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A critical review of Vitamin C for the prevention of age-related cognitive decline and Alzheimer's disease

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Abstract

Antioxidants in the diet have long been thought to confer some level of protection against the oxidative damage that is involved in the pathology of Alzheimer's disease as well as general cognitive decline in normal aging. Nevertheless, support for this hypothesis in the literature is equivocal. In the case of vitamin C (ascorbic acid) in particular, lack of consideration of some of the specific features of vitamin C metabolism has led to studies in which classification of participants according to vitamin C status is inaccurate, and the absence of critical information precludes the drawing of appropriate conclusions. Vitamin C levels in plasma are not always reported, and estimated daily intake from food diaries may not be accurate or reflect actual plasma values. The ability to transport ingested vitamin C from the intestines into blood is limited by the saturable sodium-dependent vitamin C transporter (SVCT1) and thus very high intakes, and the use of supplements are often erroneously considered to be of greater benefit that they really are. The current review documents differences among the studies in terms of vitamin C status of participants. Overall, there is a large body of evidence that maintaining healthy vitamin C levels can have a protective function against age-related cognitive decline and Alzheimer's disease, but avoiding vitamin C deficiency is likely to be more beneficial than taking supplements on top of a normal, healthy diet.

Keywords

Vitamin C; Ascorbic acid; Alzheimer's disease; Cognition

1. Introduction

Vitamin C (ascorbic acid) is arguably one of the most important single nutritional factors in terms of its influence on world history. Naval and sea battles have been won and lost based on the numbers of the naval forces sick with scurvy as well as military prowess, and the race to explore far away places like the Antarctic was made much harder for explorers like Captains Cook and Scott by the ravages of scurvy. Clinical scurvy is seen in a very small portion of the population nowadays, and scurvy can be avoided with intake of as little as 10 mg of vitamin C per day [1]. However, there is still a large portion of the population that is deficient or depleted in vitamin C as will be described in this review. The optimal level of vitamin C intake for brain function is unknown, but vitamin C plays a critical role in brain development and protection across the lifespan [2]. Several sources now suggest that recommended dietary allowance (RDA) should be as much as double the currently advised

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75–125 mg per day depending on age, gender, pregnancy and smoking habits [3–5]. The role and transport of vitamin C in the brain have previously been reviewed [2]. In the current review evidence is presented that vitamin C deficiency is far more widespread than is currently presumed, and that adequate vitamin C intake is critical in the slowing the onset and progression of Alzheimer's disease (dementias of the Alzheimer's type, DAT). For instance vitamin C depletion has been observed in up to 30 % of presumed healthy population samples [6–11]. Generally plasma concentration of <11 μM is considered to be deficient, 11–28 μM is depleted or marginally deficient, 28–40 μM is adequate, and > 40 μM is optimal [12]. However, the actual value greater than 40 μM that truly represents optimal intake is as yet unknown.

It is becoming increasing clear that oxidative stress generated by reactive oxygen species (ROS) is a critical component in the pathogenesis of DAT and other neurodegenerative disorders. This relationship has been shown in human studies and is supported in many mice models [13–16]. Reactivity of ROS can be terminated if the radical meets and reacts with an antioxidant molecule such as vitamins C or E (α-tocopherol), leading to neutralised or greatly reduced reactivity. Antioxidant molecules lose their antioxidant properties via this process and therefore must be constantly replenished or recycled. Oxidative stress arises when the production of free radicals exceeds the ability of an organism to eliminate or neutralise them and is one of multiple processes that can lead to apoptosis in neurons [17]. ROS are generated as part of normal physiological processes with the majority of endogenous ROS (including superoxide, hydrogen peroxide and the hydroxyl radical) created by the electron transport chain during the production of ATP in the mitochondria [18, 19]. Under normal circumstances, ROS are neutralized by antioxidant enzymes (superoxide dismutase, glutathione peroxidase, and catalase) and by antioxidants within the cell and the interstitial fluids (including vitamin C, vitamin E, glutathione). Current opinion suggests that efforts to maintain the antioxidant capacity of cells present a suitable arena in which to target treatments for the DAT. A diet deficient in antioxidants can also lead to impaired ability to repair neuronal DNA, which leaves the cells even more sensitive to oxidative damage [20]. Vitamin C is thought to be the most effective antioxidant in plasma, in part due to its water solubility and to the wide range of ROS that it can scavenge [21]. If found to be protective against age-related and neurological diseases, vitamin C supplements would provide an intervention of low cost and toxicity. The elderly are at high risk of malnutrition, or at least sub-clinical malnutrition, for a number of reasons including limited mobility, low income, institutionalization, reduced appetite, and poorer cognitive function. Preventing the depletion of antioxidant stores and maintaining healthy levels throughout the life in order to improve the healthspan of the population may be a more important strategy than trying to reverse ROS damage that has already occurred.

Despite this strong rationale clinical data concerning the efficacy of an antioxidant-rich diet, or antioxidant supplements, are equivocal. In the case of vitamin C in particular the lack of consensus in the literature may be due to erroneous or inappropriate analysis or classification of data. In fact, just as with vitamin D, deficiency is far more widespread in the general and aging population than is currently believed. Vitamin C is extremely important as an antioxidant owing to its ability to neutralize oxygen and nitrogen based radicals, and because it also recycles both vitamin E and tetrahydrobiopterin, which respectively have key antioxidant and enzyme cofactor functions in brain. Furthermore, vitamin C is a cofactor in a number of hydroxylation reactions where it protects hydroxylase enzymes by reducing Fe^{3+} and Cu^{2+} at their active sites, such as in the synthesis of collagen, carnitine, and norepinephrine and also in the regulation of the gene HIF1- α [2]. The main property of vitamin C that renders it such an efficient antioxidant is its low midpoint reduction potential [22], which allows ready donation of an electron to reactive oxygen species. Further, ascorbate is a one electron donor and thus forms a radical termed

