable results to EPIC-Norfolk, with the vitamin C status of men and women being 50 ± 23 and 60 ± 31 μmol/L, respectively, giving a weighted mean of 56 μmol/L for the total cohort. Of these, only 1% exhibited vitamin C deficiency, higher for men (1.8%) than women (0.4%). Dietary intakes of vitamin C were assessed from six 24-h dietary records during the first 18 months of the study, giving a weighted mean of 100 mg/day for the cohort (103 and 98 mg/day for men and women, respectively). Another smaller study was carried out at a similar time (1995–1997) in Sète in the South of France in >1900 elderly participants aged >60 years (the POLA study) [22]. This study indicated a lower mean vitamin C concentration of 36 μmol/L (32 and 40 μmol/L for men and women, respectively) and a higher prevalence of deficiency (9%; 13% for men and 6% for women). Other smaller studies carried out in France (Paris and Nancy) have shown intermediate vitamin C status between these two studies, i.e., 39–41 μmol/L for men and 53 μmol/L for women [23,24]. The men in Nancy had a correspondingly lower dietary intake of 80 mg/day, determined using a dietary questionnaire that collected data on the participant's diet history during the previous year [24].

Two studies have been carried out in Finland: one in North Karelia (1992–2002) in >1600 adults aged 25–64 years [25,26] and one in Eastern Finland (1984–1989) in >1600 men aged 42–65 years [27]. The mean vitamin C status for men ranged from 37 to 48 ± 23 μmol/L, with a prevalence of deficiency of 2.2% to 5.7% [25–27]. A small study of apparently healthy elderly participants in the Alicante province of Spain in 2000/2001 indicated comparable vitamin C status, with 38 ± 20 μmol/L in men and 51 ± 18 μmol/L in women, giving a weighted mean of 45 μmol/L [29]. The participants had relatively high intakes of vitamin C, assessed using a semiquantitative food frequency questionnaire; 125 ± 64 mg/day for men and 136 ± 70 mg/day for women. Another small study of five countries across Europe (including Spain, France, Netherlands, Northern Ireland and Republic of Ireland) indicated a weighted mean in healthy non-smoking adults (aged 25–45 years) of 59 μmol/L vitamin C (54 and 64 μmol/L for men and women, respectively) [30]. Similar values were recorded in Graz, Austria (1991–1994); 58 ± 21 μmol/L for a cohort of 786 adult men and women (50 ± 20 μmol/L for men and 64 ± 19 μmol/L for women) [28]. In Europe, the highest vitamin C values reported to date have come from the GISELA study in Giessen Germany (1994–2004), in which independently living senior citizens had a weighted mean of 71 μmol/L vitamin C (62 and 76 μmol/L for men and women, respectively) [31]. Dietary intakes, assessed using a 3-day estimated dietary record, revealed a mean intake of 90 mg/day for the cohort (84 and 93 mg/day for men and women, respectively). The researchers attributed the lower vitamin C plasma concentrations in men compared with women partly to a volumetric dilution effect due to differences in fat-free mass.

In the United States, the National Health and Nutrition Examination Surveys (NHANES) have been reporting nationally representative vitamin C status data over nearly four decades [32,40,41]. The most recent survey (2003/2004) included >4400 adults (aged ≥20 years) and indicated a mean status of 49 μmol/L vitamin C for the cohort (45 and 53-μmol/L for men and women, respectively) [32]. The prevalence of deficiency was 8.4% (10% for men and 6.9% for women), significantly higher than that observed in the larger European SU.VI.MAX and EPIC-Norfolk studies. Dietary intakes were not reported in this study, however, the earlier NHANES III (1988–1994) report indicated a mean dietary intake of ~106 mg/day (determined using 24-h dietary recalls) for a similarly aged cohort (≥18 years) [41]. The recent Canadian Health Measures Survey (2012/2013) assessed the vitamin C status of >1600 adults (aged 20–79 years) and reported a mean vitamin C status of 53 μmol/L (47 and 59 μmol/L for men and women, respectively). The prevalence of deficiency was <3%, in line with the large European studies (SU.VI.MAX and EPIC-Norfolk). Another smaller study was carried out in >900 young non-smoking Canadians (aged 20–29 years), however, the vitamin C concentrations were unusually low (31 μmol/L, with 14% deficiency), despite relatively high dietary intakes of 242 mg/day for the total cohort (140 mg/day for non-supplement users), assessed using food frequency questionnaires for dietary intake over the previous month [34]. The low vitamin C status in this study

has been attributed to the blood samples not being processed or stored appropriately for accurate vitamin C analysis [42,43].

Several studies have been carried out in high-income countries in Asia and the Pacific (Table 1). In Japan, the largest study was undertaken in 1977 (in Shibata, Niigata Prefecture) [35]. This study comprised >2100 adults aged 40–89 years and indicated a mean vitamin C status of 51 μmol/L (43 ± 19 μmol/L for men and 57 ± 17 μmol/L for women). A more recent study carried out in Tokyo (2006) in >600 elderly women (aged 70–84 years) indicated a comparable plasma vitamin C status of 51 ± 9 μmol/L. Middle-aged participants in the South Island of New Zealand had a mean vitamin C status of 44 μmol/L (41 and 47 μmol/L for men and women, respectively) and a relatively low prevalence of deficiency of 2.4% (4.0% for men and 1.0% for women). The average dietary intake of these participants was 110 mg/day (113 and 107 mg/day for men and women, respectively), determined using four day estimated food diaries [39]. In contrast, the vitamin C status of adults (aged 30–69) in Singapore was lower at a mean of 37 μmol/L (32 and 41 μmol/L for men and women, respectively), with 12% deficiency (17% for men and 6% for women) [37,38]. Lower vitamin C concentrations were reported in Indians and Malays than Chinese in this study. The authors suggested this may have been due to low intakes of fresh fruit and different traditional cooking practices and cuisines.

3.2. Low and Middle Income Countries

There have been few vitamin C status studies carried out in Central and South America (Table 2). Two studies in Mexican women have indicated low vitamin C status (19 and 30 ± 13 μmol/L) and high rates of hypovitaminosis C and deficiency (up to 32% and 39%) [44,45], despite reported mean dietary intakes of 72 mg/day, based on three 24-h recalls [44]. Hypovitaminosis C was associated with obesity in one of the studies [44]. Regional variations were seen with lower deficiency rates in Mexico City than North and South Mexico [45]. A study of 369 elderly people of low socioeconomic status in Quinto, Ecuador indicated a very low mean vitamin C status of 15 μmol/L (11 ± 9 μmol/L for men and 17 ± 10 μmol/L for women) and a high prevalence of deficiency (43%), with men exhibiting more deficiency than women (60% vs. 33%, respectively) [46]. Another small study of 117 pregnant females admitted to hospital in Sao Paulo, Brazil, showed a mean vitamin C status of 33 μmol/L and 31% to have hypovitaminosis C [47].

A limited number of studies have been performed assessing vitamin C status across Africa (Table 2), none of which are large scale epidemiological studies of the general population, having primarily investigated specific groups. In South Africa a small study assessed vitamin C concentrations in 285 elderly participants [48]. A low mean vitamin C status of 25 μmol/L was observed in these participants (23 and 25 μmol/L for men and women, respectively), and was reflected by low vitamin C intakes (mean 39 mg/day; 27 and 42 mg/day for men and women, respectively). A high prevalence of hypovitaminosis C (defined as <34 μmol/L in this study) was observed (mean 66%; 84% for men and 62% for women). A number of small studies have been carried out in Nigerian women. One study of 400 antenatal clinic patients showed low vitamin C status (20 ± 29 μmol/L) with 80% of the women exhibiting hypovitaminosis C [49]. The authors attributed this to high parity, inadequate nutrition and nutritional taboos among Northern Nigerian females. Of note, a smaller study of female hospital and university staff in South Nigeria during the rainy season reported very high mean vitamin C intakes (>680 mg/day, determined by 24-h diet history), saturating vitamin C status (74 μmol/L), and no cases of hypovitaminosis and deficiency were seen [50]. In Uganda, research into pre-eclampsia in Kampala (Malago Hospital), 400 well pregnant women were studied and showed very low vitamin C status (only 11 ± 4 μmol/L) and high rates of vitamin C deficiency (70%) [51]. Additionally, a control group of 200 well women from clinics also showed low vitamin C status (15 ± 6 μmol/L) and 28% hypovitaminosis C. The differences between these studies may be a reflection of seasonal variation and disparities in intake across socioeconomic and regions of the continent, or limitations of the assay methodologies used. Of note, clinical outbreaks of scurvy still occur in Africa such as a recent outbreak in tribesmen in Kenya [52].

Table 2. Vitamin C status and prevalence of deficiency and hypovitaminosis C of adults in low- and middle-income countries.

Country (Region) Sampling Dates	Population (Age Range)	Vitamin C Status (μmol/L) [a]	Deficiency (% <11 μmol/L)	Hypovitaminosis C (% <23 or 28 μmol/L)	Dietary Intake (mg/day) [a]	References
Upper-middle						
Russia (Pitkäranta District, Republic of Karelia) 1992–2002	1191 total (25–64 years) 579 males 612 females	9.0 [b] 5.0 13	– 79 –	– 90 –	– – –	[25,26]
China (Shanghai) 1995–2001	2031 females (30–64 years)	45 (48)	–	–	–	[53]
China (Linxian) 1999–2000	948 total (~50–79 years) 473 males 475 females	33 (14–55) [c] 27 (11–50) [c] 37 (16–57) [c]	– – –	– – –	– – –	[54]
China (Gansu, Guangxi, Shandong, Fujian) 1999–2001	734 pregnant females (20–35 years)	19 [b]	–	66	–	[55]
Thailand (Bangkok) 2003	209 total (23–68 years) 90 males 119 females	36 (0–102) [d] 37 (0–77) [d] –	– –	31 [b] 33 30	– – –	[56]
Mexico (multiple states)	855 non-pregnant females (12–49 years)	19	39	–	–	[45]
Mexico (Central-Queretaro state) 2012	580 females (37 years) [e]	30 (13)	5	32	72 [b]	[44]
Ecuador (Quito) 2003–2004	369 total (>65 years) 125 elderly males 224 elderly females	15 [b] 11 (9) 17 (10)	43 [b] 60 33	– – –	– – –	[46]
Brazil (Sao Paulo) 2008	117 pregnant females (≥15 years)	33 (2)	6	31	–	[47]
South Africa (Cape Town) 2015	285 total (≥60 years) 53 elderly males 232 elderly females	25 [b] 23 25	– – –	66 [b,f] 84 [f] 62 [f]	39 [b] 27 42	[48]
Lower-middle						
India (North-Haryana state; South-Tamil Nadu) 2004–2006	5638 total (≥60 years) 2668 North total 1283 elderly males 1385 elderly females 2970 South total 1407 elderly males 1563 elderly females	– – – – – – –	59 [b] 74 78 71 46 51 40	81 [b] 89 – – 74 – –	29 [b] 23 [b] – – 34 [b] – –	[57]

Table 2. *Cont.*

Country (Region) Sampling Dates	Population (Age Range)	Vitamin C Status (μmol/L) [a]	Deficiency (% <11 μmol/L)	Hypovitaminosis C (% <23 or 28 μmol/L)	Dietary Intake (mg/day) [a]	References
India (North-Balba-garh, Faridabad district) 2002–2003	1112 total (≥50 years) ~48% males ~52% females	~15 [b] 13 17	– – –	– – –	– – –	[58]
India (West-Maharashtra state) 1998–2000	322 total (20–45 years) 214 males 108 females	18 [b] 17 (7) 20 (7)	18 [b] 20 13	71 [b] 75 63	34 [b] 40 [b] 29 [b]	[59]
Indonesia (West Java) 2011	98 total (39–50 years) 45 males; 53 females	29 (19)	11	45	–	[60]
Nigeria (Northwest-Kano state) 2009–2011	400 pregnant females (<20–39 years)	20 (29)	–	80	–	[49]
Nigeria (South-east-Enugu) 2009	200 non-pregnant females (29 years) [b,e]	74 [b]	0	0	683 [b,g]	[50]
Low income						
Uganda (Kampala) 2008–2009	600 females (15–49 years) 400 pregnant 200 non-pregnant	12 [b] 11 (4) 15 (6)	56 [b] 70 28	– – –	– – –	[51]

[a] Data represent mean (SD), if provided, [b] For missing data, weighted means were calculated, [c] Mean or median and interquartile range, [d] Median and range; [e] Mean age; [f] Cutoff of 34 μmol/L; [g] Converted from weighted mean of 3882 μmol using Mr of 176.

Vitamin C deficiency in India has been relatively well characterized with a large population-based study (Table 2). In this study, of >5600 adults aged over 60, frank deficiency was observed in 74% of adults in North India and 46% of adults in South India, with a higher prevalence of deficiency observed in men than women [57]. This was reflected by low dietary intakes of vitamin C (determined by 24-h dietary recall) of 23 and 34 mg/day for North and South India, respectively. Very low mean vitamin C concentrations of 15 μmol/L (13 and 17 μmol/L for men and women, respectively) in villages in North India have been reported [58]. A small study of healthy adults in Western India showed comparably low vitamin C status with a mean of 18 μmol/L (17 ± 7 μmol/L for men and 20 ± 7 μmol/L for women), along with high rates of deficiency and >70% hypovitaminosis C [59]. Dietary intakes of vitamin C were low (mean 34 mg/day; 40 mg/day for men and 29 mg/day for women), as determined using a food frequency questionnaire of usual dietary intake over the previous year.

Mean vitamin C concentrations are available from several studies in China (Table 2). One in Linxian indicated relatively low mean concentrations of 33 μmol/L for people aged ≥50 years (27 and 37 μmol/L for men and women, respectively) [54]. A higher mean status of 45 μmol/L was observed in >2000 women aged 30–64 years in Shanghai [53]. In contrast, women in the third trimester of pregnancy had a low mean vitamin C status of 19 μmol/L and a high prevalence of hypovitaminosis C (66%) [55]. Research in Russia has shown extremely low mean vitamin C status (9 μmol/L), particularly in men, with high rates of deficiency (79%) and hypovitaminosis C (90%) in adult males [25,26]. A small study in Bangkok, Thailand, indicated a low mean vitamin C status (36 μmol/L) and high prevalence of hypovitaminosis C (31%) [56]. Similar values were reported in Java, Indonesia, with a mean status of 29 ± 19 μmol/L and 45% hypovitaminosis C [60]. Elsewhere in Asia seasonal severe outbreaks of clinical scurvy have been noted in the winter months in Afghanistan occurring at a prevalence rate of 6.3% towards the end of the winter months [61].

3.3. Children and Adolescents

Although vitamin C pharmacokinetics are relatively well understood in adult men and women [11,62], relatively little is known about its pharmacokinetics in children and adolescents. However, based on its newly discovered epigenetic roles, vitamin C may be particularly important for the growth and development of infants and children [63]. Global recommended intakes for children and adolescents are less than adults and are generally based on their lower body weight [10]. In well-nourished populations, such as the USA, the vitamin C status of children is higher (mean 71 μmol/L) and the prevalence of deficiency lower (1.6%) than adults [32]. However, this is not necessarily the case in LMIC, where low vitamin C status has been observed in children and adolescents (Table 3). Studies in Mexico have shown high rates of deficiency and hypovitaminosis C in children [45,64]. Mean vitamin C concentrations in school aged children were low at 28 and 24 ± 9 μmol/L, with up to 38% hypovitaminosis C and 23% deficiency. A food frequency questionnaire indicated a mean intake of 44 mg/day vitamin C [64]. Of note, rates of overweight and obese children in the more recent Mexico study were high at 44%, and vitamin C concentrations were inversely associated with body fat and abdominal fat [64].

Table 3. Vitamin C status and prevalence of deficiency and hypovitaminosis C of children and adolescents globally.

Country (Region) Sampling Dates	Population (Age Range)	Vitamin C Status (μmol/L) [a]	Deficiency (% <11 μmol/L)	Hypovitaminosis C (% <23 or 28 μmol/L)	Dietary Intake (mg/day) [a]	References
High income						
United States 2003–2004	823 children (6–11 years)	71 [b]	1.6 [b]	–	–	[32]
	400 boys	74 (60–88) [c]	1.3	–	–	
	423 girls	69 (56–87) [c]	1.8	–	–	
	2016 adolescents (12–19 years)	53 [b]	3.3 [b]	–	–	
	1037 boys	51 (37–68) [c]	2.7	–	–	
	979 girls	55 (38–76) [c]	3.9	–	–	
Upper-middle						
Mexico (multiple states) 1999	1815 children (0–11 years)	28	23	–	–	[45]
Mexico (Queretaro state) 2012	197 children (6–11 years)	24 (9)	8	38	44	[64]
Lower-middle						
India (Jaipur city)	5000 well-nourished preschool children	–	0	–	–	[65]
	1000 malnourished preschool children	–	1.1	–	–	
India (Hyderabad)	869 children (6–16 years)	–	–	60 [d]	–	[66]
India (Delhi slum) 2012–2013	775 adolescent females (11–18 years)	43 (26)	6.3	34	48 (26)	[67]
Bangladesh (Dhaka district) 2003	307 adolescent females (14–18 years)	46 (20)	2.0	11	–	[68]
Nigeria (Enugu state)	90 adolescents (13–20 years)	–	–	47 [e]	–	[69]
	Males	35–43	–	60 [e]	24–27	
	Females	47–51	–	40 [e]	15–20	

[a] Data represent mean (SD), if provided; [b] For missing data, weighted means were calculated; [c] Mean or median and interquartile range; [d] Cutoff <30 μmol/L; [e] Cutoff <40 μmol/L.

Several studies have been carried out in children of different ages in India. Malnourished preschool children in Jaipur city were shown to have a higher prevalence of deficiency than well-nourished children [65]. The low prevalence of deficiency of this cohort (1.1%) is possibly due to the young age and consequently lower body weight of these children. In contrast, a high prevalence of 60% hypovitaminosis C (defined as <30 μmol/L) has been observed in older children in Hyderabad [66]. Poor vitamin status (43 ± 26 μmol/L) and a high prevalence of hypovitaminosis C (34%) has been observed among adolescent girls in the slums of Delhi [67]. They reported a mean dietary intake of 48 ± 26 mg/day, as determined by 24-h dietary recall. A study from Bangladesh demonstrated a comparable mean vitamin C status of 46 ± 20 μmol/L in rural adolescent females (with mild to moderate anemia), however, only 11% had hypovitaminosis C [68]. This is likely due to the study being carried out during the rainy season during which time green leafy vegetables were likely to have been widely available. In a small study in Nigeria, male and female adolescents were found to have very low dietary intakes, determined by a 3-day weighed food intake in the boarding students, and correspondingly low vitamin C status [69]. Nearly 50% of the adolescents had vitamin C levels <40 μmol/L (60% in boys and 40% in girls).

Overall, there are clear disparities in vitamin C status and prevalence of deficiency between high-income countries and LMICs. This is illustrated in Figure 1. There are numerous factors that likely contribute to the observed disparities between the various populations, with differences in dietary intakes between high-income countries and LMIC likely playing a major role (Figure 2). Dietary intakes can be influenced by geographic, economic, social and cultural factors [70]. Many staple foods, particularly those that are grain-based, contain negligible vitamin C. Furthermore, various processing during food preparation and traditional cooking practices employing longer cooking times, can deplete the vitamin C content of food [71], and these are not typically accounted for in assessments of vitamin C intake.

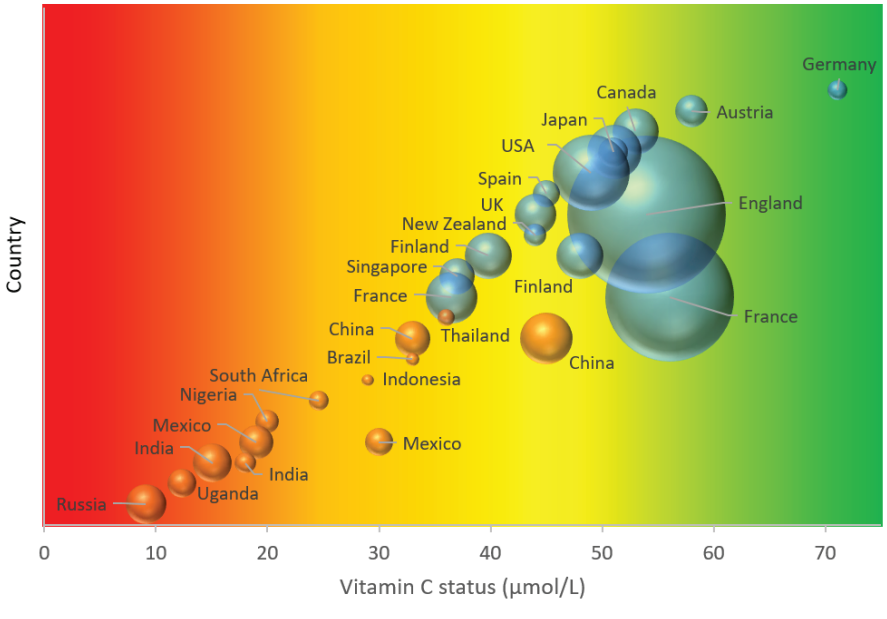


Figure 1. Global adult vitamin C status. The area of the bubble represents the size of the study. Blue bubbles represent high-income countries; orange bubbles represent low- and middle-income countries. Vitamin C status cutoffs: red—deficient (<11 μmol/L); orange—hypovitaminosis C (<23 μmol/L); yellow-inadequate (<50 μmol/L); green—adequate (>50 μmol/L).

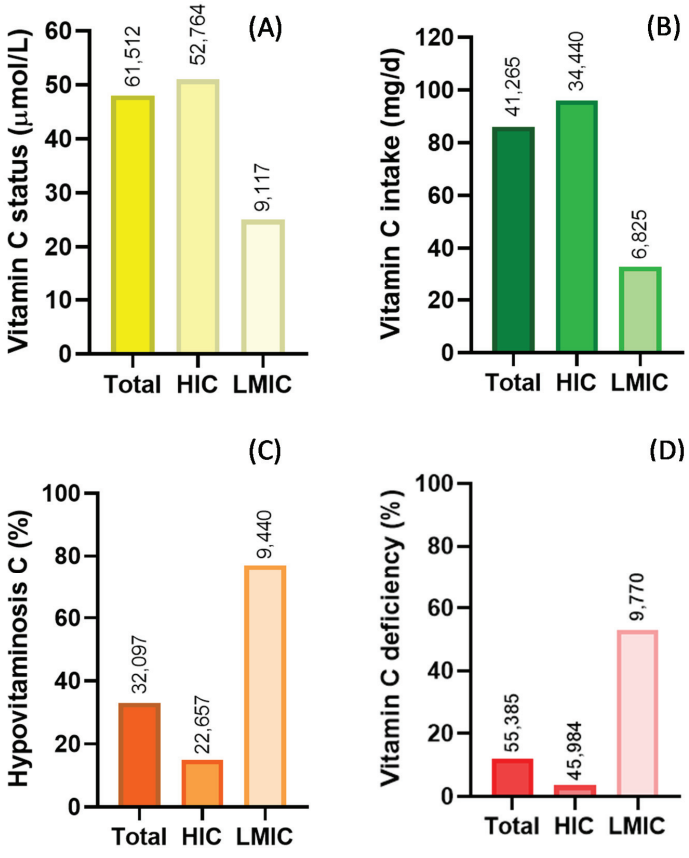


Figure 2. Summary of global vitamin C status (**A**) and intake (**B**) and prevalence of hypovitaminosis C (**C**) and vitamin C deficiency (**D**). Key: HIC—high-income countries; LMIC—low- and middle-income countries. Hypovitaminosis C, <23 μmol/L; vitamin C deficiency, <11 μmol/L. Numbers above bars indicate total number of individuals assessed.

4. Limitations of Vitamin C Status Studies

The assessment of plasma vitamin C status comes with a number of limitations. Due to the sensitivity of vitamin C to oxidation, appropriate handling, processing and storage of biological samples prior to analysis is very important for accurate determination of the vitamin [43]. Vitamin C status can be measured using a range of methods, many of which have limitations [72]. The current gold standard is HPLC analysis, however, a number of other cheaper spectrophotometric methods are available and used globally; these can be prone to interference by other compounds in the samples [72]. All of these analytical methods require meticulous attention to technique to prevent oxidation and loss of vitamin C. These methodological issues may be problematic in LMIC due to lack of adequate infrastructure and resources, particularly in rural settings, which could result in falsely low levels and as such should be interpreted with some caution. Nevertheless, assessing circulating vitamin C concentrations may be a more accurate indicator of status than dietary intake estimates alone as these have numerous methodological issues, e.g., recorder bias, which can result in an overestimate of vitamin intakes [73]. Intakes are also frequently calculated by nutritional tables that rarely factor in the effects of storage, processing and cooking on vitamins, which contribute to overestimation of intakes. Cassava, a major component of the staple diet in many LMICs, is an example of this where almost

100% of the vitamin C is lost prior to consumption [71]. The studies presented in Tables 1–3 have used a variety of dietary assessment tools, from 24-h dietary recalls to food frequency questionnaires of various durations. It is also sometimes unclear whether supplemental intakes of the vitamin have been included or excluded in the reported intakes.

Mean vitamin C status is commonly reported, which is not necessarily a good indicator as population distributions can mask significant rates of deficiency. A further issue is consensus on the cutoffs for inadequacy; vitamin C deficiency is usually defined as plasma concentrations <11 μmol/L, however, although hypovitaminosis C is often defined as <23 μmol/L, some studies report 'inadequacy' as <28 μmol/L (or even <30 or 34 μmol/L), therefore making it difficult to directly compare prevalence between studies. Vitamin C 'adequacy' has been more recently defined as >50 μmol/L [74], however, many of the older studies report concentrations >28 μmol/L as being 'adequate'. Because plasma vitamin C status is affected by recent dietary intake, particularly in replete people [75], assessment of fasting samples is preferred. However, this is not always possible to do, and some studies do not state whether fasting samples were analyzed. Nevertheless, in populations that are predominantly hypovitaminosis C, non-fasting is less likely to be an issue due to depleted body stores moderating fluctuations in plasma levels following consumption of the vitamin. Supplement intake also affects status [33], however, whether supplement use has been included or excluded in the study participants is not always reported.

A significant number of the large epidemiological studies were carried out in the 1990s and early 2000s, thus, much of the vitamin C status data are now ≥20 years out of date. Therefore, in light of increases in obesity and related chronic cardiometabolic health conditions such as diabetes and cardiovascular disease worldwide the results must be extrapolated with caution. Furthermore, many of the studies have been carried out in limited or defined regions with very few being nationwide and randomly selected. Therefore, most are not representative of the countries as a whole, particularly as status has been demonstrated to vary significantly based on geographic region. Finally, only a small percentage of the world's countries have been represented, most of which were high- and upper middle-income countries. This is of concern since there are clearly significant issues with vitamin C status and prevalence of deficiency in LMIC.

5. Associations between Vitamin C Status and Health

There are numerous factors that affect vitamin C status and requirements, including health aspects such as obesity; these have been covered in detail in other recent reviews [10,76]. Low vitamin C status is thought to be both a cause and a consequence of various communicable and non-communicable diseases. Lower vitamin C status has been observed in pre-diabetes and metabolic syndrome, indicating that depletion of the vitamin begins prior to development of the cardiometabolic disorders diabetes and cardiovascular disease [77,78]. Numerous epidemiological studies have indicated an association between vitamin C intake or status and overall mortality, including mortality due to cardiovascular disease and malignancies [79–81]. Vitamin C could simply be a marker for healthy fruit and vegetable consumption, however, it appears to have a stronger association with cancer and cardiovascular disease mortality than other micronutrients [80]. It is important to note that many prospective vitamin C intervention studies of non-communicable diseases have been carried out in predominantly replete populations, thus precluding an effect of the intervention and resulting in equivocal findings [82]. The interventional studies of vitamin C supplements or dietary interventions with positive outcomes have predominantly been in groups at high risk of insufficiency, such as institutionalized individuals in Europe [83].

The vitamin has a number of mechanistic rationales for its pleiotropic roles in human health and disease, including both antioxidant and cofactor functions [1,84]. For example, the particularly high concentrations of vitamin C in the adrenal and pituitary glands indicate important roles in hormonal regulation and the stress response [6]. Its high concentrations in the brain and associations with cognitive function also indicate important roles in the functioning of the central nervous system [39,85].

Vitamin C is widely accepted to play important roles in optimal immunological function [86]. Vitamin C deficiency has been associated with respiratory infections such as pneumonia, which is also one of the major complications and causes of death in cases of scurvy [87]. Leukocytes of both the innate and adaptive immune systems contain high concentrations of the vitamin and it is believed to play important roles in their development and immune functions [86]. While the optimum intake remains unclear, a healthy intake of at least 200 mg/day may be protective against a wide range of globally important infectious diseases [87,88]. Enhanced intakes could also potentially prevent less severe infections from developing into more severe conditions, such as sepsis, which is a major cause of communicable disease morbidity and mortality worldwide [88]. This is of particular relevance with regard to the current coronavirus (SARS-CoV-2) pandemic [89].

6. Conclusions and Future Directions

With the above limitations in mind, this review indicates that vitamin C deficiency is likely to be common globally—and particularly so in low-income groups and low-middle income countries. Despite this, the vitamin C status of many countries and populations has not yet been assessed. Therefore, further research is required to explore this in many regions of the world; to do this accurately will require improved methods for sampling, processing, storing and analyzing samples. Currently, point-of-care vitamin C monitors are in development; depending on their accuracy, these could be used directly in the field to aid in further assessment of vitamin C status globally.

Given the growing burden of non-communicable disease in low- and middle-income settings and potential protective effects of healthy vitamin C intakes against cardiovascular diseases, a range of malignancies, cataracts and multiple infectious diseases [1,84,87], improving vitamin C intake in these areas may prove to be a cheap and effective public health intervention. Despite this, recommended dietary intakes of vitamin C vary significantly between countries (Table 4), with the recommendations of some health authorities being primarily for prevention of deficiency (i.e., 40–45 mg/day), rather than optimization of health (i.e., 200 mg/day) [10]. Reassessment of vitamin C requirements has not been high on the agenda of various international health authorities, but the data indicate that it is clearly a nutrient of concern in many countries. The high vitamin C status reported in older adults in Germany, which has one of the highest dietary recommendations in the world [31,90], indicates that it is possible to achieve saturating vitamin C concentrations through the diet and/or supplementation. This calls to the need for harmonization of vitamin C recommendations globally.

Interventions to help optimize vitamin C nutriture worldwide could include: education (clinical and public, e.g., Global Nutrition and Empowerment), increased dietary recommendations (e.g., by the Food and Agriculture Organization/WHO), reduced tax on fresh fruit and vegetables, encouraging local growing of fruit and vegetables in LMICs, novel vitamin C tax reductions for products such as soft drinks that are high in vitamin C and government subsidies for supplementation (e.g., Vitamin Angels), and including regular provision to institutionalized individuals (e.g., elderly and school children). Research has indicated that the bioavailability of vitamin C from supplements is comparable to that from fruit and vegetables [91], although the latter is encouraged due to the presence of other essential nutrients and health-promoting phytochemicals. However, it is not always possible for people to obtain sufficient vitamin C through diet alone, particularly in light of the low vitamin C content of many staple foods [76]. Therefore, additional supplementation is encouraged in these situations, particularly if there are also underlying morbidities or other risk factors that increase the requirement for the vitamin [76]. Furthermore, of importance to note is that micronutrient deficiencies often do not occur in isolation, particularly in LMICs [46].

Overall, vitamin C deficiency appears common in low- and middle-income settings and not uncommon in many other settings, particularly in at risk groups. Further studies are required to confirm these findings, including in the countries not yet represented, and to fully understand the associations of vitamin C with a range of disease processes. Globally, clinicians should remain vigilant to vitamin C deficiency as a cause or contributing factor to many common presentations. Our findings

also suggest a need for interventions to prevent deficiency in a range of at risk groups and regions of the world.

Table 4. Global recommended dietary intakes for vitamin C.

Country or Authority	Males (mg/day)	Females (mg/day)
High Income		
France	110	110
DACH, European Union	110	95
Japan	100	100
Italy, Singapore	105	85
USA, Canada	90	75
Nordic, Netherlands	75	75
Spain	60	60
Australia, New Zealand, FAO/WHO	45	45
United Kingdom	40	40
Low-Middle Income		
China	100	100
South Africa	90	90
Thailand	90	75
Malaysia	70	70
Vietnam	70	65
Philippines	70	60
Indonesia	60	60
India	40	40

DACH—Germany, Austria, Switzerland; Nordic—Denmark, Finland, Iceland, Norway, Sweden; FAO/WHO—Food and Agriculture Organization/World Health Organization. Data from [10].

Author Contributions: Both authors contributed equally to the review. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: A.C.C. is a recipient of a Sir Charles Hercus Health Research Fellowship from the Health Research Council of New Zealand.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Carr, A.C.; Frei, B. Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans. *Am. J. Clin. Nutr.* **1999**, *69*, 1086–1107. [CrossRef] [PubMed]
2. Levine, M.; Padayatty, S.J.; Espey, M.G. Vitamin C: A concentration-function approach yields pharmacology and therapeutic discoveries. *Adv. Nutr.* **2011**, *2*, 78–88. [CrossRef] [PubMed]
3. Savini, I.; Rossi, A.; Pierro, C.; Avigliano, L.; Catani, M.V. SVCT1 and SVCT2: Key proteins for vitamin C uptake. *Amino Acids* **2008**, *34*, 347–355. [CrossRef] [PubMed]
4. Hornig, D. Distribution of ascorbic acid, metabolites and analogues in man and animals. *Ann. N. Y. Acad. Sci.* **1975**, *258*, 103–118. [CrossRef] [PubMed]
5. Englard, S.; Seifter, S. The biochemical functions of ascorbic acid. *Annu. Rev. Nutr.* **1986**, *6*, 365–406. [CrossRef] [PubMed]
6. Carr, A.C.; Shaw, G.M.; Fowler, A.A.; Natarajan, R. Ascorbate-dependent vasopressor synthesis: A rationale for vitamin C administration in severe sepsis and septic shock? *Crit. Care* **2015**, *19*, e418. [CrossRef]
7. Young, J.I.; Zuchner, S.; Wang, G. Regulation of the epigenome by vitamin C. *Annu. Rev. Nutr.* **2015**, *35*, 545–564. [CrossRef]
8. Vissers, M.C.; Kuiper, C.; Dachs, G.U. Regulation of the 2-oxoglutarate-dependent dioxygenases and implications for cancer. *Biochem. Soc. Trans.* **2014**, *42*, 945–951. [CrossRef]

9. Troesch, B.; Hoeft, B.; McBurney, M.; Eggersdorfer, M.; Weber, P. Dietary surveys indicate vitamin intakes below recommendations are common in representative Western countries. *Br. J. Nutr.* **2012**, *108*, 692–698. [CrossRef]
10. Carr, A.C.; Lykkesfeldt, J. Discrepancies in global vitamin C recommendations: A review of RDA criteria and underlying health perspectives. *Crit. Rev. Food Sci. Nutr.* **2020**, 1–14. [CrossRef]
11. Levine, M.; Conry-Cantilena, C.; Wang, Y.; Welch, R.W.; Washko, P.W.; Dhariwal, K.R.; Park, J.B.; Lazarev, A.; Graumlich, J.F.; King, J.; et al. Vitamin C pharmacokinetics in healthy volunteers: Evidence for a recommended dietary allowance. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 3704–3709. [CrossRef]
12. Johnston, C.S.; Corte, C. People with marginal vitamin C status are at high risk of developing vitamin C deficiency. *J. Am. Diet. Assoc.* **1999**, *99*, 854–856. [CrossRef]
13. Khan, R.M.; Iqbal, M.P. Deficiency of Vitamin C in South Asia. *Pak. J. Med. Sci.* **2006**, *22*, 347–355.
14. Dickinson, N.; Macpherson, G.; Hursthouse, A.S.; Atkinson, J. Micronutrient deficiencies in maternity and child health: A review of environmental and social context and implications for Malawi. *Environ. Geochem. Health* **2009**, *31*, 253–272. [CrossRef] [PubMed]
15. The World Bank. World Bank Country and Lending Groups. Available online: https://datahelpdesk.worldbank.org/knowledgebase/articles/906519-world-bank-country-and-lending-group (accessed on 13 January 2020).
16. Canoy, D.; Wareham, N.; Welch, A.; Bingham, S.; Luben, R.; Day, N.; Khaw, K.T. Plasma ascorbic acid concentrations and fat distribution in 19,068 British men and women in the European Prospective Investigation into Cancer and Nutrition Norfolk cohort study. *Am. J. Clin. Nutr.* **2005**, *82*, 1203–1209. [CrossRef]
17. McCall, S.J.; Clark, A.B.; Luben, R.N.; Wareham, N.J.; Khaw, K.T.; Myint, P.K. Plasma vitamin C levels: Risk factors for deficiency and association with self-reported functional health in the European Prospective Investigation into Cancer-Norfolk. *Nutrients* **2019**, *11*, 1552. [CrossRef]
18. Bates, C.J.; Prentice, A.; Cole, T.J.; Van Der Pols, J.C.; Doyle, W.; Finch, S.; Smithers, G.; Clarke, P.C. Micronutrients: Highlights and research challenges from the 1994-5 National Diet and Nutrition Survey of people aged 65 years and over. *Br. J. Nutr.* **1999**, *82*, 7–15. [CrossRef]
19. Wrieden, W.L.; Hannah, M.K.; Bolton-Smith, C.; Tavendale, R.; Morrison, C.; Tunstall-Pedoe, H. Plasma vitamin C and food choice in the third Glasgow MONICA population survey. *J. Epidemiol. Community Health* **2000**, *54*, 355–360. [CrossRef]
20. Faure, H.; Preziosi, P.; Roussel, A.M.; Bertrais, S.; Galan, P.; Hercberg, S.; Favier, A. Factors influencing blood concentration of retinol, alpha-tocopherol, vitamin C, and beta-carotene in the French participants of the SU.VI.MAX trial. *Eur. J. Clin. Nutr.* **2006**, *60*, 706–717. [CrossRef]
21. Galan, P.; Viteri, F.E.; Bertrais, S.; Czernichow, S.; Faure, H.; Arnaud, J.; Ruffieux, D.; Chenal, S.; Arnault, N.; Favier, A.; et al. Serum concentrations of beta-carotene, vitamins C and E, zinc and selenium are influenced by sex, age, diet, smoking status, alcohol consumption and corpulence in a general French adult population. *Eur. J. Clin. Nutr.* **2005**, *59*, 1181–1190. [CrossRef]
22. Birlouez-Aragon, I.; Delcourt, C.; Tessier, F.; Papoz, L. Associations of age, smoking habits and diabetes with plasma vitamin C of elderly of the POLA study. *Int. J. Vitam. Nutr. Res.* **2001**, *71*, 53–59. [CrossRef] [PubMed]
23. Drewnowski, A.; Rock, C.L.; Henderson, S.A.; Shore, A.B.; Fischler, C.; Galan, P.; Preziosi, P.; Hercberg, S. Serum beta-carotene and vitamin C as biomarkers of vegetable and fruit intakes in a community-based sample of French adults. *Am. J. Clin. Nutr.* **1997**, *65*, 1796–1802. [CrossRef] [PubMed]
24. Marangon, K.; Herbeth, B.; Lecomte, E.; Paul-Dauphin, A.; Grolier, P.; Chancerelle, Y.; Artur, Y.; Siest, G. Diet, antioxidant status, and smoking habits in French men. *Am. J. Clin. Nutr.* **1998**, *67*, 231–239. [CrossRef]
25. Paalanen, L.; Prattala, R.; Alfthan, G.; Salminen, I.; Laatikainen, T. Vegetable and fruit consumption, education and plasma vitamin C concentration in Russian and Finnish Karelia, 1992–2002. *Public Health Nutr.* **2014**, *17*, 2278–2286. [CrossRef] [PubMed]
26. Matilainen, T.; Vartiainen, E.; Puska, P.; Alfthan, G.; Pokusajeva, S.; Moisejeva, N.; Uhanov, M. Plasma ascorbic acid concentrations in the Republic of Karelia, Russia and in North Karelia, Finland. *Eur. J. Clin. Nutr.* **1996**, *50*, 115–120. [PubMed]
27. Nyyssonen, K.; Parviainen, M.T.; Salonen, R.; Tuomilehto, J.; Salonen, J.T. Vitamin C deficiency and risk of myocardial infarction: Prospective population study of men from eastern Finland. *BMJ* **1997**, *314*, 634–638. [CrossRef]

28. Sen, A.; Marsche, G.; Freudenberger, P.; Schallert, M.; Toeglhofer, A.M.; Nagl, C.; Schmidt, R.; Launer, L.J.; Schmidt, H. Association between higher plasma lutein, zeaxanthin, and vitamin C concentrations and longer telomere length: Results of the Austrian Stroke Prevention Study. *J. Am. Geriatr. Soc.* **2014**, *62*, 222–229. [CrossRef]
29. Vioque, J.; Weinbrenner, T.; Asensio, L.; Castello, A.; Young, I.S.; Fletcher, A. Plasma concentrations of carotenoids and vitamin C are better correlated with dietary intake in normal weight than overweight and obese elderly subjects. *Br. J. Nutr.* **2007**, *97*, 977–986. [CrossRef]
30. Olmedilla, B.; Granado, F.; Southon, S.; Wright, A.J.; Blanco, I.; Gil-Martinez, E.; Berg, H.; Corridan, B.; Roussel, A.M.; Chopra, M.; et al. Serum concentrations of carotenoids and vitamins A, E, and C in control subjects from five European countries. *Br. J. Nutr.* **2001**, *85*, 227–238. [CrossRef]
31. Jungert, A.; Neuhauser-Berthold, M. The lower vitamin C plasma concentrations in elderly men compared with elderly women can partly be attributed to a volumetric dilution effect due to differences in fat-free mass. *Br. J. Nutr.* **2015**, *113*, 859–864. [CrossRef]
32. Schleicher, R.L.; Carroll, M.D.; Ford, E.S.; Lacher, D.A. Serum vitamin C and the prevalence of vitamin C deficiency in the United States: 2003–2004 National Health and Nutrition Examination Survey (NHANES). *Am. J. Clin. Nutr.* **2009**, *90*, 1252–1263. [CrossRef]
33. Langlois, K.; Cooper, M.; Colapinto, C.K. Vitamin C status of Canadian adults: Findings from the 2012/2013 Canadian Health Measures Survey. *Health Rep.* **2016**, *27*, 3–10.
34. Cahill, L.; Corey, P.N.; El-Sohemy, A. Vitamin C deficiency in a population of young Canadian adults. *Am. J. Epidemiol.* **2009**, *170*, 464–471. [CrossRef]
35. Sakai, N.; Yokoyama, T.; Date, C.; Yoshiike, N.; Matsumura, Y. An inverse relationship between serum vitamin C and blood pressure in a Japanese community. *J. Nutr. Sci. Vitaminol.* **1998**, *44*, 853–867. [CrossRef] [PubMed]
36. Saito, K.; Yokoyama, T.; Yoshida, H.; Kim, H.; Shimada, H.; Yoshida, Y.; Iwasa, H.; Shimizu, Y.; Yoshitaka, K.; Handa, S.; et al. A significant relationship between plasma vitamin C concentration and physical performance among Japanese elderly women. *J. Gerontol. A Biol. Sci. Med. Sci.* **2011**. [CrossRef] [PubMed]
37. Hughes, K.; New, A.L.; Lee, B.L.; Ong, C.N. Plasma vitamins A, C and E in the general population of Singapore, 1993 to 1995. *Ann. Acad. Med. Singap.* **1998**, *27*, 149–153. [PubMed]
38. Hughes, K.; Ong, C.N. Vitamins, selenium, iron, and coronary heart disease risk in Indians, Malays, and Chinese in Singapore. *J. Epidemiol. Community Health* **1998**, *52*, 181–185. [CrossRef] [PubMed]
39. Pearson, J.F.; Pullar, J.M.; Wilson, R.; Spittlehouse, J.K.; Vissers, M.C.M.; Skidmore, P.M.L.; Willis, J.; Cameron, V.A.; Carr, A.C. Vitamin C status correlates with markers of metabolic and cognitive health in 50-year-olds: Findings of the CHALICE cohort study. *Nutrients* **2017**, *9*, 831. [CrossRef] [PubMed]
40. Smith, J.L.; Hodges, R.E. Serum levels of vitamin C in relation to dietary and supplemental intake of vitamin C in smokers and nonsmokers. *Ann. N. Y. Acad. Sci.* **1987**, *498*, 144–152. [CrossRef] [PubMed]
41. Hampl, J.S.; Taylor, C.A.; Johnston, C.S. Vitamin C deficiency and depletion in the United States: The Third National Health and Nutrition Examination Survey, 1988 to 1994. *Am. J. Public Health* **2004**, *94*, 870–875. [CrossRef]
42. Hoffer, L.J. Re: "Vitamin C deficiency in a population of young Canadian adults". *Am. J. Epidemiol.* **2010**, *171*, 387. [CrossRef]
43. Pullar, J.M.; Bayer, S.; Carr, A.C. Appropriate handling, processing and analysis of blood samples is essential to avoid oxidation of vitamin C to dehydroascorbic acid. *Antioxidants* **2018**, *7*, 29. [CrossRef]
44. Garcia, O.P.; Ronquillo, D.; Caamano Mdel, C.; Camacho, M.; Long, K.Z.; Rosado, J.L. Zinc, vitamin A, and vitamin C status are associated with leptin concentrations and obesity in Mexican women: Results from a cross-sectional study. *Nutr. Metab.* **2012**, *9*, 59. [CrossRef] [PubMed]
45. Villalpando, S.; Montalvo-Velarde, I.; Zambrano, N.; Garcia-Guerra, A.; Ramirez-Silva, C.I.; Shamah-Levy, T.; Rivera, J.A. Vitamins A, and C and folate status in Mexican children under 12 years and women 12–49 years: A probabilistic national survey. *Salud Publ. Mex.* **2003**, *45*, S508–S519. [CrossRef]
46. Hamer, D.H.; Sempertegui, F.; Estrella, B.; Tucker, K.L.; Rodriguez, A.; Egas, J.; Dallal, G.E.; Selhub, J.; Griffiths, J.K.; Meydani, S.N. Micronutrient deficiencies are associated with impaired immune response and higher burden of respiratory infections in elderly Ecuadorians. *J. Nutr.* **2009**, *139*, 113–119. [CrossRef] [PubMed]

47. Madruga de Oliveira, A.; Rondo, P.H.; Mastroeni, S.S.; Oliveira, J.M. Plasma concentrations of ascorbic acid in parturients from a hospital in Southeast Brazil. *Clin. Nutr.* **2008**, *27*, 228–232. [CrossRef] [PubMed]
48. Charlton, K.E.; Kolbe-Alexander, T.L.; Nel, J.H. Micronutrient dilution associated with added sugar intake in elderly black South African women. *Eur. J. Clin. Nutr.* **2005**, *59*, 1030–1042. [CrossRef] [PubMed]
49. Ugwa, E.A.; Iwasam, E.A.; Nwali, M.I. Low Serum Vitamin C Status Among Pregnant Women Attending Antenatal Care at General Hospital Dawakin Kudu, Northwest Nigeria. *Int. J. Prev. Med.* **2016**, *7*, 40. [CrossRef]
50. Nwagha, U.I.; Iyare, E.E.; Ejezie, F.E.; Ogbodo, S.O.; Dim, C.C.; Anyaehie, B.U. Parity related changes in obesity and some antioxidant vitamins in non-pregnant women of South-Eastern Nigeria. *Niger. J. Clin. Pract.* **2012**, *15*, 380–384. [CrossRef]
51. Kiondo, P.; Tumwesigye, N.M.; Wandabwa, J.; Wamuyu-Maina, G.; Bimenya, G.S.; Okong, P. Plasma vitamin C assay in women of reproductive age in Kampala, Uganda, using a colorimetric method. *Trop. Med. Int. Health* **2012**, *17*, 191–196. [CrossRef]
52. Halestrap, P.; Scheenstra, S. Outbreak of scurvy in Tana River County, Kenya: A case report. *Afr. J. Prim. Health Care Fam. Med.* **2018**, *10*, e1–e3. [CrossRef] [PubMed]
53. Frankenfeld, C.L.; Lampe, J.W.; Shannon, J.; Gao, D.L.; Li, W.; Ray, R.M.; Chen, C.; King, I.B.; Thomas, D.B. Fruit and vegetable intakes in relation to plasma nutrient concentrations in women in Shanghai, China. *Public Health Nutr.* **2012**, *15*, 167–175. [CrossRef] [PubMed]
54. Lam, T.K.; Freedman, N.D.; Fan, J.H.; Qiao, Y.L.; Dawsey, S.M.; Taylor, P.R.; Abnet, C.C. Prediagnostic plasma vitamin C and risk of gastric adenocarcinoma and esophageal squamous cell carcinoma in a Chinese population. *Am. J. Clin. Nutr.* **2013**, *98*, 1289–1297. [CrossRef]
55. Ma, A.G.; Schouten, E.G.; Wang, Y.; Xu, R.X.; Zheng, M.C.; Li, Y.; Wang, Q.; Sun, Y. Micronutrient status in anemic and non-anemic Chinese women in the third trimester of pregnancy. *Asia Pac. J. Clin. Nutr.* **2009**, *18*, 41–47. [PubMed]
56. Viroonudomphol, D.; Mahaisiriyodom, A.; Mingkhawn, R.; Sadomthian, P.; Korchasri, N.; Jittngamkhum, S.; Sangcakul, A. Relationship between serum antioxidant vitamins A, E, and C and lipid profiles in priest subjects at the Priest Hospital. *Southeast Asian J. Trop. Med. Public Health* **2005**, *36*, 246–253.
57. Ravindran, R.D.; Vashist, P.; Gupta, S.K.; Young, I.S.; Maraini, G.; Camparini, M.; Jayanthi, R.; John, N.; Fitzpatrick, K.E.; Chakravarthy, U.; et al. Prevalence and risk factors for vitamin C deficiency in north and south India: A two centre population based study in people aged 60 years and over. *PLoS ONE* **2011**, *6*, e28588. [CrossRef]
58. Dherani, M.; Murthy, G.V.; Gupta, S.K.; Young, I.S.; Maraini, G.; Camparini, M.; Price, G.M.; John, N.; Chakravarthy, U.; Fletcher, A.E. Blood levels of vitamin C, carotenoids and retinol are inversely associated with cataract in a North Indian population. *Invest. Ophthalmol. Vis. Sci.* **2008**, *49*, 3328–3335. [CrossRef]
59. Chiplonkar, S.A.; Agte, V.V.; Mengale, S.S.; Tarwadi, K.V. Are lifestyle factors good predictors of retinol and vitamin C deficiency in apparently healthy adults? *Eur. J. Clin. Nutr.* **2002**, *56*, 96–104. [CrossRef]
60. Amaliya, A.; Laine, M.L.; Delanghe, J.R.; Loos, B.G.; Van Wijk, A.J.; Van der Velden, U. Java project on periodontal diseases: Periodontal bone loss in relation to environmental and systemic conditions. *J. Clin. Periodontol.* **2015**, *42*, 325–332. [CrossRef]
61. Cheung, E.; Mutahar, R.; Assefa, F.; Ververs, M.T.; Nasiri, S.M.; Borrel, A.; Salama, P. An epidemic of scurvy in Afghanistan: Assessment and response. *Food Nutr. Bull.* **2003**, *24*, 247–255. [CrossRef]
62. Levine, M.; Wang, Y.; Padayatty, S.J.; Morrow, J. A new recommended dietary allowance of vitamin C for healthy young women. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 9842–9846. [CrossRef] [PubMed]
63. Camarena, V.; Wang, G. The epigenetic role of vitamin C in health and disease. *Cell. Mol. Life Sci.* **2016**, *73*, 1645–1658. [CrossRef] [PubMed]
64. Garcia, O.P.; Ronquillo, D.; del Carmen Caamano, M.; Martinez, G.; Camacho, M.; Lopez, V.; Rosado, J.L. Zinc, iron and vitamins A, C and e are associated with obesity, inflammation, lipid profile and insulin resistance in Mexican school-aged children. *Nutrients* **2013**, *5*, 5012–5030. [CrossRef] [PubMed]
65. Chainani, N.; Sharma, P.; Meena, N.; Sharma, U. Pattern of vitamin deficiencies among the malnourished preschool children in ICDS blocks of Jaipur city. *Indian J. Matern. Child Health* **1994**, *5*, 109–111. [PubMed]
66. Sivakumar, B.; Nair, K.M.; Sreeramulu, D.; Suryanarayana, P.; Ravinder, P.; Shatrugna, V.; Kumar, P.A.; Raghunath, M.; Rao, V.V.; Balakrishna, N.; et al. Effect of micronutrient supplement on health and nutritional status of schoolchildren: Biochemical status. *Nutrition* **2006**, *22*, S15–S25. [CrossRef] [PubMed]

67. Bansal, P.G.; Toteja, G.S.; Suman, R. Plasma vitamin C status of adolescent girls in a slum of Delhi. *Indian Pediatr.* **2014**, *51*, 932–933.
68. Ahmed, F.; Khan, M.R.; Banu, C.P.; Qazi, M.R.; Akhtaruzzaman, M. The coexistence of other micronutrient deficiencies in anaemic adolescent schoolgirls in rural Bangladesh. *Eur. J. Clin. Nutr.* **2008**, *62*, 365–372. [CrossRef]
69. Ene-Obong, H.N.; Odoh, I.F.; Ikwuagwu, O.E. Plasma vitamin A and C status of in-school adolescents and associated factors in Enugu State, Nigeria. *J. Health Popul. Nutr.* **2003**, *21*, 18–25.
70. World Health Organization. *Requirements of Ascorbic Acid, Vitamin D, Vitamin B12, Folate, and Iron*; World Health Organization: Geneva, Switzerland, 1970.
71. Montagnac, J.A.; Davis, C.R.; Tanumihardjo, S.A. Nutritional value of cassava for use as a staple food and recent advances for improvement. *Compr. Rev. Food Sci. Food Saf.* **2009**, *8*, 181–194. [CrossRef]
72. Washko, P.W.; Welch, R.W.; Dhariwal, K.R.; Wang, Y.; Levine, M. Ascorbic acid and dehydroascorbic acid analyses in biological samples. *Anal. Biochem.* **1992**, *204*, 1–14. [CrossRef]
73. Lins, I.L.; Bueno, N.B.; Grotti Clemente, A.P.; Pfrimer, K.; Sawaya, A.L.; De Menezes Toledo Florencio, T.M. Energy Intake in Socially Vulnerable Women Living in Brazil: Assessment of the Accuracy of Two Methods of Dietary Intake Recording Using Doubly Labeled Water. *J. Acad. Nutr. Diet.* **2016**, *116*, 1560–1567. [CrossRef] [PubMed]
74. European Food Safety Authority Panel on Dietetic Products Nutrition and Allergies. Scientific opinion on dietary reference values for vitamin C. *EFSA J.* **2013**, *11*, 3418.
75. Carr, A.C.; Bozonet, S.M.; Vissers, M.C. A randomised cross-over pharmacokinetic bioavailability study of synthetic versus kiwifruit-derived vitamin C. *Nutrients* **2013**, *5*, 4451–4461. [CrossRef] [PubMed]
76. Carr, A.C.; Rowe, S. Factors affecting vitamin C status and prevalence of deficiency: A global health perspective. *Nutrients* **2020**, *12*, 1963. [CrossRef]
77. Wilson, R.; Willis, J.; Gearry, R.; Skidmore, P.; Fleming, E.; Frampton, C.; Carr, A. Inadequate vitamin C status in prediabetes and type 2 diabetes mellitus: Associations with glycaemic control, obesity, and smoking. *Nutrients* **2017**, *9*, 997. [CrossRef] [PubMed]
78. Ford, E.S.; Mokdad, A.H.; Giles, W.H.; Brown, D.W. The metabolic syndrome and antioxidant concentrations: Findings from the Third National Health and Nutrition Examination Survey. *Diabetes* **2003**, *52*, 2346–2352. [CrossRef]
79. Khaw, K.T.; Bingham, S.; Welch, A.; Luben, R.; Wareham, N.; Oakes, S.; Day, N. Relation between plasma ascorbic acid and mortality in men and women in EPIC-Norfolk prospective study: A prospective population study. European Prospective Investigation into Cancer and Nutrition. *Lancet* **2001**, *357*, 657–663. [CrossRef]
80. Goyal, A.; Terry, M.B.; Siegel, A.B. Serum antioxidant nutrients, vitamin A, and mortality in U.S. Adults. *Cancer Epidemiol. Biomark. Prev.* **2013**, *22*, 2202–2211. [CrossRef]
81. Wang, S.M.; Fan, J.H.; Taylor, P.R.; Lam, T.K.; Dawsey, S.M.; Qiao, Y.L.; Abnet, C.C. Association of plasma vitamin C concentration to total and cause-specific mortality: A 16-year prospective study in China. *J. Epidemiol. Community Health* **2018**, *72*, 1076–1082. [CrossRef]
82. Lykkesfeldt, J.; Poulsen, H.E. Is vitamin C supplementation beneficial? Lessons learned from randomised controlled trials. *Br. J. Nutr.* **2010**, *103*, 1251–1259. [CrossRef]
83. Hemilä, H.; Louhiala, P. Vitamin C for preventing and treating pneumonia. *Cochrane Database Syst. Rev.* **2013**, *8*. [CrossRef] [PubMed]
84. Carr, A.C.; Cook, J. Intravenous vitamin C for cancer therapy—Identifying the current gaps in our knowledge. *Front. Physiol.* **2018**, *9*, 1182. [CrossRef] [PubMed]
85. May, J.M. Vitamin C transport and its role in the central nervous system. *Subcell. Biochem.* **2012**, *56*, 85–103. [CrossRef] [PubMed]
86. Carr, A.C.; Maggini, S. Vitamin C and immune function. *Nutrients* **2017**, *9*, 1211. [CrossRef]
87. Hemilä, H. Vitamin C and infections. *Nutrients* **2017**, *9*, 339. [CrossRef]
88. Carr, A.C. Vitamin C in pneumonia and sepsis. In *Vitamin C: New Biochemical and Functional Insights*; Chen, Q., Vissers, M., Eds.; CRC Press: Boca Raton, FL, USA, 2020; pp. 115–135.
89. Calder, P.C.; Carr, A.C.; Gombart, A.F.; Eggersdorfer, M. Optimal nutritional status for a well-functioning immune system is an important factor to protect against viral infections. *Nutrients* **2020**, *12*, 1181. [CrossRef]

90. German Nutrition Society. New reference values for vitamin C intake. *Ann. Nutr. Metab.* **2015**, *67*, 13–20. [CrossRef]
91. Carr, A.C.; Vissers, M.C. Synthetic or food-derived vitamin C—Are they equally bioavailable? *Nutrients* **2013**, *5*, 4284–4304. [CrossRef]

nutrients

MDPI

Review

Factors Affecting Vitamin C Status and Prevalence of Deficiency: A Global Health Perspective

Anitra C. Carr [1,*] and Sam Rowe [2]

[1] Nutrition in Medicine Research Group, Department of Pathology & Biomedical Science, University of Otago, Christchurch 8011, New Zealand

[2] Department of Clinical Sciences, Liverpool School of Tropical Medicine, Liverpool L35QA, UK; sam.rowe@lstmed.ac.uk

* Correspondence: anitra.carr@otago.ac.nz; Tel.: +64-3364-0649

Received: 3 June 2020; Accepted: 26 June 2020; Published: 1 July 2020

Abstract: A recent review of global vitamin C status has indicated a high prevalence of deficiency, particularly in low- and middle-income countries, as well as in specific subgroups within high-income countries. Here, we provide a narrative review of potential factors influencing vitamin C status globally. The in vivo status of vitamin C is primarily affected by dietary intake and supplement use, with those who supplement having a higher mean status and a lower prevalence of deficiency. Dietary intake can be influenced by cultural aspects such as traditional cooking practices and staple foods, with many staple foods, such as grains, contributing negligible vitamin C to the diet. Environmental factors can also affect vitamin C intake and status; these include geographic region, season, and climate, as well as pollution, the latter partly due to enhanced oxidative stress. Demographic factors such as sex, age, and race are known to affect vitamin C status, as do socioeconomic factors such as deprivation, education and social class, and institutionalization. Various health aspects can affect vitamin C status; these include body weight, pregnancy and lactation, genetic variants, smoking, and disease states, including severe infections as well as various noncommunicable diseases such as cardiovascular disease and cancer. Some of these factors have changed over time; therefore, we also explore if vitamin C status has shown temporal changes. Overall, there are numerous factors that can affect vitamin C status to different extents in various regions of the world. Many of these factors are not taken into consideration during the setting of global dietary intake recommendations for vitamin C.

Keywords: vitamin C; vitamin C status; vitamin C deficiency; global health; dietary intake; obesity; smoking; communicable disease; infection; noncommunicable disease

1. Introduction

Due to random genetic mutations that have occurred during our evolution, humans have lost the ability to synthesize ascorbate in our livers [1]. Therefore, instead of being a normal liver metabolite, as is the case for most other animal species, ascorbate has instead become a vitamin and must be obtained through the diet to prevent hypovitaminosis C and outright deficiency [2]. In a recent review, we described the global status of vitamin C and the prevalence of deficiency as assessed by plasma concentrations [3]. Whilst vitamin C concentrations are normally tightly controlled in humans with adequate intake, a number of studies suggest that hypovitaminosis C and deficiency are not uncommon worldwide and may be very common in some settings. Vitamin C is well absorbed in small quantities. Uptake occurs primarily through the sodium-dependent vitamin C transporter-1 (SVCT-1), which is rapidly saturated, allowing relatively limited absorption of vitamin C per serving [4]. Vitamin C is then accumulated at varying concentrations in different body tissues [5]. The vitamin is excreted unchanged in the urine, but reuptake occurs in the renal tubules [6]. Further loss of vitamin C occurs through

the oxidation of ascorbic acid to dehydroascorbic acid which may then be recycled to ascorbic acid or undergo further oxidative decomposition [7]. Thus, in vivo, vitamin C concentrations are affected by a range of factors that include dietary intake, absorption, distribution, oxidative decomposition, and elimination.

Vitamin C acts as an important antioxidant and plays a myriad of functions in optimal health and prevention of disease [8,9]. Even mild insufficiency or hypovitaminosis C can be associated with symptoms such as low mood [10]. More severe deficiency can be associated with a range of clinical presentations [11]. Prolonged severe deficiency results in the clinical syndrome of scurvy, a condition that continues to be diagnosed in individuals and occasionally in public health outbreaks to this day [12]; if left untreated, scurvy is a fatal disease. The optimum intake and concentrations of vitamin C, however, have been subject to debate and recommended values are not universal [13]. Due to a range of benefits, a number of international authorities have increased their dietary recommendations for vitamin C from those previously recommended on the basis of preventing scurvy [13]. Although these recommendations generally take into account variations in requirements based on age, sex, pregnancy and lactation, and sometimes smoking status, there are many other factors that affect vitamin C status that are not taken into consideration by these global authorities. In this review, we highlight the numerous factors that impact on vitamin C status and prevalence of deficiency globally.

2. Dietary Factors Determining Vitamin C Status

2.1. Dietary Intake

Dietary intake of vitamin C is a key determinant of body status, with the amount consumed and the frequency of consumption correlating with plasma status and prevalence of deficiency (Table 1) [14,15]. Fresh fruit and vegetables are the major dietary source of vitamin C, with fruit intake correlating more strongly with plasma vitamin C status [16,17]. Foods rich in vitamin C include oranges and other citrus fruit, guavas, kiwifruit, cranberries, strawberries, papayas, mangoes, melons, cantaloupe, spinach, Swiss chard, tomatoes, asparagus, and Brussels sprouts (Figure 1) [18]. In contrast, grains (e.g., rice, millet, wheat/couscous, corn), some starchy roots and tubers, meat (other than liver), eggs, and dairy contain very little vitamin C.

Table 1. Dietary factors determining vitamin C status.

Factor	Summary and Comments	References
Dietary intake	Dietary intake, particularly fruit intake, correlates with improved vitamin C status and decreased prevalence of deficiency; is dependent on the amount consumed, frequency of consumption, and type of food consumed as the vitamin C content of food varies. High dietary fat and sugar intake are associated with decreased vitamin C intake and status.	[14–17,19–24]
Staple foods	Staple foods such as grains (e.g., rice, millet, wheat/couscous, corn) and some starchy roots and tubers are low in vitamin C; populations who consume these staples can have lower overall vitamin C intake.	[18,25]
Traditional cooking practices	Through boiling or steaming, water-soluble vitamins may be leached from food and prolonged cooking of food can destroy vitamin C; this could lead to decreased vitamin C status in certain social or ethnic groups. Drying of leafy vegetables also decreases water-soluble vitamins.	[26,27]
Supplement use	Supplement users have significantly higher vitamin C status and negligible prevalence of deficiency. Non-users have a 2–3 fold odds ratio of insufficient and deficient vitamin C status.	[14,15,19,28–30]

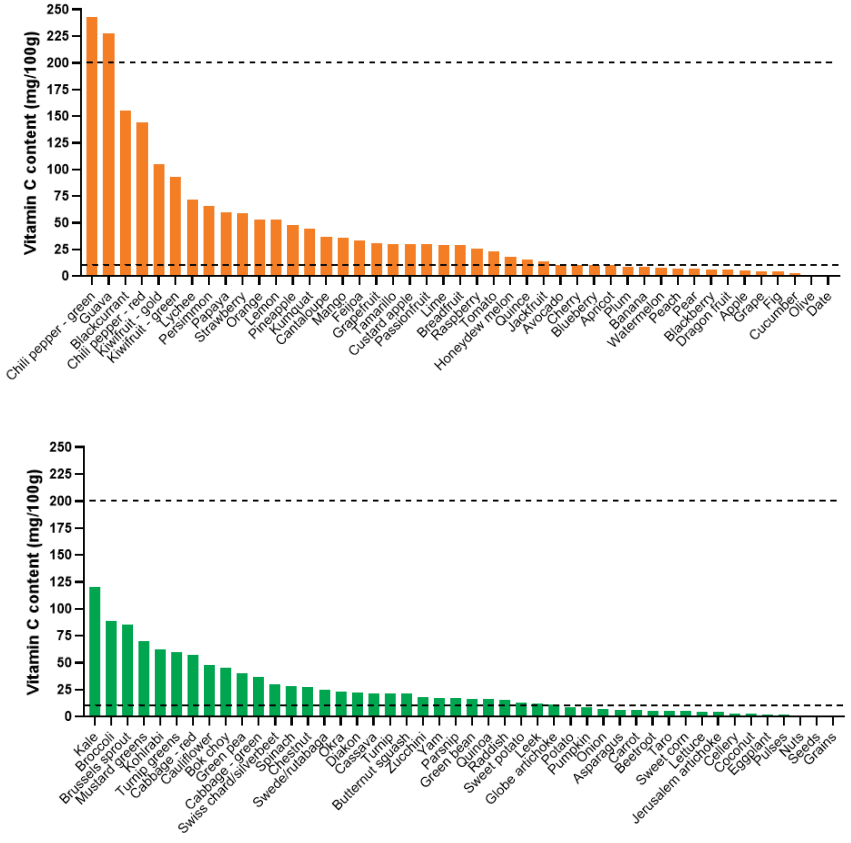


Figure 1. Estimated vitamin C content of selected fruit and vegetables. Data derived from the United States Department of Agriculture (https://fdc.nal.usda.gov/). Note that vitamin C content can vary depending on the plant variety, and cooking may decrease the vitamin C content to variable extents. Pulses include kidney beans, chickpeas, mung beans, pinto beans, soybeans, lentils, peanuts, split peas; nuts include hazelnuts, pistachios, macadamia nuts, pecans, walnuts, brazil nuts, cashew nuts; seeds include chia, flax seeds, pumpkin seeds, sunflower seeds, sesame seeds; grains include rice, millet, wheat/couscous, cornmeal. Animal products, such as meat (other than liver), eggs, and milk contain negligible vitamin C. Dotted lines: lower line indicates daily intake to prevent scurvy (10 mg/d); upper line indicates daily intake for optimal health (200 mg/d).

Suboptimal diet is an important preventable risk factor for noncommunicable diseases, and a low intake of fruit was found to be the third leading dietary risk for deaths (two million deaths) and disability-adjusted life-years (65 million DALYs) globally [31]. The World Health Organization recommends at least 400 g (i.e., five portions) of fruit and vegetables per day, excluding potatoes, sweet potatoes, cassava, and other starchy roots [32]. Historically, scurvy has been reported in refugee camps in Ethiopia; the relief food distributed to the refugees was almost completely deficient in vitamin C, and the environment where the camps were located precluded an adequate supply of fresh food [33]. However, vitamin C deficiency may even be common in fertile areas of Africa, such as Uganda [34]. There have been a number of outbreaks of clinical scurvy in recent years, including in tribesmen in Kenya and during the Afghanistan winter [35,36], and cases of clinical scurvy continue to be diagnosed related to poor intake even in high-income countries [12,37].

2.2. Cultural Aspects: Staple Foods and Traditional Cooking Practices

Food consumption is influenced by a number of factors, such as geographical, economic, social, and cultural [25]. It is well known that economic factors, such as poverty, can limit the consumption of healthy foods. Furthermore, tradition and social customs may influence the consumption of a variety of foods. For example, a study in Nepal highlighted cultural beliefs around menstruation, pregnancy, and lactation that decreased micronutrient intake and intrahousehold disfavouritism towards women in the distribution of micronutrient-rich foods [27]. In low- and middle-income countries (LMICs), geographic considerations and agricultural practices influence the production and consumption of staple foods, which can greatly influence vitamin C intake. For example, countries whose populations consume grains such as rice and millet as staple foods (e.g., in Asia and parts of Africa) tend to have lower intakes of vitamin C [18,25]. In contrast, some areas of Africa and Latin America where yams and sweet potato are staple foods, vitamin C intakes may be higher. However, the vitamin C content of food can also vary depending on the time of harvest, transportation and storage conditions, and food preparation conditions. For example, the vitamin C content of some staple foods, e.g., cassava, is significantly depleted by different processing methods, which could lead to inaccuracies in estimated vitamin C intake [38]. Vitamin C is heat-labile and can be destroyed by cooking; the lower vitamin C status of Indians and Malays living in Singapore is thought to be partly due to its destruction by more prolonged cooking [26].

