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Effects of Vitamin C on Mental Vitality in Young Adults

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Abstract

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Background

Vitamin C (L-ascorbic acid or ascorbate) is an essential nutrient that acts as a powerful reducing agent with antioxidant properties. The brain requires the highest concentration of vitamin C in the body because it is vulnerable to oxidative stress due to its high metabolic rate and abundant lipid content. In addition to acting as an antioxidant, vitamin C plays a critical role in neural differentiation and neurotransmitter synthesis and release, suggesting that it is one of the most important nutrients for maintaining brain homeostasis. Thus, vitamin C is believed to have the potential to promote psychological function, supported by previous *in vivo* studies showing that reduced levels of vitamin C are associated with various psychiatric disorders. Mental vitality, a psychological state of aliveness and alertness, provides energy to cope with taxing problems and encourages healthy psychological functioning, such as motivation and goal-seeking. Given that the

early signs of vitamin C deficiency include lethargy and low energy in the body, vitamin C appears to be involved in boosting vitality-related mental functions in humans. However, in contrast to a great deal of understanding of the antioxidant properties of vitamin C, its biological role in the brain has yet to be fully understood in humans. Moreover, causal and mechanistic relationships between vitamin C status and vitality-related psychological functions have yet to be investigated.

Objectives

This research aimed to gain deep insight into the relationship between vitamin C status and mental function in healthy young adults. **Study 1** aimed to determine the association between serum vitamin C concentrations and mental vitality using a population-based cross-sectional study. The aim of **Study 2** was to determine the causal relationship between vitamin C and mental vitality using a randomized, double-blind, placebo-controlled vitamin C supplementation trial and to explore the physiological mechanisms by which vitamin C promotes mental vitality in a healthy young population with suboptimal vitamin C status.

Methods

In **Study 1**, a cross-sectional investigation was performed in 214 healthy men and women (20–39 y). Serum vitamin C concentrations were measured using high-performance liquid chromatography. To determine the factors associated with serum vitamin C status, participants' lifestyle factors (smoking status, physical activity, alcohol consumption, dietary intake of vitamin C, and vitamin C supplement use) and a single nucleotide polymorphism (SNP) in the gene encoding

sodium-dependent vitamin C transporter 1 were investigated. Data on smoking status, alcohol consumption, physical activity, and vitamin C supplement use were collected using questionnaires. Usual dietary intake of vitamin C was assessed using a two-day dietary record. SNP rs6596473 at the solute carrier family 23 member 1 (SLC23A1) gene locus was genotyped using TaqMan™ SNP Genotyping Assay. Participants rated their mental vitality levels (fatigue and subjective attention) and mood states (stress, depression, and positive and negative affect) using validated self-reported questionnaires. Binary logistic analysis was used to determine factors associated with suboptimal serum vitamin C status with concentrations less than 50 μmol/L. The associations between serum vitamin C concentrations and mental states were determined using linear regression analysis. In **Study 2**, a randomized, double-blind, placebo-controlled vitamin C supplementation trial was performed in otherwise healthy men and women (20–39 y) whose serum vitamin C concentrations were suboptimal (<50 μmol/L). Participants were randomly allocated to receive 500 mg of vitamin C twice a day for 4 weeks (n = 24) or a placebo (n = 22). Mental vitality indicators were measured as follows: validated self-reported questionnaires determined participants' levels of fatigue, subjective attention, work engagement with a three-dimensional structure (vigor, dedication, and absorption), and self-control resources; Stroop color-word test combined with mental arithmetic tasks evaluated cognitive function of sustained attention and cognitive flexibility; and enzyme-linked immunosorbent assays (ELISA) determined serum concentrations of brain-derived neurotrophic factor (BDNF). Mood state assessments were performed to determine the levels of stress, depression, positive and negative affect, and anxiety. To explore how vitamin C promotes mental vitality through the gut-microbiota-

brain axis, bacterial 16S ribosomal RNA gene amplicons were sequenced using Next-Generation Sequencing technology. The relative abundance of specific bacterial taxa was analyzed using QIIME2. Bacterial functional abundance was predicted using PICRUSt2 based on the 16S rRNA data and a reference genome database. Based on the results from PICRUSt2, ELISA determined the serum concentrations of lipopolysaccharide (LPS)-binding protein and spermidine. The mechanistic relationship between vitamin C-induced mental vitality and gut microbiota profiles was established in concert with investigations of proinflammatory cytokines and neurotransmitters. Electrochemiluminescent multiplex immunoassay determined serum concentrations of cytokines, including interleukin (IL) 6, IL-8, IL-10, tumor necrosis factor (TNF) α, and interferon (IFN) γ. In addition, concentrations of neurotransmitters in sera, including L-DOPA, norepinephrine, and serotonin, were measured using ultrahigh-performance liquid chromatography-mass spectrometry.

Results

In the population-based cross-sectional study (**Study 1**), the binary logistic analysis showed that individuals in the lowest tertile of dietary vitamin C intake were 3.9 times more likely to have suboptimal serum vitamin C concentrations than those in the highest tertile of dietary vitamin C intake (95% CI: 1.77, 8.83). In addition, individuals who did not use vitamin C-containing supplements were 4.4 times more likely to have suboptimal serum vitamin C concentrations than those who regularly took vitamin C supplements (95% CI: 2.09, 9.40). Also, men were 4.5 times more likely than women to have suboptimal concentrations of vitamin C in their sera (95% CI: 2.10, 10.01). Regarding the SNP rs6596473 at the SLC23A1 gene locus,

individuals with the minor allele homozygote (GG) were 2.7 times more likely to have suboptimal serum vitamin C concentrations than those with the major allele homozygote (CC) (95% CI: 1.05, 7.30; adjusted for sex, dietary vitamin C intake, and vitamin C supplement use). The linear regression analysis showed that attention scores, an important indicator of mental vitality level, directly correlated with serum concentrations of vitamin C ($\beta = 0.20$, $p < 0.01$; adjusted for sex, age, BMI, current smoking, alcohol consumption, and physical activity). On the other hand, there was no significant association between serum vitamin C concentrations and fatigue levels or mood states. In the randomized, double-blind, placebo-controlled trial (**Study 2**) involving young adults (20-39 y) with suboptimal serum vitamin C concentrations (<50 μmol/L), daily supplementation with 1000 mg of vitamin C for 4 weeks increased serum vitamin C concentrations by 106% ($p < 0.001$). On the other hand, serum vitamin C concentrations in the placebo group decreased by 25% ($p < 0.01$). Notably, vitamin C supplementation significantly increased the levels of subjective attention and work absorption compared to placebo supplementation (both $p < 0.05$): subjective attention and work absorption scores in the vitamin C group increased by 27% ($p < 0.01$) and 10% ($p < 0.05$), respectively, whereas those scores in the placebo group did not significantly change. In addition, compared to the placebo group, the vitamin C group showed marginal improvement in fatigue ($p = 0.06$) and comprehensive work engagement ($p = 0.07$). On the other hand, there was no significant treatment effect of vitamin C on self-control resources, serum BDNF concentrations, and mood indices compared to the placebo treatment. In the Stroop color-word test, the participants supplemented with vitamin C showed higher sustained attention than those in the placebo group ($p < 0.05$). Furthermore, there was a direct correlation between the

endpoint serum concentration of vitamin C and cognitive performance in the Stroop test ($r = 0.28$, $p = 0.05$). Analysis of gut bacterial communities showed that vitamin C supplementation significantly increased the relative abundance of *Bacillaceae*, *Bifidobacterium*, and *Anaerotruncus* and decreased the relative abundance of *Desulfovibrio* compared to placebo supplementation (all $p < 0.05$). Moreover, cognitive performance in the Stroop test directly correlated with changes in *Bacillaceae* ($r = 0.30$, $p < 0.05$) and *Anaerotruncus* ($r = 0.35$, $p < 0.05$), while inversely correlated with changes in *Desulfovibrio* ($r = –0.31$, $p < 0.05$). The analysis of the functional abundance of gut microbiota showed that vitamin C supplementation significantly decreased the LPS- and polyamine-producing bacteria compared to the placebo (both $p < 0.05$). Accordingly, compared to the placebo group, significant decreases in the concentrations of LPS-binding protein and spermidine were observed in sera of the vitamin C group (both $p < 0.05$). Furthermore, cognitive performance in the Stroop test inversely correlated with changes in the abundance of bacterial polyamine biosynthesis ($r = –0.58$, $p < 0.001$) and the Entner-Doudoroff pathway ($r = –0.33$, $p < 0.05$), which were reduced by vitamin C supplementation. For serum cytokine concentrations, there was a significant time-by-group interaction effect in serum IL-6, IL-10, and TNF-α concentrations (all $p < 0.05$). Specifically, in the placebo group, concentrations of serum IL-6 and IL-10 increased by 46% ($p < 0.01$) and 22% ($p < 0.01$), respectively, whereas those in the vitamin C group did not significantly change. In addition, serum TNF-α concentrations in the vitamin C group decreased by 23% ($p < 0.01$), whereas those in the placebo group did not significantly change. Notably, the placebo group showed a direct correlation between poor cognitive performance in the Stroop test and increased concentrations of IL-8 ($r = 0.47$, $p < 0.05$) and IL-

10 (r = 0.45, p = 0.05), but these associations were abolished in the vitamin C group. For serum neurotransmitters, including L-DOPA, norepinephrine, and serotonin, there was no treatment effect of vitamin C on the concentration change; however, only the vitamin C group showed a robust direct correlation between an increase in L-DOPA concentrations and an increase in work engagement levels (r = 0.58, p < 0.001).

Conclusion

This study is the first to show a causal and mechanistic relationship between vitamin C and mental vitality in young adults with suboptimal vitamin C status. Optimal vitamin C status supports the normal psychological function of dopamine to promote enthusiasm and motivation for work. Sufficient vitamin C intake also inhibits endotoxin-producing gut microbes, a potential risk factor for chronic inflammation, contributing to maintaining attentional focus during cognitive tasks. Current findings suggest that optimal vitamin C status promotes gut microbiota balance and increases mental energy in young adults.

Keywords: vitamin C, mental vitality, attention, motivation, gut microbiota, gut-brain axis, proinflammatory cytokine, single nucleotide polymorphism

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List of Abbreviations

BDNF	Brain-derived neurotrophic factor
ED pathway	Entner-Doudoroff pathway
IL	Interleukin
LBP	Lipopolysaccharide-binding protein
LPS	Lipopolysaccharide
MAF	Minor allele frequency
OR	Odds ratio
OTU	Operational taxonomic unit
PA	Polyamine
PBO	Placebo
SLC23A1	Solute carrier family 23 member 1
SNP	Single nucleotide polymorphism
SVCT1	Sodium-dependent vitamin C transporter 1
TNF-α	Tumor necrosis factor α
VC	Vitamin C

I. Introduction

1. Background

Vitamin C, also known as L-ascorbic acid or ascorbate, is an essential nutrient that functions as an electron donor and cofactor in several biological reactions (Du, Cullen, and Buettner 2012). The distribution of vitamin C in the body is thought to reflect the functional requirements of those tissues and organs (Figueroa-Méndez and Rivas-Arancibia 2015). Accordingly, given that the brain has the highest concentration of vitamin C than any other organ, it can be seen that vitamin C is closely related to brain function and maintaining its homeostasis (Fiona E Harrison and May 2009b). More specifically, *in vitro* experiments and animal model studies have shown that vitamin C in the brain effectively scavenges reactive oxygen species and free radicals to protect neurons from oxidative stress and is involved in recycling other brain antioxidants such as glutathione and vitamin E (Ballaz and Rebec 2019). In addition, vitamin C induces the differentiation and maturation of neurons and regulates the synthesis or transmission of neurotransmitters, including catecholamines and serotonin (Hansen *et al.* 2018; James M May, Qu, and Meredith 2012; Seitz *et al.* 1998). Consistent with *in vitro* results, evidence from *in vivo* models has shown that vitamin C deficiency or depletion conditions are implicated in abnormal phenotypes of brain structure and function, indicating cerebral hemorrhage, neurodegeneration, mood disorders, or cognitive decline (Tveden-Nyborg 2021; Hansen *et al.* 2018; Plevin and Galletly 2020). Although a few human studies also have observed a significant relationship between vitamin C status and brain function, the currently available evidence is limited to the elderly or patient populations with clinical psychiatric symptoms (Plevin and Galletly 2020; Fiona E Harrison 2012; Moretti, Fraga, and Rodrigues 2017). Therefore, it is

highly desirable to investigate whether improvement of vitamin C status in healthy populations is also effective in better brain function, such as vitality-related psychological and cognitive domains

The gut microbiota, a collection of commensal bacteria, archaea, fungi, and viruses, is referred to as a forgotten organ in humans because the number of intestinal bacteria equals the number of cells in the human body, and their genetic repertoire is estimated to be 150 times greater than that of humans (Lloyd-Price *et al.* 2017). Accumulating evidence suggests that the gut microbiota has the potential to influence various metabolic and physiological functions of the host, including nutrient metabolism, immune responses, and disease pathology (Tilg *et al.* 2020; Rooks and Garrett 2016; Cryan and Dinan 2012; Gentile and Weir 2018; Gehrig *et al.* 2019). Hence, gut dysbiosis, a condition with the loss of beneficial microbiota and expansion of pathogenic microbes, has been linked with gastrointestinal disorders and various unhealthy outcomes affecting other distal organs (Carding *et al.* 2015). Given the overall impact of the gut microbiota on human health, the gut-microbiota-brain axis, bidirectional communication between enteric bacteria and the central nervous system, is recently considered an essential concept for understanding the pathogenesis of mental illness (Cryan *et al.* 2019). Alteration in intestinal gut bacterial communities can affect brain function through the gut-microbiota-brain axis and vice versa (Mayer, Tillisch, and Gupta 2015). Indeed, overwhelming evidence from *in vivo* and clinical studies has observed the significant link between gut microbiota imbalance and the prevalence and severity of various psychiatric disorders, such as depression, anxiety, autism, and dementia (Cryan and Dinan 2012). Therefore, it is clear that maintaining a balanced gut microbiota is an important determinant of brain health.

It is worth noting a link between dietary vitamins and the gut microbiota since vitamins are essential for brain homeostasis as well as survival of the gut microbiota and their basic biological processes (Pham, Dold, *et al.* 2021; Das, Babaei, and Nielsen 2019). Since dietary vitamins are entirely absorbed in the proximal small intestine, vitamins have not traditionally been considered to affect the gut flora (Said 2011). However, emerging animal and clinical studies over the past few years have shown that high-dose or colon-targeted vitamins can reach the large intestine, and commensal bacteria utilize significant amounts of the vitamin in the gut (Pham, Fehlbaum, *et al.* 2021; Fangmann *et al.* 2018). Therefore, recent studies focus on determining the effectiveness of vitamins as adjuvants for the prevention and treatment of various mental illness, which is achieved via the gut-microbiota-brain axis (Berding *et al.* 2021). For example, dietary riboflavin has been reported to nourish the short-chain fatty acid-producing bacteria (L. Liu *et al.* 2022). Accumulating evidence also shows that vitamin D acts as a signaling molecule that regulates neuroreceptor activity within the gut-microbiota-brain axis. In contrast to the widely investigated vitamin D or B complex, the effects of vitamin C administration on the gut microbiota have been rarely investigated, and an association with brain function remains to be established.

The mechanisms underlying bidirectional communication between gut and brain involve alterations in bacterial composition and metabolism and mediation of neuro-immuno-endocrine factors (de Vos *et al.* 2022). For example, a growing body of studies has shown that the compositional shift toward gut microbiota dysbiosis and the consequent bacterial metabolic changes trigger systemic inflammatory cascades and chronic neuroinflammation, a major cause of mental disorders (Rooks and Garrett 2016). Impaired balance in gut flora also

increases the permeability of the intestinal mucosa, increasing the likelihood of toxic bacterial fragments reaching the central nervous system (Bhattacharyya and Bhunia 2021). Additionally, clinical and experimental evidence indicates that microbial-derived metabolites not only interact locally with intestinal cells, but also exert their effects remotely via neuroendocrine and metabolic pathways (Y. Liu *et al.* 2020; Yang and Cong 2021). That is, it is likely that multiple mechanisms work together to mediate the gut-microbiota-brain axis, contributing to homeostasis of the gut, brain, and microbial community.

2. Objectives

Hypothesis

1. High serum vitamin C concentrations may be associated with high mental vitality levels in a young population.
2. Vitamin C supplementation may promote mental vitality, which is achieved by gut microbiota alteration interacting with immune and central nervous systems through the gut-microbiota-brain axis.

Aims

1. The aim of **Study 1** was to determine the association of serum vitamin C concentrations with mental vitality in young adults by conducting a population-based cross-sectional study.
2. The aim of **Study 2** was to determine whether vitamin C supplementation promotes mental vitality in otherwise healthy individuals with suboptimal serum vitamin C status by conducting a randomized, double-blind, placebo-controlled trial. The mechanisms by which vitamin C supplementation promotes mental vitality were investigated by assessing the effects of vitamin C on gut bacterial communities, inflammatory cytokines, and neurotransmitters.

II. Literature review

1. Biochemical functions of vitamin C in humans

Vitamin C (ascorbic acid) is a water-soluble carboxylic acid consisting of six carbons. Most mammals can synthesize vitamin C on their own. However, humans cannot synthesize vitamin C due to a lack of L-gulono-1,4-lactone oxidase, an enzyme responsible for the final step in vitamin C synthesis (Nishikimi and Yagi 1991). Therefore, humans are entirely supplied with vitamin C from their diet. In humans, ingested vitamin C is readily absorbed from the small intestine and distributed to the organs by sodium-dependent vitamin C transporter (SVCT) 1, which belongs to solute carrier family 23 member 1 (SLC23A1) (Sotiriou *et al.* 2002). Vitamin C is differentially accumulated by most tissues and body fluids. High concentrations of vitamin C are found in the pituitary gland, adrenal glands, lens, and brain. There are two important biological forms of vitamin C: ascorbate anion, the deprotonated ascorbic acid at physiological pH, and dehydroascorbic acid, the oxidized form of ascorbic acid (Du, Cullen, and Buettner 2012). Vitamin C acts as an electron donor, a reducing agent that exerts antioxidant activity. Most of the physiological functions of ascorbic acid depend on its redox properties (S. J. Padayatty *et al.* 2003). In addition, ascorbic acid acts as a cofactor for fifteen mammalian enzymes, such as dopamine β-monooxygenase, peptidylglycine α-amidating monooxygenase, prolyl hydroxylase, lysyl hydroxylase, asparaginyl hydroxylase, trimethyllysine hydroxylase, and γ-butyrobetaine hydroxylase (Sebastian J Padayatty and Levine 2016). Therefore, vitamin C is necessary for collagen synthesis and contributes to the formation and maintenance of connective tissues, such as tendons, bones, and cartilage in our body. Vitamin C is also involved in regulating the activity of hydroxylase in tyrosine and phenylalanine

metabolism. In addition, vitamin C plays a critical role in converting folic acid to its active form, tetrahydrofolate. Vitamin C converts ferric cation into ferrous in the intestinal tract and prevents calcium from forming insoluble salts with iron, which increases intestinal iron absorption. Vitamin C also enzymatically breaks down histamine and converts it to aspartate for detoxification.

2. Actions of vitamin C in central nervous system

Circulating vitamin C enters the brain via SVCT2, which presents its highest expression in the central nervous system (Fiona E Harrison and May 2009b). By active transport of SVCT2, ascorbic acid crosses the blood-brain barrier, choroid plexus, and neuronal membranes. In addition, dehydroascorbic acid is transported by glucose transporter (GLUT) 1 and 12 in the blood-brain barrier and astrocytic membranes (Miyata *et al.* 2022; Agus *et al.* 1997). It has been reported that oral vitamin C administration upregulates basal expression of GLUT1 in endothelial cells of the brain cortex in rat models (Iwata *et al.* 2014). In addition, neurons modify the availability of intracellular vitamin C itself in response to an increase in extracellular ascorbic acid via SVCT2 translocation to the plasma membrane, ensuring optimal vitamin C uptake (Covarrubias-Pinto *et al.* 2018). In humans, the brain contains 2-10 mmol/L of vitamin C, which is 200 times higher than the plasma concentration.

Neuroprotection

The brain is vulnerable to oxidative damage due to its high metabolic rate and an elevated content of unsaturated fatty acids in cell membranes (Niemoller and Bazan 2010). Because vitamin C has a strong reducing activity and small molecular size, it can serve as the first line of intracellular defense against free radicals produced by cellular metabolism in the brain (Ballaz and Rebec 2019). Therefore, in some brain regions, vitamin C levels are known to reach the millimolar level, which is ten times higher than the average concentration in other tissues (Figueroa-Méndez and Rivas-Arancibia 2015). Preclinical evidence

suggests that vitamin C deficiency in the brain causes oxidative stress and neurodegeneration. For example, the lack of the ability to synthesize ascorbic acid in transgenic mice promotes the pathogenesis of age-related neurodegeneration (Dixit *et al.* 2015). Dehydroascorbic acid attenuates oxidative damage that was caused by ischemia-reperfusion injury in rats (Bémeur *et al.* 2005). Vitamin C also inhibits oxidative stress triggered by neurotoxins, including ethanol and monosodium glutamate (Hashem, El-Din Safwat, and Algaidi 2012; Huang *et al.* 2002). Specifically, in the hippocampal neurons of a prenatal rat model, vitamin C protects against ethanol-induced apoptotic neurodegeneration (Huang *et al.* 2002). In addition, the antioxidant activity of vitamin C protects neurons against massive glutamate and subsequent excitotoxicity induced by the overactivation of glutamate receptors (Choi 1992). The part of the brain where vitamin C acts are divided into the intracellular and extracellular compartment. The scavenging activity of vitamin C is not limited to aqueous phase, and vitamin C participates in free radical scavenging of cell membranes and other hydrophobic compartments through interaction with vitamin E (J. M. May 1999). Thus, vitamin C protects neurons and immune cells in the brain from oxidative radicals and maintains a redox equilibrium.