monodehydroascorbate or simply the ascorbate radical. This radical is efficiently recycled to ascorbate by NAD(P)H-dependent enzymes. The ascorbate radical also prefers to react with itself rather than with cellular proteins or DNA by dismutating to form one molecule of ascorbate and one of dehydroascorbate, the two-electron oxidized form of ascorbate. Dehydroascorbate has a short half life of approximately 6 minutes in physiological buffers and can be reduced back to ascorbate, or undergo irreversible ring opening to 2,3-diketo-1-gulonic acid and be lost. Although it may seem counterintuitive, vitamin C may also have pro-oxidant roles *in vivo* as well as *in vitro*, particularly in the presence of transition metals such as copper or iron and it is not known how such roles interact with different disease states [23]. Supplemental intake greater than 500 mg per day may be injurious to patients who are prone to kidney (oxalate) stones [24]. Vitamin C can enhance iron absorption by maintaining iron in ferrous (Fe^{2+}) rather than ferric (Fe^{3+}) state. This may be beneficial in some patients but not in those that suffer from medical conditions that cause iron overload.

For the following review, the search strategy employed was to search Pubmed for all articles that specifically measured vitamin C in terms of estimated intake or blood plasma levels in healthy (young to aged) and clinical populations. Other studies that considered intake of fruits and vegetables together, without special attention to particular nutritional components were not included. Both prospective and cross-sectional studies were included. When vitamin C measurements were described, close attention was paid to the assignment of subjects into particular dietary groups as well as to the actual values reported. Critical for this review, in addition to average values, the spread of sample values from whence estimations were made of the portions of the population that might be deficient in terms of plasma level or dietary intake of vitamin C are also reported. The review focuses on the reported deficiencies that are often overlooked in summarizing such data.

2. Human clinical studies

The many studies of nutrition and cognition during healthy and abnormal aging have been reviewed in detail numerous times [25–27]. Such reviews generally conclude that there is some supporting evidence for the use of dietary fruits and vegetables and vitamin C and E supplements to slow cognitive deterioration, but that support is not universal and that more work needs to be done in this area. The present review takes a different perspective, that of analyzing the results from these studies with careful attention to methodological anomalies in measurement of vitamin C status. Vitamin C was assessed either by dietary intake questionnaires or by analysis of plasma levels. Cognitive ability in these studies was assessed via a range of neuropsychological tests. The concept of cognitive ability is a fluid and broad subject that makes it difficult to accurately compare between studies. Furthermore, the choice of cognitive test, including the type of memory and the complexity of the task is critical and can easily affect sensitivity in a non-demented population, although each has been validated in its own right [28]. There are differences in rates of decline, particularly among the healthy aging, and averaging of scores may mask indications of cognitive dysfunction. Cognitive performance on these tests, particularly in the elderly, is also easily affected by common problems with sight and hearing, and depression. For simplicity, this review considers ‘cognitive ability’ rather than focusing on the specific test in order to highlight only whether any beneficial change was recorded or not.

The current USA RDA for vitamin C is 75 mg/day in adult females, 90 mg/day in males, with amounts up to 125 mg/day recommended for pregnant or lactating women [29]. For smokers an additional 35 mg per day is already recommended to account for increased oxidative stress and vitamin C turnover [5]. The Food Standards Agency guide for UK Institutions recommends only 40 mg per day vitamin C for adults over 19 years (October 2006, www.food.gov.uk). The RDA for a given nutrient is calculated based on avoiding

deficiency rather than optimal level and thus this is unlikely to reflect the optimal intake level [30]. Humans and animals are limited in the amount of vitamin C that can be absorbed at any given time by the function of sodium-dependent vitamin C (SVCT1) transporters in the gut [31]. Therefore, doses over approximately 400 mg/day result in saturation in plasma with excretion of excess absorbed vitamin C [3] - particularly if combined with a healthy diet and/or taken more than once per day (e.g. [32]). Even a multivitamin containing only 30 or 60 mg of vitamin C should be sufficient to maintain adequate vitamin C levels in addition to dietary intake of fruits and vegetables. A plasma level of 40–60 μM VC is considered to be healthy, but this can be increased further following doses of 250 mg per day or higher [3]. Plasma levels under approximately 28 μM are considered depleted and levels below 11 μM are considered deficient [12]. At 8 μM plasma subjects were considered depleted but showed no signs of scurvy [3].

2.1 Supporting Evidence for increased vitamin C intakes

2.1.1 Elderly - non-demented subjects—A number of studies have found associations between vitamin C and E intake and cognitive ability in healthy individuals aged 60 and over. In a British sample assessed at ages 65 and over in the early 1970s, vitamin C intake (measured through food diaries) was the only dietary factor that predicted cognitive ability. It was also the best predictor of survival during the 20 year follow-up period and this relationship was supported by plasma vitamin C levels [33]. In a similar study in Albuquerque of participants aged 60–94 living independently and in good health, vitamin C was again shown to be the major dietary component that could be strongly linked to cognitive function [34]. In fact, the poorest performance on tests of verbal memory and recall were found in the bottom 10% of the group in terms of vitamin C intake. A follow up study from the same group, comparing re-test cognitive ability with nutrition at the time of testing and also from the nutritional assessment conducted 6 years previously, showed a correlation between cognitive ability and both past and present nutritional status, including vitamin C intake and plasma levels [35]. In a Swiss cohort aged 65 to 94 years, significant correlation was found between plasma vitamin C levels and cognitive performance, particularly vocabulary and recognition [36]. Similar effects were found for beta-carotene, but not plasma vitamin E. In this study antioxidant measurements were available in the same participants from a study conducted 20 years previously. The researchers found remarkable consistency in plasma antioxidant status across this time frame, indicating that the measurements taken in the older generation were truly reflective of lifetime nutrition and supplementation patterns. In a cross-sectional study in a Maryland population, an association was found between plasma vitamin C level and score on a digit-symbol substitution task, but not the Mini-Mental State Exam (MMSE) [37]. Of note is that in this population only 3% of the population had ‘sub-optimal’ vitamin C levels (<40 μmol/L) and approximately 40% of the population were taking vitamin supplements that included vitamin C. In a large cohort of Chicago elderly who were tested at baseline and 3 years later, significant associations were found with vitamin E from diet and supplements and lower rate of cognitive decline [38]. A positive but weaker relationship was found with vitamin C. Higher vitamin C intake from diet and supplements was associated with lower chance of cognitive impairment on MMSE in an Australian cohort from Sydney [39] although this relationship was not true for all cognitive tasks. The Cache County in Utah study included dietary questionnaires and repeat testing over several follow up visits for more than 3000 participants spanning an average of 7.2 years and found a significant correlation between high vitamin C intake and cognition [32, 40]. Rates of cognitive decline measured by the modified MMSE were slower in those who had a high intake of vitamin C through food and supplements, alone and in combination with vitamin E. Taking greater than 500 mg vitamin C per day made no additional difference. In another study of a group of older women (> 65 years) with or at risk for cardiovascular disease, supplements of vitamin C (500 mg/day) were administered long-

