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Gender Differences in Plasma Vitamin C Concentrations and Cognitive Function: A Pilot Cross-Sectional Study in Healthy Adults

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ABSTRACT

Background: A number of investigations have highlighted the importance of vitamin C in maintaining brain health. Biologically, vitamin C has exhibited roles in neuromodulation, neurodevelopment, vascular support, and neuroprotection. Vitamin C's contribution to cognitive function in both cognitively intact and impaired cohorts has previously been assessed, with little focus on gender variability.

Objective: The present study explored the interaction between gender and plasma vitamin C on cognitive performance, and the effect of different amounts of plasma vitamin C (adequate/inadequate) on various cognitive tasks by gender.

Methods: This retrospective analysis was conducted in healthy adults ($n = 80$, female = 52, male = 28, 24–96 y) with a range of blood plasma vitamin C concentrations. Cognitive assessments included the Swinburne University Computerized Cognitive Assessment Battery (SUCCAB) and 2 pen-and-paper tests, the Symbol Digits Modalities Test (SDMT) and the Hopkins Verbal Learning Test–Revised (HVLT-R). Food-frequency questionnaires were used to elucidate dietary consumption.

Results: After adjusting for a number of potential covariates such as age, number of prescribed medications and dose of vitamin C supplementation, results indicated a significant interaction ($P < 0.001$) between plasma vitamin C and gender on cognitive function, on both the computerized and pen-and-paper assessments. A novel finding was that the performance of males with inadequate plasma vitamin C was poorer on tasks involving components of memory (short/delayed), inhibition, and visual perception, whereas females presenting with inadequate vitamin C were more compromised on tasks involving psychomotor performance/motor speed. Additionally, females with adequate vitamin C concentrations exhibited higher performance than males on tasks involving recall, recognition, attention, and focus.

Conclusions: Further larger-scale investigations are required to establish a cause-and-effect relation and to elucidate whether differences in cognitive function between genders may be attributed to plasma vitamin C status. This trial was registered at https://www.anzctr.org.au/Trial/Registration/TrialReview.aspx?id=369440&isReview=true as ACTRN12615001140549. *Curr Dev Nutr* 2020;4:nzaa038.

Keywords: vitamin C, ascorbic acid, cognition, central nervous system, gender

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Abbreviations used: CMEM, contextual memory; CNS, central nervous system; CRT, choice reaction time; CSTR, congruent Stroop; DQES v2, Dietary Questionnaire for Epidemiological Studies, version 2; DREC, delayed recognition memory; FFQ, food-frequency questionnaire; HVLT-R, Hopkins Verbal Learning Test–Revised; IREC, immediate recognition memory; ISTR, incongruent Stroop; MANCOVA, multivariate ANCOVA; MMSE, Mini-Mental State Examination; ROS, reactive oxygen species; SDMT, Symbol Digits Modalities Test; SRT, simple reaction time; SUCCAB, Swinburne University Computerized Cognitive Assessment Battery; SVCT2, sodium-dependent vitamin C transporter 2; SWM, spatial working memory.

Introduction

There has been increasing research aimed at uncovering the roles of vitamin C on central nervous system (CNS) function and its importance in maintaining brain health (1). The reduced form of vitamin C, ascorbic acid, is a potent water-soluble antioxidant, not capable of being synthesized by any human tissue, including the brain. Studies have demonstrated a key neuromodulatory role of vitamin C within the brain (2). This function is exerted through vitamin C's involvement as a cofactor for the synthesis of catecholamines such as norepinephrine (3) and serotonin (3), alongside synaptic acetylcholine release (4) and re-uptake of glutamate (5).

Additional roles of vitamin C on the CNS include neurodevelopment (6), stimulation of brain-derived neurotrophic factor (7), cardiovascular support through collagen production, angiogenesis, reduced NO metabolism, and the biosynthesis of carnitine (8). Vitamin C's

neuroprotective roles include prevention of excitotoxicity (9), regeneration of other antioxidants (10), reduction in lipid peroxidation (11), and reduction in proinflammatory cytokines (12) and scavenger of brain reactive oxygen species (ROS) (13).

Human autopsy studies have indicated that the most saturated vitamin C brain regions include the cerebral cortex, hippocampus, and amygdala (14, 15). Ascorbic acid is maintained at concentrations 100 times higher in neurons than those present in the circulating plasma (15) and 2.5–4 times higher concentrations in cerebrospinal fluid than in plasma (16). A number of factors, including aging, have demonstrated an association with depletions of vitamin C in brain tissue, especially within the cerebral cortex, pituitary gland, and hippocampus (17, 18).

A meta-analysis consisting of multiple prospective cohort studies showed that the relative risk for dementia was significantly decreased with higher intakes of vitamin C (19), while another meta-analysis showed significantly lower values of blood vitamin C in Alzheimer's patients than those in healthy controls (20, 21).

Based on 50 studies, our recent systematic review revealed higher mean plasma vitamin C concentrations in the cognitively intact groups of participants compared with cognitively impaired groups (1). In our recent cross-sectional study, cognitively intact participants presenting with adequate plasma vitamin C concentrations, as opposed to those with inadequate concentrations, demonstrated significantly better performance on sensitive cognitive assessments involving immediate and delayed recall, attention, focus, and recognition (22). In line with these results, a recent randomized controlled trial using middle-aged adults (23) revealed significant improvements on tasks relating to verbal and visual memory, visual motor performance, and processing speed following 200 mg vitamin C supplementation for 8 wk.

Epidemiological studies have frequently shown higher vitamin C serum/plasma concentrations in women than in men (24–26) and significantly higher rates of deficiency in males (27). Furthermore, at equal vitamin C intake amounts, women achieve higher serum/plasma concentrations than men (26, 28).

Overall, no conclusive gender-related mechanistic differences in the pharmacokinetics of vitamin C have been observed in humans (29–31), despite women reaching the plasma ascorbate concentration plateau (70 μmol/L) at lower vitamin C intake amounts than men (32). Recent research has suggested that body weight is considered to be an essential factor for these gender-related differences (33), while another study (34) indicated that a higher absolute fat-free mass (muscle mass) in males, and thus a higher distribution volume of vitamin C, may contribute. Various lifestyle differences between gender groups may also play a role, such as higher levels of physical activity, alcohol consumption, and smoking among males and higher intakes of vitamin C–containing foods and supplements among females (34, 35). These differences point to increased ROS and possible vitamin utilization in males. As a consequence, studies have recommended higher daily intakes of vitamin C for males (33).

Few studies have addressed gender differences in brain tissue vitamin C concentrations. One rat model demonstrated that male rats had greater cerebral vitamin C loss in response to stress and ROS generated during ischemia than females (36). A number of additional factors may influence gender variations in brain vitamin C distribution, including variations in hydrogen peroxide, ROS (36), excitotoxicity (37), proinflammatory mediators such as TNF-α, IL-1β, IL-6, and inducible NO synthase inducible nitric oxide synthase (iNOS), and hormonal factors such as mineralocorticoids (38), 17β-estradiol (E2), and progesterone (39). A handful of studies have assessed potential differential associations between vitamin C and cognition by gender. A study conducted in a large cohort of elderly Korean participants reported a significant relation between vitamin C consumption and Mini-Mental State Examination (MMSE) scores in men but not in women (40). Another study revealed that men who reported low MMSE scores consumed less vitamin C than females with low MMSE scores; however, little variability in vitamin C consumption was observed in those who demonstrated high MMSE scores (41).