Neuronal development

Vitamin C plays a vital role in brain development. Vitamin C levels are highest in the fetal brain of rats, doubling from day 15 to day 20 of gestation and then decreasing significantly at birth (Kratzing, Kelly, and Kratzing 1985). Ascorbic acid has been demonstrated in *in vitro* experiments to enhance the differentiation of mouse embryonic stem cells into neurons and increase the proportion of

dopaminergic and serotonergic neurons (S.H. Lee *et al.* 2000). Differentially expressed genes induced by vitamin C treatment during neuronal differentiation are involved in cell adhesion and development, neurogenesis, maturation, and neurotransmission. In the absence of SVCT2, mouse hippocampal neurons display decreased dendritic branches and total dendritic length, suggesting that SVCT2 is required for the normal maturation of neurons (Qiu *et al.* 2007). It has also been reported that ascorbic acid increases the number of dopaminergic neurons in culture and *in vivo* systems by stimulating the differentiation of various neural progenitor cells of mice and humans (Bagga, Dunnett, and Fricker-Gates 2008; Chen, He, and Zhang 2009). These effects of vitamin C on lineage commitment of dopaminergic neurons are not due to the antioxidant properties of vitamin C, as alternative antioxidant agents cannot mimic these effects (Yan, Studer, and McKay 2001). Instead, the mechanisms by which ascorbic acid promotes the differentiation of dopaminergic neurons rely on epigenetic regulation in the brain (He *et al.* 2015). Specifically, ascorbic acid promotes the loss of cytosine methylation in the promoter regions of neuron-specific dopaminergic genes, giving transcription factors access to these regions and consequently differentiating neural stem cells into dopaminergic neurons. Another mouse model experiment reported that vitamin C prevents D-galactose-induced decline in hippocampal neurogenesis by promoting cell proliferation, neuronal differentiation, and neuronal maturation (Nam *et al.* 2019). Also, vitamin C contributes to the reduction of neuroinflammation in the mouse hippocampus because it increases the levels of endogenous antioxidants and the expression of Sirt1, caveolin-1, and brain-derived neurotrophic factors. Further, vitamin C is involved in synapse formation, thus improving learning and memory while reducing the risk of mood disorders and

neurodegenerative diseases in *in vivo* models (Moretti and Rodrigues 2022). Taken together, vitamin C is essential for neuronal differentiation, maturation, and synapse formation.

Neuromodulation

Vitamin C in the brain plays a variety of roles in the synthesis and regulation of biogenic amines. For example, vitamin C is an essential cofactor for dopamine β-hydroxylase, an enzyme that catalyzes the conversion of dopamine to norepinephrine (Daubner, Le, and Wang 2011). Vitamin C is also involved in the regulation of dopaminergic neurotransmission. Vitamin C release into the extracellular space prevents dopamine oxidation during activation of the substantia nigra dopaminergic system (Ballaz and Rebec 2019). Animal models of vitamin C deficiency show the marked reduction in dopamine and other catecholamine levels concomitant with behavioral changes (Hansen *et al.* 2018). In contrast, ascorbic acid administration to rats was reported to enhance dopamine-mediated behavioral effects (Ward *et al.* 2013). Additionally, extracellular vitamin C levels in the striatum increase in response to amphetamine, a drug that enhances dopamine transmission (Pierce *et al.* 1992). For example, when amphetamine is applied directly to the animal's striatal neurons, vitamin C enhances the synaptic action of dopaminergic neurons. Vitamin C participates in the catalysis of peptidyl-glycine α-amidated monooxygenase, an enzyme that synthesizes neuropeptide hormones abundant in the hypothalamus and pituitary gland (Eipper and Mains 1991). Vitamin C is also a cofactor in the formation of epoxyeicosatrienoic acid from arachidonic acid catalyzed by cytochrome P450 peroxygenase, which mediates autocrine and paracrine in the nervous system (H. Jiang *et al.* 2012). Glutamate, the

most abundant excitatory neurotransmitter in the brain, triggers the release of vitamin C from astrocytes in the striatum (Nelson *et al.* 2007). As a result, extracellular vitamin C directly regulates neuronal excitability by inhibiting T-type Ca^{2+}-channels, affecting neurotransmission. In addition, vitamin C-mediated redox reactions attenuate the activity of glutamate N-methyl-D-aspartate receptors in forebrain neurons (Sandstrom and Rebec 2007). Taken together, vitamin C affects the synthesis and release of biogenic amines in a wide range of brain regions.

3. Roles of vitamin C in mood states

Evidence from *in vitro* experiments and animal studies on the action of vitamin C in the central nervous system suggests that vitamin C promotes psychological function in humans. However, most of the studies that have investigated the role of vitamin C in brain function have focused on mood states, especially in patients with psychiatric disorders, including depression, anxiety, and schizophrenia. Therefore, the efficacy of vitamin C supplementation for other psychological domains, such as cognition and mental vitality, has been rarely reported in humans. Currently available findings regarding the role of vitamin C in mood states investigated in animal models or clinical studies are presented in **Tables 1** and **Table 2**, respectively.

Depression

Depression presents symptoms like mood disorders, psychomotor disturbances, and physical disabilities (Comstock and Helsing 1977). A number of animal studies have shown the phenotypic effects of vitamin C administration on depressive behavior assessed by the forced swimming test, tail suspension test, and novelty-suppressed feeding test (Fraga *et al.* 2020; Goyal, Gupta, and Verma 2016; Shivavedi *et al.* 2017; Shivavedi *et al.* 2019). In addition, several mechanisms by which vitamin C exerts antidepressant effects have been elucidated through *in vivo* experiments. In this regard, vitamin C has been reported to modulate the synaptic activity of various neuronal receptors and ion channels, including serotonin 1A receptors, gamma-aminobutyric acid receptors, N-methyl-D-aspartate receptors, and potassium channels (Binfaré *et al.* 2009; Acuña *et al.* 2013; Rosa *et al.* 2016).

Specifically, vitamin C activates the serotonin 1A receptor and inhibits the activity of the N-methyl-D-aspartate receptor and potassium channel. In addition, vitamin C increases the phosphorylation of S6 kinase beta-1 and extracellular signal-regulated kinases. On the other hand, vitamin C decreases the phosphorylation of p38 mitogen-activated protein kinase in the hippocampus and cortex (Fraga *et al.* 2020; Moretti *et al.* 2016; Moretti *et al.* 2015b). Furthermore, a previous mouse model experiment has shown that vitamin C modulates neurotransmitter release by increasing synapsin 1 levels in nerve endings and increases dendritic spin density in certain brain regions (Fraga *et al.* 2020). In addition to *in vivo* experiments, several epidemiological studies have shown an association between vitamin C status and depression. However, the antidepressant effects of vitamin C administration on depression in patients are not as dramatic as its effects in experimental animals.

Anxiety

Anxiety is a psychiatric disorder caused by dysregulation of the hypothalamic-pituitary-adrenal axis and dysfunction of neurotransmitters such as gamma-aminobutyric acid, serotonin, and noradrenaline (Grillon 2008). It has been suggested that there is a direct correlation between oxidative stress levels and anxiety-like behaviors (Hughes, Lowther, and van Nobelen 2011). Although the mechanisms by which vitamin C alleviates anxiety are not fully understood, preclinical studies have provided insight into the underlying actions of vitamin C. In this regard, vitamin C modulates the neurotransmitter activity, attenuates the cortisol activity, and mitigates oxidative stress in the brain. In rats, mice, and zebrafish models with anxiety symptoms, vitamin C administration significantly

alleviated or abolished anxiolytic-like effects (Fraga *et al.* 2018; de Almeida *et al.* 2014; Walia, Garg, and Garg 2019; Olajide *et al.* 2017; Narayanan *et al.* 2010; Raeis-Abdollahi *et al.* 2019; Puty *et al.* 2014). A cross-sectional study has shown that patients with anxiety have lower circulating vitamin C levels than controls (Gautam *et al.* 2012). Two intervention trials have reported that vitamin C supplementation significantly reduces the severity of anxiety compared to the placebo or other antioxidant vitamins (de Oliveira *et al.* 2015; Mazloom, Ekramzadeh, and Hejazi 2013).

Schizophrenia

Schizophrenia is a neuropsychiatric disorder estimated to affect 1% of the world's population, and its symptoms and pathophysiology are highly complex (Javitt 2012; Arroll, Wilder, and Neil 2014). Symptoms of schizophrenia include hallucinations, paranoia, delusions, lack of motivation, weakened speech, and reduced social functioning. The cause of schizophrenia is still not fully understood, and much controversy exists. However, insufficient dopamine levels due to the loss of dopamine-producing cells appear to be associated with schizophrenia (Wabaidur, ALOthman, and Naushad 2012). However, some argue that the overactivity of the dopaminergic system causes abnormalities in neurotransmission mediated by N-methyl-D-aspartate receptor receptors (Javitt 2012). In addition, there is growing evidence that oxidative stress may be associated with schizophrenic pathology (Morera-Fumero *et al.* 2017). Antipsychotics administration is known to induce a large amount of free radicals in the body, effectively suppressed by coadministration of vitamin C in the rat model (Heiser *et al.* 2010). However, there were no significant differences in plasma vitamin C

concentrations between schizophrenic patients and controls in a case-control study (Young *et al.* 2007). On the contrary, a small-scale intervention trial has reported that supplementation with antioxidants combination, including vitamin C, significantly alleviates symptoms of schizophrenia compared to baseline (Sivrioglu *et al.* 2007).

Psychological distress

Several studies have reported the effects of vitamin C supplementation on psychological distress, such as tension, anger, and stress (Pullar *et al.* 2018; M. Zhang *et al.* 2011; Wang *et al.* 2013; Kennedy *et al.* 2010; Carr *et al.* 2013; Conner *et al.* 2020; Brody *et al.* 2002). Most studies assessed psychological distress levels using the Profile of Mood States (POMS), a self-reported questionnaire. After supplementation with 500 mg of vitamin C twice daily for one week, POMS scores at the endpoint were lower than baseline in acutely hospitalized patients (M. Zhang *et al.* 2011). Another clinical trial involving acutely hospitalized patients found that daily supplementation with 500 mg of vitamin C twice daily reduced POMS scores compared to supplementation with 5000 IU of vitamin D *(*Wang *et al.* 2013). Two clinical trials reported the efficacy of vitamin C supplementation for reducing psychological distress in a healthy population. Compared to placebo, vitamin C supplementation significantly reduced mood disturbance assessed using the POMS (Conner *et al.* 2020). Brody *et al* reported that vitamin C supplementation at 3000 mg/day significantly reduced psychological stress responses to public speaking and mental arithmetic test compared to placebo (Brody *et al.* 2002).

Table 1. Animal studies investigating the role of vitamin C in mood disorders

Disease	Animal	Treatment	Main findings	References
Depression	Mouse	A single dose of 10 mg/kg, orally	↑ Antidepressant-like effect (↓ latency to feed in the novelty-suppressed feeding test); ↑ S6K1(p70S6K) phosphorylation and synapsin 1 immunocontent; ↑ dendritic spin density in the mature ventral dentate gyrus, but no effect in the dorsal dentate gyrus	(Fraga *et al.* 2020)
Depression	Mouse	A single dose of 0.065 mg/g or 0.13 mg/g, intraperitoneally	↑ Antidepressant-like effect (↓ immobility time in forced swimming test and tail suspension test)	(Goyal, Gupta, and Verma 2016)
Depression	Streptozotocin and foot-shock-induced depression/diabetes rat	25 mg/kg, orally for 11 days	↑ Antidepressant-like effect (↓ immobility period); lipid peroxidation in the prefrontal cortex; ↓ reduction of serotonin and noradrenaline levels; ↓ TNF-α and IL-6 levels in the prefrontal cortex	(Shivavedi *et al.* 2017)
Depression	Streptozotocin and foot-shock-induced depression/diabetes rat	10-400 mg/kg, orally for 11 days	↑ Antidepressant-like effect (↓ immobility dose-dependently); ↓ hypercorticosteronemia and adrenal hyperplasia; ↑ IL-10 levels and ↓ lipid peroxidation in the prefrontal cortex	(Shivavedi *et al.* 2019)

Depression	Mouse	0.1-1 mg/kg, orally by 21 days	↑ Antidepressant-like effect (↓ immobility time in tail suspension test); ↑ PKB (Akt) phosphorylation in cerebral cortex; ↓ p38 MAPK phosphorylation	(Moretti *et al.* 2015b)
Depression	Inflammation (TNF-α)-induced depression mouse	Pretreatment 0.1-10 mg/kg, orally	Prevention of TNF-α-induced depression; ↑ synergic antidepressant-like effect with classical antidepressants; ↓ p38 MAPK phosphorylation in hippocampus and cerebral cortex; ↑ ERK1 phosphorylation in the hippocampus	(Moretti *et al.* 2015a)
Depression	Chronic unpredictable stress-induced depression mouse	A single dose of 0.1 mg/kg with ketamine, orally	↓ Depressive-like behavior (↓ immobility time in tail suspension test)	(Moretti *et al.* 2019)
Anxiety	Mouse	1, 3 and 10 mg/kg, orally	↑ Anxiolytic-like effect (↑ total time spent in the open arms of elevated plus maze and in the center of the open field test; ↓ rearing responses; ↑ latency to grooming; ↓ rostral grooming; ↑ total time in light area in the light/dark preference test)	(Fraga *et al.* 2018)
Anxiety	Mouse	Acute or repeated 250 mg/kg, intraperitoneally up to 14 days	↑ Anxiolytic-like effect (↓ the number of marbles buried in the marble burying test)	(de Almeida *et al.* 2014)

Anxiety	Mouse	100 mg/kg, intraperitoneally	↑ Anxiolytic-like effect (↑ time spent in the light compartment on light/dark box paradigm and in the open arm of elevated plus maze test;	(Walia, Garg, and Garg 2019)
Anxiety	Aluminum chloride-induced AD rat	100 mg/kg daily for 15 days	↓ Anxiety behavior (↑ time spent in the open arm of elevated plus maze)	(Olajide *et al.* 2017)
Anxiety	Rat	100 mg/kg, orally for 3 weeks	Abolished Monosodium glutamate-induced anxiety (↑ time spent in the open arm of elevated plus maze)	(Narayanan *et al.* 2010)
Anxiety	Prenatal exposure of chrysotile asbestos to rat	500 mg/kg three-times repeated intraperitoneal injections with chrysotile asbestos	Abolished chrysotile asbestos-induced anxiolytic-like effects; ↓ lipid peroxidation in the hippocampus	(Raeis-Abdollahi *et al.* 2019)
Anxiety	Methylmercury-induced anxiolytic zebrafish	Pretreatment 2 mg/g, intraperitoneally	Abolished methylmercury-induced anxiolytic-like effects (improvement in light/dark preference test; ↑ extracellular serotonin level)	(Puty *et al.* 2014)
Schizophrenia	Rat	1 mM of vitamin C incubation with antipsychotics (haloperidol, clozapine and olanzapine)	↓ Antipsychotics-induced formation of reactive oxygen species in the whole blood of rats	(Heiser *et al.* 2010)

S6K1, S6 kinase beta-1; TNF-α; tumor necrosis factor α; IL-6; interleukin 6; IL-10, interleukin 10; PKB, protein kinase B; p38 MAPK, p38 mitogen-activated protein kinases; ERK, extracellular signal-regulated kinases.

Table 2. Clinical studies investigating the role of vitamin C in mood states

Mood	Population	Study design	Main findings	References
Depression	Patients (*n* = 322; ≥65 y)	Cross-sectional study	↑ Higher depressive symptoms in patients with plasma vitamin C concentrations of less than 11 μmol/L compared to those with ≥11 μmol/L	(Gariballa 2014)
Depression	Adolescent girls (*n* = 849; 12-18 y)	Cross-sectional study	↓ High vitamin C intake was associated with ↑ low depression levels	(Kim *et al.* 2015)
Depression	Controls and unipolar depression patients (*n* = 100)	Case-control study	↓ Lower serum vitamin C concentration in patients compared to controls	(Bajpai *et al.* 2014)
Depression	Controls and depression patients (*n* = 60)	Case-control study	↓ Lower vitamin C intake in MDD patients compared to controls	(Prohan *et al.* 2014)
Depression	Depression patients (*n* = 24)	A randomized, double-blind, placebo-controlled trial; fluoxetine plus 1000 mg/day of vitamin C or placebo for 6 months	↓ Lower depressive symptoms in vitamin C supplementation group compared to the placebo group	(Amr *et al.* 2013)
Depression	MDD patients (*n* = 43)	A randomized, double-blind,	No effect on depression compared to the	(Sahraian,

		placebo-controlled trial; citalopram plus vitamin C or placebo for 8 weeks	placebo groups	Ghanizadeh, and Kazemeini 2015)
Depression	Diabetic patients (*n* = 45)	A randomized, single-blind, placebo-controlled trial; 1000 mg/day of vitamin C, 400 IU/day of vitamin E, or placebo for 6 weeks	No effect on depression compared to the vitamin E or placebo group	(Mazloom, Ekramzadeh, and Hejazi 2013)
Depression	Healthy adults (*n* = 81)	A randomized, double-blind, placebo-controlled trial; 3000 mg/day of vitamin C or placebo for 14 days	No effect on depression compared to the placebo group	(Brody 2002)
Anxiety	Controls and patients with GAD and depression (*n* = 80; 20-60 y)	Case-control study	↓ Lower serum vitamin C concentrations in patients compared to controls	(Gautam *et al.* 2012)
Anxiety	High school students (*n* = 42)	A randomized, double-blind, placebo-controlled trial; 500 mg/day of vitamin C or placebo	↓ Lower anxiety levels (↓ scores in Beck Anxiety Inventory) compared to the placebo group.	(de Oliveira *et al.* 2015)
Anxiety	Diabetic patients (*n* = 45)	A randomized, single-blind,	↓ Lower anxiety levels in the vitamin C	(Mazloom,

		placebo-controlled trial; 1000 mg/day of vitamin C or 400 IU/day of vitamin E or placebo for 6 weeks	supplementation group (↓ scores in Depression Anxiety Stress Scales) compared to other groups	Ekramzadeh , and Hejazi 2013)
Schizophrenia	Controls and schizophrenia patients (*n* = 34)	Case-control study	No difference between cases and controls in plasma vitamin C concentrations	(Young *et al.* 2007)
Schizophrenia	Schizophrenia patients (*n* = 17)	Intervention study; antioxidant complex (1000 mg/day of vitamin C, 1000 mg/day of omega-3 fatty acids and 400 IU/day of vitamin E) for 16 weeks	↓ Reduction in symptoms of schizophrenia (↓ scores in Brief Psychiatric Rating Scale, Scale for the Assessment of Negative Symptoms, Simpson Angus Scale, and Barnes Akathisia Rating Scale) after the intervention	(Sivrioglu *et al.* 2007)
Psychological distress	Healthy men (*n* = 139, 18-35 y)	Cross-sectional study	↑ High plasma vitamin C concentrations were associated with ↓ low psychological distress (↓ scores in Profile of Mood States)	(Pullar *et al.* 2018)
Psychological distress	Acutely hospitalized patients (*n* = 55)	A randomized, double-blind, active-control trial; 1000 mg/day of vitamin C or 2000 IU/day of vitamin D for 10 days	↓ 34% reduction in psychological distress (↓ scores in Profile of Mood States) compared to baseline	(M. Zhang *et al.* 2011)
Psychological	Acutely hospitalized	A randomized, double-blind,	↓ Reduction in psychological distress (↓	(Wang *et al.*

distress	patients (*n* = 46)	active-control trial; 1000 mg/day of vitamin C or 5000 IU/day of vitamin D for a mean of 8-day	scores in Profile of Mood States) compared to the vitamin D supplementation group	2013)
Psychological distress	Healthy men (*n* = 215, 30-55 y)	A randomized, double-blind, placebo-controlled trial; multi-vitamin/mineral supplement (Berocca®) with B-complex, vitamin C (500 mg) and minerals or placebo for 33-day	↓ Lower stress levels (↓ scores in Perceived Stress Scale) at the endpoint compared to the placebo group	(Kennedy *et al.* 2010)
Psychological distress	Healthy adults (*n* = 167, 18-35 y)	A placebo-controlled trial; kiwifruit, 250 mg/day of vitamin C, or placebo; cross-over; 4-week intervention and 2-week wash-out	↓ Reduction in psychological distress (↓ scores in Profile of Mood States) compared to the placebo group.	(Conner *et al.* 2020)
Psychological distress	Healthy adults (*n* = 108, 20-40 y)	A randomized, double-blind, placebo-controlled trial; 3000 mg of vitamin C or placebo for 14 days	↓ Lower subjective stress responses to acute psychological stress induced by public speaking and mental arithmetic compared to the placebo group.	(Brody *et al.* 2002)

MDD, major depressive disorder. GAD, generalized anxiety disorder.

4. Recommended dietary allowance and toxicity of vitamin C

The recommended dietary allowance is defined as the dietary intake level that is sufficient to meet the nutrient requirement of nearly all healthy individuals (Krinsky *et al.* 2000). The average requirement of vitamin C for Korean adults is estimated at 75 mg/day, which is the amount that can maintain a nearly saturated level of vitamin C in white blood cells with little urinary excretion. Accordingly, the recommended intake of vitamin C was calculated as 100 mg/day for Korean adults considering the individual coefficient of variation of 10%. Recently, the German, Austrian, and Swiss nutrition societies have revised the reference values for the intake of vitamin C as 110 mg/day for men and 95 mg/day for women to compensate for metabolic losses of vitamin C and ensure a fasting plasma ascorbate concentration of 50 μmol/L. Additionally, Frei *et al* have suggested 200 mg/day as the optimal vitamin C intake to maximize the health benefits of vitamin C and minimize the risk of adverse health effects (Frei, Birlouez-Aragon, and Lykkesfeldt 2012). The Tolerable Upper Intake Level (UL) indicates the maximum daily nutrient intake without the risk of adverse health effects for nearly all individuals. For vitamin C, the UL for adults is usually set at 2 g/day. A number of clinical trials have evaluated the safety of vitamin C intake in excess of recommended dietary allowance. They have not provided a pattern of evidence to support concerns about safety other than intermittent gastrointestinal upset or mild diarrhea. Gastrointestinal disturbances, such as nausea, abdominal cramps, and diarrhea, are the most common side effects of high vitamin C intake (Hoffer 1973). Some studies have reported gastrointestinal effects, such as diarrhea and transient colic, at doses of 3 to 4 g/day in healthy adults (Cameron and Campbell 1974). In

another study evaluating the adverse effects of ascorbic acid supplementation at 1, 5, and 10 g for 5 days in healthy adults, diarrhea was reported in 2 of 15 subjects at 10 g/day (Wandzilak *et al*. 1994). Another risk of high-dose vitamin C intake is increased oxalate excretion and kidney stone formation. However, no clinical significance in urinary oxalate excretion was observed in most of epidemiological studies involving healthy subjects with 3-10 g/day of ascorbic acid consumption for at least 2 years (Tsao and Salimi 1984). Another possible side effect of high vitamin C intake is increased iron absorption, leading to iron overload. However, the literature suggests that vitamin C intake does not increase iron absorption beyond recommended levels. In healthy adults, iron stores were not increased after 2 g/day of vitamin C intake for up to 20 months (Cook *et al*. 1984). These findings suggest that vitamin C does not induce excessive iron absorption in healthy individuals. Ascorbate can act as a pro-oxidant by reducing iron and copper ions, which induce hydroxyl radical production through the Fenton reaction (Buettner and Jurkiewicz 1996). Combining ascorbic acid with unbound iron can promote lipid peroxidation *in vitro* (Laudicina and Marnett 1990). However, *in vivo* iron usually cannot perform these catalytic reactions because it binds to proteins such as transferrin and ferritin. Moreover, epidemiological studies show that high-dose vitamin C intake did not affect the levels of oxidative damage in healthy adults and is not associated with an increased risk of oxidative stress-related diseases, such as heart disease (Berger *et al*. 1997). Taken together, the evidence from clinical trials concludes that even high doses of vitamin C, up to 2000 mg per day, are safe for most adults.