term [41]. Following an average of 3.5 years of supplementation, telephone interviews were performed on a subset of the oldest participants in order to assess cognitive function. Multiple interviews were conducted with approximately two years between each interview. Vitamin C supplement users scored slightly higher than controls at the initial study and showed less cognitive decline over the study duration. However, this effect was driven by those who experienced a cardiovascular event during follow up. There were no significant effects of vitamin E (~600 IU every other day) or beta-carotene treatments.

Some studies have a stronger emphasis on the types of foods providing nutrition and not simply intake levels. In a Korean sample of independent living adults aged greater than 60 years, food diaries were examined in conjunction with cognitive testing [42]. A strong correlation was found between intake of fruits and vegetables and cognitive function. In fact, in the groups whose cognitive scores were classed as normal, fruit intake was 1.5 to 3 times greater than in the inadequate- or poor-rated groups that together comprised approximately 50% of the population. Vitamin C intake was lowest in the poor cognitive ability group. In a Spanish cohort of 260 healthy elderly with only moderate to no cognitive deterioration (7 or fewer errors on the MMSE) lower cognitive scores were associated with poorer diet including less intake of fruits and vegetables and both folic acid and vitamin C [43]. In two similar studies from the same group, subjects with fewer errors during cognitive testing had superior diets including higher intake of fruits, and also vitamin C, vitamin E and folic acid, and consumed fewer unhealthy foods such as chocolates and cakes [44, 45]. A more recent study by a French group found a positive association between intake of fruits and vegetables, particularly vitamin C rich foods, and verbal memory [46]. The same clear pattern was not seen with food intake and executive function. Food intake was assessed by multiple 24-hour food records at baseline (45 to 60 years of age). Cognitive ability was assessed 13 years later but not at the baseline stage. Of particular note in this study is that the observed associations were driven by poorer performance in those in the lowest quartile for fruit and vegetable intake supporting the idea that prevention of deficiency is potentially more important than supplementation above normal. Participants excluded for missing dietary information were more likely to be smokers and to have poorer cognitive scores. Inclusion of these participants could potentially have led to stronger associations if this group really did have poorer healthy eating behaviors. Results remained largely unchanged even taking into account vascular disease, however, other diseases were not specifically mentioned.

Fewer studies assess oxidative stress markers in similar normal populations (as opposed to disease populations). Vitamin C decreased F_2-isoprostanes, a highly sensitive measure of lipid peroxidation, in guinea pigs [47]. Polidori et al [48] studied cognition and antioxidant intake in participants aged 45 to 102 years with no previous cognitive impairment, non-smokers who took no vitamin or iron supplements or other medications. The population had either high (> 4 per day) or low (< 1 per day) intake of fruits and vegetables. The authors found significant correlation between higher MMSE scores and higher vitamin E levels but lower plasma F_2-isoprostane levels (a marker of lipid peroxidation), independent of body mass index and education level and across the range of ages measured. Unfortunately vitamin C was not measured in this sample among the range of antioxidants assayed. However, all micronutrients and vitamins that were measured were correlated with each other and thus it is possible that similar relationships would be seen with vitamin C level which is strongly related to fruit and vegetable intake. This is an area that deserves further investigation. A study that estimated ferric-reducing antioxidant power from semi-quantitative food intake diaries found inconsistent associations between cognitive ability and theoretical antioxidant capacity in the cohort of nurses > 70 years during repeated assessments [49]. If the correlation between oxidative stress and poorer cognition could be

supported more thoroughly, then that would also offer support for the taking of antioxidants that may have the potential to modify the progress of deterioration.