One study (42) has assessed the interaction between gender and plasma vitamin C concentrations on cognitive function using a cognitively intact sample. The study revealed that higher plasma concentrations were associated nonsignificantly with higher MMSE scores for men but not for women. Older age was associated with lower MMSE scores in men but not in women. However, the range of plasma vitamin C concentrations and MMSE scores was similar between the gender groups. Symbol Digits Modalities Test (SDMT) scores increased with increasing plasma concentrations for both gender groups. The authors concluded that more sensitive cognitive assessments were required to determine true associations in cognitively intact cohorts. Previous investigations which assessed vitamin C intake or plasma concentrations controlled for gender as a potential confounding factor rather than considering gender as a potential vitamin C effect modifier (43, 44).

Although efforts have been made to establish the links between plasma vitamin C concentrations and cognitive function in healthy participants and to distinguish gender differences in the vitamin's plasma and structural concentrations (using animal models), a paucity of research has explored the interaction between gender and plasma vitamin C concentrations on cognitive function. A major limitation of the research to date has been the use of a cognitive measure not suitable for distinguishing subtle differences in cognitively intact samples and the failure to consider gender as a potential vitamin C effect modifier when assessing cognition.

To our knowledge, this is the first pilot study to advance the findings from previous research by examining how plasma vitamin C concentrations interact with cognitive functions between cognitively intact males and females with the use of suitable cognitive instruments. Using a sensitive battery of computerized and pen-and-paper assessments, the present pilot study explored the interaction between gender, plasma vitamin C, and cognitive performance, assessing how gender groups with different concentrations of plasma vitamin C (adequate/inadequate) perform on various cognitive tasks.

Methods

This article is a retrospective analysis of a recently published study (22), in which we investigated the association between plasma vitamin C concentrations and cognitive function. The methodology of cognitive and vitamin C assessments, briefly outlined below, is described in more detail in the related publication (22). Here we focus only on statistical anal-

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yses relevant to the present study investigating the interaction between cognition and plasma vitamin C by gender.

This study was approved by the Human Research Ethics Committees at the National Institute of Integrative Medicine and Swinburne University of Technology. The trial was registered with the Australian and New Zealand Clinical Trial Registry (ACTRN12615001140549). Participants provided informed written consent.

Cognitively intact adults (≥18 y) were sought to participate in this cross-sectional study. Asymptomatic participants were primarily recruited from the National Institute of Integrative Medicine. We included participants with no major neurodegenerative condition (i.e., dementia; 3MS score >79), and included those likely to be displaying a range of plasma vitamin C concentrations (i.e., varying diets, supplementation, age groups). Participants were excluded if they were pregnant or lactating, color blind, or taking antidepressants, antipsychotics, anxiolytics, illicit drugs, or any cognition-enhancing drugs. Participants were also excluded if they were not able to give informed consent.

Cognitive assessments

A number of cognitive assessments were performed. These included the Hopkins Verbal Learning Test–Revised (HVLT-R) (45), SDMT (46), and the Swinburne University Computerized Cognitive Assessment Battery (SUCCAB) (47).

HVLT-R/SDMT (paper-and-pen tests).

The HVLT-R is a validated paper-and-pen test designed to examine verbal learning, immediate and delayed recall, and delayed recognition. Participants were required to recognize and recall a list of 12 words immediately (across 3 trials) and after 40 min (fourth trial). The SDMT (Sheridan, Fitzgerald et al. 2006) (46) required participants to pair numbers (1–9) with geometric figures within 90 s. This primarily assessed divided attention, tracking, and visual screening. For more information, see reference 22.

SUCCAB.

The SUCCAB is a validated cognitive test battery consisting of 8 computer-based cognitive tasks assessing various aspects of cognitive performance (47). Participants were asked to respond as quickly and accurately as possible in each task. A 4-button response box was used to complete the tasks, with buttons corresponding to task-specific response dimensions: color (red, blue, green, or yellow), "yes" or "no," or the spatial location of objects on the screen (top, bottom, left, or right). The 8 tasks that comprised the SUCCAB battery included simple reaction time (SRT), choice reaction time (CRT), incongruent Stroop (ISTR), congruent Stroop (CSTR), immediate recognition memory (IREC), spatial working memory (SWM), contextual memory (CMEM), and delayed recognition memory (DREC). Ratio performance was calculated by dividing percentage of correct responses by response times (seconds) for each task. (For more information see reference 22.)

Questionnaires

A questionnaire assessed intake of prescribed medications, dietary supplementation (dose/frequency), smoking status, highest level of education, exercise (duration/type), family history of neurodegenerative disease, and any history of an incident possibly contributing to cognitive dysfunction.

A long-term (1 y) dietary intake of a number of additional nutrients was assessed using the computerized version of the Dietary Questionnaire for Epidemiological Studies, version 2 (DQES v2) (48). The Cancer Council DQES v2 covered 5 types of dietary intake based on the previous 12 mo, incorporating 80 items. Estimated mean daily intake of a number of vitamins and minerals was derived from the consumption of these foods. Second, an "in house"–developed short-term food-frequency questionnaire (FFQ) was administered. This FFQ (based on the Cancer Council FFQ) assessed specifically what the consumption of vitamin C– and vitamin B-12–rich foods was within 2 wk prior to the testing session. The consumption of other nutrients such as zinc, magnesium, etc, was assessed due to their potential link with cognitive function (49). Serum vitamin B-12 was assessed, in particular, due to the vitamin's prominent link with cognition (50, 51).

Plasma vitamin C assessment

Fasting (8–12 h) blood tests were performed by the researcher/phlebotomist immediately after the completion of the SDMT. Blood was collected in two 6-mL lithium heparin Vacutainer blood tubes. The blood tubes were immediately wrapped in foil, kept away from light, and transported on dry ice. Each blood tube was spun in a centrifuge at 3600 rpm for 10 min at room temperature. Plasma from the heparin tubes was aliquoted into separate 3-mL tubes. These were also wrapped in foil and kept away from light. Two biochemical techniques were used to assess plasma concentrations, these included an HPLC analysis that was conducted by an external laboratory and a colorimetric analysis (52) conducted within our laboratory. For more information regarding the biochemical analysis, see reference 22.

Statistical analyses

Analyses were performed using SPSS IBM statistics version 23 package (IBM SPSS). Statistical significance was set at $P < 0.05$. Values that were 2 SDs away from their means for the cognitive assessments were excluded from analysis. Tests of normality were conducted to ensure a normal distribution of cognitive scores before any subsequent analyses were undertaken.

Primary analyses were conducted to compare cognitive performance between gender groups displaying adequate plasma vitamin C with those in the inadequate group. Average plasma vitamin C concentrations were subgrouped into established reference ranges representing adequate (≥28 μmol/L) and inadequate (also defined as depleted, <28 μmol/L) concentrations (25, 53, 54), based on expert committee recommendation (55). Cognitive performance was compared using a 2 (gender) by 2 (plasma vitamin C category) ANOVA for continuous variables.

This was followed by a 2-factor multivariate ANCOVA (MANCOVA) on cognitive performance measures that examined the interaction between gender and vitamin C group after adjusting for potential confounders. Potential confounding variables were assessed using the correlation regression analyses generated in the MANCOVA, with covariates included in the model only if they had a significant effect ($P < 0.05$) on the cognitive performance variables. A Bonferroni correction adjustment was used in the MANCOVA analyses to avoid a type 1 error.