5. Assessment of human vitamin C status

Vitamin C concentrations in plasma or serum

Physiological absorption and body storage of vitamin C can be evaluated using plasma and serum levels (Dehghan *et al.* 2007; Mitmesser *et al.* 2016). The blood vitamin C concentrations are directly correlated with usual dietary intake of vitamin C (Dehghan *et al.* 2007; Brubacher, Moser, and Jordan 2000). In particular, the fasting plasma or serum concentration of ascorbic acid indicates total body pool levels and relatively long-term vitamin C intake (Sebastian J Padayatty and Levine 2016). Therefore, fasting plasma or serum vitamin C concentrations are most frequently used to provide a criterion for vitamin C status assessment. In general, scurvy, a vitamin C deficiency disease, is clinically manifest at circulating vitamin C concentrations less than 11 μmol/L (Plevin and Galletly 2020; Hodges *et al.* 1969). According to several depletion-repletion studies, plasma vitamin C concentrations increase in a sigmoidal curve when vitamin C intake increases (Basu 1982; Sauberlich 1975; Irwin and Hutchins 1976). When 60 to 100 mg/day of vitamin C is administered to vitamin C-depleted healthy subjects, plasma concentrations show a steep rise to values of approximately 50 μmol/L (Basu 1982; Levine *et al.* 2001; Levine *et al.* 1996). With intakes of more than 100 mg of vitamin C, plasma concentrations of vitamin C increase to about 70-80 μmol/L with a progressive flattening, which is maintained by chronic vitamin C intakes of at least 200 mg/day (Levine *et al.* 1996; Carr *et al.* 2016).

Vitamin C concentrations in circulating cells

Vitamin C concentrations in blood cells, such as neutrophils, monocytes, platelets and lymphocytes, are indicative of dietary intake of vitamin C (Graumlich *et al.* 1997). In particular, the leukocyte vitamin C concentration is considered an indicator of tissue storage of ascorbic acid. Vitamin C is actively transported to leukocytes by the SVCT and is 25- and 80-fold more concentrated in neutrophils and lymphocytes, respectively, than in plasma (Jacob, Planalto, and Agee 1992). In nonsmoking adults, the circulating cellular ascorbate concentration is almost saturated at vitamin C intakes of approximately 100 mg/day. Neutrophils, monocytes, platelets and lymphocytes are saturated with vitamin C at concentrations of approximately 1300, 3000, 3500, and 3500-4000 μmol/L, respectively (Levine *et al.* 1996; Levine *et al.* 2001). Measurement of ascorbic acid in leukocytes is less common in clinical practice because it requires a large volume of blood and the measurements vary depending on the number of cells.

Urinary excretion of vitamin C

Urinary ascorbic acid levels may reflect recent dietary intakes of vitamin C (Levine *et al.* 2001). When ingested more than 1 g/day, vitamin C is mainly excreted in the urine in an unmetabolized form. Some of the absorbed vitamin C are metabolized into oxalic acid, threonic acid, and L-xylose, which are primarily excreted in the urine. When 100 mg/day of vitamin C is consumed, approximately 25% of the vitamin C intake is excreted in the urine (Levine *et al.* 1996; EFSA Panel on Dietetic Products and Allergies 2013). Urinary excretion of ascorbate increases sharply when the plasma vitamin C concentration reaches above around 50 μmol/L, which suggests near-saturation of body pools (Friedman, Sherry, and Ralli 1940; Kallner, Hartmann, and Hornig 1979; Graumlich *et al.* 1997).

6. Optimal vitamin C status in humans

When humans have steady vitamin C intake of 50 mg twice a day, fasting plasma vitamin C concentrations are approximately 45-56 μmol/L, and neutrophil and lymphocyte vitamin C concentrations are 1250 μmol/L and 3100 μmol/L, respectively (Sebastian J Padayatty and Levine 2016). *In vitro* cellular systems, plasma ascorbate concentrations at 50 μmol/L have been shown to effectively inhibit LDL oxidation (Jialal, Vega, and Grundy 1990). In addition, the antioxidant activity of vitamin C, which inhibits superoxide production by activated human neutrophils and macrophages, increases as the extracellular ascorbate concentration increases from 28 to 284 μmol/L, with the greatest rise at the concentration range from 28 to 57 μmol/L (Anderson and Lukey 1987). Further, neutralization of hypochlorous acid, a strong oxidant produced by activated phagocytic leukocytes, increases proportionally with increasing ascorbic acid concentrations at plasma concentrations of 22 to 85 μmol/L (Halliwell, Wasil, and Grootveld 1987). European Food Safety Authority has determined the average requirement of dietary vitamin C based on the quantity that allows fasting plasma concentrations of ascorbate at around 50 μmol/L considering metabolic vitamin C losses and maintenance of an adequate body pool (EFSA Panel on Dietetic Products and Allergies 2013). Furthermore, the risks of coronary heart disease, stroke, cardiovascular disease, total cancer, and mortality showed a 24-30% reduction in the relative risk when the concentration of vitamin C increased to 50 μmol/L (Aune *et al.* 2018). Hence, a fasting serum or plasma vitamin C concentration of 50 μmol/L or greater is generally considered optimal vitamin C status in saturating body pools and optimizing health status.

7. Factors associated with vitamin C status

Demographic factors

Men generally have lower vitamin C status and higher prevalence of deficiency than women (McCall *et al.* 2019). This is in part a result of the volume-dilution effect due to the higher fat-free mass in men (Jungert and Neuhäuser-Berthold 2015). Older adults appear to have an increased need for vitamin C than younger subjects due to lower intestinal absorption of vitamin C (Davies *et al.* 1984). A previous meta-analysis supported the increased requirement of vitamin C in the elderly by estimating that the corresponding value of plasma concentrations for a vitamin C intake of 60 mg/day was lower in older adults compared to younger adults (Brubacher, Moser, and Jordan 2000). However, other studies have suggested that the elderly do not show a higher prevalence of vitamin C deficiency or hypovitaminosis C than younger subjects when dietary vitamin C intake is adequate (Wrieden *et al.* 2000; Newton *et al.* 1983). It has been suggested that low vitamin C intake and comorbidities that increase vitamin C loss from the body contribute to a high prevalence of vitamin C deficiency in elderly people (Salehi *et al.* 2010).

Lifestyle factors

Vitamin C status in the body is primarily determined by dietary intake of vitamin C (Carr and Rowe 2020). Plasma vitamin C concentrations increase in a sigmoidal curve when vitamin C intake increases (Basu 1982; Sauberlich 1975; Irwin and Hutchins 1976). People who take vitamin C-containing supplements have higher average blood levels and a lower prevalence of deficiency than those who do not

take supplements (Carr and Rowe 2020). Epidemiological studies frequently have reported that vitamin C deficiency is prevalent among young adults, even in industrialized countries (L. Cahill, Corey, and El-Sohemy 2009; Schleicher *et al.* 2009). The poor vitamin C status in the young can be attributed to their lifestyle factors such as smoking, excessive drinking, and unhealthy eating habits that fail to provide a fresh and balanced diet that is rich in vitamin C. In addition, smokers have lower plasma levels of vitamin C than non-smokers with same dietary intake of vitamin C. The metabolic turnover of ascorbate in smokers is estimated about 35 mg/day greater than in nonsmokers, which is attributed to increased oxidative stress and other metabolic differences (Kallner, Hartmann, and Hornig 1979). In the Multinational Monitoring of Trends and Determinants in Cardiovascular Disease cohort, 36% of male smokers and 23% of female smokers showed vitamin C deficiency (Wrieden *et al.* 2000). In addition, people who have vigorous physical activities also show increased vitamin C utilization in the body because vitamin C is critical for fat metabolism that yields energy (Carr and Rowe 2020; EFSA Panel on Dietetic Products and Allergies 2013).

Single nucleotide polymorphism

Several recent studies of human genetic variation provide new evidence for regulating vitamin C homeostasis. In particular, interest in single nucleotide polymorphisms (SNPs), the most common genetic variation in humans, is growing. Polymorphisms in the gene encoding the sodium-dependent vitamin C transport protein are strongly associated with circulating ascorbic acid levels and have the potential to affect the vitamin C status of tissues (Michels, Hagen, and Frei 2013). Notably, more than 150 SNPs have been identified at the gene locus of solute

carrier family 23 member 1 (SLC23A1), many of which have yet to be thoroughly investigated in large populations to determine global allele frequencies. Five SNPs at the SLC23A1 gene locus were investigated at least twice in a large cohort (Amir Shaghaghi *et al.* 2014; de Jong *et al.* 2014; Timpson *et al.* 2010; Duell *et al.* 2013; L.E. Cahill and El-Sohemy 2009; Ravindran *et al.* 2019), and their phenotypic effects are summarized in **Table 3**. The British Women's Heart and Health Study (BWHHS), which involved more than 3,000 British women, found that rs33972313, rs10063949, and rs6596473 were significantly correlated with circulating vitamin C concentrations (Timpson *et al.* 2010). Among them, rs33972313 was found to lower blood vitamin C levels by 4.15 per minor allele, which was repeated in the European Prospective Investigation into Cancer and Nutrition (EPIC) Norfolk cohort. In addition, a meta-analysis of five large cohorts, including BWHHS and EPIC-Norfolk, has shown that the rs33972313-minor allele effect of reducing the blood vitamin C concentration remains valid. Conversely, rs10063949 and rs6596473 were found to increase circulating vitamin C concentrations in people carrying the minor allele. Additionally, in a German cohort, the rs6596473-minor allele increased the risk of aggressive periodontitis by 26% (de Jong *et al.* 2014). However, there was no significant correlation between rs6596473 and the risk of Crohn's disease in a Canadian cohort (Amir Shaghaghi *et al.* 2014). On the other hand, people with rs10063949-major allele were found to show the 2.5-fold increased risk of Crohn's disease in the same cohort. Despite increasing reports in Western populations, SNP studies in Asians have not been sufficiently conducted to establish the global minor allele and genotype frequencies of SNPs present at the SLC23A1 gene locus, and there are few investigations of the relationship between SNPs and vitamin C-related health outcomes.

Table 3. Phenotypic effects of SNPs in the gene encoding SVCT1 protein in humans

SNP ID	Location on chr5	Global MAF	Allele	Phenotypic effects	References
rs6596473	139374887	0.4899	C/G/A	2.86 μM ↑ in circulating vitamin C per minor allele in a British cohort; 1.01 μM ↑ in plasma vitamin C per minor allele in a Norfolk cohort; 1.26 times risk ↑ per minor allele in aggressive periodontitis in a German cohort; No association with Crohn's disease risks in a Canadian cohort; No association with gastric cancer risks in a European cohort; No association with cataract risks in an Indian cohort	(Amir Shaghaghi *et al.* 2014; Timpson *et al.* 2010; de Jong *et al.* 2014; Duell *et al.* 2013; Ravindran *et al.* 2019)
rs33972313	139379813	0.03581	G/A	4.15 μM ↓ in circulating vitamin C per minor allele in a British cohort; 8.31 μM ↓ in circulating vitamin C per minor allele in a Norfolk cohort; 5.98 μM ↓ in circulating vitamin C per minor allele in a meta-analysis of five cohorts; 2.16 times risk ↑ per minor allele in cortical cataract in an Indian cohort; No association with Crohn's disease risks in a Canadian cohort; No association with gastric cancer risks in a European cohort	(Timpson *et al.* 2010; Amir Shaghaghi *et al.* 2014; Duell *et al.* 2013)
rs10063949	139383837	0.4421	A/G	1.91 μM ↑ in circulating vitamin C per minor allele in a British cohort; 2.5 times risk ↑ in Crohn's disease per major allele in a Canadian cohort; No association with plasma vitamin C in a Norfolk cohort.	(Timpson *et al.* 2010; Amir Shaghaghi *et al.* 2014)
rs11950646	139378785	0.4027	A/G	0.14 μM ↓ in plasma vitamin C per minor allele in a European cohort; No association with gastric cancer risks in a European cohort	(L.E. Cahill and El-Sohemy 2009; Duell *et al.* 2013)
rs4257763	139378470	0.4059	G/A	Highest serum vitamin C levels in homozygous minor allele in non-smoking Caucasian cohort	(L.E. Cahill and El-Sohemy 2009)

SNP, single nucleotide polymorphism; chr, chromosome; MAF, minor allele frequency.

8. Vitamins and gut microbiota

In recent years, it has become evident that gut microbiota plays a vital role in maintaining human health and pathogenesis of various diseases, including neuropsychiatric disorders (Carding *et al.* 2015). In particular, there is growing interest in the effects of nutrient intake and supplementation on the gut microbiome and related health outcomes (Berding *et al.* 2021). Several recent epidemiological studies have reported that an imbalance in the gut microbiota and vitamin deficiencies are closely related, which consequently, may directly impact the host's health (Hibberd *et al.* 2017). For example, in a cross-sectional study that included pregnant women, vitamin D intake was inversely correlated with the alpha diversity of the gut microbiota. In addition, the relative abundance of Proteobacteria, known to induce proinflammatory responses, positively correlated with vitamin D and retinol intake while inversely correlated with vitamin E intake. Further, those with higher vitamin E and fiber intakes had lower relative abundances of *Sutterella*, which is implicated with autism (Mandal *et al.* 2016). Another cross-sectional study has observed that lower dietary niacin intake was associated with reduced alpha diversity and the abundance of Bacteroidetes in the obese population (Fangmann *et al.* 2018). A previous intervention study has reported that supplementation of 100 mg of riboflavin for 14 days increased the number of *Faecalibacterium prausnitzii* and *Roseburia* in the feces while decreasing *Escherichia coli* (L. Liu *et al.* 2022). A few epidemiological studies have been published, but most previous studies have primarily focused on vitamin D or B-complex supplementation. Therefore, the effects of vitamin C on the gut microbiota have been rarely investigated.

9. Limitations of previous studies

Overall, much remains to be done in order to clarify the relation between vitamin C status and mental health. Furthermore, few clinical trials have been conducted to provide convincing evidence to support or exclude the efficacy of vitamin C for psychological functioning. Available evidence is primarily from investigations of patients with psychiatric disorders. It is pertinent to note that suboptimal vitamin C status rarely presents with distinct clinical symptoms, which makes it liable to overlook the physiological benefits that healthy people can have after reaching optimal vitamin C levels. In addition, most previous intervention trials did not use reliable biomarkers to assess subjects' vitamin C status, which was replaced by an assessment of dietary vitamin C intake. Moreover, the attainment of optimal vitamin C status was not confirmed at the endpoint. Hence, currently available results from clinical trials are highly contradictory. Therefore, to obtain reliable findings that overcome these limitations, it is necessary to define clear criteria for vitamin C status at baseline and track changes in subjects' vitamin C status during the intervention. Collectively, well-designed intervention trials that can explain the causal relationship between vitamin C and mental function in a healthy population are highly needed.

III. Study 1

Association between vitamin C status and mental vitality: a cross-sectional study

Parts of this work have been published (Sim *et al*. 2022).

1. Introduction

Vitamin C, also known as ascorbic acid, is an indispensable nutrient that participates in several biological reactions in the body (Du, Cullen, and Buettner 2012). Moreover, since humans have lost the ability to synthesize ascorbic acid from glucose, sufficient vitamin C must be supplied from dietary food (Nishikimi and Yagi 1991). Thus, dietary intake of vitamin C is considered critical in determining the vitamin C status of the body, such as blood concentrations (Dehghan *et al.* 2007). However, the response of the vitamin C concentration to a given intake of dietary vitamin C can vary depending on the non-dietary factors, including sex, age, smoking, vigorous physical exercise, and oxidative stress-involving diseases (Carr and Rowe 2020). More recently, emerging evidence has suggested that a single nucleotide polymorphism (SNP), the common genetic variant found in at least one percent of the population, may also explain the inter-individual variation of vitamin C status (Michels, Hagen, and Frei 2013). In particular, the SNP at the solute carrier family 23 member 1 (SLC23A1) gene locus is attracting attention because *SLC23A1* encodes the sodium-dependent vitamin C transporter 1 (SVCT1) (Sotiriou *et al.* 2002). SVCT1 is primarily responsible for regulating intestinal absorption and renal reabsorption of vitamin C, ultimately contributing to whole-body vitamin C homeostasis in humans. Several previous cross-sectional studies have shown that blood vitamin C levels significantly differed depending on the genotype of a certain SNP at the SLC23A1 gene locus, such as rs10063949, rs33972313, and rs6596473 (Amir Shaghaghi *et al.* 2014; de Jong *et al.* 2014; Timpson *et al.* 2010). In addition, rs4257763 SNP, also found in the SLC23A1 gene, have been reported to modify the strength of the correlation

between dietary vitamin C and serum ascorbic acid in healthy populations (L.E. Cahill and El-Sohemy 2009). However, relevant studies are still insufficient to establish the solid evidence in respect to the effect of SNPs in the gene encoding SLC23A1 on vitamin C status in humans. Moreover, the association between the common genetic variation at the SLC23A1 gene locus and vitamin C levels has not been investigated in the Korean population.

The concentration of vitamin C is under strict homeostatic regulation throughout the body, and the distribution of vitamin C is generally thought to reflect the functional requirements of corresponding tissues (Sebastian J Padayatty and Levine 2016). Given that the brain has the highest concentration of vitamin C than any other organ, it is clear that vitamin C plays a pivotal role in maintaining brain function and homeostasis (Fiona E Harrison and May 2009b). To be more specific, preclinical experiments have shown that vitamin C in the brain acts as a major antioxidant and free radical scavenger and is involved in the recycling of other brain antioxidants such as vitamin E (Ballaz and Rebec 2019; Ishaq *et al.* 2013). Vitamin C also participates in neuron maturation, myelin formation, neurotransmitter synthesis, and neuronal signal transmission (Plevin and Galletly 2020; Moretti and Rodrigues 2022). Consistent with the *in vitro* results, animal models with vitamin C depletion or deficiency showed impairment of brain structure and function characterized by cerebral hemorrhage, neurodegeneration, mood disorder, or cognitive decline (Dixit *et al.* 2015). In addition, a few observations from human studies have suggested a relationship between suboptimal vitamin C status and mental function (Moretti, Fraga, and Rodrigues 2017). For example, it has been reported that individuals with lower plasma vitamin C concentrations had a worse cognitive performance (Pearson *et al.* 2017).

In addition to cognitive function, several clinical studies have linked poor vitamin C status with mood disorders, such as depression and anxiety (Moritz *et al.* 2020). However, the currently available evidence from human studies is still highly limited because ascorbic acid has been classically thought to be little related to mental health. Furthermore, previous results have mainly reported the association between severe vitamin C deficiency or depletion and clinical symptoms. Therefore, there has been little investigation on whether vitamin C status in healthy populations is also associated with brain functions such as vitality-related psychological domains.

In this context, the first aim of this study was to determine the association between suboptimal serum vitamin C status and a SNP at the SLC23A1 gene locus. The second aim was to determine the association between serum vitamin C concentrations and mental vitality and mood states in a healthy young population.

2. Materials and Methods

2.1. Participants

The sample size of the cross-sectional study was determined based on the calculation formula (Charan and Biswas 2013) with the prevalence of vitamin C deficiency in young adults previously reported (L. Cahill, Corey, and El-Sohemy 2009), a confidence level of 95%, and a margin of error of 5%. Participants were recruited from Seoul National University from June to August 2018. The inclusion criteria were as follows: young adults aged 20–39 with no acute or chronic disease such as hypertension, diabetes, cardiovascular disease, dyslipidemia, kidney disease, cancer, gastrointestinal disorders, and endocrine disorders. A total of 214 men and women were included in the cross-sectional study. This observational study was performed at the Department of Food and Nutrition at Seoul National University between June and August 2018, and was approved by the Institutional Review Board of Seoul National University (1806/002-009). The study was retrospectively registered at the Clinical Research Information Service on June 1st, 2020 (KCT0005074). All procedures were performed in accordance with the relevant guidelines and regulations. Written informed consent was provided by all participants prior to their inclusion in the study.

2.2. Measurement of general characteristics

All participants attended a laboratory measurement between 0800 and 1000 after a 12-hour fast, avoiding excessive exercise and alcohol consumption during the last 24 hours. General characteristics which might influence serum ascorbic acid concentrations were assessed. During the visit, participants' height (cm) and weight (kg) were measured using an automatic digital scale (BSM330; InBody Co., Ltd., Seoul, Korea). The precision of height and weight measurement was to the nearest 0.1 cm and 0.1 kg, respectively. Body mass index (BMI) was calculated as weight divided by the square of height (kg/m^2). Data on smoking status, alcohol consumption, and vitamin C supplement use were collected using a questionnaire. The International Physical Activity Questionnaire (IPAQ) determined a score of physical activity in metabolic equivalent of task (MET) minutes per week (P.H. Lee, Macfarlane, *et al.* 2011). Before visiting the laboratory, all participants were provided with a standardized two-day dietary recording sheet and reference photographs indicating the portion size. They were encouraged to record immediately after consuming food and beverages with reference to the standardized photographs to minimize errors. During the visit, a trained researcher reviewed completeness and accuracy of the dietary records. Vitamin C intake from food sources was estimated from the dietary records using CAN-Pro web ver. 5.0 (Korean Nutrition Society, Republic of Korea).

2.3. Measurement of serum vitamin C concentrations

When participants were at rest, overnight fasting venous blood samples from the antecubital fossa were collected into 4-mL serum separator tubes (BD Biosciences, Franklin Lakes, NJ, USA). To minimize the oxidation of vitamin C, whole blood samples were protected from light and centrifuged immediately. The separated sera were immediately transported to a deep freezer of -80°C. Within 24 hours after blood collection, high-performance liquid chromatography was performed using a reagent kit to measure serum vitamin C concentrations (Chromsystems Reagent Kit for the Vitamin C Measurement in sera; Chromsystems Instruments & Chemicals GmbH, Gräfelfing, Germany). In brief, 0.1 mL of serum samples were mixed with 0.1 mL of Precipitation Solution in a reaction vial. After incubation for 10 min at 4°C, the samples were centrifuged for 5 min at 9,000 g. 20 μL of supernatant was injected into the HPLC system with a UV-vis detector set at 245 nm wavelength. The column temperature was kept at 20-25°C, with a flow rate of 1.5 mL/min. Injection-to-injection time was 5 min. The assay used a single-point calibrator at 1.53 mg/dL. The intra-assay coefficient of variation (CV) was less than 2.5%, and the inter-assay CV was less than 5%.