Reporting of plasma levels: The classification of groups according to vitamin status differs greatly among these studies. In many populations the 'high vitamin C' group is not particularly high and the 'low' groups may represent severely depleted or deficient vitamin plasma levels. Alternatively, a 'high vitamin C' supplemented group may have equivalent vitamin C plasma levels to a no- vitamin C or low- vitamin C supplement group if those individuals have a healthy intake of fruits and vegetables if categorization is based solely on supplement levels. In many cases group means may appear normal, but attention to the range of data suggests that some members are much lower (or higher) than others in the group, thus potentially masking any cognitive effects present in the study data depending on method of analysis (see Table 1). In the Gale et al. study [33], the highest vitamin C intake was calculated at > 45 mg which is actually lower than the RDA, or with a blood plasma level of just 28 μM or greater which would include samples only marginally above levels typically considered to be 'depleted'. Upper limits are not provided, but examination of the data suggests that around half of the population studied had depleted or deficient vitamin C plasma levels, which were clearly linked to dietary intake. Sixty-two percent of subjects in the La Rue et al. [35] experiments took vitamin C supplements. Thus, this group was already self-selected for better health habits, and therefore cognition, which may have masked some nutrition-cognition relationships. Although the mean plasma vitamin C level was healthy, around 70 μM at both beginning and end of the study, the lowest values were clearly in the deficient level, at 9 μM in 1980 and 15 μM in 1986. The maximum value recorded was 155 μM indicating that high plasma levels can be achieved through diet and supplements. Mean vitamin C intakes in a young Canadian population were estimated at approximately 130–150 mg/day in non-supplement users to 230–270 mg/day when supplement users were considered but with very high variability in all sub-groups. Mean vitamin C levels in serum for all groups were at the lower end of normal range (29–32 μM), but with variability in each group that suggests many in the population were at risk for vitamin C depletion or deficiency [50]. In a similar population studied by the same group, 14 % of the population were found to have deficient vitamin C levels (< 11 μM) and 33 % had depleted levels (11–28 μM). Overall, approximately 17% of this population did not meet the RDA for vitamin C intake, which is an important determination, and critically, suggests that a portion of the population consumed the RDA for vitamin C but still had depleted serum levels [8]. These contradictory results of apparently adequate intake in the majority of the population, but large groups exhibiting deficient or depleted vitamin C levels could suggest overestimation of daily intake, or loss of vitamin C in samples, or that some people require greater than the RDA to maintain optimal serum vitamin C levels. Underestimation of vitamin C levels could occur if samples were not treated properly. Frequent freeze-thaw cycles or exposure to any metals (such as iron in the hemolysis of red blood cells) could both lead to rapid degradation of vitamin C in the sample. However, this does not appear to be the case in these studies [8, 50], given that samples were analyzed using appropriate methodologies in specialized facilities.

As is often the case, in the Ortega et al. study [43] group means for calculated vitamin C intake appear to be adequate (96.5 to 138.3 mg/day). However, with calculated group standard deviations ranging from 55 to 90 mg/day the implication is that there is a very broad range of intake values in the population, some of which likely fall into the deficient/depleted categories. In a similar, later study by the same group, vitamin E intake and plasma levels were significantly correlated with cognitive ability [51]. Of concern is that in this cohort of 120 non-institutionalized elderly in Madrid, 95% had a vitamin E intake level that was below the RDA for the vitamin and none was taking vitamin supplements. Even in the poorest performing group in a related study [44], the mean vitamin C intake was 103 mg/

day, but as with other studies, the range of intakes in each of the groups was great (between 46 and 79 mg/day) indicating a huge difference in population intakes. Mean vitamin E intake was calculated to be less than 50% RDA for low and high cognitive scoring groups. In an Austrian study that reported no relationship between cognition and plasma vitamin C, mean vitamin C levels were 56 μmol/L with a standard deviation of 20. This suggests that some of the population were well-supplemented with vitamin C, but some may have been depleted and no distinction was made between these groups [52]. In the Peneau et al study [46] analyses were performed according to quartiles of the population. Vitamin C was measured in plasma in a subset of 450–500 participants per quartile. Group means corresponded well with estimated average intakes of 67–135 mg per day and were all at a healthy level of 50–60 μmol/L but the greater standard deviations in plasma vitamin C and estimated vitamin C intake for the lowest two quartiles for fruit and vegetable intake (around 23 μmol/L) indicate that some members of these groups would be at depleted or deficient levels for vitamin C.

2.1.2 Alzheimer's patients—Vitamins C and E, along with other antioxidants (vitamin A, uric acid and antioxidant enzymes superoxide dismutase and glutathione peroxidase) were all lower in those with mild cognitive impairment or DAT than in controls [53]. Examination of a cohort of Japanese-American men from the Honolulu-Asia aging study revealed a significant protective effect of combined vitamin C and E supplements on cognitive functions (including attention, memory and language assessments) and particularly in cases of vascular dementia [54]. There was no significant effect of supplement use in the DAT-only group, although some DAT patients would have been included in the mixed dementia group. The study considered those taking vitamin C or vitamin E as individual supplements either alone or in combination and this group comprised 37.9% of the population studied. However, multivitamin users were disregarded because the levels of vitamin C and vitamin E in multivitamins are typically lower than in single supplements. Effects of overall diet were not assessed, suggesting that a number of healthy vitamin C level individuals may have been included in the non-users group in analyses. Indeed, multivitamin use is often associated with other healthy dietary behaviors and even low dose supplements plus a good diet can provide sufficient antioxidant protection. In a subset of participants, supplement data were available from two different time points prior to cognitive testing which permitted the consideration of long-term versus short-term supplement use. Indeed, long-term supplement use was found to be of the greatest benefit. With only one recording of cognitive data, disease progression could not be monitored. In fact many demented individuals had to be removed from analyses because they may have been impaired at the time that initial questionnaires about supplement use were recorded. In a Canadian sample, older adults who were assessed as non-demented at baseline were examined for cognitive deterioration and possible DAT and other dementias at a 5-year follow up [55]. Supplement use was associated with less cognitive deterioration at follow-up, but not fewer cases of DAT *per se*. Again, neither dosage and duration of use data, nor dietary information were available, but vitamin C and E supplement users were typically older and not heavy smokers, pointing to a generally healthier lifestyle in this group. In the Cache County study, taking vitamin C and E in combination with non-steroidal anti-inflammatory drugs was found to prevent cognitive deterioration over an 8 year follow up period [56]. However, this effect was restricted to carriers of the ApoE ε4 allele. Participants that took multivitamins were only considered vitamin C or vitamin E users if the supplement contained >400IU of vitamin E or >500mg of vitamin C. In the case of vitamin C, this is more than enough to saturate blood vitamin C levels and so some patients classified as non-users could also have been vitamin C replete. Vitamin E alone (2000 IU per day) or in combination with selegiline (a monoamine oxidase inhibitor) improved outcome (time to death, institutionalization or loss of function) over a 2-year follow-up period in patients with

DAT [57]. However, this relationship was dependent on cognitive performance at the beginning of the study, indicating a relationship between cognitive decline and general health in this already compromised (low MMSE score) population. This study highlights the difficulty of taking repeated measurements in a demented population as advanced dementia precludes testing.