2.3. Supplement Use

An optimal vitamin C intake (i.e., 200 mg/d) can be obtained from five-plus daily servings of fresh fruit and vegetables, providing that at least one or two servings are high vitamin C foods, however, this is not always possible for many people around the world. Therefore, taking supplements in addition to dietary intake can help to maintain optimal vitamin C status. There are many different forms of vitamin C supplements; however, research indicates that these generally have the same bioavailability as food-derived vitamin C [39]. Numerous studies have found that even in developed countries, where there is no shortage of fresh fruit and vegetables, those who consume supplements have significantly higher vitamin C status and/or lower prevalence of deficiency (Table 2). Two large health surveys carried out in Canada (CHMS) and the USA (NHANES IV) reported 20% supplement use in Canada and 37 to 47% supplement use, for men and women, respectively, in the USA [14,15]. It should be noted, however, that in these surveys, supplement use was considered to be as little as once in the previous month. Nevertheless, both studies found vitamin C status was at least 20 μmol/L higher in supplement users, with a low prevalence of deficiency (0–2%) in those who supplemented [14,15]. Two studies in the UK indicated that people who did not supplement had a two-fold odds ratio of having a vitamin C status of <28 μmol/L and a three-fold odds ratio of their vitamin C status being <11 μmol/L, relative to concentrations >28 μmol/L [19,28].

Table 2. Effect of socioeconomic factors on vitamin C status.

Factor	Summary and Comments	References
Socioeconomic status/deprivation	Individuals with lower socioeconomic status or higher deprivation have lower vitamin C status and a higher prevalence of deficiency; this is partly due to the higher cost of good quality, nutrient-dense food.	[15,19,21,24,28,40–45]
Education and social class	Similarly, individuals with lower education and manual occupations have lower vitamin C status.	[14,17,20,28,42]
Institutionalized	Institutionalized elderly, and other institutionalized individuals (e.g., priests, prisoners, boarding school children) have lower vitamin C status and a higher prevalence of deficiency; this is partly due to a lower dietary intake.	[41,46–48]

3. Environmental Factors Affecting Vitamin C Status

3.1. Geographic Region

Geographic differences in vitamin C status have been reported (Table 3); there are likely many factors that contribute to the observed differences. For example, significant differences in vitamin C status and prevalence of deficiency were observed between Finland and neighboring Russia (where strikingly low plasma concentrations were observed) [17]. Geographical differences in the consumption of fresh fruit and vegetables were apparent, which were associated with deficiency. Low educational status was also noted to be associated with deficiency on the Russian side of the border [17]. Another study carried out in five countries across Europe indicated a difference in the vitamin C status of women, with the biggest differences being observed between Northern Ireland and the Republic of Ireland [49]. No differences in vitamin C status were observed for men from these different countries, whilst women had higher vitamin C status than the men in three of the five countries. Mosdol et al. [19] reported that low-income participants living in Scotland and Northern Island had a lower prevalence of deficiency than those living in England, whilst those living in Wales had a higher prevalence of deficiency. As part of the SU.VI.MAX trial, France was divided into seven regions, and vitamin C status was found to be significantly lower in the Northern region [50]. The reasons for these regional differences are unknown. In a large study of elderly people in India, a higher prevalence of deficiency was observed in the north (74% deficiency) compared with the south (46% deficiency) [24]. Similarly, in Mexico, a higher prevalence of deficiency was observed in children in the north and south regions (~26% deficiency) compared with those living in Mexico City (12% deficiency) [40]. This could reflect differences in the socioeconomic status between urban and rural environments.

Table 3. Environmental factors affecting vitamin C status.

Factor	Summary and Comments	References
Geographical region	Vitamin C status varies by geographical region, both within and between countries; this could partly reflect differences in socioeconomic status and available foods.	[17,19,24,40,41,49–51]
Season	Vitamin C status varies seasonally between countries, likely reflecting different crops and thus the types and/or amounts of vitamin C-rich foods consumed.	[24,30,52,53]
Climate	Drought and harsh winter climates have been associated with outbreaks of clinical scurvy.	[36]
Pollution	Exposure to environmental pollutants, e.g., smoke, can deplete vitamin C status; this is partly due to enhanced oxidative stress.	[24,42,54–58]

3.2. Season and Climate

Since fresh fruit and vegetables are the major source of vitamin C for most people, it is perhaps not surprising that there have been reports of seasonal differences in vitamin C status (Table 3). Surprisingly, however, studies carried out in England and China have indicated that vitamin C status tends to be highest in winter and lowest in autumn, with up to 10 μmol/L difference between these seasons [30,52]. Another study in China indicated a much higher vitamin C status in winter relative to spring [53]. This likely reflects the types or amounts of vitamin C-rich foods being consumed in winter. Similar trends were observed in northern India, with less deficiency being observed in the winter months; however, in southern India, the winter months were associated with a higher prevalence of deficiency [24]. This likely reflects the different climatic and agricultural patterns across the subcontinent. It is possible that seasonal variances in plasma vitamin C concentrations are affected by baseline vitamin C status, i.e., whether the individual is already saturated or not. Bates et al. [59] reported seasonal variations in participants with higher intakes and blood status at baseline compared with little variation in those with low intakes and status, the latter likely due to a depleted body pool

and preferential uptake of the vitamin by tissues. Changes in climate, such as drought, are also likely to impact on the vitamin C status of the populations reliant on local foods in the affected region [36]. This also leads to increased reliance on staple crops like cassava that lose their vitamin content prior to consumption [38].

3.3. Pollution

Currently, over half of the world's population lives in urban areas, and WHO data indicate that more than 80% of people living in urban areas are exposed to air quality levels that exceed WHO guideline limits, with LMICs suffering from the highest exposures [60,61]. Air pollution causes an estimated seven million premature deaths worldwide every year, primarily resulting from increased mortality from strokes, heart disease, chronic obstructive pulmonary disease, lung cancer, and acute respiratory infections [61]. Environmental air pollution, such as smoke derived from burning biomass, can comprise reactive species that potentially affect in vivo antioxidant status [24]. Another example is environmental tobacco smoke, which is an underestimated pollutant in many parts of the world. Exposure to environmental tobacco smoke is associated with depleted vitamin C status in both nonsmoking adults and children [42,54–58]. Tribble et al. [54] found that the vitamin C status of passive smokers was significantly lower than that of nonexposed nonsmokers, despite comparable dietary intakes of the vitamin. Hypovitaminosis C was observed in 12% of passive smokers, but not in nonexposed nonsmokers. Analysis of the data of children from the NHANES III and IV surveys revealed a dose–response relationship between levels of tobacco exposure and serum vitamin C concentrations [55,56]. Not all studies in adults, however, have shown effects of passive smoking on vitamin C status [62–65]. Nevertheless, supplementation with vitamin C and other antioxidants was found to decrease oxidative biomarkers in participants exposed to environmental tobacco smoke [62,66].

4. Effect of Demographic Factors on Vitamin C Status

4.1. Sex

In high-income settings, females appear to have higher vitamin C status and a lower prevalence of deficiency than males [3]. According to McCall et al. [28], UK males have a four-fold odds ratio of deficiency compared with females (Table 4). The difference in vitamin C status between males and females is thought to be partly a result of a volumetric dilution effect due to the higher fat-free mass of males [29]. There are also differences in dietary intakes between men and women, with women generally having comparable or higher intakes than men in high-income countries, although this difference is less apparent in some low-income settings [3]. It should also be noted that pregnancy and lactation typically lower women's vitamin C status due to hemodilution and the needs of the developing fetus and growing infant (see below). Many countries have higher dietary recommendations for men (to take into account the differences in body mass) and for pregnant and lactating women [13].

Table 4. Effect of demographic factors on vitamin C status.

Factor	Summary and Comments	References
Sex	Males generally have lower vitamin C status, and a higher prevalence of deficiency, than females; this is partly a result of a volumetric dilution effect due to the higher fat-free mass of males. This difference is less apparent in some low- and middle-income countries.	[14,15,17,19,22,24,26,28–30,42,43,49,50,53,67–70]
Age	Both children and elderly tend to have higher vitamin C status in high-income settings; this could partly be due to lower body weight. Elderly can have a higher prevalence of vitamin C deficiency in some settings; this could be due to lower intake and/or comorbidities.	[14,15,20,24,28,29,41,50,71,72]
Race	In the US and UK, African-Caribbean and South Asian people had a lower status than Caucasians. In South Asia, Malays and Indians had a lower status than Chinese; this is thought to be partly due to differences in culinary practices. Differences are more apparent between women of different races.	[15,26,30,73,74]

4.2. Age

Some studies carried out in people aged >60 years (e.g., the French POLA study and the British National Diet and Nutrition Survey) have shown lower vitamin C status than other studies carried out in the same countries with younger age groups (e.g., the French SU.VI.MAX study and the European EPIC-Norfolk study); however, it is not ideal to compare values between different studies due to potential methodological differences. A number of studies have indicated that older age within the same study population is associated with an increased prevalence of vitamin C deficiency, particularly in men, and older men also tend to have a lower vitamin C intake than older women [20,24,28,71]. However, a counterpoint to this is that overall vitamin C status can be higher in older people [15,29,50], although not in all cases, as was found in men in Finland who had lower vitamin C status with older age [72]. Schleicher et al. found a U-shaped curve for vitamin C status over the age range of 6 to 60+ years [15]. It is possible that the lower body mass of children and elderly, due to age-related frailty, could contribute to their higher vitamin C status. However, there are, clearly, subpopulations within the aging population who have increased deficiency, likely due to lower intake and/or comorbidities.

4.3. Race

A number of studies have indicated that vitamin C status varies by race (Table 4; reviewed in [74]). The prevalence of vitamin C deficiency appeared to be highest amongst South Asians and was thought to be partly due to traditional cooking practices. It was also suggested that the high proportion of individuals with low vitamin C concentrations in South Asian populations might contribute to their higher rates of cardiovascular disease. Only a few studies have assessed different ethnic groups within the same study [15,26,30,73]. In 1980, Koh et al. [73] assessed vitamin C status in a cohort of black and white participants in Mississippi, USA. Black males and females had significantly lower vitamin C status than white males and females (up to 10 μmol/L difference for females). The more recent US NHANES IV survey also found significantly lower vitamin C status in black females compared with white females [15]. In London, the vitamin C status of black and South Asians was significantly lower than white participants (up to 15 μmol/L lower for South Asian females) [30]. Similarly, in Singapore, Malays and Asian Indians were found to have significantly lower vitamin C status than Chinese (up to 12 μmol/L for females) [26]. Studies in Uganda, South Africa, and Nigeria all showed high rates of deficiency in predominately black Africans [3].

5. Effect of Socioeconomic Factors on Vitamin C Status

5.1. Socioeconomic Status/Deprivation

Socioeconomic status affects diet quality as foods of higher quality and higher nutritional value generally cost more [75]. Therefore, it is not surprising that numerous studies have shown that lower socioeconomic status and deprivation negatively impact on vitamin C status (Table 2). In a UK study, McCall et al. [28] reported that those who were most deprived (based on the Townsend deprivation index) had a two-fold odds ratio of vitamin C deficiency relative to those with plasma values above 28 μmol/L. This finding was supported by Mosdol et al. [19], who specifically focused on the low-income population within the UK, and showed a higher prevalence of deficiency and insufficiency than has been reported for the general UK population. Bates et al. [41] reported a three-fold difference in plasma vitamin C status for people of low socioeconomic status in the UK relative to those of higher status. A two-fold difference in vitamin C intake between the two socioeconomic groups likely contributed to the observed differences in vitamin C status. Other more recent surveys have confirmed the association of low vitamin C status with low socioeconomic status [15,43]. The British National Diet and Nutrition Survey indicated a 2 μmol/L increase in vitamin C status in adults for every additional £10,000 of income [44].

5.2. Education and Social Class

Similar findings have been reported for the level of education, with the lowest level of education associated with the lowest vitamin C status [14,17]. McCall et al. [28] reported that those with the lowest level of education in the UK had a 4.5-fold odds ratio of deficiency relative to a vitamin C status of >28 μmol/L. The same authors investigated social class and found that those having manual occupations had a three-fold odds ratio of deficiency compared with those having nonmanual occupations [28]. Similar findings were reported by Wreiden et al. [20], with two- to three-fold higher prevalence of vitamin C deficiency for females and males, respectively, with manual occupations.

5.3. Institutionalization

One survey of elderly people assessed institutionalized individuals relative to free-living individuals [41]. The authors found significantly lower vitamin C status (25 vs. 44 μmol/L) and a higher prevalence of deficiency (40% vs. 14%) in institutionalized elderly compared with free-living elderly, respectively. Much of this is likely due to a higher proportion of individuals consuming less than the recommended nutrient intake for vitamin C in institutional settings [41]. This will become a growing concern with an increasingly aging population. Other institutionalized individuals, e.g., priests, prisoners, and boarding school children, have also been found to have lower vitamin C status, and higher hypovitaminosis C and severe deficiency, compared to control groups [46–48].

6. Health Aspects that Affect Vitamin C Status

6.1. Body Weight and Body Mass Index (BMI)

Bodyweight and the related BMI, waist circumference, or waist-to-hip ratio are well known to have a significant association with vitamin C status (Table 5). Numerous studies have shown an inverse association between body weight or BMI and vitamin C status, with obese individuals having the lowest vitamin C status [14–16,29,30,67,76]. Schleicher et al. [15] reported up to a 15 μmol/L difference in the vitamin C status of obese women compared with women of healthy weight (i.e., 45 versus 60 μmol/L, respectively). Pearson et al. [43] found that individuals with hypovitaminosis C (i.e., <23 μmol/L) had significantly higher weight, BMI, and waist circumference. Lower vitamin C status in those of the highest weight could be partly due to lower dietary intake of the vitamin [76,77]. In support of this, lower vitamin C status was found in those with the highest fat intake [23]. It has also been suggested that the observed differences in vitamin C status could be a result of volumetric dilution due to differences in fat-free mass [29]. This premise is supported by supplementation studies that have indicated that individuals with higher body weight do not replete as readily as those of normal body weight [78,79].

Based on these observations, Block et al. [78] proposed that recommended vitamin C intakes should be based on a dose per kg body weight or in terms of an ideal plasma concentration. This is a prudent recommendation considering the ongoing increase in body weight globally, with the prevalence of obesity exceeding 50% in some countries [80,81]. Obesity is a risk factor for numerous diseases, particularly cardiometabolic diseases, such as diabetes and cardiovascular disease, which are also associated with lower vitamin C status and a higher prevalence of deficiency [9]. Enhanced dietary fat and sugar, which are risk factors for cardiometabolic diseases, are also associated with decreased vitamin C intake and status [22,23].

Table 5. Health aspects that affect vitamin C status.

Factor	Summary and Comments	References
Bodyweight, BMI	Individuals with higher body weight or BMI have lower vitamin C status; this is likely partly due to a volumetric dilution effect.	[14–16,24,29,30,43,67,76,77,82,83]
Physical activity	Physical activity level positively correlates with vitamin C status, with inactive individuals having a 3-fold odds ratio of deficiency; this is likely partly due to associated lifestyle factors such as diet and body weight.	[28,29]
Pregnancy and lactation	Pregnancy is associated with lower vitamin C status; this is partly due to hemodilution and active transfer of vitamin C to the developing fetus and growing infant via breastmilk.	[34]
Genetic variants	Polymorphisms in the genes for the vitamin C transporter (SVCT1) and haptoglobin (Hp2-2) are associated with lower vitamin C status; the latter is thought to be due to enhanced oxidative stress.	[6,45,84–87]
Smoking	Smokers have lower vitamin C status and a higher prevalence of deficiency than nonsmokers; this is partly due to enhanced oxidative stress.	[14–16,19,20,24,28–30,43,45,50,53,54,67,71,88–91]
Disease states	Various communicable and noncommunicable diseases are associated with lower vitamin C status; this is partly due to inflammatory processes and enhanced oxidative stress.	[9,92–94]

6.2. Pregnancy and Lactation

Pregnant women typically have lower vitamin C status compared with nonpregnant women [34]. This is most likely due to hemodilution, as well as active transfer of the vitamin to the developing fetus [95]. Women with complications of pregnancy can have even lower vitamin C status [34,96]. Supplementation of pregnant women with vitamin C can potentially decrease the risk of some pregnancy-related complications [97]. Studies in high-income countries, which included women with normal vitamin C status, have failed to show benefit from supplementation [98]. However, studies in low-income settings with high rates of vitamin deficiency show potential benefits, including decreased rates of low birth weight, decreased hospital admissions, and possible decreased rates of pre-eclampsia [99]. It is noteworthy that the recently discovered epigenetic regulatory activities of vitamin C could have important roles to play in fetal development [100]. An animal model has indicated that maternal vitamin C can regulate the reprogramming of DNA methylation and germline development [101]. It is likely that lactating women also have lower vitamin C status due to the transfer of vitamin C to the growing infant via breastmilk. Many global authorities have taken the enhanced requirements of pregnant and lactating women into consideration, with recommendations above their standard dietary recommendations of +10–20 mg/d for pregnant women and +20–60 mg/d for lactating women [13].

6.3. Genetic Variants

Vitamin C status can potentially be influenced by genetic variants. The SLC23A1 gene encodes the sodium-dependent vitamin C transporter-1 (SVCT1), which is responsible for active uptake of dietary vitamin C through the intestinal epithelium and reuptake of filtered vitamin C via the kidney tubules and is essential in vitamin C-requiring animals [4,6]. A number of single nucleotide variants have

been identified in the SLC23A1 gene, and modeling of in vitro data for four of these variants indicates 40% to 75% decreases in vitamin C uptake [6,102]. These variants are relatively common in those of African descent (6–16%), and the variant with the largest decrease in vitamin C transport has a high prevalence in African Americans [6]. Meta-analysis has indicated a strong association of another of these variants with decreased vitamin C status in five cohorts in the UK (frequency of ~3%) [45]. In the British Women's Heart and Health Study, a further two variants, with relatively common frequencies of ~30%, were also found to be associated with vitamin C status [45]. Cahill et al. [84] reported that variants of SVCT1 can also modify the strength of the correlation between dietary vitamin C and serum vitamin C.

Vitamin C status could be further influenced by genetic variants affecting the metabolism of the vitamin [103]. A common variant of the hemoglobin-binding protein haptoglobin (Hp2-2) has a decreased ability to bind hemoglobin and results in increased oxidation of vitamin C in vitro [85]. Several studies have shown that this variant is associated with lower circulating vitamin C concentrations [85–87]. The Hp2-2 variant appeared to have a greater effect on individuals with dietary vitamin C intakes <90 mg/day [84]. It is noteworthy that genetic variants can show marked geographical differences; e.g., Hp2-2 is present in ~36% Northwest Europeans, 51% Iranians, 55% Thais and Chinese, and 84% Indians (where it is thought to have originated) [104]. Other genetic variants, such as those of the detoxifying enzyme glutathione S-transferase, may also have associations with vitamin C status, particularly in individuals with lower intakes, thereby resulting in an increased risk of deficiency [105]. High-dose vitamin supplementation has been shown to ameliorate certain gene variant defects [106] and is hypothesized to occur with vitamin C-related variants [107]. Therefore, individuals with genetic variants affecting vitamin C status may require higher dietary intakes.

6.4. Smoking

Smoking is a well-known source of oxidants and in vivo oxidative stress [108]. Multiple studies have shown depleted vitamin C status and a higher prevalence of deficiency in smokers compared with nonsmokers (Table 5). McCall et al. [28] reported that current smokers had over a seven-fold odds ratio of deficiency compared with nonsmokers, and Wrieden et al. [20] showed two- to three-fold more deficiency in male and female smokers, respectively. Smokers tended to have dietary intakes that were lower in vitamin C, e.g., lower fruit and vegetable intake and higher fat intake [88,89,109,110]. However, when differences in dietary intake were taken into account, smokers still exhibited lower vitamin C status and higher requirements than nonsmokers [88,89,111,112]. Kallner et al. [113] demonstrated over a 40% increase in vitamin C turnover in smokers compared with nonsmokers.

A few international regulatory authorities have taken the enhanced requirements of smokers into consideration with additional intake recommendations of 20 to 80 mg/d above their normal adult recommendations [13]. However, it is likely that these additional intakes are insufficient to compensate for the enhanced requirements of smokers [90]. Furthermore, some countries continue to show an increasing trend in smoking rates, which could potentially impact their population's vitamin C status and requirements [114]. Of note, smoking cessation results in an increase in vitamin C status [115].

6.5. Disease States

Vitamin C status can be depleted by various disease states due to inflammatory processes and enhanced oxidative stress (reviewed in [9,92,93]). A number of studies of hospitalized patients showed a high prevalence of depleted plasma vitamin C status, and concentrations were inversely correlated with inflammatory markers [116–119]. A wide range of medical conditions are associated with vitamin C insufficiency; these include noncommunicable diseases such as cardiovascular diseases (e.g., strokes, coronary artery disease and hypertension), congestive heart failure, malignancy, chronic inflammatory states (e.g., rheumatoid arthritis), metabolic disorders (e.g., diabetes), and cataracts [9,92,93]. Acute infectious diseases leading to enhanced inflammation are also associated with depleted plasma vitamin C concentrations in plasma and immune cells, as are a range of chronic infections such as HIV,

Helicobacter pylori, and tuberculosis, which are prevalent in many LMICs [94,120]. It should also be noted that requirements for vitamin C during infections increase with the severity of the infection, requiring significantly enhanced intakes to reach normal plasma status [94]. This is particularly pertinent to the current global coronavirus (SARS-CoV-2) pandemic, which causes severe pneumonia and sepsis, conditions known to be associated with significantly depleted vitamin C status [121,122]. It should also be noted that individuals with marginal vitamin C status are at higher risk of developing vitamin C deficiency [123], and once depleted, higher than recommended intakes of the vitamin are required to fully replete them again [78,79].

7. Has Vitamin C Status Changed Over Time?

Various factors have changed over time that could potentially affect vitamin C status. These include population demographics and health status, e.g., obesity and smoking rates. Public health policies, such as recommended dietary intakes for vitamin C, have changed in many countries [13]. Therefore, have these temporal changes been reflected in changing vitamin C status over time? There have been relatively few studies that have assessed vitamin C status in the same populations or regions over time. The findings can also be complicated by changes in analytical methodologies over time. Two surveys have been carried out 19 years apart on elderly people in Britain: one in 1979 by the Department of Health and Social Security, and the National Diet and Nutrition Survey in 1998 [41]. There was an increase in vitamin C status from 29 to 44 μmol/L between the two surveys. However, it should be noted that participants in the first survey were older and analytical methodologies may have varied between the two surveys. In contrast, the more recent National Diet and Nutrition Surveys (2008–2017) have shown a significant 6% increase in vitamin C deficiency over the nine-year period in women aged 19–64 [44].

Schleicher et al. [15] carried out an indepth comparison of US vitamin C status in NHANES IV (2003–2004) relative to NHANES III that was undertaken 10–15 years earlier (i.e., 1988–1994) [124]. For those aged ≥20 years, overall vitamin C deficiency decreased by about 44% (from 15% to 8%) between the two surveys. Of note, there was an increase in the recommended dietary intake for vitamin C in the US in 2000, from 60 to 90 mg/d for men and to 75 mg/d for women [125]. However, it is unlikely that this increase in dietary recommendations fully accounts for the decrease in vitamin C deficiency over time as there were also some differences in methodology between the two surveys, with the more recent survey likely being more accurate [15]. Looking back even earlier to the NHANES II survey (carried out between 1976 and 1980), vitamin C status was reported to be higher for both men and women (i.e., ≥50 μmol/L for men and ≥60 μmol/L for women), with 5% deficiency reported, despite lower dietary intakes compared to the later NHANES III survey [126]. It should be noted, however, that vitamin C in the NHANES II samples was analyzed using the 2,4-dinitrophenylhydrazine method, which can be prone to interference by other components in plasma and thus potentially overestimate vitamin C concentrations [127].

Other time course data is available from studies carried out in Finland and neighboring Russia between 1992 and 2002 [17,51]. In North Karelia, Finland, the vitamin C status of men decreased from 54 to 27 μmol/L over 5 years, then rose slightly to 37 μmol/L over the next 5 year period [17]. Nyyssonen et al. reported a small increase in baseline vitamin C values in Finish men over 6 years [72]. In Pitkäranta, Russia, the vitamin C status of women decreased from 22 to 13 μmol/L over a 5-year period, while the vitamin C status of Russian men remained in the deficiency range over a 10-year period [17]. The blood samples from the more recent survey (2002) were from fasting participants; however, it is not clear whether they were fasting in the earlier surveys (1992 and 1997).

Another set of studies was carried out in Java, Indonesia, between 2005 and 2011 [128,129]. Mean vitamin C status decreased in the subjects from 45 to 29 μmol/L over 6 years, although the percentage of deficiency decreased from 15% to 11% over this time. The decrease in mean vitamin C status could partly be explained by the earlier study assessing nonfasting plasma (as demonstrated by a higher percentage of vitamin C status >23 μmol/L, i.e., 72% versus 54%). However, there are possibly

other environmental, lifestyle, or health-related issues that could be responsible for the changes in vitamin C status over time in Indonesia, Finland, and Russia.

Not all studies have shown changes in vitamin C status over time. A survey carried out in Shanghai, China, over a five-year period (between 1995 and 2000) showed no change in vitamin C status over this time period, although there was a dip in status at the midpoint (from 43 to 34 μmol/L) [52]. Thus, there is currently no clear trend in vitamin C status over time. However, much of the vitamin C data is very dated, and thus may not accurately reflect the current situation, particularly in countries where there have been changes over time in specific factors that can affect vitamin C status.

8. Conclusions and Future Directions

This narrative review describes the findings of numerous studies which highlight the various factors that can impact on vitamin C status and prevalence of deficiency. The studies were of variable quality; ideally, multivariate analysis of the various factors highlighted in these studies would be performed to further examine the relationships discussed. Another limitation is the variable quality of the processes and methodologies used to assess vitamin C status in the studies; this has been addressed in detail in our review on global vitamin C status [3].

Global vitamin C status appears to be a cause for concern, and we believe this is primarily due to poor diet, particularly in LMICs, although also apparent in subgroups within high-income settings [3]. Vitamin C concentrations provide a useful biomarker for a healthy diet; unfortunately, accurate analysis is costly, technically challenging, and time-consuming [130]. Thus, there is a need for low cost, accurate, and commercially available methods to assess plasma concentrations. Equally, the current assessment of intake is also limited and fails to take into account that the vitamin may be almost entirely eliminated prior to consumption in some globally important crops, such as cassava [38]. Future amendments to nutrient reference tables should take into this aspect into account.

Clearly, there is a global need for increased consumption of vitamin-C-rich fresh fruit and vegetables. From our earlier review, this appears to be most notable in LMIC's; however, it is also a concern in other at-risk groups [3]. Further education is required globally in how to meet the daily vitamin C requirements with locally available crops year-round. Some staple foods such as cassava require prolonged cooking to remove toxins (in this case, cyanide); efforts have been made to reduce this content in genetically modified cassava, which could allow higher vitamin C content from this vitamin-rich plant at the time of consumption [38]. Given the high rates of deficiency in some diets, cooking practices may also require modification to help increase intake.

A number of studies have assessed the potential for vitamin C supplements to reduce a range of noncommunicable diseases and infections [9,94]. However, there remains much controversy as to the efficacy of supplementation, primarily due to badly designed studies that do not take into account the baseline vitamin C status of the participants [98]. Therefore, knowledge of the high-risk groups for deficiency should be utilized for future studies as these groups are more likely to benefit from supplementation.

In the meantime, global and local policymakers should consider the local data available on deficiency in an attempt to modify dietary intake and other modifiable risk factors. More of these risk factors should be taken into account during the review and update of global recommended dietary intakes for the vitamin. Clinicians worldwide should also remain vigilant to detect and correct hypovitaminosis C and deficiency in the at-risk groups highlighted.

Author Contributions: A.C.C. and S.R. contributed equally to the review. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Acknowledgments: A.C.C. is a recipient of a Sir Charles Hercus Health Research Fellowship from the Health Research Council of New Zealand.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Nishikimi, M.; Yagi, K. Molecular basis for the deficiency in humans of gulonolactone oxidase, a key enzyme for ascorbic acid biosynthesis. *Am. J. Clin. Nutr.* **1991**, *54*, 1203S–1208S. [CrossRef]
2. Stone, I. Homo sapiens ascorbicus, a biochemically corrected robust human mutant. *Med. Hypotheses* **1979**, *5*, 711–721. [CrossRef]
3. Rowe, S.; Carr, A.C. Global Vitamin C Status and Prevalence of Deficiency: A Cause for Concern? *Preprints*, 2020; 202005.0394.v1.
4. Savini, I.; Rossi, A.; Pierro, C.; Avigliano, L.; Catani, M.V. SVCT1 and SVCT2: Key proteins for vitamin C uptake. *Amino Acids* **2008**, *34*, 347–355. [CrossRef]
5. Hornig, D. Distribution of ascorbic acid, metabolites and analogues in man and animals. *Ann. N. Y. Acad. Sci.* **1975**, *258*, 103–118. [CrossRef] [PubMed]
6. Corpe, C.P.; Tu, H.; Eck, P.; Wang, J.; Faulhaber-Walter, R.; Schnermann, J.; Margolis, S.; Padayatty, S.; Sun, H.; Wang, Y.; et al. Vitamin C transporter Slc23a1 links renal reabsorption, vitamin C tissue accumulation, and perinatal survival in mice. *J. Clin. Investig.* **2010**, *120*, 1069–1083. [CrossRef]
7. Washko, P.W.; Wang, Y.; Levine, M. Ascorbic acid recycling in human neutrophils. *J. Biol. Chem.* **1993**, *268*, 15531–15535. [PubMed]
8. Carr, A.; Frei, B. Does vitamin C act as a pro-oxidant under physiological conditions? *FASEB J.* **1999**, *13*, 1007–1024. [CrossRef]
9. Carr, A.C.; Frei, B. Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans. *Am. J. Clin. Nutr.* **1999**, *69*, 1086–1107. [CrossRef]
10. Levine, M.; Conry-Cantilena, C.; Wang, Y.; Welch, R.W.; Washko, P.W.; Dhariwal, K.R.; Park, J.B.; Lazarev, A.; Graumlich, J.F.; King, J.; et al. Vitamin C pharmacokinetics in healthy volunteers: Evidence for a recommended dietary allowance. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 3704–3709. [CrossRef] [PubMed]
11. Deirawan, H.; Bs, J.W.F.; Zarka, M.; Bluth, M.H.; Moossavi, M. Revisiting the pathobiology of scurvy: A review of the literature in the context of a challenging case. *Int. J. Dermatol.* **2020**. [CrossRef]
12. Ceglie, G.; Macchiarulo, G.; Marchili, M.R.; Marchesi, A.; Lelia, R.A.; Di Camillo, C.; Villani, A. Scurvy: Still a threat in the well-fed first world? *Arch. Dis. Child.* **2019**, *104*, 381–383. [CrossRef] [PubMed]
13. Carr, A.C.; Lykkesfeldt, J. Discrepancies in global vitamin C recommendations: A review of RDA criteria and underlying health perspectives. *Crit. Rev. Food Sci. Nutr.* **2020**, *2020*, 1–14. [CrossRef] [PubMed]
14. Langlois, K.; Cooper, M.; Colapinto, C.K. Vitamin C status of Canadian adults: Findings from the 2012/2013 Canadian Health Measures Survey. *Health Rep.* **2016**, *27*, 3–10.
15. Schleicher, R.L.; Carroll, M.D.; Ford, E.S.; Lacher, D.A. Serum vitamin C and the prevalence of vitamin C deficiency in the United States: 2003–2004 National Health and Nutrition Examination Survey (NHANES). *Am. J. Clin. Nutr.* **2009**, *90*, 1252–1263. [CrossRef]
16. Drewnowski, A.; Rock, C.L.; Henderson, S.A.; Shore, A.B.; Fischler, C.; Galan, P.; Preziosi, P.; Hercberg, S. Serum beta-carotene and vitamin C as biomarkers of vegetable and fruit intakes in a community-based sample of French adults. *Am. J. Clin. Nutr.* **1997**, *65*, 1796–1802. [CrossRef] [PubMed]
17. Paalanen, L.; Prättälä, R.; Alfthan, G.; Salminen, I.; Laatikainen, T. Vegetable and fruit consumption, education and plasma vitamin C concentration in Russian and Finnish Karelia, 1992–2002. *Public Health Nutr.* **2013**, *17*, 2278–2286. [CrossRef] [PubMed]
18. Joint FAO/WHO Expert Group. *Human Vitamin and Mineral. Requirements*; Food and Agriculture Organization: Rome, Italy, 2001.
19. Mosdol, A.; Erens, B.; Brunner, E.J. Estimated prevalence and predictors of vitamin C deficiency within UK's low-income population. *J. Public Health* **2008**, *30*, 456–460. [CrossRef] [PubMed]
20. Wrieden, W.L.; Hannah, M.K.; Bolton-Smith, C.; Tavendale, R.; Morrison, C.; Tunstall-Pedoe, H. Plasma vitamin C and food choice in the third Glasgow MONICA population survey. *J. Epidemiol. Community Health* **2000**, *54*, 355–360. [CrossRef] [PubMed]
21. De Oliveira, A.M.; Rondo, P.R.D.C.; Mastroeni, M.F.; Oliveira, J.M. Plasma concentrations of ascorbic acid in parturients from a hospital in Southeast Brazil. *Clin. Nutr.* **2008**, *27*, 228–232. [CrossRef]
22. Charlton, K.E.; Kolbe-Alexander, T.L.; Nel, J.H. Micronutrient dilution associated with added sugar intake in elderly black South African women. *Eur. J. Clin. Nutr.* **2005**, *59*, 1030–1042. [CrossRef]

23. Carter, B.E.; Monsivais, P.; Drewnowski, A. Absorption of folic acid and ascorbic acid from nutrient comparable beverages. *J. Food Sci.* **2010**, *75*, H289–H293. [CrossRef] [PubMed]
24. Ravindran, R.D.; Vashist, P.; Gupta, S.K.; Young, I.S.; Maraini, G.; Camparini, M.; Jayanthi, R.; John, N.; Fitzpatrick, K.E.; Chakravarthy, U.; et al. Prevalence and risk factors for vitamin C deficiency in north and south India: A two centre population based study in people aged 60 years and over. *PLoS ONE* **2011**, *6*, e28588. [CrossRef] [PubMed]
25. Joint FAO-WHO Expert Group. *Requirements of Ascorbic Acid, Vitamin D, Vitamin B12, Folate, and Iron*; Report No.: 0512-3054 (Print) 0512-3054 Contract No.: 452; World Health Organization: Geneva, Switzerland, 1970.
26. Hughes, K.; Ong, C.N. Vitamins, selenium, iron, and coronary heart disease risk in Indians, Malays, and Chinese in Singapore. *J. Epidemiol. Community Health* **1998**, *52*, 181–185. [CrossRef] [PubMed]
27. Gittelsohn, J.; Thapa, M.; Landman, L.T. Cultural factors, caloric intake and micronutrient sufficiency in rural Nepali households. *Soc. Sci. Med.* **1997**, *44*, 1739–1749. [CrossRef]
28. McCall, S.J.; Clark, A.B.; Luben, R.N.; Wareham, N.J.; Khaw, K.T.; Myint, P.K. Plasma vitamin C levels: Risk factors for deficiency and association with self-reported functional health in the European Prospective Investigation into Cancer-Norfolk. *Nutrients* **2019**, *11*, 1552. [CrossRef] [PubMed]
29. Jungert, A.; Neuhäuser-Berthold, M. The lower vitamin C plasma concentrations in elderly men compared with elderly women can partly be attributed to a volumetric dilution effect due to differences in fat-free mass. *Br. J. Nutr.* **2015**, *113*, 859–864. [CrossRef] [PubMed]
30. Ness, A.R.; Cappuccio, F.P.; Atkinson, R.W.; Khaw, K.T.; Cook, D.G. Plasma vitamin C levels in men and women from different ethnic backgrounds living in England. *Int. J. Epidemiol.* **1999**, *28*, 450–455. [CrossRef]
31. GBD 2017 Diet Collaborators. Health effects of dietary risks in 195 countries, 1990–2017: A systematic analysis for the Global Burden of Disease Study 2017. *Lancet* **2019**, *393*, 1958–1972. [CrossRef]
32. World Health Organization. *Diet, Nutrition and the Prevention of Chronic Diseases: Report of a Joint WHO/FAO Expert Consultation*; Contract No.: No. 916; WHO: Geneva, Switzerland, 2003.
33. Desenclos, J.C.; Berry, A.M.; Padt, R.; Farah, B.; Segala, C.; Nabil, A.M. Epidemiological patterns of scurvy among Ethiopian refugees. *Bull. World Health Organ.* **1989**, *67*, 309–316.
34. Kiondo, P.; Tumwesigye, N.M.; Wandabwa, J.; Wamuyu-Maina, G.; Bimenya, G.S.; Okong, P. Plasma vitamin C assay in women of reproductive age in Kampala, Uganda, using a colorimetric method. *Trop. Med. Int. Health* **2012**, *17*, 191–196. [CrossRef]
35. Halestrap, P.; Scheenstra, S. Outbreak of scurvy in Tana River County, Kenya: A case report. *Afr. J. Prim. Health Care Fam. Med.* **2018**, *10*, e1–e3. [CrossRef] [PubMed]
36. Cheung, E.; Mutahar, R.; Assefa, F.; Ververs, M.-T.; Nasiri, S.M.; Borrel, A.; Salama, P.; Hartini, T.N.S.; Winkvist, A.; Lindholm, L.; et al. An epidemic of scurvy in Afghanistan: Assessment and response. *Food Nutr. Bull.* **2003**, *24*, 247–255. [CrossRef]
37. Khalife, R.; Grieco, A.; Khamisa, K.; Tinmouh, A.; McCudden, C.; Saidenberg, E. Scurvy, an old story in a new time: The hematologist's experience. *Blood Cells Mol. Dis.* **2019**, *76*, 40–44. [CrossRef]
38. Montagnac, J.A.; Davis, C.R.; Tanumihardjo, S.A. Nutritional value of cassava for use as a staple food and recent advances for improvement. *Compr. Rev. Food Sci. Food Saf.* **2009**, *8*, 181–194. [CrossRef]
39. Carr, A.C.; Vissers, M.C. Synthetic or food-derived vitamin C—Are they equally bioavailable? *Nutrients* **2013**, *5*, 4284–4304. [CrossRef] [PubMed]
40. Villalpando, S.; Montalvo-Velarde, I.; Zambrano, N.; García-Guerra, A.; Ramírez-Silva, C.I.; Shamah-Levy, T.; Rivera-Dommarco, J. Vitamins A, and C and folate status in Mexican children under 12 years and women 12–49 years: A probabilistic national survey. *Salud Pública México* **2003**, *45*, 508–519. [CrossRef]
41. Bates, C.J.; Prentice, A.; Cole, T.J.; Van Der Pols, J.C.; Doyle, W.; Finch, S.; Smithers, G.; Clarke, P.C. Micronutrients: Highlights and research challenges from the 1994–5 National Diet and Nutrition Survey of people aged 65 years and over. *Br. J. Nutr.* **1999**, *82*, 7–15. [CrossRef]
42. Chiplonkar, S.A.; Agte, V.V.; Mengale, S.S.; Tarwadi, K.V. Are lifestyle factors good predictors of retinol and vitamin C deficiency in apparently healthy adults? *Eur. J. Clin. Nutr.* **2002**, *56*, 96–104. [CrossRef]
43. Pearson, J.F.; Pullar, J.M.; Wilson, R.; Spittlehouse, J.K.; Vissers, M.C.M.; Skidmore, P.M.L.; Willis, J.; Cameron, V.A.; Carr, A.C. Vitamin C status correlates with markers of metabolic and cognitive health in 50-year-olds: Findings of the CHALICE cohort study. *Nutrients* **2017**, *9*, E831. [CrossRef]

44. Bates, B.; Collins, D.; Cox, L.; Nicholson, S.; Page, P.; Roberts, C.; Steer, T.; Swan, G. *National Diet and Nutrition Survey Years 1 to 9 of the Rolling Programme (2008/2009–2016/2017): Time Trend and Income Analyses*; Public Health England: London, UK, 2019; p. 56.
45. Timpson, N.J.; Forouhi, N.G.; Brion, M.-J.; Harbord, R.; Cook, D.G.; Johnson, P.C.D.; McConnachie, A.; Morris, R.; Rodriguez, S.; Luan, J.; et al. Genetic variation at the SLC23A1 locus is associated with circulating concentrations of L-ascorbic acid (vitamin C): Evidence from 5 independent studies with >15,000 participants. *Am. J. Clin. Nutr.* **2010**, *92*, 375–382. [CrossRef]
46. Viroonudomphol, D.; Mahaisiriyodom, A.; Mingkhawn, R.; Sadomthian, P.; Korchasri, N.; Jittngamkhum, S.; Sangcakul, A. Relationship between serum antioxidant vitamins A, E, and C and lipid profiles in priest subjects at the Priest Hospital. *Southeast Asian J. Trop. Med. Public Health* **2005**, *36*, 246–253.
47. Gould, C.; Tousignant, B.; Brian, G.; McKay, R.; Gibson, R.; Bailey, K.; Venn, B.J. Cross-sectional dietary deficiencies among a prison population in Papua New Guinea. *BMC Int. Health Hum. Rights* **2013**, *13*, 21. [CrossRef]
48. Ene-Obong, H.N.; Odoh, I.F.; Ikwuagwu, O.E. Plasma vitamin A and C status of in-school adolescents and associated factors in Enugu State, Nigeria. *J. Health Popul. Nutr.* **2003**, *21*, 18–25.
49. Olmedilla, B.; Granado, F.; Southon, S.; Wright, A.J.A.; Blanco, I.; Gil-Martinez, E.; Berg, H.V.D.; Corridan, B.; Roussel, A.-M.; Chopra, M.; et al. Serum concentrations of carotenoids and vitamins A, E, and C in control subjects from five European countries. *Br. J. Nutr.* **2001**, *85*, 227–238. [CrossRef]
50. Faure, H.; Preziosi, P.; Roussel, A.M.; Bertrais, S.; Galan, P.; Hercberg, S.; Favier, A. Factors influencing blood concentration of retinol, alpha-tocopherol, vitamin C, and beta-carotene in the French participants of the SU.VI.MAX trial. *Eur. J. Clin. Nutr.* **2006**, *60*, 706–717. [CrossRef]
51. Matilainen, T.; Vartiainen, E.; Puska, P.; Alfthan, G.; Pokusajeva, S.; Moisejeva, N.; Uhanov, M. Plasma ascorbic acid concentrations in the Republic of Karelia, Russia and in North Karelia, Finland. *Eur. J. Clin. Nutr.* **1996**, *50*, 115–120.
52. Frankenfeld, C.L.; Lampe, J.W.; Shannon, J.; Gao, D.L.; Li, W.; Ray, R.M.; Chen, C.; King, I.B.; Thomas, D.B. Fruit and vegetable intakes in relation to plasma nutrient concentrations in women in Shanghai, China. *Public Health Nutr.* **2012**, *15*, 167–175. [CrossRef]
53. Lam, T.K.; Freedman, N.D.; Fan, J.H.; Qiao, Y.L.; Dawsey, S.M.; Taylor, P.R.; Abnet, C.C. Prediagnostic plasma vitamin C and risk of gastric adenocarcinoma and esophageal squamous cell carcinoma in a Chinese population. *Am. J. Clin. Nutr.* **2013**, *98*, 1289–1297. [CrossRef]
54. Tribble, D.L.; Giuliano, L.J.; Fortmann, S.P. Reduced plasma ascorbic acid concentrations in nonsmokers regularly exposed to environmental tobacco smoke. *Am. J. Clin. Nutr.* **1993**, *58*, 886–890. [CrossRef]
55. Strauss, R.S. Environmental tobacco smoke and serum vitamin C levels in children. *Pediatrics* **2001**, *107*, 540–542. [CrossRef]
56. Wilson, K.M.; Finkelstein, J.N.; Blumkin, A.K.; Best, D.; Klein, J.D. Micronutrient levels in children exposed to secondhand tobacco smoke. *Nicotine Tob. Res. Off. J. Soc. Res. Nicotine Tob.* **2011**, *13*, 800–808. [CrossRef]
57. Preston, A.M.; Rodriguez, C.; Rivera, C.E. Plasma ascorbate in a population of children: Influence of age, gender, vitamin C intake, BMI and smoke exposure. *Puerto Rico Health Sci. J.* **2006**, *25*, 137–142.
58. Preston, A.M.; Rodriguez, C.; Rivera, C.E.; Sahai, H. Influence of environmental tobacco smoke on vitamin C status in children. *Am. J. Clin. Nutr.* **2003**, *77*, 167–172. [CrossRef]
59. Bates, C.J.; Rutishauser, I.H.; Black, A.E.; Paul, A.A.; Mandal, A.R.; Patnaik, B.K. Long-term vitamin status and dietary intake of healthy elderly subjects. 2. Vitamin, C. *Br. J. Nutr.* **1979**, *42*, 43–56. [CrossRef] [PubMed]
60. United Nations. 68% of the World Population Projected to Live in Urban Areas by 2050, Says UN 2018. Available online: https://www.un.org/development/desa/en/news/population/2018-revision-of-world-urbanization-prospects.html (accessed on 12 January 2020).
61. World Health Organization. Air Pollution. Available online: https://www.who.int/health-topics/air-pollution#tab=tab_1 (accessed on 12 January 2020).
62. Dietrich, M.; Block, G.; Benowitz, N.L.; Morrow, J.D.; Hudes, M.; Jacob, P., 3rd; Norkus, E.P.; Packer, L. Vitamin C supplementation decreases oxidative stress biomarker f2-isoprostanes in plasma of nonsmokers exposed to environmental tobacco smoke. *Nutr. Cancer* **2003**, *45*, 176–184. [CrossRef]
63. Farchi, S.; Forastiere, F.; Pistelli, R.; Baldacci, S.; Simoni, M.; Perucci, C.A.; Viegi, G. Exposure to environmental tobacco smoke is associated with lower plasma beta-carotene levels among nonsmoking women married to a smoker. *Cancer Epidemiol. Biomark. Prev.* **2001**, *10*, 907–909.

64. Forastiere, F.; Mallone, S.; Lo Presti, E.; Baldacci, S.; Pistelli, F.; Simoni, M.; Scalera, A.; Pedreschi, M.; Pistelli, R.; Corbo, G.; et al. Characteristics of nonsmoking women exposed to spouses who smoke: Epidemiologic study on environment and health in women from four Italian areas. *Environ. Health Perspect.* **2000**, *108*, 1171–1177. [CrossRef] [PubMed]
65. Ayaori, M.; Hisada, T.; Suzukawa, M.; Yoshida, H.; Nishiwaki, M.; Ito, T.; Nakajima, K.; Higashi, K.; Yonemura, A.; Ohsuzu, F.; et al. Plasma levels and redox status of ascorbic acid and levels of lipid peroxidation products in active and passive smokers. *Environ. Health Perspect.* **2000**, *108*, 105–108. [CrossRef]
66. Howard, D.J.; Ota, R.B.; Briggs, L.A.; Hampton, M.; Pritsos, C.A. Oxidative stress induced by environmental tobacco smoke in the workplace is mitigated by antioxidant supplementation. *Cancer Epidemiol. Biomark. Prev.* **1998**, *7*, 981–988.
67. Galan, P.; Viteri, F.E.; Bertrais, S.; Czernichow, S.; Faure, H.; Arnaud, J.; Ruffieux, D.; Chenal, S.; Arnault, N.; Favier, A.; et al. Serum concentrations of beta-carotene, vitamins C and E, zinc and selenium are influenced by sex, age, diet, smoking status, alcohol consumption and corpulence in a general French adult population. *Eur. J. Clin. Nutr.* **2005**, *59*, 1181–1190. [CrossRef]
68. Itoh, R.; Yamada, K.; Oka, J.; Echizen, H.; Murakami, K. Sex as a factor in levels of serum ascorbic acid in a healthy elderly population. *Int. J. Vitam. Nutr. Res.* **1989**, *59*, 365–372. [PubMed]
69. Hughes, K.; New, A.L.; Lee, B.L.; Ong, C.N. Plasma vitamins A, C and E in the general population of Singapore, 1993 to 1995. *Ann. Acad. Med. Singap.* **1998**, *27*, 149–153. [PubMed]
70. Dherani, M.; Murthy, G.V.S.; Gupta, S.K.; Young, I.S.; Maraini, G.; Camparini, M.; Price, G.M.; John, N.; Chakravarthy, U.; Fletcher, A. Blood levels of vitamin C, carotenoids and retinol are inversely associated with cataract in a North Indian population. *Investig. Ophthalmol. Vis. Sci.* **2008**, *49*, 3328–3335. [CrossRef]
71. Birlouez-Aragon, I.; Delcourt, C.; Tessier, F.J.; Papoz, L. Associations of age, smoking habits and diabetes with plasma vitamin C of elderly of the POLA study. *Int. J. Vitam. Nutr. Res.* **2001**, *71*, 53–59. [CrossRef]
72. Nyyssönen, K.; Parviainen, M.T.; Salonen, R.; Tuomilehto, J.; Salonen, J. Vitamin C deficiency and risk of myocardial infarction: Prospective population study of men from eastern Finland. *BMJ* **1997**, *314*, 634–648. [CrossRef]
73. Koh, E.T.; Chi, M.S.; Lowenstein, F.W. Comparison of selected blood components by race, sex, and age. *Am. J. Clin. Nutr.* **1980**, *33*, 1828–1835. [CrossRef]
74. Khan, R.M.; Iqbal, M.P. Deficiency of Vitamin C in South Asia. *Pak. J. Med Sci.* **2006**, *22*, 347–355.
75. Darmon, N.; Drewnowski, A. Contribution of food prices and diet cost to socioeconomic disparities in diet quality and health: A systematic review and analysis. *Nutr. Rev.* **2015**, *73*, 643–660. [CrossRef]
76. Canoy, D.; Wareham, N.; Welch, A.; Bingham, S.; Luben, R.; Day, N.; Khaw, K.T. Plasma ascorbic acid concentrations and fat distribution in 19,068 British men and women in the European Prospective Investigation into Cancer and Nutrition Norfolk cohort study. *Am. J. Clin. Nutr.* **2005**, *82*, 1203–1209. [CrossRef]
77. Garcia, O.P.; Ronquillo, D.; Caamano Mdel, C.; Camacho, M.; Long, K.Z.; Rosado, J.L. Zinc, vitamin A, and vitamin C status are associated with leptin concentrations and obesity in Mexican women: Results from a cross-sectional study. *Nutr. Metab.* **2012**, *9*, 59. [CrossRef]
78. Block, G.; Mangels, A.R.; Patterson, B.H.; Levander, O.A.; Norkus, E.P.; Taylor, P.R. Body weight and prior depletion affect plasma ascorbate levels attained on identical vitamin C intake: A controlled-diet study. *J. Am. Coll Nutr.* **1999**, *18*, 628–637. [CrossRef] [PubMed]
79. Carr, A.C.; Pullar, J.M.; Bozonet, S.M.; Vissers, M.C. Marginal ascorbate status (hypovitaminosis C) results in an attenuated response to vitamin C supplementation. *Nutrients* **2016**, *8*, E341. [CrossRef] [PubMed]
80. Ng, M.; Fleming, T.; Robinson, M.; Thomson, B.; Graetz, N.; Margono, C.; Mullany, E.C.; Biryukov, S.; Abbafati, C.; Abera, S.F.; et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: A systematic analysis for the Global Burden of Disease Study 2013. *Lancet* **2014**, *384*, 766–781. [CrossRef]
81. Yatsuya, H.; Li, Y.; Hilawe, E.H.; Ota, A.; Wang, C.; Chiang, C.; Zhang, Y.; Uemura, M.; Osako, A.; Ozaki, Y.; et al. Global trend in overweight and obesity and its association with cardiovascular disease incidence. *Circ. J.* **2014**, *78*, 2807–2818. [CrossRef] [PubMed]
82. Saito, K.; Yokoyama, T.; Yoshida, H.; Kim, H.; Shimada, H.; Yoshida, Y.; Iwasa, H.; Shimizu, Y.; Kondo, Y.; Handa, S.; et al. A significant relationship between plasma vitamin C concentration and physical performance among Japanese elderly women. *J. Gerontol. Ser. Boil. Sci. Med. Sci.* **2011**, *67*, 295–301. [CrossRef]

83. Cahill, L.; Corey, P.N.; El-Sohemy, A. Vitamin C deficiency in a population of young Canadian adults. *Am. J. Epidemiol.* **2009**, *170*, 464–471. [CrossRef]
84. Cahill, L.E.; El-Sohemy, A. Vitamin C transporter gene polymorphisms, dietary vitamin C and serum ascorbic acid. *J. Nutrigenet. Nutrigenom.* **2009**, *2*, 292–301. [CrossRef]
85. Langlois, M.R.; Delanghe, J.R.; De Buyzere, M.L.; Bernard, D.R.; Ouyang, J. Effect of haptoglobin on the metabolism of vitamin, C. *Am. J. Clin. Nutr.* **1997**, *66*, 606–610. [CrossRef]
86. Na, N.; Delanghe, J.R.; Taes, Y.E.; Torck, M.; Baeyens, W.R.; Ouyang, J. Serum vitamin C concentration is influenced by haptoglobin polymorphism and iron status in Chinese. *Clin. Chim. Acta* **2006**, *365*, 319–324. [CrossRef]
87. Cahill, L.E.; El-Sohemy, A. Haptoglobin genotype modifies the association between dietary vitamin C and serum ascorbic acid deficiency. *Am. J. Clin. Nutr.* **2010**, *92*, 1494–1500. [CrossRef]
88. Marangon, K.; Herbeth, B.; Lecomte, E.; Paul-Dauphin, A.; Grolier, P.; Chancerelle, Y.; Artur, Y.; Siest, G. Diet, antioxidant status, and smoking habits in French men. *Am. J. Clin. Nutr.* **1998**, *67*, 231–239. [CrossRef] [PubMed]
89. Schectman, G.; Byrd, J.C.; Gruchow, H.W. The influence of smoking on vitamin C status in adults. *Am. J. Public Health* **1989**, *79*, 158–162. [CrossRef] [PubMed]
90. Schectman, G.; Byrd, J.C.; Hoffmann, R. Ascorbic acid requirements for smokers: Analysis of a population survey. *Am. J. Clin. Nutr.* **1991**, *53*, 1466–1470. [CrossRef] [PubMed]
91. Boonpangrak, S.; Tantimongcolwat, T.; Treeratanapiboon, L.; Leelahakul, P.; Prachayasittikul, V. Lifestyle behaviors and serum vitamin C in the Thai population in Bangkok Metropolitan. *EXCLI J.* **2018**, *17*, 452–466.
92. McGregor, G.P.; Biesalski, H.K. Rationale and impact of vitamin C in clinical nutrition. *Curr. Opin. Clin. Nutr. Metab. Care* **2006**, *9*, 697–703. [CrossRef]
93. Carr, A.C.; Cook, J. Intravenous vitamin C for cancer therapy—Identifying the current gaps in our knowledge. *Front. Physiol.* **2018**, *9*, 1182. [CrossRef]
94. Carr, A.C. Vitamin C in pneumonia and sepsis. In *Vitamin C: New Biochemical and Functional Insights: Oxidative Stress and Disease*; Chen, Q., Vissers, M., Eds.; CRC Press/Taylor & Francis: Boca Raton, FL, USA, 2020; pp. 115–135.
95. Juhl, B.; Lauszus, F.F.; Lykkesfeldt, J. Is Diabetes Associated with Lower Vitamin C Status in Pregnant Women? A Prospective Study. *Int. J. Vitam. Nutr. Res.* **2016**, *86*, 184–189. [CrossRef]
96. Juhl, B.; Lauszus, F.F.; Lykkesfeldt, J. Poor Vitamin C Status Late in Pregnancy Is Associated with Increased Risk of Complications in Type 1 Diabetic Women: A Cross-Sectional Study. *Nutrients* **2017**, *9*, 186. [CrossRef]
97. Rumbold, A.; Ota, E.; Nagata, C.; Shahrook, S.; Crowther, C.A. Vitamin C Supplementation in Pregnancy. *Cochrane Database Syst. Rev.* 2015. [CrossRef]
98. Lykkesfeldt, J.; Poulsen, H.E. Is vitamin C supplementation beneficial? Lessons learned from randomised controlled trials. *Br. J. Nutr.* **2010**, *103*, 1251–1259. [CrossRef]
99. Ugwa, E. Vitamin C supplementation in pregnancy: A review of current literature. *Niger. J. Basic Clin. Sci.* **2015**, *12*, 1–5. [CrossRef]
100. Camarena, V.; Wang, G. The epigenetic role of vitamin C in health and disease. *Cell Mol. Life Sci.* **2016**, *73*, 1645–1658. [CrossRef] [PubMed]
101. DiTroia, S.P.; Percharde, M.; Guerquin, M.-J.; Wall, E.; Collignon, E.; Ebata, K.T.; Mesh, K.; Mahesula, S.; Agathocleous, M.; Laird, D.J.; et al. Maternal vitamin C regulates reprogramming of DNA methylation and germline development. *Nature* **2019**, *573*, 271–275. [CrossRef] [PubMed]
102. Michels, A.J.; Hagen, T.M.; Frei, B. Human genetic variation influences vitamin C homeostasis by altering vitamin C transport and antioxidant enzyme function. *Annu. Rev. Nutr.* **2013**, *33*, 45–70. [CrossRef] [PubMed]
103. Delanghe, J.R.; Langlois, M.R.; De Buyzere, M.L.; Torck, M.A. Vitamin C deficiency and scurvy are not only a dietary problem but are codetermined by the haptoglobin polymorphism. *Clin. Chem.* **2007**, *53*, 1397–1400. [CrossRef] [PubMed]
104. Langlois, M.R.; Delanghe, J.R. Biological and clinical significance of haptoglobin polymorphism in humans. *Clin. Chem.* **1996**, *42*, 1589–1600. [CrossRef]
105. Cahill, L.E.; Fontaine-Bisson, B.; El-Sohemy, A. Functional genetic variants of glutathione S-transferase protect against serum ascorbic acid deficiency. *Am. J. Clin. Nutr.* **2009**, *90*, 1411–1417. [CrossRef]

106. Ames, B.N.; Elson-Schwab, I.; Silver, E.A. High-dose vitamin therapy stimulates variant enzymes with decreased coenzyme binding affinity (increased K(m)). *Am. J. Clin. Nutr.* **2002**, *75*, 616–658. [CrossRef]
107. Levine, M.; Eck, P. Vitamin C: Working on the x-axis. *Am. J. Clin. Nutr.* **2009**, *90*, 1121–1123. [CrossRef]
108. Lykkesfeldt, J.; Viscovich, M.; Poulsen, H.E. Plasma malondialdehyde is induced by smoking: A study with balanced antioxidant profiles. *Br. J. Nutr.* **2004**, *92*, 203–206. [CrossRef]
109. Zondervan, K.T.; Ocke, M.C.; Smit, H.A.; Seidell, J.C. Do dietary and supplementary intakes of antioxidants differ with smoking status? *Int. J. Epidemiol.* **1996**, *25*, 70–79. [CrossRef] [PubMed]
110. Larkin, F.A.; Basiotis, P.P.; Riddick, H.A.; Sykes, K.E.; Pao, E.M. Dietary patterns of women smokers and non-smokers. *J. Am. Diet Assoc.* **1990**, *90*, 230–237. [PubMed]
111. Dietrich, M.; Block, G.; Norkus, E.P.; Hudes, M.; Traber, M.G.; Cross, C.E.; Packer, L. Smoking and exposure to environmental tobacco smoke decrease some plasma antioxidants and increase gamma-tocopherol in vivo after adjustment for dietary antioxidant intakes. *Am. J. Clin. Nutr.* **2003**, *77*, 160–166. [CrossRef] [PubMed]
112. Lykkesfeldt, J.; Christen, S.; Wallock, L.M.; Chang, H.H.; Jacob, R.A.; Ames, B.N. Ascorbate is depleted by smoking and repleted by moderate supplementation: A study in male smokers and nonsmokers with matched dietary antioxidant intakes. *Am. J. Clin. Nutr.* **2000**, *71*, 530–536. [CrossRef]
113. Kallner, A.B.; Hartmann, D.; Hornig, D.H. On the requirements of ascorbic acid in man: Steady-state turnover and body pool in smokers. *Am. J. Clin. Nutr.* **1981**, *34*, 1347–1355. [CrossRef]
114. GBD 2015 Tobacco Collaborators. Smoking prevalence and attributable disease burden in 195 countries and territories, 1990–2015: A systematic analysis from the Global Burden of Disease Study 2015. *Lancet* **2017**, *389*, 1885–1906. [CrossRef]
115. Lykkesfeldt, J.; Prieme, H.; Loft, S.; Poulsen, H.E. Effect of smoking cessation on plasma ascorbic acid concentration. *BMJ* **1996**, *313*, 91. [CrossRef]
116. Sharma, Y.; Miller, M.; Shahi, R.; Doyle, A.; Horwood, C.; Hakendorf, P.; Thompson, C. Vitamin C deficiency in Australian hospitalized patients: An observational study. *Intern. Med. J.* **2019**, *49*, 189–196. [CrossRef]
117. Gan, R.; Eintracht, S.; Hoffer, L.J. Vitamin C deficiency in a university teaching hospital. *J. Am. Coll. Nutr.* **2008**, *27*, 428–433. [CrossRef]
118. Gariballa, S.; Forster, S. Effects of acute-phase response on nutritional status and clinical outcome of hospitalized patients. *Nutrition* **2006**, *22*, 750–757. [CrossRef]
119. Fain, O.; Pariès, J.; Jacquart, B.; Le Moël, G.; Kettaneh, A.; Stirnemann, J.; Héron, C.; Sitbon, M.; Taleb, C.; Letellier, E.; et al. Hypovitaminosis C in hospitalized patients. *Eur. J. Intern. Med.* **2003**, *14*, 419–425. [PubMed]
120. Hemilä, H. Vitamin C and infections. *Nutrients* **2017**, *9*, E339. [CrossRef] [PubMed]
121. Carr, A.C.; Spencer, E.; Dixon, L.; Chambers, S.T. Patients with community acquired pneumonia exhibit depleted vitamin C status and elevated oxidative stress. *Nutrients* **2020**, *12*, 1318. [CrossRef] [PubMed]
122. Carr, A.C.; Rosengrave, P.C.; Bayer, S.; Chambers, S.; Mehrtens, J.; Shaw, G.M. Hypovitaminosis C and vitamin C deficiency in critically ill patients despite recommended enteral and parenteral intakes. *Crit. Care* **2017**, *21*, 300. [CrossRef] [PubMed]
123. Johnston, C.S.; Corte, C. People with marginal vitamin C status are at high risk of developing vitamin C deficiency. *J. Am. Diet. Assoc.* **1999**, *99*, 854–856. [PubMed]
124. Hampl, J.S.; Taylor, C.A.; Johnston, C.S. Vitamin C deficiency and depletion in the United States: The Third National Health and Nutrition Examination Survey, 1988 to 1994. *Am. J. Public Health* **2004**, *94*, 870–875. [CrossRef]
125. Institute of Medicine Panel on Dietary Antioxidants and Related Compounds. *Dietary Reference Intakes for Vitamin, C.; Vitamin, E.; Selenium, and Carotenoids*; National Academies Press: Washington, DC, USA, 2000; p. 529.
126. Loria, C.M.; Whelton, P.K.; Caulfield, L.E.; Szklo, M.; Klag, M.J. Agreement among indicators of vitamin C status. *Am. J. Epidemiol.* **1998**, *147*, 587–596. [CrossRef]
127. Washko, P.W.; Welch, R.W.; Dhariwal, K.R.; Wang, Y.; Levine, M. Ascorbic acid and dehydroascorbic acid analyses in biological samples. *Anal. Biochem.* **1992**, *204*, 1–14. [CrossRef]
128. Amaliya; Timmerman, M.F.; Abbas, F.; Loos, B.G.; Van der Weijden, G.A.; Van Winkelhoff, A.J.; Winkel, E.G.; Van der Velden, U. Java project on periodontal diseases: The relationship between vitamin C and the severity of periodontitis. *J. Clin. Periodontol.* **2007**, *34*, 299–304.

129. Amaliya, A.; Laine, M.L.; Delanghe, J.R.; Loos, B.G.; Van Wijk, A.J.; Van der Velden, U. Java project on periodontal diseases: Periodontal bone loss in relation to environmental and systemic conditions. *J. Clin. Periodontol.* **2015**, *42*, 325–332. [CrossRef]
130. Pullar, J.M.; Bayer, S.; Carr, A.C. Appropriate handling, processing and analysis of blood samples is essential to avoid oxidation of vitamin C to dehydroascorbic acid. *Antioxidants* **2018**, *7*, E29. [CrossRef] [PubMed]

nutrients

MDPI

Article

Trends in Vitamin C Consumption in the United States: 1999–2018

Mary Brauchla, Mark J. Dekker and Colin D. Rehm *

PepsiCo, 700 Anderson Hill Road, Purchase, NY 10577, USA; Mary.Brauchla@pepsico.com (M.B.); Mark.Dekker@pepsico.com (M.J.D.)
* Correspondence: Colin.Rehm@pepsico.com; Tel.: +1-(914)-253-2432

Abstract: Low intakes of fruits and vegetables have resulted in suboptimal intakes of several micronutrients, including vitamin C. This cross-sectional study used data from 84,902 children/adults (≥1 y) who completed a 24-h dietary recall as part of the United States National Health and Nutrition Examination Survey (1999–2018). Mean vitamin C intakes from foods/beverages were calculated as were trends in major food/beverage sources of vitamin C. Percentages below the Estimated Average Requirement (EAR) were estimated. Overall, mean vitamin C consumption declined by 23% (97–75 mg/d; *p*-value for trend < 0.001). 100% fruit juice was the leading source of vitamin C (25.6% of total or 21.7mg/d), but this declined by 48% (25–13 mg/d; *p*-value for trend < 0.001). Whole fruit increased among children/adolescents (+75.8%;10–17 mg/d; *p*-value for trend < 0.001), but not adults, while the vegetable contribution was generally unchanged. The proportion of the population below the EAR increased by 23.8% on a relative scale or 9 percentage points on an absolute scale (38.3–47.4%). Declines in vitamin C intake is driven largely by decreases in fruit juice coupled with modest increases in whole fruit. Due to associations between vitamin C intake and numerous health outcomes these trends warrant careful monitoring moving forward.

Keywords: vitamin C; dietary surveys; trends; descriptive studies; United States; fruit juice; fruit; vegetables

Citation: Brauchla, M.; Dekker, M.J; Rehm, C.D. Trends in Vitamin C Consumption in the United States: 1999–2018. *Nutrients* **2021**, *13*, 420. https://doi.org/10.3390/nu13020420

Academic Editor: Anitra Carr
Received: 11 January 2021
Accepted: 27 January 2021
Published: 28 January 2021

1. Introduction

Most of the United States (US) population currently fails to meet fruit and vegetables recommendations provided by the most recent Dietary Guidelines for Americans (DGAs) [1]. Recent data from the National Health and Nutrition Examination Survey (NHANES) suggests the average fruit and vegetable intake in US adults is 0.9 cup equivalents (cup eq.) and 1.5 cup eq., respectively, which is far below the recommended 1.5–2 cup eq. of fruits and 2–3 cup eq. of vegetables [2,3]. Low consumption of fruits and vegetables have led to low intake of several vitamins and minerals, including vitamin C [1,4]. Many fruits and vegetables contain high concentrations of vitamin C and commonly promoted sources in the US include red and green bell peppers, citrus fruit and juices, strawberries and kiwi [5]. Vitamin C is also added to foods and beverages to improve the nutrient profile or for other purposes, including flavoring and food preservation.

The health benefits of vitamin C are well documented: vitamin C is essential to normal function of the immune system [6–8], skin health and collagen synthesis [9], and function of the nervous system [10]. Meta-analyses of epidemiologic studies have shown that vitamin C measured via self-report and in sera is associated with a decreased risk of coronary heart disease, stroke and some cancers, as well as all-cause mortality [11] although whether these associations are directly related to vitamin C itself or driven by vitamin C as a marker of fruit and vegetable intake is less certain [12]. In order to ensure normal function of the body and to avoid vitamin C deficiency current US vitamin C recommendations are to consume 75 mg/day for women and 90 mg/day for men [13]. Severe vitamin C deficiency is rare but

if vitamin C consumption is limited or nonexistent for several weeks it can result in scurvy, a disease causing anorexia, poor wound healing, and tooth loss and gingival bleeding [14].

Despite the clear benefits of adequate vitamin C intake this nutrient has consistently been classified as underconsumed in the DGAs since 2005 [1,15,16], though not as a nutrient of public health concern. Surprisingly, there is little data documenting the most commonly consumed sources of vitamin C and how changes in intake of top sources affect intakes. A better understanding of vitamin C consumption patterns and trends could provide valuable information to promote intake in the future and monitor for any upticks in potential sub-optimal intakes. The purpose of this study was to track vitamin C intake, percentage of the population consuming amounts below the Estimated Average Requirement (EAR) and to identify top food and beverage sources of vitamin C in a large nationally-representative sample of the US population over a 20-year period.

2. Materials and Methods

2.1. Data Sources

This cross-sectional study was based on data from the 10 most recent 2-year cycles (1999–2018) of the continuous NHANES. NHANES data is a nationally representative survey that uses a complex multistage probability sample to create a representative sample of the noninstitutionalized civilian US population. NHANES is the flagship survey for assessing the diets of the US population. The response rates to the survey differ by year, but were 62.8% in 2017–2018 and 82% in 1999–2000 [17]. The National Center for Health Statistics (NCHS) obtained Institutional Review Board approval and informed consent was obtained from all subjects; the data have subsequently been made freely available for public use [18].

2.2. Dietary Recall Data

For primary analyses, dietary data came from a single 24-h dietary recall conducted in-person. A single 24-h recall for a large sample size will lead to an unbiased estimate of population-level average intakes [19]. Respondents reported the types and amounts of all foods and beverages consumed in the preceding 24 h, from midnight to midnight in an in-person dietary recall using a computerized assisted Automated Multiple Pass Method. The method probes for commonly forgotten foods and queries detailed information on the amounts of foods consumed using common reference units and examples [20]. For young children (<6 y) the parent was the primary respondent; for children 6–11 y, the child was the primary respondent, but a parent/guardian was present and could assist. For children aged ≥12 y, the child was the primary source of dietary recall, but could be assisted by an adult. Dietary recalls were conducted in either Spanish or English.

2.3. Analysis Approach

Secondary analyses examined the proportion of the population consuming vitamin C levels below pre-specified thresholds (e.g., Estimated Average Requirement). Because a single 24-h recall (or even the average of multiple recalls) cannot reliably estimate the population distribution of intakes we used the National Cancer Institute (NCI) method to estimate the proportion of the population consuming intakes below this threshold. As this method requires a subset of participants to have a second 24-h recall we used data from 2003-onwards for these analyses (1999–2002 public-use data do not include a second dietary recall). The second dietary recall is conducted over the telephone after the initial 24-h recall.