2.4. SNP selection and genotyping

Two SNPs, s6596473 and rs33972313 at the SCL23A1 gene locus, were selected for genotyping based on a previous meta-analysis reporting that they had a minor-allele effect on circulating ascorbic acid concentrations (Timpson *et al.* 2010). The venous blood samples from the antecubital fossa were collected into EDTA-coated tubes (BD Biosciences, Franklin Lakes, NJ, USA). Buffy coats were isolated from whole blood samples by centrifugation and immediately stored at -80°C. Genomic DNA was isolated from a buffy coat using the QIAamp® DNA Blood Kit (QIAGEN, Hilden, Germany). Briefly, 200 µL of buffy coat sample was added with 20 µL of QIAGEN Protease and 200 µL of Buffer AL. The mixture was incubated at 56°C for 10 min. After the incubation, 200 µL of 96 % of ethanol was added to the mixture. The mixture was pipetted onto the QIAamp Mini spin column and centrifuged at 14,000 rpm for 1 min. 500 µL of Buffer AW1 was pipetted onto the QIAamp Mini spin column combined with a 2 mL collection tube. The tube was centrifuged at 14,000 rpm for 1 min. 500 µL of Buffer AW2 was pipetted onto the QIAamp Mini spin column in another 2 mL collection tube. The tube was centrifuged at 14,000 rpm for 3 min. Finally, 200 µL of Buffer AE was pipetted onto the QIAamp Mini spin column in a 1.5 mL microcentrifuge tube. After incubation at room temperature for 1 min, the tube was centrifuged at 14,000 rpm for 1 min to elute the DNA. Extracted DNA was quantified by spectrophotometer NanoDrop ND-2000 (Thermo Scientific, Waltham, MA, USA). Genotyping was performed using TaqMan® SNP Genotyping Assay, Human for rs6596473 and rs33972313 (Applied Biosystems, Foster City, CA, USA) and TaqMan® Genotyping Master Mix (Applied Biosystems, Foster City, CA, USA).

Locus-specific TaqMan primers and allele-specific TaqMan probes were predesigned and supplied in an assay kit. Sequences of the primers and probes used in the genotyping is shown in **Table 1**. TaqMan® probes specific to allele 1 had FAM™ as fluorescent reporter dye at the 5' end, and those specific to allele 2 had VIC™ as fluorescent reporter dye at the 5' end. Each had a non-fluorescent quencher with a TaqMan® minor groove binder at the 3' end. Polymerase chain reaction (PCR) was performed on 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) in 25 µL of reaction volume. A total of 13.75 µL per well of the reaction mixture containing 12.5 µL of 2X TaqMan® Master Mix and 1.25 µL of 20X Assay Working Stock was prepared for each PCR. The reaction mixture was pipetted to each well of the reaction plate. The plate was sealed with adhesive film and then centrifuged briefly to bring the reaction mixture to the bottom of the well and eliminate air bubbles. After removing the adhesive film from the plate, 11.25 µL of genome DNA (20 ng) or control was added to each well. All the reactions consisted of one cycle at 95°C for 10 min followed by 40 cycles of amplification at 92°C for 15s and 60°C for 1 min.

Table 1. Sequences of the primers and probes used in the genotyping

SNP ID	Position	Alleles	TaqMan primers	TaqMan probes
rs6596473	chr5:138738475	C/G	F: CATTGAGGCTGCCACTTGAC R: TGCCCATTTAGAGGATGCTAGACT	FAM: CCTATGGGCCTGAGACA VIC: CCTATGGGCGTGAGACA
rs33972313	chr5:138743401	A/G	F: AGACCTCCAGTGCCTTCAGT R: GCAGCACGTCTGTCAAGGT	FAM: TCATGACCGTGTGGCT VIC: CATCATGACCATGTGGCT

SNP, single nucleotide polymorphism; chr, chromosome; F, forward primer; R, reverse primer.

2.5. Assessment of vitality and mood states

Participants' vitality and mood states were assessed using self-reported questionnaires. The levels of attention and fatigue were measured using two items each from the Checklist Individual Strength, which is a widely used multidimensional instrument (Worm-Smeitink *et al.* 2017) ("*When I am doing something, I can keep my thoughts on it*" and "*I find it easy to concentrate*" for attention assessment, with Cronbach's $\alpha = 0.79$; "*I feel tired*" and "*I tired easily*" for fatigue assessment, with Cronbach's $\alpha = 0.84$). Each item was scored with a seven-point Likert scale anchored by "*strongly disagree*" and "*strongly agree,*" with higher scores indicating higher levels of fatigue and attention.

The levels of stress, depression, and positive and negative affect were assessed with Stress Response Inventory (SRI), Beck's Depression Inventory (BDI), and Positive Affect and Negative Affect Schedule (PANAS), respectively. The SRI was designed to assess seven stress factors: tension, aggression, somatization, anger, depression, fatigue, and frustration (Koh *et al.* 2001). It consists of 22 items concerning psychological, physiological, and behavioral stress responses (e.g., "*My body trembles,*" "*My voice is louder than it usually is,*" and "*I have lost my self-confident*"). Each item was scored with a five-point Likert scale anchored by "*strongly disagree*" to "*strongly agree.*" The BDI is one of the most widely used instruments for determining the severity of depression; it consists of a 21-question multiple-choice inventory relating to symptoms of depression, such as hopelessness, irritability, feelings of guilt or being punished, fatigue, weight loss, and lack of interest in sex (Beck and Beamesderfer 1974). Each of the 21 questions contained four statements ordered according to the severity of symptoms (none to

very severe) and each statement was assigned a numeric value from zero to three (e.g., "*I do not feel sad*," "*I feel sad*," "*I am sad all the time and I can't snap out of It*," and "*I am so sad and unhappy that I can't stand it*"). The PANAS is a reliable and well-validated instrument that consists of two 10-item scales to measure both positive (e.g., "*attentive*," "*active*," "*excited*," and "*determined*") and negative ("*hostile*," "*irritable*," "*ashamed*," and "*guilty*") affect (Watson, Clark, and Tellegen 1988). Each item is rated on a five-point scale anchored by "*no at all*" to "*very much.*"

2.6. Statistical analysis

All statistical analyses were performed using SPSS version 28.0.1.1 (SPSS Inc., Chicago, IL, USA). Depending on the general stratification of vitamin C levels based on saturation of body pools and risk of deficiency, participants were categorized as either optimal (≥50 μmol/L) or suboptimal (<50 μmol/L) groups. According to the IPAQ criteria, physical activity was categorized as low, moderate, or high. Estimated dietary intake of vitamin C was categorized into tertiles (the lowest, middle, and the highest). Alcohol consumption frequency was categorized as ≤one day a month, 2-4 days a month, and ≥two days a week. Depending on the normality of the data, an unpaired *t*-test or Mann-Whitney *U* test was performed to compare age, height, weight, BMI, and dietary intake of vitamin C between the optimal and suboptimal groups. A Pearson's Chi-square or Fisher's exact test was performed to compare the sex ratio, the percentage of current smokers and vitamin C supplement users, and the levels of physical activity and dietary intake of vitamin C between the optimal and suboptimal groups. Hardy-Weinberg equilibrium was tested by comparing genotype distributions using a Pearson's Chi-square test. Binary logistic analysis was performed to model the probability of suboptimal serum vitamin C status (<50 μmol/L) depending on the independent variables, including general characteristics and the genotype of rs6596473. Two different models were used to analyze the association of suboptimal vitamin C concentrations with the genotype of rs6596437: Model 1 did not include covariates, whereas Model 2 included sex, dietary intake of vitamin C, and vitamin C supplement use as covariates. The logistic models calculated the odds ratio (OR) and 95% confidence interval (CI). Simple or multiple linear regression analysis

was performed to assess the association of serum vitamin C concentrations with vitality levels or mood states, using each vitality and mood score as a dependent variable and serum vitamin C concentration as an independent variable. Model 1 did not include covariates, whereas Model 2 included sex, age, BMI, physical activity level, current smoking status, and alcohol use as covariates. From linear regression, the unstandardized coefficient (B), 95% confidence interval of B, standard error (SE), and standardized coefficient (β) were calculated. $P < 0.05$ was considered statistically significant.

3. Results

3.1. General characteristics of participants

A total of 250 volunteers were assessed for eligibility based on the inclusion criteria (**Figure 1**). Of the 235 eligible subjects, 214 finally participated in the cross-sectional study, excluding 21 who withdrew their participation (Figure 1). Serum concentrations of vitamin C were analyzed in 214 participants. In the entire population, the concentrations of serum vitamin C ranged from 16.6 to 164.2 μmol/L, with mean and median values of 56.0 μmol/L and 55.5 μmol/L, respectively (**Figure 2**). Serum vitamin C concentrations in male participants ranged from 17.6 to 164.2 μmol/L, with mean and median values of 51.4 μmol/L and 48.5 μmol/L, respectively (Figure 2). For female participants, serum vitamin C concentrations ranged from 16.6 to 119.82 μmol/L, and mean and median values were 58.9 μmol/L and 58.6 μmol/L, respectively (Figure 2). According to their serum vitamin C concentrations, participants were categorized as either optimal (≥50 μmol/L) or suboptimal (<50 μmol/L) groups (**Table 2**). Among 214 participants (84 men and 130 women; 26.2 ± 3.9 y), those whose serum concentrations of vitamin C were below 50 μmol/L accounted for 38% (n = 81) (Table 2). The sex ratio was significantly different between the optimal and suboptimal groups ($p < 0.001$) (Table 2). The men in the suboptimal group had a lower height than those in the optimal group ($p = 0.003$), while no significant difference in height was found between the optimal and suboptimal groups in the female participants (Table 2). The percentage of vitamin C supplement users was higher in the optimal group compared to the suboptimal group (optimal, 41%;

suboptimal, 17%) ($p < 0.001$) (Table 2). Vitamin C intake from foods was higher in the optimal group than the suboptimal group (optimal, 71.2 ± 63.1; suboptimal, 51.3 ± 32.6) ($p = 0.018$) (Table 2). No significant difference was found between the two groups in the rest of the general characteristics, including age, weight, BMI, current smoking status, physical activity level, and alcohol consumption frequency (Table 2).

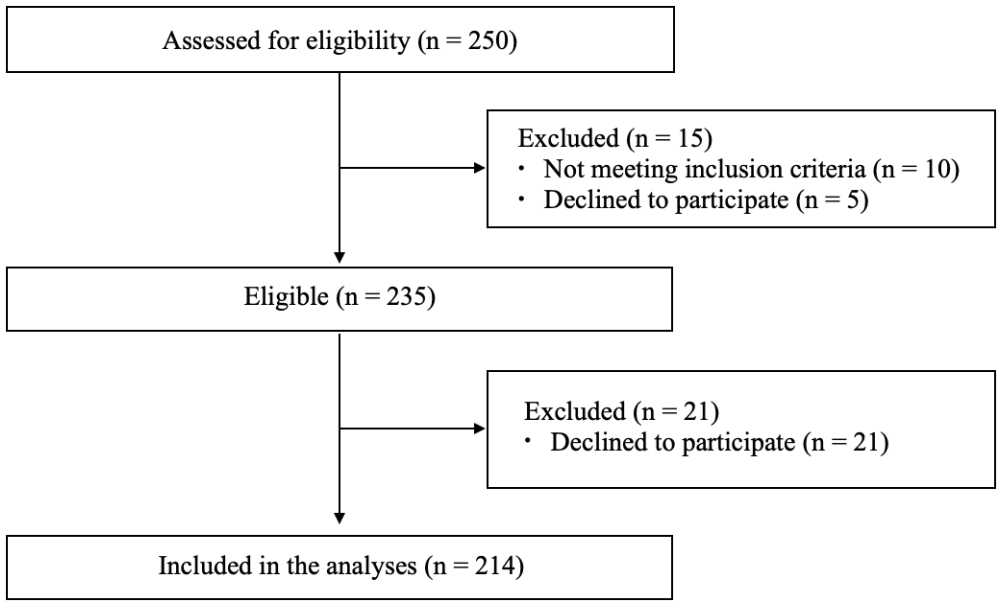

Figure 1. Flowchart of participation throughout the course of the cross-sectional study

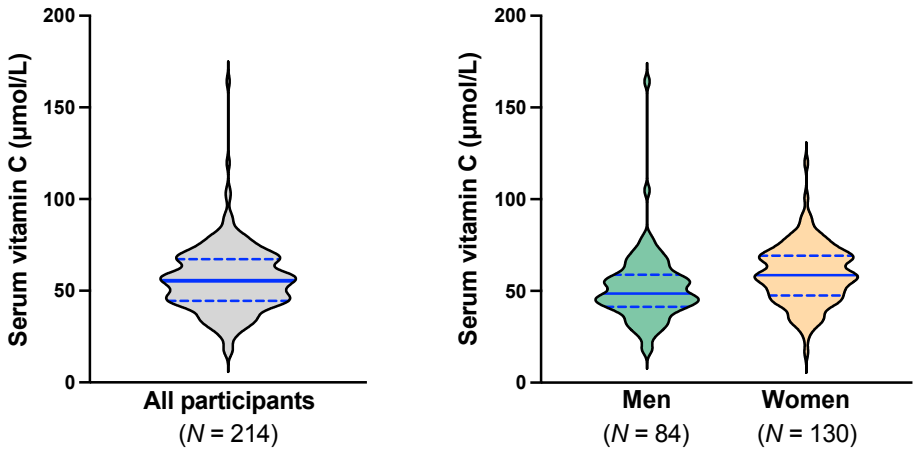

Figure 2. Distribution of serum vitamin C concentrations in the cross-sectional population

Fasting blood samples were collected from 214 healthy men (n = 84) and women (n = 130) in the resting state. The serum concentrations of vitamin C were determined using high-performance liquid chromatography within 24 hours of the blood collection. The width of the plot is proportional to the density of the values. The median values are shown in solid blue lines. Lower and upper dashed lines indicate the first and the third quartiles, respectively.

Table 2. General characteristics of participants

Characteristics	Total	Optimal	Suboptimal	*P*
Subject, *n* (%)	214 (100)	133 (62.1)	81 (37.9)	-
Sex, *n* (%)				<0.001
Men	84 (39.3)	40 (30.1)	44 (54.3)	
Women	130 (60.7)	93 (69.9)	37 (45.7)	
Age, y	26.2 ± 3.9	26.3 ± 3.7	26.1 (5.1)	0.724
Height, cm	167.0 ± 7.5	166.5 ± 7.6	167.7 (7.3)	0.260
Men	174.1 ± 5.3	175.8 ± 4.8	172.5 ± 5.4	0.003
Women	162.4 ± 4.6	162.5 ± 4.5	162.0 ± 4.9	0.571
Weight, kg	62.5 ± 13.0	61.3 ± 12.0	64.3 ± 14.4	0.177
Men	73.9 ± 11.5	74.4 ± 10.5	73.3 ± 12.5	0.666
Women	55.1 ± 7.5	55.7 ± 7.4	53.5 ± 7.6	0.129
BMI, kg/m^2	22.2 ± 3.3	22.0 ± 2.9	22.6 ± 3.8	0.274
Men	24.3 ± 3.3	24.0 ± 2.7	24.6 ± 3.8	0.430
Women	20.9 ± 2.4	21.1 ± 2.5	20.3 ± 2.3	0.116
Current smoking, *n* (%)				0.690
No	200 (93.5)	125 (94.0)	75 (92.6)	
Yes	14 (6.5)	8 (6.0)	6 (7.4)	
Physical activity, MET-min/wk	2218 (1706)	2269 (1711)	2133 (1705)	0.572
Low, *n* (%)	32 (15.0)	21 (15.8)	11 (13.6)	
Moderate, *n* (%)	114 (53.3)	70 (52.6)	44 (54.3)	0.907
High, *n* (%)	68 (31.8)	42 (31.6)	26 (32.1)	
Alcohol consumption, *n* (%)				0.527
≤1 day a month	76 (35.5)	51 (38.3)	25 (30.9)	

2-4 days a month	100 (46.7)	60 (45.1)	40 (49.4)	
≥2 days a week	38 (17.8)	22 (16.5)	16 (19.8)	
Dietary intake of vitamin C, mg/d	63.7 ± 54.4	71.2 ± 63.1	51.3 ± 32.6	0.018
Highest, *n* (%)	71 (33.2)	29 (34.5)	42 (32.3)	
Middle, *n* (%)	71 (33.2)	31 (36.9)	40 (30.8)	0.425
Lowest, *n* (%)	72 (33.6)	24 (28.6)	48 (36.9)	
Vitamin C supplement use, *n* (%)				<0.001
No	146 (68.2)	79 (59.4)	67 (82.7)	
Yes	68 (31.8)	54 (40.6)	14 (17.3)	

Values are mean ± SD or *n* (%). *P*-values were obtained by comparing the optimal and suboptimal groups using an unpaired *t*-test, Mann-Whitney *U* test, Pearson's Chi-square test, or Fisher's exact test. Dietary intake of vitamin C was categorized into tertiles: the lowest tertile (10.5–36.4 mg/d), the middle tertile (36.5–61.4 mg/d), and the highest tertile (61.5–368.32 mg/d). Optimal, participants whose serum vitamin C concentrations were ≥50 μmol/L; Suboptimal, participants whose serum vitamin C concentrations were <50 μmol/L; MET; metabolic equivalent of task.

3.2. Genotype frequency of SNP rs6596473 at the SLC23A1 gene locus and distribution of serum vitamin C concentrations in each genotype

Two hundred fourteen participants were genotyped for the single nucleotide polymorphisms of rs6596473 and rs33972313 at the SCL23A1 gene locus. For rs33972313, the minor allele frequency was determined at less than 1%; therefore, rs33972313 was excluded from the subsequent analyses.

For rs6596473, the major and minor alleles were C and G, respectively, and the minor G-allele frequency was 0.3461 in the entire population (**Table 3**). The rs6596473-CG heterozygotes accounted for 50% (n = 108) of the total study population, the highest among the three genotypes (Table 3). In the entire population, rs6596473-CC and -GG homozygotes accounted for 37% (n = 79) and 13% (n = 27), respectively (Table 3). In the optimal group, the minor allele frequency was 0.3421, comparable to that of the entire population (Table 3). In the optimal group, CC, CG, and GG for rs6596473 accounted for 41% (n = 54), 50% (n = 67), and 9% (n = 12), respectively (Table 3); and the genotype distributions of the optimal group did not significantly differ from those of the entire population (data not shown). In the suboptimal group, the MAF was 0.4383, which was relatively higher than that of the optimal group. However, the suboptimal group showed no significant difference in genotype distributions compared to the optimal group (data not shown), showing the highest frequency in CG and the lowest frequency in GG (Table 3).

In the rs6596473-CC homozygous carriers, serum vitamin C concentrations ranged from 29.8 to 89.1 μmol/L, with a median of 56.4 μmol/L (**Figure 3**). Individuals with rs6596473-CG heterozygotes had serum vitamin C

concentrations ranging from 16.6 to 119.8 μmol/L, with a median of 55.2 μmol/L (Figure 3). Serum concentrations of vitamin C in the rs6596473-GG homozygous carriers ranged from 29.5 to 164.2 μmol/L (Figure 3). The median vitamin C concentration in the GG group was 48.8 μmol/L, which was relatively lower than that in the genotypes CC and CG (Figure 3). However, there was no significant difference in serum concentrations of vitamin C between genotypes (data not shown).

Table 3. Minor allele and genotype frequencies of SNP rs6596473 at the SLC23A1 gene locus

rs6596473	Total (*n* = 214)	Optimal (*n* = 133)	Suboptimal (*n* = 81)
MAF	0.3461	0.3421	0.4383
Genotype, *n* (%)			
CC	79 (36.9)	54 (40.6)	25 (30.9)
CG	108 (50.5)	67 (50.4)	41 (50.6)
GG	27 (12.6)	12 (9.0)	15 (18.5)

Each frequency of the rs6596473 genotype is presented as *n* (%). SNP, single nucleotide polymorphism; SLC23A1, solute carrier family 23 member 1; Optimal, participants whose serum vitamin C concentrations were ≥50 μmol/L; Suboptimal, participants whose serum vitamin C concentrations were <50 μmol/L; MAF, minor allele frequency (G-allele is a minor allele for rs6596473).

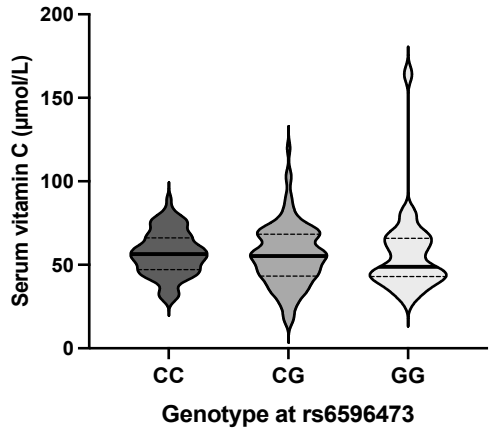

Figure 3. Distribution of serum vitamin C concentrations according to the genotype of rs6596473

Violin plots show the distribution of serum concentrations of vitamin C according to the genotype of rs6596473 at the gene encoding a solute carrier family 23 member 1 (CC, *n* = 79; CG, *n* = 108; GG, *n* = 27). The width of the plot is proportional to the density of the values. The median values are shown in solid lines. Lower and upper dashed lines indicate the first and the third quartiles, respectively.

3.3. Association between suboptimal serum vitamin C concentrations and SNP rs6596473 at the SLC23A1 gene locus

First, the association between suboptimal serum vitamin C concentrations and general characteristics was analyzed (**Table 4**). As expected, dietary intake of vitamin C and vitamin C supplement use were strongly associated with suboptimal serum vitamin C concentrations. More specifically, individuals with dietary intake of vitamin C in the middle tertile were 2.9 times more likely to have suboptimal serum vitamin C concentrations than those with dietary intake of vitamin C in the highest tertile (OR, 2.888; 95% CI, 1.291–6.461; $p = 0.010$) (Table 4). Individuals with dietary intake of vitamin C in the lowest tertile were four times more likely to have suboptimal serum vitamin C concentrations than those with dietary intake of vitamin C in the highest tertile (OR, 3.957; 95% CI, 1.773–8.832; $p < 0.001$) (Table 4). In addition, individuals who did not use vitamin C-containing supplements were 4.4 times more likely to have suboptimal serum vitamin C concentrations than those who regularly took vitamin C supplements (OR, 4.443; 95% CI, 2.099–9.405; $p < 0.001$) (Table 4). Also, men were 4.6 times more likely than women to have suboptimal concentrations of vitamin C in their sera (OR, 4.586; 95% CI, 2.101–10.01; $p < 0.001$) (Table 4). Suboptimal serum vitamin C status was not associated with age, BMI, physical activity level, alcohol consumption frequency, and current smoking status (Table 4).