Plasma levels of vitamin E and C were both lower in patients with mild cognitive impairment and DAT compared to controls [58]. This difference existed in patients who were not taking any antioxidant supplements, but in whom a nutritional assessment ruled out the possibility of general under-nutrition. Other antioxidants, including uric acid, vitamin A and enzymes superoxide dismutase and glutathione peroxidase were also lower in mild cognitive impaired and DAT subjects. Also, in this group approximately one third of patients were ApoE ε4 carriers, whereas none of the control group was. Whether oxidative stress associated with the disease was responsible for the reduction of antioxidants, or whether the low antioxidants contributed to the progression of the disease cannot be deduced from this study. Nevertheless, the association between cognitive impairment and low antioxidant status is clear. In another study, plasma lipid-soluble vitamins E and A (retinol) were lower, and malondialdehyde higher in DAT patients with poor MMSE scores, compared to age matched control subjects with normal MMSE scores [59]. All subjects were non-smokers and had equivalent nutritional intake and stable weights. These data suggest that greater oxidative stress and free radical production in the DAT patients was responsible for the lowered plasma antioxidants given that intake did not vary between the groups.

Rather than plasma vitamin C levels alone, it has been suggested that a higher CSF:plasma ratio for vitamin C in DAT patients versus age-matched controls may be reflective of increased antioxidant requirements in the brain depleting peripheral vitamin C levels [60]. In a short 1 year follow-up study, Bowman et al. found that although neither plasma nor cerebrospinal fluid (CSF) VC levels *per se* predicted cognitive decline in mild to moderate AD cases, differences at baseline in the ratio between CSF and plasma vitamin C were predictive [61]. This relationship suggests either that the ability to maintain high vitamin C in the CSF from where it is available for transport into brain protects against cognitive decline, or, as the authors suggest, healthier brains that are better able to maintain the high vitamin C levels in CSF through intact blood brain barrier function are also less affected by the progression of DAT pathology. Although blood brain barrier integrity may affect retention of vitamin C in the brain if seriously impaired, vitamin C is transported into brain from CSF via SVCT2 transporters in the choroid plexus and not via the blood brain barrier. Combined with the fact that vitamin C levels were only measured at baseline and so the rate of cognitive decline cannot be compared with change in CSF:plasma ratio across the same time period, and the lack of a control group it is difficult to fully interpret these potentially very interesting findings. Data from non-demented individuals who should, in theory, have higher plasma and CSF vitamin C could lead to additional conclusions regarding cognitive function and vitamin C levels. Vitamin C intake was not recorded in the subjects, although 34% took some form of vitamin C supplement. The group mean for plasma vitamin C was only 41 μM, which is at the lower edge of normal range. With a standard deviation of 30 μM, and given that one third of the group were taking vitamin C supplements, the implication is that some of the other subjects had very low vitamin C intakes.

2.2 Non-supporting evidence for role of vitamin C

2.2.1 Elderly – non-demented subjects—In a longitudinal study concerning eye disease, participants aged 61–87 years were given multiple supplements. One group received an antioxidant that included vitamin C (500 mg/day), vitamin E (400 IU/day), and beta

carotene (15 mg/day), a second group received zinc and copper supplements together, a third group received all of the supplements together in combination and the final control group received placebo [62]. Following an average treatment time of 7 years, cognitive tests were administered and no differences were found among the groups. However, this study did not include a baseline measurement before treatments were initiated and so decline in cognitive function cannot be assessed. Furthermore, the participants that agreed to take part in the cognitive testing were on average younger, better educated, and with better vision and overall health than those that declined to take part. This combination of factors is already known to offer an advantage in combating cognitive decline, making the detection of all but the most robust differences less likely. In a group of independent-living elderly in New Mexico, vitamin C serum levels were lower in Hispanics than in non-Hispanic whites, and lower in men than in women [63]. Although the lower vitamin C levels were not significantly associated with cognitive function, there was a trend in that direction and low vitamin C was associated with a history of depression. The cut-off point for low vitamin C was 57 μM, which is well within the healthy range. A very low vitamin C subgroup was not considered separately.

A novel experiment in a Scottish cohort study of 176 volunteers that compared cognition at age 77 with dietary intake and supplement use had the added advantage of IQ scores measured at age 11 [64]. Men who had a higher IQ at age 77 were more likely to be current dietary supplement users, but supplement use did not predict current cognitive ability. However, the authors considered all dietary supplements together, which included vitamin C and vitamin E, but the most common supplement was fish oil. Only 27% of the population took any form of supplement. Dietary intake was also calculated. Although average vitamin C intake (84 mg/day) was calculated at close to the RDA in the non-supplement users and slightly higher in users, mean plasma vitamin C levels were low in each group. In supplement users and female non-users plasma vitamin C was 40–50 μmol/L with a standard deviation of 20–30 μmol/L, indicating that a portion of the population had depleted levels. The mean in male non-users was a surprisingly low 26.5 μmol/L with a standard deviation of 23 suggesting dangerously low levels in some users. The lack of clarification of supplement type may have precluded the authors from finding meaningful differences, and the low numbers of subjects that participated in all parts of the study (any participation, cognitive testing, dietary questionnaire, blood sample) may also have masked potential findings. A double-blind, placebo-controlled study of 6 months antioxidant supplementation that included 150 mg vitamin C per day and 36 mg per day vitamin E showed no benefits to cognition in a sample of 220 German women aged 60–91 years [65]. Although serum vitamin C levels are reported to have increased as expected following supplementation, neither baseline nor final levels are reported. Overall the women were described as healthy and well nourished and so the percentage of subjects who might have received maximal benefit from vitamin supplementation is likely to be low.