To examine trends in vitamin C intake stratified by food/beverage source (hereafter referred to as source) data were broken into five mutually exclusive groups: 100% fruit juice, whole fruit, vegetables, fruit drinks and other sources. These categories were created based on preliminary analyses examining the top sources of vitamin C in the diet. Each category was defined based on the prefix code within the Food and Nutrient Database for Dietary Studies [21]. For example, foods starting with a "7" were identified as vegetables (including vegetable juices), those starting as "611", "642" and "643" were identified as

100% fruit juice, and "9252", "9253", "9255" among others were identified as fruit drinks. Because mixed dishes may contain many of these food groups (e.g., apple pie or mixed meat dishes would be classified as "other" but would include vitamin C from apples and vegetables respectively). As such, these categories should be considered as generally representative and not a full accounting of the total contribution of fruits, vegetables or fruit juices. It was not possible to use the What We Eat in America food categories as this database is only available from 2005 onwards.

Analyses were conducted for the total population age ≥1 y who had at least one valid 24-h dietary recall as defined by NCHS staff. Per standard analysis procedures of 24-h dietary recall data, there was no additional exclusion of individuals based on extremely low or high energy intakes. Pregnant and lactating women were included in the analysis of dietary recalls.

2.4. Biochemical Indicators of Vitamin C Intake

Secondary analyses compared biochemical indicators of vitamin C measured in sera for 2003–2006 (pooled from two cycles) and 2017–2018 among individuals with valid serum vitamin C levels (age ≥ 6 y) participating in the laboratory assessment of NHANES. Pregnant women were excluded. The protocols for data collection and analyses were generally comparable across the years and included both fasted and unfasted samples [22,23]. Consistent with prior analyses, vitamin C levels were transformed using a square-root transformation and back-transformed for data presentation [24,25]. Because multiple factors can influence vitamin C measurements and these factors may have changed over time (e.g., decreasing smoking and increasing obesity) both crude and adjusted analyses were conducted [26,27]. Adjusted analyses included age (using unrestricted splines), gender, race/ethnicity, family income-to-poverty ratio (with a missing indicator), measured body mass index (BMI) and an indicator of smoking status derived from serum cotinine levels. As analyses included both children and adults, a combined BMI measure was created that grouped people into an underweight (<18.5 for adults; <5% ile for children), healthy weight (18.5–24.9; 5–84.9% ile), overweight (25–29.9; 85–94.9% ile) and obese (≥30; ≥95% ile) based on standard cut-points for adults and percentiles from the Centers for Disease Control and Prevention (CDC) growth charts for children. Current smokers were defined as individuals with a serum cotinine level ≥10 ng/mL consistent with the prior literature, with all others considered non-smokers [24]. Serum cotinine data was used instead of self-report data as consistent self-report data was not available for children/adolescents and smoking status is very strongly associated with serum vitamin C levels. Use of supplements and consumption of vitamin C in the diet was not adjusted for as they are on the causal pathway in assessing any potential trends in vitamin C levels.

2.5. Statistical Analyses

For each 2-year cycles, the survey-weighted mean and corresponding 95% confidence interval of average vitamin C intakes from sources was calculated. A survey-weighted linear regression model was then used to determine if there was a significant linear trend in intakes of vitamin C. Analyses were repeated for age/sex groups defined by the Dietary Reference Intakes (DRI) publication [28]. Analyses by source of vitamin C were conducted in a similar manner and for the overall population, children and adolescents (age 1–18 y) and adults (≥19 y) separately. The NCI method was used to estimate the proportion of the population both overall and by age/sex population sub-groups from the DRI [29]. For analyses of serum vitamin C both crude and adjusted models were implemented using survey-weighted linear regression models of the square-root transformed serum vitamin C level, with results back-transformed for interpretation. A complete case analysis approach was used for the serum vitamin C analysis (with missing indicators for BMI and poverty status). Given the large sample size and number of statistical tests being employed a two-sided α-level of 0.01 was used to determine statistical significance. Primary analyses used Stata 16.0 (College Station, TX, USA) and implementation of the NCI method was done in SAS 9.4 (SAS Institute Inc.,

Cary, NC, USA) using macros developed and publicly available on the NCI website [30]. All analyses appropriately accounted for the complex survey design of NHANES data.

3. Results

For primary analyses, the sample size was 84,902 (range across survey cycles 7284 to 9322. Overall sample characteristics across the 20-year study period are shown in Table 1, which as per design of the survey show the data are representative of the US population. The mean vitamin C levels are shown for each socio-demographic group for reference. Young children and adolescents and younger adults had the highest average intakes of vitamin C, and males consumed about 15.2% more vitamin C (though this difference is explained by energy intakes; on an energy-adjusted basis, female participants consumed more vitamin C [87.4 mg/d vs. 74.8 mg/d per 2000 kcal]). By race/ethnicity, the non-Hispanic white population consumed the least vitamin C and the Mexican-American population consumed the most, though these differences are largely attributable to differences in the underlying age distributions of the different populations. The highest income individuals consumed marginally more vitamin C than other groups, but there was no clear gradient by income.

Table 1. Population characteristics, 1999–2018.

	N	Weighted %	Mean Vitamin C, mg/d (95% CI)
Total	84,902	100.0	85 (83, 86)
Age group			
1–3	6605	4.0	88 (85, 91)
4–8	8534	6.8	81 (78, 84)
9–13	9219	6.9	75 (73, 78)
14–18	9976	7.1	81 (77, 84)
19–30	11,211	17.0	87 (84, 90)
31–50	16,322	27.6	86 (83, 89)
51–70	15,214	22.4	85 (83, 88)
≥71	7821	8.1	85 (82, 88)
Sex			
Female	43,138	51.2	79 (77, 80)
Male	41,764	48.8	91 (89, 93)
Race/ethnicity			
Non-Hispanic White	32,117	65.3	80 (78, 82)
Non-Hispanic Black	20,007	12.0	94 (92, 97)
Mexican-American	18,577	9.8	95 (91, 98)
Other Hispanic	6801	5.7	92 (89, 96)
Other race/mixed race	7400	7.1	87 (83, 90)
Family income-to-poverty ratio [a]			
<1.00 [lower income]	20,621	16.2	85 (82, 88)
1.00–1.99	20,957	20.1	80 (77, 83)
2.00–3.99	19,880	26.5	81 (79, 83)
≥4.00 [higher income]	16,625	30.2	90 (87, 93)
Missing	6819	6.9	87 (94, 91)

[a] Interpreted as ratio of family income to the federal poverty guidelines. In 2018, the federal poverty level for a family of four in the contiguous United States was $25,100.

Trends in mean vitamin C intakes over the 20-year period are shown in Table 2. On average, vitamin C levels decreased by about 2.3 mg/d for each 2-year period or by 22.6% overall (p-value for trend < 0.001). Declines were particularly dramatic among children 1–3 y (change from 1999–2000 to 2017–2018: −32.1%), 14–18y females (−39.6%), 14–18y males (−33.6%), women 51–70 y (−30.7%), and men 51–70 y (−25.7%). The only subgroups not experiencing a statistically significant decline were 9–13y males and females, 31–50 y females and older adults (≥71 y). Because other changes in the composition of the population can affect trends in dietary intakes, sensitivity analyses evaluated overall trends adjusted for age, sex, race/ethnicity and energy intakes. There was no observable difference between adjusted and unadjusted results (see Table 3).

Table 2. Trends in population mean vitamin C consumption from foods and beverages (mg/d) overall and by Dietary Reference Intake age and sex groups, 1999–2018.

		Vitamin C mg/d (95% CI)										*p*-Value for Trend
	EAR (mg)	**1999–2000**	**2001–2002**	**2003–2004**	**2005–2006**	**2007–2008**	**2009–2010**	**2011–2012**	**2013–2014**	**2015–2016**	**2017–2018**	
Total population (age ≥ 1 y)	-	97 (90, 105)	94 (87, 100)	88 (83, 94)	86 (83, 89)	84 (77, 91)	86 (84, 89)	83 (77, 90)	78 (75, 80)	77 (72, 81)	75 (71, 80)	<0.001
Age/sex groups												
1–3	22	109 (95, 123)	92 (86, 98)	105 (93, 116)	84 (75, 94)	102 (91, 112)	81 (74, 88)	85 (71, 99)	81 (75, 88)	68 (61, 76)	74 (63, 84)	<0.001
4–8	39	93 (85, 101)	80 (71, 90)	91 (83, 99)	80 (70, 91)	86 (77, 94)	79 (71, 86)	84 (76, 92)	72 (66, 79)	67 (55, 78)	79 (68, 90)	<0.001
9–13, female	56	77 (68, 87)	82 (71, 92)	84 (74, 94)	76 (66, 86)	67 (53, 81)	73 (62, 85)	69 (58, 80)	64 (57, 72)	64 (59, 68)	80 (62, 98)	0.08
9–13, male	63	81 (68, 94)	81 (69, 92)	77 (67, 87)	73 (63, 83)	87 (73, 101)	68 (55, 81)	80 (70, 90)	80 (69, 92)	73 (61, 84)	70 (56, 83)	0.21
14–18, female	60	91 (84, 99)	78 (63, 92)	77 (68, 86)	74 (64, 84)	77 (65, 89)	73 (62, 85)	59 (54, 64)	65 (54, 76)	66 (54, 79)	55 (46, 63)	<0.001
14–18, male	75	107 (89, 125)	97 (84, 110)	106 (80, 132)	98 (87, 108)	79 (69, 90)	98 (83, 114)	96 (58, 135)	79 (66, 92)	70 (60, 80)	71 (54, 88)	<0.001
19–30, female	60	87 (76, 97)	88 (77, 98)	82 (70, 94)	78 (71, 85)	80 (66, 95)	80 (72, 89)	80 (69, 92)	71 (66, 75)	75 (65, 85)	71 (62, 80)	0.002
19–30, male	75	100 (84, 116)	123 (93, 154)	94 (81, 108)	106 (90, 121)	96 (82, 111)	101 (86, 116)	96 (78, 114)	87 (75, 100)	87 (75, 99)	71 (61, 81)	<0.001
31–50, female	60	87 (72, 102)	82 (69, 96)	85 (73, 96)	76 (63, 89)	75 (63, 87)	80 (71, 88)	76 (68, 84)	74 (66, 82)	73 (64, 82)	74 (67, 82)	0.028
31–50, male	75	109 (96, 122)	107 (89, 125)	99 (84, 115)	95 (87, 103)	95 (83, 108)	97 (85, 108)	86 (73, 99)	76 (70, 82)	84 (74, 95)	85 (72, 98)	<0.001
51–70, female	60	101 (89, 112)	94 (87, 101)	78 (68, 87)	78 (71, 85)	82 (72, 91)	91 (78, 105)	76 (69, 82)	75 (69, 80)	74 (66, 82)	70 (62, 77)	<0.001
51–70, male	75	109 (96, 122)	101 (92, 110)	87 (80, 94)	98 (89, 108)	85 (75, 95)	91 (84, 98)	99 (83, 114)	83 (75, 90)	83 (73, 93)	81 (72, 90)	<0.001
≥71, female	60	94 (85, 103)	85 (75, 94)	83 (76, 89)	81 (75, 88)	74 (68, 80)	78 (72, 85)	81 (71, 92)	85 (78, 92)	76 (64, 87)	75 (63, 87)	0.031

Table 2. *Cont.*

	Vitamin C mg/d (95% CI)										*p*-Value for Trend
	EAR (mg)	**1999–2000**	**2001–2002**	**2003–2004**	**2005–2006**	**2007–2008**	**2009–2010**	**2011–2012**	**2013–2014**	**2015–2016**	**2017–2018**	
≥71, male	75	112 (99, 125)	90 (82, 99)	87 (79, 96)	98 (84, 112)	86 (78, 95)	89 (75, 103)	83 (69, 96)	97 (85, 109)	88 (74, 101)	85 (71, 99)	0.06

EAR refers to Estimated Average Requirement.

Table 3. Sensitivity analyses for overall trend adjusted for covariates.

	Vitamin C mg/d (95% CI)										*p*-Value for Trend
	1999–2000	**2001–2002**	**2003–2004**	**2005–2006**	**2007–2008**	**2009–2010**	**2011–2012**	**2013–2014**	**2015–2016**	**2017–2018**	
Unadjusted	97 (90, 105)	94 (87, 100)	88 (83, 94)	86 (83, 89)	84 (77, 91)	86 (84, 89)	83 (77, 90)	78 (75, 80)	77 (72, 81)	75 (71, 80)	<0.001
Age-adjusted	97 (90, 105)	94 (88, 100)	88 (83, 94)	86 (83, 90)	84 (77, 91)	86 (84, 89)	83 (77, 90)	78 (75, 80)	76 (72, 81)	75 (71, 79)	<0.001
Age/sex-adjusted	97 (90, 105)	94 (88, 100)	88 (83, 94)	86 (83, 90)	84 (77, 92)	86 (84, 89)	83 (77, 90)	77 (75, 80)	76 (72, 81)	75 (71, 80)	<0.001
Age/sex/race adjusted	98 (91, 105)	94 (89, 100)	89 (84, 94)	87 (83, 90)	85 (78, 91)	86 (84, 89)	83 (77, 89)	77 (75, 80)	76 (71, 80)	74 (70, 79)	<0.001
Energy-adjusted	94 (87, 101)	90 (84, 96)	84 (78, 89)	83 (79, 86)	83 (76, 90)	85 (82, 87)	80 (74, 87)	76 (74, 78)	76 (71, 80)	73 (69, 77)	<0.001
Fully-adjusted	94 (88, 101)	91 (85, 96)	84 (80, 89)	83 (79, 87)	83 (77, 89)	85 (82, 87)	79 (73, 86)	75 (73, 78)	74 (70, 79)	72 (67, 76)	<0.001

Age defined as DRI age groups, race/ethnicity as five groups (non-Hispanic white, non-Hispanic black, Mexican-American, other Hispanic and other/mixed race). Energy parameterized as 2000 kcal. Estimated as adjusted marginal means.

Trends in sources of vitamin C are provided in Figure 1. Overall (Figure 1A), the decline in vitamin C intakes appears to be driven by decreases in vitamin C from 100% fruit juice and fruit drinks. In 1999–2000, 100% fruit juice was the second leading source of vitamin C (just below fruit), but in 2017–2018, it was the fourth leading source of vitamin C, declining by nearly half (−48.4%). At the same time, vitamin C from vegetables was generally unchanged and vitamin C from whole fruit increased by about 25.8%. The contribution of fruit drinks to vitamin C intakes also declined dramatically by more than half (−50.7%). Vitamin C from other sources did not materially change. Some differences in trends by sources were observed when data were stratified by age. Among children/adolescents (Figure 1B), the contribution of whole fruit to vitamin C intakes increased, whereas no increase was observed for adults (Figure 1C). While the contribution of juice declined in both groups, the decline was more pronounced among adults. For children/adolescents there was a modest decline in vitamin C from vegetables and some suggestion of the same for adults, but not statistically significant.

3.1. EAR Results

For the overall population, the proportion of the population below the EAR increased from 38.3% to 47.4% from 2003–2004 to 2017–2018, a 24% relative increase (Figure 2A). Figure 2B shows the proportion of the population below the EAR by age/sex group in 2003–2004 compared to 2017–2018. The most marked increase in the proportion below the EAR was among the youngest (1–3 y), females 14–18 y, males 14–18 y, males 19–30 y, males 31–50 y and older males. The only sub-group with a decrease in the proportion below the EAR were children 4–8 y.

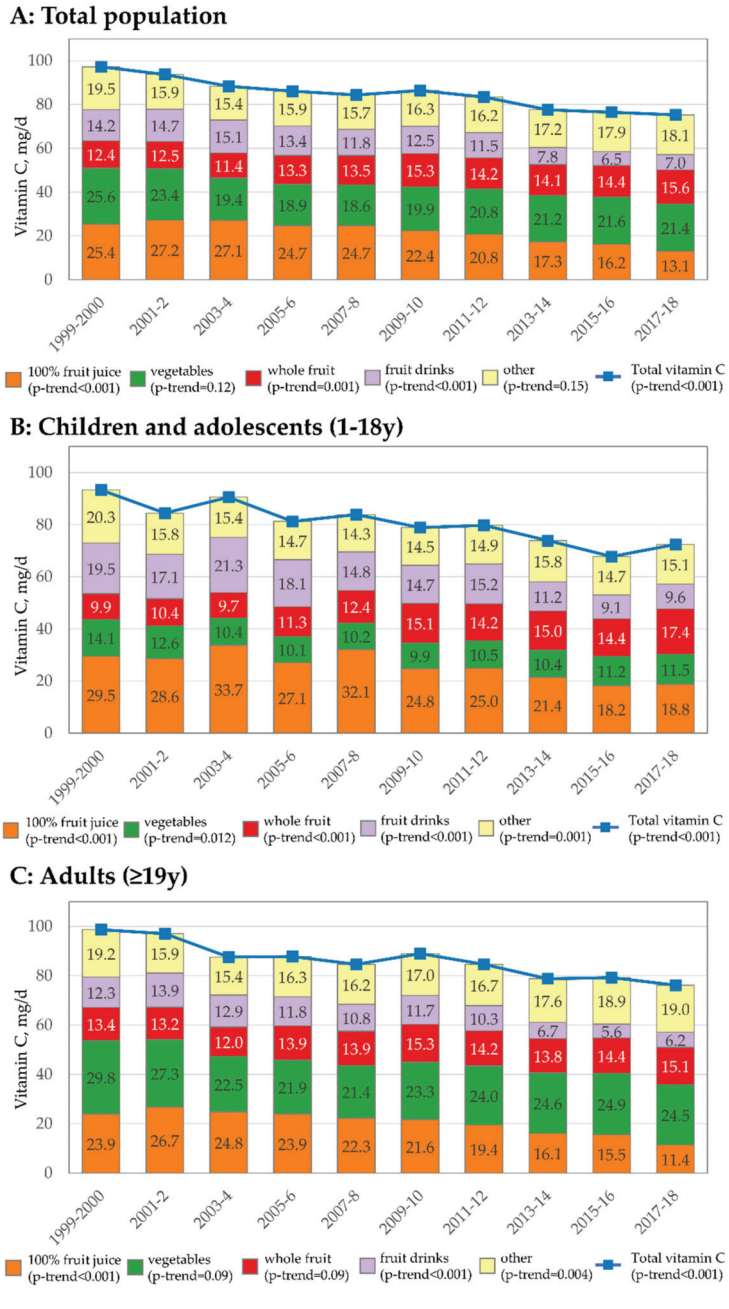


Figure 1. Trend in vitamin C intakes overall and by specific food/beverage source overall (**A**), among children/adolescents 1–18 y (**B**), and adults ≥ 19 y (**C**), 1999–2018.

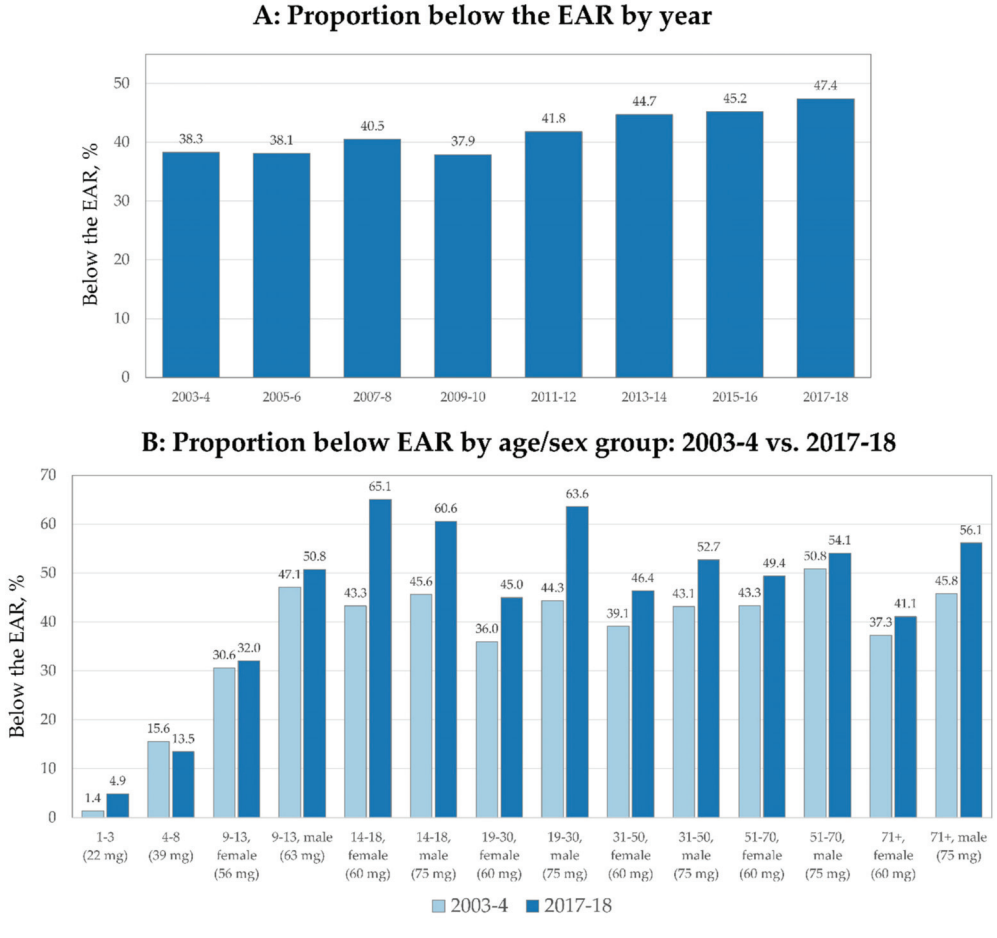


Figure 2. Trends in proportion of population below the Estimated Average Requirement (EAR) overall (**A**) and by age/sex group (**B**).

3.2. Trends Based on Biomarkers of Vitamin C

A total of 20,675 individuals age $\geq$ 6 y (6696 in 2017–2018 and 13,979 in 2003–2006) were included in the analysis of serum vitamin C levels. The results of the serum vitamin C analysis diet are shown in Figure 3. Overall, there was a non-significant decline in serum vitamin C levels in fully-adjusted analyses of approximately 4.8% from 2003–2006 to 2017–2018. There was little suggestion of a difference in serum vitamin C levels for children and adolescents. For adults, there was some evidence of a decrease (declined by approximately 5.5%), but like the overall analysis, this difference was not statistically significant.

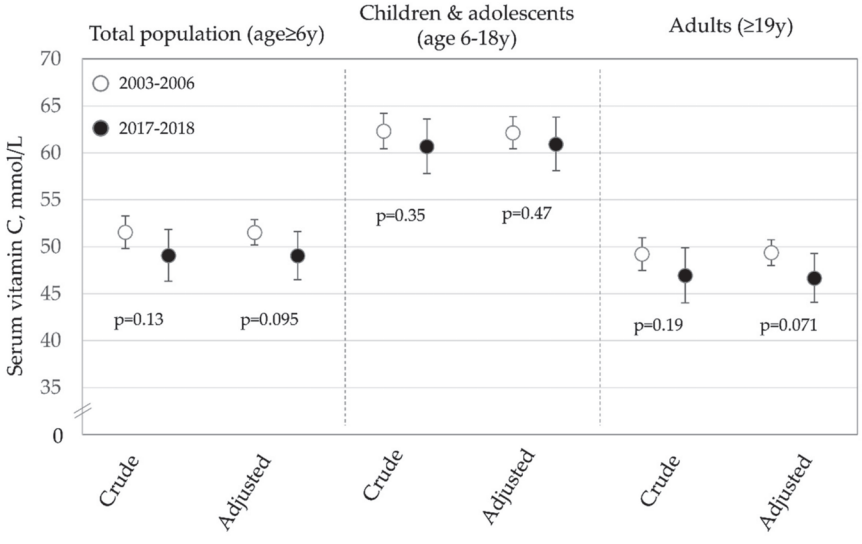


Figure 3. Mean serum vitamin C concentrations in 2003–2006 compared to 2017–2018 overall, among children/adolescents and adults (Adjusted model includes covariates for age group, race/ethnicity, sex, BMI, family income-to-poverty ratio and serum cotinine levels. Overall sample size is 20,675, 6821 for children/adolescents and 13,854 for adults).

4. Discussion

In this large nationally-representative survey of more than 84,000 participants with high-quality dietary data collected over a 20-year period we observed a 22.6% decrease in vitamin C intakes. This decrease was observed amongst all but a limited number of age/sex groups and appears to be driven by reduced contribution of vitamin C from 100% fruit juice and no marked change in vitamin C from vegetables. Vitamin C from whole fruits increased for children/adolescents, but not adults. From 2003–2004 to 2017–2018, the proportion of individuals with intakes below the EAR increased from 38.3% to 47.4%. Analyses of serum vitamin C observed a non-significant decrease in vitamin C levels, particularly for adults, but the proportional decline was less than was observed for diet.

While far from optimal, prior population-based studies have shown that the US-diet has modestly improved over the past 15–20 years. Notable changes include increased whole grain, fiber, polyunsaturated fat and whole fruit consumption and decreased consumption of added sugars and solid fats [31]. Fiber (+16.6% for adults; +25.3% for children/adolescents) and calcium (+20.1% for adults; +21.2% for children), two nutrients of public health concern, increased among adults and children from 1999–2012 and 1999–2016, respectively [31,32]. Vitamin D intakes have not meaningfully changed among adults [31] and no major trend was observed for potassium. The current finding of fairly-dramatic decreases in vitamin C intakes in the context of improving diet quality overall represents somewhat of a paradox.

One possible explanation for these findings is the reduced consumption of 100% fruit juices. Our results show that in 1999–2000, about 26% of vitamin C consumed in the diet came from 100% fruit juice, declining to 17% by 2017–2018. This finding is consistent with a large body of research demonstrating decreased consumption of 100% fruit juice [31–35]. To date, studies documenting changes in fruit juice consumption have not formally assessed the potential impact on nutrient intakes. While many professional organizations recommend whole fruit over 100% fruit juice, juice is a beverage and is more practically replaced by another beverage such as water, juice drinks and other beverages or none-at-all rather than a direct exchange for whole fruit [36,37]. Outside of milk (where

consumption has dropped precipitously), 100% fruit juices tend to have a very high nutrient density compared to other beverages [38].

While not directly quantifiable, much of these consumption changes may be due to messaging around the sugar content of juice and purported links between sugar and adverse health outcomes [39] which have also translated to recomendations from the American Academy of Pediatrics suggesting limiting juice intake in children to certain age-specific levels [40]. The observed change in whole fruit intake is less consistent on the population level with children increasing intake far more than adults. This difference may be due to national efforts such as revamping the school lunch program and initiatives like "Let's Move" and "Play 60" ad campaigns that are specifically targeted towards children. Curiously, trends in whole fruit consumption show increasing intakes among US adults, at least through 2011–2012. This suggests that the increase in whole fruit was not for vitamin C rich fruits (e.g., citrus, berries or tropical fruits) and may have been for fruits with relatively little vitamin C (e.g., apples or bananas), the latter of which are amongst the most commonly consumed fruits in the US [41]. This observation highlights the need to conduct detailed analyses that not only examine trends in dietary intakes by broad food sources but using as fine-resolution as possible to truly understand the dietary changes being made in the population.

While the changes in vitamin C sources from juice and whole fruit were compelling it is worth noting that we observed no marked change in vitamin C contributions from vegetables, which is consistent with relatively flat consumption of vegetables over this study period. For children, we observed a modest trend towards less vitamin C from vegetables and for adults there was some suggestion of such a trend (p-value for trend = 0.09). The generally flat intake of vegetables represents an important public health challenge as vegetables have numerous health benefits and are nutrient dense and energy sparse. Multiple campaigns and policies have been implemented to encourage vegetable consumption but there remain numerous barriers to intake. The high cost of whole fruit and vegetables, particularly fresh versions, is one often-cited barrier [42,43]. Descriptive studies show that on a per-calorie basis these are amongst the costliest foods [44] and qualitative studies show that consumers, especially those with lower-incomes, are highly attuned to their cost [45]. Beyond their direct cost, availability challenges are noteworthy, along with concerns regarding taste, quality, and time of preparation/convenience [46–48]. In part due to these barriers, individuals with higher socioeconomic status (SES) routinely consume greater amounts of whole fruits and vegetables than individuals of lower SES [45,49]. However, all groups consume too few fruits and vegetables, so simply eliminating disparities in intake will not be adequate to bring population-level intakes in-line with recommendations. On the other-hand numerous relatively inexpensive 100% fruit juices, are widely available. United States Department of Agriculture estimates show a median price per fruit cup equivalent of $0.32 for frozen juices from concentrate and $0.42 for ready-to-drink juices, compared to $0.72, $1.18, $0.94 and $0.91 for fresh, frozen, canned and dried fruits respectively [50]. In terms of food loss, the percent of consumer losses for fresh fruits and vegetables stands at 33 and 30% respectively, compared to 13% for processed fruit (a broader category which includes juice) [51]. Future studies should be conducted to unpack the specific mechanisms informing dietary choices for vitamin C dense foods and beverages.

This analysis focused on vitamin C from foods and beverages and deliberately did not include vitamin C from supplements. This choice was made because numerous professional organizations, including the American Heart Association and American Institute for Cancer Research encourage individuals to meet their micronutrient recommendations through diet alone [52,53]. The Academy of Nutrition and Dietetics and the US Preventive Services Task Force do not go quite this far but both discourage supplement use for the purposes of disease prevention [54,55] which stands in contrast to the most commonly reported reason reported for supplement use, which is to "improve/maintain health" [56]. An analysis of NHANES data from 1999–2012 among adults, showed the 30-day use of

vitamin C supplements, including from multi-vitamins decreased significantly from 42% to 36% [57], but more recent data are not available. Published data on trends among children/adolescents are not available but data from 2017–2018 NHANES show only 3.0% were taking a single ingredient vitamin C supplement and 23.8% were taking some type of multivitamin/multimineral supplement, which usually contain vitamin C [58].

It is important to consider the declines in vitamin C intakes observed here in the context of dietary adequacy. The issue with declining trends in vitamin C intakes from foods/beverages is not a concern of acute deficiency (e.g., scurvy), but rather on its potential impact on the immune system, skin health and collagen synthesis, nervous system and potentially on reactive oxidative species [59]. On a comparative basis the proportion below the EAR for vitamin C is higher than some nutrients of public health concern. Cowan et al. show in sex-stratified analyses that the proportion of men with vitamin C intakes less than the EAR (50.8% in 2011–2014) is higher than calcium (26%) and potassium (35%), but much lower than vitamin D (91.5%) [60]. For women, the proportion below the EAR was higher for vitamin C than for potassium, though more women were below the EAR for calcium [60]. Another reason to monitor this decline is the connection between vitamin C intake and iron bioavailability. In the latest Dietary Guidelines for Americans, iron was identified as an under-consumed nutrient for certain groups [1] and vitamin C is known to increase the bioavailability of non-heme iron [61]. As consumer interest in a "flexitarian" or "plant-based" diet has increased along with decreasing trends of some animal foods this is an issue meriting careful assessment [62,63]. Given these concerns, the decrease in mean vitamin C intakes and more importantly, the increase in the proportion of individuals with intakes below the EAR is of potential concern and merits careful monitoring looking forward.

Study limitations include the use of self-reported dietary data which is subject to systematic and random errors [64]. Despite this, professionally collected 24-h recalls are considered amongst the strongest dietary assessment instruments and form the cornerstone of dietary surveillance in the US and elsewhere. Further, a single 24-h recall can provide an unbiased estimate of population average intakes. Our analysis of biochemical indicators of diet may be subject to residual confounding, via covariate measurement error or inefficient parameterization of variables, though we did adjust for variables previously shown to be strongly related to serum vitamin C and both BMI and serum cotinine are objective measures not subject to self-report bias, though they may be subject to other forms of measurement error. Strengths of the study included a very large sample size, which allowed us to examine statistically reliable data by two-year cycle and present data stratified by age and sex. A further strength of the study is looking at the specific sources of changes in vitamin C, which allow us to start identifying the potential reasons for trends rather than simply describing them.

5. Conclusions

In this large population-based study we observed a dramatic decline in vitamin C intakes that appears to be driven by declines in fruit juice consumption. A modest increase in vitamin C from whole fruit could not make up the gap due to declines in 100% fruit juice consumption. While at the population-level there is little risk of severe vitamin C deficiency, given observed relationships and associations between vitamin C intake and numerous health outcomes these trends are of potential concern. The proportion of individuals with vitamin C intakes less than the EAR appear to be greater than for other nutrients of public health concern including calcium (for men) and potassium. There is tremendous need to increase fruit and vegetable consumption in the United States and multiple approaches will likely be necessary to impart these changes. Looking forward, vitamin C intakes should be continually monitored and identifying specific sub-populations at greater risk for inadequate vitamin C intakes could be identified. Close examinations of dietary trends for specific dietary constituents of interest beyond the overall topline trend can help us understand why the diet may be changing and identify potential strategies to improve diet.

Author Contributions: Author contributions were as follows: M.B., M.J.D. and C.D.R. designed the research; all analyses were completed by C.D.R.; manuscript was written by C.D.R. and M.B. All authors have read and agreed to the published version of the manuscript.

Funding: This study was funded by PepsiCo Inc.

Institutional Review Board Statement: Not applicable as this study used fully de-identified publicly-available data which is not considered human subject research. The National Center for Health Statistics received approval from their Ethics Review Board.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study by the National Center for Health Statistics.

Data Availability Statement: All data for this project are publicly available on the National Center for Health Statistics website, available at: https://wwwn.cdc.gov/nchs/nhanes/Default.aspx.

Acknowledgments: The views expressed in this manuscript are those of the authors and do not necessarily reflect the position or policy of PepsiCo Inc.

Conflicts of Interest: All authors are currently employed by PepsiCo, Inc. The authors have no additional conflicts of interest to report.

References

1. U.S. Department of Health and Human Services; U.S. Department of Agriculture. *2015–2020 Dietary Guidelines for Americans*; U.S. Department of Health and Human Services; U.S. Department of Agriculture: Washington, DC, USA, 2015.
2. Hoy, M.K.; Clemens, J.C.; Martin, C.L.; Moshfegh, A.J. Fruit and Vegetable Consumption of US Adults by Level of Variety, What We Eat in America, NHANES 2013–2016. *Curr. Dev. Nutr.* **2020**, *4*, nzaa014. [CrossRef] [PubMed]
3. U.S. Department of Agriculture. Choose My Plate. Available online: https://www.choosemyplate.gov/ (accessed on 30 September 2020).
4. Taylor, C.A.; Hampl, J.S.; Johnston, C.S. Low Intakes of Vegetables and Fruits, Especially Citrus Fruits, Lead to Inadequate Vitamin C Intakes among Adults. *Eur. J. Clin. Nutr.* **2000**, *54*, 573–578. [CrossRef] [PubMed]
5. National Institutes of Health. Vitamin C Fact Sheet for Consumers. Available online: https://ods.od.nih.gov/pdf/factsheets/VitaminC-Consumer.pdf (accessed on 4 October 2020).
6. Granger, M.; Eck, P. Dietary Vitamin C in Human Health. *Adv. Food Nutr. Res.* **2018**, *83*, 281–310. [PubMed]
7. Gombart, A.F.; Pierre, A.; Maggini, S. A Review of Micronutrients and the Immune System-Working in Harmony to Reduce the Risk of Infection. *Nutrients* **2020**, *12*, 236. [CrossRef] [PubMed]
8. Mousavi, S.; Bereswill, S.; Heimesaat, M.M. Immunomodulatory and Antimicrobial Effects of Vitamin C. *Eur. J. Microbiol. Immunol. (Bp)* **2019**, *9*, 73–79. [CrossRef]
9. Pullar, J.M.; Carr, A.C.; Vissers, M.C.M. The Roles of Vitamin C in Skin Health. *Nutrients.* **2017**, *9*, 866. [CrossRef]
10. Figueroa-Méndez, R.; Rivas-Arancibia, S. Vitamin C in Health and Disease: Its Role in the Metabolism of Cells and Redox State in the Brain. *Front. Physiol.* **2015**, *6*, 397. [CrossRef]
11. Aune, D.; Keum, N.; Giovannucci, E.; Fadnes, L.T.; Boffetta, P.; Greenwood, D.C.; Tonstad, S.; Vatten, L.J.; Riboli, E.; Norat, T. Dietary Intake and Blood Concentrations of Antioxidants and the Risk of Cardiovascular Disease, Total Cancer, and All-Cause Mortality: A Systematic Review and Dose-Response Meta-Analysis of Prospective Studies. *Am. J. Clin. Nutr.* **2018**, *108*, 1069–1091. [CrossRef]
12. Woodside, J.V.; Draper, J.; Lloyd, A.; McKinley, M.C. Use of Biomarkers to Assess Fruit and Vegetable Intake. *Proc. Nutr. Soc.* **2017**, *76*, 308–315. [CrossRef]
13. Institute of Medicine. *Dietary Reference Intakes for Vitamin C, Vitamin E, Selenium, and Caroteinoids*; Food and Nutrition Board; National Academy Press: Washington, DC, USA, 2000.
14. Maxfield, L.; Crane, J.S. *Vitamin C Deficiency*; StatPearls Publishing: Treasure Island, FL, USA, 2020.
15. U.S. Department of Agriculture; U.S. Department of Health and Human Services. *Dietary Guidelines for Americans*; Government Printing Office: Washington, DC, USA, 2005.
16. United States Department of Agriculture; United States Department of Health and Human Services. Dietary Guidelines for Americans. Available online: https://health.gov/sites/default/files/2020-01/DietaryGuidelines2010.pdf (accessed on 15 October 2020).
17. Centers for Disease Control and Prevention. NHANES Response Rates and Population Totals. Available online: https://wwwn.cdc.gov/nchs/nhanes/responserates.aspx#response-rates (accessed on 13 December 2020).
18. Centers for Disease Control and Prevention. National Health and Nutrition Examination Survey. Available online: https://www.cdc.gov/nchs/nhanes/index.htm (accessed on 22 October 2020).
19. Freedman, L.S.; Commins, J.M.; Willett, W.; Tinker, L.F.; Spiegelman, D.; Rhodes, D.; Potischman, N.; Neuhouser, M.L.; Moshfegh, A.J.; Kipnis, V.; et al. Evaluation of the 24-Hour Recall as a Reference Instrument for Calibrating Other Self-Report

Instruments in Nutritional Cohort Studies: Evidence from the Validation Studies Pooling Project. *Am. J. Epidemiol.* **2017**, *186*, 73–82. [CrossRef]
20. Centers for Disease Control and Prevention. National Health and Nutrition Examination Survey (NHANES), MEC in-Person Dietary Interviewers Procedures Manual. Available online: https://wwwn.cdc.gov/nchs/data/nhanes/2017-2018/manuals/2017_MEC_In-Person_Dietary_Interviewers_Manual.pdf (accessed on 5 October 2020).
21. United States Department of Agriculture. Food and Nutrient Database for Dietary Studies. Available online: https://www.ars.usda.gov/northeast-area/beltsville-md-bhnrc/beltsville-human-nutrition-research-center/food-surveys-research-group/docs/fndds-download-databases/ (accessed on 13 December 2020).
22. Centers for Disease Control and Prevention. National Health and Nutrition Examination Survey 2005–2006 Data Documentation, Codebook and Frequencies Vitamin C. Available online: https://wwwn.cdc.gov/nchs/nhanes/2005-2006/VIC_D.htm (accessed on 13 December 2020).
23. Centers for Disease Control and Prevention. National Health and Nutrition Examination Survey 2017–2018 Data Documentation, Codebook, and Frequencies. Available online: https://wwwn.cdc.gov/Nchs/Nhanes/2017-2018/VIC_J.htm (accessed on 13 December 2020).
24. Schleicher, R.L.; Carroll, M.D.; Ford, E.S.; Lacher, D.A. Serum Vitamin C and the Prevalence of Vitamin C Deficiency in the United States: 2003–2004 National Health and Nutrition Examination Survey (NHANES). *Am. J. Clin. Nutr.* **2009**, *90*, 1252–1263. [CrossRef] [PubMed]
25. Kim, S. Overview of Cotinine Cutoff Values for Smoking Status Classification. *Int. J. Environ. Res. Public Health* **2016**, *13*, 1236. [CrossRef] [PubMed]
26. Drope, J.; Liber, A.C.; Cahn, Z.; Stoklosa, M.; Kennedy, R.; Douglas, C.E.; Henson, R.; Drope, J. Who's Still Smoking? Disparities in Adult Cigarette Smoking Prevalence in the United States. *CA Cancer J. Clin.* **2018**, *68*, 106–115. [CrossRef] [PubMed]
27. Hales, C.M.; Fryar, C.D.; Carroll, M.D.; Freedman, D.S.; Aoki, Y.; Ogden, C.L. Prevalence of Obesity and Severe Obesity among Adults: United States, 2017–2018. *NCHS Data Brief* **2020**, *360*, 1–8.
28. Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium. *Dietary Reference Intakes for Calcium and Vitamin D*; Taylor, C.L., Ross, A.C., Yaktine, A.L., Eds.; National Academies Press: Washington, DC, USA, 2011.
29. Tooze, J.A.; Midthune, D.; Dodd, K.W.; Freedman, L.S.; Krebs-Smith, S.M.; Subar, A.F.; Guenther, P.M.; Carroll, R.J.; Kipnis, V. A New Statistical Method for Estimating the Usual Intake of Episodically Consumed Foods with Application to Their Distribution. *J. Am. Diet. Assoc.* **2006**, *106*, 1575–1587. [CrossRef] [PubMed]
30. National Cancer Institute, Division of Cancer Control & Population. Usual Dietary Intakes: SAS Macros for the NCI Method. Available online: https://epi.grants.cancer.gov/diet/usualintakes/macros.html (accessed on 13 December 2020).
31. Rehm, C.D.; Peñalvo, J.L.; Afshin, A.; Mozaffarian, D. Dietary Intake among Us Adults, 1999–2012. *Jama* **2016**, *315*, 2542–2553. [CrossRef] [PubMed]
32. Liu, J.; Rehm, C.D.; Onopa, J.; Mozaffarian, D. Trends in Diet Quality among Youth in the United States, 1999–2016. *Jama* **2020**, *323*, 1161–1174. [CrossRef]
33. Miller, G.; Merlo, C.; Demissie, Z.; Sliwa, S.; Park, S. Trends in Beverage Consumption among High School Students—United States, 2007–2015. *MMWR Morb. Mortal. Wkly. Rep.* **2017**, *66*, 112–116. [CrossRef]
34. Miles, G.; Siega-Riz, A.M. Trends in Food and Beverage Consumption among Infants and Toddlers: 2005–2012. *Pediatrics* **2017**, *139*, e20163290. [CrossRef]
35. Nicklas, T.A.; O'Neil, C.E.; Saab, R.; Fulgoni, V.L., III. Trends in Orange Juice Consumption and Nutrient Adequacy in Children 2003–2016. *Lifesci. Global* **2020**, *9*, 100–114. [CrossRef]
36. Mintel. Juice and Juice Drinks: Including Impact of Covid-19—Us—June. 2020. Available online: https://store.mintel.com/report/juice-and-juice-drinks-incl-impact-of-covid-19-us-june-2020 (accessed on 8 December 2020).
37. Neves, M.F.; Trombin, V.G.; Marques, V.N.; Martinez, L.F. Global Orange Juice Market: A 16-Year Summary and Opportunities for Creating Value. *Trop. Plant Pathol.* **2020**, *45*, 166–174. [CrossRef]
38. Smedman, A.; Lindmark-Månsson, H.; Drewnowski, A.; Edman, A.K.M. Nutrient Density of Beverages in Relation to Climate Impact. *Food Nutr. Res.* **2010**, *54*, 5170. [CrossRef] [PubMed]
39. Guasch-Ferré, M.; Hu, F.B. Are Fruit Juices Just as Unhealthy as Sugar-Sweetened Beverages? *Jama Netw. Open* **2019**, *3*, e193109. [CrossRef] [PubMed]
40. Heyman, M.B.; Abrams, S.A. Fruit Juice in Infants, Children, and Adolescents: Current Recommendations. *Pediatrics* **2017**, *139*, e20170967. [CrossRef] [PubMed]
41. Brauchla, M.; Rehm, C.D.; Fulgoni, V.L., III. Cost-Effective Options for Increasing Consumption of Underconsumed Food Groups and Nutrients in the US. *Public Health Nutr.* **2021**, in press.
42. Miller, V.; Yusuf, S.; Chow, C.K.; Dehghan, M.; Corsi, D.J.; Lock, K.; Popkin, B.; Rangarajan, S.; Khatib, R.; Lear, S.A.; et al. Availability, Affordability, and Consumption of Fruits and Vegetables in 18 Countries across Income Levels: Findings from the Prospective Urban Rural Epidemiology (Pure) Study. *Lancet Glob. Health* **2016**, *4*, e695–e703. [CrossRef]
43. Mook, K.; Laraia, B.A.; Oddo, V.M.; Jones-Smith, J.C. Food Security Status and Barriers to Fruit and Vegetable Consumption in Two Economically Deprived Communities of Oakland, California, 2013–2014. *Prev. Chronic Dis.* **2016**, *13*, E21. [CrossRef]
44. Rehm, C.D.; Monsivais, P.; Drewnowski, A. Relation between Diet Cost and Healthy Eating Index 2010 Scores among Adults in the United States 2007–2010. *Prev. Med.* **2015**, *73*, 70–75. [CrossRef]

45. Jack, D.; Neckerman, K.; Schwartz-Soicher, O.; Lovasi, G.S.; Quinn, J.; Richards, C.; Bader, M.; Weiss, C.; Konty, K.; Arno, P.; et al. Socio-Economic Status, Neighbourhood Food Environments and Consumption of Fruits and Vegetables in New York City. *Public Health Nutr.* **2013**, *16*, 1197–1205. [CrossRef]
46. Blitstein, J.L.; Snider, J.; Evans, W.D. Perceptions of the Food Shopping Environment Are Associated with Greater Consumption of Fruits and Vegetables. *Public Health Nutr.* **2012**, *15*, 1124–1129. [CrossRef]
47. Haynes-Maslow, L.; Parsons, S.E.; Wheeler, S.B.; Leone, L.A. A Qualitative Study of Perceived Barriers to Fruit and Vegetable Consumption among Low-Income Populations, North Carolina, 2011. *Prev. Chronic. Dis.* **2013**, *10*, E34. [CrossRef] [PubMed]
48. Lucan, S.C.; Barg, F.K.; Long, J.A. Promoters and Barriers to Fruit, Vegetable, and Fast-Food Consumption among Urban, Low-Income African Americans—A Qualitative Approach. *Am. J. Public Health* **2010**, *100*, 631–635. [CrossRef] [PubMed]
49. Lee-Kwan, S.H.; Moore, L.V.; Blanck, H.M.; Harris, D.M.; Galuska, D. Disparities in State-Specific Adult Fruit and Vegetable Consumption—United States, 2015. *MMWR Morb. Mortal. Wkly Rep.* **2017**, *66*, 1241–1247. [CrossRef] [PubMed]
50. U.S. Department of Agriculture. Interactive Chart: Most and Least Expensive Fruits and Vegetables. Available online: https://www.ers.usda.gov/data-products/fruit-and-vegetable-prices/interactive-chart-most-and-least-expensive-fruits-and-vegetables/ (accessed on 13 December 2020).
51. United States Department of Agriculture. *The Estimated Amount, Value, and Calories of Postharvest Food Losses at the Retail and Consumer Levels in the United States*; Wells, H.F., Buzby, J.C., Hyman, F., Eds.; United States Department of Agriculture: Washington, DC, USA, 2014. Available online: https://www.ers.usda.gov/webdocs/publications/43833/43680_eib121.pdf?v=9667.8 (accessed on 10 December 2020).
52. American Heart Association. Vitamin Supplements: Hype or Help for Healthy Eating. Available online: https://www.heart.org/en/healthy-living/healthy-eating/eat-smart/nutrition-basics/vitamin-supplements-hype-or-help-for-healthy-eating (accessed on 8 December 2020).
53. American Institute for Cancer Research. Supplements and Nutrients, Accept No Substitutes. Available online: https://www.aicr.org/cancer-prevention/healthy-eating/supplements-nutrients/ (accessed on 13 December 2020).
54. Marra, M.V.; Boyar, A.P. Position of the American Dietetic Association: Nutrient Supplementation. *J. Am. Diet. Assoc.* **2009**, *85*, 257S–264S.
55. US Preventive Services Task Force. Vitamin Supplementation to Prevent Cancer and CVD: Preventive Medication. Available online: https://www.uspreventiveservicestaskforce.org/uspstf/document/RecommendationStatementFinal/vitamin-supplementation-to-prevent-cancer-and-cvd-counseling (accessed on 23 November 2020).
56. Bailey, R.L.; Gahche, J.J.; Miller, P.E.; Thomas, P.R.; Dwyer, J.T. Why US Adults Use Dietary Supplements. *JAMA Intern. Med.* **2013**, *173*, 355–361. [CrossRef]
57. Kantor, E.D.; Rehm, C.D.; Du, M.; White, E.; Giovannucci, E.L. Trends in Dietary Supplement Use among US Adults from 1999–2012. *Jama* **2016**, *316*, 1464–1474. [CrossRef]
58. Stierman, B.; Mishra, S.; Gahche, J.J.; Potischman, N.; Hales, C.M. Dietary Supplement Use in Children and Adolescents Aged $\leq$ 19 Years—United States, 2017–2018. *MMWR Morb. Mortal. Wkly. Rep.* **2020**, *69*, 1557–1562. [CrossRef]
59. Lobo, V.; Patil, A.; Phatak, A.; Chandra, N. Free Radicals, Antioxidants and Functional Foods: Impact on Human Health. *Pharmacogn. Rev.* **2010**, *4*, 118–126. [CrossRef]
60. Cowan, A.E.; Jun, S.; Tooze, J.A.; Eicher-Miller, H.A.; Dodd, K.W.; Gahche, J.J.; Guenther, P.M.; Dwyer, J.T.; Potischman, N.; Bhadra, A.; et al. Total Usual Micronutrient Intakes Compared to the Dietary Reference Intakes among U.S. Adults by Food Security Status. *Nutrients* **2019**, *12*, 38. [CrossRef]
61. Teucher, B.; Olivares, M.; Cori, H. Enhancers of Iron Absorption: Ascorbic Acid and Other Organic Acids. *Int. J. Vitam. Nutr. Res.* **2004**, *74*, 403–419. [CrossRef]
62. Aschemann-Witzel, J.; Gantriis, R.F.; Fraga, P.; Perez-Cueto, F.J. Plant-Based Food and Protein Trend from a Business Perspective: Markets, Consumers, and the Challenges and Opportunities in the Future. *Crit. Rev. Food Sci. Nutr.* **2020**, 1–10. [CrossRef] [PubMed]
63. Shan, Z.; Rehm, C.D.; Rogers, G.; Ruan, M.; Wang, D.D.; Hu, F.B.; Mozaffarian, D.; Zhang, F.F.; Bhupathiraju, S.N. Trends in Dietary Carbohydrate, Protein, and Fat Intake and Diet Quality among Us Adults, 1999–2016. *JAMA* **2019**, *322*, 1178–1187. [CrossRef] [PubMed]
64. Willett, W. *Nutritional Epidemiology*, 3rd ed.; New York Oxford University Press: New York, NY, USA, 2012.

nutrients

MDPI

Communication

Estimation of Vitamin C Intake Requirements Based on Body Weight: Implications for Obesity

Anitra C. Carr [1,*], Gladys Block [2] and Jens Lykkesfeldt [3]

[1] Nutrition in Medicine Research Group, Department of Pathology and Biomedical Science, University of Otago, Christchurch 8011, New Zealand
[2] School of Public Health, University of California, Berkeley, Berkeley, CA 94720-7360, USA; gblock@berkeley.edu
[3] Faculty of Health & Medical Sciences, University of Copenhagen, DK-1870 Frederiksberg C, Denmark; jopl@sund.ku.dk
* Correspondence: anitra.carr@otago.ac.nz; Tel.: +643-364-0649

Abstract: Higher body weight is known to negatively impact plasma vitamin C status. However, despite this well-documented inverse association, recommendations on daily vitamin C intakes by health authorities worldwide do not include particular reference values for people of higher body weight. This suggests that people of higher body weight and people with obesity may be receiving insufficient vitamin C in spite of ingesting the amounts recommended by their health authorities. The current preliminary investigation sought to estimate how much additional vitamin C people with higher body weights would need to consume in order to attain a comparable vitamin C status to that of a lower weight person consuming an average Western vitamin C intake. Data from two published vitamin C dose-concentration studies were used to generate the relationship: a detailed pharmacokinetic study with seven healthy non-smoking men and a multiple depletion–repletion study with 68 healthy non-smoking men of varying body weights. Our estimates suggest that an additional intake of 10 mg vitamin C/day is required for every 10 kg increase in body weight to attain a comparable plasma concentration to a 60 kg individual with a vitamin C intake of ~110 mg/day, which is the daily intake recommended by the European Food Safety Authority (EFSA). Thus, individuals weighing e.g., 80 and 90 kg will need to consume ~130 and 140 mg vitamin C/day, respectively. People with obesity will likely need even higher vitamin C intakes. As poor vitamin C status is associated with increased risk of several chronic diseases including cardiovascular disease, these findings may have important public health implications. As such, dose-finding studies are required to determine optimal vitamin C intakes for overweight and obese people.

Keywords: vitamin C; ascorbate; obesity; body weight; vitamin C intake; plasma ascorbate concentrations; vitamin C requirements; dietary vitamin C

Citation: Carr, A.C.; Block, G.; Lykkesfeldt, J. Estimation of Vitamin C Intake Requirements Based on Body Weight: Implications for Obesity. *Nutrients* **2022**, *14*, 1460. https://doi.org/10.3390/nu14071460

Academic Editor: Carol Johnston

Received: 13 March 2022
Accepted: 28 March 2022
Published: 31 March 2022

1. Introduction

Higher body weight is known to negatively impact vitamin C status, with an inverse association observed between body weight and plasma ascorbate concentrations reported in numerous epidemiological studies [1,2]. Several factors may contribute to this relationship. Firstly, increased body weight correlates with an increased volume of distribution, which from a purely volumetric perspective results in a lower plasma concentration from the same intake with increased body weight [3,4]. In fact, this relationship constitutes the basis of the lower recommended daily intake for women compared to men in some countries [5]. Secondly, obesity per se has been associated with adipocyte dysregulation and systemic low-grade inflammation presumably resulting in increased oxidative stress and potentially an increased turnover of vitamin C [6,7]. The volumetric dilution premise is supported by a well-conducted depletion–repletion study carried out by Block et al. [8]. This study showed decreasing vitamin C status with increasing body weight in participants receiving

an identical dietary intake of vitamin C. We have also previously shown that the lack of response to low dose vitamin C supplementation in participants with hypovitaminosis C was partly due to body weight [9].

Current global vitamin C intake recommendations for adults are typically based on a 'one size fits all' paradigm [5]. However, with increasing obesity rates becoming a global pandemic [10], the negative impact of higher body weight on vitamin C status should be taken into consideration in public health recommendations. Furthermore, obesity is a major risk factor for severe cardiometabolic diseases such as type 2 diabetes and cardiovascular disease [11]. These diseases have also been associated with depleted vitamin C status, although body weight per se also appears to be a major predictor of vitamin C status in these conditions [12,13].

Vitamin C has numerous important functions in vivo through acting as a cofactor for a family of biosynthetic and regulatory metalloenzymes, including hydroxylating enzymes involved in gene transcription regulation and epigenetic modifications [14,15]. Of note, optimal kinetics of these vitamin C-dependent enzymes are dependent on adequate in vivo vitamin C concentrations [15,16]. Thus, the decreased vitamin C concentrations observed in people with overweight and obesity may compromise in vivo enzyme functions and thereby increase the already elevated disease risk even further.

In this preliminary investigation, dose-concentration data from two vitamin C supplementation studies, the Block depletion-repletion study [8], and the Levine pharmacokinetic study [17], were used to estimate how much additional vitamin C intake may be required for people of higher body weight to attain a vitamin C status comparable to that of a lower weight individual ingesting an adequate amount of vitamin C.

2. Materials and Methods

The analyses were based on vitamin C dose-concentration data from two studies: Block et al. [8] and Levine et al. [17]. The Block study [8] comprised 68 healthy men who underwent two vitamin C depletion and repletion phases. During the one-month depletion phases they consumed a controlled diet providing 9 mg/day of vitamin C, and each one-month repletion phase provided an additional 108 mg/day of vitamin C (i.e., a total of 117 mg vitamin C/day), from either supplement, fruit, or vegetables. The source of vitamin C did not affect the plasma ascorbate concentrations obtained [18]. The participants did not smoke, drink alcohol, or take aspirin during the study, and consumed only the food and drink provided. Participants were weighed weekly, and caloric intake was adjusted to prevent weight gain or loss during the study. Data from the second repletion phase was used for the current investigation as it was the better fit to the Levine pharmacokinetic data [17]. The final regression model for the second repletion phase was as follows: $AUC = -168.5 + 7.6 \times AA + 95.7 \times dose$, $R^2 = 0.55$, where AUC is the area under the plasma ascorbate repletion curve (for phase 2); AA is the plasma ascorbic acid values at the beginning of the second repletion cycle; and dose is the vitamin C intake per kg of body weight [8].

The Levine study [17] comprised seven healthy men who underwent a depletion and dose response repletion study over 4–6 months in a hospital metabolic unit. Exclusion criteria were cigarette smoking, use of regular medications, history of kidney stones, glucose-6-phosphate dehydrogenase deficiency, diabetes mellitus, bleeding disorders, or family history of iron overload/hemochromatosis. During depletion they consumed a diet of <5 mg/d vitamin C. Steady-state plasma concentrations were determined at sequential vitamin C doses of 30, 60, 100, 200, 400, 1000, and 2500 mg/day. For the current investigation GraphPad Prism 9 (GraphPad Software, San Diego, CA, USA) was used to fit a variable slope (four parameters) curve to the steady-state plasma ascorbate concentrations as a function of dose. This curve displayed sigmoidal kinetics: $AA = 7.4 + (dose^{4.3}) \times 61.5/(dose^{4.3} + 109 \times 10^6)$, where AA is the ascorbic acid concentration and dose is the vitamin C intake. This was used to estimate dose (mg/day) based on

predicted plasma concentrations (μmol/L), as well as estimate plasma ascorbate concentrations (μmol/L) based on dose (mg/day).

3. Results

The Block study [8] quantified the effect of body weight on plasma ascorbate concentrations attained on a given dose of vitamin C in 68 healthy non-smoking men of mean age 41 years (range 30–59 years) and mean weight 81 kg (range 59–101 kg). The mean plasma ascorbate concentration attained following depletion was 13 ± 2 μmol/L, and repletion with 117 mg/day vitamin C provided a mean plasma concentration of 49 ± 14 μmol/L. Based on the regression model derived from the plasma ascorbate values following supplementation, the predicted plasma ascorbate concentrations that would be attained after various time intervals in men with various body weights (e.g., 59, 68, 82, and 91 kg) were calculated (Figure 1) [8]. These resulted in predicted plasma ascorbate concentrations of 59, 54, 46, and 41 μmol/L after 4 weeks of supplementation. The difference in plasma ascorbate concentrations between the lowest and highest body weight categories was 18 μmol/L.

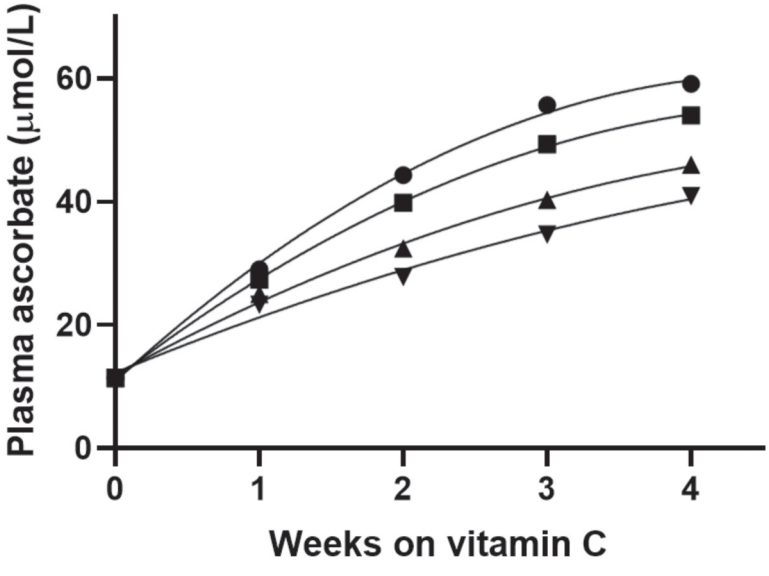


Figure 1. Predicted plasma ascorbate concentration after repletion with vitamin C (117 mg/day) by body weight. The body weights used in the model were: 59 kg (130 lbs; ●), 68 kg (150 lbs; ■), 82 kg (180 lbs; ▲), 91 kg (200 lbs; ▼). The model assumed that all participants started at the same depleted level. Data were obtained from Table 3 in reference [8].

For the current investigation, the sigmoidal vitamin C dose-concentration curve from the Levine pharmacokinetic study [17] was used to estimate the vitamin C dose required to obtain the predicted plasma ascorbate concentrations derived in the Block study (Figure 2). This resulted in estimated doses of 109, 97, 84, and 77 mg/day (to obtain the predicted plasma concentrations of 59, 54, 46, and 41 μmol/L, respectively). The 117 mg/day dose administered in the Block study equated to an estimated 61 μmol/L plasma ascorbate concentration using the Levine curve, i.e., a mere 2 μmol/L more than the plasma concentration predicted in the Block study for the lowest body weight (i.e., 59 μmol/L for 59 kg person), suggesting that the predicted data for the low body weight category in the Block study was an excellent fit to the Levine dose-concentration data.

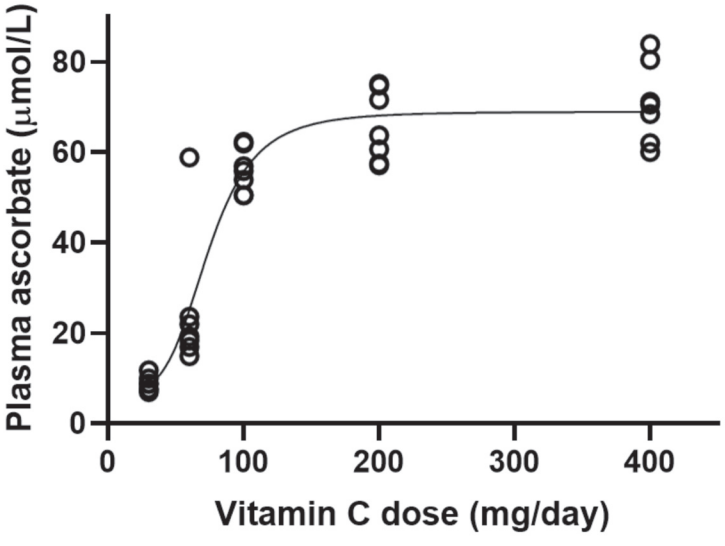


Figure 2. Steady-state plasma ascorbate concentrations in volunteers as a function of daily dose. Data were obtained from Table 1 in reference [17] and a sigmoidal curve was fitted to the data.

The difference in estimated vitamin C doses between the lowest and highest predicted plasma concentrations was 32 mg/day (Table 1). Thus, the data suggest that a 91 kg person would need to consume an extra 32 mg of vitamin C to reach the same plasma ascorbate concentration as a 59 kg person consuming 109 mg, i.e., a total of 140 mg/day. A linear relationship was observed between weight and change in plasma concentration resulting from a 117 mg/d daily intake (Figure 3A). However, when comparing the increase in dose required with increasing weight to attain a plasma concentration similar to a 59 kg individual, a non-linear relationship was observed, particularly when extrapolated to higher body weights (Figure 3B).

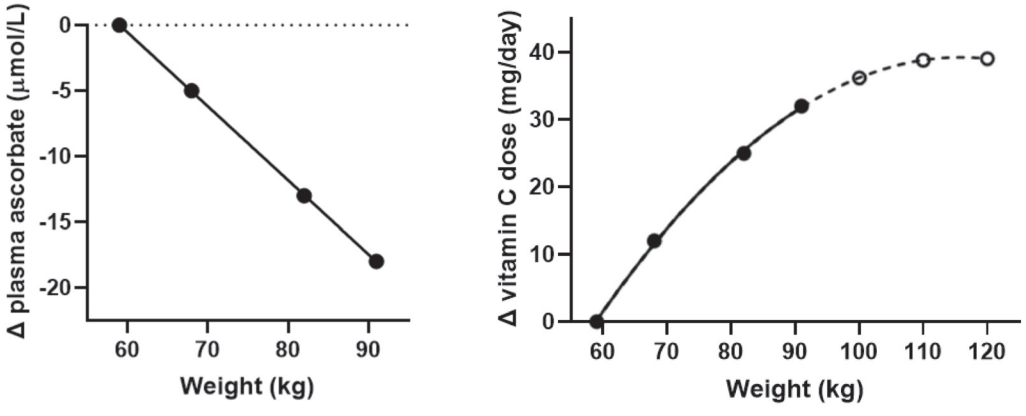


Figure 3. Decreased steady-state ascorbate concentrations with weight (**A**) and increased vitamin C requirements with weight (**B**). The dashed line represents extrapolation of the weight data points.

Table 1. Estimated vitamin C doses based on body weight categories.

	Body Weight Category, kg (lbs)			
	59 (130)	**68 (150)**	**82 (180)**	**91 (200)**
Dose (mg)/kg body weight [1]	2.0	1.7	1.4	1.3
Predicted plasma ascorbate (μmol/L) [2]	59	54	46	41
△ Plasma ascorbate (μmol/L)	0	−5	−13	−18
Estimated vitamin C dose (mg) [3]	109	97	84	77
△ Vitamin C dose (mg)	0	12	25	32
Total vitamin C dose	109	121	134	141

[1] Supplemented with 117 mg/day [8]; [2] After 4 weeks of vitamin C supplementation [8]; [3] Vitamin C dose was estimated using the curve from Figure 2 above.

4. Discussion

This investigation, drawing on previously published data [8], shows a considerable body weight dependency when translating the same daily intake of vitamin C to the corresponding steady-state plasma ascorbate concentration. Our estimates suggest that within a weight range of approximately 60–90 kg, an additional 10 mg/day vitamin C intake is required for each 10 kg increase in body weight to attain a comparable plasma concentration to that of a 60 kg individual ingesting 110 mg vitamin C/day. This corresponds to 140 mg/day for a 90 kg person, which was the highest body weight data available for this study. However, extrapolating the relationship between body weight and dose beyond 90 kg suggested that the 10 mg/10 kg increase in requirement tapers off when approaching 120 kg body weight. This is probably due to fat mass increasing with increased obesity. Consequently, the volume of distribution for ascorbate, being a highly water-soluble molecule, is unlikely to increase proportionally to weight gain over the full range of obesity. Thus, correcting exclusively for volumetric dilution of vitamin C may not be appropriate for weights over about 110 kg.

However, the estimated 40 mg/day additional requirement for those weighing 110+ kg (Figure 3B) is likely an underestimate because people with obesity will likely need even higher vitamin C intakes for other reasons. As obesity and fat mass increase, so does low grade inflammation mediated by adipokines and oxidative stress [7]. As inflammation and oxidative stress conditions are generally recognised to increase vitamin C turnover, a further increase in vitamin C intake would be required to compensate for obesity. However, the present study does not allow for conclusions regarding vitamin C requirements in people with obesity, as the weight range was too limited.

The present findings have implications for public health policy and nutrition recommendations. A vitamin C intake of 110 mg/day is the recommended dietary intake of the European Food Safety Authority (EFSA) and other European countries (e.g., France, Germany, Austria, and Switzerland) [5]. This intake is to attain a plasma ascorbate concentration of 50 μmol/L, which is considered adequate [19]. Of note, even with a slightly higher intake (i.e., 117 mg/day), participants in the Block study with body weights >80 kg had predicted plasma vitamin C concentrations <50 μmol/L [8]. Many countries globally have recommended vitamin C intakes well below 110 mg/day, some as low as 40 mg/day [5], which provide inadequate plasma concentrations [17], even in people of lower body weight, such as 59 kg [20].

According to the World Health Organisation, more than 1.9 billion (39%) of adults were overweight in 2016 and of these over 650 million (13%) were obese [21]. In some regions of the world, these percentages are considerably higher, e.g., 61% in the Americas, 55% in Europe, and 46% in the Eastern Mediterranean [11]. Furthermore, obesity prevalence of >50% is particularly prevalent in specific genders in a number of low-middle income countries [22]. In 2005, the average global body weight was estimated to be 62 kg, however, the average weight in North America was estimated to be 81 kg [23]. Currently, the average body weight in the USA for adult males is 91 kg [24], with 74% of US adults

being overweight, and of these 42% are obese [25]. Therefore, future studies are needed to determine the vitamin C requirements for overweight and obese people in particular.

Other than body weight, the most significant contributors to attainable vitamin C status among non-smokers appear to be baseline vitamin C status and how long the person has been depleted [8]. Such depletion is common in low-income populations and is also a result of chronic diseases or frequent acute illnesses, such as respiratory infections [1]. Smoking is another important factor that results in depletion of vitamin C status due to elevated oxidative stress [5]. These factors will negatively impact not only the attainable vitamin C status, but also the length of time to reach steady-state levels. Of note, in the Block study [8], overweight non-smoking participants did not appear to have reached steady-state values even after one month of daily supplementation with 117 mg/day. Other potential confounders include differences in total body water (which also correlates with total body fat and fat-free mass) between men and women and also between people of different ethnicities [26], suggesting there could be ethnic differences in vitamin C requirements based on volumetric effects. As such, even within the same weight category, there is likely to be significant variation in vitamin C requirements due to the presence of various confounders.

There are several limitations of this preliminary investigation, including the assumption that body weight was the sole contributor to the differences in plasma vitamin C status in the Block study data, as some of the factors mentioned above may have had an impact on the predicted plasma concentrations in the Block study, although the investigators did rule out any major impact from environmental smoke exposure, physical and emotional stress, minor illnesses, and physical activity [8]. As mentioned above, the higher weight participants may not have reached steady-state values, however, the participants also had not been depleted for as long as those in the Levine pharmacokinetic study [17]. The Levine study did not report the ages and weights of the participants included in the study, although they were stated to be healthy non-smoking men [17]. Therefore, the equivalency of the data from these participants to the low body weight category data generated in the Block study is uncertain. Finally, the data used for this investigation is derived from only one dose of vitamin C (117 mg/day). Although this is a highly relevant dose and close to the daily intake recommended by EFSA, other doses may offer different vitamin C requirement profiles based on where the resultant plasma ascorbate concentrations fall on the sigmoidal Levine dose-concentration curve. As such, further dose-finding studies are required to determine optimal vitamin C intakes in overweight and obese people.

5. Conclusions

The role of body weight as a significant contributor to attained steady-state plasma ascorbate concentrations should be considered in making public health recommendations. Because recommended vitamin C intakes expressed as mg/day will result in lower plasma ascorbate concentrations in higher weight relative to lower weight people, more than 20 years ago Block et al. [8] indicated that dietary recommendations should be based on a dose per kg body weight, or in terms of a desirable plasma concentration. Alternatively, targeted recommendations are needed for people with higher body weight. It is noteworthy that authorities of many countries have already issued special increased recommended daily intakes of vitamin C for subpopulations with increased risk of vitamin C deficiency such as smokers and pregnant and lactating women [5].