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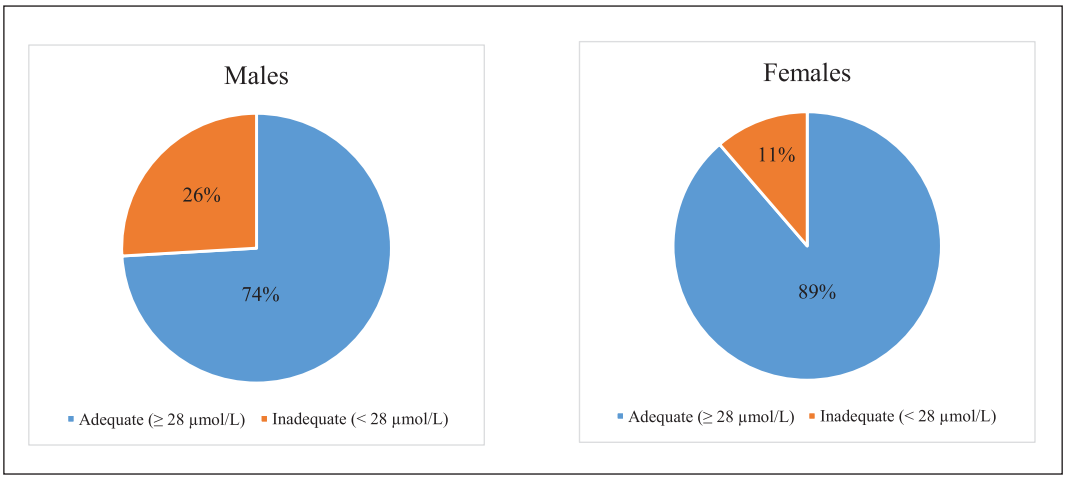


FIGURE 1 Proportion of males and females presenting with adequate (≥28 μmol/L) and inadequate/depleted (<28 μmol/L) plasma vitamin C concentrations. The inadequate group included 3 participants (2 males, 1 female) who displayed deficient vitamin C concentrations (<11 μmol/L).

For the SUCCAB, mean response time and percentage accuracy were calculated for each task. The accuracy percentage was divided by the mean response time (seconds) to provide the overall performance ratio score. This ratio also assisted in accounting for the issue of speed versus accuracy trade-off that exists with increasing age (56).

A number of cognitive variables were derived from the HVLT-R assessment. Trials 1–3, delayed recall (trial 4), total recall, and recognition index were scored based on the number of correct responses. HVLT-R is the sum of correct responses in trials 1–4. SDMT performance was scored out of 110 points.

Sample size calculation

Our initial study indicated that a sample size calculation of 80 participants was sufficient (with a 10% attrition rate) to detect a significant mean difference on the spatial working memory task on the SUCCAB, with 80% power and 95% confidence (22). This was based on a study that established aged-related mean SUCCAB values for each task using cognitively intact adults (47).

The literature suggests that a pilot study sample should consist of 10% of the projected sample for the larger study ($n = 80$) (57, 58). The sample size for the present pilot study comprised 13 participants in the inadequate group and 47 in the adequate group.

In order to confirm whether this pilot study was sufficiently powered, we ran nonparametric analyses (Mann-Whitney test), which took medians into account. This demonstrated a number of significant trends that were consistent with our MANCOVA presented below. The Mann-Whitney subgroup analysis revealed that even with a small sample size in those with inadequate vitamin C concentrations, we had sufficient power to detect differences in cognitive function between gender and vitamin C groups. A parametric MANCOVA was conducted in the present study due to this analysis being a more powerful analysis than a nonparametric analysis assessing for an interaction while also controlling for a number of key covariates, such as age.

Results

ANOVA of all participants revealed a marginal, nonsignificant difference in mean plasma vitamin C concentrations between genders [males (mean, plus/minus SD): 44.46 ± 4.43, 8–111 μmol/L; females: 48.45 ± 3.13, range: 8–116 μmol/L; $P = 0.46$]. The percentage of males presenting with inadequate plasma vitamin C concentrations (26%) was higher than the percentage of female participants (11%) (**Figure 1**). The inadequate group included 3 participants who displayed deficient vitamin C concentrations (<11 μmol/L). **Table 1** shows the descriptive statistics comparing the adequate and inadequate plasma vitamin C categories by gender. An ANOVA was conducted between gender groups for each vitamin C group separately and a 2 × 2 factorial ANOVA (gender by vitamin C group) was conducted and is shown in Table 1.

A 1-factor ANOVA revealed no significant differences on any of the variables in Table 1 between males and females exhibiting inadequate plasma vitamin C concentrations. Females in the inadequate plasma vitamin C group displayed a borderline significant higher mean zinc supplementation dose ($P = 0.06$) and mean serum vitamin B-12 concentrations ($P = 0.07$) than males. In those with adequate plasma vitamin C concentrations, females undertook significantly more vigorous intensity exercise, on average, per week than males ($P = 0.03$) and presented with significantly higher mean serum vitamin B-12 concentrations than males ($P = 0.023$). Males with adequate plasma vitamin C concentrations presented with significantly higher mean BMIs ($P = 0.023$) and a significantly higher average daily alcohol consumption than females ($P < 0.001$). Additionally, a chi-square likelihood ratio test using Fisher's exact test revealed a significant interaction between smoking status and plasma vitamin C category in both genders, with both male and female smokers less likely to display adequate vitamin C concentrations ($P = 0.013$).

A 2 × 2 factorial ANOVA revealed no significant associations between gender and vitamin C group among the analyzed descriptive variables. Trends revealed higher mean BMIs in both males with adequate and inadequate vitamin C, and more prescribed medications in the male group in both vitamin C groups. Higher mean dietary intake of vita-

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TABLE 1 Descriptive characteristics between adequate and inadequate plasma vitamin C categories for gender groups[1]