Data from the Rotterdam study found a beneficial effect of Beta-carotene intake but no such association for vitamins C and E [66]. Intake was estimated from food diaries but the lowest scoring individuals for cognitive testing and institutionalized individuals were not given dietary questionnaires because of concerns about the accuracy of their responses. This would have eliminated cognitively impaired individuals from the analyses, except for 11 mildly demented individuals. Furthermore, the lowest 15 % of the population for vitamin C intake was estimated at <70 mg/day which is borderline for appropriate intake and no further differentiation is given for those that may have been seriously affected by hypovitaminosis. This limits the power of the conclusions regarding vitamin C. In a large Austrian community sample, plasma levels of vitamin E and beta-carotene were found to correlate significantly with cognitive ability, with the lowest quartile for cognitive ability having the lowest plasma vitamin E and beta-carotene levels [52]. The same relationship was not true for vitamin C.

Performance on memory tasks in a North American sample was significantly correlated with serum levels of vitamin E. Serum vitamin C, vitamin A, folate and carotenoids were also measured but the same relationships were not preserved [67]. Despite these negative findings, it is important to remember that where antioxidant function is involved, vitamin C is required for the neutralization of ROS and also for recycling of the α-tocopherol radical. Thus the beneficial effects of vitamin E are often at least partially dependent on vitamin C level even, if vitamin C does not appear to have a direct effect.

2.2.2 Alzheimer's disease patients—In a population of over 2000 North Carolina elderly aged over 75 years, 11% were supplement users (including vitamin C and E) but only 3% of the population were defined as long-term users. Current use of antioxidants conferred a protective advantage against cognitive decline over a 7-year follow up period [68]. Dose and detailed duration of use information were not available and in fact the doses were simply classed as high if from a single supplement and low if from a multivitamin. Dietary intake was not considered. In a sub-group of the same population, 616 elderly participants were followed over 14 years. Vitamin E or C supplement use was not found to have a protective effect against developing DAT [69]. No record was made of dietary intake, or indeed of the levels contained in the supplements used or frequency of use. Given that in this population less than 9% took any form of supplement of vitamins E or C, suggesting low adoption of healthy nutrition behaviors, it is difficult to draw firm conclusions from these data. Subjects that developed dementia were typically older, less educated, and less likely to be married than non-demented subjects. In a study of 25 patients with probable DAT and 41 controls (age-matched close friends or relatives of the DAT population), Vitamin E levels were significantly lower in the DAT group than controls. Vitamin C levels did not differ significantly between groups [70]. As is typical for such studies, participants were excluded if they took any medications that may interfere with plasma antioxidant levels, or had any other major disease, but as comorbidity of diseases is so prevalent in an older population the elimination of these confounders is likely to obscure results by excluding the populations most at risk for vitamin C deficiency. In fact, in this study it was determined that no participant excluded fresh fruits and vegetables from their diet, and a control subject with mild cognitive impairment (MMSE <27) was specifically excluded from the group. In a large middle-aged population (>12,000) aged 48–67 years from several North American states, participants were administered three different tests of cognitive ability and completed food questionnaires including questions about antioxidant vitamin supplement use [71]. The researchers found no significant correlations between antioxidant intake (vitamins E, C, and A, or multivitamin supplements) and cognition. There was a very low incidence of poor cognition in this group that may have prevented the detection of any beneficial effects of antioxidant treatment. The lowest vitamin C intake group was reported as <65 mg/day, which is around the RDA, but the range that included the lowest intakes is not given. It would be very interesting if this same group could be followed up throughout the next 40 years to assess the benefits of a lifetime of good or poor nutrition. Data from the Rotterdam study found an association for vitamin E and lower levels of Alzheimer's disease at follow up an average of 9.6 years later but not for vitamin C intake [72]. Supplement use included single supplements and multivitamins but main intake measures were estimated from food diaries. Median intake in the lowest tertile for vitamin C intake was 80 mg/day, similar to the Jama et al. study described earlier in this population. The exclusion of intake information from the most cognitively impaired individuals and the lack of information regarding the portion of the population that may have been at risk for vitamin C deficiency limits the strength of the conclusions regarding the relationship between vitamin C and development of dementia in this potentially most affected portion of the population.

3. Discussion & Conclusions

Confounding factors are endemic in human population studies. Dementia is often worse in the less educated and in lower socio-economic classes and it is thought that this is due to the building of a “cognitive reserve” in a more intellectually active group. Without completely repudiating this theory, it should also be noted that those from low socioeconomic and low education backgrounds are far more likely to have poor dietary intake of fruits and vegetables, are less likely to take nutritional supplements, are more likely to have a high fat diet (which can decrease vitamin C [73]), are more likely to smoke, and have likely been at this disadvantage throughout life. Unhealthy behaviors such as smoking, poor diet, low amounts of exercise and also complete alcohol abstinence during midlife not only tend to cluster in individuals, but have a cumulative effect such that poorer cognition is found in those that engage in more of these behaviors [74]. Dietary questionnaires also often lack detail as to lifetime dietary habits. Documentation of intake is onerous and it is inadvisable to overburden participants. In a young (20–29 years) sample approximately 7% (93/1277) of participants had to be excluded from a dietary study due to over- or under-reporting daily calorie intake (<800 or >3500 calories/day)[50]. This is representative of the recall errors that can affect data collected in this manner. Even when food types and amounts are recalled correctly, differences in storage and cooking can decrease the vitamin C level in the food. Recall and accuracy are even more likely to be compromised in the elderly as memory fails and furthermore, cognitively impaired individuals may find it more difficult to obtain, prepare and consume adequately nutritious food. Thus, lower than expected vitamin C levels in blood could be due to inaccurate food intake reporting, to a poorer diet, or to increased metabolism of vitamin C due to the oxidative demands of the disease. It is difficult to disambiguate these possibilities in cross-sectional studies. Diet and supplement use can also change over the course of experiments, which necessarily comprise several years between baseline and follow-up but may not assess nutrient intake at all time points. Some studies are able to make use of precise weighing methods, but this is a lot of work and is typically only possible in institutionalized subjects where food intake can be controlled [45]. This approach also makes the assumption that antioxidant levels in plasma directly reflect levels in the brain. Further, high levels of vitamin C gained from dietary sources will often be accompanied by higher levels of a number of other beneficial compounds (vitamins, phytochmicals) also found from the same sources. Thus it is possible that vitamin C is a surrogate marker for another more active compound. Given the specific roles of vitamin C described above this possibility is unlikely to provide the entire explanation.