The results from the current investigation suggest that to attain comparable plasma concentrations to a 60 kg person consuming an average vitamin C intake of 110 mg/day, i.e., the daily intake recommended by most European countries, an additional 10 mg/day of vitamin C would be required for each additional 10 kg increase in body weight. As such, higher weight people (e.g., 80–90 kg) will require estimated vitamin C intakes of 130–140 mg/day. Because vitamin C uptake is comparable between synthetic and food sources of the vitamin [27], any advice around obtaining vitamin C through food sources

should include recommendations for higher numbers of servings of fruits and vegetables for overweight and obese people.

Author Contributions: Conceptualization, A.C.C.; methodology, A.C.C.; formal analysis, A.C.C.; writing—original draft preparation, A.C.C.; writing—review and editing, G.B. and J.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: All available data is reported in the paper.

Acknowledgments: The author would like to thank Helen Lunt for her helpful feedback.

Conflicts of Interest: The author declares no conflict of interest.

References

1. Carr, A.C.; Rowe, S. Factors Affecting Vitamin C Status and Prevalence of Deficiency: A Global Health Perspective. *Nutrients* **2020**, *12*, 1963. [CrossRef] [PubMed]
2. Tveden-Nyborg, P.; Lykkesfeldt, J. Does vitamin C deficiency increase lifestyle-associated vascular disease progression? Evidence based on experimental and clinical studies. *Antioxid. Redox Signal.* **2013**, *19*, 2084–2104. [CrossRef] [PubMed]
3. Jungert, A.; Neuhäuser-Berthold, M. The lower vitamin C plasma concentrations in elderly men compared with elderly women can partly be attributed to a volumetric dilution effect due to differences in fat-free mass. *Br. J. Nutr.* **2015**, *113*, 859–864. [CrossRef] [PubMed]
4. Rowe, S.; Carr, A.C. Global vitamin C status and prevalence of deficiency: A cause for concern? *Nutrients* **2020**, *12*, 2008. [CrossRef]
5. Carr, A.C.; Lykkesfeldt, J. Discrepancies in global vitamin C recommendations: A review of RDA criteria and underlying health perspectives. *Crit. Rev. Food Sci. Nutr.* **2021**, *61*, 742–755. [CrossRef]
6. Fernández-Sánchez, A.; Madrigal-Santillán, E.; Bautista, M.; Esquivel-Soto, J.; Morales-González, Á.; Esquivel-Chirino, C.; Durante-Montiel, I.; Sánchez-Rivera, G.; Valadez-Vega, C.; Morales-González, J.A. Inflammation, Oxidative Stress, and Obesity. *Int. J. Mol. Sci.* **2011**, *12*, 3117–3132. [CrossRef]
7. Codoñer-Franch, P.; Valls-Bellés, V.; Arilla-Codoñer, A.; Alonso-Iglesias, E. Oxidant mechanisms in childhood obesity: The link between inflammation and oxidative stress. *Transl. Res.* **2011**, *158*, 369–384. [CrossRef]
8. Block, G.; Mangels, A.R.; Patterson, B.H.; Levander, O.A.; Norkus, E.P.; Taylor, P.R. Body weight and prior depletion affect plasma ascorbate levels attained on identical vitamin C intake: A controlled-diet study. *J. Am. Coll. Nutr.* **1999**, *18*, 628–637. [CrossRef]
9. Carr, A.C.; Pullar, J.M.; Bozonet, S.M.; Vissers, M.C.M. Marginal Ascorbate Status (Hypovitaminosis C) Results in an Attenuated Response to Vitamin C Supplementation. *Nutrients* **2016**, *8*, 341. [CrossRef]
10. GBD 2015 Obesity Collaborators. Health effects of overweight and obesity in 195 countries over 25 Years. *N. Engl. J. Med.* **2017**, *377*, 13–27. [CrossRef]
11. Yatsuya, H.; Li, Y.; Hilawe, E.H.; Ota, A.; Wang, C.; Chiang, C.; Zhang, Y.; Uemura, M.; Osako, A.; Ozaki, Y.; et al. Global Trend in Overweight and Obesity and Its Association with Cardiovascular Disease Incidence. *Circ. J.* **2014**, *78*, 2807–2818. [CrossRef] [PubMed]
12. Wilson, R.; Willis, J.; Gearry, R.; Skidmore, P.; Fleming, E.; Frampton, C.; Carr, A. Inadequate Vitamin C Status in Prediabetes and Type 2 Diabetes Mellitus: Associations with Glycaemic Control, Obesity, and Smoking. *Nutrients* **2017**, *9*, 997. [CrossRef] [PubMed]
13. Carr, A.C.; Spencer, E.; Heenan, H.; Lunt, H.; Vollebregt, M.; Prickett, T.C.R. Vitamin C Status in People with Types 1 and 2 Diabetes Mellitus and Varying Degrees of Renal Dysfunction: Relationship to Body Weight. *Antioxidants* **2022**, *11*, 245. [CrossRef] [PubMed]
14. Camarena, V.; Wang, G. The epigenetic role of vitamin C in health and disease. *Cell. Mol. Life Sci.* **2016**, *73*, 1645–1658. [CrossRef] [PubMed]
15. Kuiper, C.; Vissers, M.C. Ascorbate as a co-factor for Fe- and 2-oxoglutarate dependent dioxygenases: Physiological activity in tumor growth and progression. *Front. Oncol.* **2014**, *4*, 359. [CrossRef]
16. Lykkesfeldt, J.; Tveden-Nyborg, P. The Pharmacokinetics of Vitamin C. *Nutrients* **2019**, *11*, 2412. [CrossRef]
17. Levine, M.; Conry-Cantilena, C.; Wang, Y.; Welch, R.W.; Washko, P.W.; Dhariwal, K.R.; Park, J.B.; Lazarev, A.; Graumlich, J.F.; King, J.; et al. Vitamin C pharmacokinetics in healthy volunteers: Evidence for a recommended dietary allowance. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 3704–3709. [CrossRef]
18. Mangels, A.R.; Block, G.; Frey, C.M.; Patterson, B.H.; Taylor, P.R.; Norkus, E.P.; Levander, O.A. The bioavailability to humans of ascorbic acid from oranges, orange juice and cooked broccoli is similar to that of synthetic ascorbic acid. *J. Nutr.* **1993**, *123*, 1054–1061.

19. European Food Safety Authority Panel on Dietetic Products Nutrition and Allergies. Scientific opinion on dietary reference values for vitamin C. *EFSA J.* **2013**, *11*, 3418.
20. Levine, M.; Wang, Y.; Padayatty, S.J.; Morrow, J. A new recommended dietary allowance of vitamin C for healthy young women. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 9842–9846. [CrossRef]
21. World Health Organization. Obesity and Overweight. 2021. Available online: https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight (accessed on 5 February 2022).
22. Ng, M.; Fleming, T.; Robinson, M.; Thomson, B.; Graetz, N.; Margono, C.; Mullany, E.C.; Biryukov, S.; Abbafati, C.; Abera, S.F.; et al. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: A systematic analysis for the Global Burden of Disease Study 2013. *Lancet* **2014**, *384*, 766–781. [CrossRef]
23. Walpole, S.C.; Prieto-Merino, D.; Edwards, P.; Cleland, J.; Stevens, G.; Roberts, I. The weight of nations: An estimation of adult human biomass. *BMC Public Health* **2012**, *12*, 439. [CrossRef] [PubMed]
24. Fryar, C.D.; Carroll, M.D.; Gu, Q.; Afful, J.; Ogden, C.L. Anthropometric reference data for children and adults: United States, 2015–2018. *Vital Health Stat.* **2021**, *3*, 1–44.
25. Fryar, C.D.; Carroll, M.D.; Afful, J. Prevalence of Overweight, Obesity, and Severe Obesity among Adults Aged 20 and over: United States, 1960–1962 through 2017–2018; *NCHS Health E-Stats*. 2020. Available online: https://www.cdc.gov/nchs/data/hestat/obesity-adult-17-18/obesity-adult.htm (accessed on 5 February 2022).
26. Chumlea, W.C.; Guo, S.S.; Zeller, C.M.; Reo, N.V.; Baumgartner, R.N.; Garry, P.J.; Wang, J.; Pierson, R.N.; Heymsfield, S.B.; Siervogel, R.M. Total body water reference values and prediction equations for adults. *Kidney Int.* **2001**, *59*, 2250–2258. [CrossRef] [PubMed]
27. Carr, A.C.; Vissers, M.C.M. Synthetic or Food-Derived Vitamin C—Are They Equally Bioavailable? *Nutrients* **2013**, *5*, 4284–4304. [CrossRef] [PubMed]

nutrients

MDPI

Article

Prevalence of Hypovitaminosis C and its Relationship with Frailty in Older Hospitalised Patients: A Cross-Sectional Study

Yogesh Sharma [1,2,*], Alexandra Popescu [3], Chris Horwood [4], Paul Hakendorf [4] and Campbell Thompson [5]

[1] College of Medicine & Public Health, Flinders University, Adelaide 5042, Australia
[2] Department of General Medicine, Division of Medicine, Cardiac & Critical Care, Flinders Medical Centre, Adelaide 5042, Australia
[3] Department of Geriatrics & Rehabilitation, Flinders Medical Centre, Adelaide 5042, Australia; alexandra.popescu@sa.gov.au
[4] Department of Clinical Epidemiology, Flinders Medical Centre, Adelaide 5042, Australia; chris.horwood@sa.gov.au (C.H.); paul.hakendorf@sa.gov.au (P.H.)
[5] Discipline of Medicine, The University of Adelaide, Adelaide 5005, Australia; campbell.thompson@adelaide.edu.au
* Correspondence: yogesh.sharma@flinders.edu.au; Tel.: +61-8-82046694

Abstract: Frailty is common in older hospitalised patients and may be associated with micronutrient malnutrition. Only limited studies have explored the relationship between frailty and vitamin C deficiency. This study investigated the prevalence of vitamin C deficiency and its association with frailty severity in patients ≥75 years admitted under a geriatric unit. Patients (n = 160) with a mean age of 84.4 ± 6.4 years were recruited and underwent frailty assessment by use of the Edmonton Frail Scale (EFS). Patients with an EFS score <10 were classified as non-frail/vulnerable/mildly frail and those with ≥10 as moderate–severely frail. Patients with vitamin C levels between 11–28 µmol/L were classified as vitamin C depleted while those with levels <11 µmol/L were classified as vitamin C deficient. A multivariate logistic regression model determined the relationship between vitamin C deficiency and frailty severity after adjustment for various co-variates. Fifty-seven (35.6%) patients were vitamin C depleted, while 42 (26.3%) had vitamin C deficiency. Vitamin C levels were significantly lower among patients who were moderate–severely frail when compared to those who were non-frail/vulnerable/mildly frail ($p < 0.05$). After adjusted analysis, vitamin C deficiency was 4.3-fold more likely to be associated with moderate–severe frailty (aOR 4.30, 95% CI 1.33-13.86, $p = 0.015$). Vitamin C deficiency is common and is associated with a greater severity of frailty in older hospitalised patients.

Keywords: frailty; vitamin C deficiency; elderly; hospitalisation

Citation: Sharma, Y.; Popescu, A.; Horwood, C.; Hakendorf, P.; Thompson, C. Prevalence of Hypovitaminosis C and its Relationship with Frailty in Older Hospitalised Patients: A Cross-Sectional Study. *Nutrients* **2021**, *13*, 2117. https://doi.org/10.3390/nu13062117

Academic Editors: Anitra Carr and Adrian Gombart

Received: 26 May 2021
Accepted: 17 June 2021
Published: 20 June 2021

1. Introduction

Worldwide, with an aging population, there is a global interest in aging processes and age-related diseases. Frailty represents a precise measurement of aging-related symptoms and constitutes a syndrome taking into account physical disability, low energy levels and loss of cognition [1]. Frailty is associated with poor clinical outcomes such as falls, poor health related quality of life (HRQoL), nursing home placement and death [1,2]. This syndrome has been shown to be potentially preventable and can be reversed if targeted in earlier stages [3]. The prevalence of frailty in acutely hospitalised older patients can be up to 48% [4] while as many as 84% of patients admitted to a geriatric evaluation and management (GEM) unit may be frail [3].

While protein energy malnutrition is a major risk factor for the development of frailty, the role of micronutrient deficiencies in the development of frailty is less clear [5]. Micronutrient deficiencies are far more common and often precede development of overt malnutrition [6]. Micronutrient deficiencies can potentially increase the risk of frailty

through multiple mechanisms such as an increase in oxidative stress and inflammation, the impairment of bone and muscle metabolism and a reduction in immunity [7]. Vitamin C is a powerful antioxidant and 40% of the total body pool is present in the skeletal muscles [8].

Previous studies suggest that micronutrient deficiency (especially vitamin D and vitamin B12 deficiency) may be associated with frailty, but the relationship between biochemical vitamin C deficiency and frailty is unclear [7]. To date, only limited studies [9,10] have explored the relationship between frailty and vitamin C deficiency. The aims of the current research were to determine the prevalence of vitamin C deficiency in older frail patients admitted to a GEM unit and explore the relationship between vitamin C deficiency and severity of frailty. The hypothesis for this research was that older hospitalised patients will have a high prevalence of vitamin C deficiency and low vitamin C levels will be associated with a greater severity of frailty.

Outcomes: The primary outcome for this study was to determine the prevalence of biochemical vitamin C deficiency in older patients admitted to a GEM unit, and the secondary outcome was to determine whether biochemical vitamin C deficiency is a predictor of severe frailty.

2. Materials and Methods

Patients $\geq$ 75 years who were admitted to the GEM unit of Flinders Medical Centre between May–December 2020 were recruited by convenience sampling in this research. A written informed consent was obtained from the participants, and in the case of cognitive impairment, consent was obtained from the legal guardian. A member of the research team approached the participants and provided them with a participant information sheet in addition to verbal information about the research project. The participants were given sufficient time to read and discuss their participation with their caretakers as well as the treating medical team. If the participants were agreeable, they were asked to sign a consent form.

The exclusion criteria were a lack of a valid consent, patients receiving end of life care and those on vitamin C replacement. Ethical approval for this study was granted by the Southern Adelaide Human Clinical Research Ethics Committee, and this study was registered with the Australia and New Zealand Clinical Trial Registry.

Frailty assessment was performed by use of the Edmonton Frail Scale (EFS). The EFS is a valid and reliable instrument for the identification of frailty in hospitalised patients and predicts clinical outcomes [11,12]. The EFS contains nine components and is scored out of 17. Individual components include: cognition, general health status, self-reported health, functional independence, social support, polypharmacy, mood, continence and functional performance. The component scores are summed, and the following cut-off scores are used to classify the severity of frailty: not frail (0–5), apparently vulnerable (6–7), mild frailty (8–9), moderate frailty (10–11) and severe frailty (12–17). Comorbidities were assessed by use of the Charlson comorbidity index (CCI), which is a score based on various diseases such as myocardial infarction, congestive heart failure, diabetes, renal failure, cerebrovascular disease, peripheral vascular disease, chronic lung disease, liver disease, peptic ulcer and acquired immunodeficiency syndrome (AIDS) and is a valid and reliable method of measuring comorbidity [13,14].

The physical functioning was assessed by use of the Short Physical Performance Battery (SPPB) test, which is a validated measure of lower limb function in older adults and uses tasks that mimics activities of daily living [15]. This test comprises of three subtests: standing balance, four-metre gait speed (4 m GS) and five sit-to-stand (5 STS) tests. The subtests are scored from 0–4 and summated to give a total SPPB score (ranging from 0–12) with higher scores being indicative of a better physical performance [16]. Fall risk was assessed using the Timed Up and Go (TUG) test [17]. In this test, the patient is asked to rise from a seated position, walk 3 metres, turn around and return and sit in the starting point chair while timed. Patients who are unable to complete this test in < 12 seconds are considered to have at high risk of falls [18].

Cognitive status was determined by the use of the Mini Mental State Examination (MMSE) [19] and mood was assessed by using the Geriatric Depression Scale (GDS) [20]. The GDS is a 15-item tool that has been validated for screening depressive symptoms in the older population, including acutely hospitalised medical patients [21,22]. Nutrition risk was determined by use of the Malnutrition Universal Screening Tool (MUST) [23] and the HRQoL was determined by the European Quality of Life 5-Dimension 5-Level (EQ5D-5L) questionnaire [24]. The activities of daily living (ADL) were assessed by use of the Hospital Admission Risk Profile (HARP) score [25], which predicts patients at high risk of discharge to a facility.

Fasting venous blood samples were drawn by a trained phlebotomist. The sample for vitamin C level was wrapped in an aluminium foil and immediately placed on ice for transport to a central laboratory. High performance liquid chromatography (HPLC) was used to determine vitamin C levels. HPLC has been previously validated for the rapid and specific measurement of vitamin C [26]. Plasma vitamin C levels correlate with dietary vitamin C intake and unlike leucocyte vitamin C levels, plasma vitamin C levels are not influenced by changes in the white blood cell (WBC) count and thus represent an accurate measure of vitamin C status [26,27]. According to Johnston's criteria [28], vitamin C levels $\geq$ 28 μmol/L are classified as normal, 11–27 μmol/L as vitamin C depletion and < 11 μmol/L as vitamin C deficiency. For this study, patients with vitamin C levels $\geq$ 28 μmol/L were defined as vitamin C replete and all those with levels < 28 μmol/L as hypovitaminosis C. Vitamin C levels were also divided into quintiles, and we compared clinical outcomes such as length of hospital stay (LOS), in hospital mortality and readmissions within 30 days of hospital discharge in different quintiles. In addition, the phlebotomist collected venous blood samples for determination of haemoglobin, creatinine, C-reactive protein (CRP), albumin, vitamin D and vitamin B12 levels. The technique of spectrophotometry was used to determine haemoglobin, creatinine and albumin levels, while rapid immunoassays, Roche Diagnostics (www.roche.com), determined the C-RP, vitamin D and vitamin B12 levels in the central laboratory.

Statistics

The normality of the data was assessed by visual inspection of histograms. Continuous variables were assessed by use of the Student's t-tests or rank sum tests and categorical variables by Chi squared statistics or Fisher's exact test as appropriate. A Kruskal–Wallis H test was used to compare the LOS in different quintiles of vitamin C. Patients with EFS scores $\geq$10 were classified as moderately to severely frail while those with EFS scores <10 as non-frail, vulnerable or mildly frail. We correlated vitamin C levels with EFS scores. A logistic regression analysis was used to determine whether vitamin C deficiency was associated with a greater severity of frailty after adjustment for the following co-variates: age, sex, Charlson index, smoking status, MUST score, MMSE, GDS, fruit and vegetable intake, socioeconomic status (determined by annual income), HARP score, vitamin D and vitamin B12 levels. We determined any effect modification by use of interaction terms with vitamin D and vitamin B12 in the model. We performed a sensitivity analysis and determined the bias-corrected estimates using the jackknife resampling method as suggested by Nemes et al [29]. Prediction graphs with 95% confidence intervals were plotted to determine whether vitamin C levels were associated with increasing severity of frailty using the marginsplot command in STATA. Polypharmacy was defined as being on five or more medications.

The sample size was based on a recent study [30], which suggested a high prevalence (>70%) of biochemical vitamin C deficiency in hospitalised patients. Assuming a prevalence of 70% and a precision level of 10%, the calculated sample size for this study was 140, and assuming 10% missing data, a total of 160 patients were deemed sufficient for this study. All statistical analyses were conducted using Stata version 17.0 (StataCorp, College Station, TX, USA).

3. Results

Six hundred and three patients were admitted under the GEM unit between May and December 2020, of whom, 176 patients were approached by convenience sampling for participation, and 160 patients were recruited for this study while 16 patients were excluded because of various reasons (Figure 1).

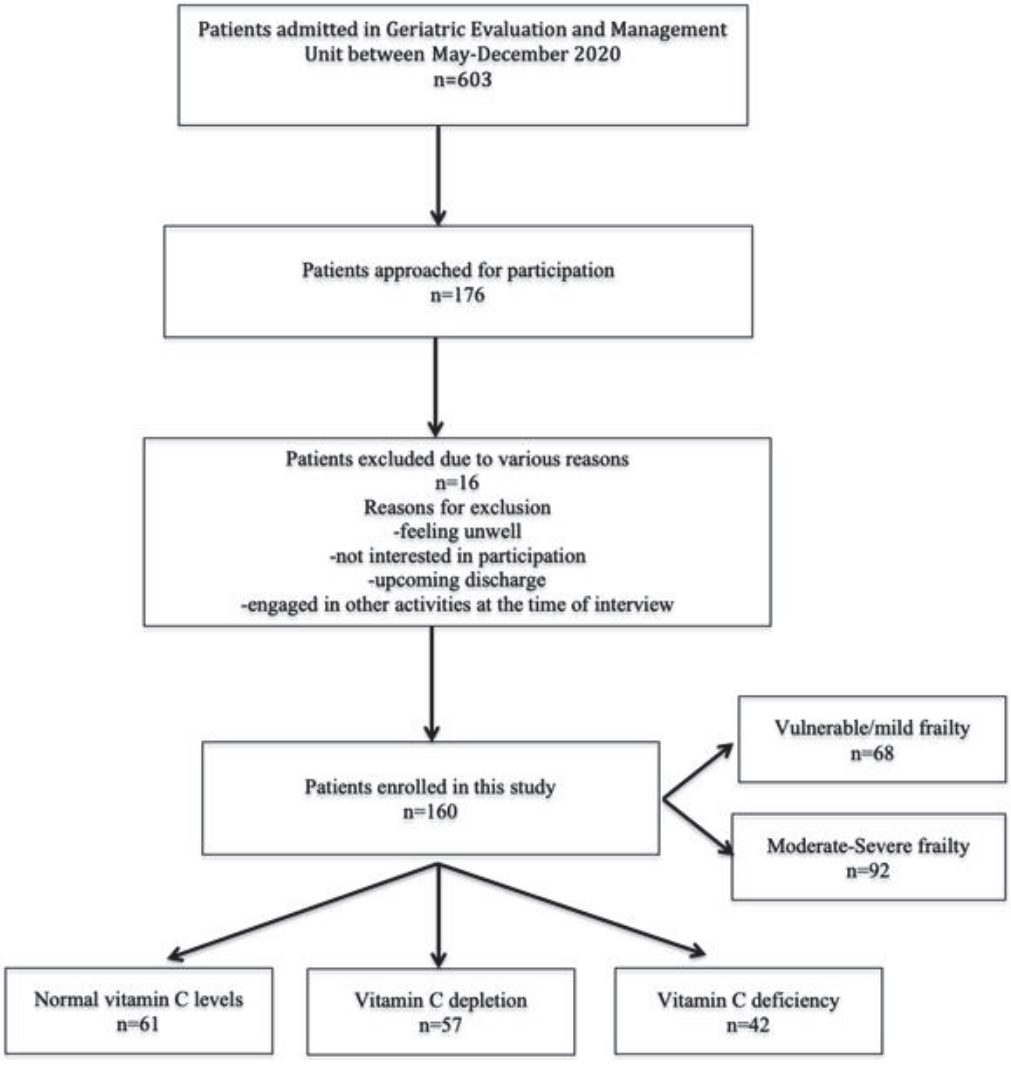


Figure 1. Study flow diagram.

There was no missing data and vitamin C results were available for all the participants. The mean (SD) age was 84.4 (6.4) years (range: 75–105 years) and 96 (60%) were females. All patients were residing in their own homes and 78 (48.7%) were living with their partners. The mean Charlson index was 8.4 (2.6), and the majority of patients were on polypharmacy 130 (81.3%) and many were admitted with falls as the principal diagnosis (69, 43.1%). The mean (SD) vitamin C levels were 26.8 (23.0) µmol/L, (range: 3–148). The median (IQR) time

from hospital admission to the collection of vitamin C sample was 4 (4, 4) days. According to Johnston's criteria, 61 (38.1%) had a normal vitamin C status, 57 (35.6%) were vitamin C depleted and while 42 (26.3%) had vitamin C deficiency (Figure 1). Overall, 99 (61.9 %) were classified as having hypovitaminosis C, i.e., vitamin C levels below 28 μmol/L. The mean (SD) EFS score was 9.8 (2.1) (range: 5–16). Sixty-eight (42.5%) patients were classified as non-frail/vulnerable or with mild frailty (EFS < 10) and 92 (57.5%) were classified with moderate to severely frailty (EFS $\geq$ 10). Patients who had moderate to severe frailty were more likely to be older, with a higher Charlson index, a lower mean MMSE score and were more likely to be depressed than those who were non-frail/vulnerable or had mild frailty (Table 1). Patients with moderate to severe frailty had poor physical functioning, as reflected by a longer TUG test and lower SPPB scores, and had a poorer health related quality of life, as reflected by the lower EQ5D index when compared to those who were non-frail/vulnerable or had mildly frailty (Table 1). Both LOS and in-hospital mortality was significantly higher among patients who were moderately to severely frail in comparison with the non-frail/vulnerable/mildly frail group. However, there was no difference in 30-day readmissions between the two groups.

Table 1. Characteristics of vulnerable/mildly frail patients compared to moderate/severely frail patients.

Variable	Non-Frail/Vulnerable/Mild Frailty	Moderate–Severe Frailty	*p* = Value
Number (%)	68 (42.5)	92 (57.5)	
Age, mean (SD)	82.8 (5.7)	85.7 (6.7)	0.004
Sex female, *n* (%)	39 (57.4)	57 (61.9)	0.557
Charlson index mean (SD)	7.8 (2.5)	8.8 (2.6)	0.001
Medications mean (SD)	6.9 (3.9)	7.9 (3.3)	0.070
Residence home alone *n* (%)	32 (47.1)	50 (54.4)	0.362
Education diploma/university *n* (%)	31 (45.6)	34 (36.9)	0.272
Income <40k/year	34 (50.8)	60 (65.2)	0.067
Medications mean (SD)	6.9 (3.9)	7.9 (3.3)	0.070
MMSE mean (SD)	25.8 (3.4)	23.8 (3.2)	<0.001
Smokers *n* (%)	41 (60.3)	51 (55.4)	0.539
GDS mean (SD)	3.5 (2.1)	5.2 (3.1)	<0.001
MUST score mean (SD)	0.86 (1.1)	0.99 (1.2)	0.511
Fruits/Vegetable intake/day mean (SD)	1.3 (0.6)	1.2 (0.6)	0.187
HARP score mean (SD)	2.4 (1.2)	3.5 (0.6)	<0.001
TUG score in seconds mean (SD)	25.3 (15.4)	40.3 (20.5)	<0.001
Vitamin C μmol/L mean (SD)	31.8 (24.4)	22.9 (21.4)	0.015
Hypovitaminosis C *n* (%)	37 (54.4)	62 (67.4)	0.095
Vitamin C deficient *n* (%)	11 (16.2)	31 (33.7)	0.013
Vitamin D nmol/L mean (SD)	62.9 (27.3)	72.4 (33.4)	0.058
Vitamin B12 pmol/L mean (SD)	442.9 (300.7)	502.5 (351.8)	0.262
Albumin g/L mean (SD)	35.6 (30.0)	31.3 (5.2)	0.180
EFS scores mean (SD)	7.7 (1.1)	11.3 (1.3)	<0.001
SPPB scores total mean (SD)	5.5 (2.8)	2.7 (2.3)	<0.001
EQ5D index mean (SD)	0.78 (0.13)	0.68 (0.16)	<0.001
LOS median (IQR)	11.5 (14)	22.7 (17)	0.004
In-hospital mortality *n* (%)	0	7 (7.6)	0.02
30-day readmissions *n* (%)	15 (22.1)	20 (21.7)	0.961

SD, Standard Deviation; MMSE, Mini Mental State Examination; GDS, Geriatric Depression Scale; MUST, Malnutrition Universal Screening Tool; HARP, Hospital Admission Risk Profile; TUG, Timed Up and Go Test; EFS, Edmonton Frail Scale; SPPB, Short Physical Performance Battery; EQ5D, European Quality of Life questionnaire; LOS, Length of Hospital Stay; IQR, Interquartile Range.

There was no difference in the median (IQR) time in days from hospital admission to the collection of the vitamin C sample between the moderate to severely frail and the non-frail/vulnerable/mildly frail groups (4 (3,4) vs. 4 (4, 4) days, $p > 0.05$, respectively). The mean (SD) vitamin C levels were significantly lower among patients who had moderate to severe frailty when compared to those who were non-frail/vulnerable or had mild frailty

(22.9 (21.4) vs. 31.8 (24.4), p = value = 0.015). There was a weak negative correlation between vitamin C levels and EFS score (correlation coefficient = - 0.14, p = 0.081). The logistic regression analysis suggested that patients with vitamin C deficiency were more likely to be associated with moderate to severe frailty after adjustment for age, sex, Charlson index, smoking status, MUST score, MMSE, GDS, fruit and vegetable intake, socioeconomic status, HARP score, vitamin D and vitamin B12 levels when compared to those who were non-frail/vulnerable or mildly frail (aOR 4.30, 95% CI 1.33–13.86, p == 0.015) (Table 2). The senstivity analysis using the jackknife resampling method confirmed these estimates (aOR 4.30, SE 3.01, 95% CI 1.07–17.12, p = 0.039). There was no effect modification (p > 0.05) by use of interaction terms with vitamin B12 and vitamin D. The margins plot indicated that low plasma vitamin C levels predicted moderate to severe frailty (Figure 2). Clinical outcomes such as LOS, inhospital mortality and 30-day readmissions were not significantly different according to different vitamin C quintiles (> 0.05).

Table 2. Logistic regression model showing adjusted odds ratios in moderate–severely frail patients compared to non-frail/vulnerable/mildly frail patients with normal vitamin C status as the baseline.

Variable.	aOR	95% CI	*p* Value
Vitamin C deficiency	4.30	1.33–13.86	0.015
Vitamin C depletion	1.83	0.66–5.08	0.243
Age	0.94	0.87–1.02	0.158
Sex male	0.95	0.34–2.673	0.924
Charlson index	1.03	0.85–1.27	0.715
Smokers	0.68	0.28–1.66	0.395
MUST score	1.17	0.77–1.78	0.463
MMSE score	0.89	0.78–1.02	0.104
GDS score	1.33	1.09–1.61	0.004
Fruits/Vegetable intake	0.94	0.84–1.06	0.350
HARP score	4.78	2.43–9.40	<0.001
Income <40k/year	1.67	0.63–4.44	0.302
Creatinine	0.99	0.98–1.00	0.069
Vitamin D levels	1.01	0.99–1.02	0.281
Vitamin B12 levels	1.00	0.99–1.00	0.344

aOR, adjusted odds ratio; MUST, Malnutrition Universal Screening Tool; MMSE, Mini Mental State Examination; GDS, Geriatric Depression Scale; HARP, Hospital Admission Risk Profile.

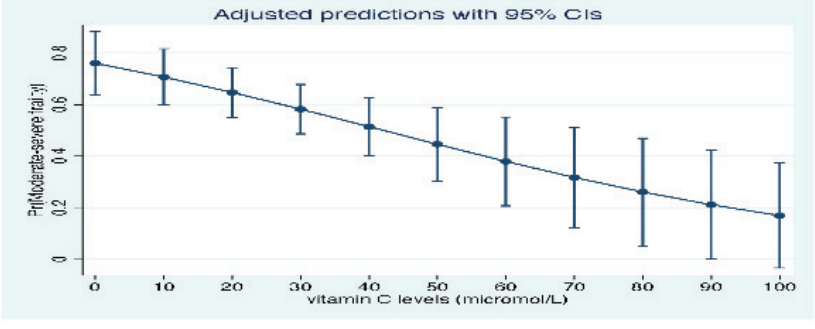


Figure 2. Prediction of frailty severity according to vitamin C status.

4. Discussion

The results of this study suggest that there is a high prevalence of hypovitaminosis C in older hospitalised patients with a quarter of patients being vitamin C deficient. Patients with moderate to severe frailty demonstrated a significantly lower vitamin C levels when compared to those who were non-frail/vulnerable or were mildly frail even after adjustment for various co-variates.

The prevalence of hypovitaminosis C in this study was 61.9%, which confirms the findings of a recent Australian study [30] that also found that a high proportion (>70%) of acutely hospitalised older patients were vitamin C depleted. Similary, a study [31] on patients who were referred for an elective surgical procedure found that 43.1% had vitamin C depletion. The slightly lower prevalence of vitamin C deficiency in the above study could be related to the lower mean age (62 (15.3) years vs. 84.4 (6.4) years) of patients in this study because two previous studies [32,33] have suggested that vitamin C levels decline with aging.

Previous studies indicate that older people have inadequate intake of micronutrients such as vitamin A, D, E, B6, B12, folate and zinc [34–36]. The intake of vitamin C and carotenoids may also decline with older age [37,38]. Older people are at a high risk of micronutrient malnutrition due to a range of factors including anorexia of ageing and social factors, such as difficulty with shopping or the preparation of meals due to physical disabilities [32,39,40]. Our study also found that the risk of malnutrition, as determined by the MUST score, was not significantly higher among patients who had hypovitaminosis C. These findings corroborrate previous evidence that micronutrient deficiencies in older patients may exist even without overt signs of clinical malnutrition [7].

This study indicates that vitamin C deficiency is associated with a greater severity of frailty in older hospitalised patients. This could be related to a higher degree of oxidative stress in ascorbic acid deficient skeletal muscles leading to muscle dysfunction. Previous evidence indicates that there is a relationship between oxidative stress and frailty [41,42]. Reactive oxygen species (ROS) are continuously produced in skeletal muscles [43], and the production of ROS is increased by exercise, which promotes oxidative stress due to induction of potentially damaging biomolecules, such as proteins, lipids and DNA [44]. Recent research shows that physical inactivity also induces ROS production and may lead to muscle atrophy [45,46]. Furthermore, elevated ROS levels have been found in subjects with ageing-related sarcopenia and muscular diseases [42,47].

In addition, vitamin C is involved in the synthesis of carnitine and collagen [48]. While carnitine is required for the metabolism of long chain fatty acids during physical activity, collagen is a key structural component of skeletal muscles and tendons [49,50]. Animal studies have suggested that ascorbic acid deficiency in skeletal muscles caused muscle atrophy, concomitant with a high expression of muscle atrophy-related genes with a reduction in physical performance [8,51]. Interestingly, ascorbic acid supplementation restored physical performance and reduced the expression of muscle atrophy-related genes [8].

Limited clinical studies have determined a relationship between vitamin C deficiency and frailty. A Japanese study [9] involving 655 community dwelling older women (mean (SD) age 75.4 (4.1) years) suggested that plasma vitamin C levels positively correlate with muscle strength and physical performance measured in terms of handgrip strength, length of time standing on one leg and gait speed. Similarly, a study in the UK [52] involving 628 community dwelling patients aged 63–73 years found that a higher vitamin C intake was associated with a better physical performance measured by short chair rising time. Another European study [48], which included >13000 men and women aged 42–82 years, found that both dietary intake and plasma vitamin C levels had a positive association with fat-free mass (FFM) using bioelectrical impedance analysis.

Fruits and vegetables are rich sources of antioxidants including carotenoids, flavonoids, vitamin C and other polyphenols [53]. Research suggests that a higher intake of fruits and vegetables is protective against inflammation, cardiovascular disease and mortality [54–56]. It is also possible that in addition to the high oxidant stress, vitamin C deficiency also indirectly contributes to the increasing severity of frailty due to the higher prevalence of cardiovascular diseases in this population.

Although previous studies have indicated negative health outcomes with vitamin C deficiency [57,58], this was not evident in our study and clinical outcomes such as LOS, mortality and readmissions were similar across different vitamin C quintiles. Our

study was, however, not powered to detect clinical outcomes. In addition, evidence to date is unconvincing that vitamin C supplementation is beneficial in improving either cardiovascular outcomes [57] or sepsis-related mortality [10,59]. However, the evidence is of low quality because of limitations such as the use of a small sample size and shorter duration of interventions. It is possible that vitamin C supplementation will be more effective for a subgroup of patients such as older people, those with a higher baseline cardiovascular risk and those with lower baseline vitamin C status. Future trials are needed avoiding the abovementioned limitations and targeting subgroup of population who are at a high risk of vitamin C deficiency to clarify beneficial effects of vitamin C supplementation.

5. Limitations

This study used convenient sampling for recruitment of participants, and it is possible that the sample may not be a true representative of patients who were admitted under the GEM unit. In addition, the sample is not representative of the general elderly population but of those individuals who were referred to a geriatric unit and were therefore more likely to have geriatric syndromes. The findings of this study cannot be used to suggest causality because of the cross-sectional design of the study. We were unable to quantify the intake of energy and protein of our patients in this study. There was a delay in obtaining vitamin C levels immediately following hospitalisation because micronutrient levels decline during hospitalisation due to a range of factors such as anorexia, inflammation and polypharmacy [60,61].

6. Conclusions

This study confirms that a high prevalence of hypovitaminosis C in older hospitalised patients and vitamin C deficiency is associated with a greater severity of frailty. Further studies are needed to confirm this association and to determine whether vitamin C replacement may be beneficial in prevention or reversal of frailty.

Author Contributions: Conceptualization, Y.S. and C.T.; methodology, Y.S.; software, Y.S., C.H. and P.H.; validation, Y.S. and A.P.; formal analysis, Y.S.; investigation, Y.S.; resources, Y.S.; data curation, Y.S., A.P. and C.H.; writing—original draft preparation, Y.S.; writing—review and editing, Y.S. and C.T.; visualization, Y.S.; supervision, Y.S. and C.T.; project administration, Y.S. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Southern Adelaide Human Clinical Research Ethics Committee (SAHREC) no. 64.19 on 9 August 2019.

Informed Consent Statement: Written informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author only after permission is granted by the ethics committee.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Clegg, A.; Young, J.; Iliffe, S.; Rikkert, M.O.; Rockwood, K. Frailty in elderly people. *Lancet* **2013**, *381*, 752–762. [CrossRef]
2. Morley, J.E.; Vellas, B.; Van Kan, G.A.; Anker, S.D.; Bauer, J.M.; Bernabei, R.; Cesari, M.; Chumlea, W.; Doehner, W.; Evans, J.; et al. Frailty Consensus: A Call to Action. *J. Am. Med Dir. Assoc.* **2013**, *14*, 392–397. [CrossRef]
3. Hammami, S.; Zarrouk, A.; Piron, C.; Almas, I.; Sakly, N.; Latteur, V. Prevalence and factors associated with frailty in hospitalized older patients. *BMC Geriatr.* **2020**, *20*, 144–148. [CrossRef] [PubMed]
4. Ibrahim, K.; Howson, F.F.A.; Culliford, D.J.; Sayer, A.A.; Roberts, H.C. The feasibility of assessing frailty and sarcopenia in hospitalised older people: A comparison of commonly used tools. *BMC Geriatr.* **2019**, *19*, 1–7. [CrossRef]
5. Artaza-Artabe, I.; Sáez-López, P.; Sánchez-Hernández, N.; Fernández-Gutierrez, N.; Malafarina, V. The relationship between nutrition and frailty: Effects of protein intake, nutritional supplementation, vitamin D and exercise on muscle metabolism in the elderly. A systematic review. *Matur.* **2016**, *93*, 89–99. [CrossRef] [PubMed]

6. Raynaud-Simon, A.; Lesourd, B. Dénutrition du sujet âgé. Conséquences cliniques [Malnutrition in the elderly. Clinical conse-quences]. *Presse Med.* **2000**, *29*, 2183–2190.
7. Semba, R.D.; Bartali, B.; Zhou, J.; Blaum, C.; Ko, C.-W.; Fried, L.P. Low Serum Micronutrient Concentrations Predict Frailty Among Older Women Living in the Community. *J. Gerontol. Ser. A Boil. Sci. Med. Sci.* **2006**, *61*, 594–599. [CrossRef] [PubMed]
8. Takisawa, S.; Funakoshi, T.; Yatsu, T.; Nagata, K.; Aigaki, T.; Machida, S.; Ishigami, A. Vitamin C deficiency causes muscle atrophy and a deterioration in physical performance. *Sci. Rep.* **2019**, *9*, 1–10. [CrossRef]
9. Saito, K.; Yokoyama, T.; Yoshida, H.; Kim, H.; Shimada, H.; Yoshida, Y.; Iwasa, H.; Shimizu, Y.; Kondo, Y.; Handa, S.; et al. A Significant Relationship between Plasma Vitamin C Concentration and Physical Performance among Japanese Elderly Women. *J. Gerontol. Ser. A Biol. Sci. Med. Sci.* **2012**, *67*, 295–301. [CrossRef]
10. Scholz, S.S.; Borgstedt, R.; Ebeling, N.; Menzel, L.C.; Jansen, G.; Rehberg, S. Mortality in septic patients treated with vitamin C: A systematic meta-analysis. *Crit. Care* **2021**, *25*, 1–10. [CrossRef]
11. Perna, S.; Francis, M.D.; Bologna, C.; Moncaglieri, F.; Riva, A.; Morazzoni, P.; Allegrini, P.; Isu, A.; Vigo, B.; Guerriero, F.; et al. Performance of Edmonton Frail Scale on frailty assessment: Its association with multi-dimensional geriatric conditions assessed with specific screening tools. *BMC Geriatr.* **2017**, *17*, 1–8. [CrossRef]
12. Dent, E.; Kowal, P.; Hoogendijk, E.O. Frailty measurement in research and clinical practice: A review. *Eur. J. Intern. Med.* **2016**, *31*, 3–10. [CrossRef] [PubMed]
13. Frenkel, W.J.; Jongerius, E.J.; Uitert, M.J.M.-V.; Van Munster, B.C.; De Rooij, S.E. Validation of the Charlson Comorbidity Index in Acutely Hospitalized Elderly Adults: A Prospective Cohort Study. *J. Am. Geriatr. Soc.* **2014**, *62*, 342–346. [CrossRef] [PubMed]
14. Murray, S.B.; Bates, D.W.; Ngo, L.; Ufberg, J.W.; Shapiro, N.I. Charlson Index Is Associated with One-year Mortality in Emergency Department Patients with Suspected Infection. *Acad. Emerg. Med.* **2006**, *13*, 530–536. [CrossRef]
15. De Villar, L.O.-P.; Martínez-Olmos, F.J.; Junqué-Jiménez, A.; Amer-Cuenca, J.J.; Gramage, J.M.; Mercer, T.; Segura-Ortí, E. Test-retest reliability and minimal detectable change scores for the short physical performance battery, one-legged standing test and timed up and go test in patients undergoing hemodialysis. *PLoS ONE* **2018**, *13*, e0201035. [CrossRef]
16. Larsson, P.; Borge, C.R.; Nygren-Bonnier, M.; Lerdal, A.; Edvardsen, A. An evaluation of the short physical performance battery following pulmonary rehabilitation in patients with chronic obstructive pulmonary disease. *BMC Res. Notes* **2018**, *11*, 1–7. [CrossRef]
17. Nightingale, C.J.; Mitchell, S.N.; Butterfield, S.A. Validation of the Timed Up and Go Test for Assessing Balance Variables in Adults Aged 65 and Older. *J. Aging Phys. Act.* **2019**, *27*, 230–233. [CrossRef] [PubMed]
18. Shumway-Cook, A.; Brauer, S.; Woollacott, M. Predicting the Probability for Falls in Community-Dwelling Older Adults Using the Timed Up & Go Test. *Phys. Ther.* **2000**, *80*, 896–903. [CrossRef]
19. Folstein, M.F.; Robins, L.N.; Helzer, J.E. The Mini-Mental State Examination. *Arch. Gen. Psychiatry* **1983**, *40*, 812. [CrossRef]
20. Bellón, J.Á.; Lardelli, P.; Luna, J.D.D.; Delgado, A. Validity of self reported utilisation of primary health care services in an urban population in Spain. *J. Epidemiol. Community Health* **2000**, *54*, 544–551. [CrossRef]
21. Shimada, H.; Park, H.; Makizako, H.; Doi, T.; Lee, S.; Suzuki, T. Depressive symptoms and cognitive performance in older adults. *J. Psychiatr. Res.* **2014**, *57*, 149–156. [CrossRef]
22. Dennis, M.; Kadri, A.; Coffey, J. Depression in older people in the general hospital: A systematic review of screening instruments. *Age Ageing* **2012**, *41*, 148–154. [CrossRef] [PubMed]
23. Sharma, Y.; Thompson, C.; Kaambwa, B.; Shahi, R.; Miller, M. Validity of the Malnutrition Universal Screening Tool (MUST) in Australian hospitalized acutely unwell elderly patients. *Asia Pac. J. Clin. Nutr.* **2017**, *26*, 994–1000. [PubMed]
24. Balestroni, G.; Bertolotti, G. EuroQol-5D (EQ-5D): An instrument for measuring quality of life. *Monaldi Arch. Chest Dis.* **2012**, *78*, 155–159. [CrossRef] [PubMed]
25. Liu, S.K.; Montgomery, J.; Yan, Y.; Mecchella, J.N.; Bartels, S.J.; Masutani, R.; Batsis, J.A. Association Between Hospital Admission Risk Profile Score and Skilled Nursing or Acute Rehabilitation Facility Discharges in Hospitalized Older Adults. *J. Am. Geriatr. Soc.* **2016**, *64*, 2095–2100. [CrossRef] [PubMed]
26. Tessier, F.; Birlouez-Aragon, I.; Tjani, C.; Guilland, J.C. Validation of a micromethod for determining oxidized and reduced vitamin C in plasma by HPLC-fluorescence. *Int. J. Vitam. Nutr. Res.* **1996**, *66*, 166–170.
27. Mitmesser, S.H.; Ye, Q.; Evans, M.; Combs, M. Determination of plasma and leukocyte vitamin C concentrations in a randomized, double-blind, placebo-controlled trial with Ester-C®. *SpringerPlus* **2016**, *5*, 1–11. [CrossRef]
28. Johnston, C.S.; Corte, C. People with Marginal Vitamin C Status are at High Risk of Developing Vitamin C Deficiency. *J. Am. Diet. Assoc.* **1999**, *99*, 854–856. [CrossRef]
29. Nemes, S.; Jonasson, J.M.; Genell, A.; Steineck, G. Bias in odds ratios by logistic regression modelling and sample size. *BMC Med. Res. Methodol.* **2009**, *9*, 56. [CrossRef] [PubMed]
30. Sharma, Y.; Miller, M.; Shahi, R.; Doyle, A.; Horwood, C.; Hakendorf, P.; Thompson, C. Vitamin C deficiency in Australian hospitalized patients: An observational study. *Intern. Med. J.* **2018**. [CrossRef]
31. Ravindran, P.; Wiltshire, S.; Das, K.; Wilson, R.B. Vitamin C deficiency in an Australian cohort of metropolitan surgical patients. *Pathology* **2018**, *50*, 654–658. [CrossRef] [PubMed]
32. McCall, S.J.; Clark, A.B.; Luben, R.N.; Wareham, N.J.; Khaw, K.-T.; Myint, P.K. Plasma Vitamin C Levels: Risk Factors for Deficiency and Association with Self-Reported Functional Health in the European Prospective Investigation into Cancer-Norfolk. *Nutrients* **2019**, *11*, 1552. [CrossRef] [PubMed]

33. Carr, A.C.; Rowe, S. Factors Affecting Vitamin C Status and Prevalence of Deficiency: A Global Health Perspective. *Nutrients* **2020**, *12*, 1963. [CrossRef]
34. Jacques, P.F.; Felson, D.T.; Tucker, K.L.; Mahnken, B.; Wilson, P.W.; Rosenberg, I.H.; Rush, D. Plasma 25-hydroxyvitamin D and its determinants in an elderly population sample. *Am. J. Clin. Nutr.* **1997**, *66*, 929–936. [CrossRef]
35. Maras, J.E.; Bermudez, O.I.; Qiao, N.; Bakun, P.J.; Boody-Alter, E.L.; Tucker, K.L. Intake of α-tocopherol is limited among US adults. *J. Am. Diet. Assoc.* **2004**, *104*, 567–575. [CrossRef]
36. Wright, J.D.; Bialostosky, K.; Gunter, E.W.; Carroll, M.D.; Najjar, M.F.; A Bowman, B.; Johnson, C.L. Blood folate and vitamin B12: United States, 1988-94. *Vital-Health Stat. Ser. 11 Data Natl. Health Surv.* **1998**, *11*, 1–78.
37. Nowjack-Raymer, R.; Sheiham, A. Association of edentulism and diet and nutrition in US adults. *J. Dent. Res.* **2003**, *82*, 123–126. [CrossRef] [PubMed]
38. Michelon, E.; Blaum, C.; Semba, R.D.; Xue, Q.L.; Ricks, M.O.; Fried, L.P. Vitamin and carotenoid status in older women: Associations with the frailty syndrome. *J. Gerontol. A Biol. Sci. Med. Sci.* **2006**, *61*, 600–607. [CrossRef] [PubMed]
39. Sanford, A.M. Anorexia of aging and its role for frailty. *Curr. Opin. Clin. Nutr. Metab. Care* **2017**, *20*, 54–60. [CrossRef]
40. Ter Borg, S.; Verlaan, S.; Hemsworth, J.; Mijnarends, D.M.; Schols, J.M.G.A.; Luiking, Y.C.; De Groot, L.C.P.G.M. Micronutrient intakes and potential inadequacies of community-dwelling older adults: A systematic review. *Br. J. Nutr.* **2015**, *113*, 1195–1206. [CrossRef] [PubMed]
41. Saum, K.-U.; Dieffenbach, A.K.; Jansen, E.H.; Schöttker, B.; Holleczek, B.; Hauer, K.; Brenner, H. Association between Oxidative Stress and Frailty in an Elderly German Population: Results from the ESTHER Cohort Study. *Gerontology* **2015**, *61*, 407–415. [CrossRef] [PubMed]
42. Moylan, J.S.; Reid, M.B. Oxidative stress, chronic disease, and muscle wasting. *Muscle Nerve* **2007**, *35*, 411–429. [CrossRef]
43. Arbogast, S.; Reid, M.B. Oxidant activity in skeletal muscle fibers is influenced by temperature, CO2 level, and muscle-derived nitric oxide. *Am. J. Physiol. Integr. Comp. Physiol.* **2004**, *287*, R698–R705. [CrossRef]
44. Winterbourn, C.C. Reconciling the chemistry and biology of reactive oxygen species. *Nat. Chem. Biol.* **2008**, *4*, 278–286. [CrossRef]
45. Kondo, H.; Nakagaki, I.; Sasaki, S.; Hori, S.; Itokawa, Y. Mechanism of oxidative stress in skeletal muscle atrophied by immobilization. *Am. J. Physiol. Metab.* **1993**, *265*, E839–E844. [CrossRef]
46. Rodríguez-Hernández, Á.; Cordero, M.D.; Salviati, L.; Artuch, R.; Pineda, M.; Briones, P.; Izquierdo, L.G.; Cotán, D.; Navas, P.; Sánchez-Alcázar, J.A. Coenzyme Q deficiency triggers mitochondria degradation by mitophagy. *Autophagy* **2009**, *5*, 19–32. [CrossRef]
47. Siu, P.M.; Pistilli, E.E.; Alway, S.E. Age-dependent increase in oxidative stress in gastrocnemius muscle with unloading. *J. Appl. Physiol.* **2008**, *105*, 1695–1705. [CrossRef]
48. Lewis, L.N.; Hayhoe, R.P.G.; A Mulligan, A.; Luben, R.N.; Khaw, K.-T.; A Welch, A. Lower Dietary and Circulating Vitamin C in Middle- and Older-Aged Men and Women Are Associated with Lower Estimated Skeletal Muscle Mass. *J. Nutr.* **2020**, *150*, 2789–2798. [CrossRef] [PubMed]
49. Chapman, M.; Meza, R.; Lieber, R.L. Skeletal muscle fibroblasts in health and disease. *Differentiation* **2016**, *92*, 108–115. [CrossRef] [PubMed]
50. Rebouche, C.J. Ascorbic acid and carnitine biosynthesis. *Am. J. Clin. Nutr.* **1991**, *54*, 1147S–1152S. [CrossRef] [PubMed]
51. Barreiro, E.; de la Puente, B.; Busquets, S.; López-Soriano, F.J.; Gea, J.; Argiles, J.M. Both oxidative and nitrosative stress are associated with muscle wasting in tumour-bearing rats. *FEBS Lett.* **2005**, *579*, 1646–1652. [CrossRef]
52. Martin, H.; Sayer, A.A.; Jameson, K.; Syddall, H.; Dennison, E.M.; Cooper, C.; Robinson, S. Does diet influence physical performance in community-dwelling older people? Findings from the Hertfordshire Cohort Study. *Age Ageing* **2011**, *40*, 181–186. [CrossRef]
53. Fung, T.; Stuijk, E.; Rodriguez-Artalejo, F.; Hagan, K.; Hu, F.; Lopez-Garcia, E. Fruits and Vegetables Intake and Risk of Frailty in Women Aged 60 and Older (OR33-08-19). *Curr. Dev. Nutr.* **2019**, *3*. [CrossRef]
54. Zhu, F.; Du, B.; Xu, B. Anti-inflammatory effects of phytochemicals from fruits, vegetables, and food legumes: A review. *Crit. Rev. Food Sci. Nutr.* **2018**, *58*, 1260–1270. [CrossRef]
55. Zhao, C.-N.; Meng, X.; Li, Y.; Li, S.; Liu, Q.; Tang, G.-Y.; Li, H.-B. Fruits for Prevention and Treatment of Cardiovascular Diseases. *Nutrients* **2017**, *9*, 598. [CrossRef]
56. Wang, X.; Ouyang, Y.; Liu, J.; Zhu, M.; Zhao, G.; Bao, W.; Hu, F.B. Fruit and vegetable consumption and mortality from all causes, cardiovascular disease, and cancer: Systematic review and dose-response meta-analysis of prospective cohort studies. *BMJ* **2014**, *349*, g4490. [CrossRef] [PubMed]
57. Cook, N.R. A Randomized Factorial Trial of Vitamins C and E and Beta Carotene in the Secondary Prevention of Cardiovascular Events in Women. *Arch. Intern. Med.* **2007**, *167*, 1610–1618. [CrossRef]
58. Loria, C.M.; Klag, M.J.; E Caulfield, L.; Whelton, P.K. Vitamin C status and mortality in US adults. *Am. J. Clin. Nutr.* **2000**, *72*, 139–145. [CrossRef] [PubMed]
59. Ashor, A.W.; Brown, R.; Keenan, P.D.; Willis, N.D.; Siervo, M.; Mathers, J.C. Limited evidence for a beneficial effect of vitamin C supplementation on biomarkers of cardiovascular diseases: An umbrella review of systematic reviews and meta-analyses. *Nutr. Res.* **2019**, *61*, 1–12. [CrossRef]

60. Allard, J.P.; Keller, H.; Teterina, A.; Jeejeebhoy, K.N.; Laporte, M.; Duerksen, D.R.; Gramlich, L.; Payette, H.; Bernier, P.; Davidson, B.; et al. Factors associated with nutritional decline in hospitalised medical and surgical patients admitted for 7 d or more: A prospective cohort study. *Br. J. Nutr.* **2015**, *114*, 1612–1622. [CrossRef]
61. Lima, J.; Teixeira, P.P.; Eckert, I.D.C.; Burgel, C.F.; Silva, F.M. Decline of nutritional status in the first week of hospitalisation predicts longer length of stay and hospital readmission during 6-month follow-up. *Br. J. Nutr.* **2021**, *125*, 1132–1139. [CrossRef] [PubMed]

nutrients

MDPI

Article

KiwiC for Vitality: Results of a Placebo-Controlled Trial Testing the Effects of Kiwifruit or Vitamin C Tablets on Vitality in Adults with Low Vitamin C Levels

Tamlin S. Conner [1,*], Benjamin D. Fletcher [1], Jillian J. Haszard [2], Juliet M. Pullar [2], Emma Spencer [3], Louise A. Mainvil [4] and Margreet C. M. Vissers [2]

[1] Department of Psychology, University of Otago, Dunedin 9054, New Zealand; ben.fletcher@postgrad.otago.ac.nz

[2] Centre for Free Radical Research, Department of Pathology and Biomedical Science, University of Otago, Christchurch 8140, New Zealand; jill.haszard@otago.ac.nz (J.J.H.); juliet.pullar@otago.ac.nz (J.M.P.); margreet.vissers@otago.ac.nz (M.C.M.V.)

[3] Department of Pathology and Biomedical Science, University of Otago, Christchurch 8140, New Zealand; emma.spencer@otago.ac.nz

[4] Department of Human Nutrition, University of Otago, Dunedin 9054, New Zealand; louise.mainvil@icloud.com

* Correspondence: tamlin.conner@otago.ac.nz; Tel.: +64-3-479-7624

Received: 13 July 2020; Accepted: 18 September 2020; Published: 22 September 2020; Corrected: 30 September 2022

Abstract: Consumption of vitamin C-rich fruits and vegetables has been associated with greater feelings of vitality. However, these associations have rarely been tested in experimental trials. The aim of the current study was to test the effects of eating a vitamin C-rich food (kiwifruit) on subjective vitality and whether effects are driven by vitamin C. Young adults (n = 167, 61.1% female, aged 18–35 years) with plasma vitamin C < 40 µmol/L were allocated to three intervention conditions: kiwifruit (2 SunGold™ kiwifruit/day), vitamin C (250 mg tablet/day), placebo (1 tablet/day). The trial consisted of a two-week lead-in, four-week intervention, and two-week washout. Plasma vitamin C and vitality questionnaires (total mood disturbance, fatigue, and well-being) were measured fortnightly. Self-reported sleep quality and physical activity were measured every second day through smartphone surveys. Nutritional confounds were assessed using a three-day food diary during each study phase. Plasma vitamin C reached saturation levels within two weeks for the kiwifruit and vitamin C groups. Participants consuming kiwifruit showed a trend of improvement in mood disturbance, significantly decreased fatigue, and significantly improved well-being after two weeks of the intervention. Improvements in well-being remained elevated through washout. Consumption of vitamin C tablets alone was associated with improved well-being after two weeks, and additionally improved mood and fatigue for participants with consistently low vitamin C levels during lead-in. Diet records showed that participants consuming kiwifruit reduced their fat intake during the intervention period. Intervention effects remained significant when adjusting for condition allocation groupings, age, and ethnicity, and were not explained by sleep quality, physical activity, BMI, or other dietary patterns, including fat intake. There were no changes in plasma vitamin C status or vitality in the placebo group. Whole-food consumption of kiwifruit was associated with improved subjective vitality in adults with low vitamin C status. Similar, but not identical changes were found for vitamin C tablets, suggesting that additional properties of kiwifruit may contribute to improved vitality.

Keywords: Vitamin C status; energy; well-being; kiwifruit; mood

1. Introduction

There is growing evidence that increased consumption of fruit and vegetables is associated with subjective feelings of vitality, which constitutes feelings of less fatigue, improved mood, and a "zest for life" [1–3], in conjunction with decreased levels of depression, anxiety, and stress [2,4,5]. Fruit and vegetables are rich in numerous essential nutrients and are the main source of vitamin C for humans and other species that cannot synthesize this compound in the liver [6,7]. Vitamin C availability determines intracellular levels throughout the body and saturation in most organs is achieved when plasma concentrations are >60 μmol/L [8–10]. Tissue vitamin C content is variable and some organs such as the brain and adrenals preferentially accumulate high concentrations of the vitamin even when plasma levels are below saturation [10,11]. High intracellular concentrations inside these organs coincide with a demand for ascorbate as a cofactor to support Cu- and Fe-containing enzymes, including those responsible for the synthesis of adrenalin, peptide hormones and collagen [12–15].

Insufficient intake of vitamin C results in lowered plasma ascorbate status. Levels below 11 μmol/L are indicative of the deficiency disease, scurvy [16,17], which is accompanied by impaired collagen synthesis resulting in skin changes, bruising, bleeding gums, and poor wound healing [7,18]. Early symptoms of vitamin C deficiency, including fatigue, depression, mental weariness, social introversion, hysteria, hypochondriasis, and reduced motivation and arousal [18–22], become apparent when plasma levels are <23 μmol/L, a condition described as hypovitaminosis C [20,21].

Several studies have suggested that increasing vitamin C intake to ensure plasma and tissue saturation can decrease the fatigue-related symptoms of vitamin C insufficiency and improve physical activity levels [16,23,24]. We, and others, have reported improvements in positive mood and vitality in association with increased fruit and vegetable intake [3,25–28]. Improved vitamin C status is closely aligned with increased fruit and vegetable consumption [29–31] and, given the role of vitamin C in neurotransmitter, norepinephrine, and peptide hormone synthesis [12–15], it is possible that many of the beneficial effects of fruit and vegetables could be attributed to this component. To determine the contribution of vitamin C to a food-related health benefit requires a specifically targeted and well-controlled study and this was the primary aim of our placebo-controlled intervention study.

We have previously reported pilot evidence of vitality improvements in young men with low baseline plasma vitamin C levels following a six-week intervention with kiwifruit, a high vitamin C food [32]. Although kiwifruit consumption resulted in the expected plasma saturation, we could not determine the relative contributions of the whole fruit or the vitamin C component to the observed decreased fatigue and improved vigour scores [32]. The current study was designed as a placebo-controlled trial to investigate the effects of increased vitamin C consumption via a food (kiwifruit), or tablets, on vitality changes in a sample of healthy young adults with low baseline vitamin C levels. The primary vitality outcome measures were self-reported mood disturbance, fatigue, and well-being, which were measured fortnightly during the study. Smartphone surveys were also administered every second night during the study to track day-level changes in sleep and physical activity while also minimising retrospective recall. We hypothesised that participants who consumed kiwifruit or vitamin C tablets would show greater improvements in their mood, fatigue, and well-being over time, relative to those who consumed placebo tablets. As blood plasma vitamin C levels were monitored fortnightly throughout the study, we also hypothesised that changes in vitamin C levels would mirror the improved psychological outcomes reported from consumption of kiwifruit or vitamin C tablets, relative to placebo.

2. Materials and Methods

2.1. Trial Design

The study was preregistered with the Australian and New Zealand Clinical Trial Registry (Trial ID: ACTRN12617001031358) and approved by the New Zealand Health and Disability Ethics Committee (17/NTB/104). The study was a three-armed, randomized (1:1:1 ratio), parallel arm, placebo-controlled

trial (Figure 1). Participants were selected for the study following extensive screening that included measurement of plasma vitamin C status (see Screening section). The baseline plasma vitamin C status and vitality for enrolled participants was assessed before and after a two-week lead-in period. During a four-week intervention period the participants consumed, daily, either two SunGold™ kiwifruit, a chewable vitamin C tablet, or a chewable placebo tablet matched for appearance and flavour. A wash-out period established whether participants returned to baseline following the intervention. Plasma vitamin C levels and vitality outcomes were measured fortnightly across the study.

2.2. Participant Recruitment and Screening

Participants were recruited and enrolled from university and polytechnic campuses in Dunedin, New Zealand, between July 2017 and April 2018. Eligibility criteria shown in Table 1 were used to recruit a cohort of young adults with low baseline vitamin C status. Participants completed an online screening questionnaire that accessed all eligibility criteria (except for vitamin C levels) prior to being invited to a nutrition clinic at the University of Otago where a registered nurse took blood samples to determine participants' vitamin C levels. The flow of participants is represented in Figure 2. We screened 726 eligible individuals, measuring plasma vitamin C levels from fasting blood samples. Participants with plasma vitamin C concentration of ≤40 μmol/L were invited into the study (n = 180) and subsequently enrolled (n = 170) and randomized to a treatment arm (n = 167).

Table 1. Inclusion and exclusion criteria.

Inclusion Criteria (All Required for Inclusion)	Exclusion Criteria (Only One Required for Exclusion)
Males and females aged 18–35 years	Taking prescription medication (within past three months)
Plasma vitamin C levels <40 μmol/L	Allergy/intolerance to kiwifruit
Non-smoker	Recent smoker (within previous year)
Currently a student	Taking vitamin C supplements (within past three months)
	High fruit/juice & vegetable consumption (≥5 servings/day)
	Excessive alcohol consumption (>21 standard drinks/week)
	Diabetes mellitusBleeding disorders
	Fainting due to fear of needles

2.3. Condition Allocation

Our intention was to avoid participants being exposed to the other conditions during allocation of the intervention (e.g., participants seeing others receiving kiwifruit when they received tablets or vice versa). This was achieved by group randomisation based on scheduled clinic, either on separate days or across days, to ensure that everyone attending the same clinic would receive either kiwifruit or tablets. Participants receiving tablets at a given clinic were typically a random mix of the active and placebo groups. The allocation of clinics was determined by the lead author using a random number generator. As allocation occurred after participants were already enrolled into their clinic day (usually Mon–Thurs), the clinical study co-ordinator and prospective participants were unaware of their allocation when booking clinic appointments and did not learn of their allocation until they attended the clinic. A delay in the delivery of tablets meant that clinics in the first and second weeks were non-randomly allocated to the kiwifruit condition, and those in the third and fourth weeks, to the tablet conditions (randomised to placebo or vitamin C), with the remaining clinics randomised as intended. A randomisation schedule is shown in Supplementary Figure S1. Randomisation groups were accounted for in the statistical analyses (see Statistical Analyses, below). Tablets were bottled and labelled by the lead author (TSC) with the label including only the participant's first and last name and tablet instructions to "chew one tablet daily, store in a dark dry place, and return bottle and unused tablets on next visit". All research assistants and participants were double- blinded to their tablet condition. It was not possible to blind research assistants or participants to the kiwifruit condition. The separation of allocation clinics, however, meant that these participants were unaware of the nature

of the other treatment conditions. Condition information was kept in an electronic password-protected document by the lead author (TSC) and unblinded following data collection and entry.

2.4. Procedure

Participants enrolled in the study attended one clinic session every two weeks during the eight-week study (5 clinic visits). At clinic visit 1 of the study, height, and weight were recorded to calculate BMI (kg/m^2), a fasting blood sample was taken for vitamin C analysis, and the participants completed the vitality questionnaires. During clinic visits 2 and 3, participants were provided with a two-week supply of placebo tablets, vitamin C tablets, or kiwifruit. In addition, a comprehensive three-day food and beverage record was distributed during clinic visits 1, 3, and 4, and returned at the following clinic visits. Weight was also recorded following the intervention to calculate post-intervention BMI. See Figure 1 for the study timeline.

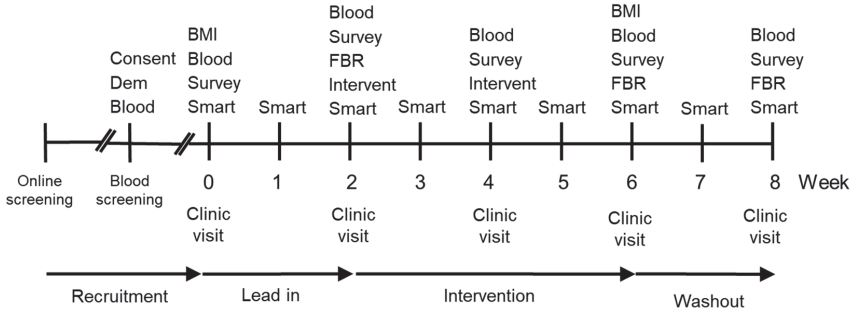


Figure 1. Timeline of study design. Key Consent-Consent form and study information; Dem-Demographic questionnaire; Blood-Blood sample; BMI-Body mass index; Survey–Survey measuring mood disturbance (POMS), fatigue (MFSI-SF), and well-being (WEMWBS); Smart-Smartphone survey, every second day during week; FBR–3-day Food and Beverage Record is returned; Intervent–Intervention provided with a two week supply of supplement (placebo, vitamin C, or kiwifruit).

Throughout the entire eight-week trial, participants were sent brief smartphone surveys every alternate night to measure secondary covariates of self-reported sleep and physical activity, plus other outcomes not discussed in this report. A total of 29 smartphone surveys were sent to participants every other night around 6 p.m. for the duration of the study, starting the day of their first clinic visit and ending on the day of their last clinic visit. They were asked to respond to the survey that night before going to bed. If participants missed a smartphone survey, they were sent an email the following evening, which included a link to complete a make-up smartphone survey for the following day. If another consecutive survey was missed, a research assistant called the participant to confirm that there were no technical issues, and to ensure that the participant was still happy to participate.

2.5. Intervention

Participants were supplemented with either two SunGold™ kiwifruit per day, chewable vitamin C tablets, or chewable placebo tablets, for four weeks. SunGold™ kiwifruit contain 160 ± 31 mg of vitamin C per 100 g (Mean ± SD), and two SunGold™ kiwifruit (~150g combined weight) contain a total of approximately 250 mg of vitamin C [33]. To help preserve the SunGold™ kiwifruit, participants were instructed to store their kiwifruit in their refrigerator. Our independent analyses indicated that storage conditions did not affect the vitamin C content of the fruit. Each vitamin C tablet contained 250 mg of vitamin C and was identical in flavour and appearance with placebo tablets that contained no active vitamin C ingredients (both manufactured by Tishcon Corporation, Salisbury, MD, USA). Participants were instructed to store their tablets in a cool, dry place, such as in a cupboard.

2.6. Demographic and Health Characteristics

Demographic measures collected at the onset of the study included age, gender (male, female, and gender diverse), ethnicity (New Zealand Census categories, multiple endorsements allowed), year at university (1 to 6), and current socioeconomic status (measured by three items ("I have enough money to buy things I want", "I don't need to worry too much about paying my bills", and "I don't think I'll have to worry about money too much in the future"), answered from 1 (strongly disagree) to 7 (strongly agree) (averaged, $\alpha = 0.79$) [34]). Health information collected at the onset of the study included past and current smoking status, typical weekly alcohol use, typical daily fruit and vegetable consumption ("How many days a week do you eat fruit/vegetables?" (0 to 7 days), "On days you eat fruit/vegetables, how many servings do you usually eat?" (0.5 to 6+ servings), multiplied and divided by 7).

2.7. Primary Outcome Vitality Measures

The fortnightly survey consisted of three validated scales that measured experiences of vitality "during the past week, including today". The Profile of Mood States questionnaire short form (POMS-SF) [35] is a 35-item measure where mood items are rated on a five-point Likert-type scale ranging from 0 (not at all) to 4 (extremely). The mood items reflect six factors of tension-anxiety, depression-dejection, anger-hostility, vigour-activity, fatigue-inertia, and confusion-bewilderment. Using these six factors, a total mood disturbance (TMD) was calculated (minimum to maximum score of −20 to 100) by summing the scores from the negative mood factors and subtracting the score from the vigor factor (Cronbach's αs = 0.87–0.92). The Multidimensional Fatigue Symptom Inventory—short form (MFSI-SF) [36] is a 30-item measure that assesses general fatigue, physical fatigue, emotional fatigue, mental fatigue, and vigour (each with a maximum score of 24), rated on a five-point Likert-type scale ranging from 0 (not at all) to 4 (extremely). The vigour score was deducted from remaining sum scores to give a total fatigue score (minimum to maximum score of −24 to 96) (αs =0.89–0.96 across the 5 time points). The Warwick–Edinburgh Mental Well-being Scale (WEMWBS) [37] comprises 14 positive psychological well-being and vitality statements, which include feelings of cheerfulness, interest in new things, and spare energy levels, rated on a five-point Likert-type scale ranging from 1 (none of the time) to 5 (all of the time). All 14 items were summed to give a total well-being score (minimum to maximum score of 14 to 70) (αs = 0.91–0.93).