Next, binary logistic analysis was performed to determine whether SNP rs6596473 at the SLC23A1 gene locus was associated with suboptimal serum vitamin C concentrations (**Table 5**). In Model 1, no adjustment was performed. In Model 2, variables in sex, dietary intake of vitamin C, and vitamin C supplement

use were designated as covariates based on the finding that they were associated with suboptimal serum vitamin C status. Individuals with rs6596473 heterozygotes (CG) did not differ in the possibility to have suboptimal serum vitamin C concentrations compared to those with rs6596473-major allele homozygotes (CC) both in the unadjusted and adjusted models (Table 5). On the other hand, rs6596473-minor allele homozygous carriers (GG) were 2.7 times more likely to have suboptimal serum vitamin C concentrations than rs6596473-major allele homozygous carriers (CC) in the unadjusted model (Model 1: OR, 2.700; 95% CI, 1.103–6.608; $p = 0.030$) (Table 5). After adjusting for sex, vitamin C intake levels, and vitamin C supplement use, individuals with rs6596473-GG homozygotes were 2.8 times more likely to have suboptimal serum vitamin C concentrations than those with rs6596473-CC homozygotes (Model 2: OR, 2.780; 95% CI, 1.058–7.304; $p = 0.038$) (Table 5).

Table 4. Association between suboptimal serum vitamin C concentrations and general characteristics

Variable (unit or no.)	OR	95% CI	*P*
Sex			
Women (130)	Reference	Reference	-
Men (84)	4.586	2.101, 10.01	<0.001
Age (y)	0.986	0.909, 1.071	0.746
BMI (kg/m^2)	0.958	0.857, 1.071	0.455
Physical activity			
Low (32)	Reference	Reference	-
Moderate (114)	1.398	0.554, 3.526	0.478
High (68)	1.001	0.359, 2.793	0.997
Alcohol consumption			
≤1 day a month (76)	Reference	Reference	-
2-4 days a month (100)	0.984	0.478, 2.026	0.965
≥2 days a week (38)	1.063	0.428, 2.645	0.895
Current smoking			
No (200)	Reference	Reference	-
Yes (14)	0.722	0.202, 2.577	0.616
Dietary intake of vitamin C			
Highest (71)	Reference	Reference	-
Middle (71)	2.888	1.291, 6.461	0.010
Lowest (72)	3.957	1.773, 8.832	<0.001
Vitamin C supplement			
Users (68)	Reference	Reference	-
Nonusers (146)	4.443	2.099, 9.405	<0.001

Statistics were obtained by analyzing the association between suboptimal serum vitamin C concentrations (<50 μmol/L) and general characteristics using binary logistic analysis. OR, odds ratio; CI, confidence interval.

Table 5. Association between suboptimal serum vitamin C concentrations and SNP rs6596473 at the SLC23A1 gene locus

rs6596473 genotype (*n*)	OR	95% CI	*P*
Model 1			
CC (79)	Reference	Reference	-
CG (108)	1.322	0.716, 2.440	0.372
GG (27)	2.700	1.103, 6.608	0.030
Model 2			
CC (79)	Reference	Reference	-
CG (108)	1.205	0.613, 2.370	0.589
GG (27)	2.780	1.058, 7.304	0.038

Statistics were obtained by analyzing the association between suboptimal serum vitamin C concentrations (<50 μmol/L) and SNP rs6596473 at the SLC23A1 gene locus using binary logistic analysis. Model 1, unadjusted; Model 2, adjusted for sex (men or women), dietary intake of vitamin C (the lowest, middle, or the highest), and vitamin C supplement use (no or yes); SNP, single nucleotide polymorphism; SLC23A1, solute carrier family 23 member 1; OR, odds ratio; and CI, confidence interval**.**

3.4. Association between serum vitamin C concentrations and mental vitality and mood states

Next, linear regression analysis was performed to determine whether serum concentrations of vitamin C were associated with vitality levels and mood states. Vitality indicators included attention and fatigue. Mood indicators included stress, depression, and positive and negative affect. Simple or multiple linear regression analysis was performed using each score of vitality and mood states as a dependent variable and the serum vitamin C concentration and other covariates as an independent variable. No covariates were included in Model 1, whereas in Model 2, sex, age, BMI, current smoking status, physical activity, and alcohol use were included as covariates. As shown in **Table 6**, fatigue scores were not significantly correlated with serum concentrations of vitamin C both in the unadjusted and adjusted models. On the other hand, simple linear regression analysis showed that attention scores directly correlated with serum concentrations of vitamin C (Model 1, β = 0.161 and p = 0.018) (Table 6). Furthermore, after adjusting for the covariates, including sex, age, BMI, current smoking status, physical activity, and alcohol use, serum vitamin C concentrations showed a more robust association with attention scores (Model 2, β = 0.203 and p = 0.003) (Table 6). On the other hand, as shown in **Table 7**, stress, depression, and positive and negative affect scores were not associated with serum vitamin C concentrations in both the unadjusted and adjusted models.

Table 6. Association between serum vitamin C concentrations and mental vitality

	B (SE)	95% CI	*β*	t	*P*
Model 1					
Attention	0.018 (0.008)	0.003, 0.034	0.161	2.374	0.018
Fatigue	–0.008 (0.008)	–0.025, 0.009	–0.066	–0.959	0.339
Model 2					
Attention	0.023 (0.008)	0.008, 0.039	0.203	2.919	0.003
Fatigue	–0.013 (0.009)	–0.030, 0.004	–0.104	–1.502	0.135

Statistics were obtained using simple or multiple linear regression analysis. Model 1, unadjusted; Model 2, adjusted for sex, age (y), BMI (kg/m^2), current smoking status (no or yes), physical activity level (<median or ≥median), and alcohol use (no or yes); B, unstandardized coefficient; SE, standard error; CI, confidence interval for B; *β,* standardized coefficient. Adapted from previous publication (Sim *et al.* 2022).

Table 7. Association between serum vitamin C concentrations and mood states

	B (SE)	95% CI	*β*	t	*P*
Model 1					
Stress	–0.098 (0.104)	–0.303, 0.108	–0.064	–0.937	0.350
Depression	–0.012 (0.025)	–0.062, 0.037	–0.034	–0.493	0.622
Positive affect	0.035 (0.030)	–0.023, 0.094	0.082	1.193	0.234
Negative affect	–0.018 (0.026)	–0.070, 0.034	–0.048	–0.693	0.489
Model 2					
Stress	–0.187 (0.102)	–0.388, 0.015	–0.123	–1.827	0.069
Depression	–0.041 (0.024)	–0.088, 0.006	–0.112	–1.718	0.087
Positive affect	0.049 (0.029)	–0.009, 0.107	0.114	1.681	0.094
Negative affect	–0.035 (0.026)	–0.086, 0.017	–0.091	–1.326	0.186

Statistics were obtained using simple or multiple linear regression analysis. Model 1, unadjusted; Model 2, adjusted for sex, age (y), BMI (kg/m^2), current smoking status (no or yes), physical activity level (<median or ≥median), and alcohol use (no or yes); B, unstandardized coefficient; SE, standard error; CI, confidence interval for B; *β,* standardized coefficient. Adapted from previous publication (Sim *et al.* 2022).

4. Discussion

The objectives of this study were to determine *1)* whether a common polymorphism in the vitamin C transporter gene was associated suboptimal serum vitamin C status and *2)* whether vitamin C status was associated with mental vitality levels and mood states. Therefore, serum ascorbic acid concentrations, genotypes of rs6596473 at the SLC23A1 gene locus, mental vitality levels, and mood states were determined in 214 healthy men and women. As a result, homozygous minor allele carriers (GG) for SNP rs6596473 were three times more likely to have suboptimal serum vitamin C concentrations than minor allele non-carriers (CC). Furthermore, analysis of the association between serum vitamin C concentrations and psychological functions showed that serum vitamin C concentrations were directly correlated with attention scores.

Fasting serum vitamin C concentration, a reliable biomarker for whole body pool of vitamin C, is generally stratified to be suboptimal (10-50 μmol/L) or optimal (≥50 μmol/L) based on saturation of vitamin C body pools and risk of deficiency ("New Reference Values for Vitamin C Intake" 2015). In addition, plasma vitamin C concentrations above 50 μmol/L have been reported to benefit cardiovascular disease and cancer risk (Gey *et al.* 1993). Thus, participants' vitamin C status was evaluated as either optimal (≥50 μmol/L) or suboptimal (<50 μmol/L) according to their serum vitamin C concentrations. Surprisingly, nearly 40% of the current population was assessed to have suboptimal concentrations of vitamin C in their sera. A large body of epidemiological studies has shown that various demographic factors and health aspects modify human vitamin C status, for example, sex, age, BMI, disease states, smoking, and alcohol consumption (Carr

and Rowe 2020; Granger and Eck 2018). Specifically, lower plasma ascorbic acid levels are observed in males, the elderly, the obese, people with infection, smokers, and alcoholics, which is attributed to reduced intakes of vitamin C-providing foods, a high level of oxidative stress in the body, or comorbidities. Likewise, dietary intake of vitamin C was a significant determinant of serum vitamin C concentrations in the current study. Notably, men were 4.6 times more likely to have suboptimal serum vitamin C status than women. Consistent with this, previous epidemiological studies have frequently reported that lower vitamin C serum/plasma concentrations and higher rates of deficiency are observed in men compared to women (Langlois, Cooper, and Colapinto 2016; Travica *et al.* 2020). The high fat-free mass in men, which exhibits a volumetric dilution effect, partly explains why men have lower levels of vitamin C than women (Jungert and Neuhäuser-Berthold 2015).

Meanwhile, there is growing evidence that specific single nucleotide polymorphisms in the cellular vitamin C transporter genes may also explain the inter-individual variation in vitamin C status (Niforou, Konstantinidou, and Naska 2020). SVCT1 is highly expressed in kidney and intestinal epithelial cells and actively transports most vitamin C in those organs (Sotiriou *et al.* 2002). Maintaining optimal systemic vitamin C levels depends on the balance between intestinal absorption and renal reabsorption; therefore, genetic variation in the SLC23A1 gene, such as rs6596473, may affect circulating ascorbic acid concentrations and related health outcomes. A few other studies have investigated the effects of rs6596473 on vitamin C status or disease risks (Duell *et al.* 2013; Amir Shaghaghi *et al.* 2014; de Jong *et al.* 2014). Contrary to the current results, the minor allele of rs6596473 is allele-C in other previous genotyping studies

(Timpson *et al.* 2010). Furthermore, the phenotypic effects of rs6596473 remains equivocal. In the multi-ethnocultural nonsmoking individuals aged 20-29, rs6596473 showed no effect on serum ascorbic acid concentrations (L.E. Cahill and El-Sohemy 2009). Similarly, Senthilkumari *et al.* did not observe a significant difference in plasma vitamin C concentrations between rs6596473 genotypes in the order Indian population (Senthilkumari *et al.* 2014). However, in the same cohort, minor allele effects on the decrease in aqueous humor ascorbate concentrations were observed; the per allele difference in aqueous humor vitamin C for rs6596473 was –217 μmol/L. In addition, rs6596473-minor allele homozygotes had an 80% increased risk for follicular lymphoma in a large San Francisco cohort, but no association was observed in a German population (Skibola *et al.* 2008). A meta-analysis of five large cohorts has reported a minor allele effect of rs6596473 on plasma vitamin C concentrations, which showed a mean decrease of 1.01 mmol/L per minor C-allele (Timpson *et al.* 2010). In the current healthy population aged 20-39, rs6596473-GG homozygotes were three times more likely to have suboptimal vitamin C status than CC homozygotes with no minor allele. Furthermore, this association remained valid despite controlling sex and dietary intake of vitamin C-containing foods or supplements. These observations, for the first time, support the possibility that polymorphisms in the vitamin C transporter gene may explain inter-individual variation in vitamin C status in the Korean population; however, this should be further verified in subsequent epidemiologic studies. Understanding of various nutritional responses depending on genetic variation is expected to contribute to future strategies for personalized nutrition.

Early clinical studies reported increased fatigue, mood disturbances, and decreased arousal and motivation in people with scurvy (Hirschmann and Raugi

1999). The biological functions that vitamin C performs in the brain and alterations in the psychological state caused by vitamin C depletion allow us to infer the association between vitamin C status in the body and mental function in the brain. Indeed, this association has been partly supported by several studies reporting that increased consumption of vegetables and fruits was associated with favorable mental outcomes, such as low levels of fatigue and a feeling of positive emotion (Mujcic and J. Oswald 2016; Rooney, McKinley, and Woodside 2013; Głąbska *et al.* 2020). However, the beneficial effects of fruits and vegetables can be attributed to a variety of nutritional components in addition to vitamin C; therefore, it is necessary to investigate the sole contribution of vitamin C on vitality and mood states.

In this study, there was a clear trend that serum vitamin C concentrations were directly correlated with positive affect scores and were inversely correlated to fatigue and negative mood levels, including stress and depression. Consistent with this, a previous cross-sectional study conducted in young male tertiary students showed inverse correlations of plasma vitamin C concentrations with depression, anger, and confusion levels (Pullar *et al.* 2018). Moreover, individuals with optimal vitamin C concentrations greater than 50 μmol/L had lower total mood disturbance scores than those with vitamin C levels of less than 50 μmol/L. Another cohort study of Italian women aged 60-90 found that lower serum ascorbic acid concentrations (<23 μmol/L) were associated with higher depression levels (Marazzi *et al.* 1990). The current study showed for the first time that serum concentrations of vitamin C were positively correlated with subjective attention levels in a healthy young population. Moreover, such a significant association was stable despite adjusting for various demographic and lifestyle confounding factors,

such as sex, age, BMI, smoking, alcohol consumption, and physical activity. Notably, serum vitamin C concentration was the most vital determinant of the attention level than other confounding variables. Similarly, in a previous cohort that included cognitively intact individuals, there was a direct correlation between plasma vitamin C concentrations and cognitive performance on tasks requiring attentional focus (Travica *et al.* 2019). Attention is a cognitive process that makes it possible to respond continuously to relevant stimuli (Lindsay 2020). This psychological ability is crucial in a wide range of daily activities, including professional activities at work. As a result, optimal vitamin C status is supposed to enhance a variety of desirable mental functions, such as work performance and goal achievement, thereby promoting enthusiasm for life. A possible explanation for the link between vitamin C status and mental vitality is that vitamin C has a modulating effect on neurotransmitters and hormones that enhance alertness, vigilance, and motivational salience (F. E. Harrison and May 2009a). For example, vitamin C stimulates the production of acetylcholine and increases the number of acetylcholine receptors in the brain (Kuo *et al.* 1979; Rebec and Pierce 1994). In particular, it is well established that vitamin C is essential for the catecholamine system (Ballaz and Rebec 2019). Specifically, vitamin C contributes to the conversion of dopamine to norepinephrine in the brain by mediating the catalytic action of dopamine-β-hydroxylase (Daubner, Le, and Wang 2011). Furthermore, dopamine signaling increases vitamin C release and bioavailability in neurons (Pierce and Rebec 1990). Besides, plausible biological mechanisms by which vitamin C affects brain functions are well established in the literature. However, most have been obtained from *in vitro* and animal experiments; therefore, the

question still remains as to whether it is possible to translate preclinical results into humans.

In conclusion, these findings support that genetic variations in the vitamin C transporter gene can be a significant determinant of vitamin C status in a Korean population. Further, vitamin C status is associated with mental vitality, especially attentional ability. Therefore, elaborately designed clinical trials are highly required to elucidate the causal effect of vitamin C on mental vitality in pursuit of extensive investigations of underlying mechanisms.

IV. Study 2

Effects of vitamin C supplementation on mental vitality: a randomized, double-blind, placebo-controlled trial

Parts of this work have been published (Sim *et al*. 2022).

1. Introduction

Vitamin C (L-ascorbic acid or ascorbate) is an essential nutrient that functions as an indispensable electron donor and a cofactor in various biological reactions, such as collagen hydroxylation, carnitine biosynthesis, and tyrosine metabolism (Njus *et al.* 2020). Notably, vitamin C presents its highest concentrations in the brain, and animal model and *in vitro* studies have reported that vitamin C performs critical roles in brain functions (Fiona E Harrison and May 2009b). For example, vitamin C protects neurons from oxidative stress, induces differentiation and maturation of neurons, and regulates the synthesis or release of neuro-modulating factors, including serotonin, catecholamines, and glutamate (Figueroa-Méndez and Rivas-Arancibia 2015; Moretti and Rodrigues 2022). Accordingly, vitamin C is inferred to be essential for maintaining normal mental health.

Humans rely on dietary supply to obtain vitamin C due to the absence of a gene encoding L-gulonolactone oxidase, which is critical for vitamin C synthesis from glucose. Although a daily intake of one to two servings of citrus fruits or vegetables provides adequate amounts of vitamin C, previous reports have shown that suboptimal vitamin C status is prevalent among young adults, even in industrialized countries (L. Cahill, Corey, and El-Sohemy 2009; Langlois, Cooper, and Colapinto 2016; Schleicher *et al.* 2009). The poor vitamin C status in the young may be attributed to external factors such as smoking, excessive alcohol use, and unhealthy eating habits that fail to provide a balanced diet rich in vitamin C (McCall *et al.* 2019). Thus, even healthy young individuals can be at risk of suboptimal vitamin C status, which could disrupt brain function activity. However, compared to the elderly or patients, vitamin C status in the young is liable to be

considered unimportant.

Vitality is defined as a subjective feeling of energy and aliveness, which highlights the psychological aspects (Rozanski and Kubzansky 2005). Vitality decline is the earliest sign of scurvy, a clinical symptom of severe vitamin C deficiency; it manifests in fatigue, decreases in arousal and motivation, and cognitive impairment (Kinsman and Hood 1971). Feeling vital is a key component in healthy psychological functioning, such as self-regulation, work performance, and goal-seeking (Rozanski and Kubzansky 2005). Given that professional and social participation is highest among young people, it is necessary to investigate whether improvement of vitamin C status helps to promote their vitality and work performance. However, the link between vitamin C status and vitality-related psychological and cognitive functions at a young age is equivocal, and their causal relationship has rarely been examined.

The gut microbiota, a collection of intestinal bacteria, archaea, fungi, and viruses, is often referred to as the forgotten organ because gut microorganisms represent the most excellent density and abundance in the human body (Grice and Segre 2012). A growing body of evidence has shown that gut bacterial communities have the potential to sense and modify large amounts of chemical signals derived from the internal or external environment (Thursby and Juge 2017). It is now evident through a vast amount of research both on the animal and human gut microbiome that intestinal bacteria are vitally concerned with various physiological functions of the host, such as nutrient metabolism, immune responses, and disease pathology (Hou *et al.* 2022). Hence, it is unsurprising that maintaining a balance in the gut microbiota is a critical factor in determining health status in humans.

Considering the overarching influence of the gut bacterial communities on human health, the gut-microbiota-brain axis, bidirectional communication between the gut bacteria and the central nervous system, has been recently considered an essential concept for understanding the pathogenesis of mental disorders (Cryan *et al.* 2019). Disruptions in the gut-microbiota-brain axis are associated with various psychiatric symptoms, such as depression, anxiety, autism, and dementia (Dinan and Cryan 2017). Potential mechanisms by which the gut microbiota affects brain function include alterations in bacterial composition and metabolism, production of microbial metabolites, immune activation, and neuromodulation (de Vos *et al.* 2022; Makris *et al.* 2021). For example, it has been extensively reported that significant changes in the bacterial composition and consequent dysbiosis trigger a systemic inflammatory cascade and increase the likelihood that toxic bacterial fragments can reach the central nervous system (Tilg *et al.* 2020). Furthermore, a growing body of evidence points out that microbial-derived metabolites can remotely exert their effects on various host metabolic reactions, such as immune responses and neuromodulation (Rooks and Garrett 2016; Yang and Cong 2021; Cryan *et al.* 2019). In other words, multiple mechanisms likely act in concert to mediate the gut-microbiota-brain axis, thereby contributing to homeostasis of the gut, brain, and microbial communities.

Dietary vitamins are essential not only for human brain functions but also for the survival of the gut microbiota and their fundamental biological processes (Uebanso *et al.* 2020; LeBlanc *et al.* 2017). Because vitamins are fully absorbed in the proximal small intestine, dietary vitamins were not previously thought to affect the gut flora (Said 2011). However, there is an increasing observation in human studies that high doses of vitamins may escape complete absorption and reach the

distal gut (Fangmann *et al.* 2018; Steinert *et al.* 2016). Furthermore, it has been reported that significant amounts of vitamins that reach the large intestine are taken up by non-vitamin-producing gut microbes, directly affecting the symbiotic relationship between the gut microbiota (Magnúsdóttir *et al.* 2015). Hence, growing attention is being paid to the impacts of vitamin administration on the gut-microbiota-brain axis to determine the efficacy of vitamins as adjuncts for the prevention and treatment of various mood disorders (Mörkl *et al.* 2018; Berding *et al.* 2021). However, most previous studies have primarily focused on vitamin D or B-complex supplementation; therefore, the effects of vitamin C on the gut-microbiota-brain axis are not fully understood.

The aim of this study was to determine the causal relationship between vitamin C status and mental vitality by assessing the effects of vitamin C supplementation on mental function using a randomized, double-blind, placebo-controlled trial. Further, the underlying mechanisms by which vitamin C promotes mental vitality through the gut-microbiota-brain axis were investigated by assessing the changes in the gut bacterial community, proinflammatory cytokine, and neurotransmitter profiles.

2. Materials and Methods

2.1. Participants

Participants were recruited from September to October 2019 from Seoul National University. Men and women who met the following criteria volunteered for participation: *1)* 20–39 years of age; *2)* no medical history of acute or chronic diseases such as hypertension, diabetes, cardiovascular disease, dyslipidemia, kidney disease, cancer, gastrointestinal disorders, and endocrine disorders; and *3)* no use of vitamin C-containing supplements within a month. Next, an assessment of serum vitamin C concentration was carried out to screen volunteers with suboptimal vitamin C status, which was defined as a blood concentration of less than 50 μmol/L. Eligible volunteers attended a screening visit between 0800 and 1000. During the screening, all participants completed anthropometric measurements (body height and weight), followed by blood sample collection. Height and weight were measured using the automatic scale (BSM330; InBody Co., Ltd., Seoul, Korea). Fasting venous blood samples from the antecubital fossa were collected into 4-mL serum separator tubes (BD Biosciences, Franklin Lakes, NJ, USA) to determine the fasting vitamin C concentration. All measurements of vitamin C concentration were performed within 24 hours of the blood collection using a high-performance liquid chromatography kit (Chromsystems Instruments & Chemicals GmbH, Gräfelfing, Germany; intra-assay coefficient of variation [CV] <2.5%, inter-assay CV <5%) under the manufacturer's protocol.

The sample size of the intervention study was calculated as fifty based on the attention scores derived from the previous cross-sectional study with $\alpha = 0.05$

and a power of 80%. Finally, a total of 50 individuals (28 men and 22 women) who had shown a vitamin C concentration of less than 50 μmol/L were included in the vitamin C supplementation trial.

This intervention study was conducted in the Department of Food and Nutrition at Seoul National University between September and December 2019, and was approved by the IRB of Seoul National University (1909/002-011) and was registered at the CRIS on September 4th, 2019 (KCT0004276). All steps were performed in accordance with the relevant guidelines and regulations. Written informed consent was obtained from all participants before their inclusion in the study.