Variable	Adequate plasma vitamin C (≥28 μmol/L)					Inadequate plasma vitamin C (<28 μmol/L)					*P* (2 × 2 factorial ANOVA)
	Males		Females		*P* (M vs. F adequate)	Males		Females		*P* (M vs. F inadequate)	
	n	Values	*n*	Mean ± SE		*n*	Mean ± SE	*n*	Mean ± SE		
Age, y	20	65 ± 3	47	57 ± 2	0.08	7	73 ± 6	6	63 ± 22	NS	0.25
Gender, %	20	30	47	70	$\chi^2 = 0.001$	7	53.8	6	46.2	NS	$\chi^2 = 0.09$
BMI, kg/m^2	20	27 ± 1	47	25 ± 0.4	0.024	7	27 ± 1	6	25 ± 1	NS	NS
Smoker, %	1	5	0	0	NS	2	28.6	1	16.7	NS	$\chi^2 = 0.013$
Prescribed medications	20	2 ± 1	47	1 ± 0.2	0.08	7	2 ± 1	6	1 ± 0.2	NS	NS
Education, y	20	13 ± 0.3	47	13 ± 0.2	NS	7	13 ± 1	6	13 ± 1	NS	NS
Exercise, min/wk											
Moderate intensity	8	146 ± 40	25	138 ± 23	NS	2	145 ± 81	2	120 ± 81	NS	NS
Vigorous intensity	4	143 ± 35	8	250 ± 25	0.03	1	90 ± 71	1	180 ± 71	NA	NS
Nutrient intake											
Dose of vitamin C supplementation, mg/d	7	800 ± 233	13	892 ± 168	NS	0	0	0	0	NA	NA
Vitamin C dietary intake, mg/d	20	86 ± 5	47	85 ± 3	NS	7	76 ± 8	6	72 ± 8	NS	NS
Plasma vitamin C, μmol/L	20	54 ± 4	47	53 ± 3	NS	7	18 ± 7	6	15 ± 8	NS	NS
Vitamin B-12 supplementation, μg/d	4	210 ± 124	17	211 ± 60	NS	0	0	0	0	NA	NA
Vitamin B-12 dietary, μg/d	20	3 ± 0.2	47	3 ± 0.1	NS	7	3 ± 0.4	5	3 ± 0.4	NS	NS
Serum vitamin B-12, pmol/L	20	439 ± 71	47	648 ± 119	0.02	7	269 ± 129	6	429 ± 60	0.07	NS
Vitamin D supplementation, IU/d	6	1417 ± 188	18	1556 ± 108	NS	1	1000 ± 460	0	0	NA	NA
Vitamin D dietary, μg/d	10	4 ± 1	28	3 ± 0.3	NS	4	3 ± 1	5	4 ± 1	NS	NS
Magnesium supplementation, mg/d	7	621 ± 74	13	685 ± 54	NS	1	500 ± 194	0	0	NA	NA
Magnesium dietary, mg/d	10	485 ± 68	28	517 ± 40	NS	4	593 ± 107	5	452 ± 96	NS	NS
Fish oil/krill oil supplementation, mg/d	4	1250 ± 144	8	1313 ± 92	NS	3	1167 ± 167	0	0	NA	NA
Dietary omega-3, mg/d	10	386 ± 94	28	594 ± 56	0.07	4	466 ± 149	5	101 ± 133	NS	NS
Vitamin E, mg/d	10	16 ± 3	28	14 ± 1	NS	4	12 ± 4	5	13 ± 2	NS	NS
Vitamin B-1, mg/d	10	2 ± 0.3	28	2 ± 0.2	NS	4	2 ± 1	5	2 ± 0.3	NS	NS
Vitamin B-2, mg/d	10	2 ± 0.3	28	2 ± 0.2	NS	4	2 ± 1	5	2 ± 1	NS	NS
Vitamin B-3, mg/d	10	23 ± 3	28	23 ± 2	NS	4	28 ± 5	5	25 ± 4	NS	NS
Vitamin B-5, mg/d	10	4 ± 0.4	28	3 ± 0.2	NS	4	3 ± 1	5	4 ± 1	NS	NS
Vitamin B-6, mg/d	10	1 ± 0.2	28	1 ± 0.1	NS	4	1 ± 0.3	5	1 ± 0.3	NS	NS
Vitamin B-7, mg/d	10	36 ± 5	28	33 ± 3	NS	4	32 ± 7	5	40 ± 6	NS	NS
Iron, mg/d	10	12 ± 2	28	12 ± 1	NS	4	13 ± 2	5	13 ± 2	NS	NS
Zinc, mg/d	10	11 ± 1	28	10 ± 1	NS	4	8 ± 2	5	12 ± 2	0.06	NS
Iodine, μg/d	10	135 ± 15	28	116 ± 9	NS	4	111 ± 24	5	125 ± 21	NS	NS
Alcohol, g/d	10	15 ± 3	28	3 ± 2	<0.001	4	1 ± 4	5	1 ± 4	NS	0.07
Caffeine, mg/d	10	488 ± 114	28	557 ± 68	NS	4	742 ± 180	5	322 ± 161	NS	0.08

[1]Values are means ± SEs unless otherwise indicated. Moderate exercise = 50–60% of maximum heart rate; vigorous exercise = 70–85% of maximum heart rate. NS: $P > 0.05$. NA, not applicable.

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min C was evident in both males and females who displayed adequate vitamin C concentrations than in those with inadequate concentrations. Serum vitamin B-12 concentrations tended to be higher in males displaying adequate vitamin C concentrations than in those presenting with inadequate concentrations, and were higher in females with adequate vitamin C concentrations. The highest mean daily alcohol consumption was found in males presenting with adequate vitamin C concentrations ($P = 0.07$) and the highest average daily caffeine consumption in males with inadequate vitamin C concentrations ($P = 0.08$), with more than twice the daily caffeine consumption in this latter group.

Results of a MANCOVA examining the interaction of gender and plasma vitamin C group on SUCCAB ratio performance are shown in **Table 2**. Missing values were reported on a number of SUCCAB tasks due to a coding programming error during score generation. As a result, the MANCOVA model for SUCCAB performance was performed with data missing from 8 participants displaying adequate plasma vitamin C concentrations ($n = 72$). The correlation regression analyses generated in the MANCOVA examined whether the variables in Table 1 had a significant contribution to the MANCOVA model. Covariates were controlled for if they had a significant contribution to the model. Age had a significant, negative impact on CSTR ratio ($P = 0.02$), ISTR ratio ($P = 0.031$), and SWM ratio ($P = 0.006$). Number of medications had a significant, negative impact on IREC ($P = 0.04$) and on DREC ($P = 0.02$). After adjusting for the significant covariates, there was a statistically significant gender by plasma vitamin C group interaction [$F(32, 219) = 7.38$; $P < 0.001$, Wilks' Lambda = 0.068] on the SUCCAB task ratios. The adjusted model displayed an $R^2 > 89\%$ for each of the SUCCAB ratio tasks, indicating a large proportion of the variance on the SUCCAB ratios accounted for by the gender and vitamin C interaction effects in the MANCOVA model after covariate adjustment.

Overall, both male and female participants in the adequate vitamin C group performed better with higher performance ratios than those in the inadequate group. A gender by vitamin C interaction was observed in all individual SUCCAB task ratios (**Table 3** and **Figure 2**).

Graphically, the nonparallel lines and/or lines that crossed demonstrated greater differences between males and females and were indicative of a prominent gender by vitamin C interaction on the task. This was demonstrated on the CRT (males = 161.22 vs. 177.63), IREC (males = 60.91 vs. 79.32), CSTR (males = 114.81 vs. 121.01), and DREC (males = 59.31 vs. 71.13) tasks (Figure 2 and Table 3). For each specific task, there was a marginal disparity in cognitive ratios between the inadequate and adequate vitamin C groups in females. However, males in the inadequate vitamin C group exhibited lower ratio performance than females in the inadequate group. Besides the CSTR task, females in the adequate vitamin C group displayed higher performance ratios than males in the adequate group. For SWM and CMEM, the parallel lines indicated similar rates of reduced performance ratio in the inadequate vitamin C group for both genders (Figure 2 and Table 3).

Conversely, there was a disparity in SRT ratio performance (322.73 vs. 307.95) in males between the vitamin C groups. However, disparity between females displaying inadequate and adequate vitamin C concentrations (321.35 vs. 290.69) was clearer than in males on the SRT ratio performance (307.95 vs. 322.73). Overall, these results suggest a lower performance in males with inadequate vitamin C than females with inadequate concentrations, specifically on CRT, IREC, CSTR, and DREC. Females with inadequate vitamin C were predominantly lower in SRT performance than males with inadequate concentrations.

Additionally, a MANCOVA examined the interaction of gender and plasma vitamin C group on HVLT-R scores and the SDMT (**Table 4**). Covariates were only considered if they had a significant contribution to the model. Vitamin C supplementation dose had a significant positive impact on trial 2 ($P = 0.03$), trial 3 ($P = 0.006$), and delayed recall ($P = 0.01$). Additionally, age had a significant impact on delayed recall ($P = 0.002$), recognition index ($P = 0.04$), and SDMT ($P < 0.001$). After adjusting for the significant covariates, there was a statistically significant MANOVA [$F(28, 246) = 11.74$; $P < 0.001$; Wilks' Lambda = 0.047] for a gender by plasma vitamin C category interaction on the HVLT-R tasks and SDMT. The adjusted model displayed R^2 values >95% for each of the SUCCAB ratio tasks, indicating a large proportion of the variance on the HVLT-R and SDMT tasks was accounted for by the gender and vitamin C interaction MANCOVA model after adjusting for covariates.

Similarly to the SUCCAB tasks, participants in the adequate vitamin C group displayed higher performance on all the HVLT-R and SDMT tasks than those in the inadequate group in both males and females. A gender by vitamin C interaction was observed on all individual HVLT-R tasks and on the SDMT (**Table 5**, **Figures 3** and **4**).