2.8. Smartphone Surveys

Automated smartphone surveys asked participants questions about their sleep and physical activity as secondary covariates. Sleep duration was measured using the single question "Approximately how many hours did you sleep last night?", participants answered using a pulldown menu on their smartphone, which ranged from 0 to 24 h, increasing in increments of 30 min. Sleep quality was assessed with a single question on a 10-point Likert scale, "Please rate the quality of your sleep last night: 0 ("worst possible sleep") to 10 ("best possible sleep")". Physical activity was assessed with the single item activity questionnaire, "Today, have you done a total of 30 min or more of physical activity, enough to raise your breathing rate? (sport, exercise, brisk walking, or cycling)", answered with a "yes" (1) or a "no" (0).

2.9. Dietary Intake

Participants completed a diet and beverage record on three non-consecutive days, over a two-week period, at each phase of the study (lead-in, intervention, and washout). Diet records were used to calculate macronutrients to determine if dietary intake was equivalent between conditions over time. The food and beverage records were calculated using Diet Cruncher software (version 1.6, Way Down South Software, Dunedin, New Zealand) and the New Zealand FOODfiles Food Composition Database (2006).

2.10. Vitamin C Analysis

Fasting blood samples were kept on ice and processed within 2 h of collection as previously described [38]. Briefly, blood samples were centrifuged at 1000× *g* for 10 min at 4 °C, 700 μL of plasma removed and mixed with 700 μL cold 0.54 mol/L perchloric acid/DTPA solution, vortexed, and placed on ice for ten minutes. Precipitated protein was removed by centrifugation at 13,000× *g* for two minutes, and two samples of 500 μL supernatant stored at −80 °C. Samples were incubated with the reducing agent tris(2-carboxyethyl)phosphine hydrochloride (TCEP) to convert any oxidised ascorbate to the reduced form and vitamin C content was determined by high performance liquid chromatography (HPLC) with electrochemical detection [38,39]. Vitamin C concentration was calculated relative to an ascorbic acid standard curve run on the same day.

2.11. Missing Data Treatment and Statistical Analyses

Data were analysed with SPSS (version 26) and GraphPad Prism (version 8) with the alpha level set at 0.05 and two-tailed tests of significance. All participants randomized (n = 167) to a condition were included in the analyses following the "once randomized, always analysed principle" [40]. Specifically, participants from the randomized sample (n = 167) with any data at any point were included in the descriptive statistics, figures, and statistical analyses, reflecting an available cases approach, using all observed data with pairwise deletion only. We used this available cases approach with recommended sensitivity analysis comparisons because the amount of missing data was relatively small (3.36% missing data values) and below the 5% missingness rule-of-thumb [41–43]. Missing data were mainly due to drop out over the study duration, resulting in a monotone pattern, although this was broken by a general pattern of missingness for some data. Our data were determined to be not missing completely at random (Little's MCAR test = χ^2 (490) = 597.57, p = 0.001). Due to mental health outcomes being the primary measure, there was some concern that individuals with poorer mental health may be more likely to drop out of the study resulting in data missing not at random (MNAR). There was no evidence for this concern because the baseline distributions in total mood disturbance, fatigue, and well-being between participants who completed washout and participants who dropped out were relatively similar. Thus, we proceeded under the missing at random assumption (MAR) and completed sensitivity analysis to test the robustness of assumptions.

Descriptive statistics for participant characteristics were computed and the study outcome measures were plotted over time for each condition (vitamin C, total mood disturbance, fatigue, and well-being across weeks 0, 2, 4, 6, and 8 for the three conditions). Differences between outcome measures at baseline and intervention within each condition were determined with paired t-tests, comparing each intervention point at weeks 4 and 6, and the washout point at week 8, with the baseline measure at week 2 (second lead-in). Differences between conditions between baseline and intervention time points were investigated with ANOVA (mixed ANOVAs with condition (placebo, vitamin C, and kiwifruit) and time (Study Week 2, 4; Study Week 2, 6; or, Study Week 2, 4, 6) as factors) and ANCOVA (adding to the above analysis of variance models any covariates reflecting significant between-condition differences in demographic, health, or dietary factors). One-way ANOVA was used to determine any demographic, health, or dietary differences between conditions at each phase of the study. Post hoc analyses with Tukey's HSD (α = 0.05) were used to determine differences between conditions. If the homogeneity of variance was violated, then Welch's F test and post hoc analyses with Games-Howell were conducted. All ANOVA analyses were repeated for sensitivity analysis comparisons to assess the robustness of the sample due to missing data. Using pattern mixture models, different distributions between participants who dropped out and who remained in the study were analysed (see Online Supplementary Material).

3. Results

As shown in Figure 2, a total of 170 participants enrolled in the study and attended the baseline session (Week 0). Three people withdrew or were excluded from the study prior to randomization, leaving 167 participants randomized into conditions and included in the primary available cases analysis. After randomization, five people never received their intervention (two for no contact/no show, both assigned to placebo condition; one withdrew/no longer available, assigned to vitamin C condition; two disliked assigned condition and were unwilling to adhere, one assigned to vitamin C condition and one assigned to kiwifruit condition). A further three people withdrew or were excluded for health or medical reasons prior to completing the four-week intervention period (two for health reasons, both in vitamin C condition; one started medication, in kiwifruit condition). Thus, a total of 159 participants completed the four-week intervention and 155 completed the full washout.

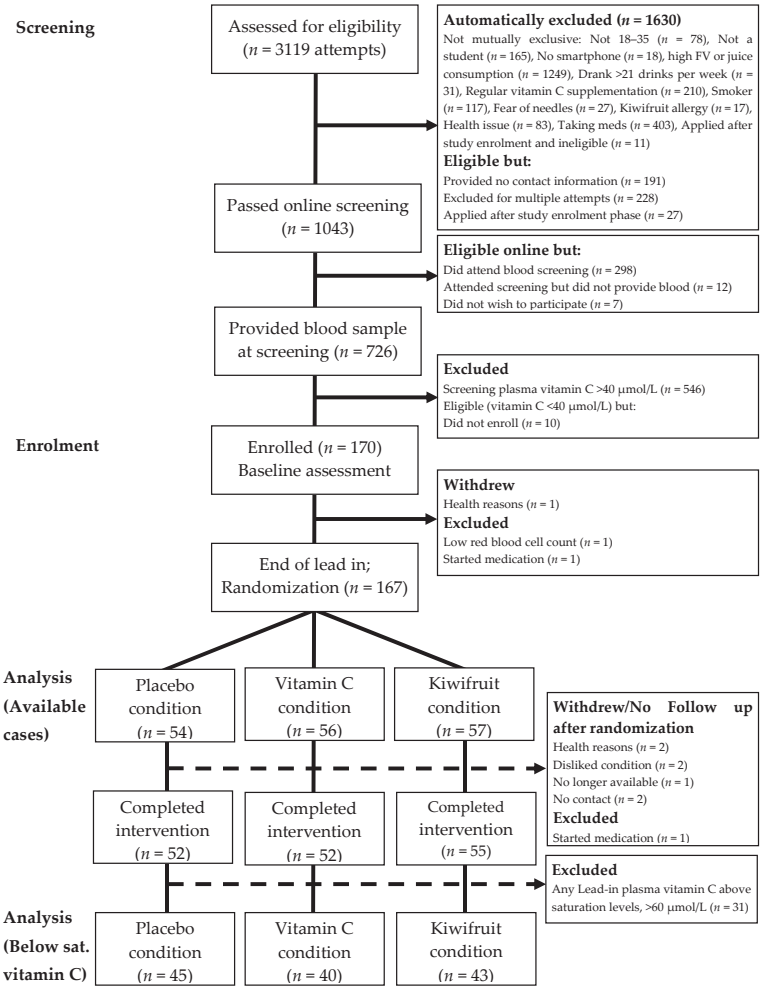


Figure 2. Participant flow diagram illustrating recruitment processes, inclusions and reasons for exclusion, allocated intervention condition, and withdrawals/loss-to-follow up. Below sat. vitamin C = Below saturation vitamin C status [selecting participants with vitamin C levels <60 μmol/L].

For more detail, see Table 2. These numbers reflect a post-randomization attrition rate of 4.79% for completing the four-week intervention (placebo = 3.70%; vitamin C = 7.14%; and kiwifruit = 3.51%), and 7.19% for completing the full study including washout (placebo = 5.56%; vitamin C = 8.93%; kiwifruit = 7.02%). Attrition rates did not differ by condition (*p*s = 0.599 and 0.760).

Table 2. Number of participants who completed the vitality outcome measures each visit.

		Condition		
	Total Sample	**Placebo**	**Vitamin C**	**Kiwifruit**
Week 0 (Lead in)	$n = 170$			
Randomization	$n = 167$	$n = 54$	$n = 56$	$n = 57$
Week 2 (Lead in)	$n = 164$	$n = 52$	$n = 55$	$n = 57$
Week 4 (Intervention)	$n = 161$	$n = 52$	$n = 53$	$n = 56$
Week 6 (Intervention)	$n = 159$	$n = 52$	$n = 52$	$n = 55$
Week 8 (Washout)	$n = 155$	$n = 51$	$n = 51$	$n = 53$

Repeated measurement of plasma vitamin C status during the lead-in period showed considerable variation in a number of individuals, with many presenting above initial recruitment levels (40 μmol/L) and some increasing to well above saturation levels (60 μmol/L) prior to intervention (Figure 3). This meant that some participants with adequate vitamin C levels were receiving the intervention. Therefore, we ran two additional subgroups analyses to try to address our original research hypothesis of testing the intervention on people with low vitamin C. We ran a per protocol (PP) analysis on 92 participants with plasma vitamin C level <40 μmol/L during the lead-in period, and a second analysis on 128 participants who maintained below saturation levels of vitamin C (<60 μmol/L) during the lead-in period, as this latter analysis achieved sufficient power and still assessed individuals with low vitamin C levels (a priori power analyses indicated that 120 participants (40 per condition) provided 80% power to detect an effect size of 0.7). Demographic characteristics for the PP subgroup ($n = 92$) and the below saturation vitamin C subgroup ($n = 128$) are reported in the Online Supplementary Material. For all analysis, participants were analysed according to their originally assigned condition.

The participant baseline characteristics for the sample overall and by condition are detailed in Table 3, with additional descriptive statistics in Supplemental Tables S1 and S2. The recruited cohort was 40/60% men/women, was ethnically heterogeneous and of slightly above average socio-economic status. The average BMI was 24 ± 4.4 (Mean ± SD), with most participants falling within the healthy range; range was from 13 to 43. The sample was not especially distressed as shown by the mean scores on the vitality measures from the two lead-in clinic visits (Supplemental Table S1). The average total mood disturbance reported at lead-in was relatively low (mean (M) = 5.68–5.38, SD = 14.12–16.63), with higher scores indicating worse mood on a scale from −20 to 100. Average fatigue was also relatively low (M = 2.45–1.18, SD = 14.24–15.57), with higher scores indicating higher fatigue on a scale from −24 to 96. Average well-being was moderate to high (M = 47.85–48.52, SD = 8.65–8.96), with higher scores indicating greater well-being on a scale from 14 to 70. The three conditions were mostly equivalent in demographic characteristics, baseline health characteristics, baseline vitality measures, and baseline vitamin C levels (ANOVAs or Chi-Squares not significant), except that the kiwifruit condition had younger participants ($F(2.164) = 8.906$, $p < 0.001$) with fewer years at university ($F(2.164) = 3.305$, $p = 0.039$) and a trend towards fewer Asian participants (χ^2 (2.167) = 5.230, $p = 0.073$) than the other conditions. Moreover, the placebo condition had lower lead-in sleep quality than the vitamin C condition ($F(2.163) = 3.054$, $p = 0.050$). Accordingly, these variables were controlled for in ANCOVA.

Plasma vitamin C levels were significantly affected by both the kiwifruit and vitamin C tablet interventions and reached saturation levels (>60 μmol/L) within two weeks (Table 4 and Figure 3). The vitamin C levels were mostly equivalent for the kiwifruit and vitamin C conditions during weeks 2 to 8, except that the vitamin C condition had a trend towards higher levels of vitamin C than the kiwifruit condition at week 6 (81 vs. 75 μmol/L, $t(105) = 1.860$, $p = 0.066$). Levels were maintained at

saturation for the duration of the intervention in both conditions. In contrast, there was no change in vitamin C status throughout the study for those in the placebo condition. Vitamin C levels for the intervention conditions decreased significantly following a two-week washout (Week 8) but were still higher than the baseline levels and the placebo group (both different from Week 2 at $p < 0.001$) (Figure 3).

Table 3. Baseline characteristics for the total sample ($n = 167$) and each condition.

	Total Sample ($n = 167$)	**Placebo ($n = 54$)**	**Vitamin C ($n = 56$)**	**Kiwifruit ($n = 57$)**
Age (years)	21.69 (3.54)	21.87 (3.31)	22.95 (4.46)	20.28 (1.87)
Gender:				
Male	64 (38.3%)	20 (37.0%)	22 (39.3%)	22 (38.6%)
Female	102 (61.1%)	34 (63.0%)	34 (60.7%)	34 (59.6%)
Gender diverse	1 (0.6%)	0 (0.0%)	0 (0.0%)	1 (1.8%)
Ethnicity:				
European	64 (38.3%)	19 (35.2%)	19 (33.9%)	26 (45.6%)
Asian	67 (40.1%)	25 (46.3%)	26 (46.4%)	16 (28.1%)
Indian	15 (9.0%)	5 (9.3%)	6 (10.7%)	4 (7.0%)
Māori & Pasifika	9 (5.4%)	2 (3.7%)	2 (3.6%)	5 (8.8%)
Other & multiple	12 (7.2%)	3 (5.6%)	3 (5.4%)	6 (10.5%)
Year of study at university	2.80 (1.51)	2.81 (1.53)	3.16 (1.74)	2.44 (1.17)
Socioeconomic status (1 to 7) [1]	4.64 (1.34)	4.81 (1.25)	4.58 (1.34)	4.52 (1.41)
Height (cm)	168.49 (10.31)	168.21 (11.09)	168.51 (9.91)	168.73 (10.10)
Weight (kg):				
Pre-intervention	68.01 (15.71)	67.72 (18.83)	67.82 (14.92)	68.47 (13.29)
Post-intervention [2]	68.08 (15.89)	67.70 (19.12)	68.01 (15.12)	68.50 (13.30)
BMI (kg/m^2):				
Pre-intervention	23.84 (4.44)	23.85 (5.73)	23.70 (3.76)	23.98 (3.65)
Post-intervention [2]	23.88 (4.44)	24.00 (5.86)	23.66 (3.66)	23.95 (3.53)
No History of Smoking:	150 (89.8%)	49 (90.7%)	49 (87.5%)	52 (91.2%)
Vegetables servings/day	1.00 (0.756)	0.97 (0.80)	0.99 (0.72)	1.02 (0.76)
Fruit servings/day	0.39 (0.38)	0.37 (0.36)	0.38 (0.32)	0.43 (0.46)
Alcoholic standards /week	3.43 (5.23)	2.60 (4.02)	3.04 (5.97)	4.59 (5.36)
Sleep duration (hours) [3]	7.18 (1.04)	7.07 (1.11)	7.35 (0.86)	7.11 (1.11)
Sleep quality (1 to 10) [3]	6.54 (1.29)	6.21 (1.42)	6.80 (1.29)	6.61 (1.12)
Physical activity (30 min/ day): % of days [3]	46.1%	47.5%	45.5%	45.5%

[1] $n = 165$; Placebo $n = 54$; Vitamin C $n = 56$; Kiwifruit $n = 55$ [2] $n = 158$; Placebo $n = 52$; Vitamin C $n = 51$; Kiwifruit $n = 55$. [3] $n = 166$; Placebo $n = 54$; Vitamin C $n = 55$; Kiwifruit $n = 57$.

Table 4. Total sample plasma vitamin C concentrations (μmol/L) by condition during the study period.

	Placebo		**Vitamin C**		**Kiwifruit**	
	n	**Mean (SD)**	***n***	**Mean (SD)**	***n***	**Mean (SD)**
Week 0 (Lead-in)	54	31.93 (15.33)	56	35.10 (19.6)	56	40.08 (17.7)
Week 2 (Lead-in)	52	38.19 (20.82)	55	39.10 (20.96)	57	37.90 (17.88)
Week 4 (Intervention)	52	36.82 (18.89)	53	76.34 (17.57) ****[a]	55	78.91 (20.21) ****[a]
Week 6 (Intervention)	52	39.25 (20.82)	52	80.84 (17.14) ****[a]	55	74.88 (16.03) ****[a] *[b]
Week 8 (Washout)	51	38.27 (17.66)	51	54.94 (16.52) ****[abc]	53	50.35 (16.15) ****[abc]

Note. Results shown are means (standard deviations). [a] Comparison with Week 2 (Lead-in); [b] Comparison with Week 4 (Intervention); [c] Comparison with Week 6 (Intervention). * $p < 0.05$, **** $p < 0.0001$.

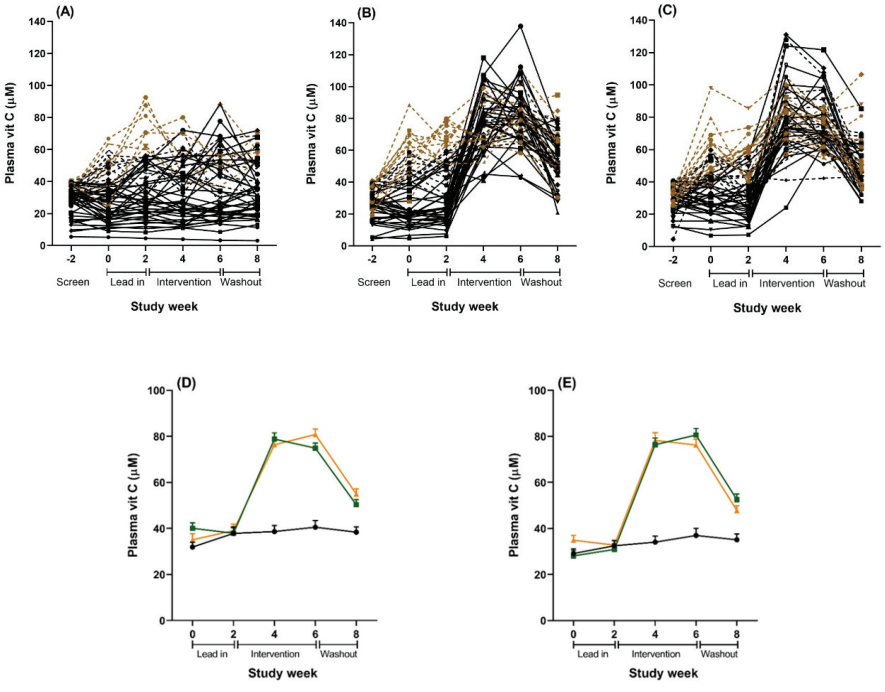


Figure 3. Plasma vitamin C concentrations over the study period for the total sample (n = 167). (**A**–**C**): Individual results for all participants randomized to (**A**) placebo, (**B**) vitamin C tablet, and (**C**) kiwifruit conditions. Solid black lines are individuals in the per protocol (PP) analyses with lead-in plasma vitamin C levels <40 μmol/L (n = 92). Combined black lines (solid and dotted) are individuals in the below saturation analyses with lead-in plasma vitamin C levels <60 μmol/L (n = 128). Brown dotted lines are individuals with lead-in plasma vitamin C levels >60 μmol/L excluded from the below saturation analysis (n = 39). (**D**): Combined results of all plasma vitamin C levels for the total sample (n = 167). Black = placebo tablet, n = 54; orange = vitamin C tablet, n = 56; green = kiwifruit, n = 57. (**E**): Combined results of all plasma samples for the cohort sub-set with vitamin C concentrations <60 μmol/L at baseline and lead in (n = 128). Black = placebo tablet, n = 45; orange = vitamin C tablet, n = 40; green = kiwifruit, n = 43. Data are the raw unadjusted means ± SE.

The changes in the primary vitality outcomes by condition for the total sample (n = 167) are shown in Figure 4 and Supplemental Table S1, which compares two weeks of intervention (Study Week 4), the end of the intervention (Study Week 6), and washout (Study Week 8), to the end of the lead-in period (Study Week 2). For the POMS total mood disturbance, only participants in the kiwifruit condition (Figure 4) showed significantly reduced total mood disturbance after two weeks of intervention (Study Week 4), and at the end of the intervention (Study Week 6) which returned to baseline at washout (Study Week 8). Participants in the vitamin C condition showed non-significant reductions in their POMS total mood disturbance (Figure 4) and those in the placebo condition were unchanged over time (Figure 4). Participants in the kiwifruit condition showed a trend reduction in fatigue after two weeks of supplementation (Figure 4) but not at four weeks of supplementation (Study Week 6). For participants in the vitamin C condition, fatigue increased significantly upon withdrawal of vitamin C during washout ($p < 0.05$). No changes in fatigue were seen in the placebo condition. For well-being, there was a significant increase in well-being after two weeks of kiwifruit supplementation, which remained elevated after four weeks of supplementation and did not decrease at washout (Figure 4). Participants in the vitamin C group also showed a trend increase in well-being at the end of the

intervention (Figure 4), which was eliminated at washout. There was no change in well-being in the placebo group except for an increase between Study Week 4 and 6 of placebo supplementation, which was mainly due to well-being returning to baseline levels (Supplemental Table S1). There was no reported or observed harm or adverse events as a result of any intervention arm in this study.

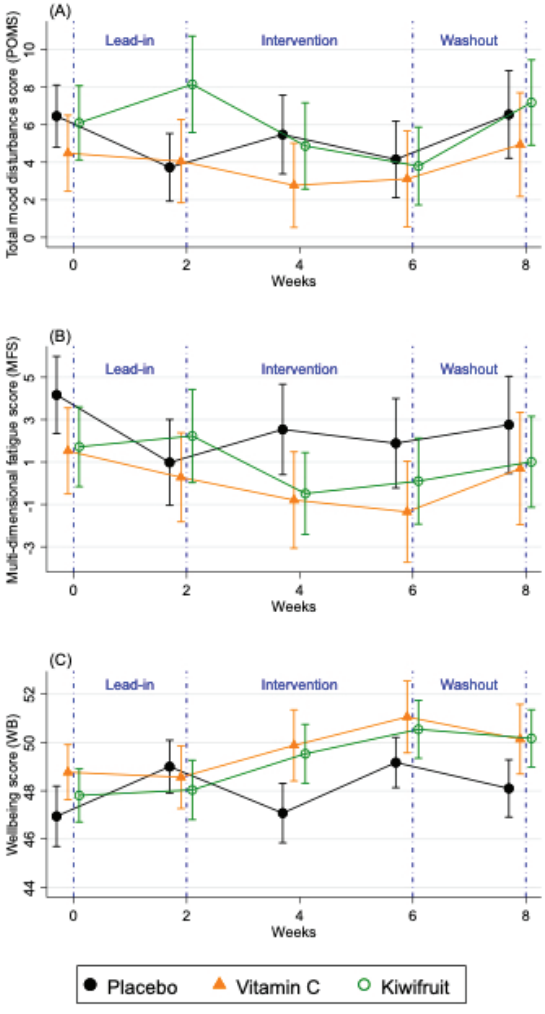


Figure 4. Changes in fortnightly (**A**) total mood disturbance scores (POMS), (**B**) multidimensional fatigue score (MFS), and (**C**) well-being (WB) over the study period for the total sample (n = 167). Results are presented as means ± SE for participants allocated to placebo tablet (black lines), vitamin C tablet (orange lines) and kiwifruit (green lines) conditions. Lead-in Week 2 served as baseline, which was compared against Week 4 and Week 6 of the intervention.

The analysis of variance results is presented in Table 5. The available cases analysis (far right columns) showed significant condition x time effects when comparing Study Week 2 (end of lead-in) with Study Week 4 (second week of intervention) for POMS total mood disturbance (p = 0.035, partial eta squared (η_p^2) = 0.041) and well-being (p = 0.009, η_p^2 = 0.058) and a trend for fatigue (p = 0.063, η_p^2 = 0.034). These effects remained significant when controlling for the demographic and health

covariates. However, there were no significant condition x time effects when comparing Study Week 2 (end of lead-in) with Study Week 6 (end of intervention; all ns), and there was only a trend for well-being when comparing all three time points together ($p = 0.054$, $\eta_p^2 = 0.029$). Results were similar in the adjusted models.

The intervention effects were not due to differences between conditions in BMI (Welch's F(2.99.036) = 0.104, $p = 0.901$), physical activity (intervention weeks 3–4, F(2.158) = 0.285, $p = 0.752$; intervention weeks 5–6, F(2.157) = 0.029, $p = 0.972$), sleep quality (intervention weeks 3–4, F(2.158) = 0.657, $p = 0.520$; intervention weeks 5–6, F(2.157) = 0.643, $p = 0.527$), sleep quantity (intervention weeks 3–4, F(2.158) = 0.370, $p = 0.692$), dietary factors such as total protein intake (F (2.156) = 0.556, $p = 0.575$), or total carbohydrate intake (F (2.156) = 2.198, $p = 0.114$). There was a trend difference between conditions for sleep quantity (intervention weeks 5–6, F(2.157) = 2.496, $p = 0.086$), which was driven by a trend for participants in the vitamin C condition to sleep for marginally longer compared to the placebo condition ($p = 0.095$), but not the kiwifruit condition ($p = 0.194$). There was also a trend difference between conditions for total energy intake (KJ) (F (2.156) = 2.706, $p = 0.070$). A post hoc Tukey HSD test indicated a trend for lower total energy intake (KJ) for the kiwifruit condition during the second half of the intervention (weeks 5–6) compared to the vitamin C condition ($p = 0.073$). This difference between conditions was driven by total fat intake (Welch's F (2.96.204) = 4.016, $p = 0.021$). A post hoc Games-Howell test indicated significantly lower total fat intake (grams) for participants in the kiwifruit condition during the second half of the intervention (weeks 5–6) compared to participants in the vitamin C condition ($p = 0.019$; Supplemental Table S2). Importantly, as shown in Table 5, adjusting for the difference in sleep quantity and fat intake did not change the condition × time effects.

Sensitivity analysis using pattern mixture models based on monotone missing data for the primary outcomes suggested that the observed cases analysis was mostly robust (see Online Supplementary Material). When looking at different patterns of missing data, the results maintained the same patterns (Supplemental Table S12). Sensitivity analysis of total mood disturbance, fatigue, and well-being did not weaken results.

The changes in the vitality outcomes by condition for the below saturation vitamin C sample ($n = 128$) are shown in Supplemental Table S5, Supplemental Figure S1, and Table 5. When testing individuals with consistently below saturation levels of vitamin C during the lead-in phase, participants in the vitamin C arm showed reductions in fatigue after supplementing with vitamin C for two weeks ($p = 0.065$) and four weeks ($p = 0.017$), which returned to baseline levels at washout ($p = 0.379$). ANOVA results from Table 5 also showed significant condition x time effects for fatigue in the low vitamin C group ($n = 128$). These patterns for fatigue were not observed when analysing the full sample suggesting that vitamin C benefits fatigue in those with low vitamin C levels. By contrast, the effects of kiwifruit were similar in the PP, below saturation vitamin C, and observed cases analyses and tended to occur early in the intervention at two weeks, whereas the effects from vitamin C alone tended to build up through the intervention. Results for the PP sample ($n = 92$) mostly mirrored results for the below saturation vitamin C sample and are shown in Supplemental Table S9, Supplemental Figure S2, Tables 5 and 6.

Table 5. Effect of Vitamin C tablet or Kiwifruit on mood disturbance, fatigue, and well-being after 2 and 4 weeks of intervention, compared to week 2 of baseline (end of Lead in) ($n = 161$).

	Placebo Group ($n = 52$)		Vitamin C Tablet Group ($n = 53$)				Kiwifruit Group ($n = 56$)			
	Baseline Mean (SD)	Mean Change from Baseline (SD)	Baseline Mean (SD)	Mean Change from Baseline (SD)	Mean Difference in Change (95% CI) Compared to Placebo [a]	*p*-Value	Baseline Mean (SD)	Mean Change from Baseline (SD)	Mean Difference in Change (95% CI) Compared to Placebo [a]	*p*-Value
POMS total score [b]										
2 weeks of intervention	3.7 (13.0)	1.8 (13.1)	3.9 (16.7)	−1.1 (10.1)	−3.0 (−6.9, 0.8)	0.121	8.6 (19.4)	−3.7 (8.9)	−4.0 (−8.1, 0.2)	0.062
4 weeks of intervention [c]	3.7 (13.0)	0.4 (9.9)	3.9 (16.9)	−0.8 (14.8)	−1.2 (−5.5, 3.2)	0.604	7.3 (17.0)	−3.5 (11.4)	−3.0 (−7.3, 1.3)	0.177
Fatigue score [d]										
2 weeks of intervention	1.0 (14.6)	1.6 (8.9)	0.2 (15.8)	−1.0 (9.2)	−2.8 (−6.3, 0.6)	0.108	2.4 (16.7)	−2.8 (10.7)	−3.8 (−7.4, −0.2)	0.038
4 weeks of intervention [c]	1.0 (14.6)	0.9 (8.9)	0.1 (16.0)	−1.5 (12.8)	−2.6 (−6.4, 1.3)	0.194	1.6 (15.9)	−1.5 (9.7)	1.5 (−4.4, 7.4)	0.617
Well-being score [e]										
2 weeks of intervention	49.0 (7.0)	−1.9 (8.3)	49.0 (9.4)	0.8 (4.4)	2.8 (0.5, 5.1)	0.018	47.8 (9.3)	1.7 (5.4)	3.4 (1.2, 5.7)	0.003
4 weeks of intervention [c]	49.0 (7.9)	0.2 (6.6)	49.2 (9.4)	1.8 (7.8)	1.7 (−0.8, 4.3)	0.180	48.2 (9.0)	2.4 (7.0)	2.0 (−0.5, 4.5)	0.124

Note: POMS = Profile of Mood States questionnaire. [a] Mean differences, 95% CI, and *p*-values determined using a mixed effects regression model adjusted for baseline scores and with the two randomisation clusters as random effects. [b] Higher score means higher mood disturbance overall (worse mood) (minimim possible score = −20, maximum = 100). [c] One participant in each of the vitamin C and kiwifruit groups did not have data at 4 weeks of intervention. [d] Higher multi-dimensional fatigue score means higher fatigue (minimum possible score = −24, maximum = 96). [e] Higher well-being score means higher well-being (minimum possible score = 14, maximum = 70).

Table 6. Effect of Vitamin C tablet or Kiwifruit on mood disturbance, fatigue, and well-being after 2 and 4 weeks of intervention, compared to week 2 of baseline (end of lead-in), for those with plasma vitamin C below saturation < 60 μmol/L before intervention ($n = 128$) and those with plasma vitamin C below 40 μmol/L before the intervention, as per protocol ($n = 92$).

	Plasma Vitamin C Below Saturation ($n = 128$)				Per Protocol ($n = 92$)			
	Vitamin C Tablet Group ($n = 40$)		Kiwifruit Group ($n = 43$)		Vitamin C Tablet Group ($n = 29$)		Kiwifruit Group ($n = 27$)	
	Mean Difference in Change (95% CI) Compared to Placebo [a]	*p*-Value	Mean Difference in Change (95% CI) Compared to Placebo [a]	*p*-Value	Mean Difference in Change (95% CI) Compared to Placebo [a]	*p*-Value	Mean Difference in Change (95% CI) Compared to Placebo [a]	*p*-Value
POMS total score [b]								
2 weeks of intervention	−4.2 (−8.5, 0.2)	0.060	−3.9 (−8.5, 0.6)	0.087	−7.9 (−13.2, −2.6)	0.003	−4.5 (−10.3, 1.2)	0.121
4 weeks of intervention [c]	−3.5 (−7.8, 0.7)	0.105	−2.8 (−7.3, 1.6)	0.212	−3.6 (−8.4, 1.2)	0.137	−3.6 (−8.7, 1.6)	0.174
Fatigue score [c]								
2 weeks of intervention	−3.7 (−7.2, −0.2)	0.036	−4.4 (−8.0, −0.8)	0.017	−5.9 (−9.9, −1.8)	0.005	−4.1 (−8.5, 0.4)	0.073
4 weeks of intervention [c]	−4.7 (−8.5, −0.9)	0.016	−1.4 (−5.3, 2.6)	0.505	−4.4 (−8.9, 0.1)	0.057	−0.7 (−5.5, 4.2)	0.790
Well-being score [d]								
2 weeks of intervention	2.5 (0.01, 5.0)	0.049	4.8 (2.2, 7.4)	< 0.001	3.0 (0.0, 6.0)	0.047	4.1 (0.7, 7.6)	0.017
4 weeks of intervention [c]	2.3 (−0.3, 4.9)	0.077	2.7 (−0.01, 5.4)	0.051	2.2 (−0.7, 5.1)	0.143	2.6 (−0.6, 5.7)	0.112

Note: POMS = Profile of Mood States questionnaire; [a] Mean differences, 95% CI, and p-values determined using a mixed effects regression model adjusted for baseline scores, age, ethnicity, years of university study, and plasma vitamin C levels before the intervention, and with the two randomisation clusters as random effects. [b] Higher score means higher mood disturbance overall (worse mood) (minimim possible score = −20, maximum = 100). [c] Higher multi-dimensional fatigue score means higher fatigue (minimum possible score = −24, maximum = 96). [d] Higher well-being score means higher well-being (minimum possible score = 14, maximum = 70).

4. Discussion

Our randomised controlled trial aimed to investigate the extent to which vitamin C contributes to the mood improvements reported in association with increased fruit and vegetable intake [3,25–28]. We were able to compare the effects of increased vitamin C intake from a fruit source (SunGold™ kiwifruit) with the equivalent amount of vitamin C in tablet form and with a placebo tablet on subjective vitality in healthy young adults. Improvements in mood and well-being were apparent for the group consuming kiwifruit, whereas improvements in fatigue and well-being were apparent for the group consuming vitamin C but only if they had low levels of vitamin C as selected in the below saturation vitamin C or per protocol subgroups analyses. Importantly, there were no changes in any outcomes in the placebo group.

These results indicate that the vitamin C content of the fruit may be a significant component contributing to improvements in vitality. However, the kiwifruit-mediated effects were broader (affecting more outcomes for more people), and occurred earlier in the intervention at two weeks, whereas the effects from vitamin C alone were narrower (affecting fewer outcomes for specific types of people) and tended to occur throughout the intervention. These differences are notable, particularly given that the kiwifruit and tablet interventions resulted in a comparable increase in plasma vitamin C levels and there are no known differences in uptake and bioavailability of "synthetic" versus "natural" vitamin C [44]. In this regard, our study provides novel insight into the potential differences between single-nutrient supplementation with a vitamin C tablet versus provision of the same content of vitamin C from a whole fruit. It appears that additional and maybe extended benefits result from whole fruit consumption. This finding reinforces the potential health and well-being benefit from whole food rather than single-nutrient dietary supplementation, although neither intervention showed any harmful side-effects.

Other compounds in whole kiwifruit that could account for their broader effects include fibre, folate, or potassium, all of which are relatively high in SunGold™ kiwifruit [33,45,46]. Increased dietary fibre intake can benefit the gut microbiome, which has been linked to changes in mental health [47–49]. Low folate intake has been associated with negative mental health outcomes, such as depression [50], and a low sodium and high potassium diet is associated with greater vigour and lower levels of depression, tension, and POMS total mood disturbance [51]. However, the relationship between dietary factors and mental health is complex and is likely mediated by several micronutrient interactions. Further research is required to determine the full impact that dietary factors have on mental health in isolation. Interestingly, we found that supplementing with kiwifruit was associated with reductions in the intake of dietary fat. This may be due to participants substituting kiwifruit for high-fat snacks and/or reductions in appetite from consuming a nutrient-dense whole food. Controlling for fat intake did not attenuate or eliminate the intervention effect, suggesting that reduced fat intake is unlikely to be driving the differences in vitality between the kiwifruit and vitamin C or placebo tablet groups.

Our study design had numerous strengths. The use of placebo and control groups is frequently problematic for food intervention studies and determination of the active ingredient in any particular food is challenging [52]. By assigning control arms that included a matched vitamin C tablet and a placebo tablet, we were able to include a blinded intervention (tablets) that avoided the complexities associated with the assignment of a different control food that can introduce potentially confounding nutrients. Use of the vitamin C tablet and placebo arms therefore assessed potential benefits of vitamin C on mental health in isolation to other dietary components [53]. Additional strengths included the length of the eight-week trial with lead-in and washout periods, extensive screening to identify people with low vitamin C levels that ensured that the intervention was appropriately targeted towards individuals in whom vitamin C supplementation would improve baseline status, strict inclusion and exclusion criteria to omit people with conditions that might affect vitamin C processing, and objective measurement of blood vitamin C levels at five time points.

At below-saturation levels, plasma vitamin C concentrations are very sensitive to changes in dietary intake [16]. Our observation that a number of individuals with an initial plasma concentration

<40 µmol/L subsequently showed saturation levels >60 µmol/L during lead-in is consistent with a variable dietary intake for occasional fruit and vegetable eaters. This is potentially a limitation of the study. Running a per protocol (PP) and below saturation vitamin C analysis of those individuals whose plasma vitamin C levels were consistently low or below saturation during the lead-in period ensured that we could still understand how our intervention affected this susceptible population. This enabled us to show that single-nutrient supplementation with a vitamin C tablet was mainly beneficial in those below saturation levels of vitamin C. Further analysis of those with below saturation levels of vitamin C during lead-in allowed us to also assess the intervention on an adequately powered group (n = 128) in whom increases in vitamin C status was possible. The consistent monitoring of vitamin C levels is therefore a strength of this study and has enabled us to avoid the pitfalls of many other vitamin supplementation studies that have not monitored vitamin status, which potentially confounds interpretation [21,54]. Lastly, we noted a trend ethnicity imbalance between the kiwifruit group and the other conditions. Controlling for this imbalance reduced but did not eliminate our intervention effects, which suggest potential ethnicity-linked differences in the effects of vitamin C on vitality worthy of follow up. However, this remains an exploratory question for future research adequately powered to test for differences.

The similarities between the effects of vitamin C and kiwifruit on vitality outcomes suggests that the vitamin contributes to the beneficial effects of fruit consumption. This has often been suggested, but not directly tested. Vitamin C supports numerous functions in vivo that could account for these effects. It is an essential cofactor for two enzymes that synthesise carnitine that is necessary for fatty acid metabolism and energy production [55,56]. Vitamin C availability affects carnitine production in animals and humans and has been shown to affect energy production via fat metabolism in healthy individuals [24,55,57,58].

Vitamin C is also essential for the synthesis of neurotransmitters and peptide hormones in the nervous system and adrenal glands. The Cu-containing enzyme dopamine β-hydroxylase catalyses the conversion of dopamine to norepinephrine [14,59,60] and vitamin C deficiency results in decreased norepinephrine production in animals [61–63]. In addition, vitamin C is required for the regeneration of tetrahydrobiopterin, the enzyme cofactor for the tyrosine hydroxylase that produces dopamine [64]. Dopamine is a neurotransmitter important for contributing "zest to life" [65], suggesting a possible linking between vitamin C and vitality. The biosynthesis of many amidated peptide-based hormones, including oxytocin, vasopressin, thyrotropin-releasing hormone, and substance P, is catalysed by the vitamin C-dependent peptidylglycine α-amidating monooxygenase (PAM) [13,66–69]. Many of these hormones affect neural processes and could influence both physical and emotional well-being. In particular, oxytocin release is associated with many positive mood enhancing effects [70]. Strikingly, tissues where these catecholamines and peptide hormones are synthesised contain the highest levels of vitamin C in the body, i.e., pituitary, neurons, ovaries, testes, eyes, adrenals, placenta, thymus, and pancreas [13,69,71]. Based on the above research, it is clear that vitamin C has the potential to influence the nervous system.

However, it is also clear from our study that there are differences in effects between vitamin C alone and whole fruit. This is in line with other studies that have shown vitality gains with fruit and vegetable consumption independently of vitamin C status [25]. Together with the results from the current study, it would appear that there are indeed additional health benefits that are measurable in a healthy population and that can be gained from ensuring that essential micronutrients such as vitamin C are delivered through whole food rather than a supplement. Future research should also consider the wider mechanisms linking consumption of whole fruits and vegetables to subjective vitality.

Supplementary Materials: The following are available online at http://www.mdpi.com/2072-6643/12/9/2898/s1, Figure S1: Below saturation vitamin C sample (n = 128) changes in fortnightly measures of vitality (total mood disturbance, fatigue, and well-being) over the study period, Figure S2: Per protocol (PP) sample (n = 92) changes in fortnightly measures of vitality (total mood disturbance, fatigue, and well-being) over the study period, Table S1: Available cases sample (n = 167) raw mean scores (and standard deviations) of the primary outcome measures and secondary covariates overall and by condition, Table S2: Available cases sample (n = 167) macronutrient diet record

data (kilojoules of intake daily) for the placebo, vitamin C, and kiwifruit conditions, Table S3: Below saturation vitamin C sample (n = 128) participant characteristics overall and by condition, Table S4: Below saturation vitamin C sample (n = 128) plasma vitamin C concentrations (μmol/L) by condition during the study period, Table S5: Below saturation vitamin C sample (n = 128) raw mean scores (and standard deviations) of primary outcome measures and secondary covariates overall and by condition, Table S6: Below saturation vitamin C sample (n = 128) macronutrient diet record data for the placebo, vitamin C, and kiwifruit conditions, Table S7: Per protocol sample (n = 92) participant characteristics overall and by condition, Table S8: Per protocol sample (n = 92) plasma vitamin C concentrations (μmol/L) by condition during the study period, Table S9: Per protocol sample (n = 92) raw mean scores (and standard deviations) of the primary outcome measures and secondary covariates overall and by condition, Table S10: Per protocol sample (n = 92) macronutrient diet record data for the placebo, vitamin C, and kiwifruit conditions, Table S11: Discontinued participants (cumulative percentage (n)), and Table S12: Sensitivity analysis using pattern mixture models.

Author Contributions: Conceptualization, T.S.C. and M.C.M.V.; methodology, T.S.C. and M.C.M.V.; statistical analyses, J.J.H., T.S.C., B.D.F., and M.C.M.V.; investigation, T.S.C., B.D.F., J.M.P., and E.S.; resources, T.S.C., L.A.M., and M.C.M.V.; writing—original draft preparation, T.S.C., B.D.F., and M.C.M.V.; writing—review and editing, T.S.C., B.D.F., J.M.P., and M.C.M.V.; visualization, J.J.H. and M.C.M.V.; supervision, T.S.C. and M.C.M.V.; funding acquisition, T.S.C. and M.C.M.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Zespri International Ltd., grant number HN1788, and the University of Otago.

Acknowledgments: We thank Anitra C. Carr for input on the study design. We also thank Lauren Alessi, Jayde A. M. Flett, Nadia Norazizan, and Rosemary Stamm for their assistance as coordinators of the study, and Natasha Amarasekara, Jessica Barton, and Shay-Ruby Wickett for their assistance with data collection. We are grateful to our registered nurse Margi Bryant who collected all blood samples during clinic visits.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Blanchflower, D.G.; Oswald, A.J.; Stewart-Brown, S. Is psychological well-being linked to the consumption of fruit and vegetables? *Soc. Indic. Res.* **2012**, *114*, 785–801. [CrossRef]
2. Piqueras, J.A.; Kuhne, W.; Vera-Villarroel, P.; Van Straten, A.; Cuijpers, P. Happiness and health behaviours in Chilean college students: A cross-sectional survey. *BMC Public Health* **2011**, *11*, 443. [CrossRef] [PubMed]
3. White, B.A.; Horwath, C.C.; Conner, T.S. Many apples a day keep the blues away-daily experiences of negative and positive affect and food consumption in young adults. *Br. J. Health Psychol.* **2013**, *18*, 782–798. [CrossRef] [PubMed]
4. Crichton, G.E.; Bryan, J.; Hodgson, J.M.; Murphy, K.J. Mediterranean diet adherence and self-reported psychological functioning in an Australian sample. *Appetite* **2013**, *70*, 53–59. [CrossRef]
5. Głąbska, D.; Guzek, D.; Groele, B.; Gutkowska, K. Fruit and vegetable intake and mental health in adults: A systematic review. *Nutrients* **2020**, *12*, 115. [CrossRef]
6. Block, G.; Norkus, E.; Hudes, M.; Mandel, S.; Helzlsouer, K. Which plasma antioxidants are most related to fruit and vegetable consumption? *Am. J. Epidemiol.* **2001**, *154*, 1113–1118. [CrossRef]
7. Granger, M.; Eck, P. Dietary Vitamin C in human health. *Adv. Food Nutr. Res.* **2018**, *83*, 281–310.
8. Levine, M.; Rumsey, S.C.; Wang, Y.; Park, J.; Kwon, O.; Amano, N. In situ kinetics: An approach to recommended intake of vitamin C. *Methods Enzymol.* **1997**, *281*, 425–437.
9. Rumsey, S.; Levine, M. Absorption, transport, and disposition of ascorbic acid in humans. *Nutr. Biochem.* **1998**, *9*, 116–130. [CrossRef]
10. Levine, M.; Wang, Y.; Padayatty, S.J.; Morrow, J. A new recommended dietary allowance of vitamin C for healthy young women. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 9842–9846. [CrossRef]
11. Hasselholt, S.; Tveden-Nyborg, P.; Lykkesfeldt, J. Distribution of vitamin C is tissue specific with early saturation of the brain and adrenal glands following differential oral dose regimens in guinea pigs. *Br. J. Nutr.* **2015**, *113*, 1539–1549. [CrossRef] [PubMed]
12. Meredith, M.E.; May, J.M. Regulation of embryonic neurotransmitter and tyrosine hydroxylase protein levels by ascorbic acid. *Brain Res.* **2013**, *1539*, 7–14. [CrossRef] [PubMed]
13. Prigge, S.T.; Mains, R.E.; Eipper, B.A.; Amzel, L.M. New insights into copper monooxygenases and peptide amidation: Structure, mechanism and function. *Cell. Mol. Life Sci.* **2000**, *57*, 1236–1259. [CrossRef]

14. Levine, M. Ascorbic acid specifically enhances dopamine beta-monooxygenase activity in resting and stimulated chromaffin cells. *J. Biol. Chem.* **1986**, *261*, 7347–7356. [PubMed]
15. Pihlajaniemi, T.; Myllyla, R.; Kivirikko, K.I. Prolyl 4-hydroxylase and its role in collagen synthesis. *J. Hepatol.* **1991**, *13* (Suppl. S3), S2–S7. [CrossRef]
16. Levine, M.; Conry-Cantilena, C.; Wang, Y.; Welch, R.W.; Washko, P.W.; Dhariwal, K.R.; Park, J.B.; Lazarev, A.; Graumlich, J.F.; King, J.; et al. Vitamin C pharmacokinetics in healthy volunteers: Evidence for a recommended dietary allowance. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 3704–3709. [CrossRef]
17. Carr, A.C.; Frei, B. Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans. *Am. Soc. Clin. Nutr.* **1999**, *69*, 1086–1107. [CrossRef]
18. Agarwal, A.; Shaharyar, A.; Kumar, A.; Bhat, M.S.; Mishra, M. Scurvy in pediatric age group—A disease often forgotten? *J. Clin. Orthop. Trauma* **2015**, *6*, 101–107. [CrossRef]
19. Harrison, F.E.; May, J.M. Vitamin C function in the brain: Vital role of the ascorbate transporter SVCT2. *Free Radic. Biol. Med.* **2009**, *46*, 719–730. [CrossRef]
20. Kinsman, R.A.; Hood, J. Some behavioral effects of ascorbic acid deficiency. *Am. J. Clin. Nutr.* **1971**, *24*, 455–464. [CrossRef]
21. Lykkesfeldt, J.; Poulsen, H.E. Is vitamin C supplementation beneficial? Lessons learned from randomised controlled trials. *Br. J. Nutr.* **2010**, *103*, 1251–1259. [CrossRef] [PubMed]
22. Léger, D. Scurvy. *Can. Fam. Physician* **2008**, *54*, 1403–1406. [PubMed]
23. Cheraskin, E.; Ringsdorf, W.M.J.; Medford, F.H. Daily vitamin C consumption and fatigability. *J. Am. Geriatr. Soc.* **1976**, *24*, 136–137. [CrossRef] [PubMed]
24. Johnston, C.S.; Barkyoumb, G.M.; Schumacher, S.S. Vitamin C supplementation slightly improves physical activity levels and reduces cold incidence in men with marginal vitamin C status: A randomized controlled trial. *Nutrients* **2014**, *6*, 2572–2583. [CrossRef]
25. Conner, T.S.; Brookie, K.L.; Carr, A.C.; Mainvil, L.A.; Vissers, M.C. Let them eat fruit! The effect of fruit and vegetable consumption on psychological well-being in young adults: A randomized controlled trial. *PLoS ONE* **2017**, *12*, e0171206. [CrossRef]
26. Mujcic, R.; Oswald, A.J. Evolution of well-being and happiness after increases in consumption of fruit and vegetables. *Am. J. Public Health* **2016**, *106*, 1504–1510. [CrossRef]
27. Rooney, C.; McKinley, M.C.; Woodside, J.V. The potential role of fruit and vegetables in aspects of psychological well-being: A review of the literature and future directions. *Proc. Nutr. Soc.* **2013**, *72*, 420–432. [CrossRef]
28. Jacka, F.N.; Pasco, J.A.; Mykletun, A.; Williams, L.J.; Hodge, A.M.; O'Reilly, S.L.; Nicholson, G.C.; Kotowicz, M.A.; Berk, M. Association of western and traditional diets with depression and anxiety in women. *Am. J. Psychiatry* **2010**, *211*, 55–68. [CrossRef]
29. Choi, J.E.; Ainsworth, B.E. Associations of food consumption, serum vitamins and metabolic syndrome risk with physical activity level in middle-aged adults: The National Health and Nutrition Examination Survey (NHANES) 2005–2006. *Public Health Nutr.* **2016**, *19*, 1674–1683. [CrossRef]
30. Paschalis, V.; Theodorou, A.A.; Kyparos, A.; Dipla, K.; Zafeiridis, A.; Panayiotou, G.; Vrabas, I.S.; Nikolaidis, M.G. Low vitamin C values are linked with decreased physical performance and increased oxidative stress: Reversal by vitamin C supplementation. *Eur. J. Nutr.* **2016**, *55*, 45–53. [CrossRef]
31. Harding, A.H.; Wareham, N.J.; Bingham, S.A.; Khaw, K.; Luben, R.; Welch, A.; Forouhi, N.G. Plasma vitamin C level, fruit and vegetable consumption, and the risk of new-onset type 2 diabetes mellitus: The European prospective investigation of cancer—Norfolk prospective study. *Arch. Intern. Med.* **2008**, *168*, 1493–1499. [CrossRef] [PubMed]
32. Carr, A.C.; Bozonet, S.M.; Pullar, J.M.; Vissers, M.C. Mood improvement in young adult males following supplementation with gold kiwifruit, a high-vitamin C food. *J. Nutr. Sci.* **2013**, *2*, e24. [CrossRef]
33. Nishiyama, I.; Yamashita, Y.; Yamanaka, M.; Shimohashi, A.; Fukuda, T.; Oota, T. Varietal difference in vitamin C content in the fruit of kiwifruit and other actinidia species. *J. Agric. Food Chem.* **2004**, *52*, 5472–5475. [CrossRef] [PubMed]
34. Griskevicius, V.; Delton, A.W.; Robertson, T.E.; Tybur, J.M. Environmental contingency in life history strategies: The influence of mortality and socioeconomic status on reproductive timing. *J. Personal. Soc. Psychol.* **2011**, *100*, 241–254. [CrossRef] [PubMed]
35. McNair, D.M.; Heuchert, J.W.P. *Profiles of Mood States—Technical Update*; Multi-Health Systems Inc.: Toronto, ON, Canada, 2005.

36. Stein, K.D.; Jacobsen, P.B.; Blanchard, C.M.; Thors, C. Further Validation of the Multidimensional Fatigue Symptom Inventory-Short Form. *J. Pain Symptom Manag.* **2004**, *27*, 14–23. [CrossRef] [PubMed]
37. Tennant, R.; Hiller, L.; Fishwick, R.; Platt, S.; Joseph, S.; Weich, S.; Parkinson, J.; Secker, J.; Stewart-Brown, S. The Warwick-Edinburgh Mental Well-being Scale (WEMWBS): Development and UK validation. *Health Qual. Life Outcomes* **2007**, *5*, 63. [CrossRef]
38. Pullar, J.M.; Bayer, S.; Carr, A.C. Appropriate handling, processing and analysis of blood samples is essential to avoid oxidation of vitamin C to dehydroascorbic acid. *Antioxidants* **2018**, *7*, 29. [CrossRef]
39. Carr, A.C.; Bozonet, S.M.; Pullar, J.M.; Simcock, J.W.; Vissers, M.C. A randomized steady-state bioavailability study of synthetic versus natural (kiwifruit-derived) vitamin C. *Nutrients* **2013**, *5*, 3684–3695. [CrossRef]
40. Kruse, R.L.; Alper, B.S.; Reust, C.; Stevermer, J.J.; Shannon, S.; Williams, R.H. Intention-to-treat analysis: Who is in? Who is out? *J. Fam. Pract.* **2002**, *51*, 969–971.
41. Jakobsen, J.C.; Gluud, C.; Wetterslev, J.; Winkel, P. When and how should multiple imputation be used for handling missing data in randomised clinical trials–a practical guide with flowcharts. *BMC Med Res. Methodol.* **2017**, *17*, 162. [CrossRef]
42. Dettori, J.R.; Norvell, D.C.; Chapman, J.R. The sin of missing data: Is all forgiven by way of imputation? *Glob. Spine J.* **2018**, *8*, 892–894. [CrossRef] [PubMed]
43. National Research Council. The prevention and treatment of missing data in clinical trials. In *Panel on Handling Missing Data in Clinical Trials*; National Academies Press: Washington, DC, USA, 2010.
44. Carr, A.C.; Vissers, M.C. Synthetic or food-derived vitamin C-are they equally bioavailable? *Nutrients* **2013**, *5*, 4284–4304. [CrossRef] [PubMed]
45. Richardson, D.P.; Ansell, J.; Drummond, L.N. The nutritional and health attributes of kiwifruit: A review. *Eur. J. Nutr.* **2018**, *57*, 2659–2676. [CrossRef]
46. U.S. Department of Agriculture. *National Nutrient Database for Standard Reference Legacy Release*; Agricultural Research Service: Beltsville, MD, USA, 2018.
47. Dash, S.; Clarke, G.; Berk, M.; Jacka, F.N. The gut microbiome and diet in psychiatry: Focus on depression. *Curr. Opin. Psychiatry* **2015**, *28*, 1–6. [CrossRef] [PubMed]
48. Scott, K.P.; Duncan, S.H.; Flint, H.J. Dietary fibre and the gut microbiota. *Nutr. Bull.* **2008**, *33*, 201–211. [CrossRef]
49. Tap, J.; Furet, J.-P.; Bensaada, M.; Philippe, C.; Roth, H.; Rabot, S.; Lakhdari, O.; Lombard, V.; Henrissat, E.; Corthier, G.; et al. Gut microbiota richness promotes its stability upon increased dietary fibre intake in healthy adults. *Environ. Microbiol.* **2015**, *17*, 4954–4964. [CrossRef]
50. Morris, M.S.; Fava, M.; Jacques, P.F.; Selhub, J.; Rosenberg, I.H. Depression and folate status in the US population. *Psychother. Psychosom.* **2003**, *72*, 80–87. [CrossRef]
51. Torres, S.J.; Nowson, C.A.; Worsley, A. Dietary electrolytes are related to mood. *Br. J. Nutr.* **2008**, *100*, 1038–1045. [CrossRef]
52. Staudacher, H.M.; Irving, P.M.; Lomer, M.C.; Whelan, K. The challenges of control groups, placebos and blinding in clinical trials of dietary interventions. *Proc. Nutr. Soc.* **2017**, *76*, 203–212. [CrossRef]
53. Adan, R.A.; van der Beek, E.M.; Buitelaar, J.K.; Cryan, J.F.; Hebebrand, J.; Higgs, S.; Schellekens, H.; Dickson, S.L. Nutritional psychiatry: Towards improving mental health by what you eat. *Eur. Neuropsychopharmacol.* **2019**, *29*, 1321–1332. [CrossRef]
54. Michels, A.J.; Frei, B. Myths, artifacts, and fatal flaws: Identifying limitations and opportunities in vitamin C research. *Nutrients* **2013**, *5*, 5161–5192. [CrossRef]
55. Dunn, W.A.; Rettura, G.; Seifter, E.; Englard, S. Carnitine biosynthesis from gamma-butyrobetaine and from exogenous protein-bound 6-N-trimethyl-L-lysine by the perfused guinea pig liver. Effect of ascorbate deficiency on the in situ activity of gamma-butyrobetaine hydroxylase. *J. Biol. Chem.* **1984**, *259*, 10764–10770.
56. Rebouche, C.J. Ascorbic acid and carnitine biosynthesis. *Am. J. Clin. Nutr.* **1991**, *54* (Suppl. S6), 1147S–1152S. [CrossRef] [PubMed]
57. Nelson, P.J.; Pruitt, R.E.; Henderson, L.L.; Jenness, R.; Henderson, L.M. Effect of ascorbic acid deficiency on the in vivo synthesis of carnitine. *Biochim. Biophys. Acta* **1981**, *672*, 123–127. [CrossRef]
58. Noland, R.C.; Koves, T.R.; Seiler, S.E.; Lum, H.; Lust, R.M.; Ilkayeva, O.; Stevens, R.D.; Hegardt, F.G.; Muoio, D.M. Carnitine insufficiency caused by aging and overnutrition compromises mitochondrial performance and metabolic control. *J. Biol. Chem.* **2009**, *284*, 22840–22852. [CrossRef] [PubMed]

59. Rush, R.A.; Geffen, L.B. Dopamine beta-hydroxylase in health and disease. *Crit. Rev. Clin. Lab. Sci.* **1980**, *12*, 241–277. [CrossRef] [PubMed]
60. May, J.M.; Qu, Z.C.; Nazarewicz, R.; Dikalov, S. Ascorbic acid efficiently enhances neuronal synthesis of norepinephrine from dopamine. *Brain Res. Bull.* **2013**, *90*, 35–42. [CrossRef]
61. Hoehn, S.K.; Kanfer, J.N. Effects of chronic ascorbic acid deficiency on guinea pig lysosomal hydrolase activities. *J. Nutr.* **1980**, *110*, 2085–2094. [CrossRef]
62. Deana, R.; Bharaj, B.S.; Verjee, Z.H.; Galzigna, L. Changes relevant to catecholamine metabolism in liver and brain of ascorbic acid deficient guinea-pigs. *Int. J. Vitam. Nutr. Res.* **1975**, *45*, 175–182.
63. Bornstein, S.R.; Yoshida-Hiroi, M.; Sotiriou, S.; Levine, M.; Hartwig, H.G.; Nussbaum, R.L.; Eisenhofer, G. Impaired adrenal catecholamine system function in mice with deficiency of the ascorbic acid transporter (SVCT2). *FASEB J.* **2003**, *17*, 1928–1930. [CrossRef]
64. May, J.M.; Qu, Z.C.; Meredith, M.E. Mechanisms of ascorbic acid stimulation of norepinephrine synthesis in neuronal cells. *Biochem. Biophys. Res. Commun.* **2012**, *426*, 148–152. [CrossRef]
65. Kringelbach, M.L.; Berridge, K.C. Toward a functional neuroanatomy of pleasure and happiness. *Trends Cogn. Sci.* **2009**, *13*, 479–487. [CrossRef] [PubMed]
66. Englard, S.; Seifter, S. The biochemical functions of ascorbic acid. *Annu. Rev. Nutr.* **1986**, *6*, 365–406. [CrossRef] [PubMed]
67. Eipper, B.A.; Mains, R.E. The role of ascorbate in the biosynthesis of neuroendocrine peptides. *Am. J. Clin. Nutr.* **1991**, *54* (Suppl. S6), 1153S–1156S. [CrossRef]
68. Kukucka, M.A.; Misra, H.P. HPLC determination of an oxytocin-like peptide produced by isolated guinea pig Leydig cells: Stimulation by ascorbate. *Arch. Androl.* **1992**, *29*, 185–190. [CrossRef] [PubMed]
69. Sheldrick, E.L.; Flint, A.P. Post-translational processing of oxytocin-neurophysin prohormone in the ovine corpus luteum: Activity of peptidyl glycine alpha-amidating mono-oxygenase and concentrations of its cofactor, ascorbic acid. *J. Endocrinol.* **1989**, *122*, 313–322. [CrossRef]
70. Ishak, W.W.; Kahloon, M.; Fakhry, H. Oxytocin role in enhancing well-being: A literature review. *J. Affect. Disord.* **2011**, *130*, 1–9. [CrossRef]
71. Luck, M.R.; Jungclas, B. Catecholamines and ascorbic acid as stimulators of bovine ovarian oxytocin secretion. *J. Endocrinol.* **1987**, *114*, 423–430. [CrossRef]

nutrients

MDPI

Article

Initial Evidence of Variation by Ethnicity in the Relationship between Vitamin C Status and Mental States in Young Adults

Benjamin D. Fletcher [1], Jayde A. M. Flett [2], Shay-Ruby Wickham [1], Juliet M. Pullar [3], Margreet C. M. Vissers [3] and Tamlin S. Conner [1,*]

[1] Department of Psychology, University of Otago, Dunedin 9016, New Zealand; ben.fletcher@postgrad.otago.ac.nz (B.D.F.); shay.wickham@postgrad.otago.ac.nz (S.-R.W.)
[2] Independent Researcher, Wellington 6011, New Zealand; jayde.flett@gmail.com
[3] Centre for Free Radical Research, Department of Pathology and Biomedical Science, University of Otago, Christchurch 8011, New Zealand; juliet.pullar@otago.ac.nz (J.M.P.); margreet.vissers@otago.ac.nz (M.C.M.V.)
* Correspondence: tamlin.conner@otago.ac.nz; Tel.: +64-3479-7624

Abstract: Higher fruit and vegetable intake has been associated with improved mood, greater vitality, and lower stress. Although the nutrients driving these benefits are not specifically identified, one potentially important micronutrient is vitamin C, an important co-factor for the production of peptide hormones, carnitine and neurotransmitters that are involved in regulation of physical energy and mood. The aim of our study was to investigate the cross-sectional relationship between blood plasma vitamin C status and mood, vitality and perceived stress. A sample of 419 university students (aged 18 to 35; 67.8% female) of various ethnicities (49.2% European, 16.2% East Asian, 8.1% Southeast/Other Asian, 9.1% Māori/Pasifika, 11.5% Other) provided a fasting blood sample to determine vitamin C status and completed psychological measures consisting of the Profile of Mood States Short Form (POMS-SF), the vitality subscale of the Rand 36-Item Short Form (SF-36), and the Perceived Stress Scale (PSS). Participants were screened for prescription medication, smoking history, vitamin C supplementation, fruit/juice and vegetable consumption, kiwifruit allergies, excessive alcohol consumption and serious health issues, and provided age, gender, ethnicity, and socioeconomic status information, which served as covariates. There were no significant associations between vitamin C status and the psychological measures for the sample overall. However, associations varied by ethnicity. Among Māori/Pasifika participants, higher vitamin C was associated with greater vitality and lower stress, whereas among Southeast Asian participants, higher vitamin C was associated with greater confusion on the POMS-SF subscale. These novel findings demonstrate potential ethnicity-linked differences in the relationship between vitamin C and mental states. Further research is required to determine whether genetic variation or cultural factors are driving these ethnicity differences.

Keywords: psychology; mental health; well-being; nutrition; micronutrients; healthy adults; ethnicity; Māori; Pasifika; Pacific; Asian

Citation: Fletcher, B.D.; Flett, J.A.M.; Wickham, S.-R.; Pullar, J.M.; Vissers, M.C.M.; Conner, T.S. Initial Evidence of Variation by Ethnicity in the Relationship between Vitamin C Status and Mental States in Young Adults. *Nutrients* **2021**, *13*, 792. https://doi.org/10.3390/nu13030792

Academic Editor: Carol S. Johnston

Received: 16 January 2021
Accepted: 24 February 2021
Published: 27 February 2021

1. Introduction

Higher fruit and vegetable intake is associated with various mental health benefits, including improved mood, vitality, and well-being, as well as decreased depression, anxiety, and stress [1–6]. However, the mechanisms by which fruit and vegetable intake confers mental health benefits remain unknown. Higher levels of micronutrients such as vitamin C have been associated with improved mood [1,7,8]. Individuals supplemented with high vitamin C foods, such as kiwifruit or vitamin C tablets, have also reported decreased total mood disturbance and fatigue, and increased levels of well-being and vitality [1,9]. Furthermore, mental health benefits are closely related to changes in vitamin C levels, but not other micronutrient levels, suggesting vitamin C may be an important factor underlying improvements [1,7].

Vitamin C is a required co-factor for a number of enzymes involved in the synthesis of various neurotransmitters, hormones, and catecholamines that may have an impact on mood states [10]. Humans are one of only a few species unable to synthesise vitamin C and, for us, it is therefore a dietary requirement, making us vulnerable to vitamin C deficiency [11]. One of the first signs of vitamin C insufficiency is the onset of lethargy and psychological symptoms, supporting the idea that vitamin C plays a role in mood outcomes. Low vitamin C intake can lead to hypovitaminosis C (<23 μmol/L) and scurvy (<11 μmol/L), a fatal condition characterised by early symptoms of depression, fatigue, and irritability, in addition to poor wound healing and gum disease [10,12–16].

A limited number of studies have specifically assessed the impact of vitamin C on mood in isolation from other micronutrients. Several case studies have reported that vitamin C supplementation in participants with various morbid and co-morbid diseases leads to improvements in measures of depression, fatigue, total mood disturbance, vigour, global health status, and physical, emotional, cognitive and social functioning [8,17–22]. However, healthy individuals need to be assessed to determine the generalisability of these associations. Dietary intervention with vitamin C has been reported to result in greater well-being and decreased levels of fatigue and anxiety in healthy participants [9,23,24]. In addition, a cross-sectional study of 139 healthy men (aged 18 to 35) found a small but statistically significant relationship between blood plasma vitamin C levels and mood, measured using the POMS questionnaire [7]. Participants with higher vitamin C levels reported a lower total mood disturbance, which was driven by reduced levels of depression, anger, and confusion on the POMS subscales. However, only men from a relatively homogenous sample of NZ Europeans (76%; Māori (9%); Unspecified (15%)) were assessed, and confounding variables which can influence vitamin C status and mood, such as smoking and alcohol use, were not measured. Several studies have indicated that ethnicity is a factor in variations in the prevalence of mood disturbance, although causation for these differences is by no means established [25,26].

The aim of the current study was to determine the association between blood plasma vitamin C and mood-related outcomes (mood disturbance, vitality, and perceived stress) in a larger and more ethnically heterogeneous sample of healthy adult men and women. We extended previous research by screening out participants with characteristics that might affect, and potentially confound, their vitamin C levels and mood-related outcomes (e.g., smoking, excessive alcohol consumption, prescribed medication, or pre-existing health conditions, such as diabetes [27–30]) and controlled for demographic and health characteristics related to both mental health and vitamin C outcomes. Questionnaire measures of mood (POMS-short form), vitality (SF-36), and perceived stress (PSS), as well as fasting blood plasma vitamin C levels, were collected. It was hypothesised that vitamin C levels would be positively associated with mood and vitality scores, and negatively associated with perceived stress, when controlling for covariates. Exploratory analyses tested gender and ethnicity as moderators of the relationship between vitamin C and mood, as less is known about these associations in non-European and mixed gendered samples.

2. Materials and Methods

2.1. Participants and Procedure

Participants were recruited during screening for the KiwiC for Vitality Intervention Study (see Conner et al., 2020)) [9]. The trial was preregistered with the Australian and New Zealand Clinical Trial Registry (Trial ID: ACTRN12617001031358) and approved by the New Zealand Health and Disability Ethics Committee (17/NTB/104). Psychological measures were added to the screening protocol for the second wave of recruitment and these participants were included in this analysis. For full protocol and methods used, see Conner et al. (2020) [9]. Recruitment for the present dataset began in February 2018 and ended in April 2018, and used advertisements placed around the University of Otago and Otago Polytechnic campuses in Dunedin, New Zealand. Students interested in participating signed up through a brief online survey to pre-assess their eligibility (see Table 1).

Those who met eligibility criteria were invited to a 30-minute clinic visit in the Department of Human Nutrition clinic located at the University of Otago.

Table 1. Eligibility criteria assessed in the online screening and screening appointment.

Inclusion Criteria (All Required for Inclusion)	Exclusion Criteria (Only One Required for Exclusion)
Any gender aged 18–35 years	Taking prescription medication (within past three months)
Non-smoker	Allergy/intolerance to kiwifruit
Currently a student	Recent smoker (within past year)
	Taking vitamin C supplements (within past three months)
	High fruit/juice and vegetable consumption (≥5 servings/day)
	Excessive alcohol consumption (>21 standard drinks/week)
	Serious health issues, such as diabetes mellitus, kidney disease, bleeding disorders, or clinical depression
	Fainting due to fear of needles

During the clinic visit, participants signed informed consent before completing a one-page re-screening survey asking about health conditions, medication use, fear of needles, fruit and vegetable consumption, vitamin C supplement usage, juice intake, age, and fasting status, to ensure eligibility criteria were still met. Participants then gave a fasted blood sample, after which they completed the rest of the survey detailing measures of demographic characteristics, mood (POMS), vitality (SF-36), and stress (PSS; see Measures section). After completing the survey, participants were provided with breakfast and reimbursed $20.

2.2. Measures

2.2.1. Vitamin C Analysis

Participants provided one 9 mL sample of fasting blood, collected into a BD EDTA Vacutainer that was immediately placed on ice and processed at 4 °C within 2 h of collection. Plasma was separated by centrifugation at 1000× *g* for 10 min and 700 µL was extracted with 700 µL of cold 0.54 mol/L perchloric acid/DTPA solution [31]. Samples were vortexed and centrifuged at 13,000× *g* for two minutes to pellet the protein precipitate. Two samples of 500 µL supernatant were stored at −80 °C. Vitamin C was measured by High Performance Liquid Chromatography with electrochemical detection (HPLC-ECD) [31].