Participants in these studies, particularly ones that involve repeated testing, are more likely to be well-educated, younger, have better cognitive abilities, have higher vitamin C and E intakes, have higher income, and have fewer depressive symptoms than those who declined participation or who were lost to follow-up testing [35, 37, 38]. In fact, those with preexisting illness or severe cognitive dysfunction are typically not included in such studies [39, 45], which can lead to ceiling effects with narrow ranges of results in cognitive testing [48]. Some disease populations, such as diabetics, may represent a low vitamin C group in which support of a positive link between supplementation and cognitive protection would be even more beneficial. Diabetes is a known risk factor for DAT, and diabetics also have lower vitamin C levels, presumably due to increased oxidative stress in addition to impaired glucose and insulin signaling [75–78]. Far from being a confounding factor, subjects with such a disease comprise a critical sub-group to study. Many of these features are also correlated with ethnic group and socio-economic status [67]. Thus, the design of a typical population study self-selects for healthier, more cognitively-able population. This effectively minimizes several confounding factors, but narrows the chance of detecting cognitive effects. As can be seen from Table 1, many studies include high numbers of vitamin supplement users and yet vitamin C deficiency persists even in these populations. Thus, it

can be concluded that in less healthy populations the proportion of those at risk for vitamin C deficiency will be even greater, particularly in aging populations with problems maintaining independent living skills such as obtaining and preparing food. In studies in which plasma vitamin C has been measured, there is the assumption that it reflects a lifetime at this approximate level and while some studies do suggest that this relationship is true [36], current habits are not a guarantee of past situation, particularly if healthy behaviors are instigated as the result of a disease diagnosis.

Initial dietary recommendations of 60 mg per day were determined based on the idea that the dose at which excretion in urine occurs signifies saturation in tissues, however, this is not the case [30]. Very little vitamin C is excreted following a single dose of 15–50 mg in female subjects at steady-state following controlled intake [3]. In the same study doses of between 200 and 400 mg per day were required to saturate neutrophils, monocytes, platelets, lymphocytes, and plasma [3]. Future studies could include urinary analysis to confirm intakes that are less than optimal (<50 mg per day). The findings of similar saturation point, although not absolute level, in plasma and other cells within the blood indicate that plasma is an appropriate sample type for vitamin C as long as samples are stored and assayed correctly to avoid loss due to oxidation. However, organs retain vitamin C differentially [79, 80] and so plasma levels may not accurately reflect status of all organs.

The aim of this review is not disparage the evidence collected up to this point, nor to dismiss negative findings in these studies, nevertheless, in many cases there is a vital impediment to full interpretation of the data and it is critical to consider that these confounding factors may have masked potential beneficial effects of antioxidant treatments in specific at-risk groups. This is even more important in the case of vitamin C where metabolism, including saturable transport into blood from intestines via the SVCT1, and into tissues via the SVCT2, is often not considered in interpretations [31]. Individual differences that exist within the population that govern the effectiveness of vitamin C transporters should also be considered. These differences could render food diary information even less accurate as perceived intake may not be equivalent to absorption. Plasma vitamin C differs according to polymorphisms of SVCT2 and SVCT1 despite equivalent vitamin C intake indicating that SVCT1&2 genotype may determine the strength of the association between vitamin C intake and circulating vitamin C levels [50]. Improved performance of SVCT1 may affect either absorption in gut or recovery via reabsorption in the kidney. Experiments designed to study the effects of severe vitamin C deficiency in participants in controlled environments, such as prison studies [1, 81, 82] and those of conscientious objectors in World War 2 [83], carry a number of important ethical considerations that make repetition undesirable and unlikely. Nevertheless, careful control of study data, inclusion of a wider range of participants, healthy and unhealthy, will help further clarify this issue. Future studies should take care to measure nutrient intake at more than one time-point, including detailed information on any supplements taken, and to support these data with analyses of blood samples. Participants who may already be affected with DAT, other dementias, or potentially confounding diseases should not be excluded from the study but should be included with care as to statistical techniques. At least the data for intakes and nutrient levels in blood and cognitive ability should be reported even if not included in the main analyses. The ability to control these factors renders investigation of the cognitive effects of vitamin C and E in animal studies critical to our understanding of the neurochemistry. Mouse models now exist that have deficiencies in vitamin C synthesis [84] and transport of both SVCT1 and SVCT2 [85, 86], which make these studies feasible. In support of these ideas, vitamin C supplementation did not have any large effects on psychological performance, personality dimensions or current mental state, in young men (17–29 years) *except in cases where supplements corrected an existing deficiency* (our italics) [87]. Long-term vitamin C deprivation, evidenced by low plasma levels in men with normal diets, was linked to nervousness,

depression and emotional lability. In the literature there is overwhelming evidence that a lifetime of good nutrition, thus avoiding sub-clinical deficiency in vitamin C and other antioxidants, is necessary to restrict the accumulation of damage, particularly in cases of disease. That similar relationships between antioxidant nutrition and cognition have been found in populations throughout the world in vastly differing populations suggests that this is an area that requires closer consideration.

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Abbreviations

DAT	Dementias of the Alzheimer type
ROS	Reactive oxygen species
MMSE	Mini Mental State Exam
RDA	Recommended dietary allowance

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Table 1

Detailed report of plasma VC values for studies described in this review, where such data are available.