Nonparallel lines related to significant interaction/gender differences were observed on trial 1, trial 2, recognition index, total recall, and the SDMT (Figures 3 and 4, Table 5). Females with adequate vitamin C displayed higher SDMT scores and HVLT-R scores than males in the adequate group on all measures besides the delayed recall, on which males in the adequate group demonstrated higher scores.

For trial 1 (male = 6.89 vs. 6.82, female = 7.73 vs. 6.99) and recognition index (male = 10.84 vs. 10.59, female = 11.56 vs. 10.29) a smaller discrepancy was demonstrated between the adequate and inadequate vitamin C groups in males than in females (Table 5). For trial 2 (7.96 vs. 9.80) and the SDMT (40.87 vs. 49.76), females in the inadequate vitamin C group scored lower than females in the adequate group, whereas there was less disparity on these tasks in males between the adequate and inadequate vitamin C groups (trial 2: 9.56 vs. 8.67; SDMT: 47.98 vs. 47.46). Furthermore, males displayed a larger disparity on delayed recall between the adequate and inadequate vitamin C groups (9.98 vs. 7.72) than females (9.54 vs. 8.33). Taken together, these results suggest higher performance in males presenting with inadequate plasma vitamin C on the SDMT than females with an inadequate plasma vitamin C. Results further revealed lower performance on delayed recall in males presenting with inadequate plasma vitamin C concentrations than females, and females presenting with adequate vitamin C concentrations had higher performance on the recognition index than males in the adequate group.

Discussion

In summary, our pilot study found that females with adequate plasma vitamin C performed better than males on tasks involving recall, recognition, attention and focus, SDMT scores (assessing psychomotor performance), and HVLT-R scores (assessing verbal learning, immediate and delayed recall, and recognition). Females with inadequate plasma vitamin C concentrations performed better than

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TABLE 2 MANCOVA: associated univariate *F* tests for gender by group interaction for SUCCAB performance[1]

Model	Task	*F*-test interaction *P* value	R^2	Covariate name	$\beta \pm$ SE	Covariate *P* value
Gender by adequate/inadequate plasma group interaction term	Simple reaction time (SRT)	<0.001	0.97	—	—	—
	Choice reaction time (CRT)	<0.001	0.95	—	—	—
	Immediate recognition memory (IREC)	<0.001	0.97	No. of Meds	−3.06 ± 1.47	0.04
	Congruent Stroop (CSTR)	<0.001	0.96	Age	−0.47 ± 0.02	0.02
	Incongruent Stroop (ISTR)	<0.001	0.92	Age	−0.43 ± 0.19	0.03
	Spatial working memory (SWM)	<0.001	0.89	Age	−0.53 ± 0.19	0.006
	Contextual memory (CMEM)	<0.001	0.96	—	—	—
	Delayed recognition memory (DREC)	<0.001	0.96	No. of Meds	−2.65 ± 1.11	0.02

[1]MANCOVA, multivariate ANCOVA; SUCCAB, Swinburne University Computerized Cognitive Assessment Battery; β, β-slope coefficient related to the covariates accounted for in the MANCOVA.

males on IREC/DREC, CSTR, and CRT, whereas males with inadequate vitamin C performed better on tasks involving psychomotor performance/motor speed (SDMT task/SRT). Given that our results are derived from a small, retrospective analysis, our findings are hypothesis generating, with larger-scale studies being required for more conclusive results and mechanisms of action to be identified.

Based on previous research, it has been observed that those with adequate plasma vitamin C concentrations performed better on a series of cognitive tasks than those displaying inadequate and deficient concentrations (22). To our knowledge, this pilot study is the first to extend these previous investigations and demonstrate that gender may play a role on how vitamin C concentrations affect cognitive performance.

TABLE 3 Means, SEs, and 95% CIs for gender by group interaction for SUCCAB ratios across both plasma vitamin C groups and gender categories[1]

Cognitive measure	Plasma category (adequate/inadequate)	Gender	Sample size, *n*	Mean	SE	95% CI
Simple reaction time (SRT)	Adequate	Male	18	307.95↓	14.1	279, 336
		Female	41	321.35	9.60	303, 340
	Inadequate	Male	7	322.73	23.3	276, 369
		Female	6	290.69↓	24.29	242, 339
Choice reaction time (CRT)	Adequate	Male	18	187.08	7.88	171, 203
		Female	41	185.78↓	5.31	175, 196
	Inadequate	Male	7	161.22↓	12.97	135, 187
		Female	6	177.63	13.5	150, 205
Immediate recognition memory (IREC)	Adequate	Male	18	75.39↓	4.67	66, 85
		Female	41	85.44	3.14	79, 92
	Inadequate	Male	7	60.91↓	7.70	46, 76
		Female	6	79.32	7.98	63, 95
Congruent Stroop (CSTR)	Adequate	Male	18	143.00	5.80	131, 154
		Female	41	131.95↓	3.91	124, 140
	Inadequate	Male	7	114.81↓	9.54	96, 133
		Female	6	121.01	9.94	101, 146
Incongruent Stroop (ISTR)	Adequate	Male	18	107.47↓	5.69	96, 119
		Female	41	112.01	3.84	104, 120
	Inadequate	Male	7	114.81	9.37	95, 133
		Female	6	104.72↓	9.78	85, 124
Spatial working memory (SWM)	Adequate	Male	18	81.76	5.49	71, 93
		Female	41	79.30↓	3.71	72, 87
	Inadequate	Male	7	75.63	9.05	58, 94
		Female	6	63.84↓	9.41	45, 83
Contextual memory (CMEM)	Adequate	Male	18	74.41↓	6.42	62, 87
		Female	41	76.64	4.33	68, 85
	Inadequate	Male	7	66.70	10.56	46, 88
		Female	6	65.33↓	11.00	43, 87
Delayed recognition memory (DREC)	Adequate	Male	18	71.33↓	3.51	64, 78
		Female	41	71.93	2.37	67, 77
	Inadequate	Male	7	59.31↓	5.76	48, 71
		Female	6	71.13	6.01	59, 83

[1]MANCOVA, multivariate ANCOVA; SUCCAB, Swinburne University Computerized Cognitive Assessment Battery; ↓, lower mean value between genders (male/female) for vitamin C subgroup (adequate/inadequate).