2.2.2. Demographics

The survey was used to record age, gender (male, female, gender diverse), ethnicity (based on New Zealand census categories, tick all that apply: New Zealand European, Māori, Samoan, Cook Island Māori, Tongan, Niuean, Chinese, Indian, Another stated ethnicity with free text response), and current socioeconomic status (SES) using three items ("I have enough money to buy the things I want"; "I don't need to worry too much about paying my bills"; "I don't think I'll have to worry about money too much in the future") rated from 1 (strongly disagree) to 7 (strongly agree) which were averaged (Cronbach's $\alpha = 0.762$) [32].

2.2.3. Mood

The Profile of Mood States Short Form (POMS-SF) [33] is a 35-item measure of mood experienced "during the past week, including today". The items cover six dimensions of mood states: Tension, depression, anger, fatigue, confusion, and vigour and are rated on a five-point Likert scale (1 = not at all to 5 = extremely). The positive items for vigour were summed together and subtracted from the sum of the negative items (tension, depression, anger, fatigue and confusion) to determine an overall mood score, called total mood disturbance (TMD). TMD scores for the POMS short form range from −20 to 100, with higher scores indicative of greater total mood disturbance (Cronbach's $\alpha = 0.899$). Subscales

were also analysed separately (αs tension = 0.843, depression = 0.825, anger = 0.799, fatigue = 0.860, confusion = 0.773, vigor = 0.828).

2.2.4. Vitality

The Rand 36-Item Short Form (SF-36) [34] is a 36-item patient report survey measuring overall health. The four-item subscale measuring vitality "during the past week including today" was used in the present study (did you feel: Full of life, have a lot of energy, worn out, and tired). Items were rated on a six-point Likert scale (0 = none of the time to 5 = all of the time). Scores were recoded to a 0 to 100 scale (0, 20, 40, 60, 80, 100; reverse scoring worn out and tired), and averaged, with higher scores indicating greater vitality (Cronbach's $\alpha = 0.740$).

2.2.5. Stress

The four-item version of the Perceived Stress Scale (PSS) [35] was used to measure the degree to which situations in one's life are appraised as stressful "during the past week, including today". Items were rated on a five-point Likert scale (0 = never to 4 = very often). Scores range from 0 to 16, with higher scores indicating higher perceived stress (Cronbach's $\alpha = 0.645$).

2.3. Analysis

Data were analysed with SPSS (IBM SPSS Statistics, version 26) and R (version 3.6.0) with the alpha level set at 0.05. To determine the relationship between vitamin C levels and demographic factors, one-way ANOVAs were used for categorical variables, independent t-tests were used for binomial variables, and Pearson's r was used for continuous variables. Initially, scatterplots of the relationship between vitamin C and the three psychological measures (total mood disturbance, vitality, and perceived stress) for the entire sample were created. An unadjusted regression line was fitted for each plot. Multiple regression was then conducted to determine the adjusted association between vitamin C and the three psychological measures controlling for gender (0 = male, 1 = female), age (centred), SES (centred), and ethnicity (dummy coded). To dummy code ethnicity, participants were grouped into five categories (Europeans, East Asians, Southeast and other Asians, Māori and Pasifika, Other). Originally, all participants of Asian descent were grouped together. However, due to finding heterogeneity in the relation between vitamin C and mood outcomes for participants from different Asiatic regions, we subsequently separated East Asian participants from Southeast and Other Asian participants (Table 2). Ethnicity was therefore entered as a set of four dummy codes: Ethnicity D1 compared European vs. East Asians, Ethnicity D2 compared European vs. Southeast and Other Asians, Ethnicity D3 compared European vs. Māori/Pasifika and Ethnicity D4 compared Europeans with all remaining minority ethnicities.

Next, we examined whether the relationship between vitamin C and the outcomes varied by ethnicity. Scatterplots of the relationship between vitamin C and the three psychological measures (total mood disturbance, vitality, and perceived stress) for the five ethnicity groups were created separately and an unadjusted regression line was fitted for each plot. A fully adjusted moderation model was then used to determine whether there were ethnicity differences in the relationship between vitamin C and each outcome, adjusting for the covariates as above. In the fully adjusted moderation model, the interaction terms for vitamin C (centred) by ethnicity dummy codes were added. Initially, Europeans were set as the reference group, with subsequent models changing the reference group to test for all differences between groups. Gender as a moderator was also tested in the fully adjusted moderation model, by entering the vitamin C (centred) by gender (male = 0, female = 1) interaction term.

Table 2. Sample characteristics (n = 419).

	Mean (SD)	Minimum	Maximum
Age	21.13 (3.33)	18.00	35.00
SES	4.72 (1.22)	1.00	7.00
Vitamin C in μmol/L	54.90 (20.19)	4.29	118.91
Total mood disturbance	9.88 (14.42)	−18.00	71.00
Vitality	60.93 (15.09)	15.00	95.00
Perceived stress	5.31 (2.06)	0.00	11.00
			***n* (% of sample)**
Gender	Female		284 (67.78%)
	Male		135 (32.22%)
	Gender diverse		0 (0.00%)
Ethnicity	European		206 (49.16%)
	NZ European		187 (44.63%)
	Other European		19 (4.53%)
	Asian		129 (30.78%)
	East Asian [a]		68 (16.23%)
	Southeast Asian [b]		34 (8.11%)
	Other Asian [c]		27 (6.44%)
	Māori or Pasifika [d]		38 (9.07%)
	Other Ethnicities		46 (10.98%)
	Indian		25 (5.97%)
	Multiple Ethnicities		12 (2.86%)
	Middle Eastern		4 (0.95%)
	African		3 (0.72%)
	Not Specified		2 (0.48%)

SES = socioeconomic status [a] Includes Chinese, Hong Kongese, Korean, Japanese and Taiwanese ethnicities. [b] Includes Malaysian, Thai, and Singaporean ethnicities. [c] Includes Vietnamese, Bangladeshi, Indonesian, Pakistani, Filipino, Siberian, Sri Lankan, mixed Asian and not specified Asian ethnicities. [d] Includes Māori, Samoan, Tokelauan, Tongan, Tuvaluan, Fijian, mixed Māori and European (n = 21) and mixed Samoan and European (n = 3).

3. Results

3.1. Descriptive Statistics

Figure 1 shows the flow diagram of participants. A total of 424 participants attended the clinic visit and signed informed consent, after which two participants were excluded before blood was taken due to taking prescription medication and three participants were unable to give blood. The final sample for analysis was 419 participants (135 males, 284 females) aged between 18- and 35-years-old, with a mean age of 21.13 years (Standard deviation (SD) = 3.33), of various ethnicities (see Table 2). Table 2 presents the sample characteristics. Half of the participants identified as European (49.16%), with others identifying as Asian (30.78%), Māori or Pasifika (9.07%), Indian (5.97%), Middle Eastern (0.95%), African (0.72%), Unspecified (0.48%) or Other/Multiple ethnicities endorsed (2.86%). Asian participants were predominantly either East Asian (Chinese 13.6%) or Southeast Asian (Malaysian 7.6%). Participants average SES score was 4.72 (SD = 1.22), indicating that the sample was not financially stressed and did not feel as though they were deprived of resources at the present moment or in the foreseeable future. Vitamin C scores were normally distributed (range 4.29–118.91 μmol/L) and were consistent with the population average, with a mean of 54.90 μmol/L (SD = 20.19) [36]. One percent of the sample had deficient vitamin C (less than 11 μmol/L), 4.5% had low levels of vitamin C (11–23 μmol/L), 33.4% had inadequate vitamin C levels (23–50 μmol/L), and 61.1% had adequate or saturated levels of vitamin C (50+ μmol/L; Supplementary Table S1).

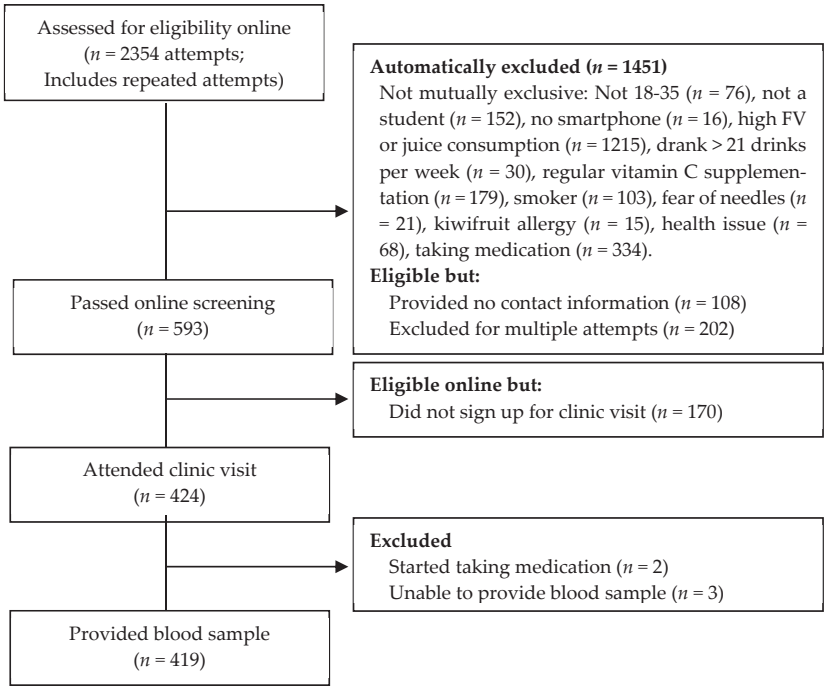


Figure 1. Participant flow diagram.

Prior to the main analyses, vitamin C differences by demographic factors and covariates were identified, such that differences in vitamin C were found for gender, ethnicity, age, and fruit and vegetable consumption. Specifically, men had a trend lower average vitamin C level (M = 52.55, SD = 18.46) than women (M = 56.02, SD = 20.90) t (295.11) = −1.721, p = 0.086 (unequal variances). Vitamin C also varied by ethnicity (Supplementary Table S1). Vitamin C levels were highest among Māori and Pasifika (M = 60.96, SD = 16.80) and Europeans (M = 59.35, SD = 19.51), and significantly lower in other ethnicities (M = 52.76, SD = 20.77), East Asians (M = 51.23, SD = 19.16), and Southeast/Other Asians (M = 41.83, SD = 18.62), F (4,414) = 11.49, $p < 0.001$ (Figure 2 and Supplementary Table S1). Age was negatively correlated with vitamin C levels (r = −0.133, p = 0.006), whereby older participants (up to age 35) had lower vitamin C levels. Fruit and vegetable consumption was also significantly positively correlated with vitamin C level (r = 0.201, $p < 0.001$). There were also ethnicity differences in fruit and vegetable consumption that paralleled the ethnicity differences in vitamin C level. Māori/Pasifika reported the highest fruit and vegetable consumption (M = 1.89 serves per day, SD = 1.01), followed by Europeans (M = 1.68, SD = 0.86), East Asians, (M = 1.29, SD = 0.72), Other (M = 1.28, SD = 0.75), and Southeast/Other Asians (M = 1.03, SD = 0.59: *Welch's F* (4,127.68) = 14.00, $p < 0.001$). SES was not significantly correlated with vitamin C level (r = −0.079, p = 0.105).

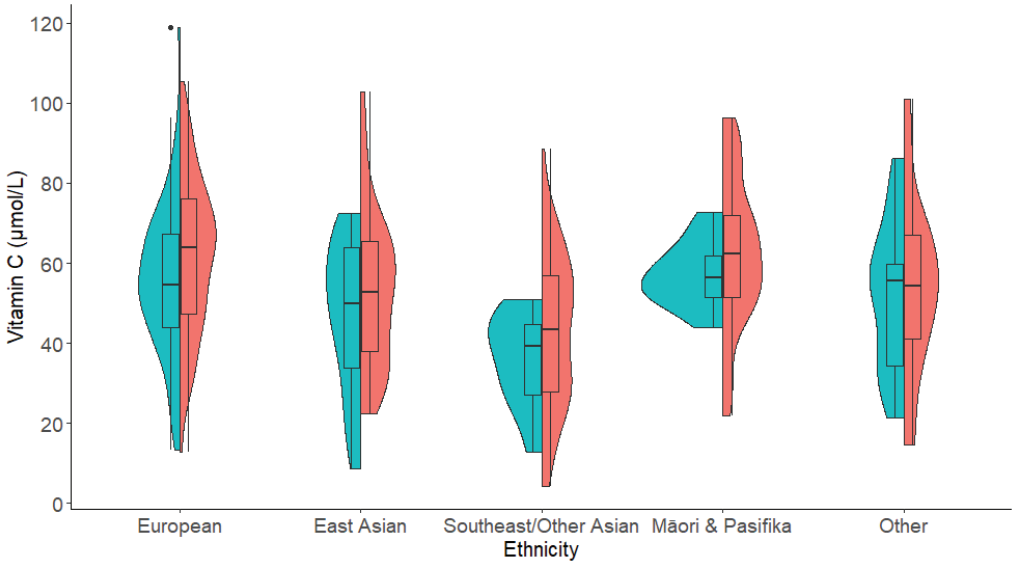


Figure 2. Violin plot showing the vitamin C levels for each ethnicity by gender (male = blue (left) and female = red (right)). Overlaid boxplots show median vitamin C levels for each gender for each ethnicity, interquartile range, and any outliers. See Supplementary Table S2 for the mean plasma vitamin C levels for each ethnicity by gender subgroup. Europeans (n = 206, 80 male, 126 female), East Asians (n = 68, 17 male, 51 female), Southeast/Other Asian (n = 61, 9 male, 52 female), Māori & Pasifika (n = 38, 10 male, 28 female), other ethnicities (n = 46, 19 male, 27 female).

Average total mood disturbance was relatively low in this sample (Mean (M) = 9.88, SD = 14.42), with scores above 16 indicative of mood disturbance. Average vitality scores were relatively high (M = 60.93, SD = 15.09), whereby a score of 100 represents high energy with no fatigue, and a score of 0 represents no energy and high fatigue. Finally, the average perceived stress scores were relatively low in this sample (M = 5.31, SD = 2.06), where the scale ranged from 0 to 16, with higher scores representing higher perceived stress. POMS Total Mood Disturbance, vitality, and perceived stress were correlated (r = −0.604 between POMS TMD and vitality; r = 0.582 between POMS TMD and perceived stress; r = −0.431 between vitality and perceived stress; all $p < 0.001$).

3.2. Relationship between Vitamin C and Mood, Vitality, and Stress

3.2.1. Overall Sample

Figure 3 shows scatterplots of the relationship between vitamin C and the three mood-related outcomes for all participants. Vitamin C was not significantly associated with total mood disturbance or vitality for the total sample (Table 3). These patterns did not change in the adjusted models. There was a trend for higher vitamin C being associated with lower perceived stress for the total sample when controlling for gender, age and socioeconomic status (p = 0.098). When testing the POMS subscales, vitamin C was not significantly associated with tension, depression, fatigue, confusion or vigour, either as an unadjusted predictor or adjusted (See Table 4). There was a trend for higher vitamin C being associated with less anger (p = 0.076), which was maintained when adjusting for covariates (p = 0.090).

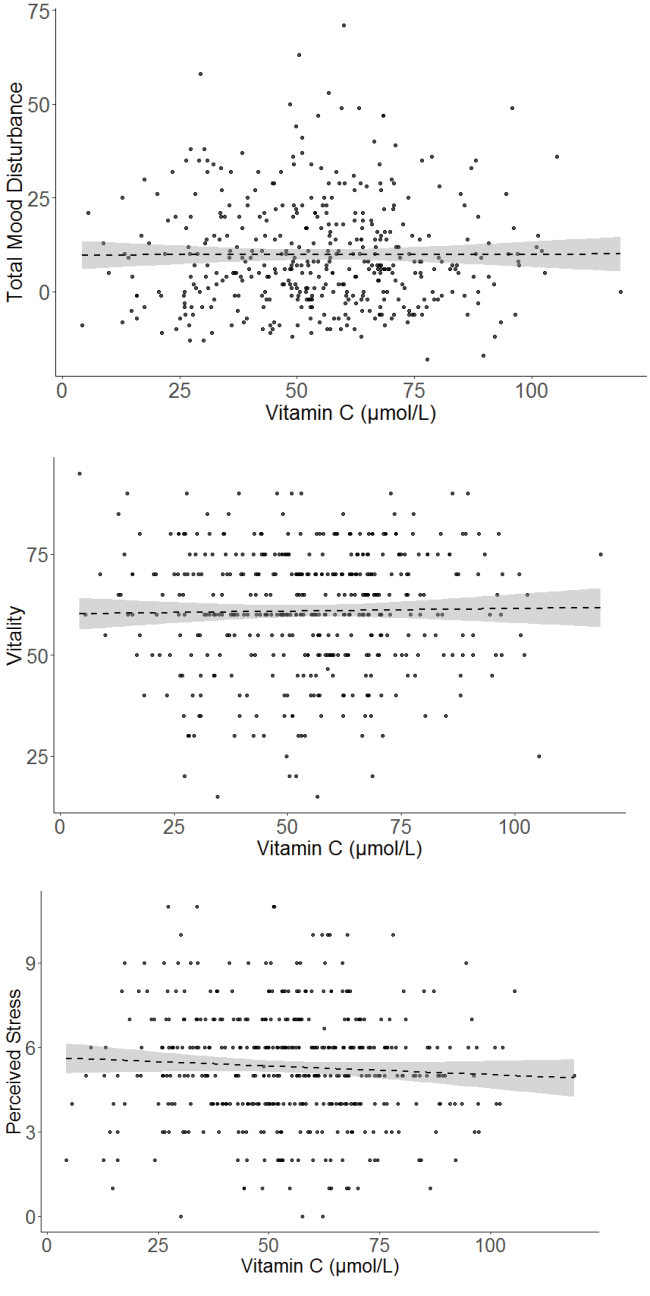


Figure 3. Simple scatterplots of the unadjusted relationship between vitamin C and the three main outcomes (POMS Total Mood Disturbance, vitality and perceived stress) for the sample overall (n = 419). Dashed line indicates the line of best fit. Grey shading indicates 95% confidence intervals.

Table 3. Stress for the Total Sample and for Different Ethnicity Groups, Unadjusted for Covariates (top), and Adjusted for Covariates (middle) and Adjusted for Covariates and Moderators (bottom).

Unadjusted associations	**Total Mood Disturbance b (SE)**	**Vitality b (SE)**	**Perceived Stress b (SE)**
Total Sample	0.002 (0.035)	0.013 (0.037)	−0.006 (0.005)
European	0.006 (0.047)	0.071 (0.052)	0.003 (0.007)
East Asian	0.033 (0.084)	0.001 (0.086)	−0.006 (0.012)
Southeast/Other Asian	0.150 (0.104)	−0.254 (0.099) *	0.016 (0.015)
Māori/Pasifika	−0.138 (0.144)	0.309 (0.167) †	−0.039 (0.021) †
Other	−0.066 (0.137)	0.135 (0.112)	−0.019 (0.017)
Adjusted associations [1]	**b (SE)**	**b (SE)**	**b (SE)**
Total Sample	−0.010 (0.035)	0.031 (0.037)	−0.008 (0.005) †
European	0.019 (0.048)	0.070 (0.053)	0.004 (0.007)
East Asian	0.022 (0.080)	0.002 (0.086)	−0.009 (0.011)
Southeast/Other Asian	0.119 (0.097)	−0.223 (0.095) *	0.012 (0.014)
Māori/Pasifika	−0.138 (0.151)	0.318 (0.174) †	−0.042 (0.021) †
Other	−0.114 (0.130)	0.151 (0.116)	−0.026 (0.016)
Adjusted + Moderators [2]	**b (SE)**	**b (SE)**	**b (SE)**
European	0.008 (0.073)	0.121 (0.076)	−0.004 (0.010)
East Asian	0.029 (0.107)	0.058 (0.111)	−0.016 (0.015)
Southeast/Other Asian	0.149 (0.121)	−0.188 (0.125)	0.005 (0.017)
Māori/Pasifika	−0.130 (0.157)	0.373 (0.163) *	−0.048 (0.022) *
Other	−0.092 (0.140)	0.201 (0.118) †	−0.031 (0.016) *

b (SE) = unstandardised coefficient (Standard Error), † p <0.10; * p <0.05. [1] Different ethnic groups adjusted for gender, age, and socioeconomic status. [2] Full model, adjusted for gender, age, socioeconomic status, ethnicity, gender by vitamin C, and ethnicity by vitamin C interactions. See Supplementary Tables S3, S5 and S7 for full models.

Table 4. Associations between Vitamin C and the Profile of Mood Scale (POMS) subscales for the Total Sample and for Different Ethnicity Groups, Unadjusted for Covariates (top), and Adjusted for Covariates (middle) and Adjusted for Covariates and Moderators (bottom).

Unadjusted associations	**Tension b (SE)**	**Depression b (SE)**	**Anger b (SE)**	**Fatigue b (SE)**	**Confusion b (SE)**	**Vigor b (SE)**
Total Sample	0.004 (0.008)	−0.006 (0.006)	−0.012 (0.007) †	0.013 (0.009)	0.001 (0.008)	−0.001 (0.009)
European	0.018 (0.012)	0.005 (0.008)	−0.011 (0.008)	0.000 (0.012)	0.001 (0.011)	0.007 (0.012)
East Asian	−0.012 (0.020)	−0.006 (0.018)	0.005 (0.017)	0.011 (0.021)	0.007 (0.020)	−0.029 (0.022)
Southeast/Other Asian	0.028 (0.023)	0.001 (0.019)	−0.007 (0.020)	0.061 (0.032) †	0.046 (0.022) *	−0.021 (0.026)
Māori/Pasifika	−0.043 (0.035)	−0.007 (0.021)	−0.007 (0.029)	−0.017 (0.042)	−0.037 (0.031)	0.027 (0.036)
Other	0.012 (0.031)	−0.010 (0.025)	−0.014 (0.027)	0.010 (0.029)	−0.014 (0.029)	0.049 (0.027) †
Adjusted associations [1]	**b (SE)**	**b (SE)**	**b (SE)**	**b (SE)**	**b (SE)**	**b (SE)**
Total Sample	0.002 (0.008)	−0.006 (0.006)	−0.011 (0.007) †	0.010 (0.009)	−0.002 (0.008)	0.003 (0.009)
European	0.021 (0.012) †	0.009 (0.008)	−0.008 (0.008)	0.001 (0.013)	0.004 (0.011)	0.008 (0.013)
East Asian	−0.015 (0.019)	−0.008 (0.017)	0.003 (0.017)	0.010 (0.022)	0.004 (0.018)	−0.027 (0.022)
Southeast/Other Asian	0.021 (0.022)	−0.003 (0.018)	−0.009 (0.020)	0.054 (0.032) †	0.043 (0.022) †	−0.012 (0.026)
Māori/Pasifika	−0.037 (0.036)	−0.007 (0.022)	−0.014 (0.029)	−0.022 (0.044)	−0.031 (0.032)	0.027 (0.038)
Other	−0.003 (0.030)	−0.016 (0.024)	−0.017 (0.027)	0.005 (0.029)	−0.029 (0.027)	0.055 (0.028) †
Adjusted + Moderators [2]	**b (SE)**	**b (SE)**	**b (SE)**	**b (SE)**	**b (SE)**	**b (SE)**
European	0.026 (0.018)	−0.002 (0.013)	−0.012 (0.014)	−0.008 (0.020)	0.011 (0.016)	0.006 (0.018)
East Asian	−0.005 (0.026)	−0.016 (0.019)	0.002 (0.020)	0.001 (0.029)	0.016 (0.024)	−0.030 (0.027)
Southeast/Other Asian	0.037 (0.029)	−0.010 (0.022)	−0.011 (0.023)	0.051 (0.032)	0.057 (0.027) *	−0.025 (0.030)
Māori/Pasifika	−0.031 (0.038)	−0.018 (0.028)	−0.010 (0.030)	−0.027 (0.042)	−0.021 (0.034)	0.022 (0.040)
Other	0.013 (0.028)	−0.022 (0.021)	−0.018 (0.022)	−0.002 (0.031)	−0.013 (0.025)	0.050 (0.029) †

b (SE) = unstandardised coefficient (Standard Error), † p <0.10; * p <0.05. [1] Adjusted for gender, age, and socioeconomic status. [2] Full model, adjusted for gender, age, socioeconomic status, ethnicity, gender by vitamin C, and ethnicity by vitamin C interactions, see Supplementary Tables S4, S6 and S8.

3.2.2. Moderation by Gender

There were no gender differences in the relationship between vitamin C and any of the psychological outcomes (Supplementary Tables S3 and S4, Vitamin C x Gender coefficients).

3.2.3. Moderation by Ethnicity

There were ethnicity differences in the relationship between vitamin C and vitality and perceived stress, but not POMS total mood disturbance. The general pattern was that higher vitamin C was associated with better outcomes for Māori/Pasifika, but poorer outcomes for Southeast/Other Asians (Figures 4–6; Table 3). Among Māori/Pasifika, higher vitamin C was associated with greater vitality (adjusted simple slope b (SE) = 0.373 (0.163), p = 0.023; Supplementary Table S5), which was significantly different from the pattern found for Southeast/Other Asians (contrast b (SE) = −0.560 (0.175), p = 0.001), but not significantly different from the other groups (Table 3; Supplementary Table S5). Southeast/Other Asian participants also significantly differed from Europeans (contrast b (SE) = −0.309 (0.116), p = 0.008; Supplementary Table S3; Table S7), and Other Ethnicity participants (contrast b (SE) = 0.389 (0.147), p = 0.009; Supplementary Table S7).

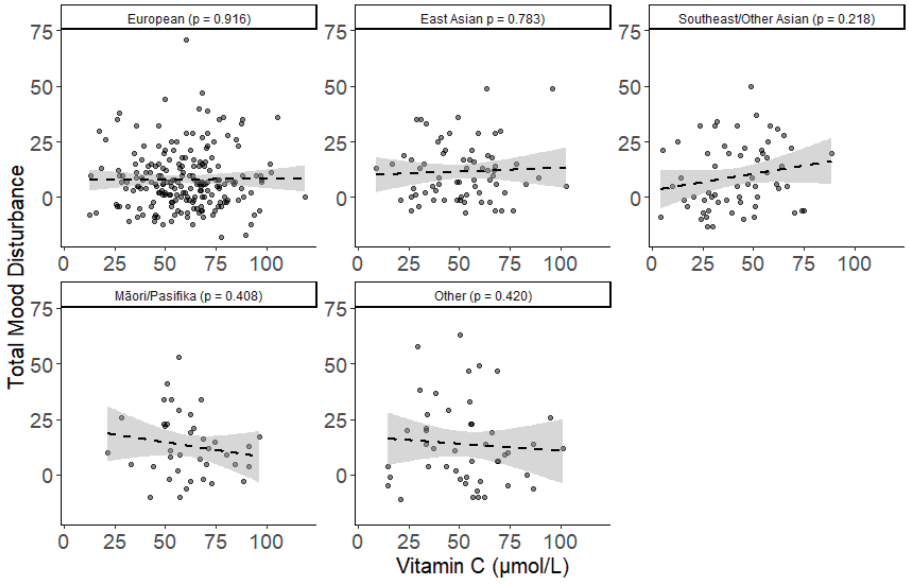


Figure 4. Simple scatterplots of the unadjusted relationship between vitamin C and Profile of Mood States (POMS) Total Mood Disturbance between different ethnic groups (Europeans, East Asians, Southeast/Other Asians, Māori/Pasifika and Other). Dashed line indicates the line of best fit. Grey shading indicates 95% confidence intervals. p values for fully adjusted simple slopes.

Similar ethnicity differences were found for perceived stress, as shown in Figure 6. Among Māori/Pasifika participants, higher vitamin C was associated with lower perceived stress (adjusted simple slope b (SE) = −0.048 (0.022), p = 0.025; Supplementary Table S5), which was significantly different from the pattern found for Southeast/Other Asians (contrast b (SE) = 0.053 (0.023), p = 0.022), and Europeans (contrast b (SE) = 0.044 (0.020), p = 0.031), but not significantly different from the other groups. A similar pattern emerged for the other ethnicity group, in which higher vitamin C was associated with lower perceived stress (adjusted simple slope b (SE) = −0.031 (0.016), p = 0.048), although it was not significantly different from patterns for any of the other groups (Supplementary Tables S3, S5, S7).

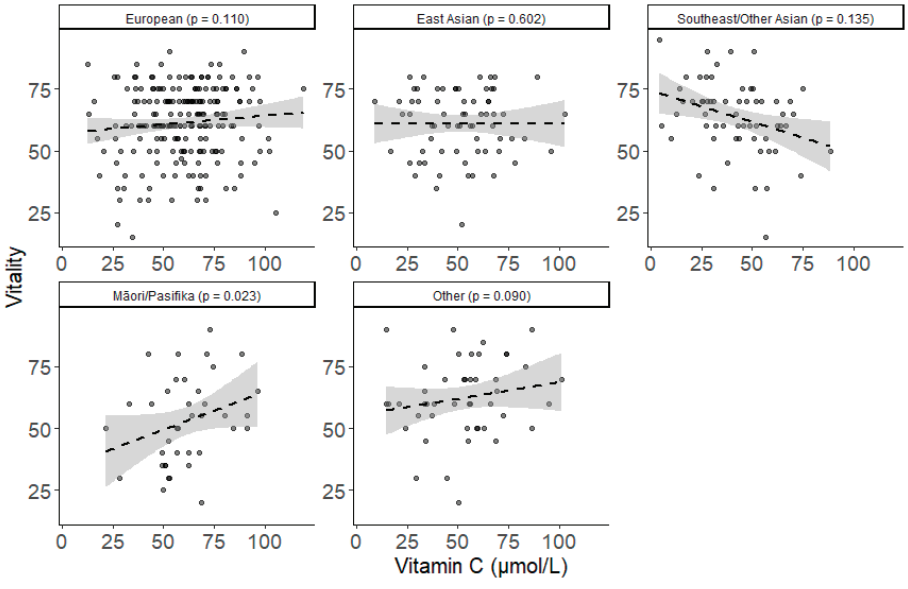


Figure 5. Simple scatterplots of the unadjusted relationship between vitamin C and vitality between different ethnic groups (Europeans, East Asians, Southeast/Other Asians, Māori/Pasifika and Other). Dashed line indicates the line of best fit. Grey shading indicates 95% confidence intervals. *p* values for fully adjusted simple slopes.

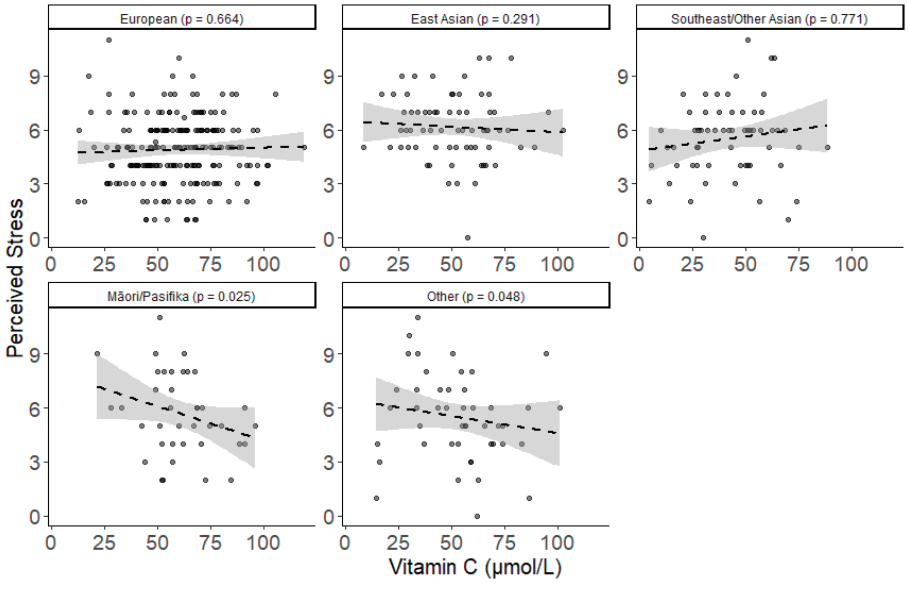


Figure 6. Simple scatterplots of the unadjusted relationship between vitamin C and perceived stress between different ethnic groups (Europeans, East Asians, Southeast/Other Asians, Māori/Pasifika and Other). Dashed line indicates the line of best fit. Grey shading indicates 95% confidence intervals. *p* values for fully adjusted simple slopes.

There were no ethnicity differences for POMS Total Mood Disturbance (Figure 4; Supplementary Tables S3, S5, S7), but there were differences in the POMS confusion

subscale (Table 4). Among Southeast/Other Asians, higher vitamin C was associated with greater confusion (adjusted simple slope b (SE) = 0.057 (0.027), $p = 0.031$; Supplementary Table S8), which was significantly different from the pattern found for Māori/Pasifika (contrast b (SE) = −0.079 (0.037), $p = 0.034$) and the other ethnicity group (adjusted simple slope b (SE) = −0.071 (0.031), $p = 0.024$; Supplementary Table S8).

4. Discussion

This study is the first to examine the relationship between blood plasma vitamin C levels and mental states by ethnicity. We noted an unexpected variation by ethnicity; to our knowledge there has been no prior report indicating an ethnic difference in the relationship between vitamin C status and mood, vitality and perceived stress. The most notable finding was that only Māori/Pasifika people showed a significant positive association between vitamin C levels and vitality, and a negative association with perceived stress. Higher vitamin C levels for Māori/Pasifika were associated with feeling more "full of life", energetic and less stressed. In contrast, Southeast Asians and Other Asians tended to report opposing relationships between vitamin C and vitality, perceived stress, and confusion, although the patterns were much weaker than those found for Māori/Pasifika. Whereas the contrast tests between Southeast/Other Asians and Māori/Pasifika were significant due to their opposing patterns, when testing Southeast/Other Asians alone, only the POMS subscale of confusion was statistically significant in adjusted models (with higher vitamin C predicting greater confusion for Southeast/Other Asians).

Average vitamin C status also differed with ethnicity: Māori/Pasifika participants had the highest average plasma vitamin C levels, followed by Europeans, Other ethnicities, East Asians and Southeast Asian/Other Asian participants. Other research has shown that individuals of African or Asian descent tend to have a lower vitamin C status than individuals of European descent [37,38] and a recent review has documented potential regional differences in vitamin C status across the globe [39]. No data were reported for Pacific regions and little data was available for Asian regions. Determining the reasons for the variations in vitamin C status is a challenge, as differences can be due to dietary composition, smoking status, health variables, socioeconomic status and inaccuracies in measurement of vitamin C in plasma samples [31]. In our study, we pre-selected a relatively healthy non-smoking population with similar education levels and socioeconomic status which raises the question of what factors, such as genetic differences or variation in dietary habits, might affect vitamin C requirements and the potential link between vitamin C and mental functioning.

One possible factor affecting vitamin C status is genetic variation affecting gene expression or polymorphisms in proteins involved in vitamin C metabolism within the body. There are polymorphisms in the sodium-dependent vitamin C transport proteins (SVCTs) that regulate uptake and distribution of the vitamin to the tissues and these polymorphisms could affect vitamin C availability throughout the body ascorbate levels [40]. However, little information is available on how this might affect different ethnic groups. Another possibility could be genetic variation in proteins involved in protection against oxidative stress such as haptoglobin and glutathione-S-transferases. Inefficiencies in the detoxification of oxidants could affect ascorbate levels in the body. For example, haptoglobin is present as three main phenotypes, Hp1-1, Hp2-1 and Hp2-2 [41]. Haptoglobin binds to free haemoglobin to prevent oxidative damage from haemoglobin-iron peroxidation and therefore could affect vitamin C levels indirectly by modulating turnover due to oxidative loss [41]. The Hp2-2 isoform binds haemoglobin poorly in comparison to Hp1-1, and is therefore likely to be less effective in limiting haem-induced oxidative stress and potentially decreasing vitamin C levels [41–44]. The Hp2-1 phenotype is a combination of Hp1 and Hp2 alleles, which is an intermediate step between the opposing functional properties of Hp1-1 and Hp2-2 [45]. Simply put, vitamin C oxidation likely occurs at a higher rate in individuals with the Hp2-2 phenotype than Hp2-1 and Hp1-1 phenotypes, contributing to a lowering of vitamin C status [41]. Māori/Pasifika, who are more likely to possess the

Hp1 allele [46], were found to generally have higher plasma status, which is consistent with a potentially decreased oxidative stress load. More detailed studies involving a direct correlation of the ascorbate status and Hp phenotype would be required to test this hypothesis.

Whether the reported mental states are associated with Hp phenotype is unknown. Previously, Hp phenotype variations have been associated with psychological disorders, including depression, schizophrenia, psychoses and familial epilepsy [45], but whether Hp phenotype changes are responsible for ethnic differences in the relationship between vitamin C and mental states is unknown.

Another possible explanation driving the ethnicity differences in our study could be variations in the availability of other micronutrients, in addition to vitamin C, that have a role in brain functioning and mood. The current study did not incorporate other dietary measures, such as diet records or biomarkers, to assess other micronutrients or diet types as possible confounding factors. For example, a Japanese diet, categorised by higher consumption of soy and fruit and vegetables has been associated with reduced depression [47]. In contrast, so called "Western Diets", which consist of refined grains, high sugar intake, processed foods, beer, and fried foods, have been linked to poorer mental health [48,49]. More specifically, B vitamins are associated with reduced stress levels and vitamin D has been associated with lower levels of depression and fatigue [50,51]. Cultural variation in diet and food preparation may affect the variation in micronutrients that are typically consumed, which may account for some of the ethnicity linked mental health differences.

In the current study, we did not find a significant relationship between vitamin C status and any mood outcomes for Europeans, which contrasts with the cross-sectional study by Pullar and colleagues [7]. This may reflect differences in sample characteristics between these studies, either driven by differences in sample size (n = 419 in our study vs. n = 139) or exclusion criteria. In the current study the exclusion criteria included smoking, excessive alcohol consumption, prescription medication and pre-existing health conditions, which can all impact mental health and vitamin C status. For example, individuals who smoke tend to have lower levels of vitamin C than individuals who do not smoke [28]. In addition, smoking has been associated with poorer mental health outcomes, such as higher levels of depression, anxiety, and stress [52,53]. Thus, some previous vitamin C studies may have found a relationship between vitamin C and mental health outcomes which was driven by other health factors rather than a direct effect of vitamin C on mental health. It is also possible that our stringent exclusion criteria made our sample relatively less representative than if we had not screened for these factors. However, the fact that we had a wide range of vitamin C levels that mirrored population estimates makes this less of an issue.

More generally, the current study found no association between mental health and vitamin C status for the total sample. These findings support an earlier New Zealand based cross-sectional study, which addressed a wide range of covariates in 50-year-old adults, suggesting that these findings may be applicable regardless of age [54]. Although, the sample in the earlier cross-sectional study consisted largely of NZ Europeans (83.7%), which may have driven the null result. It may be possible that variations by ethnicity in the relationship between mental health and vitamin C are inadvertently missed, or masked, when samples are homogenous.

Previously, we have indicated in an 8-week randomised controlled trial, that Asian ethnicity may play a role in the relationship between vitamin C and mood over time in response to a vitamin C or kiwifruit intervention [9]. However, one limitation of the current study is that the English versions of the measures have not been verified for all ethnicities. It is possible that interpretations of items varied between ethnic groups, which could influence the current findings. Nevertheless, English comprehension was assumed to be satisfactory because all participants in the current study were enrolled at university in an English-speaking country, all participants indicated understanding of the study (as

per the information and consent form, and interactions with the research assistant), and no participants required an interpreter. In addition, it has also been suggested that the POMS-SF can be generalised across ethnic groups [55]. The English version of SF-36 has been validated in many European populations, as well as NZ Europeans, younger Māori (under 45 years of age), and Asian participants (with lupus) [56–58]. However, although Pacifika populations may interpret vitality items of the SF-36 to be more representative of mental health than physical health [56], the taxonomy of these items are more reflective of mental health [59]. The PSS has been validated in many countries and languages; however, the use of the English version in a diverse sample may require further investigation [60]. In the current study, all Cronbach alpha levels were acceptable for the respective measures. Thus, ethnic differences in the relationship between vitamin C and, which may be worthy of follow-up.

The current study was strengthened by including a large sample size, an objective measurement of blood vitamin C levels rather than dietary estimates, an ethnically diverse sample, and strict exclusion criteria to eliminate potential confounding factors. Limitations included the cross-sectional correlational design, which limits drawing of causal inferences. We also recruited young adult English speaking university students who consumed fewer than five servings of fruit and vegetables per day, which reduces the generalisability of these findings. However, the average vitamin C level of our sample was consistent with the population average [36], and vitamin C levels in the current sample ranged from deficient to well beyond saturated (4.29–118.91μmol/L), suggesting the sample was representative. To improve generalisability, future research needs to be conducted in older adults of various ethnicities, possibly in different countries or environments with specific measures that are verified for each population of interest. Additionally, in the current study, we did not assess BMI. Higher BMI scores are associated with lower vitamin C [61]. Interestingly, New Zealand Māori and Pasifika populations tend towards having a higher BMI, which would be expected to result in lower vitamin C levels [62]. Asian adults tend to have the lowest rates of obesity, which would be expected to be associated with higher vitamin C levels [62]. However, this was not evident in the current study, which may suggest that BMI was not a driving factor of vitamin C levels. Food preparation methods are also likely to influence vitamin C uptake, as cooking destroys the vitamin. Our study was not intended to analyse the contribution of culinary styles to vitamin C status. However, future studies should consider including additional health measures, such as BMI to control for possible confounds. We were also limited by the number of participants in each ethnic subgroup (Table 2); as ethnicity differences were unexpected, this was not a factor in recruitment. Subsequent studies may need to focus on larger samples of specific ethnicities to thoroughly assess the relationship between ethnicity, vitamin C and mental states.

5. Conclusions

To our knowledge, this is the first study to indicate that there may be opposing relationships between vitamin C levels and vitality and perceived stress for different ethnicities. Understanding this relationship could have implications for dietary recommendations to improve mental health and well-being. The differences in vitality and perceived stress predicted from vitamin C levels between ethnic groups present a complex interaction, which may be a result of a differences in dietary habits or differing combinations of micronutrients, rather than vitamin C alone. Alternatively, the relationship between vitamin C and mental health may be moderated by genetic factors, which may indicate differences in the ideal level of vitamin C for optimal mood and mental health for different ethnicities. Further research is needed to replicate and explore the ethnicity differences in the relationship between vitamin C and mood and mental health.

Supplementary Materials: The following are available online at https://www.mdpi.com/2072-6643/13/3/792/s1, Table S1. Number of Participants with Deficient (<11 μmol/L), Marginal (11–23 μmol/L), Inadequate (23–50 μmol/L), Adequate (50–70 μmol/L), or Saturated (70+ μmol/L) Vitamin C Levels for the Total Sample and Each Ethnicity and Gender Subgroup, Table S2. Number

of Participants and Mean Vitamin C levels (μmol/L) for the Total Sample and Each Ethnicity by Gender Subgroup, Table S3. Regression Models Predicting Total Mood Disturbance, Vitality and Perceived Stress from the Demographic Covariates and Vitamin C Levels, using Europeans as the Reference Group, with Moderators, Table S4. Regression Models Predicting the Profile of Mood States (POMS) Subscales (tension, depression, anger, fatigue, confusion, and vigour) from the Demographic Covariates and Vitamin C Levels, using Europeans as the Reference Group, with Moderators, Table S5. Regression Models Predicting Total Mood Disturbance, Vitality and Perceived Stress from the Demographic Covariates and Vitamin C Levels, using Māori/Pasifika as the Reference Group, with Moderators, Table S6. Regression Models Predicting the Profile of Mood States (POMS) Subscales (tension, depression, anger, fatigue, confusion, and vigour) from the Demographic Covariates and Vitamin C Levels, using Māori/Pasifika as the Reference Group, with Moderators, Table S7. Regression Models Predicting Total Mood Disturbance, Vitality and Perceived Stress from the Demographic Covariates and Vitamin C Levels, using Southeast/Other Asians as the Reference Group, with Moderators, Table S8. Regression Models Predicting the Profile of Mood States (POMS) Subscales (tension, depression, anger, fatigue, confusion, and vigour) from the Demographic Covariates and Vitamin C Levels, using Southeast/Other Asians as the Reference Group, with Moderators.

Author Contributions: Conceptualisation, B.D.F., S.-R.W., M.C.M.V. and T.S.C.; methodology, B.D.F., S.-R.W., M.C.M.V. and T.S.C.; formal analysis, B.D.F. and T.S.C.; investigation, B.D.F., S.-R.W., J.A.M.F. and J.M.P.; resources, M.C.M.V. and T.S.C.; data curation, B.D.F.; writing—original draft preparation, B.D.F.; writing—review and editing, B.D.F., J.M.P., M.C.M.V. and T.S.C.; visualisation, B.D.F.; supervision, M.C.M.V. and T.S.C.; project administration, B.D.F. and J.A.M.F.; funding acquisition, M.C.M.V. and T.S.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Zespri International Ltd., grant number HN1788, and the University of Otago.

Institutional Review Board Statement: The trial was preregistered with the Australian and New Zealand Clinical Trial Registry (Trial ID: ACTRN12617001031358) and approved by the New Zealand Health and Disability Ethics Committee (17/NTB/104).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy and ethical reasons.

Acknowledgments: We thank Emma Spencer for assistance with processing the vitamin C samples and Nadia Norazizan for assistance with coordinating the study. We are grateful to our registered nurse Margi Bryant who collected all blood samples during the clinic visit.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Carr, A.C.; Bozonet, S.M.; Pullar, J.M.; Vissers, M.C. Mood improvement in young adult males following supplementation with gold kiwifruit, a high-vitamin C food. *J. Nutr. Sci.* **2013**, *2*, e24. [CrossRef] [PubMed]
2. Conner, T.S.; Brookie, K.L.; Carr, A.C.; Mainvil, L.A.; Vissers, M.C. Let them eat fruit! The effect of fruit and vegetable consumption on psychological well-being in young adults: A randomized controlled trial. *PLoS ONE* **2017**, *12*, e0171206. [CrossRef] [PubMed]
3. Blanchflower, D.G.; Oswald, A.J.; Stewart-Brown, S. Is psychological well-being linked to the consumption of fruit and vegetables? *Soc. Indic. Res.* **2013**, *114*, 785–801. [CrossRef]
4. McMartin, S.E.; Jacka, F.N.; Colman, I. The association between fruit and vegetable consumption and mental health disorders: Evidence from five waves of a national survey of Canadians. *Prev. Med.* **2013**, *56*, 225–230. [CrossRef] [PubMed]
5. Mujcic, R.; Oswald, A.J. Evolution of well-being and happiness after increases in consumption of fruit and vegetables. *Am. J. Public Health.* **2016**, *106*, 1504–1510. [CrossRef]
6. White, B.A.; Horwath, C.C.; Conner, T.S. Many apples a day keep the blues away-daily experiences of negative and positive affect and food consumption in young adults. *Br. J. Health Psychol.* **2013**, *18*, 782–798. [CrossRef]
7. Pullar, J.M.; Carr, A.C.; Bozonet, S.M.; Vissers, M. High vitamin C status is associated with elevated mood in male tertiary students. *Antioxidants* **2018**, *7*, 91. [CrossRef] [PubMed]

8. Wang, Y.; Liu, X.J.; Robitaille, L.; Eintracht, S.; MacNamara, E.; Hoffer, L.J. Effects of vitamin C and vitamin D administration on mood and distress in acutely hospitalized patients. *Am. J. Clin. Nutr.* **2013**, *98*, 705–711. [CrossRef] [PubMed]
9. Conner, T.S.; Fletcher, B.D.; Pullar, J.M.; Spencer, E.; Mainvil, L.A.; Vissers, M.C.M. KiwiC for Vitality: Results of a randomized placebo-controlled trial testing the effects of kiwifruit or vitamin C tablets on vitality in adults with low vitamin C levels. *Nutrients* **2020**, *12*, 2898. [CrossRef]
10. Harrison, F.E.; May, J.M. Vitamin C function in the brain: Vital role of the ascorbate transporter SVCT2. *Free Radic. Biol. Med.* **2009**, *46*, 719–730. [CrossRef] [PubMed]
11. Nishikimi, M.; Fukuyama, R.; Minoshima, S.; Shimizu, N.; Yagi, K. Cloning and chromosomal mapping of the human nonfunctional gene for L-gulono-gamma-lactone oxidase, the enzyme for L-ascorbic acid biosynthesis missing in man. *J. Biol. Chem.* **1994**, *269*, 13685–13688. [CrossRef]
12. Agarwal, A.; Shaharyar, A.; Kumar, A.; Bhat, M.S.; Mishra, M. Scurvy in pediatric age group-A disease often forgotten? *J. Clin. Orthop. Trauma.* **2015**, *6*, 101–107. [CrossRef]
13. Carr, A.C.; Frei, B. Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans. *Am. Soc. Clin. Nutr.* **1999**, *69*, 1086–1107. [CrossRef]
14. Kinsman, R.A.; Hood, J. Some behavioral effects of ascorbic acid deficiency. *Am. J. Clin. Nutr.* **1971**, *24*, 455–464. [CrossRef] [PubMed]
15. Léger, D. Scurvey. *Can. Fam. Physician* **2008**, *54*, 1403–1406. [PubMed]
16. Lykkesfeldt, J.; Poulsen, H.E. Is vitamin C supplementation beneficial? Lessons learned from randomised controlled trials. *Br. J. Nutr.* **2010**, *103*, 1251–1259. [CrossRef] [PubMed]
17. Carr, A.C.; Vissers, M. Good nutrition matters: Hypovitaminosis C associated with depressed mood and poor wound healing. *N. Z. Med. J.* **2012**, *125*, 107–109.
18. Carr, A.; Vissers, M.; Cook, J. Parenteral vitamin C relieves chronic fatigue and pain in a patient presenting with rheumatoid arthritis and mononeuritis multiplex secondary to CNS vasculitis. *Case Rep. Clin Path.* **2015**, *2*, 57–61.
19. Carr, A.C.; Vissers, M.; Cook, J. Relief from cancer chemotherapy side effects with pharmacologic vitamin C. *N. Z. Med. J.* **2014**, *127*, 66–70. [PubMed]
20. Carr, A.C.; Vissers, M.; Cook, J.S. The effect of intravenous vitamin C on cancer-and chemotherapy-related fatigue and quality of life. *Front. Oncol.* **2014**, *4*, 283. [CrossRef]
21. Gariballa, S. Poor vitamin C status is associated with increased depression symptoms following acute illness in older people. *Int. J. Vitam Nutr Res.* **2014**, *84*, 12–17. [CrossRef]
22. Zhang, M.; Robitaille, L.; Eintracht, S.; Hoffer, L.J. Vitamin C provision improves mood in acutely hospitalized patients. *Nutrients* **2011**, *27*, 530–533. [CrossRef] [PubMed]
23. Cheraskin, E.; Ringsdorf, W.M.J.; Medford, F.H. Daily vitamin C consumption and fatigability. *J. Am. Geriatr. Soc.* **1976**, *24*, 136–137. [CrossRef] [PubMed]
24. de Oliveira, I.; de Souza, V.; Motta, V.; Da-Silva, S. Effects of oral vitamin C supplementation on anxiety in students: A doubleblind, randomized, placebo-controlled trial. *Pak. J. Biol. Sci.* **2015**, *18*, 11–18. [CrossRef]
25. Vilsaint, C.L.; NeMoyer, A.; Fillbrunn, M.; Sadikova, E.; Kessler, R.C.; Sampson, N.A.; Alvarez, K.; Green, J.G.; McLaughlin, K.A.; Chen, R. Racial/ethnic differences in 12-month prevalence and persistence of mood, anxiety, and substance use disorders: Variation by nativity and socioeconomic status. *Compr. Psychiatry* **2019**, *89*, 52–60. [CrossRef] [PubMed]
26. Lee, C.H.; Duck, I.M.; Sibley, C.G. Ethnic inequality in diagnosis with depression and anxiety disorders. *N. Z. Med. J.* **2017**, *130*, 10–20. [PubMed]
27. Fain, O.; Pariés, J.; Jacquart Bt Le Moël, G.; Kettaneh, A.; Stirnemann, J.; Héron, C.; Sitbon, M.; Taleb, C.; Letellier, E. Hypovitaminosis C in hospitalized patients. *Eur. J. Intern. Med.* **2003**, *14*, 419–425. [CrossRef]
28. Lykkesfeldt, J.; Christen, S.; Wallock, L.M.; Chang, H.H.; Jacob, R.A.; Ames, B.N. Ascorbate is depleted by smoking and repleted by moderate supplementation: A study in male smokers and nonsmokers with matched dietary antioxidant intakes. *Am. J. Clin. Nutr.* **2000**, *71*, 530–536. [CrossRef] [PubMed]
29. Cunningham, J.J.; Ellis, S.L.; McVeigh, K.L.; Levine, R.E.; Calles-Escandon, J. Reduced mononuclear leukocyte ascorbic acid content in adults with insulin-dependent diabetes mellitus consuming adequate dietary vitamin C. *Metabolism* **1991**, *40*, 146–149. [CrossRef]
30. Chen, H.; Karne, R.J.; Hall, G.; Campia, U.; Panza, J.A.; Cannon, R.O., III; Wang, Y.; Katz, A.; Levine, M.; Quon, M.J. High-dose oral vitamin C partially replenishes vitamin C levels in patients with Type 2 diabetes and low vitamin C levels but does not improve endothelial dysfunction or insulin resistance. *Am. J. Physiol. Heart Circ. Physiol.* **2006**, *290*, H137–H145. [CrossRef] [PubMed]
31. Pullar, J.M.; Bayer, S.; Carr, A.C. Appropriate handling, processing and analysis of blood samples is essential to avoid oxidation of vitamin C to dehydroascorbic acid. *Antioxidants* **2018**, *7*, 29. [CrossRef] [PubMed]
32. Griskevicius, V.; Delton, A.W.; Robertson, T.E.; Tybur, J.M. Environmental contingency in life history strategies: The influence of mortality and socioeconomic status on reproductive timing. *J. Personal. Soc. Psychol.* **2011**, *100*, 241–254. [CrossRef] [PubMed]
33. Heuchert, J.; McNair, D. *POMS 2*; Multi-Health Systems Incorporated: North Tonawanda, NY, USA, 2012.
34. Ware, J.E., Jr.; Sherbourne, C.D. The MOS 36-item short-form health survey (SF-36): I. Conceptual framework and item selection. *Med. Care* **1992**, *30*, 473–483. [CrossRef] [PubMed]

35. Cohen, S.; Kamarck, T.; Mermelstein, R. Perceived stress scale. *Meas. Stress Guide Health Soc. Sci.* **1994**, *10*, 1–2.
36. Schleicher, R.L.; Carroll, M.D.; Ford, E.S.; Lacher, D.A. Serum vitamin C and the prevalence of vitamin C deficiency in the United States: 2003–2004 National Health and Nutrition Examination Survey (NHANES). *Am. J. Clin. Nutr.* **2009**, *90*, 1252–1263. [CrossRef] [PubMed]
37. Khan, R.M.; Iqbal, M.P. Deficiency of vitamin C in South Asia. *Pak. J. Med Sci.* **2006**, *22*, 347.
38. Ness, A.R.; Cappuccio, F.P.; Atkinson, R.W.; Khaw, K.-T.; Cook, D.G. Plasma vitamin C levels in men and women from different ethnic backgrounds living in England. *Int. J. Epidemiol.* **1999**, *28*, 450–455. [CrossRef]
39. Rowe, S.; Carr, A.C. Global Vitamin C Status and Prevalence of Deficiency: A Cause for Concern? *Nutrients* **2020**, *12*, 2008. [CrossRef] [PubMed]
40. May, J.M. The SLC23 family of ascorbate transporters: Ensuring that you get and keep your daily dose of vitamin C. *Br. J. Pharmacol.* **2011**, *164*, 1793–1801. [CrossRef]
41. Langlois, M.R.; Delanghe, J.R.; De Buyzere, M.L.; Bernard, D.R.; Ouyang, J. Effect of haptoglobin on the metabolism of vitamin C. *Am. J. Clin. Nutr.* **1997**, *66*, 606–610. [CrossRef]
42. Michels, A.J.; Hagen, T.M.; Frei, B. Human genetic variation influences vitamin C homeostasis by altering vitamin C transport and antioxidant enzyme function. *Annu. Rev. Nutr.* **2013**, *33*, 45–70. [CrossRef]
43. Cahill, L.E.; El-Sohemy, A. Haptoglobin genotype modifies the association between dietary vitamin C and serum ascorbic acid deficiency. *Am. J. Clin. Nutr.* **2010**, *92*, 1494–1500. [CrossRef]
44. Delanghe, J.R.; Langlois, M.R.; De Buyzere, M.L.; Torck, M.A. Vitamin C deficiency and scurvy are not only a dietary problem but are codetermined by the haptoglobin polymorphism. *Clin. Chem.* **2007**, *53*, 1397–1400. [CrossRef] [PubMed]
45. Langlois, M.R.; Delanghe, J.R. Biological and clinical significance of haptoglobin polymorphism in humans. *Clin. Chem.* **1996**, *42*, 1589–1600. [CrossRef]
46. Carter, K.; Worwood, M. Haptoglobin: A review of the major allele frequencies worldwide and their association with diseases. *Int. J. Lab. Hematol.* **2007**, *29*, 92–110. [CrossRef] [PubMed]
47. Nanri, A.; Kimura, Y.; Matsushita, Y.; Ohta, M.; Sato, M.; Mishima, N.; Sasaki, S.; Mizoue, T. Dietary patterns and depressive symptoms among Japanese men and women. *Eur. J. Clin. Nutr.* **2010**, *64*, 832–839. [CrossRef]
48. Jacka, F.N.; Pasco, J.A.; Mykletun, A.; Williams, L.J.; Hodge, A.M.; O'Reilly, S.L.; Nicholson, G.C.; Kotowicz, M.A.; Berk, M. Association of western and traditional diets with depression and anxiety in women. *Am. J. Psychiatry* **2010**, *211*, 55–68. [CrossRef]
49. Jacka, F.N.; Kremer, P.J.; Leslie, E.R.; Berk, M.; Patton, G.C.; Toumbourou, J.W.; Williams, J.W. Associations between diet quality and depressed mood in adolescents: Results from the Australian Healthy Neighbourhoods Study. *Aust. N. Z. J. Psychiatry* **2010**, *44*, 435–442. [CrossRef]
50. Young, L.M.; Pipingas, A.; White, D.J.; Gauci, S.; Scholey, A. A systematic review and meta-analysis of B vitamin supplementation on depressive symptoms, anxiety, and stress: Effects on healthy and 'at-risk'individuals. *Nutrients* **2019**, *11*, 2232. [CrossRef] [PubMed]
51. Shah, J.; Gurbani, S. Association of Vitamin D Deficiency and Mood Disorders: A Systematic Review. *Vitam. D Defic.* **2019**. [CrossRef]
52. Jorm, A.F.; Rodgers, B.; Jacomb, P.A.; Christensen, H.; Henderson, S.; Korten, A.E. Smoking and mental health: Results from a community survey. *Med. J. Aust.* **1999**, *170*, 74–77. [CrossRef]
53. Taylor, G.; McNeill, A.; Girling, A.; Farley, A.; Lindson-Hawley, N.; Aveyard, P. Change in mental health after smoking cessation: Systematic review and meta-analysis. *BMJ* **2014**, *348*, g1151. [CrossRef]
54. Pearson, J.F.; Pullar, J.M.; Wilson, R.; Spittlehouse, J.K.; Vissers, M.; Skidmore, P.M.; Willis, J.; Cameron, V.A.; Carr, A.C. Vitamin C status correlates with markers of metabolic and cognitive health in 50-year-olds: Findings of the CHALICE cohort study. *Nutrients* **2017**, *9*, 831. [CrossRef]
55. Lin, S.; Hsiao, Y.-Y.; Wang, M. *Test Review: The Profile of Mood States*, 2nd ed.; SAGE Publications: Los Angeles, CA, USA, 2014.
56. Scott, K.M.; Sarfati, D.; Tobias, M.I.; Haslett, S.J. A challenge to the cross-cultural validity of the SF-36 health survey: Factor structure in Māori, Pacific and New Zealand European ethnic groups. *Soc. Sci. Med.* **2000**, *51*, 1655–1664. [CrossRef]
57. Thumboo, J.; Fong, K.; Ng, T.; Leong, K.; Feng, P.; Thio, S.; Boey, M. Validation of the MOS SF-36 for quality of life assessment of patients with systemic lupus erythematosus in Singapore. *J. Rheumatol.* **1999**, *26*, 97–102. [PubMed]
58. Ware, J.E., Jr.; Kosinski, M.; Gandek, B.; Aaronson, N.K.; Apolone, G.; Bech, P.; Brazier, J.; Bullinger, M.; Kaasa, S.; Leplège, A. The factor structure of the SF-36 Health Survey in 10 countries: Results from the IQOLA Project. *J. Clin. Epidemiol.* **1998**, *51*, 1159–1165. [CrossRef]
59. Ware, J.E., Jr. SF-36 health survey update. *Spine* **2000**, *25*, 3130–3139. [CrossRef] [PubMed]
60. Lee, E.-H. Review of the psychometric evidence of the perceived stress scale. *Asian Nurs. Res.* **2012**, *6*, 121–127. [CrossRef] [PubMed]
61. Kimmons, J.E.; Blanck, H.M.; Tohill, B.C.; Zhang, J.; Khan, L.K. Associations between body mass index and the prevalence of low micronutrient levels among US adults. *Medscape Gen. Med.* **2006**, *8*, 59.
62. Ministry of Health. *Annual Update of Key Results 2015/16: New Zealand Health Survey*; Ministry of Health Wellington: Wellington, New Zealand, 2016.

nutrients

MDPI

Article

Vitamin D Status among First-Generation Immigrants from Different Ethnic Groups and Origins: An Observational Study Using the Canadian Health Measures Survey

Said Yousef [1,2,*], Douglas Manuel [3,4,5], Ian Colman [1], Manny Papadimitropoulos [6,7], Alomgir Hossain [1,4], MoezAlIslam Faris [8] and George A. Wells [1,2]

1 School of Epidemiology and Public Health, Faculty of Medicine, University of Ottawa, Ottawa, ON K1H 8M5, Canada; icolman@uottawa.ca (I.C.); alhossain@ottawaheart.ca (A.H.); gawells@ottawaheart.ca (G.A.W.)
2 Cardiovascular Research Methods Centre, University of Ottawa Heart Institute, Ottawa, ON K1Y 4W7, Canada
3 Ottawa Hospital Research Institute, Ottawa, ON K1Y 4E9, Canada; dmanuel@ohri.ca
4 Institute for Clinical Evaluative Sciences, Ottawa, ON K1Y 4E9, Canada
5 Department of Family Medicine, Faculty of Medicine, University of Ottawa, Ottawa, ON K1H 8M5, Canada
6 Eli Lilly Canada Inc., Toronto, ON M5X 1B1, Canada; papadimitropoulos_manny@lilly.com
7 Faculty of Pharmacy, University of Toronto, Toronto, ON M5S 3M2, Canada
8 Department of Clinical Nutrition and Dietetics, College of Health Sciences/Research Institute of Medical and Health Sciences (RIMHS), University of Sharjah, Sharjah 27272, United Arab Emirates; mfaris@sharjah.ac.ae or moezfaris@hotmail.com
* Correspondence: sabde029@uottawa.ca or sabdelrazeq@ottawaheart.ca

Citation: Yousef, S.; Manuel, D.; Colman, I.; Papadimitropoulos, M.; Hossain, A.; Faris, M.; Wells, G.A. Vitamin D Status among First-Generation Immigrants from Different Ethnic Groups and Origins: An Observational Study Using the Canadian Health Measures Survey. *Nutrients* **2021**, *13*, 2702. https://doi.org/10.3390/nu13082702

Academic Editors: Anitra Carr and Adrian Gombart

Received: 5 July 2021
Accepted: 3 August 2021
Published: 5 August 2021

Abstract: One in five Canadians are first-generation immigrants. Evidence suggests the baseline risk for vitamin D (vitD) deficiency is increased among immigrants who move from equatorial to northern countries. We investigated the prevalence and determinants of vitD deficiency/insufficiency among first-generation immigrants compared with native-born Canadians and identified explanatory covariables. We used a cross-sectional design with data from the national Canadian Health Measures Survey (Cycles 3 and 4) (11,579 participants aged 3–79 years). We assessed serum 25-hydroxyvitamin D (S-25(OH)D) levels, sociodemographic and environmental factors, immigration status, length of time in Canada, vitD-rich food intake, ethnicity, and place of birth. Immigrants had lower mean S-25(OH)D than non-immigrants (51.23 vs. 62.72 nmol/L, $p < 0.001$). Those with younger age at the time of immigration (<18 years) had a high risk for low vitD, and S-25(OH)D levels increased with the length of time they had lived in Canada. The highest deficiency levels were in immigrants born in Morocco, India, and Lebanon compared with native-born Canadians. Ethnicity was the factor most strongly associated with S-25(OH)D. Compared with the white ethnic grouping, the Japanese had the highest level of vitD deficiency, followed by Arabs and Southeast Asians. Ethnic variations, dietary intake, and lifestyle factors are the main predictors of/explanatory factors for vitD status among Canadian immigrants.

Keywords: vitamin D; serum 25-hydroxyvitamin D; first-generation immigrants; ethnicity; melanin; dietary intake

1. Introduction

Vitamin D (vitD) plays a crucial role in physiological functions, including skeletal and non-skeletal health [1]. Vit D has two main metabolites, namely 25-hydroxyvitamin D (25-OH) and 1, 25 dihydroxy vitamin D. The dietary sources (vitamin D2 or ergocalciferol) and the animal-based foods (vitamin D3 or cholecalciferol) are the two main forms of vitamin D found in the human body [2–4]. The primary source of vitD in the human body, however, is through skin exposure to sunlight (cutaneous synthesis

of vitD3) [2,5]. Vitamin D2 and D3 are considered to have equal biological value. The total serum 25-hydroxyvitamin D (S-25(OH)D) concentration is the sum of the 25(OH) D2 and 25(OH) D3 concentrations [6]. The concentration of S-25(OH)D represents the combined contributions of the cutaneous synthesis and dietary intake of vitD and is considered the top clinical marker for overall S-25(OH)D level [7–9]. The S-25(OH)D is expressed in nanomoles per liter (nmol/L) and has a stable half-life (up to three weeks) in the human body [7].

The mean of S-25(OH)D (nmol/L) and/or the ranges for the thresholds (a deficiency, <25–30 nmol/L; insufficiency, 25–49 nmol/L; sufficiency, ≥50–<75 nmol/L) were commonly reported and used in studies to describe the status of vitD [8]. The insufficient status (<50 nmol/L) is more frequently used to describe hypovitaminosis D [10]. However, the optimal S-25(OH)D concentration for skeletal health is controversial. The Institute of Medicine (IOM) recommends maintaining S-25(OH)D concentration levels above 50 nmol/L, whereas other experts favor the concentration between 50 to 100 nmol/L [11–13].

Multiple factors affect the body's ability to synthesize vitD, including dietary intake, coexisting disease conditions (especially liver and kidney diseases), and sun-related vitD production (e.g., sun exposure) [8,9,14,15]. Other implicated factors include sociodemographic factors (e.g., socioeconomic status, age, and sex), geographical and environmental factors (e.g., season and latitude), cultural and religious aspects (e.g., clothing and prolonged breastfeeding time), and health and genetic factors (e.g., melanin levels and obesity) [8,10,16]. A global review of evidence from six regions (Europe, North America, Latin America, Asia, the Middle East/Africa, and Oceania) showed the leading risk factors for vitD deficiency were older age, female sex, higher latitude, winter season, darker skin pigmentation, less sunlight exposure, poor dietary habits, and absence of vitD fortification [10].

Hypovitaminosis D is a worldwide public health problem to which immigrants are particularly vulnerable, with a high baseline risk among populations with darker skin who migrate from equatorial regions to northern latitudes [17,18]. Migration is considered a significant risk factor for low S-25(OH)D levels attributable to lifestyle and environmental changes, including dietary intake, physical activity, sun exposure, clothing, and a move from low to high latitude countries [8,17,18]. Immigrants in Canada and other Western countries have a high prevalence of vitD deficiency and insufficiency compared with Western people, with a deficiency prevalence of 19.3–80% among different ethnic minorities [8,15,19]. A meta-analysis of 36 studies reported variation in vitD deficiency among dark-skinned immigrants was attributable to the length of residence in the host country, age at immigration, nutritional barriers, and geographic origins and ethnicities of the studied populations [18]. Moreover, the estimated pooled prevalence of vitD deficiency was 77% (95% confidence interval [CI], 70–83%) after adjustment for latitude [18].

The immigrant population in Canada has increased in recent decades. One in five Canadians is foreign-born, with these people originating from over 200 ethnic groups or origin countries [20]. Research evidence suggests the presence of significant differences in sociocultural contexts and various outcomes between first-generation and second or third generation immigrants [21,22]. Moreover, researchers recommended studying and comparing ethnic groups from the same generation [21–24].

There is a paucity of information about the status of vitD among Canadian immigrants from different ethnic groups and origins. Therefore, we intend to study first-generation immigrants as identified in the Canadian Health Measures Survey (CHMS) data (foreign-born) to investigate vitD status among immigrants compared with the non-immigrant (native-born) population. Most of the previous studies on immigrants' vitamin D involved aggregated generations of immigrants (first, second, or more generations), resulting in high heterogeneity in the study population. Moreover, they focused on specific groups from relatively few ethnicities or few countries of origin. However, this study is the first to report vitD status among large sample of first-generation immigrants from 13 major ethnic

groups and who were migrated from 153 countries of origin compared with white ethnic group and native-born Canadians.

The study aimed to estimate the prevalence and the leading determinants of vitD deficiency/insufficiency among immigrants from different ethnicities and regions/countries of birth compared with white and native-born Canadians. Moreover, to understand the effects of the environment, lifestyle, and acculturation on immigrants' vitD, we aimed to investigate vitD status to the length of time after immigration. We hypothesized that significant differences exist in vitD status between immigrant and non-immigrant Canadians, with immigrants having lower vitD status.

2. Materials and Methods

The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement was adopted in planning, implementing, and reporting this study [25].

2.1. Study Design and Participants

The CHMS is a cross-sectional population-based survey conducted by Statistics Canada in collaboration with Health Canada and the Public Health Agency of Canada [26]. The CHMS provided the first national data on vitD for the Canadian population including immigrants [26]. The CHMS was executed in cycles biannually. Compared to the 2006 Census data and the 2011 National Household Survey, CHMS data has unique information in direct physical health measures, representing the Canadian immigrant population, and addressing the gaps in their existing national information [27].

This study used data for Cycles 3 (2012–2013) and 4 (2014–2015) of CHMS data, which included randomly selected individuals aged 3–79 years [26]. Cycles-3 and -4 were selected as being the most thematic consistent cycles in reporting vitD -rich foods and other vitD determinants than the other cycles of CHMS data. Therefore, we combined Cycles 3 and 4 based on instructions provided by Statistics Canada. By merging the two cycles, we aimed to provide a larger sample size and increase the number of primary sampling units to allow for greater precision in estimating small prevalence and providing more detailed analyses.