Authors	Population	Plasma VC μM (μmol/l) Mean ± S.D. Group N	Percent of total study population with depleted or deficient VC (<28 μM) or for alternative cut- offs (as provided)	Percent of population taking a supplement containing VC
Cahill et al. [8]	20–29 years, healthy young men and women Toronto Nutrigenomics and Health Study	Deficient (< 11) 6.2 ± 10.4[a] N=133	46.8 %	30 %
		Suboptimal (11–28) 21.0 ± 10.9[a] N=325		34 %
		Adequate (> 28) 42.9 ± 9.1[a] N=521 [a] S.D. calculated from S.E.M. and N provided in [8] Table 1		40 %
Gale et al. [33]	65 years and over, non- institutionalized, UK	<11.91, N = 275	67.8 %	
		11.91–27.82, N = 302		
		>27.82, N = 274		
Gan et al. [9]	14–89 years, Hospitalized and normally-nourished controls, Canada	Reference group 52.7 ± 22.5	16 %	36 % (normal serum vitamin C group) 21 % (depleted vitamin C group) 7 % (deficient vitamin C group)
		Hospitalized group 27.6 ± 19.2	60 %	
Garry et al. [4] (VC data from population described by Goodwin et al. [34])	60 years and over non- institutionalized, no other illnesses, USA	Male - No supplements 51.7 ± 21.0, N = 55	4 %	
		Female - No supplements 64.7 ± 20.4, N= =58		
		Male - supplements 74.4 ± 23.9, N = 70		56 %
		Female - supplements 80.1 ± 17.6, N = 85		59 %
Hampl et al. [12]	12–74 years Community sample, NHANES III, USA	Male – 65–74 years (example age group) 44.9 ± 33.99[b] N = 955 Total male population N = 7355	34 %	29 %
		Female – 65–74 years (example age group) 55.1 ± 34.21[b] N = 967 Total female population N = 8414 [b] S.D. calculated from S.E.M. and N provided in [12] Table 1	27 %	34 %
Johnston & Thompson [7]	6–92 years Generally healthy outpatient population, (subjects presenting for	Overall mean 33.5 ± 14.8	33.9%	

Authors	Population	Plasma VC μM (μmol/l) Mean ± S.D. Group N	Percent of total study population with depleted or deficient VC (<28 μM) or for alternative cut- offs (as provided)	Percent of population taking a supplement containing VC
	general check-up or gynecological exam only), USA	N = 124		
		Deficient (<11.4) 7.3 ± 1.9 N = 8 (out of 124)		
		Depleted (11.4 to < 28.4) 20.5 ± 4.7 N = 34 (out of 124)		
Johnston et al. [6]	College students, USA	October sample 44.7 ± 15.9 N = 134	17 %	
		February sample 41.5 ± 13.1 N = 98	14 %	
La Rue et al. [35] (Follow-up study in same population as Garry et al. and Goodwin et al. [4, 34])	60 years and over, community dwelling, healthy, USA New Mexico Aging Process Study	1980: 69.3 ± 19.7, (range 9.1–134.6) N = 122		59 %
		1986: 70.9 ± 20.3, (range 14.8–154.4) N = 122		63 %
Lindemann et al. [63]	65 years and older, community dwelling, USA	Male Hispanic No supplement 49 ± 19, N = 121 Supplement 70 ± 25, N = 74		38 %
		Male Non-Hispanic White No supplement 57 ± 23, N = 93 Supplement 80 ± 28, N = 133		59 %
		Female Hispanic No supplement 60 ± 24, N = 89 Supplement 80 ± 31, N = 81		48 %
		Female Non-Hispanic White No supplement 70 ± 24, N = 72 Supplement 89 ± 26, N = 130		64 %
Mosdøl et al. [11]	>19 years, Community sample of 15% most deprived households, Low Income Diet and Nutrition study (LIDNS), UK	Male N=433	46.3 %	20.7 %
		Female N=876	34.6 %	
Perrig et al. [36]	65 – 94 years, Switzerland	59.4 ± 21.4, N = 442		
Richardson et al. [88]	In-patient admissions to Care of Elderly ward 65–97 years, Australia	N = 37	30 % < 11 μM 73 % < 40 μM	
Rinaldi et al. [58]	Patients with Mild Cognitive Impairment (MCI) Patients with Alzheimer's disease Control, Italy	52.4 ± 16.5, N = 25		0%
		24.9 ± 2.4, N = 63		
		25.9 ± 8.9, N = 53		
Schmidt et al. [52]	50 – 75 years, community dwelling, Austrian Stroke Prevention Study	56.6 ± 20.3, N = 1769		
Sinclair et al [70]	Mean 73–75 years, community dwelling, UK	Alzheimer patients 47.16 (IQ range 35.2– 59.1),		0 %

Authors	**Population**	**Plasma VC μM (μmol/l) Mean ± S.D. Group N**	**Percent of total study population with depleted or deficient VC (<28 μM) or for alternative cut- offs (as provided)**	**Percent of population taking a supplement containing VC**
		N=25		
		Controls 56.82 (IQ Range 47.6– 65.9), N=41		
Sato et al. [37]	65 years and older, community dwelling, USA	Normal MMSE 74.9 (IQ range 57.9– 90.8), N = 111	3 % < 40 μM (total population) 25 % < 59 μM MMSE<27	40 %
	Cardiovascular Health Study (CHS)	MMSE <27 78.9 (IQ range 64.2– 99.4), N = 433		
Whalley et al. [64]	~77 years, healthy, non- demented, UK	Male Supplement 48.7 ± 23.7, N = 9		8.5 %
		Male No Supplement 26.5 ± 23.1, N = 38		
		Female Supplement 47.6 ± 27.6, N = 22		
		Female No supplement 40.9 ± 29.2, N = 41		
Wrieden et al. [10]	25–74 years, Community sample, MONICA study, UK	N = 1276	44 % < 22.7 μM	0 % *285 of original sample of 1958 (14.5 %) excluded for vitamin supplement use*

Values reported as mg/dL in the literature have been converted to μM (μmol/l) using the conversion factor for ascorbic acid of 56.78. Values reported as μg/ml have been converted to μM (μmol/l) using the conversion factor 0.176 based on the molecular weight of ascorbic acid. Data shown are group means ± S.D., and where indicated, data range, or inter-quartile (IQ) range. MMSE, Mini Mental State Exam.