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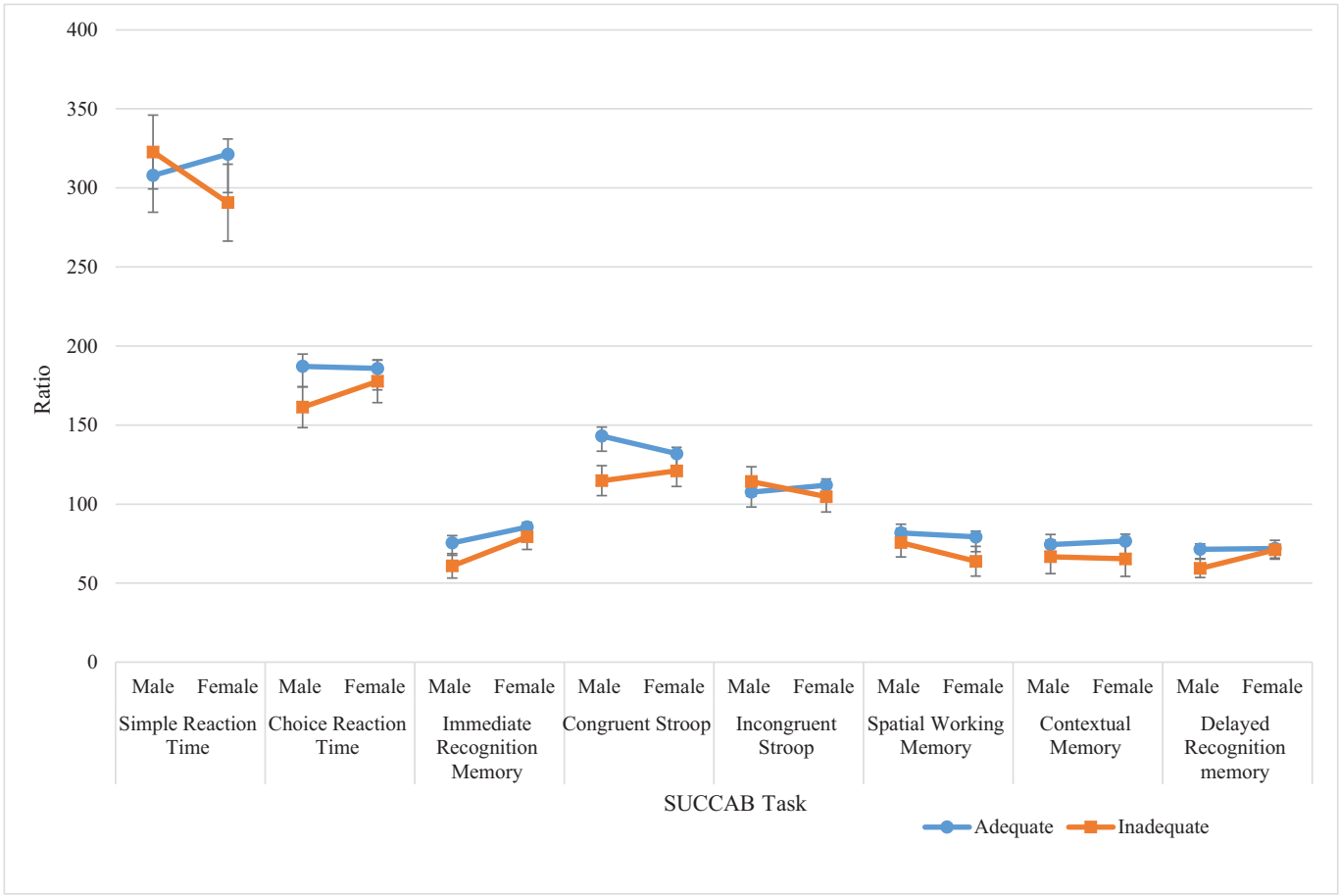


FIGURE 2 Adequate versus inadequate vitamin C groups for SUCCAB ratios. Largest interaction (largest difference between gender groups) is represented by nonparallel and/or crossing lines: ratio = accuracy ÷ response time. Adequate concentrations: ≥28 μmol/L; inadequate: <28 μmol/L. The inadequate/depleted group included 3 participants who displayed deficient vitamin C concentrations (<11 μmol/L). SUCCAB, Swinburne University Computerized Cognitive Assessment Battery.

The observation of females performing better than males on tasks involving memory (short/delayed), inhibition, and visual perception may be explained by possible variations in the distribution of brain vitamin C between genders. Due to a larger abundance of cell bodies, ascorbate concentrations are higher in gray matter than in white matter (15). Differences in gray matter volume are thought to be due to the combination of the neuroprotective effect of estrogen (which is more prominent in females) tending to decrease the loss of gray matter (59), particularly within the hippocampus (60).

Our findings of females with inadequate vitamin C concentrations demonstrating superior performance (higher performance ratios and scores) than males on CRT, IREC, CSTR, and DREC tasks may also

TABLE 4 MANCOVA: associated univariate *F* tests for gender by group interaction for HVLT-R and SDMT task performance[1]

Model	Task	*F*-test interaction *P* value	R^2	Covariate	$\beta \pm$ SE	Covariate *P* value
Gender by adequate/inadequate plasma group interaction term	Trial 1	<0.001	0.95	—	—	—
	Trial 2	<0.001	0.97	Vit C dose	0.001 ± 0.001	0.03
	Trial 3	<0.001	0.98	Vit C dose	0.001 ± 0.001	0.006
	Total recall	<0.001	0.98	Vit C dose	0.002 ± 0.001	0.02
	Delayed recall	<0.001	0.97	Age	−0.04 ± 0.013	0.003
				Vit C dose	0.001 ± 0.001	0.01
	Recognition index	<0.001	0.99	Age	−0.019 ± 0.009	0.04
	SDMT	<0.001	0.97	Age	−0.36 ± 0.06	<0.001

[1]HVLT-R, Hopkins Verbal Learning Test–Revised; MANCOVA, multivariate ANCOVA; SDMT, Symbol Digits Modalities Test; Vit, vitamin; β, β-slope coefficient related to the covariates accounted for in the MANCOVA.

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TABLE 5 Means, SEs, and 95% CIs for gender by group interaction for HVLT-R and SDMT scores across both plasma vitamin C groups and gender categories[1]

Cognitive measure	Plasma category (adequate/inadequate)	Gender	Sample size, *n*	Mean	SE	95% CI
Trial 1	Adequate	Male	27	6.89↓	0.38	6.15, 7.65
		Female	53	7.73	0.25	7.32, 8.23
	Inadequate	Male	7	6.82↓	0.66	5.61, 8.03
		Female	6	6.99	0.69	5.51, 8.57
Trial 2	Adequate	Male	27	9.56↓	0.39	8.78, 10.32
		Female	53	9.80	0.26	9.30, 10.31
	Inadequate	Male	27	8.67	0.62	7.44, 9.90
		Female	53	7.96↓	0.78	6.40, 9.53
Trial 3	Adequate	Male	27	10.55↓	0.30	9.96, 11.13
		Female	53	10.64	0.20	10.25, 11.03
	Inadequate	Male	7	9.29↓	0.47	8.36, 10.23
		Female	6	9.35	0.60	8.16, 10.15
Total recall	Adequate	Male	27	26.95↓	0.88	25.20, 28.70
		Female	53	28.26	0.58	27.10, 29.41
	Inadequate	Male	7	24.70	1.41	21.90, 27.51
		Female	6	24.26↓	1.78	20.70, 27.81
Delayed recall	Adequate	Male	27	9.98	0.39	9.21, 10.76
		Female	53	9.54↓	0.26	9.02, 10.05
	Inadequate	Male	7	7.72↓	0.68	6.36, 9.08
		Female	6	8.33	0.71	6.90, 9.75
Recognition index	Adequate	Male	27	10.84↓	0.26	10.32, 11.36
		Female	53	11.56	0.17	11.22, 11.90
	Inadequate	Male	7	10.59	0.46	9.68, 11.50
		Female	6	10.29↓	0.48	9.34, 11.24
SDMT	Adequate	Male	27	47.98↓	1.79	44.42, 52.14
		Female	53	49.76	1.18	47.41, 52.11
	Inadequate	Male	7	47.46	3.13	41.25, 53.71
		Female	6	40.87↓	3.67	33.34, 46.36

[1]HVLT-R, Hopkins Verbal Learning Test–Revised; SDMT, Symbol Digits Modalities Test; ↓, lower mean value between genders (male/female) for vitamin C subgroup (adequate/inadequate).

be explained by nutritional and health differences between the gender groups. Females in the inadequate plasma vitamin C group displayed a borderline significant higher mean zinc supplementation dose and mean serum vitamin B-12 concentrations than males. Based on our assessment of potential covariates, females with adequate vitamin C concentrations undertook significantly more vigorous intensity exercise, on average, per week than males, and presented with significantly higher mean serum vitamin B-12 concentrations. Although these variables did not have a significant impact on the analyses as covariates, this is suggestive of a healthier lifestyle in the female sample, and could explain the greater performance in females.