For each cycle, Statistics Canada determined the sample size to produce reliable and representative estimates at the national level for sex and age groups. The survey covered approximately 96% of the Canadian population, each cycle collected from sixteen collection sites spread across Canada and stratified into five regions: namely British Columbia, the Prairies (Alberta, Manitoba, and Saskatchewan), Ontario, Quebec, and the Atlantic provinces (Newfoundland and Labrador, Prince Edward Island, Nova Scotia, and New Brunswick). A dwelling stratification stage was applied and followed by a roster list of all persons living in the household, and individuals aged 3 to 79 years were randomly selected [26]. All participants provided written informed consent, and the CHMS survey was approved by the Health Canada Research Ethics Board [26].

The CHMS sample population weight was adjusted for age group and sex across Canada's five standard geographic regions [27]. It is worth mentioning that except for the total number of subjects included in the analysis, the number of participants in each group and the unweighted results cannot be published due to Statistics Canada's restrictions policy, thus the results are presented as weighted results. Detailed information about merging the two cycles and the CHMS data are presented in Appendix A and on the Statistics Canada website (http://www.statcan.gc.ca, accessed on 24 February 2021) [28,29].

2.2. Measures

The S-25(OH)D was measured using chemiluminescence immunoassay technology (DiaSorin®, Ltd., Stillwater, MN, USA). The analytical detection limit for S-25(OH)D was 10–375 nmol/L. Data for S-25(OH)D in the two cycles were extracted and used as the outcome-dependent factors.

We used four cut-offs for S-25(OH)D to identify vitD status as follows: (<30 (nmol/L); (<50 (nmol/L); (<75 (nmol/L); and (≥75 (nmol/L). First, <30 nmol/L is used to identify

deficient people. Second, (<50 nmol/L) is a cut-off for the insufficient 25(OH) D, which includes the deficient and insufficient people (it is an accumulating value and not range for the insufficient people). Third, (<75 nmol/L) is a cut-off and accumulating value for deficient, insufficient, and sufficient. Fourth (≥75 (nmol/L), is the "no added value" or the "optimal" as defined by IOM or other experts [8,11–13]. The remaining last two cut-offs (<75 nmol/L and ≥75 nmol/L) cover 100% of the total population. We assumed that reporting the prevalence of vitD deficiency, insufficiency, sufficiency, and the no added value or optimal categories using these cut-offs is important for clinicians and readers to see the proportion of participants under each stratified category. Moreover, we used ranges of S-25(OH)D for the above-mentioned cut-offs (<30 nmol/L; 30–49 nmol/L; 50–74 nmol/L and ≥75 nmol/L) for sub-groups of participants.

Independent variables were factors associated with the risk for developing vitD deficiency/insufficiency: immigration status, sex, age, income, education level, body mass index (BMI, kg/m^2), smoking status, alcohol consumption, age at immigration, length of time in Canada, sun exposure, sunscreen use, season and month of blood sampling, clothing type, physical activity, region and country of birth, ethnicity, skin pigmentation (melanin level), intake of vitD-rich foods, and vitD supplements/medications used.

We created BMI norms for adults aged ≥ 18 years according to Health Canada's national standards for weight classification [30]. For children aged 3–17 years, body weight classification was based on World Health Organization percentiles [31]. We used the Canadian Physical Activity Guidelines index to classify participants (aged 5–17 years and ≥18 years) as meeting/not meeting physical activity recommendations [32]. The season of blood sampling was categorized by the month of testing: winter (December–February), spring (March–May), summer (June–August), and fall (September–November). Region of birth was classified using five major regions based on the number of participants: Canada and North America; South/Central America and the Caribbean; Europe; Africa; and Asia. We used Statistics Canada's geographic classifications to identify the country of birth (153 countries; Appendix A), and selected the top 20 countries based on the number of participants (≥30 participants from each) to include in the analysis. These countries were Canada, China, USA, France, Jamaica, UK, Algeria, Mexico, Pakistan, Netherlands, India, the Philippines, Romania, Hong Kong, Germany, Colombia, Morocco, Italy, Iran, and Lebanon. All other countries were grouped into one category (Others). The 153 countries (excluding Canada) were also combined in another category to represent all immigrants. Statistics Canada identifies ethnicity as white and non-white. The non-white group included different major ethnic groups; namely Aboriginal, South Asian, Chinese, Black, Filipino, Latin American, Arab, Southeast Asian, West Asian, Korean, Japanese, multiple ethnicities, and others. As reported by Statistics Canada, melanin levels were used as an indicator for skin pigmentation, with higher ranks indicating darker skin.

The steps used to combine the two cycles and to calculate the variables of interest are described in Table A1, Appendix A.

2.3. Statistical Analyses

We summarized the data using numerical and graphical descriptive statistics. All statistical comparisons used the mean (standard error, SE) for continuous variables and proportions with 95% CIs for categorical variables. Data were stratified for analysis based on the variables of interest. To account for the unequal probability of selection and represent an accurate estimate of the Canadian population, we used the survey command, recommended sample weight, and degrees of freedom in the analyses. All results were weighted values. We used continuous values for S-25(OH)D in the linear regression models. A univariate analysis was used to identify independent covariates. Multivariable analyses were performed to clarify the relationship between S-25(OH)D and immigration status and highlight the factors most strongly associated with lower concentration levels of S-25(OH)D among immigrants.

We used the backward elimination method in a linear regression model adjusted for several independent covariates; namely age, sex, household income, education, BMI, season, sun exposure, sunscreen, country of birth, melanin levels, ethnicity, vitD medication and supplements, and food consumption variables. In our analysis, we applied several evaluation models. In which we determined, by excluding variables with a high percentage of missing data (e.g., margarine consumption) and variables that are influenced by S-25(OH)D levels (e.g., serum calcium), improvements in the model's estimation which better represents the study population compared to other models. Statistical significance was set at $p \leq 0.05$. Analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA) and Stata version 16.0 (StataCorp, College Station, TX, USA).

3. Results

3.1. Study Description and Participants Characteristics

The analyses were based on the total number of participants in both cycles. The combined response rate at the Canadian level (response rate for all study components like the household visit, blood samples, and activity monitor) for Cycle-3 was 51.7%, and Cycle-4 was 53.7% [33,34]. Demographic characteristics for the two cycles are presented in Table A2, Appendix B.

There were 11,579 participants (5785 from Cycle-3 and 5794 from Cycle-4) aged 3–79 years, with a mean (standard error [SE]) age of 39.23 (0.085) years. S-25(OH)D levels were available for 11,009 participants and were normally distributed with an overall weighted mean of 60.28 nmol/L. Nearly 10.30% of Canadians were vitD deficient, 63.64% had insufficient vitD, 76.1% had sufficient vitD, and 23.90% had optimal vitD status.

Table 1 presents the main characteristics of immigrant and non-immigrant Canadians. Immigrants represented 21.9% of the Canadian population (about 53% female), and the majority were of non-white ethnicity. Compared with the non-immigrant population, they were older (overrepresented in the group aged $\geq$ 18 years) and had lower household income, smoking, alcohol consumption, BMI (obesity), sun exposure during peak time, and sunscreen use. However, immigrants were more likely to have higher education levels, wear concealing clothing, and have traveled to sunny/warm climates in the two months before blood sampling than non-immigrants.

The weighted mean melanin level (index values) was higher among immigrants than non-immigrants (17.08 vs. 16.29, $p = 0.004$). Immigrants also had lower household income, and serum calcium and phosphorus levels than non-immigrants. Despite the high statistical difference of calcium ($p = 0.004$) and phosphorus ($p = 0.006$), the biological significance of the mean differences between immigrants and non-immigrants for calcium (2.40 vs. 2.42, respectively) and phosphorus (1.32 vs. 1.36, respectively) were negligible (Table 1). Non-immigrants reported more frequent consumption of vitD-rich foods (red/processed meats, liver, milk, cheese, dairy products, fortified margarine, and fatty fish) (Table A3, Appendix B).

3.2. S-25(OH)D Concentration and VitD Status by Sociodemographic, Lifestyle, and Immigration Features

Table 2 shows the weighted mean S-25(OH)D levels and vitD status by sociodemographic and lifestyle factors. Youth aged 12–17 years had the lowest S-25(OH)D levels. There were significant differences in sex, BMI (obesity), vitD supplement/medication use, sunscreen use, sun exposure during peak time (<30 min/day) and traveling to a sunny/warm climate in the two months before blood sampling. Differences were also found in income, smoking, alcohol consumption, physical activity, and clothing type as shown in Table A3 (Appendix B).

Immigrants had lower mean S-25(OH)D levels than non-immigrants (62.72 vs. 51.23 nmol/L, $p < 0.001$). Age at immigration was associated with 25(OH)D levels; younger generations (<18 years) had a higher risk for lower vitD than older people ($\geq$18 years). Moreover, years of residency after immigration was associated with S-25(OH)D levels, especially at 5 and 10 years, indicating that immigrants had higher S-25(OH)D levels the

longer they had lived in Canada. The mean S-25(OH)D level was higher among sunscreen users than non-users (62.86 vs. 54.78 nmol/L, $p < 0.001$).

Nearly 64% of participants had insufficient S-25(OH)D levels. VitD deficiency was twice as common among immigrants as non-immigrants. Moreover, compared with non-immigrants, more immigrants had insufficient vitD (52.82% vs. 31.75%) and fewer had optimal vitD (13.89% vs. 26.83%; $p < 0.001$). The more years immigrants had lived in Canada, the greater the proportion with optimal levels at 5 (15.41% vs. 7.65%, $p = 0.002$) and 10 (17.37% vs. 7.95%, $p = 0.022$) years after immigration (Table 2). The S-25(OH)D and vitD status by household income, education, smoking habits, alcohol consumption, physical activity, pregnancy, and clothing type are presented in Table A4, Appendix B.

Table 1. Weighted prevalence of immigrants and non-immigrants by sociodemographic and lifestyle factors using Cycles 3 and 4 of Canadian Health Measures Survey data.

		Non-Immigrants (78.9%), %	**Immigrants (21.9%), %**	**All Participants (100%), %**	***p*-Value**
Sex	Female	49.37	52.85	50.13	0.142
Age (years)	<5	2.84	0.42	2.31	<0.001
	5–11	9.37	3.38	8.05	
	12–17	8.20	4.57	7.41	
	18–64	69.10	75.41	70.48	
	>64	10.49	16.22	11.75	
Household income (CAD)	<50,000	33.15	43.93	35.51	0.008
	50,000–100,000	37.07	34.65	36.54	
	>100,000	29.79	21.43	27.95	
Education	>Secondary school	47.26	64.63	51.07	<0.001
BMI (kg/m^2)	Underweight	2.19	2.34	2.22	0.023
	Normal weight	41.40	41.75	41.48	
	Overweight	30.21	37.01	31.70	
	Obese	26.21	18.89	24.60	
Ethnic group	Non-white	11.76	62.67	22.91	<0.001
VitD-supplement and/or analog use	Yes	5.39	4.84	5.27	0.664
Smoking status	Current smoker	22.65	14.75	20.79	0.003
Alcohol	Current drinker	82.90	65.03	78.69	<0.001
Meet the physical activity recommendations	Yes	35.41	30.17	34.21	0.127
Sun exposure (10 am to 4 pm)	≥30 min/day	91.21	78.59	88.46	<0.001
Sunscreen use	Yes	73.94	58.57	70.64	<0.001
Clothing	Typically covered	34.66	52.54	38.51	<0.001
Traveled to sunny/warm climate	Yes	10.56	16.48	11.85	0.018
Weighted means		Mean (SE)	Mean (SE)	(95% CI)	*p*-value
	Age (years)	37.51 (0.26)	45.17 (0.58)	(−9.05, −6.26)	<0.001
	Income (CAD)	91,985 (3831)	76,317 (4004)	(8087, 23,248)	<0.001
	Calcium (mmol/L)	2.42 (0.00)	2.40 (0.01)	(0.01, 0.04)	0.004
	Phosphorus (mmol/L)	1.36 (0.01)	1.32 (0.01)	(0.01, 0.06)	0.006
	Melanin (index values)	16.29 (0.29)	17.08 (0.25)	(−1.29, −0.28)	0.004

BMI, body mass index; CAD, Canadian dollars; SE, standard error; CI, confidence interval. *p*-value: $p \leq 0.05$.

Table 2. Weighted mean S-25(OH)D (nmol/L) and vitamin D status by sociodemographic and lifestyle factors using Cycles 3 and 4 of Canadian Health Measures Survey data.

		S-25(OH)D (nmol/L)			S-25(OH)D Status			
		Mean (SE)	**(95% CI)**	***p*-Value**	**<30 (nmol/L (10.3%),%**	**<50 (nmol/L (63.64%),%**	**<75 (nmol/L (76.1%),%**	**≥75 (nmol/L (23.9%),%**
Immigration status	Non-immigrant †	62.72 (1.73)	-		7.82	31.75	73.21	26.83
	Immigrant	51.23 (1.41)	(8.37, 14.62)	<0.001	19.01 ***	52.82 ***	86.11	13.89 ***
Age at immigration (years)	<18 †	46.54 (1.63)	-		19.17	64.04	91.37	8.63
	≥18	56.33 (2.34)	(−15.14, −4.44)	0.001	18.80	41.23 **	80.76	19.24 **
5 years after immigration	≤5 †	45.94 (2.22)	-		19.55	62.54	92.35	7.65
	>5	52.77 (1.73)	(−12.58, −1.08)	0.022	18.59	50.04 *	84.59	15.41 *
10 years after immigration	≤10 †	47.03 (1.83)	-		19.55	59.83	92.05	7.95
	>10	53.98 (1.83)	(−11.79, −2.12)	0.007	18.59	48.26	82.63	17.37 **
Sex	Male †	57.92 (1.67)	-		11.13	39.65	79.62	20.38
	Female	62.53 (1.82)	(−6.30, −2.93)	<0.001	9.45	33.04 ***	72.58	27.42 ***
Age group (years)	<5	69.94 (1.64)	(−13.94, −9.01)	<0.001	1.99	15.70	67.1	32.9
	5–11	62.49 (2.16)	(−5.91, −2.29)	<0.001	4.04	27.41	77.9	22.1
	12–17	55.76 (1.93)	(0.44, 4.81)	0.021	10.49	40.38	84.36	15.64
	18–64 †	58.45 (1.81)	-		12.1	39.79	78.24	21.76
	>64	71.07 (1.20)	(−15.63, −9.55)	<0.001	4.59	22.39 ***	58.69	41.31 ***
BMI (kg/m^2)	Underweight	62.19 (4.05)	(−5.82, 7.51)	0.795	12.65	33.0	71.07	28.93
	Normal weight †	63.03 (2.04)	-		9.22	31.79	73.07	26.93
	Overweight	61.02 (1.68)	(−0.56, 4.58)	0.119	7.91	35.32	75.24	24.76
	Obese	54.48 (1.74)	(5.50, 11.60)	<0.001	15.25 ***	45.15 ***	82.36	17.64 **

Table 2. *Cont.*

		S-25(OH)D (nmol/L)			S-25(OH)D Status			
		Mean (SE)	**(95% CI)**	***p*-Value**	**<30 (nmol/L (10.3%),%**	**<50 (nmol/L (63.64%),%**	**<75 (nmol/L (76.1%),%**	**≥75 (nmol/L (23.9%),%**
VitD-supplement and/or analog use	No †	56.97 (1.74)	-		40.52		80.56	19.44
	Yes	83.46 (5.25)	(−35.92, −17.06)	<0.001	9.80 ***		43.16	56.84 ***
Sun exposure (10 am to 4 pm)	<30 min/day †	55.92 (2.20)	-		15.58	48.72	76.51	23.49
	≥30 min/day	60.82 (1.69)	(−7.81, −1.99)	0.002	9.60 **	34.65 ***	76.04	23.96
Sunscreen use	Never †	54.78 (1.85)	-		15.04	48.05	80.92	19.08
	Always, occasionally	62.86 (1.74)	(−10.16, −5.99)	<0.001	7.87 ***	30.23 ***	73.99	26.01 ***
Traveled to sunny/warm climate	No †	59.33 (1.83)	-		10.81	37.99	77.59	22.41
	Yes	67.03 (2.10)	(−11.85, −3.54)	0.001	6.49	23.71 ***	65.08	34.92 ***

Note: Following the Statistics Canada guideline, some cells merged due to the low number of participants. † Reference value; SE, standard error; CI, confidence interval; BMI, body mass index. *p*-values: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

3.3. S-25(OH)D Concentration and VitD Status by Participants' Ethnicity and Country of Origin

Differences were observed in mean S-25(OH)D levels between all ethnic groups compared with the white grouping (mean 64.48 nmol/L), except for the West Asian and Korean groups (Table 3). The lowest mean was reported for the Japanese (33.16 nmol/L; $p < 0.001$), followed by Arabs (37.14 nmol/L; $p < 0.001$), and Southeast Asians (40.92 nmol/L; $p < 0.001$). Compared with the white grouping (6.61%), more than half (55%) of the Japanese ethnic group had vitD deficiency, with similar proportions found for Arabs (52%) and Southeast Asians (41.1%). Moreover, the Japanese had the highest insufficiency level (85%), followed by Koreans (81.0%), and Southeast Asians (73.8%). The ranges for insufficiency (30–49 nmol/L) and sufficiency (50–74 nmol/L) for each ethnic group are also presented in Figure A1, Appendix B.

Table 4 highlights the marked differences among those born in Africa, Asia, and South/Central America, and Caribbean regions. The highest rates of deficient and insufficient vitD were found among immigrants from Africa (31.2% and 68.6%, respectively) and Asia (24.5% and 69.7%, respectively) compared with those born in Canada and North America (7.7% and 31%, respectively). The ranges for insufficiency (30–49 nmol/L) and sufficiency (50–74 nmol/L) for the above mentioned five regions are presented in Figure A2, Appendix B.

Table 3. Weighted mean S-25(OH)D (nmol/L) and vitamin D status by ethnicity using Cycles 3 and 4 of Canadian Health Measures Survey data.

		S-25(OH)D (nmol/L)			S-25(OH)D Status			
		Mean (SE)	**(95% CI)**	***p*-Value**	**<30 (nmol/L (10.3%),%**	**<50 (nmol/L (63.64%),%**	**<75 (nmol/L (76.1%),%**	**≥75 (nmol/L (23.9%),%**
White †		64.48 (1.73)	-		6.61	29.10	71.25	28.75
Non-white		47.67 (1.31)	(13.85, 19.77)	<0.001	21.09 ***	60.49 ***	90.39	9.61 ***
	Aboriginal	55.55 (1.77)	(4.36, 13.50)	0.001	10.63	40.35 **	82.51	17.49 *
	South Asian	45.56 (2.48)	(13.83, 24.02)	<0.001	22.11 ***	66.73 ***	91.28	8.72 ***
	Chinese	46.03 (1.56)	(14.17, 22.74)	<0.001	17.18 ***	61.84 ***	94.89	5.11 ***
	Black	43.82 (2.96)	(15.07, 26.26)	<0.001	28.87 ***	67.72 ***	91.73	8.27 ***
	Filipino	49.39 (3.93)	(5.90, 24.29)	0.003	7.51	58.85 *	90.41	9.59 ***
	Latin American	52.64 (2.87)	(5.20, 18.50)	0.001	13.43	38.66	92.13	7.87 *
	Arab	37.14 (4.62)	(18.15, 36.54)	<0.001	51.98 ***	72.17 ***	94.40	5.60 ***
	Southeast Asian	40.92 (4.43)	(13.88, 33.24)	<0.001	41.08 ***	73.84 ***	91.19	8.81 **
	West Asian	53.74 (6.64)	(−3.29, 24.78)	0.127	13.83	58.27 **	83.35	16.65
	Korean	44.26 (15.06)	(−10.07, 50.51)	0.180	25.12 *	81.04 ***	18.96	
	Japanese	33.16 (8.59)	(14.12, 48.52)	0.001	55.30 ***	86.27 ***	13.83	
	Multiple ethnicities	53.04 (2.44)	(6.45, 16.44)	<0.001	14.48 **	46.58 ***	88.83	11.17 ***
	Other ethnicities	50.02 (5.17)	(3.33, 25.61)	0.013	62.13 ***	88.36	11.64	62.13 ***

Note: following the Statistics Canada guideline, some cells merged due to the low number of participants. † Reference value; SE, standard error; CI, confidence interval. *p*-values: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

The highest mean S-25(OH)D level was found in those born in the Netherlands, followed by the UK and Germany (72.7, 68.9, and 68.5 nmol/L, respectively). The lowest mean levels of S-25(OH)D were found in those from Algeria (34.7 nmol/L, $p < 0.001$), Lebanon (39.4 nmol/L, $p = 0.008$), and Morocco (39.4 nmol/L, $p = 0.003$). The highest deficient levels were found in those from Morocco (55.9%), India (34.4%), and Lebanon (about 30%), with the highest insufficient levels in those from Algeria (87.9%), Romania

(80.7%), and Lebanon (78.7%). The ranges for insufficiency (30–49 nmol/L) and sufficiency (50–74 nmol/L) for each birth country are presented in Figure A3, Appendix B.

Table 4. Weighted mean S-25(OH)D (nmol/L) and vitamin D status by geographical region and country of birth using Cycles 3 and 4 of Canadian Health Measures Survey data.

		S-25(OH)D (nmol/L)			S-25(OH)D Status			
		Mean (SE)	**(95% CI)**	***p*-Value**	**<30 (nmol/L (10.3%),%**	**<50 (nmol/L (63.64%),%**	**<75 (nmol/L (76.1%),%**	**≥75 (nmol/L (23.9%),%**
Region of birth	Canada and North America †	63.10 (1.74)	-	-	7.66	30.95	72.74	27.26
	South/Central America, and the Caribbean	53.20 (3.13)	(2.47, 17.33)	0.011	14.65	43.29	86.51	13.49
	Europe	62.61 (1.83)	(−2.98, 3.95)	0.775	8.62	31.24	77.46	22.54
	Africa	42.74 (3.50)	(13.08, 27.64)	<0.001	31.16 ***	68.55 ***	90.21	9.79 **
	Asia	43.67 (1.67)	(15.51, 23.34)	<0.001	24.50 ***	69.67 ***	93.41	6.59 ***
Country of birth	Canada †	63.23 (1.74)	-		7.45	30.73	72.64	27.36
	China	48.62 (2.08)	(10.21, 19.01)	<0.001	10.53	63.14 ***	91.50	8.50 ***
	USA	58.67 (4.07)	(−4.36, 13.47)	0.301	15.44 *	39.01	75.69	24.31
	France	56.98 (5.19)	(−4.17, 16.67)	0.226	34.10		90.52	9.48
	Jamaica	51.72 (7.47)	(−5.03, 28.05)	0.163	23.13 *	48.04	77.07	22.93
	UK	68.88 (3.16)	(−12.11, 0.81)	0.083	11.73	21.95	66.34	33.66
	Algeria	34.73 (6.35)	(15.49, 41.50)	<0.001	87.90 ***		12.10	
	Mexico	45.67 (11.69)	(−5.28, 40.40)	0.125	56.61		87.81	12.19
	Pakistan	41.60 (7.35)	(4.35, 38.90)	0.017	25.96 *	68.38	31.60	
	Netherlands	72.69 (5.75)	(−20.12, 1.20)	0.079	20.06		45.01	54.99 ***
	India	42.40 (4.69)	(11.64, 30.02)	<0.001	34.42 ***	73.64	93.64	6.36 ***
	Philippine	48.14 (3.55)	(7.25, 22.92)	0.001	11.20	61.20 *	91.32	8.68 **
	Romania	42.95 (5.25)	(9.26, 31.29)	0.001	80.72 ***		19.30	
	Hong Kong	53.90 (7.90)	(−8.08, 26.74)	0.278	32.02		68.00	
	Germany	68.53 (6.84)	(−19.37, 8.77)	0.443	29.44		70.19	29.81
	Colombia	60.53 (9.88)	(−19.08, 24.48)	0.800	9.39		77.36	22.64
	Morocco	39.39 (7.25)	(8.77, 38.90)	0.003	55.90 ***	68.53 ***	31.50	
	Italy	63.12 (5.43)	(−10.52, 10.73)	0.984	8.07	24.55	72.49	27.51
	Iran	49.99 (6.87)	(−1.77, 28.23)	0.081	6.18	73.32 **	87.94	12.06
	Lebanon	39.38 (8.18)	(6.89, 40.80)	0.008	29.97 **	78.70 **	21.30	
	Others	49.28 (1.57)	(10.58, 17.31)	<0.001	21.97 ***	54.45 ***	90.19	9.8%
	All (153 Countries)	51.09 (1.40)	(9.01, 15.27)	<0.001	18.91 ***	53.42 ***	86.66	13.34 ***

Note: following the Statistics Canada guideline, some cells merged due to the low number of participants. † Reference value; SE, standard error; CI, confidence interval. *p*-values: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

3.4. S-25(OH)D Concentration and VitD Status by Immigration Status and Season

The S-25(OH)D increased substantially during summer and fall compared with winter. Compared with January, the lowest weighted mean was found in February and the highest in September. The highest percentage of change (−37%) from the optimal level (75 nmol/L) was in February (Figure 1) and (Table A5, Appendix B). Non-immigrants had higher S-25(OH)D in all seasons, with significant increments in spring, summer, and fall compared with the winter season (Figure 2) and (Table A6, Appendix B).

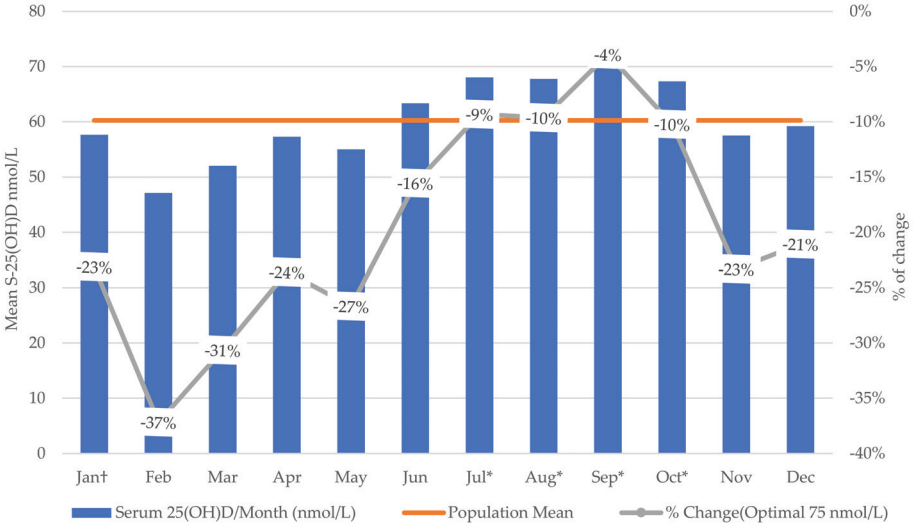


Figure 1. Weighted mean S-25(OH)D (nmol/L)/month of test and percentage of change from the optimal level (75 nmol/L) using Cycles 3 and 4 of Canadian Health Measures Survey data († reference value * significant at $p \leq 0.05$).

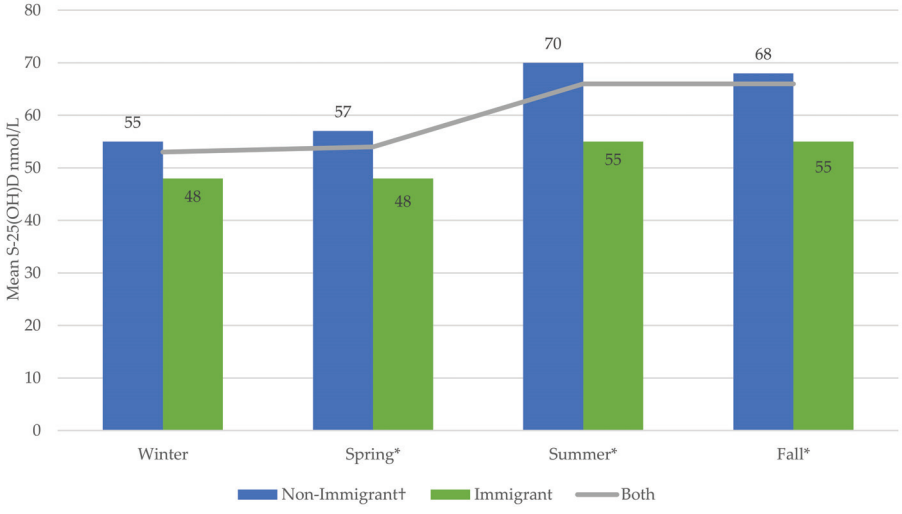


Figure 2. Weighted seasonal variation in mean S-25(OH)D (nmol/L) based on immigration status using Cycles 3 and 4 of Canadian Health Measures Survey data († reference value * significant at $p \leq 0.05$).

3.5. Results of Multivariate Analysis

The final multi-linear regression model showed that immigrants had lower S-25(OH)D levels compared with non-immigrants (beta-estimate: −5.28, 95% CI: −7.48, −3.09, $p < 0.001$) after adjusting for all covariates (Table 5). Ethnicity was the strongest predictor of S-25(OH)D in immigrants compared with non-immigrants. Other predictors such as consumption of dairy products (milk, cheese, and yogurt) and traveling to a sunny/warm climate in the two months before blood sampling were also strongly associated with differences in S-25(OH)D. Overall, the model showed a robust association between immigration status and concentrations of S-25(OH)D.

Table 5. Multivariate analysis-backward elimination method based on S-25(OH)D (nmol/L) and immigration status using Cycles 3 and 4 of Canadian Health Measures Survey data.

	S-25(OH)D		
	Beta Estimate (SE)	**(95% CI)**	***p*-Value**
Immigration status	−5.28 (1.06)	−7.48, −3.09	<0.001
Sex	4.54 (0.88)	2.71, 6.36	<0.001
Season	3.44 (0.97)	1.42, 5.46	0.002
Age	0.14 (0.04)	0.07, 0.22	0.001
Traveled to a warm/sunny climate	6.31 (1.67)	2.85, 9.78	0.001
BMI	−4.89 (0.64)	−6.11, −3.48	<0.001
Dairy products (milk, cheese, yogurt)	5.79 (0.74)	4.25, 7.32	<0.001
Skin pigmentation (melanin)	1.36 (0.28)	0.79, 1.93	<0.001
Sunscreen use	4.95 (1.12)	2.63, 7.28	<0.001
Ethnicity	−15.11 (1.61)	−18.46, −11.78	<0.001
VitD-supplement and/or analog use	0.52 (0.16)	0.17, 0.86	0.005
-Const.	29.01 (5.91)	17.91, 40.12	<0.001

Adjusted linear regression for age, sex, income, education, BMI, season, sun exposure, sunscreen use, country of birth, melanin levels, ethnicity, vitD-medication/supplements, and food consumption variables. Significant at $p \leq 0.05$. BMI, body mass index; SE, standard error; CI, confidence interval.

4. Discussion

This is the first national Canadian study to report vitD status among immigrants from different ethnic groups and origins compared with non-immigrants and has a global impact. A previous systematic review noted the need to assess vitD status and its determinants (including lifestyle factors) among subgroups living in the same country [35]. Moreover, research has highlighted the importance of comparing the same generation of immigrants rather than an aggregated generation [22], and gathering evidence, and formulating recommendations specific to sub-populations that may differ from the overall immigrant population [16].

The higher overall prevalence of vitD insufficiency among immigrants than non-immigrants in this study was consistent with global evidence suggesting that immigrants in Western countries have lower S-25(OH)D levels than non-immigrant populations [8,10,36,37]. Our multivariate analyses found ethnic background, having traveled to a sunny/warm climate, and low dairy product consumption were strong predictors of low S-25(OH)D levels among immigrants in Canada. Consistent with our findings, associations between S-25(OH)D levels and low income, winter season, low sunlight exposure, conservative dress, BMI (obesity), vitD supplements use, low physical activity, less traveling to a sunny/warm climate, non-white ethnicity, place of birth, and skin pigmentation were reported in previous studies [1,10,18].

While females are usually at higher risk for vitD deficiency [8,36–38], females in our study had markedly higher serum S-25(OH)D levels than males. In 2015, Statistics Canada reported that females had more elevated serum of vitD than males, more frequently using supplements than males (41% vs. 28%, respectively), and nearly 85% of vitD supplement users were above the cut-off (<50 nmol/L) compared with non-users (59%) [15]. McCormack et al. 2017, reported that females ≥ 19 years in Canada were using nutritional supplements (including vitD) more than males [39]. However, the higher levels of S-25(OH)D could be partly explained by the finding that females used vitD supplements more than males (66% vs. 34%, $p = 0.019$) (data not shown). In compliance with our finding, studies demonstrated that women who wear concealing clothes had lower S-25(OH)D levels than women dressed according to Western-style. It was well documented that the type of clothing determines the degree of sun exposure and supports/eliminates vitD's epidermal synthesis [8,10,18,40,41]. In this study, the effect of dress style on S-25(OH)D levels reduced (95% CI: −0.67 15.82; $p = 0.070$) in winter when both immigrants and non-immigrants wear winter clothes that cover all the body, while the difference remained statistically significant for spring, summer, and fall which may be due to different type of clothing during these seasons.

The current study found that immigrants with low household income were more linked with lower S-25(OH)D levels. A global vitD review reported that higher family income in developing countries was found inversely associated with hypovitaminosis D [10]. Other studies confirmed that lower-income immigrants were found with lower levels of S-25(OH)D than higher incomes [42,43]. Consistent with our findings, global and Canadian vitD studies reported that obese adults had significantly lower S-25(OH)D than normal/underweight and overweight [1,8,15,44]. Studies suggested that this may be due to vitD displacement in adipose tissue, which results in lower circulating S-25(OH)D levels in the blood [8].

Dairy products contain essential nutrients including vitD and calcium. Previous studies suggested insufficient consumption of these products was strongly associated with lower S-25(OH)D levels [10,42,45,46]. Furthermore, milk in Canada is fortified with vitD, indicating a possibly magnified contribution of dairy products to serum vitD for both immigrant and non-immigrant consumers. Nonetheless, consumption of fortified milk and dairy products was relatively higher in non-immigrants than immigrants, which may partially explain the difference in S-25(OH)D levels.

Canada regions have different weather conditions; nonetheless, it is expected that days in fall and winter are shorter than in summer and spring, with fewer hours of sunlight overall in Canada. In the present study, immigrants had less sun exposure than non-immigrants and had lower S-25(OH)D levels in winter than in summer. The seasonal variation in S-25(OH)D between immigrants and non-immigrants may be explained by the role of ultraviolet-B (UVB) light exposure in the endogenous synthesis of vitD. Previous studies also reported seasonal variations in vitD status [10,19,47]. Other studies reported the lowest levels of S-25(OH)D in winter [8,48], which was consistent with our findings.

The non-sunscreen users had a higher prevalence of deficiency and insufficiency in vitD status than did the frequent sunscreen users. However, such difference could be partly explained by the fact that sunscreens are used as photoprotector as it minimizes ultraviolet-B (UVB) light exposure, hence lowers endogenous vitD activation, and thus increases the likelihood of developing vitD deficiency [49]. Nonetheless, in practice, sometimes the irregular use of sunscreen or inadequate amounts may not be enough to secure the body from all UVB light. Furthermore, sunscreen users might feel sunburn protected by blocking UVB light and, hence, tend to expose themselves more to the sun than non-users, increasing S-25(OH)D levels. In line with our finding, 25% of studies in a recent review concluded that sunscreen use was associated with higher S-25(OH)D concentration, while 10% of studies reported lower levels and 35% with no association [49,50]).

Our data were obtained from immigrants from more than 150 countries, over 13 major ethnic groups, and therefore present a unique contribution to understanding Canada's ethnocultural diversity concerning vitD status. Irrespective of the length of time living in Canada, immigrants who were born in Africa and Asia had lower S-25(OH)D levels than those born in North America. The lowest levels by country of birth were among immigrants born in Algeria, Lebanon, and Morocco. In terms of ethnic origins, the lowest S-25(OH)D levels were in Japanese, followed by Arab and Southeast Asian ethnic groups. Our adjusted linear regression model indicated ethnicity was the most important indicator of low S-25(OH)D levels among immigrants compared with non-immigrants. Similarly, previous research reported vitD deficiency was more common in people from specific racial backgrounds. For example, Asian (South Asia, Southeast), African, and Middle Eastern immigrant populations had lower S-25(OH)D than their counterparts in Western countries [8,10,48]. Middle Eastern immigrants (including Lebanese and Iranian people) were more frequently reported to have deficient/insufficient levels than other ethnic groups and non-immigrant populations in Western countries [10]. A systematic review of 112 studies (168,389 participants from 44 countries) reported insufficient levels in one-third of the included studies, with the highest levels among participants living in North America and lower levels among those living in the Middle East and African regions [35].

Dark-skinned individuals have a rich amount of melanin, which acts as a biological shield against UV radiation [8]. In the present study, skin pigmentation was used to rank

participants based on melanin index values, where higher degrees of melanin indicated darker skin pigmentation. We found that immigrants had higher melanin levels than non-immigrants. Brook et al. reported the median 25(OH)D concentrations were higher in whites compared with non-whites in Canada [51] and several other studies have documented associations between skin pigmentation, ethnicity, geographical origins, and S-25(OH)D levels [8,18,52]. Research suggests that dark-skinned immigrants are at higher risk for vitD deficiency the longer time they spend in the host country [18]. In contrast, we found immigrants had markedly higher S-25(OH)D levels the longer they had lived in Canada after immigration. However, integration into a new life in Canada may reflect physical and behavioral adaptation to the surrounding environment, including lifestyle, diet, and climate changes. Immigration studies on acculturation and vitD found the length of time since immigration was a crucial indicator of lifestyle acculturation, and higher acculturation levels were associated with significantly higher S-25(OH)D levels [21,53]. However, we found immigrants had less sun exposure and less frequent consumption of vitD-rich foods than non-immigrants, and the length of time since immigration reflected reasonable lifestyle acculturation in Canada. Moreover, genomic studies showed that vitD interacts and influences the human genome through vitD receptor-mediated gene regulation and drives evolution for genomic adaptations in the context of skin color lightening [54]. The biological adaptation process is part of micronutrient gene-regulation that supports vitD3 synthesis in regions with lower UVB radiation [54]. Therefore, epigenetic adaptations may partially explain why immigrants in Canada were prone to vitD deficiency in the short term after immigration, and levels of S-25(OH)D increased over time. Furthermore, our study showed that ethnic differences played a fundamental role in S-25(OH)D levels. Common genetic polymorphisms in the vitD receptor and vitD-binding protein may partly explain differences in vitD status between different ethnicities [55]. Therefore, vitD insufficiency and deficiency among immigrants may be associated with genetic variations combined with dietary and other environmental factors; this issue warrants further investigation.

To our knowledge, this was the first study assessing S-25(OH)D levels among Canadian immigrants' different ethnic groups and origins compared with the non-immigrant population at the national level. The findings highlighted the need for further research investigating immigrants' health deterioration in the context of vitD. In addition, building knowledge of the relationship between health deterioration and vitD status along with similar biomarkers has important implications for responding to chronic and infectious diseases, including COVID-19.

Our study should be interpreted with consideration of its strengths and limitations. The CHMS data have many strengths. First, the CHMS provided the first national, comprehensive, and representative data for the Canadian population on vitD. Moreover, it used several clinical and laboratory measures to overcome the limitations of self-reported data [27]. Second, the study design and large sample size provided a nationally representative sample of immigrants in Canada. Third, it captured the ethnocultural diversity of immigrants from more than 150 countries and represented more than 13 major ethnic groups. Fourth, reported melanin level is a reliable indication of skin pigmentation. Fifth, S-25(OH)D levels were collected over the entire year, which enabled adjustment for seasonal variations in UVB light exposure, and offered a more accurate and representative measure of S-25(OH)D at the national level. Finally, the data were validated against other national data such as the Canadian Community Health Survey, Census data, and the 2011 National Household Survey [27]. However, immigrants were identified as landed people without detailed information about the immigration-specific status (e.g., refugees or asylum seekers) [27]. Research suggests refugees have a higher risk for vitD deficiency than other population groups [18,19,56,57]. The lack of data on refugees and asylum seekers limited further subgroup analyses. Another limitation of CHMS data is that the levels of biologically activated form 1,25(OH)2Dwas not measured in the study participants.

5. Conclusions

In conclusion, vitD deficiency is more common among Canadian immigrants than non-immigrants. Immigrants originating from African (Algeria and Morocco) and Asian (Lebanon) countries have a particularly low mean of S-25(OH)D. This study highlights ethnicity as a pivotal predictor of immigrants' lower S-25(OH)D levels; the Japanese, Arab, and Southeast Asian ethnic groups have a high prevalence of vitD deficiency. Short-term prevention strategies in community and clinical settings may be warranted, including vitD supplementation and awareness for people at higher risk. A long-term intervention plan may include developing/updating Canadian immigrant guidelines to incorporate evidence-based improvement strategies for vitD implementation.

Author Contributions: S.Y. and G.A.W. conceived the study. With close supervision from G.A.W., S.Y. managed the data, verified the analytical methods, and prepared the tables and figures. A.H. verified the underlying data at the Research Data Center. S.Y. and M.F. interpreted the results and prepared the final draft. All authors including D.M., I.C. and M.P. contributed to the design and analysis of the research, and the substantive review of the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Institutional Review Board Statement: All components of the national survey were reviewed and authorized annually by the Health Canada/Public Health Agency of Canada Research Ethics Board (REB# 2005-0025). The CHMS was approved by the Health Canada Research Ethics Board.

Informed Consent Statement: Written consents were collected by Statistics Canada from all participants for a personal interview, physical measures, and biospecimens collection.

Data Availability Statement: Data described in the manuscript, codebook, and analytic code will not be made publicly available because the data are confidential national survey data hosted by Statistics Canada.

Acknowledgments: The authors acknowledge the team from the Carleton, Ottawa, Outaouais Local Research Data Centre for assistance with access to CHMS data. CHMS data were obtained and supported by funding to the Canadian Research Data Centre Network from the Social Sciences and Humanities Research Council, the Canadian Institute for Health Research, the Canadian Foundation for Innovation, and Statistics Canada. Although the research and analysis were based on data from Statistics Canada, the opinions expressed do not represent the views of Statistics Canada.

Conflicts of Interest: The authors declare no personal or financial conflicts of interest.

Appendix A

Appendix A: Supporting information regarding the steps used to combine Cycles 3 and 4, and detailed information about the calculation of the variables of interest (Table A1).

Table A1. Variables of interest in Cycles 3 and 4 of the Canadian Health Measures Survey.

Variable	Description
Dependent variable	We used the contentious variable serum 25(OH)D (mmol/L). We categorized vitD status using the following cut-offs for S-25(OH)D: (<30 (nmol/L); (<50 (nmol/L); (<75 (nmol/L); and (≥75 (nmol/L). First, <30 nmol/L is used to identify deficient people. Second, (<50 nmol/L) is a cut-off for the insufficient 25(OH) D, which includes the deficient and insufficient people (it is an accumulating value and not range for the insufficient people). Third, (<75 nmol/L) is a cut-off and accumulating value for deficient, insufficient, and sufficient. Fourth (≥75 (nmol/L), it is the "no added value" or the "optimal" as defined by IOM or other experts [11–13]. Moreover, we used ranges of S-25(OH)D for the above mentioned cut-offs (<30 nmol/L; 30–49 nmol/L; 50–74 nmol/L and ≥75 nmol/L) for sub-groups of participants.

Table A1. *Cont.*

Variable	Description
Independent variables	
Immigration status	Landed immigrant (yes, no).
Age at time of immigration	Age at immigration was categorized as <18 years or ≥18 years.
Time since immigration	We used the continuous variable "length of time since first came to live in Canada" to categorize time since imigration at two cutoff points (≤5 years and >5 years; ≤10 years and >10 years). We used these points as indications of recent immigrants and long-term immigrants.
Sex	Male/female.
Age	Contentious and categorical variables.
Education	Education was classified as ≤secondary school or >secondary school.
Household income	Household income was derived and imputed by Statistics Canada. The contentious variable and categorical variables (<50,000, 50,000–100,000, and >100,000) were used as appropriate.
Body mass index	Calculated as weight divided by height squared (kg/m^2). We used body mass index norms for adults (≥18 years) based on national standards for weight classification [30]. For children (3–17 years), body mass index was classified according to World Health Organization percentiles [31].
Smoking	We categorized smoking status into two groups (former/non-smoker vs. current smoker).
Alcohol	We categorized alcohol consumption into two groups (former/non-drinker vs. current drinker).
Vitamin D-containing supplements/medications	All prescription medications, over-the-counter, and herbal remedies taken in the past month (including vitD supplements and medications) were recorded in the CHMS data. The Anatomical Therapeutic Chemicals (ATCs) is a system that classifies products according to the organ and their chemical, pharmacological, and therapeutic properties. The vitD -related ATC codes were selected (A11CC01–05). These ATC codes represent ergocalciferol (vitD 2), dihydrotachysterol (synthetic vitD analog), alfacalcidol (analog of vitD), calcitriol, and cholecalciferol (vitD 3). Users of any of these were recorded as "yes," non-users as "no," and not respondents as "missing."
Dietary intake	The CHMS collected dietary intake of different types of each item (red meat, liver, fish, egg, milk, cheese, yogurt, margarine) based on the previous month's consumption. Statistics Canada prepared derived variables for each item to describe the number of times that item had been consumed/year. These derived variables were used to calculate consumption per week or day as needed. Different types of each item were merged into a single category. For example, all kinds of milk (plain, flavored, omega-3) were merged as "all milk." The same procedure was used for "all meats" (e.g., red meat, liver, sausage, hotdog), as well as "all cheese," "all yogurt," "all eggs," and "all margarine." Another category was used for all dairy products, called "all diary." We also used fish consumption in the past two months as (yes vs. no). Values of ≤1 time/week vs. >1 time/week were used for all categories, except all milk and all dairy, which used ≤7 times/week vs. >7 times/week.
Calcium and phosphorus	Contentious variables (mmol/L).
Pregnancy	Pregnancy was classified as Yes or No.
Ethnicity	Broadly, ethnicity was classified into two main groupings as white or non-white. Statistics Canada categorized non-white into the 12 largest ethnic groups (Aboriginal, South Asian, Chinese, Black, Filipino, Latin American, Arab, South East Asian, West Asian, Korean, Japanese, Multiple ethnicities), and another category for all other ethnicities.
Season of blood sampling	The season variable was created based on the derived variable of "month of blood sampling." We categorized the season as winter (December–February), spring (March–May), summer (June–August), and fall (September–November).
Regions of birth	We used five regions: Canada and North America; South and Central America and the Caribbean; Europe; Africa; and Asia.

Table A1. *Cont.*

Variable	Description
Countries of birth	We used the geographic classifications of Statistics Canada to identify the country of birth for all CHMS participants (153 countries) [58]. We ranked and selected the top 20 countries based on the number of participants (≥30 participants from each). These countries were Canada, China, the US, France, Jamaica, the UK, Algeria, Mexico, Pakistan, the Netherlands, India, the Philippines, Romania, Hong Kong, Germany, Colombia, Morocco, Italy, Iran, and Lebanon. We combined all other countries in one category, "Other," and combined the 153 countries (excluding Canada) in another class to represent all foreign-born immigrants.
Sun exposure (10 am to 4 pm)	Sun exposure was classified as <30 min\day or ≥30 min\day.
Sunscreen application	Sunscreen application was classified as yes or no.
Clothing type	The clothing type was based on coverage and classified as yes or no. Yes indicated typically covered (face, ears, neck or arms and legs).
Physical activity	Physical activity for adults (≥18 years) was calculated based on the Canadian Physical Activity Guidelines (CPAG). The CPAG recommendation for adults is to accumulate at least 150 min of moderate-to-vigorous intensity aerobic physical activity per week, in bouts of ≥10 min. The CPAG recommendation for children (5–17 years) is to accumulate at least 60 min of moderate-to-vigorous intensity physical activity daily for at least three days per week [32].
Traveled to a sunny/warmer place (in the past two months)	Travelling to a sunny/warmer place in the past two months was classified as yes or no.
Skin pigmentation (melanin levels)	Melanin is the component that gives skin its natural color. Melanin levels were measured from the back of the hand three times; a fourth measurement was required if the difference between the first three melanin values deviated by more than 10 units. The final average calculated by Statistics Canada indicates the absolute index values of melanin, where the higher the value, the more melanin present in the epidermal. The device used for measurement was the DSM II ColorMeter (Cortex Technology, Hadsund, Denmark).

Appendix A.1. Combining Cycles 3 and 4

The CHMS is the first national population-based survey on vitamin D (vitD) conducted in Canada. Data were collected in six cycles biannually between 2007–2019. Compared with other cycles, Cycle 3 (2012–2013) and Cycle 4 (2014–2015) are considered the most thematically consistent in terms of vitD-related sociodemographic characteristics, lifestyle factors, vitD-rich foods consumption, and behavioral, environmental, and biological determinants. Immigrant status was defined as being born outside Canada [27]. In this study, we combined data from Cycles 3 and 4 to increase the sample size and provide a more stable and precise estimate of sampling variability at the national level than was possible using a single data cycle estimate. The combination of the two cycles followed Statistics Canada's guideline for combining CHMS cycles. As recommended, we used Bootstrap 1–Bootstrap 500, full-weight or applicable weight, and the required degrees of freedom to correct variance estimations. Therefore, the pre-specified combined full-sample weights and degrees of freedom for the two cycles were used in our analyses. The single-cycle weight was dropped from the dataset as it was a cycle-specific weight. The combined response rate (response rate for all study components, such as household visit, blood samples, and activity monitor) at the Canadian level for Cycle 3 was 51.7%, and that for Cycle 4 was 53.7% [33,34].

Appendix A.2. Population, Comparators, Measures, and Outcome

The population of interest in this study was first-generation Canadian immigrants (foreign-born) from different origins and ethnicities. The comparators were non-immigrants (native-born population) and the white ethnic grouping. The S-25(OH)D was measured using chemiluminescence immunoassay technology (DiaSorin®, Ltd., Stillwater, Minnesota, USA). The analytical detection limit for S-25(OH)D was 10–375 nmol/L. The inter-assay coefficients of variation for the S-25(OH)D was 13.0% and the precision for <20 nmol/L, 20–100 nmol/L and >100 nmol/L levels were 15.0%, 10.0% and 12.0%, respectively [45].

The results are presented as weighted means or proportions only. The weighted results and proportions were based on the total number of 11,579 participants. The number of participants for each group cannot be published because of Statistics Canada's restrictions policy relates to publishing weighted results. The weighting technique for complex survey results refers to statistical adjustments that have been used to correct and improve the accuracy of the survey estimates. The survey weights adjust for unequal probabilities of selection that often have occurred during sampling design and to help compensate for survey non-response weights. Thus, by using the survey weights to create estimates should yield approximately unbiased national prevalence estimates and reduce non-response bias that can be introduced due to random missing values. The study outcomes were the serum 25(OH)D levels and vitD status.

Appendix A.3. Variables of Interest

The data manipulation and all derived variables were consistent with Statistics Canada's instructions based on the specific cycle user guide, data codebook, instructions for combining more than one cycle, and the derived variable specifications [33]. The CHMS microdata are hosted in secure centers overall in Canada. These data were accessed and analyzed at the Carleton, Ottawa, Outaouais Local Research Data Centre (COOLRDC), located in the Morisset Library at the University of Ottawa.

Appendix B

Appendix B: supporting information including (Tables A2–A6).

This Appendix sets out the steps followed in combining Cycles 3 and 4 of the Canadian Health Measures Survey (CHMS), variables of interest, critical data manipulation stages, and other methodological aspects.

This Appendix contains supplementary material for the manuscript entitled "*Vitamin D Status among First-Generation Immigrants from Different Ethnic Groups and Origins: An Observational Study using the Canadian Health Measures Survey.*"

Table A2. Weighted prevalence and mean S-25(OH)D (nmol/L) levels and basic study characteristics using Cycles 3 and 4 of Canadian Health Measures Survey data.

		Cycle 3 (49.52%) %	**Cycle 4 (50.48%)** %	**Total (100%)** %
Immigration status	Immigrant	24.5	19.38	21.90
Ethnic group	White	75.10	78.87	76.98
Sex	Female	50.00	50.10	50.08
Age (years)	<5	2.47	2.14	2.30
	5–11	7.92	8.17	8.04
	12–17	7.58	7.21	7.39
	18–64	70.70	70.29	70.54
	>64	11.20	12.20	11.72
Household income (CAD)	<50,000	38.10	32.91	35.45
	50,000–100,000	36.00	37.26	36.61
	>100,000	26.00	29.84	27.93
S-25(OH)D status (nmol/l)	<50	65.04	62.27	63.64
Weighted Mean		Mean (SE)	Mean (SE)	Mean (SE)
	Age (years)	39.08 (0.15)	39.35 (0.09)	39.23 (0.085)
	S-25(OH)D (nmol/L)	61.29 (2.78)	59.16 (1.99)	60.28 (1.69)

SE, standard error.

Table A3. Weighted prevalence of vitamin D-rich food/fish consumption for immigrants and non-immigrants using Cycles 3 and 4 of Canadian Health Measures Survey data.

		Non-Immigrants (78.9%), %	Immigrants (21.9%), %	All Participants (100%), %	*p*-Value
All meats (red meat, liver, hotdog, and sausage)	>1 time/week	90.47	78.72	87.89	<0.001
All eggs (yolk/omega-3)	>1 time/week	59.68	66.95	61.26	0.067
All milk (plain, flavored, and omega-3)	>7 times/week	54.63	48.38	53.27	0.027
All cheese (cottage and other)	>1 time/week	86.70	63.26	81.57	<0.001
All yogurt	>1 time/week	72.19	70.15	71.75	0.577
All dairy products (milk, yogurt, and cheese)	>7 times/week	83.38	75.38	81.64	<0.001
All margarine (plain and omega-3)	>1 time/week	84.11	70.95	81.90	0.007
Fortified juice with calcium/vitamin D	>1 time/week	7.01	6.12	6.81	0.452
Fish consumption/past 2 months	Yes (No †)	25.18	17.09	23.43	0.046

Reference values: ≤1 time/week; ≤7 times/week. † Reference value; *p*-values ≤ 0.05.

Table A4. Weighted means for S-25(OH)D (nmol/L) and vitamin D status by sociodemographic and lifestyle factors using Cycles 3 and 4 of Canadian Health Measures Survey data.

		S-25(OH)D (nmol/L)			S-25(OH)D Status			
		Mean (SE)	(95% CI)	*p*-Value	<30 (nmol/L (10.3%),%	<50 (nmol/L (63.64%),%	<75 (nmol/L (76.1%),%	≥75 (nmol/L (23.9%),%
Household income (CAD)	<50,000 †	56.18 (1.90)	-		13.95	45.10	79.43	20.57
	50,000–100,000	61.44 (1.81)	(−7.73, −2.79)	<0.001	9.67	33.14	76.02	23.98
	>100,000	63.66 (1.79)	(−9.97, −4.98)	<0.001	6.53 ***	29.64 ***	72.09	27.91 **
Education	≤Secondary school †	59.41 (1.64)			9.57	36.81	77.58	22.42
	>Secondary school	61.00 (1.86)	(−3.59, 0.41)	0.113	10.87	35.83	74.97	25.03
Pregnancy	No†	60.35 (2.15)	-		11.72	36.88	75.29	24.71
	Yes	51.22 (7.40)	(−6.69, 24.94)	0.244	2.11	45.70	93.21	6.79 *
Smoking habits	Former/non-smoker †	61.59 (1.66)	-		9.98	34.12	73.82	26.18
	Current smoker	53.09 (2.12)	(5.74, 11.24)	<0.001	14.88 *	50.71 ***	85.02	14.98 ***
Alcoholic beverages	Former/non-drinker †	54.25 (2.17)	-		15.72	38.15	81.44	18.56
	Current drinker	61.33 (1.67)	(−10.49, 3.66)	<0.001	9.73 **	34.70 ***	74.70	25.30 **
Meet the physical activity recommendations	No †	58.79 (1.54)	-		11.32	39.34	77.91	22.09
	Yes	63.80 (2.34)	(−8.26, 1.76)	0.004	8.01	29.54 ***	72.86	27.14 *
Clothing	Uncovered †	62.07 (1.69)	-		8.23	31.13	74.85	25.15
	Typically covered	57.91 (2.00)	(1.46, 6.85)	0.004	12.86 **	42.58 ***	77.86	22.14

† Reference value; SE, standard error; CI, confidence interval; BMI, body mass index. *p*-values: * $p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$.

Table A5. Weighted means for S-25(OH)D (nmol/L) by season and month of blood test using Cycles 3 and 4 of Canadian Health Measures Survey data.

		S-25(OH)D (nmol/L)		*p*-Value
		Mean (SE)	(95% CI)	
Season	Winter †	53.80 (2.47)	-	
	Spring	54.63 (1.41)	(−7.16, 5.49)	0.786
	Summer	66.13 (2.38)	(−18.50, −6.16)	<0.001
	Fall	66.00 (3.63)	(−20.59, −3.83)	0.006
Month	January †	57.67 (9.25)	-	
	February	47.13 (6.17)	(−25.73, 4.65)	0.164
	March	52.02 (9.57)	(−28.63, 18.86)	0.674
	April	57.31 (1.64)	(−23.46, 3.10)	0.126
	May	55.00 (2.17)	(−21.38, 5.63)	0.240
	June	63.34 (8.61)	(−38.14, 5.73)	0.140
	July	68.02 (5.41)	(−37.88, −3.89)	0.018
	August	67.78 (7.31)	(−40.77, −0.53)	0.045
	September	72.08 (10.24)	(−49.86, −0.04)	0.050
	October	67.34 (5.39)	(−37.99, −2.42)	0.028
	November	57.52 (2.80)	(−24.80, −4.02)	0.149
	December	59.20 (13.18)	(−42.89, 18.75)	0.425

† Reference value; SE, standard error; CI, confidence interval; *p*-values ≤ 0.05.

Table A6. Weighted mean S-25(OH)D concentrations (nmol/L) by immigration status and season using Cycles 3 and 4 of Canadian Health Measures Survey data.

		Non-Immigrant †	Immigrant		
		Mean (SE)	Mean (SE)	(95% CI)	*p*-Value
Season	Winter	55.36 (2.33)	47.79 (4.55)	(−0.67, 15.82)	0.070
	Spring	56.94 (1.62)	47.71 (3.07)	(2.27, 16.19)	0.012
	Summer	70.03 (2.23)	55.21 (1.76)	(9.28, 20.36)	<0.001
	Fall	68.11 (3.55)	54.87 (3.95)	(8.00, 18.47)	<0.001

† Reference value; SE, standard error; CI, confidence interval; *p*-values ≤ 0.05.

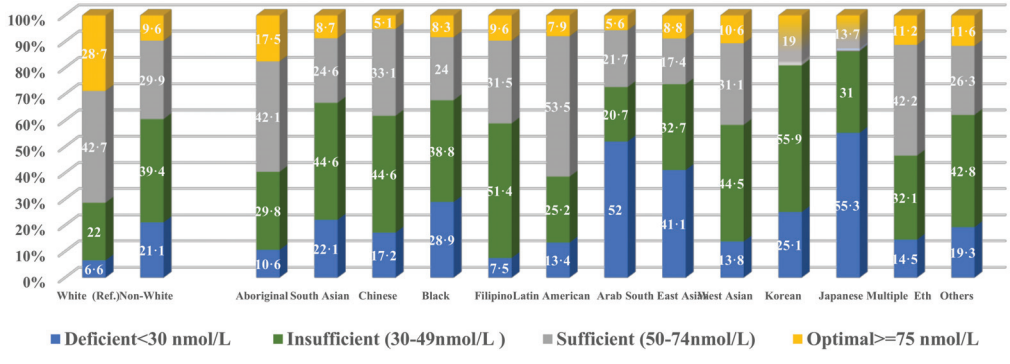


Figure A1. Weighted prevalence of S-25(OH)D status for immigrants from different ethnic groups using Cycles 3 and 4 of Canadian Health Measures Survey data.

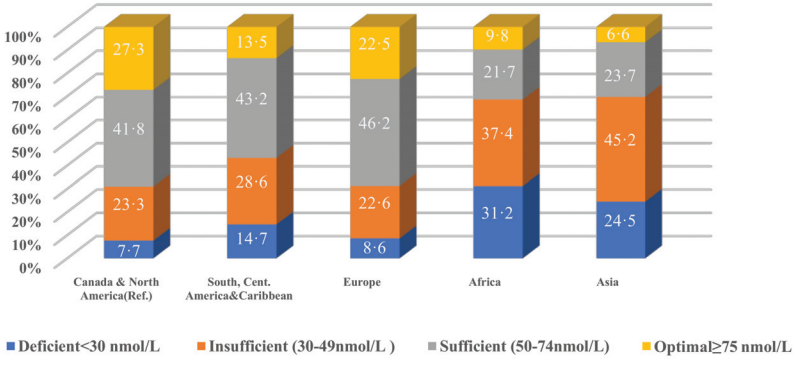


Figure A2. Weighted prevalence of S-25(OH)D status for immigrants by region of birth using Cycles 3 and 4 of Canadian Health Measures Survey data.

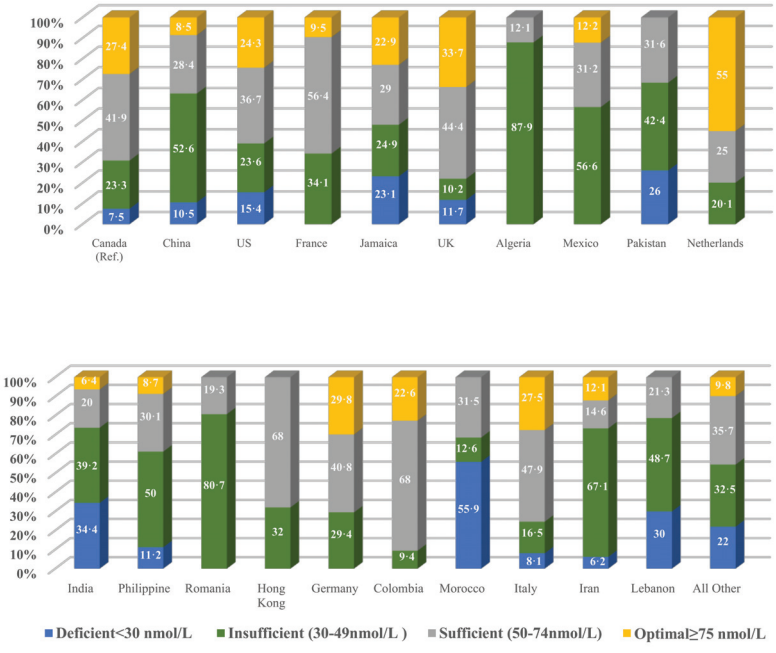


Figure A3. Weighted prevalence of S-25(OH)D status by country of birth using Cycles 3 and 4 of Canadian Health Measures Survey data.

References

1. Charoenngam, N.; Shirvani, A.; Holick, M.F. Vitamin D for skeletal and non-skeletal health: What we should know. *J. Clin. Orthop. Trauma* **2019**, *10*, 1082–1093. [CrossRef]
2. Shin, S.Y.; Kwon, M.J.; Song, J.; Park, H.; Woo, H.Y. Measurement of Serum Total Vitamin D (25-OH) Using Automated Immunoassay in Comparision With Liquid Chromatography Tandem-Mass Spectrometry. *J. Clin. Lab. Anal.* **2013**, *27*, 284–289. [CrossRef]
3. Atef, S.H. Vitamin D assays in clinical laboratory: Past, present and future challenges. *J. Steroid Biochem. Mol. Biol.* **2018**, *175*, 136–137. [CrossRef] [PubMed]
4. Martens Immunoassay for Free Vitamin D. 2018. Available online: https://patentimages.storage.googleapis.com/57/7e/64/9c4548d4ed8ce1/US9897615.pdf (accessed on 24 February 2021).