2.2. Study design

This study was a four-week, randomized, double-blind, parallel, placebo-controlled trial. A total of 50 participants were randomly assigned to either a vitamin C supplementation group (500 mg of vitamin C twice a day for 4 weeks) (n = 25) or a placebo supplementation group (n = 25), and the random assignment was stratified by sex using a pseudo-random number generator (http://www.randomizer.org). All participants, experimental staff and investigators involved in the random assignment, measurement of outcomes, and data analysis were blinded to the allocation until all analyses were complete. Participants in both the vitamin C and placebo groups were advised to maintain their physical activities and usual diet, including fruits and vegetables, and to avoid consuming any other dietary products fortified with vitamin C. To monitor their dietary intake, participants were asked to complete a two-day dietary record at the baseline and the endpoint, respectively. The dietary intake was analyzed using CAN-Pro 5.0 (Web ver., Korean Nutrition Society, Korea).

A total of two visits were carried out: the day before the supplementation started (baseline; week 0) and the day after the supplementation was completed (endpoint; week four). All participants attended laboratory measurements during the morning hours (i.e., between 0800 and 1000) after a 12-hour fast, avoiding excessive exercise and alcohol consumption during the previous 24 hours. During each visit, all participants completed anthropometric measurements (body height and weight), vitality and mood assessment, and blood collection. Stroop test was performed only at the week 4 visit. Height and weight were measured using the automatic scale (BSM330; InBody Co., Ltd., Seoul, Korea). Data on smoking

status and dietary supplement use were collected using a questionnaire, and physical activity was determined using the International Physical Activity Questionnaire (IPAQ). A schematic diagram of participants' schedules during each visit is shown in **Figure 1**.

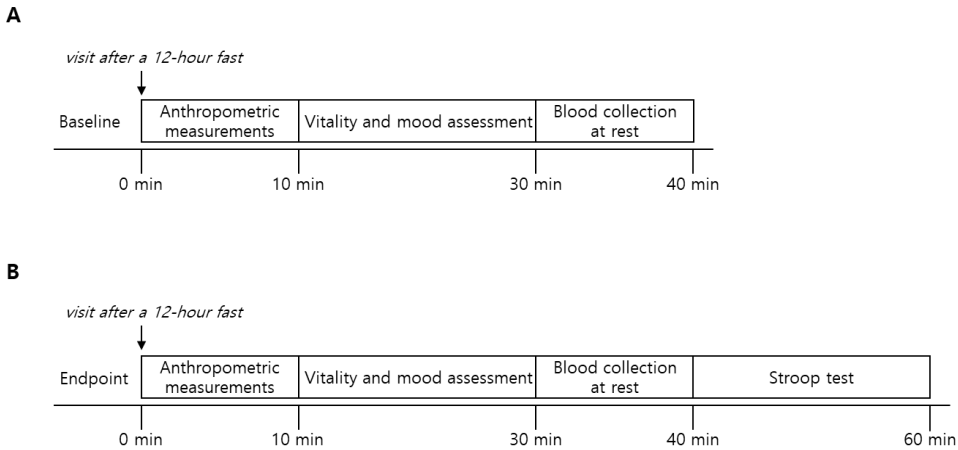

Figure 1. Schematic diagram of participants' schedules during the visit

The schematic diagram depicts laboratory schedules at the (A) baseline (week 0) and (B) endpoint (week 4). Anthropometric measurements included body height and weight. Vitality and mood assessment consisted of measurements of fatigue, subjective attention, work engagement, self-control, stress, depression, positive and negative affect, and anxiety.

2.3. Intervention

The supplementation was carried out with 2 different drink conditions: *1)* 100 mL of the vitamin C drink contained 50 kcal of energy, 11 g of sugar, 1.2 mg of vitamin B_2, 30 mg of sodium, and 500 mg of vitamin C; and *2)* 100 mL of the placebo drink had the identical nutritional content as the vitamin C drink, except for the vitamin C (0 mg). Participants were provided with either a vitamin C drink or a placebo for four weeks. Each drink was packaged in a pouch of 100-mL, and participants were instructed to daily consume the two pouches with a four-hour interval to maximize the intestinal absorption of the vitamin C. The colors and flavors of the two types of drink were identical to blind the participants to their allocation. A label was attached to each pouch, but it included only the participant's code and the date of manufacturing. The participants were instructed to record their daily consumption of the drinks, and the records were reviewed weekly to check and encourage their compliance with the trial. All of the experimental drinks were produced and supplied by Kwang Dong Pharmaceutical Co., Ltd.

2.4. Sample collection

Blood samples were collected during the participants' visits. Fasting venous blood samples from the antecubital fossa was collected into 8-mL BD Vacutainer® SST™ II Advance Plus Blood Collection Tubes (BD Biosciences, San Jose, CA, USA) and 4-mL EDTA-coated tubes in the resting state. Sera and buffy coats were separated from the whole blood samples via centrifugation for later biochemical analysis and single nucleotide polymorphism genotyping, respectively. Preparation of sera and buffy coats was performed without delay, and the separated samples were aliquoted into 1.5-mL ep tubes (Eppendorf, Hamburg, Germany) and immediately stored at -80°C for later analysis. In addition, participants collected their stool samples prior to their visit. Fresh stools were collected in a sterilized plastic tube containing DNA stabilizing preservative reagent (Norgen Biotek, Thorold, ON, Canada) and delivered to the laboratory within two days of collection. Aliquots of 200 mg of stool samples were immediately stored at -80°C for later gut microbiota analysis.

2.5. Mental vitality assessment

2.5.1. Subjective mental vitality

Fatigue, subjective attention, work engagement (vigor, dedication and absorption), and self-control resources were assessed as indicators of vitality using validated self-reported questionnaires. Fatigue and subjective attention were measured using two items each from the Checklist Individual Strength, which allowed participants to rate the subjective belief regarding their fatigue and attentional ability (Vercoulen *et al.* 1994) ("*I feel tired*" and "*I tired easily*" for fatigue assessment, with Cronbach's $\alpha = 0.84$; "*When I am doing something, I can keep my thoughts on it*" and "*I find it easy to concentrate*" for attention assessment, with Cronbach's $\alpha = 0.79$). Each item was scored with a seven-point Likert scale anchored by "*strongly disagree*" and "*strongly agree,*" with higher scores indicating higher levels of fatigue and attention.

Work engagement and self-control were assessed using the Utrecht Work Engagement Scale and State Ego Depletion Scale, respectively. Work engagement is defined as a positive and fulfilling state of mind toward work, and it can also be applied to the academic activity of a student (Seppälä *et al.* 2009). The scale includes a total of 17 items categorized into 1 of 3 constituting aspects of work engagement: vigor, dedication, and absorption. Vigor is defined as a high level of energy and mental resilience while working, which contributes to being willing to work hard and investing perseverance in one's work even in the face of difficulties. Being dedicated in work means passion, inspiration, pride, and a spirit of challenge. Absorption is a state in which one feels that time goes by quickly and

that it is not easy to get out of work by concentrating on work and having fun. The ability of self-control is a trait that can be depleted and replenished, which is associated with psychological well-being and better achievement at school or workplace (Tangney, Baumeister, and Boone 2004). In this study, five items of the State Ego Depletion Scale were used (e.g., "*I feel like my willpower is gone*"), and reverse-scored to assess self-control resources (Barnes, Miller, and Bostock 2017). All assessment was individually conducted in a separate and quiet room under the supervision of the investigator.

2.5.2. Cognitive performance

The Stroop color-word is a neuropsychological test to evaluate the ability to suppress cognitive interference that occurs when the processing of a specific stimulus interferes with the simultaneous processing of a second stimulus attribute, known as the Stroop effect (MacLeod 1991). The Stroop color-word test is extensively used to measure cognitive functions such as attention, cognitive flexibility, and information processing speed. A mental arithmetic test was conducted just before the Stroop test to assess sustained attention under cognitive fatigue and mental stress (Ushiyama *et al.* 1991). The arithmetic expression included two Arabic numerals and one operator, for example, "five figures + five figures," "five figures – five figures," "three figures × double figures," and "four figures ÷ three figures." A total of 20 questions were presented, and a maximum of one minute was given to calculate each question. Subsequently, a computer-assisted Stroop color-word test was performed based on a previous study (Zalonis *et al.* 2009). In an incongruent condition, the words were colored in one of four different colors of ink (blue, red, yellow, and green), and each word was not

colored in the respective color (e.g., the word BLUE was not colored in blue ink). Participants were asked to match the written color names of the words independently of the color of the ink by pressing the colored keys (blue, red, yellow, and green) as quickly and accurately as possible. The Stroop color-word test consisted of 128-word items in the four trials, which included 32 items each. Stroop test performance was assessed by calculating the sum or average reaction times for the correct responses out of the 128 items (Amato *et al.* 2006). All cognitive tests were programmed in the open-source software Psychopy2 (version 1.81, Peirce, 2007), and the correctness and reaction time of responses to the Stroop task were recorded by the program. Each participant completed the assessment under the supervision of a well-trained investigator in a separate and quiet room. After providing sufficient guidance on the cognitive assessment, the investigator did not intervene throughout the examination once it began. A schematic diagram of the cognitive test is shown in **Figure 2**.

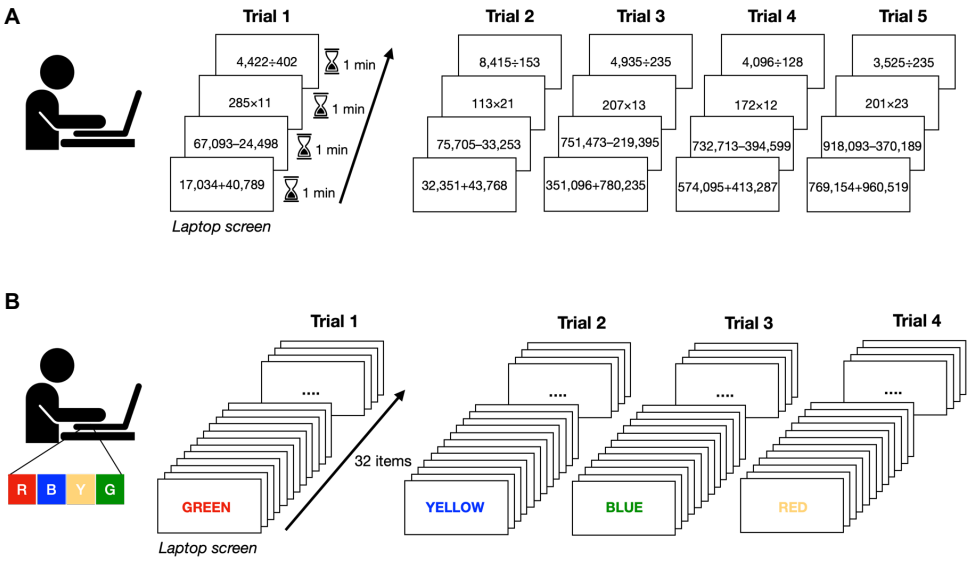

Figure 2. Schematic illustration of the cognitive test

The Stroop color-word test was performed to measure cognitive functions including attention, cognitive flexibility, and information processing speed. A mental arithmetic test was conducted just before the Stroop test to induce cognitive fatigue and mental stress. (A) The arithmetic expression included two Arabic numerals and one operator, for example, "five figures + five figures," "five figures – five figures," "three figures × double figures," and "four figures ÷ three figures." A total of 20 questions were presented, and a maximum of one minute was given to calculate each question. (B) After completing the mental arithmetic tasks, Stroop color-word test was performed immediately. The words were colored in one of four different colors of ink (blue, red, yellow, and green), and each word was not colored in the respective color (e.g., the word BLUE was not colored in blue ink). Participants were asked to match the written color names of the words independently of the color of the ink by pressing the colored keys (blue, red, yellow, and green) as quickly and accurately as possible. The Stroop color-word

test consisted of 128-word items in the four trials, which included 32 items each. Stroop test performance was assessed by calculating the sum or average reaction times for the correct responses out of the 128 items.

2.6. Mood state assessment

The levels of stress, depression, positive and negative affect, and state anxiety were determined with Stress Response Inventory (SRI), Beck's Depression Inventory (BDI), Positive Affect and Negative Affect Schedule (PANAS), and State-Trait Anxiety Inventory-X-1 (STAI-X-1), respectively. The SRI was designed to assess seven stress factors: tension, aggression, somatization, anger, depression, fatigue, and frustration (Koh *et al.* 2001). It consists of 22 items concerning psychological, physiological, and behavioral stress responses (e.g., "*My body trembles,*" "*My voice is louder than it usually is,*" and "*I have lost my self-confident*"). Each item was scored with a five-point Likert scale anchored by "*strongly disagree*" to "*strongly agree.*" The BDI is one of the most widely used instruments for determining the severity of depression; it consists of a 21-question multiple-choice inventory relating to symptoms of depression, such as hopelessness, irritability, feelings of guilt or being punished, fatigue, weight loss, and lack of interest in sex (Beck and Beamesderfer 1974). Each of the 21 questions includes four statements ordered according to the severity of the symptom (from absent to very severe) and each statement was assigned a numeric value from zero to three (e.g., "*I do not feel sad*," "*I feel sad*," "*I am sad all the time and I can't snap out of It*," and "*I am so sad and unhappy that I can't stand it*"). The PANAS is a reliable and well-validated instrument that consists of two 10-item scales to measure both positive (e.g., "*attentive*," "*active*," "*excited*," and "*determined*") and negative ("*hostile*," "*irritable*," "*ashamed*," and "*guilty*") affect (Watson, Clark, and Tellegen 1988). Each item is rated on a five-point scale anchored by "*no at all*" to "*very much.*" State anxiety is defined as temporary conditions of fear, nervousness, discomfort,

and physiological activation of the autonomic nervous system (Julian 2011). State anxiety was assessed using the STAI-X-1 that consists of 20 items on a 4-point Likert Scale.

2.7. Gut microbiota analysis

Bacterial genomic DNA extraction

Total bacterial DNA was isolated from the stool specimens using the QIAamp® Fast DNA Stool Mini Kit (QIAGEN, Hilden, Germany) according to the kit protocol. Briefly, 200 mg of fecal material was homogenized with sterilized steel beads with a diameter of 5mm in ASL buffer using TissueLyser (QIAGEN, Hilden, Germany) at 30 Hz for 5 min. Then, the suspension was heated at 95°C to lyse gram-positive bacterial cells. After the incubation, purified DNA was eluted and quantified by spectrophotometer NanoDrop ND-2000 (Thermo Scientific, Waltham, MA, USA).

16S rRNA gene amplification and sequencing

The V3-V4 regions of 16S ribosomal ribonucleic acid (rRNA), a marker gene of bacteria, was selectively amplified using polymerase chain reaction (PCR). In brief, PCR was performed using BioFACT™ F-Star Taq DNA Polymerase (BioFACT™, Seoul, Korea). In brief, 50 μL of PCR reaction mix contained 20 ng of DNA template, 5 μL of 10X taq buffer, 1 μL of 10mM dNTP mix, 2 μL of forward and reverse barcoded primers, and 0.25 μL of DNA polymerase. PCR reaction mix was amplified using a GeneAmp® PCR system 9700 (Applied Biosystems, Foster City, CA, USA). The PCR program was set as follows: an initial hold at 94°C for 5 min, then 28 cycles of denaturation at 95°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s, followed by a final extension for 10 min at 72°C and holding at 4°C. The PCR product was confirmed using 1% agarose gel electrophoresis and visualized under a Gel Doc system

(BioRad, Hercules, CA, USA). The amplified products were purified with PureLink Quick Gel Extraction and PCR Purification Combo Kit (Invitrogen, Carlsbad, CA, USA) and quantified by the Qubit 2.0 fluorometer (Introvigen, Carlsbad, CA, USA). The size of the library was assessed by BioAnalyzer (Agilent Technologies, Santa Clara, CA, USA). Finally, the amplicons were sequenced using an Illumina MiSeq sequencing system (Illumina, San Diego, CA, USA).

Bioinformatic analysis of sequencing data

The sequencing data were analyzed using QIIME2 (version 2021.8). First, microbial sequences were denoised to remove the low-quality sequences and chimeras using the DADA2 plugin. Denoised sequences were clustered into Operational Taxonomic Units (OTUs). Then OUT representative sequences were aligned to the SILVA rRNA database (version 132) at 99% sequence identity with scikit-learn Naïve Bayes-based machine learning classifier. A phylogenetic tree was generated using MAFFT and FastTree method for diversity analysis. Alpha diversity, including Observed features, Faith's phylogenetic diversity, and the Shannon index, was estimated to evaluate the richness and evenness of individual microbial communities. Bray-Curtis dissimilarity, an indicator of beta diversity, was estimated to quantify the compositional dissimilarity of gut microbiota structure, and was compared between groups using permutational multivariate analysis of variance (PERMANOVA).

2.8. Serum biochemical analysis

2.8.1. Vitamin C

Fasting blood samples were collected from all participants at rest. To minimize the oxidation of vitamin C, whole blood samples were protected from light and centrifuged immediately. The separated serum was immediately transferred to a -80°C freezer. Serum vitamin C concentration was measured within 24 hours after blood collection. High-performance liquid chromatography was performed using a Chromsystems Reagent Kit (Chromsystems Instruments & Chemicals GmbH, Gräfelfing, Germany) to determine vitamin C concentration in sera. Briefly, 0.1 mL of serum sample was mixed with 0.1 mL of precipitation solution in a reaction vial. After incubation at 4°C for 10 min, samples were centrifuged at 9,000 g for 5 min. Then, 20 μL of supernatant was injected into an HPLC system with a UV-vis detector set at a wavelength of 245 nm. The column temperature was maintained at 20-25°C with a flow rate of 1.5 mL/min. The time from injection to injection was 5 minutes. The analysis used a single-point calibrator of 1.53 mg/dL. The intra-analysis coefficient of variation (CV) was less than 2.5%, and the inter-analysis CV was less than 5%.

2.8.2. Brain-derived neurotrophic factor

Serum concentrations of brain-derived neurotrophic factor (BDNF) were determined using solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) with Human/Mouse BDNF DuoSet ELISA (R&D Systems, Minneapolis, Minnesota, USA). In brief, each well of a 96-well microplate was coated with 100

μL of Capture Antibody, which was diluted to the working concentration in PBS. The plate was sealed and incubated overnight at room temperature. After aspirating each well, the 96-well microplate was washed with Wash Buffer three times. Then, 300 μL of Reagent Diluent was added into each well, and incubation was carried out at room temperature for 1 h. After completing plate preparation, 100 μL of serum sample or standards in Reagent Diluent were added into each well of the 96-well microplate. Next, the plate was covered with an adhesive strip and incubated at room temperature for 2 h, followed by three times the aspiration and wash step. Next, 100 μL of Detection Antibody in Reagent Diluent was pipetted into each well, and then the plate was incubated again. After completing the aspiration and wash step, 100 μL of the working dilution of Streptavidin-HRP was added into each well, and the plate was incubated for 20 min at room temperature with direct avoidance light, followed by the aspiration and wash step. Then, 50 μL of Substrate Solution was pipetted into each well, and incubation was performed for 20 min at room temperature, being protected from direct light. After the incubation, 50 μL of Stop Solution was added into each well, followed by immediate determination of the optical density of each well using a microplate reader set to 450 nm.

2.8.3. Lipopolysaccharide-binding protein

For quantitative detection of lipopolysaccharide (LPS)-binding protein (LBP) in sera, a Human LBP Enzyme-Linked Immunosorbent Assay kit (Abcam, Waltham, MA, USA) was used based on standard sandwich ELISA technology. Briefly, 100 μL of standard or serum sample was added to appropriate wells of a 96-well microplate, and incubation was carried out at 37°C for 90 min. After the incubation, the plate content was completely discarded. Then, 100 μL of

biotinylated Antibody was added into each well, and the plate was incubated at 37°C for 60 min, followed by three times of washing steps with 300 μL of 0.01M PBS. Then, 100°C of ABC working solution was added to each well, and another incubation was performed at 37°C for 30 minutes. After five times of wash with 300 μL of 0.01M PBS, 90 μL of TMB was added to each well. Final incubation was performed at 37°C for 30 min in the dark, followed by the addition of 100 μL-TMB solutions. The optical density of each well was immediately determined using a microplate reader set to 450 nm.

2.8.4. Spermidine

The serum concentration of spermidine was determined using the All Species Spermidine ELISA Kit (LSBio, Seattle, WA, USA) based on the competition ELISA principle. Briefly, 50 μL of each standard, blank, and sample were pipetted into the appropriate wells of a 96-well microplate and immediately added 50 μL of Detection Reagent A working solution to each well. The plate was then incubated at 37° C. for 1 h. After the first incubation, liquid from each well was aspirated and washed three times with 350 μL of Wash Buffer each. Next, 100 μL of Detection Reagent B working solution was added to each well, and another incubation was performed at 37° C for 30 min. After completing three wash steps, 90 μL of TMB substrate solution was added to each well and the plate was incubated at 37°C for 20 min while being protected from light. Finally, upon optimal color development was achieved, 50 μL of Stop Solution was added to each well and the optical density of each well was immediately determined using a microplate reader set to 450 nm.

2.8.5. Cytokines

A total of 10 cytokines were quantified in serum samples: interferon γ (IFN-γ), interleukin (IL) 1 β (IL-1β), IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13 and tumor necrosis factor α (TNF-α). For the simultaneous detection of 10 cytokines, the Proinflammatory Panel 1 (human) Kit (Mesoscale Discovery, Rockville, MD, USA) was used based on Mesoscale Discovery (MSD) electrochemiluminescence system. Briefly, 25 µL of assay diluent was added to each well, and the plate was incubated for 30 s at room temperature. Each 25 µL of sample, standard, and control were added to appropriate wells, and another incubation was carried out for 2 h at room temperature. After the incubation, liquid from each well was aspirated and washed three times using 200 µL of PBS and with 0.05 % TWEEN 20. 25 µL of Detection Antibody was added to each well, and the plate was incubated for 1 h at room temperature, followed by three times wash steps. Finally, 150 µL of MSD Read Buffer was added to each well, and the MSD plate was placed on the MSD Sector Imager 2400 plate reader. The Electrochemiluminescence signal of each well was detected by photodetectors and analyzed using the Discovery Workbench 3.0 software (Mesoscale Discovery, Rockville, MD, USA).

2.8.6. Neurotransmitters

Serum concentrations of L-DOPA, dopamine, norepinephrine, epinephrine, and serotonin were determined based on ultra-high-performance liquid-chromatography-mass spectrometry (UPLC-MS) using a Xevo® TQ-XS Triple Quadrupole Mass Spectrometry instrument (Waters, Milford, MA, USA) combined with an ACQUITY UPLC® I-Class PLUS system (Waters, Milford, MA, USA). Briefly, separation was performed at 25°C on an Imtakt Scherzo SM-C18 column

(2.1 × 100 mm with 3.0-µm particles) (Imtakt USA, Portland, OR, USA) using 0.2% formic acid in water (solvent A) and methanol (solvent B). The flow rate was maintained at 0.2 mL/min. The linear gradient was as follows: 98% A for 0 min, 98% A for 3 min, 10% A for 8 min, 10% A for 10 min, 98% A for 11 min, and 98% A for 13 min. For mass spectrometry analysis, the mass spectrometer was operated with electrospray ionization in positive ion mode, and the mass range was set to m/z 50-1500. Mass accuracy was maintained using an automated Calibrant Delivery System (AB Sciex, Concord, Canada) connected to the second inlet of the DuoSpray source. All MS data, including retention time, m/z, and ion intensity, were extracted with Markerview software (AB Sciex, Concord, Canada) incorporated within the instrument.