In this study, male participants may have had higher oxidative stress, more prescribed medications, less vitamin C supplementation, and higher alcohol consumption than females. Although these variables did not have a significant impact on the analyses as covariates, they reveal a trend of a possibly lower overall health status in the male sample and elevated oxidative stress (61, 62). Studies have indicated a link between alcohol consumption and elevated oxidative stress, which is associated with gray matter volume reduction in specific regions, such as the frontal lobe (63), with these reductions in gray matter volume observed only in men.

Recent research has indicated that the hippocampus in guinea pigs requires more dietary vitamin C to become saturated than other brain regions (64). Due to the possibility of increased cerebral oxidative stress, males would require greater dietary vitamin C intake and plasma vitamin C to reach hippocampal saturation, making males more vulnerable to cognitive decline in scenarios of depleted concentrations of vitamin C.

Female performance on IREC/DREC, CSTR, and CRT with inadequate vitamin C concentrations was superior to that of males with inadequate concentrations. Although studies (65) have indicated a possible plateau in ascorbic cerebrospinal fluid once concentrations reach 45 μmol/L, the concentration of this plateau may be lower for females. Male mice have been shown to experience more dramatic age-associated decline in reduced glutathione (GSH) content in the brain (66), possibly affecting the brain's ability to recycle ascorbate. This may imply that the female brain might require less vitamin C to function sufficiently. The observation that females depleted of vitamin C perform similarly on these tests to those females displaying adequate concentrations is consistent with the retention of vitamin C in brain tissue.

There may be a number of explanations accounting for females presenting with inadequate vitamin C to exhibit lower performance on tasks involving psychomotor performance/motor speed (SDMT task/SRT). Recent research has demonstrated that the cerebellum of female guinea pigs, a structure that is involved in the timing and accuracy of movements, becomes saturated at relatively low intakes of vitamin C

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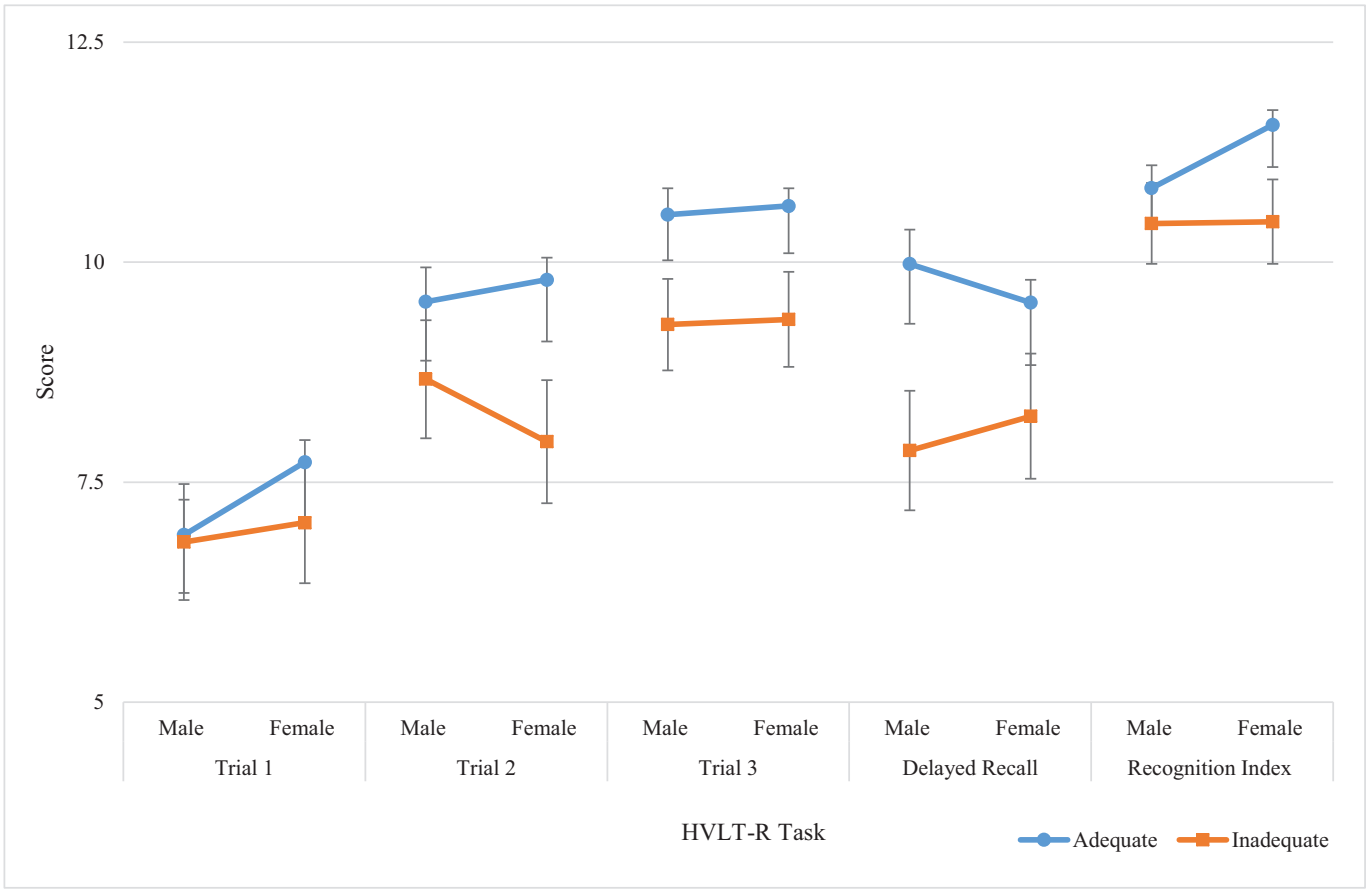


FIGURE 3 Adequate versus inadequate vitamin C groups for the HVLT-R. Largest interaction (largest difference between gender groups) is represented by nonparallel and/or crossing lines. Adequate concentrations: ≥28 μmol/L; inadequate/depleted: <28 μmol/L. The inadequate/depleted group included 3 participants who displayed deficient vitamin C concentrations (<11 μmol/L). HVLT-R, Hopkins Verbal Learning Test–Revised.

(64). Although not previously examined, this saturation threshold may be lower for males, enabling this brain region to efficiently utilize ascorbate at lower plasma concentrations.

Additionally, one animal model (using genetically modified mice unable to synthesize vitamin C like humans) revealed that during states of restricted ascorbate intake and depleted intracellular cerebellum ascorbate, sodium-dependent vitamin C transporter 2 (SVCT2) protein expression was increased within the cerebellum (67), possibly to upregulate the absorption of ascorbate. Despite the study not taking potential gender differences into account, males may be capable of expressing more cerebellar SVCT2 proteins during states of depletion, consequently absorbing and utilizing more ascorbate during depleted states.

In a cohort of 1004 adults, males showed significantly larger volumes for globus pallidus and putamen of the basal ganglia while controlling for total gray matter volume and age of the participants (68). These regions have been associated with higher order movement, providing a connection between motor centers and cortical areas for higher brain function (69).

Additional neuroimaging investigations have highlighted larger cerebellar hemispheres in men (70). Given these larger volumes, males may have larger ascorbate demands in these regions and be better at maintaining ascorbate concentrations in these regions. Vitamin C brain distribution and the observed cognitive performance in our male sample may further be accounted for by differences in regional gray matter (which contains the largest amount of vitamin C) distribution.

A large cross-sectional MRI study that explored how gender interacts with gray matter structure revealed that the cerebellar gray matter volume in males was significantly larger in the anterior and middle posterior lobes (59). A recent study also detected more gray matter volume within subcortical temporal structures in men, which included the putamen, anterior cerebellum, and premotor cortex (71). The premotor cortex is involved in movement planning and the execution of higher-order motor skills (72).