5. Nowson, C.A.; McGrath, J.J.; Ebeling, P.R.; Haikerwal, A.; Daly, R.M.; Sanders, K.M.; Seibel, M.J.; Mason, R.S. Vitamin D and health in adults in Australia and New Zealand: A position statement. *Med. J. Aust.* **2012**, *196*, 686–687. [CrossRef]
6. Heaney, R.P.; Recker, R.R.; Grote, J.; Horst, R.L.; Armas, L.A. Vitamin D3 is more potent than vitamin D2 in humans. *J. Clin. Endocrinol. Metab.* **2011**, *96*, E447–E452. [CrossRef] [PubMed]
7. De Boer, I.H.; Kestenbaum, B.; Shoben, A.B.; Michos, E.D.; Sarnak, M.J.; Siscovick, D.S. 25-hydroxyvitamin D levels inversely associate with risk for developing coronary artery calcification. *J. Am. Soc. Nephrol.* **2009**, *20*, 1805–1812. [CrossRef]
8. Spiro, A.; Buttriss, J. Vitamin D: An overview of vitamin D status and intake in E urope. *Nutr. Bull.* **2014**, *39*, 322–350. [CrossRef]
9. Zmijewski, M.A. Vitamin D and Human Health. *Int. J. Mol. Sci.* **2019**, *20*, 145. [CrossRef] [PubMed]
10. Mithal, A.; Wahl, D.A.; Bonjour, J.-P.; Burckhardt, P.; Dawson-Hughes, B.; Eisman, J.A.; Fuleihan, G.E.-H.; Josse, R.G.; Lips, P.; Morales-Torres, J. Global vitamin D status and determinants of hypovitaminosis D. *Osteoporos. Int.* **2009**, *20*, 1807–1820. [CrossRef]
11. Ross, A.; Taylor, C.; Yaktine, A.; Del Valle, H. *Dietary Reference Intakes: Calcium and Vitamin D. Committee to Review Dietary Reference Intakes for Vitamin D and Calcium Food and Nutrition Board*; National Academy of Sciences: Washington, DC, USA, 2011.
12. Vieth, R. Why the minimum desirable serum 25-hydroxyvitamin D level should be 75 nmol/L (30 ng/mL). *Best Pract. Res. Clin. Endocrinol. Metab.* **2011**, *25*, 681–691. [CrossRef]
13. Dawson-Hughes, B. *Vitamin D Deficiency in Adults: Definition, Clinical Manifestations, and Treatment*; UpToDate: Waltham, MA, USA, 2014.
14. Pham, T. Vitamin D Status of Immigrant and Ethnic Minority Children Ages 2 to 5 y in Montréal. Master's Thesis, McGill University, Montreal, QC, Canada, 2012.
15. Janz, T.; Pearson, C. *Vitamin D Blood Levels of Canadians*; Statistics Canada Ottawa (Canada): Ottawa, ON, Canada, 2013.
16. Yousef, S.; Elliott, J.; Manuel, D.; Colman, I.; Papadimitropoulos, M.; Hossain, A.; Leclair, N.; Wells, G.A. Study protocol: Worldwide comparison of vitamin D status of immigrants from different ethnic origins and native-born populations—A systematic review and meta-analysis. *Syst. Rev.* **2019**, *8*, 211. [CrossRef]
17. Cashman, K.D.; Dowling, K.G.; Škrabáková, Z.; Gonzalez-Gross, M.; Valtueña, J.; De Henauw, S.; Moreno, L.; Damsgaard, C.T.; Michaelsen, K.F.; Mølgaard, C. Vitamin D deficiency in Europe: Pandemic? *Am. J. Clin. Nutr.* **2016**, *103*, 1033–1044. [CrossRef]
18. Martin, C.A.; Gowda, U.; Renzaho, A.M. The prevalence of vitamin D deficiency among dark-skinned populations according to their stage of migration and region of birth: A meta-analysis. *Nutrition* **2016**, *32*, 21–32. [CrossRef]
19. Eggemoen, Å.R.; Knutsen, K.V.; Dalen, I.; Jenum, A.K. Vitamin D status in recently arrived immigrants from Africa and Asia: A cross-sectional study from Norway of children, adolescents and adults. *BMJ Open* **2013**, *3*, e003293. [CrossRef] [PubMed]
20. Chui, T.; Statistics Canada. *Immigration and Ethnocultural Diversity in Canada: National Household Survey*; Statistics Canada: Ottawa, ON, Canada, 2011.
21. Salant, T.; Lauderdale, D.S. Measuring culture: A critical review of acculturation and health in Asian immigrant populations. *Soc. Sci. Med.* **2003**, *57*, 71–90. [CrossRef]
22. Zhu, L. Depression risks and correlates among different generations of Chinese Americans: The effects of relationships with friends and relatives. *Soc. Sci.* **2017**, *6*, 56. [CrossRef]
23. Stephens, W.; Klimiuk, P.; Warrington, S.; Taylor, J.; Berry, J.; Mawer, E. Observations on the natural history of vitamin D deficiency amongst Asian immigrants. *QJM Int. J. Med.* **1982**, *51*, 171–188.
24. Stern, P.J. Generational differences. *J. Hand Surg.* **2002**, *27*, 187–194. [CrossRef]
25. Von Elm, E.; Altman, D.G.; Egger, M.; Pocock, S.J.; Gøtzsche, P.C.; Vandenbroucke, J.P. The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement: Guidelines for reporting observational studies. *Bull. World Health Organ.* **2007**, *85*, 867–872. [CrossRef] [PubMed]
26. Day, B.; Langlois, R.; Tremblay, M.; Knoppers, B.-M. Canadian Health Measures Survey: Ethical, legal and social issues. *Health Rep.* **2007**, *18*, 37–51. [PubMed]
27. Ng, E. Canadian Health Measures Survey: A tool for immigrant health research? *Health Rep.* **2015**, *26*, 3. [PubMed]
28. Statistics Canada. Canadian Health Measures Survey, Cycle 4, 2014–2015—Privacy Impact Assessment Summary. Available online: https://www.statcan.gc.ca/eng/about/pia/chmsc4 (accessed on 14 February 2021).
29. Statistics Canada. 2012–2013 Canadian Health Measures Survey, Cycle 3—Privacy Impact Assessment. Available online: https://www.statcan.gc.ca/eng/about/pia/chmsc3 (accessed on 14 February 2021).
30. Health Canada. *Canadian Guidelines for Body Weight Classification in Adults—Quick Reference Tool for Professionals*; Health Canada: Ottawa, ON, Canada, 2003.
31. Onis, M.d.; Onyango, A.W.; Borghi, E.; Siyam, A.; Nishida, C.; Siekmann, J. Development of a WHO growth reference for school-aged children and adolescents. *Bull. World Health Organ.* **2007**, *85*, 660–667. [CrossRef] [PubMed]
32. Canadian Physical Activity. *Sedentary Behaviour Guidelines*; Canadian Society for Exercise Physiology: Ottawa, ON, Canada, 2012.
33. Statistics Canada. Canadian Health Measures Survey (CHMS)Data User Guide: Cycle 3. 2015. Available online: https://usermanual.wiki/Pdf/CHMSUserGuideCycle3E.484319040/help (accessed on 24 February 2021).
34. Statistics Canada. Canadian Health Measures Survey (CHMS)—Cycle 4. Available online: https://www23.statcan.gc.ca/imdb/p2SV.pl?Function=getSurvey&Id=148760 (accessed on 25 January 2021).
35. Hilger, J.; Friedel, A.; Herr, R.; Rausch, T.; Roos, F.; Wahl, D.A.; Pierroz, D.D.; Weber, P.; Hoffmann, K. A systematic review of vitamin D status in populations worldwide. *Br. J. Nutr.* **2014**, *111*, 23–45. [CrossRef]

36. Pludowski, P.; Grant, W.B.; Bhattoa, H.P.; Bayer, M.; Povoroznyuk, V.; Rudenka, E.; Ramanau, H.; Varbiro, S.; Rudenka, A.; Karczmarewicz, E. Vitamin D status in central Europe. *Int. J. Endocrinol.* **2014**, *2014*. [CrossRef] [PubMed]
37. Lips, P. Vitamin D status and nutrition in Europe and Asia. *J. Steroid Biochem. Mol. Biol.* **2007**, *103*, 620–625. [CrossRef]
38. Alshahrani, A.A. Vitamin D Deficiency and Possible Risk Factors among Middle Eastern University Students in London, Ontario, Canada. Master's Thesis, The University of Western Ontario, London, ON, Canada, 2014.
39. McCormack, D.; Mai, X.; Chen, Y. Determinants of vitamin D supplement use in Canadians. *Public Health Nutr.* **2017**, *20*, 1768–1774. [CrossRef]
40. Knoss, R.; Halsey, L.G.; Reeves, S. Ethnic dress, vitamin D intake, and calcaneal bone health in young women in the United Kingdom. *J. Clin. Densitom.* **2012**, *15*, 250–254. [CrossRef]
41. Ojah, R.C.; Welch, J.M. Vitamin D and musculoskeletal status in Nova Scotian women who wear concealing clothing. *Nutrients* **2012**, *4*, 399–412. [CrossRef] [PubMed]
42. Mark, S. Vitamin D Status and Recommendations to Improve Vitamin D Status in Canadian Youth. Master's Thesis, McGill University, Montreal, QC, Canada, 2010.
43. Naugler, C.; Zhang, J.; Henne, D.; Woods, P.; Hemmelgarn, B.R. Association of vitamin D status with socio-demographic factors in Calgary, Alberta: An ecological study using Census Canada data. *BMC Public Health* **2013**, *13*, 316. [CrossRef]
44. Ghasemian, R.; Shamshirian, A.; Heydari, K.; Malekan, M.; Alizadeh-Navaei, R.; Ebrahimzadeh, M.A.; Jafarpour, H.; Shahmirzadi, A.R.; Khodabandeh, M.; Seyfari, B. The Role of Vitamin D in The Age of COVID-19: A Systematic Review and Meta-Analysis. *MedRxiv* **2020**. [CrossRef]
45. Munasinghe, L.L.; Yuan, Y.; Willows, N.D.; Faught, E.L.; Ekwaru, J.P.; Veugelers, P.J. Vitamin D deficiency and sufficiency among Canadian children residing at high latitude following the revision of the RDA of vitamin D intake in 2010. *Br. J. Nutr.* **2017**, *117*, 457–465. [CrossRef]
46. Wang, L.; Manson, J.E.; Buring, J.E.; Lee, I.-M.; Sesso, H.D. Dietary intake of dairy products, calcium, and vitamin D and the risk of hypertension in middle-aged and older women. *Hypertension* **2008**, *51*, 1073–1079. [CrossRef]
47. Thuesen, B.; Husemoen, L.; Fenger, M.; Jakobsen, J.; Schwarz, P.; Toft, U.; Ovesen, L.; Jørgensen, T.; Linneberg, A. Determinants of vitamin D status in a general population of Danish adults. *Bone* **2012**, *50*, 605–610. [CrossRef]
48. Renzaho, A.M.; Halliday, J.A.; Nowson, C. Vitamin D, obesity, and obesity-related chronic disease among ethnic minorities: A systematic review. *Nutrition* **2011**, *27*, 868–879. [CrossRef] [PubMed]
49. Neale, R.; Khan, S.; Lucas, R.; Waterhouse, M.; Whiteman, D.; Olsen, C. The effect of sunscreen on vitamin D: A review. *Br. J. Dermatol.* **2019**, *181*, 907–915. [CrossRef] [PubMed]
50. Young, A.; Narbutt, J.; Harrison, G.; Lawrence, K.; Bell, M.; O'Connor, C.; Olsen, P.; Grys, K.; Baczynska, K.; Rogowski-Tylman, M. Optimal sunscreen use, during a sun holiday with a very high ultraviolet index, allows vitamin D synthesis without sunburn. *Br. J. Dermatol.* **2019**, *181*, 1052–1062. [CrossRef]
51. Brooks, S.P.; Greene-Finestone, L.; Whiting, S.; Fioletov, V.E.; Laffey, P.; Petronella, N. An analysis of factors associated with 25-hydroxyvitamin D levels in white and non-white Canadians. *J. AOAC Int.* **2017**, *100*, 1345–1354. [CrossRef] [PubMed]
52. Nimitphong, H.; Holick, M.F. Vitamin D status and sun exposure in Southeast Asia. *Derm. Endocrinol.* **2013**, *5*, 34–37. [CrossRef]
53. Sanou, D.; O'Reilly, E.; Ngnie-Teta, I.; Batal, M.; Mondain, N.; Andrew, C.; Newbold, B.K.; Bourgeault, I.L. Acculturation and nutritional health of immigrants in Canada: A scoping review. *J. Immigr. Minority Health* **2014**, *16*, 24–34. [CrossRef]
54. Carlberg, C. Nutrigenomics of vitamin D. *Nutrients* **2019**, *11*, 676. [CrossRef]
55. Leandro, A.; Rocha, M.; Cardoso, C.; Bonecini-Almeida, M. Genetic polymorphisms in vitamin D receptor, vitamin D-binding protein, Toll-like receptor 2, nitric oxide synthase 2, and interferon-γ genes and its association with susceptibility to tuberculosis. *Braz. J. Med Biol. Res.* **2009**, *42*, 312–322. [CrossRef] [PubMed]
56. Aucoin, M.; Weaver, R.; Thomas, R.; Jones, L. Vitamin D status of refugees arriving in Canada: Findings from the Calgary Refugee Health Program. *Can. Fam. Physician* **2013**, *59*, e188–e194. [PubMed]
57. Baauw, A.; Kist-van Holthe, J.; Slattery, B.; Heymans, M.; Chinapaw, M.; van Goudoever, H. Health needs of refugee children identified on arrival in reception countries: A systematic review and meta-analysis. *BMJ Paediatr. Open* **2019**, *3*, e000516. [CrossRef] [PubMed]
58. Statistics, C. Standard Classification of Countries and Areas of Interest (SCCAI). Available online: https://publications.gc.ca/site/eng/9.856211/publication.html (accessed on 24 February 2020).

nutrients

MDPI

Article

Vitamin D, Vitamin D-Binding Proteins, and VDR Polymorphisms in Individuals with Hyperglycaemia

Rajiv Erasmus [1], Setjie Maepa [1], Ian Machingura [2], Saarah Davids [2], Shanel Raghubeer [2,*] and Tandi Matsha [2]

[1] Department of Pathology, Chemical Pathology Division, National Health Laboratory Service (NHLS) and Stellenbosch University, Tygerberg Hospital, Cape Town 7505, South Africa; rte@sun.ac.za (R.E.); setjiew@gmail.com (S.M.)

[2] SAMRC/CPUT/Cardiometabolic Health Research Unit, Department of Biomedical Science, Faculty of Health & Wellness Sciences, Cape Peninsula University of Technology, Cape Town 7530, South Africa; ianmachingura@gmail.com (I.M.); davidss@cput.ac.za (S.D.); matshat@cput.ac.za (T.M.)

* Correspondence: raghubeers@cput.ac.za; Tel.: +27-21-959-6015

Abstract: Vitamin D reportedly plays an important role in the pathogenesis of diabetes mellitus; however, this role is unclear and debated. This study investigated the association between 25(OH) vitamin D, vitamin D-binding proteins, and vitamin D receptor (VDR) polymorphisms in healthy individuals and those with prediabetes and type 2 diabetes mellitus (T2D) from South Africa. A cross-sectional study was conducted involving subjects of mixed ancestry aged ≥20 years. Males presented with higher mean 25(OH) vitamin D levels than females, while females exhibited significantly higher serum vitamin D-binding protein levels. Significant differences in mean 25(OH) vitamin D levels were observed in normo-glycaemic, prediabetes, screen-detected DM, and known DM individuals. Vitamin D receptor SNPs *Fok1* and *Taq1* were not associated with glycaemic status. *Fok1* was not associated with 25(OH) vitamin D deficiency, while *Taq1* was associated with vitamin D insufficiency. This study showed a high prevalence of vitamin D deficiency/insufficiency in this South African population, with decreased vitamin D levels observed in hyperglycaemic individuals, which was not linked to either vitamin D-binding protein or polymorphisms in *Fok1* of the *VDR* gene. These results may be used as a platform for further research into diagnosis and treatment of hyperglycaemia.

Keywords: hyperglycaemia; South Africa; vitamin D; vitamin D receptor; vitamin D-binding protein

Citation: Erasmus, R.; Maepa, S.; Machingura, I.; Davids, S.; Raghubeer, S.; Matsha, T. Vitamin D, Vitamin D-Binding Proteins, and VDR Polymorphisms in Individuals with Hyperglycaemia. *Nutrients* **2022**, *14*, 3147. https://doi.org/10.3390/nu14153147

Academic Editor: Adrian Gombart

Received: 29 June 2022
Accepted: 28 July 2022
Published: 30 July 2022

1. Introduction

The role of vitamin D in non-communicable chronic diseases and the potential impact of vitamin D supplementation as a preventive and therapeutic measure is controversial and debated [1–4]. In cross-sectional epidemiological studies, insufficient vitamin D status is associated with the development of obesity, metabolic syndrome, and type 2 diabetes (T2D) in several but not all reports [5–8]. A recent systematic review reported that vitamin D supplementation at a minimum dose of 100 µg/d (4000 IU/d) may significantly reduce serum fasting plasma glucose (FPG), glycosylated haemoglobin (HbA1c), and homeostatic model assessment of insulin resistance (HOMA-IR) index in type 2 diabetic patients [9]. However, in a randomized trial, Pittas et al. concluded that vitamin D supplementation does not lower the risk of T2D [8]. Recently, Pilz et al. critically appraised several vitamin D randomized controlled trials and suggested that many researchers should carefully investigate the cohorts included in these studies, as cohort choice may bias the results obtained during these studies [10]. Furthermore, Xu et al. reported on a greatly improved sample size and concluded that genetically increased vitamin D concentration decreased T2D risk, suggesting that vitamin D supplementation deserves further investigation in interventional studies [11].

Vitamin D-binding protein is a serum glycoprotein, which is the major carrier protein of vitamin D sterols, and is essential for the intracellular metabolism of vitamin D.

Variations in vitamin D-binding proteins are postulated to influence the amount and activity of vitamin D, which in turn affect insulin secretion, β-cell dysfunction, and glucose metabolism [12]. Vitamin D exerts its effects on target tissues by binding to the cytosolic/nuclear vitamin D receptor (VDR), a member of the steroid/thyroid hormone receptor family. The VDR is expressed in the pancreas, and four polymorphisms of the vitamin D receptor, namely *FokI*, *BsmI*, *ApaI*, and *TaqI*, have been identified to be associated with insulin secretion and sensitivity. However, some studies have found no associations with these polymorphisms [13].

The mixed-ancestry population of Bellville South, Cape Town, exhibits a high prevalence of T2D [14], and vitamin D deficiency may contribute to the high prevalence of diabetes observed in this population group. In this study, we investigated the association between 25(OH) vitamin D levels, vitamin D-binding protein, and VDR polymorphisms in subjects with prediabetes and T2D in a mixed-ancestry South African population, as research is lacking in this population group.

2. Materials and Methods

2.1. Ethical Approval

The study forms part of the ongoing Vascular and Metabolic Health study (VMH), which received ethical approval from the research ethics committees of the Cape Peninsula University of Technology (CPUT) and Stellenbosch University (NHREC: REC-230 408–014 and N14/01/003, respectively). The current study also received ethical approval from the Stellenbosch University Health Research Ethics committee (0719) and Cape Peninsula University of Technology, Faculty of Health and Wellness Sciences Research Ethics committee (CPUT/HW REC2015/H01). Written informed consent was sought from all study participants following explanation of study procedures in their language of choice. All methods were performed in accordance with the Declaration of Helsinki and all relevant regulations.

2.2. Study Population and Design

This cross-sectional study comprised 1989 participants of mixed ancestry aged ≥ 20 years residing in Bellville South, Cape Town, South Africa. For the final analysis, individuals with incomplete data, acutely ill individuals, and pregnant females were excluded from the study. Therefore, the final sample size for this study was 968, from a total of 1989 participants. A detailed description of the survey and procedures conducted in this study have been published [14].

2.3. Clinical Data

The demographic and clinical data have been previously described, and were collected using a questionnaire [15]. The weight was measured to the nearest 0.1 kg using the Omron body fat meter HBF511 digital bathroom scale with participants wearing light clothing without shoes. The stadiometer was used to measure body height to the nearest centimetre with the study participants standing on a flat surface. The body mass index was calculated as weight per square meter (kg/m^2). The waist circumference was measured using a non-elastic tape at the level of the narrowest part of the torso, as seen from the anterior view, whilst in obese participants the narrowest circumference between the ribs and the iliac crest were measured. Hip circumference was measured at the maximal circumference over the buttock using a non-elastic tape. Body mass index was used to classify participants as underweight (<18.5 kg/m^2), normal (18.5–24.99 kg/m^2), overweight (≥25 kg/m^2), or obese (≥30 kg/m^2) according to the World Health Organisation (WHO) criteria [16]. Participants who did not have a medical history of diagnosis with diabetes mellitus underwent a 2 h oral glucose tolerance test (OGTT), as recommended by the WHO. The OGTT was used to group the study participants as normoglycaemic or prediabetic (including impaired fasting glycaemia, impaired glucose tolerance, or a combination of both) using the WHO criteria [17]. The Joint Interim Statement of the International Diabetes Federation Task Force on Epidemiology and Prevention, National Heart, Lung and Blood Institute, American

Heart Association, World Heart Federation, International Atherosclerosis Society, and International Association for the Study of Obesity (JIS) was used to classify metabolic syndrome (MetS) [18].

2.4. Biochemical Analysis

Blood samples were collected from all participants after fasting overnight. Plasma glucose was measured using the hexokinase method (Cobas 6000, Roche Diagnostics; Mannheim, Germany), HbA1c using high-performance liquid chromatography (HPLC; Bio-Rad Variant Turbo, Bio-Rad, South Africa), which was National Glycohaemoglobin Standardization Program (NGSP) certified, insulin using the paramagnetic particle chemiluminescence assay (Beckman DXI, Beckman Coulter, South Africa), fructosamine using a colorimetric test with nitro blue tetrazolium (Cobas c311, Roche Diagnostics), low-density lipoprotein cholesterol (LDL-C; mmol/L) using enzymatic selective protection-end point (Beckman AU, Beckman Coulter), HDL-C (mmol/L) using enzymatic immune-inhibition—end point (Beckman AU), and triglycerides (TG; mmol/L) using glycerol phosphate oxidase-peroxidase—end point (Beckman AU). The 25(OH) vitamin D levels were measured using the paramagnetic particle chemiluminescence test (Beckman DXI), and vitamin D-binding protein (VDBP) was determined using the Human Vitamin D BP Quantikine ELISA kit (DVDBP0; R&D Systems, Minneapolis, MN, USA).

2.5. Definition of Vitamin D Deficiency

Vitamin D deficiency was defined using either the 2011 Endocrine Clinical Society Practice Guidelines as 25 hydroxyvitamin D (25(OH) vitamin D) below 20 ng/mL (50 nmol/L) and vitamin D insufficiency as 25(OH) vitamin D between 20 and 29 ng/mL (50–75 nmol/L) [19] or the Global Consensus Recommendations on Prevention and Management of Nutritional Rickets, with vitamin D deficiency defined as 25(OH) vitamin D below 12 ng/mL (30 nmol/L) and vitamin D insufficiency as 25(OH) vitamin D between 12 and 20 ng/mL (30–50 nmol/L) [20].

2.6. Genetic Analysis

Genomic DNA was extracted from whole blood samples collected in EDTA tubes using the salt extraction method, then quantified using the NanoDrop ND-1000 instrument (Nanodrop Technologies, Wilmington, USA). *VDR* single-nucleotide polymorphisms, *Fok1* (*rs2228570*), *Apa1* (*rs7975232*), and *Taq1* (*rs731236*), were genotyped using high-throughput real-time polymerase chain reaction on the Bio-Rad Optica platform (Bio-Rad, Hercules, CA, USA) using TaqMan™ genotyping assays. Primers were predesigned TaqMan™ SNP genotyping assays. All primers and kits comply with the minimum information for publication of quantitative RT-PCR experiments (MIQE). All primer sequences can be accessed via the Thermo Fisher Scientific (Waltham, MA, USA) website. Thereafter, all samples were submitted to Inqaba Biotechnical Industries (Pretoria, South Africa) for further verification by an independent laboratory. The conventional polymerase chain reaction followed by direct DNA sequencing was performed for analytical validation of genotyping.

2.7. Data Analysis

Data were analysed using Statistica 13.3 (StatSoft, Pretoria, South Africa). Categorical variables were summarized as count and percentages, while quantitative variables were indicated as mean (standard deviation) or median (25th–75th percentiles). Variable comparisons across the glycaemic status were conducted using the chi-squared test. The Pearson chi-square test was used to determine association between single-nucleotide polymorphism genotypes and/or allele frequencies and vitamin D deficiency, obesity, and glycaemia categories. A multiple linear regression model was used to establish possible associations between vitamin D and other test results. A p-value < 0.05 was considered statistically significant.

3. Results

3.1. Participant Characteristics and Vitamin D Deficiency

A total of 968 study participants were recruited, 79.2% of which were female. Table 1 indicates the characteristics of study participants and vitamin D categories (deficiency, insufficiency, and optimal) using the 2011 Endocrine Clinical Society Practice Guidelines ($p = 0.004$) and the Global Consensus Recommendations on Prevention and Management of Nutritional Rickets ($p = 0.003$), varied according to sex. Male participants were older, exhibited greater waist/hip ratios and higher glucose/insulin ratios and levels of serum creatinine, urine creatinine, aspartate aminotransferase, serum albumin, urine sodium, and cotinine. Female participants exhibited greater waist and hip circumference measurements, higher levels of two-hour postprandial blood glucose, HbA1c, fasting blood insulin, two-hour postprandial blood insulin, HDL cholesterol, LDL cholesterol, cholesterol, parathormone, calcium corrected, and phosphate, and included a greater percentage of smokers. Vitamin D and vitamin D-binding protein levels varied according to sex, with males exhibiting higher mean 25(OH) vitamin D levels than females (24 ± 8 vs. 22 ± 8 ng/mL, $p = 0.0006$, respectively), whilst females displayed significantly higher serum vitamin D-binding protein levels (323 ± 81 vs. 306 ± 74 µg/mL, $p = 0.007$).

Table 1. Participant characteristics categorized according to sex.

	Total, *N* = 968	**Males, *N* = 201**	**Females, *N* = 767**	
		Mean ± SD		***p*-Value**
Hyperglycaemia, Yes, N (%)	379 (39.1)	69 (34.3)	310 (40.4)	0.12
DM, Yes, N (%)	204 (21.1)	42 (20.9)	162 (21.1)	Combined 0.14
Pre-DM, Yes, N (%)	175 (18.1)	27 (13.4)	148 (19.3)	
Vitamin D—using Endocrine Clinical Society Practice GuidelinesVitamin D—using Endocrine Clinical Society Practice Guidelines				
Deficient, N (%)	431 (44.5)	73 (36.3)	358 (46.7)	Combined **0.004**
Insufficient, N (%)	411 (42.5)	90 (44.8)	321 (41.9)	
Optimal, N (%)	126 (13.0)	38 (18.9)	88 (11.5)	
Vitamin D—using Global Consensus Recommendations on Prevention and Management of Nutritional Rickets				
Deficient, N (%)	54 (5.6)	3 (1.5)	51 (6.6)	Combined **0.003**
Insufficient, N (%)	379 (39.2)	71 (35.3)	308 (40.2)	
Optimal, N (%)	535 (55.3)	127 (63.2)	408 (53.2)	
Participant characteristics				
Age (years)	51 ± 14	52 ± 15	51 ± 14	**<0.0001**
BMI (kg/m^2)	31 ± 8	26 ± 7	32 ± 8	0.7
WaistC (cm)	97 ± 16	91 ± 17	98 ± 16	**<0.0001**
HipC (cm)	109 ± 16	99 ± 12	112 ± 15	**<0.0001**
WHR	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	**<0.0001**
Vitamin D (25OH) (ng/mL)	22 ± 7.6	24 ± 8	22 ± 7.5	**0.0006**
Vitamin D BP (µg/mL)	320 ± 80	306 ± 74	323 ± 81	**0.007**
FBG (mmol/L)	5.9 ± 3	5.7 ± 2.5	6 ± 3	0.2
Post 2 HRs BG (mmol/L)	6.9 ± 2.9	6.3 ± 3.5	7 ± 2.7	**0.001**

Table 1. *Cont.*

	Total, *N* = 968	**Males, *N* = 201**	**Females, *N* = 767**	
		Mean ± SD		***p*-Value**
HbA1c (%)	6.3 ± 1.6	6.1 ± 1.3	6.4 ± 1.7	**0.03**
FBI (mIU/L)	10 ± 10	8 ± 11	11 ± 10	**0.006**
Post 2 HRs BI (mIU/L)	64 ± 57	41 ± 44	70 ± 59	**<0.0001**
Glucose/Insulin ratio	0.9 ± 0.9	1.3 ± 1	0.9 ± 0.9	**<0.0001**
Triglycerides (mmol/L)	1.6 ± 1.5	1.6 ± 1.9	1.5 ± 1.4	0.4
HDL Chol (mmol/L)	1.3 ± 0.3	1.2 ± 0.3	1.3 ± 0.3	**<0.0001**
LDL Chol (mmol/L)	3.3 ± 1	3 ± 1	3.4 ± 1	**<0.0001**
Chol (mmol/L)	5.3 ± 1.2	5 ± 1	5 ± 1.2	**<0.0001**
Chol/HDL ratio	4.3 ± 1.2	4.3 ± 1.3	4.3 ± 1.1	0.6
Gamma GT-S (IU/L)	43 ± 54	48 ± 70	42 ± 49	0.2
Creatinine-S (µmol/L)	65 ± 27	80 ± 37	61 ± 22	**<0.0001**
Creatinine-U (mmol/L)	14 ± 8.3	16 ± 8.7	13 ± 8.1	**<0.0001**
ALT (SGPT) (IU/L)	22 ± 23	25 ± 21	22 ± 24	0.1
AST (SGOT) (IU/L)	25 ± 13	28 ± 15	24 ± 13	**0.002**
MDRD (mL/min/1.73 m^2)	84 ± 12	84 ± 14	84 ± 12	0.9
Parathormone (pmol/L)	5.5 ± 3.4	4.9 ± 3.7	5.6 ± 3.4	**0.01**
Albumin-S (g/L)	43 ± 3.3	44 ± 3.8	43 ± 3.2	**0.0003**
Calcium corrected (mmol/L)	2.3 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	**0.0003**
Calcium-S (mmol/L)	2.4 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	0.5
Phosphate-S (mmol/L)	1.1 ± 0.2	1 ± 0.2	1.1 ± 0.2	**<0.0001**
Sodium-U (mmol/L)	109 ± 54	121 ± 57	105 ± 53	**0.0002**
CRP (mg/L)	8.8 ± 16	8.8 ± 18	8.7 ± 16	0.96
Cotinine (ng/mL)	128 ± 161	159 ± 159	120 ± 160	**0.002**
Smoking, Yes, N (%)	408 (42.2)	111 (55.2)	297 (38.8)	**<0.0001**

DM, diabetes mellitus; BMI, body mass index; WaistC, waist circumference; HipC, hip circumference; WHR, waist-to-hip-ratio; Vitamin D BP, vitamin D binding protein; FBG, fasting blood glucose; BG, blood glucose; HbA1c, glycated haemoglobin; FBI, fasting blood insulin; BI, blood insulin; HDL, high-density lipoprotein; LDL, low-density lipoprotein; Chol, cholesterol; GT-S glutamyl transferase-serum; ALT, alanine aminotransferase; SGPT, serum glutamic-pyruvic transaminase; AST, aspartate aminotransferase; SGOT, serum glutamic-oxaloacetic transaminase; MDRD, modification of diet in renal disease; CRP, c-reactive protein. Bold *p* values indicates significant differences in the analysed data.

Using the 2011 Endocrine Clinical Society Practice Guidelines, the prevalence of vitamin D deficiency and insufficiency amongst all participants was 44.5% and 42.5%, respectively. As expected, when using the Global Consensus Recommendations on Prevention and Management of Nutritional Rickets, the prevalence of vitamin D deficiency and insufficiency amongst all study participants was 5.6% and 39.2%, respectively (Table 2). The Institute of Medicine (IOM) recommended a minimum vitamin D level of ≥20 ng/mL for adults in 2011; thus, the mean vitamin D level amongst all study participants fell within this recommended level [1,21].

Table 2. Vitamin D deficiency, insufficiency, and optimal level cut-offs used to assess vitamin D status.

Vitamin D (ng/mL), Total Group *N* = 968					
Vitamin D—Endocrine Clinical Society Practice Guidelines for vitamin D deficiency			Vitamin D—Global Consensus Recommendations on Prevention and Management of Nutritional Rickets		
Category	**Cut-off value (ng/mL)**	***N* (%)**	**Category**	**Cut-off value (ng/mL)**	***N*(%)**
Deficiency	<20	431 (44.5%)	Deficiency	<12	54/968 (5.6%)
Insufficiency	20–29	411 (42.5%)	Insufficiency	12 to 20	379/968 (39.2%)
Optimal levels	≥30	126 (13.0%)	Optimal levels	>20	535/968 (55.3%)

3.2. Glycaemic Status and Vitamin D Deficiency

Table 3 shows the characteristics of the study participants according to their glycaemic status. Using the 2011 Endocrine Clinical Society Practice Guidelines for vitamin D deficiency, vitamin D varied according to the glycaemic status, with normoglycaemic participants displaying higher vitamin D levels than prediabetes mellitus, screen-detected diabetes mellitus, and known diabetes mellitus participants ($p = 0.002$).

Using the Global Consensus Recommendations on Prevention and Management of Nutritional Rickets, there was no significant difference in the prevalence of deficient, insufficient, and optimal 25(OH) vitamin D. Further, levels of vitamin D-binding protein were significantly decreased in the deficient vitamin D group compared to the insufficient vitamin D group and the optimal vitamin D group ($p = 0.007$), according to the 2011 Endocrine Clinical Society Practice Guidelines for vitamin D deficiency (Figure 1). Similarly, vitamin D-binding protein levels were significantly decreased in the deficient vitamin D group compared to insufficient and sufficient vitamin D groups ($p = 0.003$), according to the Global Consensus Recommendations on Prevention and Management of Nutritional Rickets vitamin D deficiency (Figure 2).

3.3. Genotype Distribution of VDR Polymorphisms

The Hardy–Weinberg Equilibrium (HWE) test was used to determine SNP frequency. Table 4 shows genotype distribution and minor allele frequencies within the study population. The allele percentage was calculated at 26.5% for *Fok1* (*rs2228570*; HWE $p = 0.1$), 28.9% for *Taq1* (*rs731236*; HWE $p = 0.6$, and 39% for *Apa1* (*rs7975232*; HWE $p = 0.02$). In the dominant (GG versus AA + AG), recessive (GG + AG versus AA), and additive models for SNP *rs2228570*, the determinants, sex, age, glycaemic status, MetS, and vitamin D, were not significant (Table 5). However, results from the additive model indicate that obesity was a significant determinant, with a two-fold increase in the GG genotype [OR (95% CI): 2.04 (1.02; 4.10), $p = 0.045$].

Table 6 shows that in the dominant (AA versus GG + AG), recessive (AA + AG versus GG), and additive (AA vs. AG vs. GG) models for the SNP *rs731236*, the determinants, sex, age, glycaemic status, and MetS, were not significant. However, in the recessive model, the SNP *rs731236* was associated with insufficient vitamin D ($p = 0.04$). In the additive model, there was a lower likelihood of patients being overweight [OR (95% CI):0.65 (0.43; 0.97), $p = 0.04$] in the genotype AG, while the genotype AA had a 1.82 likelihood of being vitamin D-insufficient [OR (95% CI):1.82 (1.07; 3.07), $p = 0.03$].

Table 3. Participant characteristics according to glycaemic status.

	Total Group, *N* = 968						
	Normo-Glycaemic, *N* = 589	**Pre-Diabetes Mellitus *N* = 175**	**Screen-Detected Diabetes Mellitus, *N* = 64**	**Known Diabetes Mellitus, *N* = 140**	**Gender**	**Diagnosis**	**Gender * Diagnosis**
	Mean ± SD				***p*-Value**	***p*-Value**	***p*-Value**
Vitamin D—using Endocrine Clinical Society Practice Guidelines for vitamin D deficiency							
Deficient, N (%)	240 (40.7)	89 (50.9)	31 (48.4)	71 (50.7)			Combined **0.009 ***
Insufficient, N (%)	255 (43.3)	68 (38.9)	30 (46.9)	58 (41.4)			
Optimal, N (%)	94 (16.0)	18 (10.3)	3 (4.7)	11 (7.9)			
Vitamin D—using Global Consensus Recommendations on Prevention and Management of Nutritional Rickets							
Deficient, N (%)	31 (5.3)	11 (6.3)	5 (7.8)	7 (5.0)			Combined 0.17 *
Insufficient, N (%)	211 (35.8)	78 (44.6)	26 (40.6)	64 (45.7)			
Optimal, N (%)	347 (58.9)	86 (49.1)	33 (51.6)	69 (49.3)			
Participant characteristics							
Age (years)	48 ± 14	56 ± 14	57 ± 11	58 ± 11	0.2	**<0.0001**	0.8
BMI (kg/m2)	29 ± 8	32 ± 8	34 ± 7.5	32 ± 7	**<0.0001**	**<0.0001**	0.3
WaistC (cm)	93 ± 16	101 ± 15	105 ± 14	103 ± 14	**0.006**	**<0.0001**	0.07
HipC (cm)	107 ± 15	112 ± 16	113 ± 15	111 ± 15	**<0.0001**	**0.007**	0.6
WHR	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	**<0.0001**	**<0.0001**	**0.008**
Vitamin D (25OH) (ng/mL)	23 ± 8	21 ± 7.3	20 ± 6.2	21 ± 6.2	0.2	**0.002**	**0.02**
Vitamin D BP (µg/mL)	321 ± 81	322 ± 74	312 ± 81	317 ± 81	**0.003**	0.4	0.3
FBG (mmol/L)	4.8 ± 0.5	5.3 ± 0.6	7.7 ± 4	10.6 ± 4.8	0.8	**<0.0001**	**<0.0001**
Post 2 HRs BG (mmol/L)	5.6 ± 1.3	8.9 ± 1.1	14 ± 3.7	NA	**0.001**	**<0.0001**	**<0.0001**
HbA1c (%)	5.6 ± 0.4	6 ± 0.5	7.2 ± 1.8	9.2 ± 2.3	**0.002**	**<0.0001**	**<0.0001**
FBI (mIU/L)	8.2 ± 6.5	12 ± 13	14 ± 14	14 ± 16	0.7	**<0.0001**	**0.03**
Post 2 HRs BI (mIU/L)	52 ± 49	100 ± 70	70 ± 46	NA	**0.002**	**<0.0001**	0.9
Glucose/Insulin ratio	0.9 ± 0.6	0.8 ± 0.6	0.9 ± 1.1	1.5 ± 1.6	**0.01**	**<0.0001**	**0.0006**

Table 3. *Cont.*

	Total Group, *N* = 968						
	Normo-Glycaemic, *N* = 589	**Pre-Diabetes Mellitus *N* = 175**	**Screen-Detected Diabetes Mellitus, *N* = 64**	**Known Diabetes Mellitus, *N* = 140**	**Gender**	**Diagnosis**	**Gender * Diagnosis**
		Mean ± SD			***p*-Value**	***p*-Value**	***p*-Value**
Triglycerides (mmol/L)	1.3 ± 1	1.8 ± 2.5	2.1 ± 2.3	2.1 ± 1.4	**0.001**	**<0.0001**	**<0.0001**
HDL Chol (mmol/L)	1.3 ± 0.3	1.3 ± 0.3	1.3 ± 0.3	1.2 ± 0.3	**0.0005**	0.09	0.9
LDL Chol (mmol/L)	3.2 ± 1	3.5 ± 1	3.6 ± 1	3.4 ± 1.1	**0.02**	**0.008**	0.1
Chol (mmol/L)	5.2 ± 1.2	5.5 ± 1.1	5.6 ± 1.2	5.4 ± 1.3	**0.02**	**0.002**	**0.02**
Chol/HDL ratio	4.2 ± 1.2	4.4 ± 1.1	4.6 ± 1.2	4.6 ± 1.2	0.2	**0.0003**	0.07
Gamma GT-S (IU/L)	41 ± 63	42 ± 33	53 ± 40	47 ± 41	0.7	0.8	0.6
Creatinine-S (umol/L)	63 ± 21	64 ± 22	67 ± 36	71 ± 45	**<0.0001**	**0.001**	0.1
Creatinine-U (mmol/L)	14 ± 8	14 ± 9.2	16 ± 9.8	12 ± 7.5	**0.002**	0.2	0.1
ALT (SGPT) (IU/L)	21 ± 21	22 ± 12	34 ± 58	22 ± 15	0.9	0.3	0.2
AST (SGOT) (IU/L)	25 ± 13	24 ± 7.6	31 ± 27	23 ± 11	0.9	0.3	**0.02**
MDRD (mL/min/1.73 m^2)	85 ± 11	83 ± 12	83 ± 15	80 ± 17	0.3	**0.006**	0.7
Parathormone (pmol/L)	5.3 ± 3.3	5.5 ± 3	6.2 ± 4	5.8 ± 4	0.9	**0.02**	0.09
Albumin-S (g/L)	43 ± 3.5	43 ± 2.8	42 ± 2.8	43 ± 3.3	**0.02**	0.5	0.6
Calcium corrected (mmol/L)	2.3 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	2.3 ± 0.1	**0.002**	0.2	0.05
Calcium-S (mmol/L)	2.4 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	0.3	0.7	0.4
Phosphate-S (mmol/L)	1.1 ± 0.2	1.1 ± 0.2	1.1 ± 0.2	1.1 ± 0.2	**0.002**	0.7	0.4
Sodium-U (mmol/L)	116 ± 56	104 ± 54	90 ± 46	93 ± 44	0.08	**<0.0001**	0.1
CRP (mg/L)	7.4 ± 15	10 ± 15	14 ± 21	11 ± 22	0.2	**0.002**	0.2
Cotinine (ng/mL)	142 ± 163	130 ± 164	77 ± 134	92 ± 150	0.3	**0.0001**	0.2
Smoking, Yes % (N)	280 (47.7)	74 (42.3)	16 (25)	38 (27.1)	-	-	**<0.0001** *

* denotes data analysis according to gender; Bold *p* values indicates significant differences in the analysed data.

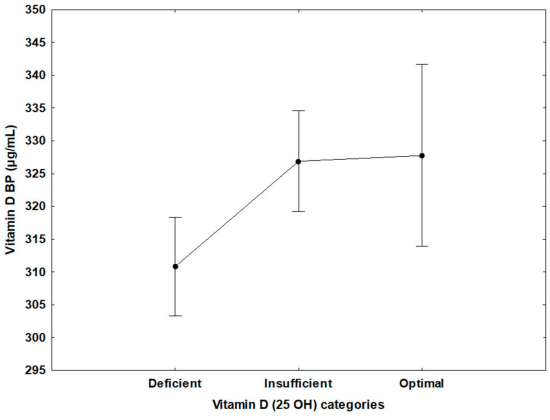


Figure 1. Using the Endocrine Clinical Society Practice Guidelines for vitamin D deficiency, vitamin D-binding protein (µg/mL) levels were found to be significantly decreased in the deficient vitamin D group, mean ± SD, 311 ± 75 (µg/mL) (*N* = 431), compared to the insufficient vitamin D group, 327 ± 85 (*N* = 408), and the optimal vitamin D group, 328 ± 75 (*N* = 126), (*p* = 0.007). BP, binding protein.

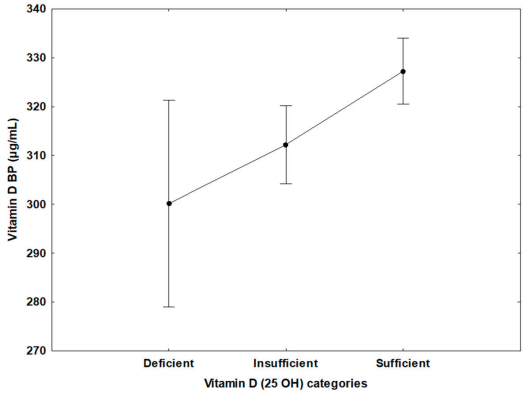


Figure 2. Using the Global Consensus Recommendations on Prevention and Management of Nutritional Rickets vitamin D deficiency, vitamin D-binding protein (µg/mL) levels were found to be significantly decreased in the deficient vitamin D group, mean ± SD, 300 ± 57 (µg/mL) (*N* = 54), compared to the insufficient vitamin D group, 312 ± 77 (*N* = 379), and the sufficient vitamin D group, 327 ± 83 (*N* = 126), (*p* = 0.003). BP, binding protein.

Table 4. Genotype distributions and minor allele frequencies (HWE: Hardy–Weinberg Equilibrium).

Fok1 Rs2228570		**Taq1 rs731236**		**Apa1 rs7975232**	
G/G, *n* (%)	532/968 (55)	A/A, *n* (%)	486/968 (50.2)	A/A, *n* (%)	377/968 (38.9)
A/G, *n* (%)	358 (37)	A/G, *n* (%)	404 (41.7)	A/C, *n* (%)	426 (44)
A/A, *n* (%)	78 (8.1)	G/G, *n* (%)	78 (8.1)	C/C, *n* (%)	165 (17)
A, *n* (%)	514 (26.5)	G, *n* (%)	560 (28.9)	C, *n* (%)	756 (39)
HWE (*p*-value)	0.1	HWE (*p*-value)	0.6	HWE (*p*-value)	**0.02**

Bold *p* values indicates significant differences in the analysed data.

Table 5. Logistic regression for dominant, recessive, and additive models for single-nucleotide polymorphisms *rs2228570*.

	Dominant		Recessive		Additive					
					AG		GG		AA	
	OR (95% CI)	*p*-Value	OR (95% CI)	*p*-Value	OR (95% CI)	*p*-Value	OR (95% CI)	*p*-Value	OR (95% CI)	*p*-Value
Sex										
Male	1		1		1		1		1	
Female	1.12 (0.79; 1.6)	0.5	1.32 (0.7; 2.5)	0.4	1.26 (0.64; 2.48)	0.5	1.36 (0.7; 2.62)	0.4	1.08 (0.74; 1.56)	0.7
Age	1 (0.99; 1.01)	0.4	1.01 (0.99; 1.03)	0.2	0.99 (0.97; 1.01)	0.3	0.99 (0.97; 1.01)	0.2	1 (0.99; 1.01)	0.6
Glycaemic status										
Normal	1		1		1		1		1	
Pre-DM	0.85 (0.59; 1.22)	0.4	0.81 (0.4; 1.65)	0.6	1.14 (0.54; 2.41)	0.7	1.31 (0.63; 2.72)	0.5	0.87 (0.59; 1.27)	0.5
DM	0.8 (0.56; 1.15)	0.2	1.13 (0.6; 2.14)	0.7	0.74 (0.38; 1.46)	0.4	0.98 (0.51; 1.88)	1	0.75 (0.51; 1.11)	0.2
JIS Criteria										
No	1		1		1		1		1	
Yes	0.81 (0.6; 1.11)	0.2	0.97 (0.55; 1.72)	0.9	0.9 (0.49; 1.64)	0.7	1.13 (0.63; 2.02)	0.7	1.25 (0.91; 1.73)	0.2
Obesity status										
Normal	1		1		1		1		1	
Overweight	0.92 (0.63; 1.36)	0.7	0.82 (0.43; 1.59)	0.6	1.18 (0.59; 2.37)	0.6	1.24 (0.63; 2.45)	0.5	0.95 (0.63; 1.44)	0.8
Obese	0.81 (0.55; 1.19)	0.3	0.51 (0.26; 1)	0.1	1.85 (0.9; 3.78)	0.1	2.04 (1.02; 4.1)	0.045	0.9 (0.6; 1.35)	0.6
Vitamin D/ES										
Sufficient	1		1		1		1		1	
Insufficient	1.16 (0.88; 1.52)	0.3	1.26 (0.77; 2.07)	0.4	0.85 (0.5; 1.44)	0.5	0.76 (0.45; 1.26)	0.3	1.12 (0.84; 1.5)	0.4
Deficient	0.81 (0.44; 1.48)	0.5	1.15 (0.39; 3.42)	0.8	0.73 (0.23; 2.34)	0.6	0.96 (0.32; 2.9)	0.9	0.76 (0.4; 1.46)	0.4

Table 6. Logistic regression for dominant, recessive, and additive models for single-nucleotide polymorphisms *rs731236*.

	Dominant		Recessive		Additive					
					AG		GG		AA	
	OR (95% CI)	*p*-Value	OR (95% CI)	*p*-Value	OR (95% CI)	*p*-Value	OR (95% CI)	*p*-Value	OR (95% CI)	*p*-Value
Sex										
Male	1		1		1		1		1	
Female	0.83 (0.59; 1.18)	0.3	1.32 (0.66; 2.66)	0.4	1.28 (0.89; 1.84)	0.2	0.84 (0.41; 1.73)	0.6	0.66 (0.32; 1.36)	0.3
Age	1.01 (1; 1.02)	0.1	1 (0.98; 1.02)	0.9	1.01 (1; 1.02)	0.1	1 (0.98; 1.02)	0.8	0.99 (0.98; 1.01)	0.6
Glycaemic status										
Normal	1		1		1		1		1	
Pre-DM	1.12 (0.78; 1.61)	0.5	0.54 (0.25; 1.15)	0.1	1.25 (0.86; 1.82)	0.2	0.6 (0.27; 1.3)	0.2	0.48 (0.22; 1.05)	0.06
DM	1.09 (0.76; 1.55)	0.6	0.85 (0.44; 1.62)	0.6	1.13 (0.78; 1.64)	0.5	0.9 (0.46; 1.75)	0.7	0.79 (0.4; 1.56)	0.5
JIS Criteria										
No	1		1		1		1		1	
Yes	1.17 (0.86; 1.58)	0.3	0.99 (0.56; 1.74)	1	1.19 (0.86; 1.65)	0.3	1.06 (0.59; 1.9)	0.8	0.89 (0.49; 1.62)	0.7
Obesity status										
Normal	1		1		1		1		1	
Overweight	0.68 (0.46; 1.01)	0.06	1.09 (0.52; 2.28)	0.8	0.65 (0.43; 0.97)	0.04	0.91 (0.42; 1.95)	0.8	1.41 (0.64; 3.08)	0.4
Obese	1 (0.68; 1.46)	1	1.19 (0.58; 2.44)	0.6	0.97 (0.65; 1.44)	0.9	1.18 (0.56; 2.46)	0.7	1.22 (0.58; 2.58)	0.6
Vitamin D										
Sufficient	1		1		1		1		1	
Insufficient	0.96 (0.73; 1.27)	0.8	1.68 (1.02; 2.77)	0.04	0.87 (0.65; 1.16)	0.3	1.58 (0.94; 2.65)	0.09	1.82 (1.07; 3.07)	0.03
Deficient	0.87 (0.48; 1.56)	0.6	1.61 (0.59; 4.37)	0.4	0.78 (0.41; 1.46)	0.4	1.44 (0.51; 4.05)	0.5	1.86 (0.64; 5.41)	0.3

4. Discussion

Vitamin D deficiency is one of the most prevalent nutritional deficiencies in the world. Vitamin D, which was previously known to be involved only in calcium homeostasis, is now known to have several other functions in the human body [22]. Subclinical and asymptomatic vitamin D deficiency is associated with increased risk of multiple malignancies, metabolic and cardiovascular diseases, diabetes, and immune disorders [23]. Studies regarding vitamin D supplementation in African populations are limited [24,25]. Over the last decade, low vitamin D levels have emerged as a risk factor for T2D, but this has not been investigated in South African populations. In this community-based study, we examined the association between serum 25-hydroxy vitamin D levels and glycaemic indicators in diabetic, prediabetic, and healthy subjects from a population at high risk of developing diabetes residing in an urban area of Cape Town, South Africa [14]. Due to differences in opinions regarding cut-off levels of vitamin D deficiency [26], we used both the 2011 Endocrine Clinical Society Practice Guidelines and the more recent Global Consensus Recommendations on Prevention and Management of Nutritional Rickets to define vitamin D deficiency as either a 25(OH) vitamin D level below 20 ng/mL (50 nmol/L) or 12 ng/mL (30 nmol/L) [20]. Similar to reports from Germany and Japan, we found a mean 25(OH) vitamin D level of 22 ng/mL, which was within the optimal levels of the Global Consensus Recommendations, but considered to be insufficient according to the Endocrine Society Guidelines [19]. We observed significant differences in the overall prevalence of vitamin D deficiency, with 44.5% of the participants classified as deficient according to the Endocrine Clinical Society Practice Guidelines, but only 5.6% were found to be deficient when using the Global Consensus Recommendations. Only 13% had optimal levels, whilst 44.7% had sub-optimal levels when using the latter criteria.

We observed vitamin D deficiency in subjects with either prediabetes, screen-detected diabetes mellitus, or known diabetes mellitus compared to normoglycaemic subjects using the former criteria. Surprisingly, when using the Global Consensus Criteria, which uses a much lower cut-off to define vitamin D deficiency, the percentage of deficient subjects was similar in each of the glycaemic groups (Table 3). This raises an important question: what levels must we use to define vitamin D deficiency? When comparing our results with earlier studies that have used higher cut-offs endorsed by the Endocrine Society, similar conclusions were arrived at: vitamin D deficiency is associated with prediabetes and diabetes. In a study from Japan, which used a cut-off of 50 nmol/L, vitamin D deficiency was found in 54% of participants, whilst it was 90.9% when a cut-off of 75 nmol/L was used. The high prevalence of vitamin D insufficiency in Japanese populations was attributed to darker skin and rare use of vitamin D supplements [27]. Similarly, studies from Egypt and Bangladesh have reported lower vitamin D levels in T2D patients [28,29] compared to healthy controls. Further research is required regarding the influence of skin colour on vitamin D levels.

A study from China examined if higher plasma 25(OH) vitamin D concentrations were associated with lower risks of diabetes in 82500 participants and further tested the relevance of 25(OH) vitamin D in T2D subjects using genetically instrumented differences in plasma 25(OH) vitamin D concentrations to ascertain causality. The concordant results of both the observational and genetic studies indicate that a higher vitamin D status is associated with a lower risk of diabetes and provide support for a causally protective effect of higher vitamin D in the prevention of T2D [30]. Abbasi et al. showed that subjects with prediabetes and low circulating 25(OH) vitamin D levels were mostly insulin-resistant, had impaired β-cell function, and were most likely to develop T2D [31].

In our study, both obesity and overweight were commonly observed. A higher body mass index (BMI) has been associated with lower vitamin D levels. Obesity affects insulin secretion, tissue sensitivity to insulin, and systemic inflammation, but this may not account for differences seen in the levels of vitamin D deficiency between the glycaemic groups, as BMIs were similar. A meta-analysis that examined 55 observational studies

showed an inverse relationship between vitamin D levels and BMI in both diabetic and non-diabetic subjects [32]. Studies in low- and middle-income countries have consistently demonstrated that women have lower average 25(OH) vitamin D levels than their male counterparts, which is largely thought to be due to differences in occupation, clothing, and cultural practices, which predisposes women in these countries to lower vitamin D status and is not related to biological differences in vitamin D metabolism between males and females [33]. Similarly, in this study we found lower vitamin D levels in females than in male study participants, although females displayed higher vitamin D-binding protein levels. These sex differences may partially be attributed to the higher BMIs observed in females. Surprisingly, no sex differences were observed in CRP levels. Several studies have suggested that the lower the vitamin D level, the greater the benefit of supplementation in preventing diabetes [34,35]. Thus, it may be prudent to consider the benefit of vitamin D supplementation in this population group, which is at high risk of developing diabetes.

The *VDR* gene is highly polymorphic, widely distributed, located on chromosome 12q13.1 [36], and controls genes related to bone metabolism, inflammation, oxidative damage, and chronic diseases [37]. Vitamin D and its receptor complex play a role in the regulation of insulin secretion from beta cells [38,39]. *VDR* gene variations are associated with the development, progression, and complications of T2D [40,41]. If the vitamin D-binding protein gene is mutated, vitamin D would decrease in serum and target tissues, although sufficient sun exposure or supplementation may ameliorate this. Four common single-nucleotide polymorphisms of the *VDR* gene have been postulated to be associated with T2D in different ethnic populations, namely *FokI* (*rs2228570*), *BsmI* (*rs1544410*), *ApaI* (*rs7975232*), and *TaqI* (*rs731236*). The full-length human *VDR* gene is ~63.5 kb.

In this study, the *VDR* single-nucleotide polymorphisms *Fok1* (*rs2228570*) and *Taq1* (*rs731236*) were not associated with glycaemic status. *Fok1* was also not associated with vitamin D level, although *Taq1* was associated with insufficient vitamin D. Amongst a population in Saudi Arabia, no significant association between the *Fok1* and *Taq1* single-nucleotide polymorphisms and vitamin D deficiency was observed [42]. A study conducted in Russia showed no difference in serum 25(OH) vitamin D concentration between *Taq1* and *Fok1* genotypes [43]. However, in a population from Bangladesh at high risk of T2D, the *ApaI* polymorphism was associated with insulin secretion and a higher prevalence of vitamin D deficiency. The *ApaI* polymorphism was correlated with fasting blood glucose levels, and glucose intolerance was evident among individuals with symptoms of diabetes at the pre-diagnosis stage [44]. As ethnicity reportedly influences *VDR* gene variations [45], the variations observed in our study may be explained by this and the participants' exposure to environmental factors [46]. Our results indicate that *Fok1* was associated with obesity, similar to observations in T2D Egyptian patients. Patients with mutant recessive homozygous TT genotype C>T polymorphisms exhibited higher waist circumference and BMIs than individuals with the homozygous CC genotype [46]. In our study, the GG genotype of the *Fok1* polymorphism was associated with a two-fold increased risk of developing obesity, similar to subjects harbouring the T allele in Greece [47].

Our study, like others, is not without limitations. The comparison of vitamin D status between different studies is difficult due to the lack of an evidence-based consensus regarding optimal levels of serum 25(OH) vitamin D, since cut-offs used to evaluate vitamin D status vary across studies. Although serum 25(OH) vitamin D measurement is a valid and commonly used biomarker of vitamin D status, its measurement still lacks standardization. Thus, the measurement of 25(OH) vitamin D differs between studies due to differences in analytical methods, assays, and devices used [48]. There are also seasonal variations in serum 25(OH) vitamin D levels, with the highest levels observed towards the end of summer and lowest levels toward the end of winter, but tracking 25(OH) vitamin D concentration over time reveals that a single measurement of serum 25(OH) vitamin D at a given point provides an estimate of future 25(OH) vitamin D levels [10]. Furthermore, this study did not take dietary choices or physical activity into consideration. As such, behavioural differences may influence the interpretation of the results. Further studies are

required to determine the influence of diet in this cohort, as well as in a cohort with similar dietary preferences and levels of physical activity.

5. Conclusions

We observed that vitamin D deficiency or insufficiency is relatively common in this mixed-ancestry population from Cape Town. Furthermore, we found that vitamin D levels were decreased in individuals with hyperglycaemia, which was not linked to either vitamin D-binding protein or *Fok1* polymorphisms in the vitamin D receptor gene, although *Taq1* was associated with an insufficient vitamin D status. The mechanisms affecting lower vitamin D levels in individuals with hyperglycaemia require further investigation. Finally, a clinical trial of vitamin D supplementation to either revert prediabetes to normoglycaemia or prevent its progression to diabetes in this population group is highly desirable and recommended.

Author Contributions: Conceptualization, R.E. and T.M.; Data curation, S.D. and T.M.; Formal analysis, R.E., S.M., I.M. and S.D.; Funding acquisition, R.E. and T.M.; Investigation, R.E., S.M., I.M., S.D., S.R. and T.M.; Methodology, R.E., S.M., I.M., S.D., S.R. and T.M.; Project administration, S.R. and T.M.; Supervision, R.E. and T.M.; Visualization, S.D.; Writing—original draft, R.E., I.M. and S.R.; Writing—review and editing, R.E., S.M. and S.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research project was funded by the South African Medical Research Council with funds from the National Treasury under its Economic Competitiveness and Support Package (MRC-RFA-UFSP-01–2013/VMH Study) and the South African National Research Foundation (Grant no. 115450). Any opinion, finding, and conclusion or recommendation expressed in this material is that of the authors, and the Medical Research Council does not accept any liability in this regard.

Institutional Review Board Statement: The study forms part of the ongoing Vascular and Metabolic Health study (VMH), which received ethical approval from the research ethics committees of the Cape Peninsula University of Technology (CPUT) and Stellenbosch University (NHREC: REC-230 408-014 and N14/01/003, respectively). The current study also received ethical approval from the Stellenbosch University Health Research Ethics committee (0719) and Cape Peninsula University of Technology, Faculty of Health and Wellness Sciences Research Ethics committee (CPUT/HW REC2015/H01). Written informed consent was sought from all study participants following explanation of study procedures in their language of choice. All methods were performed in accordance with the Declaration of Helsinki and all relevant regulations.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The sequence data used to support the findings of this study have been deposited in the SRA BioProject database (accession number: PRJNA723337).

Acknowledgments: The authors would like to thank the community of Bellville South.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

References

1. Ross, A.C.; Manson, J.E.; Abrams, S.A.; Aloia, J.F.; Brannon, P.M.; Clinton, S.K.; Durazo-Arvizu, R.A.; Gallagher, J.C.; Gallo, R.L.; Jones, G.; et al. The 2011 report on dietary reference intakes for calcium and vitamin D from the Institute of Medicine: What clinicians need to know. *J. Clin. Endocrinol. Metab.* **2011**, *96*, 53–58. [CrossRef] [PubMed]
2. Pludowski, P.; Holick, M.F.; Pilz, S.; Wagner, C.L.; Hollis, B.W.; Grant, W.B.; Shoenfeld, Y.; Lerchbaum, E.; Llewellyn, D.J.; Kienreich, K.; et al. Vitamin D effects on musculoskeletal health, immunity, autoimmunity, cardiovascular disease, cancer, fertility, pregnancy, dementia and mortality—A review of recent evidence. *Autoimmun. Rev.* **2013**, *12*, 976–989. [CrossRef] [PubMed]
3. Wilson, L.R.; Tripkovic, L.; Hart, K.H.; Lanham-New, S.A. Vitamin D deficiency as a public health issue: Using vitamin D2 or vitamin D3 in future fortification strategies. *Proc. Nutr. Soc.* **2017**, *76*, 392–399. [CrossRef] [PubMed]
4. Al-Ishaq, R.K.; Kubatka, P.; Brozmanova, M.; Gazdikova, K.; Caprnda, M.; Büsselberg, D. Health implication of vitamin D on the cardiovascular and the renal system. *Arch. Physiol. Biochem.* **2021**, *127*, 195–209. [CrossRef]

5. Gulseth, H.L.; Gjelstad, I.M.F.; Birkeland, K.I.; Drevon, C.A. Vitamin D and the metabolic syndrome. *Curr. Vasc. Pharmacol.* **2013**, *11*, 968–984. [CrossRef]
6. Pittas, A.G.; Chung, M.; Trikalinos, T.; Mitri, J.; Brendel, M.; Patel, K.; Lichtenstein, A.H.; Lau, J.; Balk, E.M. Systematic review: Vitamin D and cardiometabolic outcomes. *Ann. Intern. Med.* **2010**, *152*, 307–314. [CrossRef]
7. Mitri, J.; Muraru, M.D.; Pittas, A.G. Vitamin D and type 2 diabetes: A systematic review. *Eur. J. Clin. Nutr.* **2011**, *65*, 1005–1015. [CrossRef]
8. Pittas, A.G.; Dawson-Hughes, B.; Sheehan, P.; Ware, J.H.; Knowler, W.C.; Aroda, V.R.; Brodsky, I.; Ceglia, L.; Chadha, C.; Chatterjee, R.; et al. Vitamin D Supplementation and Prevention of Type 2 Diabetes. *N. Engl. J. Med.* **2019**, *381*, 520–530. [CrossRef]
9. Yazdanpanah, S.; Rabiee, M.; Tahriri, M.; Abdolrahim, M.; Rajab, A.; Jazayeri, H.E.; Tayebi, L. Evaluation of glycated albumin (GA) and GA/HbA1c ratio for diagnosis of diabetes and glycemic control: A comprehensive review. *Crit. Rev. Clin. Lab. Sci.* **2017**, *54*, 219–232. [CrossRef]
10. Pilz, S.; Zittermann, A.; Trummer, C.; Theiler-Schwetz, V.; Lerchbaum, E.; Keppel, M.H.; Grübler, M.R.; März, W.; Pandis, M. Vitamin D testing and treatment: A narrative review of current evidence. *Endocr. Connect.* **2019**, *8*, R27–R43. [CrossRef] [PubMed]
11. Xu, Y.; Zhou, Y.; Liu, J.; Wang, C.; Qu, Z.; Wei, Z.; Zhou, D. Genetically increased circulating 25(OH)D level reduces the risk of type 2 diabetes in subjects with deficiency of vitamin D: A large-scale Mendelian randomization study. *Medicine* **2020**, *99*, e23672. [CrossRef] [PubMed]
12. Wang, G.; Li, Y.; Li, L.; Yu, F.; Cui, L.; Ba, Y.; Li, W.; Wang, C. Association of the vitamin D binding protein polymorphisms with the risk of type 2 diabetes mellitus: A meta-analysis. *BMJ Open* **2014**, *4*, e005617. [CrossRef] [PubMed]
13. Li, L.; Wu, B.; Liu, J.-Y.; Yang, L.-B. Vitamin D receptor gene polymorphisms and type 2 diabetes: A meta-analysis. *Arch. Med. Res.* **2013**, *44*, 235–241. [CrossRef] [PubMed]
14. Erasmus, R.T.; Soita, D.J.; Hassan, M.S.; Blanco-Blanco, E.; Vergotine, Z.; Kegne, A.P.; Matsha, T.E. High prevalence of diabetes mellitus and metabolic syndrome in a South African coloured population: Baseline data of a study in Bellville, Cape Town. *S. Afr. Med. J.* **2012**, *102*, 841–844. [CrossRef] [PubMed]
15. Kengne, A.P.; Erasmus, R.T.; Levitt, N.S.; Matsha, T.E. Alternative indices of glucose homeostasis as biochemical diagnostic tests for abnormal glucose tolerance in an African setting. *Prim. Care Diabetes* **2017**, *11*, 119–131. [CrossRef] [PubMed]
16. World Health Organization. *Obesity: Preventing and Managing the Global Epidemic: Report of a WHO Consultation*; World Health Organization: Geneva, Switzerland, 2000; Volume 894.
17. World Health Organization. *Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycemia: Report of a WHO/IDF Consultation*; World Health Organization: Geneva, Switzerland, 2006.
18. Alberti, K.G.M.M.; Eckel, R.H.; Grundy, S.M.; Zimmet, P.Z.; Cleeman, J.I.; Donato, K.A.; Fruchart, J.-C.; James, W.P.T.; Loria, C.M.; Smith, S.C.J. Harmonizing the metabolic syndrome: A joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International. *Circulation* **2009**, *120*, 1640–1645. [CrossRef]
19. Holick, M.F.; Binkley, N.C.; Bischoff-Ferrari, H.A.; Gordon, C.M.; Hanley, D.A.; Heaney, R.P.; Murad, M.H.; Weaver, C.M. Evaluation, treatment, and prevention of vitamin D deficiency: An Endocrine Society clinical practice guideline. *J. Clin. Endocrinol. Metab.* **2011**, *96*, 1911–1930. [CrossRef]
20. Munns, C.F.; Shaw, N.; Kiely, M.; Specker, B.L.; Thacher, T.D.; Ozono, K.; Michigami, T.; Tiosano, D.; Mughal, M.Z.; Mäkitie, O.; et al. Global Consensus Recommendations on Prevention and Management of Nutritional Rickets. *J. Clin. Endocrinol. Metab.* **2016**, *101*, 394–415. [CrossRef]
21. Lugg, S.T.; Howells, P.A.; Thickett, D.R. Optimal Vitamin D Supplementation Levels for Cardiovascular Disease Protection. *Dis. Markers* **2015**, *2015*, 864370. [CrossRef]
22. Randev, S.; Kumar, P.; Guglani, V. Vitamin D Supplementation in Childhood—A Review of Guidelines. *Indian J. Pediatr.* **2018**, *85*, 194–201. [CrossRef]
23. Baeke, F.; Takiishi, T.; Korf, H.; Gysemans, C.; Mathieu, C. Vitamin D: Modulator of the immune system. *Curr. Opin. Pharmacol.* **2010**, *10*, 482–496. [CrossRef] [PubMed]
24. Anyanwu, A.C.; Fasanmade, O.A.; Odeniyi, I.A.; Iwuala, S.; Coker, H.B.; Ohwovoriole, A.E. Effect of Vitamin D supplementation on glycemic control in Type 2 diabetes subjects in Lagos, Nigeria. *Indian J. Endocrinol. Metab.* **2016**, *20*, 189–194. [CrossRef] [PubMed]
25. Kibirige, D.; Lumu, W.; Jones, A.G.; Smeeth, L.; Hattersley, A.T.; Nyirenda, M.J. Understanding the manifestation of diabetes in sub Saharan Africa to inform therapeutic approaches and preventive strategies: A narrative review. *Clin. Diabetes Endocrinol.* **2019**, *5*, 2. [CrossRef] [PubMed]
26. Parva, N.R.; Tadepalli, S.; Singh, P.; Qian, A.; Joshi, R.; Kandala, H.; Nookala, V.K.; Cheriyath, P. Prevalence of Vitamin D Deficiency and Associated Risk Factors in the US Population (2011–2012). *Cureus* **2018**, *10*, e2741. [CrossRef]
27. Nakamura, K.; Kitamura, K.; Takachi, R.; Saito, T.; Kobayashi, R.; Oshiki, R.; Watanabe, Y.; Tsugane, S.; Sasaki, A.; Yamazaki, O. Impact of demographic, environmental, and lifestyle factors on vitamin D sufficiency in 9084 Japanese adults. *Bone* **2015**, *74*, 10–17. [CrossRef]

28. El Gendy, H.I.; Sadik, N.A.; Helmy, M.Y.; Rashed, L.A. Vitamin D receptor gene polymorphisms and 25(OH) vitamin D: Lack of association to glycemic control and metabolic parameters in type 2 diabetic Egyptian patients. *J. Clin. Transl. Endocrinol.* **2019**, *15*, 25–29. [CrossRef] [PubMed]
29. Rahman, M.M.; Hosen, M.B.; Faruk, M.O.; Hasan, M.M.; Kabir, Y.; Howlader, M.Z.H. Association of vitamin D and vitamin D binding protein (DBP) gene polymorphism with susceptibility of type 2 diabetes mellitus in Bangladesh. *Gene* **2017**, *636*, 42–47. [CrossRef]
30. Lu, L.; Bennett, D.A.; Millwood, I.Y.; Parish, S.; McCarthy, M.I.; Mahajan, A.; Lin, X.; Bragg, F.; Guo, Y.; Holmes, M.V.; et al. Association of vitamin D with risk of type 2 diabetes: A Mendelian randomisation study in European and Chinese adults. *PLoS Med.* **2018**, *15*, e1002566. [CrossRef] [PubMed]
31. Abbasi, F.; Blasey, C.; Feldman, D.; Caulfield, M.P.; Hantash, F.M.; Reaven, G.M. Low circulating 25-hydroxyvitamin D concentrations are associated with defects in insulin action and insulin secretion in persons with prediabetes. *J. Nutr.* **2015**, *145*, 714–719. [CrossRef] [PubMed]
32. Rafiq, S.; Jeppesen, P.B. Body Mass Index, Vitamin D, and Type 2 Diabetes: A Systematic Review and Meta-Analysis. *Nutrients* **2018**, *10*, 1182. [CrossRef]
33. Roth, D.E.; Abrams, S.A.; Aloia, J.; Bergeron, G.; Bourassa, M.W.; Brown, K.H.; Calvo, M.S.; Cashman, K.D.; Combs, G.; De-Regil, L.M.; et al. Global prevalence and disease burden of vitamin D deficiency: A roadmap for action in low- and middle-income countries. *Ann. N. Y. Acad. Sci.* **2018**, *1430*, 44–79. [CrossRef] [PubMed]
34. Barbarawi, M.; Zayed, Y.; Barbarawi, O.; Bala, A.; Alabdouh, A.; Gakhal, I.; Rizk, F.; Alkasasbeh, M.; Bachuwa, G.; Manson, J.E. Effect of Vitamin D Supplementation on the Incidence of Diabetes Mellitus. *J. Clin. Endocrinol. Metab.* **2020**, *105*, 2857–2868. [CrossRef] [PubMed]
35. Zhang, Y.; Tan, H.; Tang, J.; Li, J.; Chong, W.; Hai, Y.; Feng, Y.; Lunsford, L.D.; Xu, P.; Jia, D.; et al. Effects of Vitamin D Supplementation on Prevention of Type 2 Diabetes in Patients With Prediabetes: A Systematic Review and Meta-analysis. *Diabetes Care* **2020**, *43*, 1650–1658. [CrossRef] [PubMed]
36. Bikle, D. Nonclassic actions of vitamin D. *J. Clin. Endocrinol. Metab.* **2009**, *94*, 26–34. [CrossRef] [PubMed]
37. Haussler, M.R.; Haussler, C.A.; Bartik, L.; Whitfield, G.K.; Hsieh, J.-C.; Slater, S.; Jurutka, P.W. Vitamin D receptor: Molecular signaling and actions of nutritional ligands in disease prevention. *Nutr. Rev.* **2008**, *66* (Suppl. 2), S98–S112. [CrossRef]
38. Knekt, P.; Laaksonen, M.; Mattila, C.; Härkänen, T.; Marniemi, J.; Heliövaara, M.; Rissanen, H.; Montonen, J.; Reunanen, A. Serum vitamin D and subsequent occurrence of type 2 diabetes. *Epidemiology* **2008**, *19*, 666–671. [CrossRef] [PubMed]
39. Pittas, A.G.; Dawson-Hughes, B. Vitamin D and diabetes. *J. Steroid Biochem. Mol. Biol.* **2010**, *121*, 425–429. [CrossRef]
40. Vural, H.C.; Maltas, E. RT-qPCR assay on the vitamin D receptor gene in type 2 diabetes and hypertension patients in Turkey. *Genet. Mol. Res.* **2012**, *11*, 582–590. [CrossRef] [PubMed]
41. Liu, Z.; Liu, L.; Chen, X.; He, W.; Yu, X. Associations study of vitamin D receptor gene polymorphisms with diabetic microvascular complications: A meta-analysis. *Gene* **2014**, *546*, 6–10. [CrossRef]
42. Al-Daghri, N.M.; Al-Attas, O.S.; Alkharfy, K.M.; Khan, N.; Mohammed, A.K.; Vinodson, B.; Ansari, M.G.A.; Alenad, A.; Alokail, M.S. Association of VDR-gene variants with factors related to the metabolic syndrome, type 2 diabetes and vitamin D deficiency. *Gene* **2014**, *542*, 129–133. [CrossRef]
43. Karonova, T.; Grineva, E.; Belyaeva, O.; Bystrova, A.; Jude, E.B.; Andreeva, A.; Kostareva, A.; Pludowski, P. Relationship Between Vitamin D Status and Vitamin D Receptor Gene Polymorphisms with Markers of Metabolic Syndrome among Adults. *Front. Endocrinol.* **2018**, *9*, 448. [CrossRef] [PubMed]
44. Ogunkolade, B.-W.; Boucher, B.J.; Prahl, J.M.; Bustin, S.A.; Burrin, J.M.; Noonan, K.; North, B.V.; Mannan, N.; McDermott, M.F.; DeLuca, H.F.; et al. Vitamin D receptor (VDR) mRNA and VDR protein levels in relation to vitamin D status, insulin secretory capacity, and VDR genotype in Bangladeshi Asians. *Diabetes* **2002**, *51*, 2294–2300. [CrossRef] [PubMed]
45. Haddad, S. Vitamin-D receptor (VDR) gene polymorphisms (Taq-I & Apa-I) in Syrian healthy population. *Meta Gene* **2014**, *2*, 646–650. [CrossRef] [PubMed]
46. Mackawy, A.M.H.; Badawi, M.E.H. Association of vitamin D and vitamin D receptor gene polymorphisms with chronic inflammation, insulin resistance and metabolic syndrome components in type 2 diabetic Egyptian patients. *Meta Gene* **2014**, *2*, 540–556. [CrossRef] [PubMed]
47. Vasilopoulos, Y.; Sarafidou, T.; Kotsa, K.; Papadimitriou, M.; Goutzelas, Y.; Stamatis, C.; Bagiatis, V.; Tsekmekidou, X.; Yovos, J.G.; Mamuris, Z. VDR TaqI is associated with obesity in the Greek population. *Gene* **2013**, *512*, 237–239. [CrossRef]
48. Rabenberg, M.; Scheidt-Nave, C.; Busch, M.A.; Rieckmann, N.; Hintzpeter, B.; Mensink, G.B.M. Vitamin D status among adults in Germany–results from the German Health Interview and Examination Survey for Adults (DEGS1). *BMC Public Health* **2015**, *15*, 641. [CrossRef]

MDPI
St. Alban-Anlage 66
4052 Basel
Switzerland
Tel. +41 61 683 77 34
Fax +41 61 302 89 18
www.mdpi.com

Nutrients Editorial Office
E-mail: nutrients@mdpi.com
www.mdpi.com/journal/nutrients

MDPI
St. Alban-Anlage 66
4052 Basel
Switzerland

Tel: +41 61 683 77 34

www.mdpi.com

ISBN 978-3-0365-6118-9