2.9. rs6596473 genotyping

Based on previous cross-sectional findings that the homozygous minor allele of the single nucleotide polymorphism rs6596473 was associated with serum vitamin C status, genotyping of rs6596473 was performed to determine whether there was a significant difference in genotype distribution between the vitamin C and placebo groups. Genomic DNA was isolated from a buffy coat using the QIAamp® DNA Blood Kit (QIAGEN, Hilden, Germany). Briefly, 200 µL of buffy coat sample was added with 20 µL of QIAGEN Protease and 200 µL of Buffer AL. The mixture was incubated at 56°C for 10 min. After the incubation, 200 µL of 96 % of ethanol was added to the mixture. The mixture was pipetted onto the QIAamp Mini spin column and centrifuged at 14,000 rpm for 1 min. 500 µL of Buffer AW1 was pipetted onto the QIAamp Mini spin column combined with a 2 mL collection tube. The tube was centrifuged at 14,000 rpm for 1 min. 500 µL of Buffer AW2 was pipetted onto the QIAamp Mini spin column in another 2 mL collection tube. The tube was centrifuged at 14,000 rpm for 3 min. Finally, 200 µL of Buffer AE was pipetted onto the QIAamp Mini spin column in a 1.5 mL microcentrifuge tube. After incubation at room temperature for 1 min, the tube was centrifuged at 14,000 rpm for 1 min to elute the DNA. Extracted DNA was quantified by spectrophotometer NanoDrop ND-2000 (Thermo Scientific, Waltham, MA, USA). Genotyping was performed using TaqMan® SNP Genotyping Assay Human for rs6596473 (Applied Biosystems, Foster City, CA, USA) and TaqMan® Genotyping Master Mix (Applied Biosystems, Foster City, CA, USA). Locus-specific TaqMan primers and allele-specific TaqMan probes were predesigned and supplied in an assay kit. TaqMan® probes specific to allele 1 had FAM™ as fluorescent reporter

dye at the 5’ end, and those specific to allele 2 had VIC™ as fluorescent reporter dye at the 5’ end. Each had a non-fluorescent quencher with a TaqMan® minor groove binder at the 3’ end. Polymerase chain reaction (PCR) was performed on 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) in 25 μL of reaction volume. A total of 13.75 μL per well of the reaction mixture containing 12.5 μL of 2X TaqMan® Master Mix and 1.25 μL of 20X Assay Working Stock was prepared for each PCR. The reaction mixture was pipetted to each well of the reaction plate. The plate was sealed with adhesive film and then centrifuged briefly to bring the reaction mixture to the bottom of the well and eliminate air bubbles. After removing the adhesive film from the plate, 11.25 μL of genome DNA (20 ng) or control was added to each well. The PCR program consisted of one cycle at 95°C for 10 min followed by 40 cycles of amplification at 92°C for 15s and 60°C for 1 min.

2.10. Statistical analysis

Per-protocol analysis was performed including only participants who had completed the whole treatment protocol and whose compliance was above 80%. Between-group differences in baseline characteristics were analyzed using an unpaired *t*-test for continuous variables and a Pearson's chi-square test or Fisher's exact test for categorical variables. Within-group and between-group differences in dietary intake were analyzed using a paired *t*-test and unpaired *t*-test, respectively. A paired *t*-test or Wilcoxon's signed rank test was used for the analysis of the within-group difference between the week 0 and week four measures in scores of mental vitality and mood states and concentrations of LBP, spermidine, cytokines, and neurotransmitters. For analysis of the between-group differences in mental vitality and mood indices, a repeated-measures ANOVA with Bonferroni correction was used to determine the *p*-value of time-by-group interaction, with time (week 0 versus week 4) as the within-groups factor and treatment (vitamin C versus placebo) as the between-groups factor. The Stroop color-word test performance between the groups was compared using an unpaired *t*-test, and the correlation with the endpoint vitamin C concentration was determined using Pearson's correlation analysis. Differential abundance analysis of the gut microbiota was performed using DESeq2. The estimated *p*-values of time-by-group interaction were corrected to control for the false discovery rate (FDR) for multiple tests according to the Benjamini and Hochberg procedure. PICRUSt2 software and the reference genome database Metacyc were used to predict the functional abundance of gut bacterial communities from 16S rRNA sequence data. For differential abundance analysis of the predicted bacterial metabolic function, *p*-values of time-by-group interaction

were estimated using linear mixed-effect modeling, with time-by-group as a fixed effect and subject as a random effect. The estimated *p*-values were corrected based on the FDR to control multiple tests. Linear mixed-effect modeling was used to analyze the between-group differences in the serum concentrations of cytokines and neurotransmitters. Correlation coefficients were obtained using Pearson's or Spearman correlation analysis. Statistical analyses using linear mixed-effect modeling were performed using the Statistical Package for R studio version 2022.07.0, and all other statistical analyses were performed using SPSS version 28.0.1.1 (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

3. Results

3.1. General characteristics of participants at baseline

A total of 155 volunteers were assessed for eligibility based on the inclusion criteria (**Figure 3**). As a result, 91 subjects were screened to determine their serum vitamin C concentration. Finally, 50 participants with serum vitamin C concentrations lower than 50 μmol/L were included in the intervention study and randomly assigned to either the vitamin C supplementation group (n = 25) or the placebo group (n = 25) (Figure 3). A total of four participants were excluded from the final analysis due to the withdrawal of participation or low adherence to treatment (Figure 3). The baseline characteristics of the participants are summarized in **Table 1**. There were no significant differences between the vitamin C group and placebo groups in all baseline characteristics, including sex ratio, age, BMI, physical activity and proportions of occupations, current smokers, dietary supplement users, and genotype of rs6596473 (Table 1).

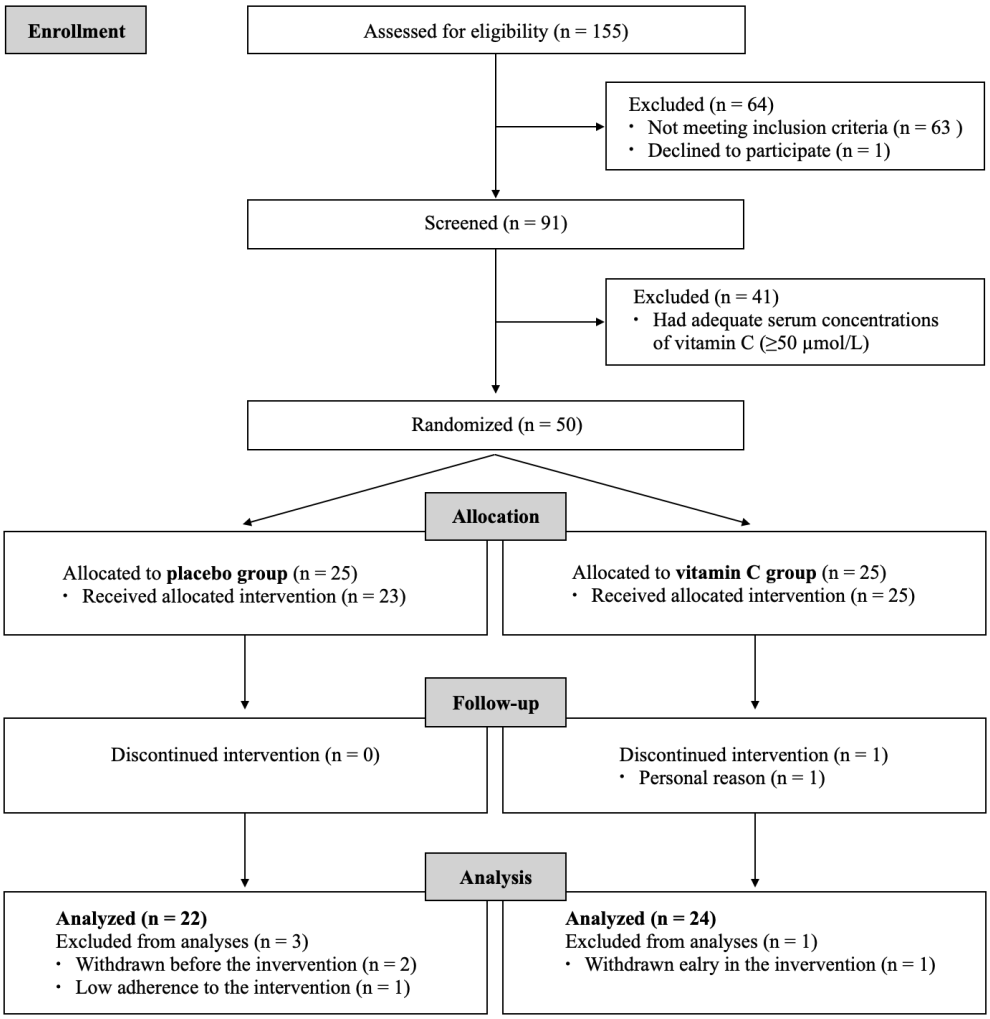

Figure 3. Flowchart of participation throughout the course of the intervention trial

Adapted from previous publication (Sim *et al*. 2022).

Table 1. General characteristics of participants at baseline

	Placebo (n = 22)			Vitamin C (n = 24)			Vitamin C *vs.* Placebo
	Total	**Men**	**Women**	**Total**	**Men**	**Women**	***P*[a]**
No. of subjects	22	12	10	24	14	10	0.79
Age (y)	23.7 (1.9)	24.0 (2.0)	23.3 (1.8)	24.6 (3.5)	24.9 (3.6)	24.1 (3.5)	0.27
BMI (kg/m^2)	22.3 (3.3)	23.5 (2.0)	20.8 (4.1)	22.6 (3.0)	24.2 (2.6)	20.3 (1.9)	0.74
Physical activity (MET-min/week)	1889 (1379)	2022 (1354)	1730 (1465)	2057 (1369)	2219 (1205)	1831 (1612)	0.68
Occupation (n)							0.89
Undergraduate student	17	9	8	17	10	7	
Graduate student	3	2	1	3	2	1	
Employee	2	1	1	4	2	2	
Current smoker (n)	3	2	1	1	1	0	0.60
Dietary supplement user (n)	2	2	0	3	3	0	>0.99
Protein	1	1	0	2	2	0	
Vitamin D	1	1	0	1	1	0	

Genotype of rs6596473[b] (*n*)							0.80
CC	6	2	4	8	3	5	
CG	14	8	6	13	9	4	
GG	2	0	2	3	2	1	

Values are mean (SD) or categorical total.

[a] *P*-values were obtained by comparing the vitamin C group with the placebo group using an unpaired *t*-test, Pearson's Chi-square test, or Fisher's exact test.

[b] rs6596473 is a single nucleotide polymorphism in the gene encoding a solute carrier family 23 member 1.

Adapted from previous publication (Sim *et al*. 2022).

3.2. Dietary intake

Dietary intakes of energy and nutrients at each baseline and endpoint were assessed using two-day dietary records (**Table 2**). At baseline, average energy intake was 1763.4 and 1725.3 kcal/d in the placebo and vitamin C groups, respectively; and there was no statistical difference between groups. For dietary intake of macronutrients, the placebo group had 227.8, 68.8, and 57.6 g/d of carbohydrates, proteins, and fats, respectively. The intakes of carbohydrate, protein, and fat in the vitamin C group were 221, 66.9, and 60.3 g/d, respectively. There was no significant difference between groups in the intakes of carbohydrates, proteins, and fats. In addition, dietary intake of fiber was 17.2 and 15.5 g/d in the placebo and vitamin C groups, respectively, and the intake of the vitamin C group did not differ from that of the placebo group at baseline. At baseline, dietary intake of vitamin C was 52.5 and 41.3 g/d in the placebo and vitamin C groups, respectively, with no significant difference. No significant difference between the placebo and vitamin C groups was found in each change in dietary intakes of energy and nutrients, including vitamin C.

Table 2. Dietary intakes of energy and nutrients

	Placebo (*n* = 22)			Vitamin C (*n* = 24)			Vitamin C *vs.* Placebo	
	Week 0	Week 4	Change	Week 0	Week 4	Change	*P*[a]	*P*[b]
Energy (kcal/d)	1763.4 (505.0)	1784.3 (351.2)	20.9 (506.3)	1725.3 (444.3)	1802.1 (503.1)	76.8 (611.0)	0.78	0.73
Carbohydrate (g/d)	227.8 (72.6)	222.1 (53.5)	–5.7 (78.6)	221.0 (61.3)	221.9 (61.6)	0.9 (98.5)	0.73	0.80
Protein (g/d)	68.8 (23.9)	72.0 (22.3)	3.2 (27.0)	66.9 (17.8)	73.2 (27.3)	6.3 (31.5)	0.75	0.72
Fat (g/d)	57.6 (21.0)	60.4 (21.2)	2.8 (27.8)	60.3 (25.5)	62.6 (28.7)	2.3 (33.1)	0.69	0.95
Fiber (g/d)	17.2 (7.5)	14.4 (5.4)	–2.8 (9.2)	15.5 (5.8)	15.9 (7.1)	0.5 (8.9)	0.46	0.22
Vitamin C (mg/d)	52.5 (57.8)	39.0 (29.6)	–13.5 (66.2)	41.3 (43.8)	34.1 (26.8)	–7.2 (56.8)	0.34	0.73

Values are presented as mean (SD).

[a] *P*-values were obtained by comparing each baseline intake between the placebo and vitamin C groups using an unpaired *t*-test.

[b] *P*-values were obtained by comparing each intake change (week 4 – week 0) between the groups using an unpaired *t*-test.

Adapted from previous publication (Sim *et al*. 2022).

3.3. Serum vitamin C concentration

At baseline, the vitamin C group did not differ from the placebo group in serum vitamin C concentrations (**Figure 4**). At the endpoint, however, only the vitamin C group showed a dramatic increase in serum vitamin C concentrations; the average serum vitamin C concentration in the vitamin C group more than doubled after the intervention (week 0, 42.5 ± 11.7 μmol/L; week 4, 87.7 ± 14.7 μmol/L; $p < 0.0001$) (Figure 4). In addition, all participants in the vitamin C group had over 50 μmol/L of serum concentrations. On the other hand, serum vitamin C concentrations in the placebo group decreased by 30% (week 0, 40.2 ± 17.2 μmol/L; week 4, 30.3 ± 11.8 μmol/L; $p < 0.01$) (Figure 4). These findings suggest that four weeks of vitamin C supplementation successfully improved vitamin C status.

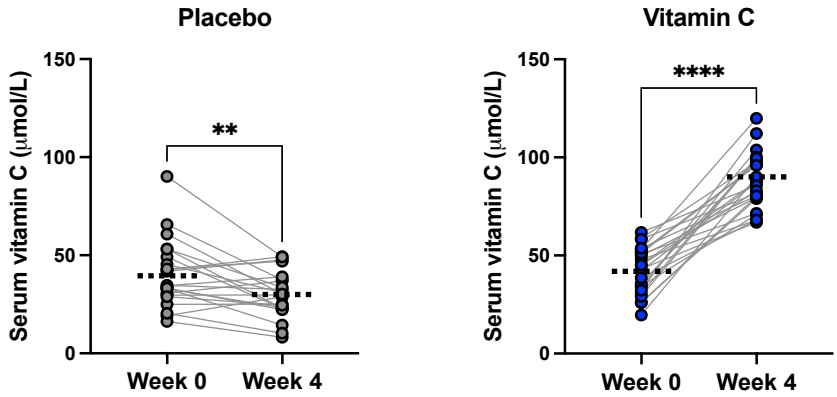

Figure 4. Effects of vitamin C supplementation on serum vitamin C concentrations

Fasting blood samples were collected from participants at rest. The serum concentrations of vitamin C were determined using high-performance liquid chromatography within 24 hours of the blood collection. Comparison of serum vitamin C concentration between the week 0 and week 4 measures within each group was performed using a paired *t*-test (**p<0.01 and ****p<0.001). Mean values are presented as black dashed lines. Adapted from previous publication (Sim *et al.* 2022).

3.4. Mental vitality

Levels of fatigue, subjective attention, work engagement (vigor, dedication, and absorption), and self-control resources were assessed as indicators of mental vitality (**Table 3**). At baseline, the vitamin C group did not differ from the placebo group in all the mental vitality scores (Table 3). However, the vitamin C group showed a greater increase in subjective attention scores than the placebo group (placebo, $\Delta = 0.3 \pm 2.5$; vitamin C, $\Delta = 1.9 \pm 2.7$; $p = 0.03$) (Table 3). In addition, vitamin C supplementation significantly increased work absorption compared to placebo supplementation (placebo, $\Delta = 0.4 \pm 9.7$; vitamin C, $\Delta = 5.8 \pm 10.2$; $p = 0.03$) (Table 3). Also, compared to the placebo group, the vitamin C group showed marginal improvement in fatigue (placebo, $\Delta = -0.05 \pm 2.6$; vitamin C, $\Delta = -1.5 \pm 2.5$; $p = 0.06$) and comprehensive work engagement (placebo, $\Delta = 0.4 \pm 9.7$; vitamin C, $\Delta = 5.8 \pm 10.2$; $p = 0.07$) (Table 3). On the other hand, there was no significant treatment effect on self-control resources (Table 3).

The Stroop color-word test, combined with the mental arithmetic tasks, was performed at week four to evaluate sustained attention, cognitive flexibility, and information processing speed under cognitive fatigue and stress. The number of correct responses did not significantly differ between the placebo and vitamin C groups (placebo group, $n = 126.3 \pm 1.0$; vitamin C group, $n = 125.8 \pm 2.5$) (data not shown). In terms of aggregated reaction time taken to find the correct answer, however, the vitamin C group showed a significantly shorter reaction time than the placebo group ($p = 0.04$) (**Figure 5**A). In addition, there was an inverse correlation between the endpoint serum concentration of vitamin C and the reaction time in the Stroop test ($r = -0.28$, $p = 0.05$) (Figure 5B). These findings suggest that

participants in the vitamin C group successfully avoided distraction under a high level of mental stress, which is attributed to improvement in the vitamin C status of the body.

Concentrations of BDNF, a neurotrophic factor implicated with cognitive function in the brain, was determined in sera. At baseline, there was no significant difference between the vitamin C and placebo groups (data not shown). No significant change in the serum BDNF concentrations was observed within each group (**Figure 6**). In addition, no significant time-by-group interaction was found (Figure 6).

Table 3. Effects of vitamin C supplementation on subjective vitality

	Placebo (n = 22)			Vitamin C (n = 24)			Vitamin C *vs.* Placebo	
	Week 0	**Week 4**	**Change**	**Week 0**	**Week 4**	**Change**[a]	***P***[b]	***P***[c]
Fatigue	8.9 (2.1)	8.9 (1.9)	–0.05 (2.6)	9.3 (2.4)	7.8 (2.7)	–1.5 (2.5)**	0.41	0.06
Attention	7.7 (2.1)	8.0 (2.0)	0.3 (2.5)	7.1 (1.8)	9.0 (2.4)	1.9 (2.7)**	0.34	0.03
Work engagement	74.3 (13.9)	74.7 (14.1)	0.4 (9.7)	68.7 (14.9)	74.4 (16.1)	5.8 (10.2)*	0.62	0.07
Vigor	25.4 (5.4)	27.0 (5.7)	1.5 (4.0)	23.2 (5.7)	25.8 (6.0)	2.5 (4.2)**	0.30	0.41
Dedication	24.5 (5.3)	23.9 (5.4)	–0.6 (4.1)	23.0 (6.8)	24.1 (6.6)	1.0 (4.0)	0.96	0.16
Absorption	24.3 (5.1)	23.8 (5.9)	–0.5 (4.1)	22.4 (4.9)	24.6 (5.5)	2.2 (4.0)*	0.27	0.03
Self-control resources	16.1 (4.1)	17.5 (4.4)	1.4 (4.8)	16.5 (4.3)	18.5 (5.3)	2.0 (4.7)*	0.68	0.72

Values are presented as mean (SD).

[a] Week 0 and week 4 measures differed significantly within the vitamin C group ($^{*}p < 0.05$, $^{**}p < 0.01$; paired t-test or Wilcoxon's signed rank test).

[b] P-values were obtained by comparing each baseline measure between the placebo and vitamin C groups using an unpaired t-test or Mann-Whitney U test.

[c] P-values were obtained by estimating time-by-group interactions using a repeated-measures ANOVA and Bonferroni correction.

Adapted from previous publication (Sim *et al*. 2022).

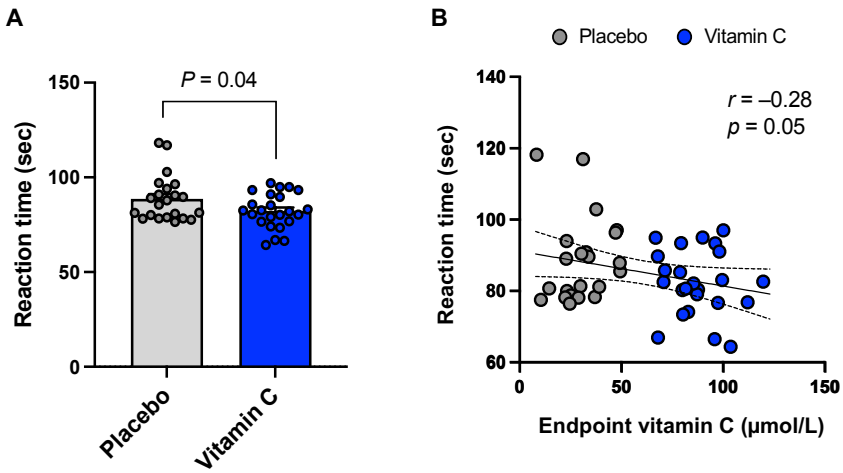

Figure 5. Performance difference in the Stroop test between the vitamin C and placebo groups

The Stroop color-word test was performed to measure cognitive function, such as attention and cognitive flexibility. A mental arithmetic test was conducted just before the Stroop test to assess sustained attention under cognitive fatigue and mental stress. In the Stroop color-word test, the words were colored in one of four different colors of ink (blue, red, yellow, and green), and each word was not colored in the respective color (e.g., the word BLUE was not colored in blue ink). Participants were asked to match the corresponding color of the word as quickly and accurately as possible. Cognitive performance in the Stroop test was assessed by calculating the sum of reaction times taken to report correct answers out of 128 items. Shorter reaction times indicate better cognitive performance on the Stroop tasks. (A) *P*-value was obtained using an unpaired *t*-test. Each dot indicates individual's reaction time in the Stroop test. (B) Correlation coefficient and *p*-value were obtained using Pearson's correlation analysis. The correlation plot is shown with a regression line and 95% confidence interval**.** Adapted from previous publication (Sim *et al*. 2022).