Males with inadequate vitamin C concentrations, on the other hand, may preserve their vitamin C more efficiently for psychomotor tasks involving neuromodulatory functions such as synaptic acetylcholine release (4) and sympathetic nerve activity (which is stimulated during tasks involving movement) (73). Elevated sympathetic activation in men (74) has been linked to increases in the secretion of the antidiuretic peptide hormone vasopressin (75), which is more present in men (76)

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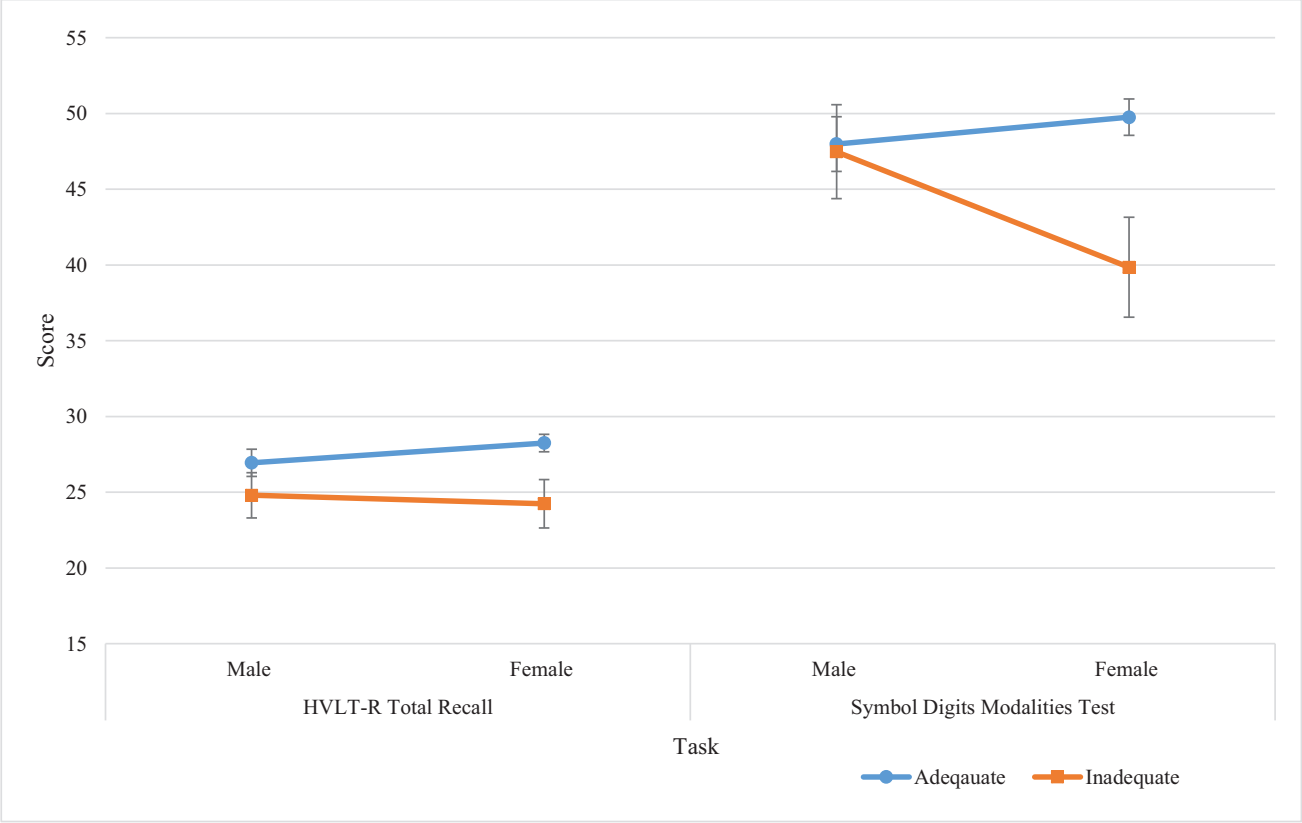


FIGURE 4 Adequate versus inadequate vitamin C groups for HVLT-R total recall and Symbol Digits Modalities Test. Largest interaction (largest difference between gender groups) is represented by nonparallel and/or crossing lines. Adequate concentrations: ≥28 μmol/L; inadequate/depleted: <28 μmol/L. The inadequate/depleted group included 3 participants who displayed deficient vitamin C concentrations (<11 μmol/L). HVLT-R, Hopkins Verbal Learning Test–Revised.

and requires the involvement of ascorbate for the synthesis of the peptide (77).

There are a number of limitations to the present pilot study. It is difficult to ascertain a causative relation based on the cross-sectional design. Furthermore, due to the study design, it is difficult to predict the duration of inadequate/adequate plasma vitamin C concentrations. Inadequate plasma concentrations can be maintained from a diet consisting of <45 mg vitamin C/d. Multiple assessments of participants over time of vitamin C and cognitive assessments would help to determine the length of depletion and the effects of this length on cognitive performance.

To elucidate the effects of inadequate concentrations of vitamin C on the brain, increased knowledge about the distribution of vitamin C to the brain and within different brain regions during varying plasma vitamin C concentrations between gender groups would be advantageous for interpretation of findings. Assessing levels of oxidative stress through superoxide dismutase, glutathione peroxidase, and malondialdehyde would give a clearer indication on the variability between the gender groups and whether this could account for variances in the way vitamin C is being utilized.

Given that our subsample with inadequate plasma vitamin C concentrations consisted of only 7 males and 6 females, our results need to be interpreted with caution, in particular those relating to participants with inadequate plasma vitamin C. There was a discrepancy in the number of males and females included in the study, particularly in those presenting with adequate concentrations. Future research should assess larger numbers of adults with inadequate plasma vitamin C concentrations and more equal numbers of males and females with adequate concentrations for more generalizable results.

Additionally, physiological differences between gender groups may have played a role. These include cardiovascular, immune, and hormonal biomarkers, alongside oxidative stress, all of which may have an impact on both cognitive performance and vitamin C concentrations and should be considered in future research. While our study measured plasma vitamin C and vitamin B-12 concentrations biochemically, additional nutrients important for cognitive health, such as a range of B vitamins, vitamin D, and homocysteine, should also be assessed in future studies (78). Measuring these through blood tests will assess nutritional absorption as well as avoid any potential recall and response bias associated with FFQs (79).

In conclusion, the results from this pilot study extend the findings from our previous study and provide an avenue of exploration for future studies examining the link between plasma vitamin C and cognition. This pilot study demonstrated differences in the effects of vitamin C on cognition between males and females after controlling for potential covariates such as age, numbers of prescribed medications, and vitamin

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C supplementation dose. One novel finding was that females with inadequate plasma vitamin C performed better than males on IREC/DREC, CSTR, and CRT, whereas males with inadequate vitamin C performed better on tasks involving psychomotor performance/motor speed. The results further suggest that, in the group with adequate plasma vitamin C concentrations, females perform better than males on tasks involving recall, recognition, attention, and focus. In adults with inadequate vitamin C concentrations, females performed better on tasks involving components of memory (short/delayed), inhibition, and visual perception, whereas males performed better on tasks involving psychomotor performance/motor speed (SDMT task/SRT). The findings from this study warrant future cohort or longitudinal, randomized controlled trials to determine a cause-and-effect relation more conclusively and the way in which cognition is affected by varying plasma vitamin C concentrations in both males and females.

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The authors' responsibilities were as follows—A Sali and NT: conceptualized the study in discussion with KR and AP; IH: provided software and statistical expertise; NT: undertook data analysis and interpreted findings in discussion with KR and prepared the manuscript with contributions from all co-authors (KR, AP, A Sali, IH, and A Scholey); and all authors: read and approved the final manuscript.

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