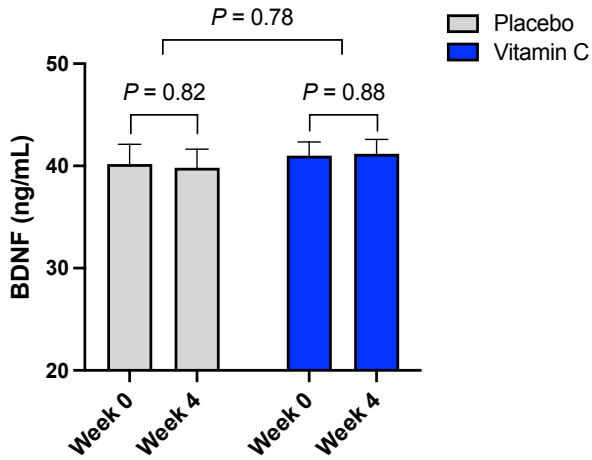

Figure 6. Changes in serum concentrations of BDNF

Statistical difference between the week 0 and week 4 measures within each group was tested using a paired *t*-test. For analysis of the vitamin C supplementation effect on the BDNF concentration, estimation of a time-by-group interaction was performed using a repeated-measures ANOVA. BDNF, brain-derived neurotrophic factor. Adapted from previous publication (Sim *et al*. 2022).

3.5. Mood state

The levels of stress, depression, positive and negative affect, and state anxiety were assessed using self-reported questionnaires. At baseline, the vitamin C group did not differ from the placebo group in all measures of mood states. After four weeks of intervention, the vitamin C group showed a significant decrease in stress scores at the endpoint ($\Delta = -4.7 \pm 10.4$; $p < 0.05$), whereas the placebo group did show a significant change ($\Delta = -3.5 \pm 10.5$; $p > 0.05$) (**Table 4**). However, there was no significant treatment effect of vitamin C on stress (Table 4). Both the placebo and vitamin C groups showed a significant decrease in depression scores at week 4 (placebo, $\Delta = -3.3 \pm 5.2$; vitamin C, $\Delta = -3.1 \pm 4.2$; both $p < 0.01$), without significant between-group difference in score changes (Table 4). For the levels of positive and negative affect and state anxiety, no significant effect of vitamin C was observed (Table 4).

Table 4. Effects of vitamin C supplementation on mood states

	Placebo (*n* = 22)			Vitamin C (*n* = 24)			Vitamin C *vs.* Placebo	
	Week 0	**Week 4**	**Change**[a]	**Week 0**	**Week 4**	**Change**[a]	***P***[b]	***P***[c]
Stress	18.8 (11.5)	15.3 (9.4)	–3.5 (10.5)	18.1 (11.3)	13.4 (12.2)	–4.7 (10.4)*	0.82	0.71
Depression	6.9 (3.9)	3.5 (3.4)	–3.3 (5.2)**	8.1 (6.2)	5.0 (5.1)	–3.1 (4.2)**	0.41	0.86
Positive affect	18.2 (8.3)	18.7 (9.2)	0.5 (6.6)	18.4 (7.5)	20.7 (9.3)	2.3 (6.2)	0.93	0.35
Negative affect	10.0 (7.5)	8.8 (7.4)	–1.2 (4.8)	10.2 (8.5)	10.3 (8.2)	0.0 (5.9)	0.93	0.43
State anxiety	38.9 (10.5)	36.2 (8.4)	–2.6 (10.4)	43.3 (10.6)	38.1 (10.8)	–5.2 (14.3)	0.16	0.49

Values are presented as mean (SD).

[a] Week 0 and week 4 measures differed significantly within each group ($^{*}p < 0.05$, $^{**}p < 0.01$; paired *t*-test).

[b] *P*-values were obtained by comparing each baseline measure between the placebo and vitamin C groups using an unpaired *t*-test.

[c] *P*-values were obtained by estimating time-by-group interactions using a repeated-measures ANOVA and Bonferroni correction.

Adapted from previous publication (Sim *et al*. 2022).

3.6. Gut microbiota

3.6.1. Rarefaction curves of species richness

Gut microbiome profiling was performed to determine whether changes in the intestinal bacterial communities had driven the mental vitality improvement in the vitamin C group. Bacterial genomic DNAs were extracted from stool samples collected at week 0 (baseline) and week four (endpoint) and sequenced using the 16S rRNA sequencing technology. A total of 4,270,913 feature counts were obtained, with an average of 53,386 per sample. Rarefaction curves were constructed based on observed features, Faith's phylogenetic diversity, and the Shannon index to evaluate whether the samples were sequenced enough to represent species richness. As shown in **Figure 7**, each rarefaction curve converged towards a horizontal asymptote, indicating that sequencing depth was sufficient to get a true estimate of alpha diversity.

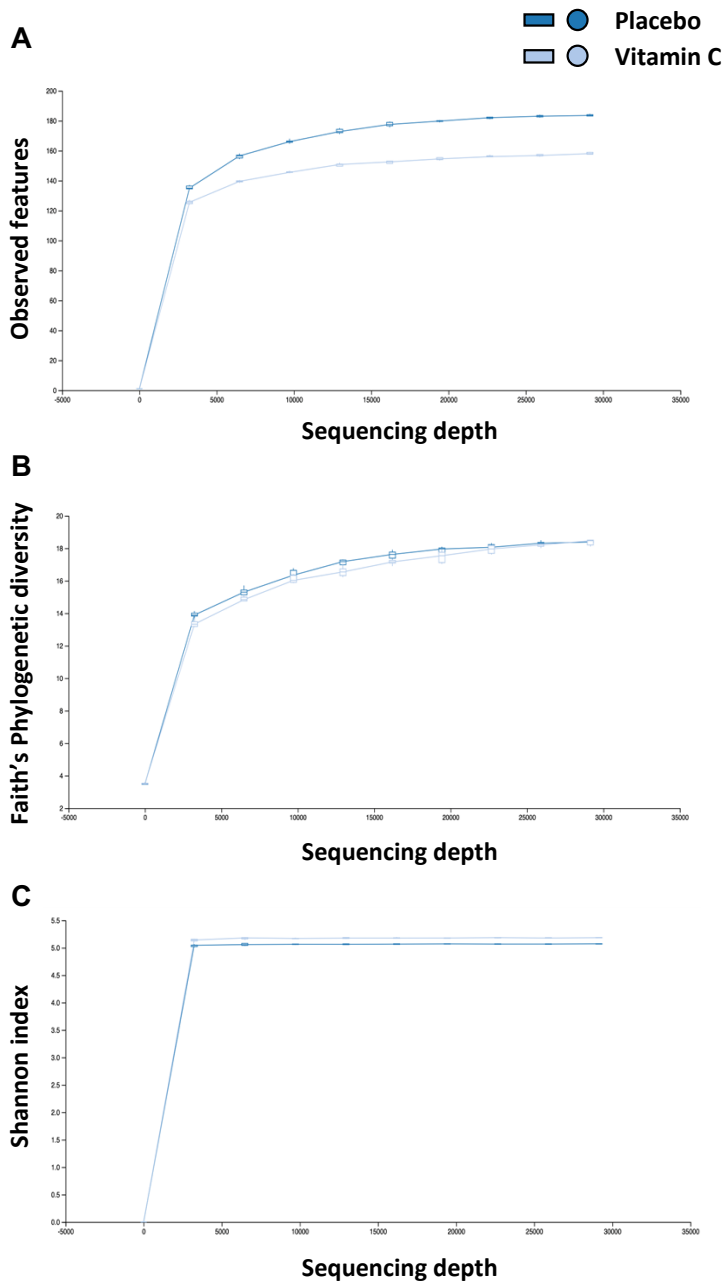

Figure 7. Rarefaction curves of alpha diversity indices of gut bacterial communities

Rarefaction curves were constructed based on (A) observed features, (B) Faith's phylogenetic diversity, and (C) Shannon index to evaluate whether the samples were sequenced enough to represent species richness.

3.6.2. Gut microbial diversity

To examine the effects of vitamin C supplementation on the gut microbial alpha diversity, observed features, Faith's phylogenetic diversity, and the Shannon index were calculated (**Figure 8**). There were no significant differences between the week 0 and week four measures within each group for all indices (Figure 8). As well, no significant time (week 0 versus week 4) × group (vitamin C versus placebo) interactions were discovered for every index (Figure 8). Next, Bray-Curtis dissimilarity was used to quantify the compositional dissimilarity of gut microbiota structure across the groups (**Figure 9**). The PERMANOVA of Bray-Curtis dissimilarity showed that vitamin C supplementation did not significantly alter the beta diversity compared to placebo supplementation (Figure 9).

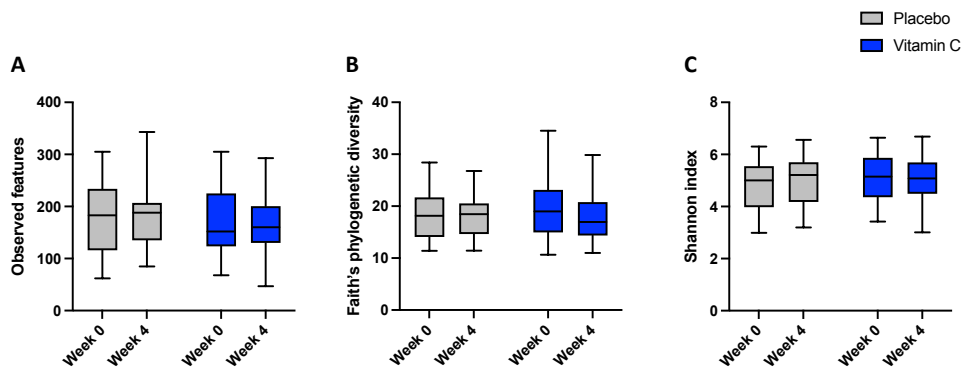

Figure 8. Changes in alpha diversity indices of gut microbiota

Each change in (A) Observed features, (B) Faith's phylogenetic diversity, and (C) Shannon index is shown as a box and whisker plot. Within each box, horizontal lines indicate median values. Boxes extend from the 25th to the 75th percentile of the distribution of values. Vertical extending lines indicate the most extreme values within 1.5 interquartile range of the 25th and 75th percentile.

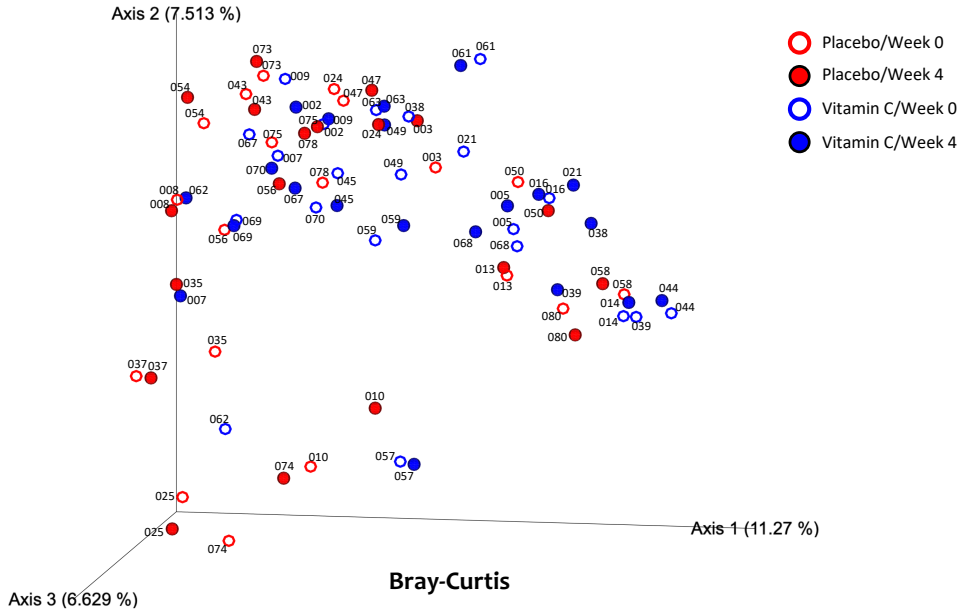

Figure 9. Bray-Curtis dissimilarity of gut microbiota

Gut microbiota profiles were analyzed at week 0 and week 4. Each circle indicates the gut microbiota profile from each individual. Each axis represents percentage of data explained by each coordinate dimension.

3.6.3. Relative abundance of gut microbiota

The relative abundance of specific bacterial taxa was compared between the placebo and vitamin C groups at the phylum and genus levels. As shown in **Figure 10**, the most abundant phylum was Bacteroidetes which were followed by Firmicutes, Proteobacteria, and Actinobacteria. In the relative abundance of Bacteroidetes and Firmicutes, the vitamin C group showed an increasing trend in Bacteroidetes while decreasing in Firmicutes (**Figure 11**A and 11B). On the other hand, the relative abundance of the two dominant phyla in the placebo group remained stable during the intervention period (Figure 11A and 11B). Despite the different pattern of change, no significant time-by-group interactions were discovered in the relative abundance of both Bacteroides and Firmicutes and the Firmicutes to Bacteroidetes ratio (Figure 11).

Next, the relative abundance of gut microbiota was analyzed at the genus level. At week 0, there was no significant difference between the vitamin C and placebo groups in the relative abundance of *Bacillaceae*, *Bifidobacterium*, *Anaerotruncus*, and *Desulfovibrio* (**Figure 12**). However, significant time-by-group interactions were discovered in these four bacterial genera (Figure 12). Compared to placebo supplementation, vitamin C supplementation significantly increased the relative abundance of *Bacillaceae* ($p = 0.002$), *Bifidobacterium* ($p = 0.01$), and *Anaerotruncus* ($p = 0.02$), classified by Gram-positive bacteria (**Figure 13**). On the other hand, the relative abundance of *Desulfovibrio*, a genus of Gram-negative, was significantly reduced in the vitamin C group compared to the placebo group ($p = 0.01$) (Figure 13).

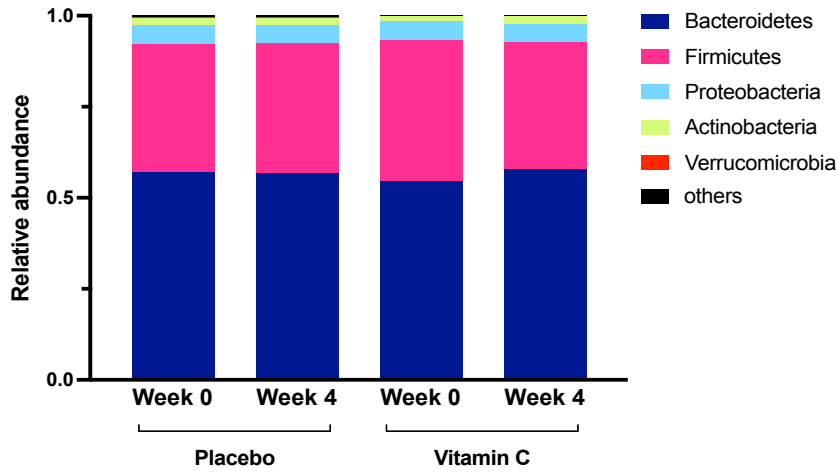

Figure 10. Changes in the relative abundance of gut microbiota at the phylum level

Changes in the relative abundances of gut bacterial phyla are presented with the stacked bar chart. The stacked bar represents the percentage of each phylum.

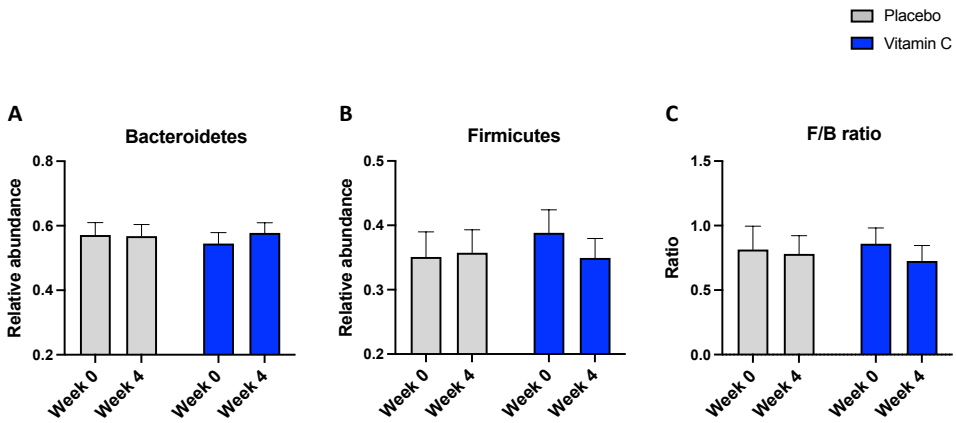

Figure 11. **Relative abundance of Bacteroidetes and Firmicutes, and the Firmicutes to Bacteroidetes ratio**

Changes in the relative abundance of the phylum (A) Bacteroidetes and (B) Firmicutes, and (C) the Firmicutes to Bacteroidetes ratio are shown. Data are presented as mean and SEM. F/B ratio, Firmicutes to Bacteroidetes ratio.

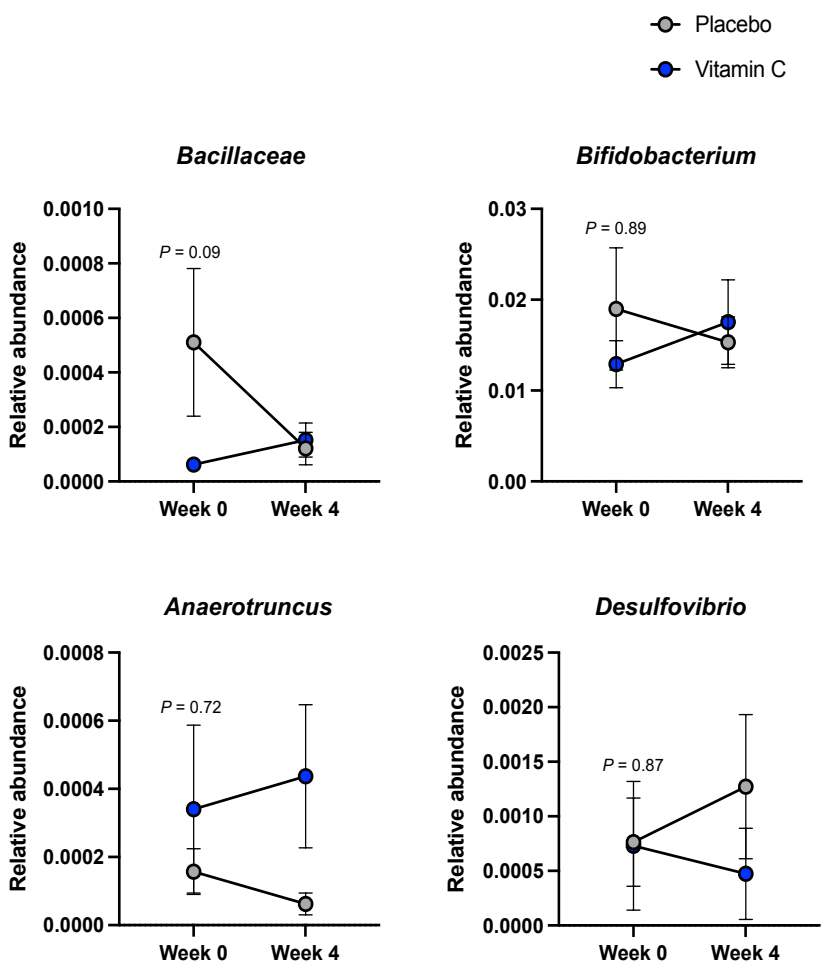

Figure 12. Changes in the relative abundance of gut microbiota at the genus level

Interaction plots show changes in the relative abundance of bacterial genera with significant time-by-group interactions based on DESeq2. Indicated *p*-values were obtained by comparing the week 0 measures between the vitamin C and placebo groups based on DESeq2. Data are presented as mean and SEM.

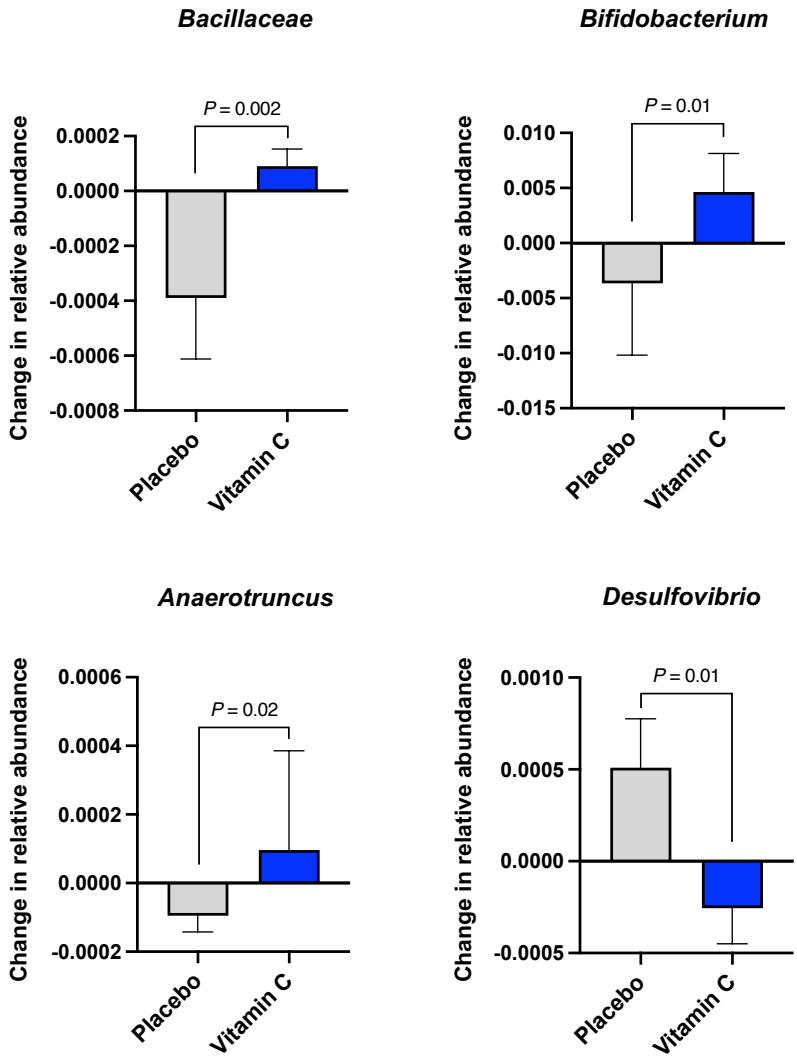

Figure 13. Effects of vitamin C supplementation on the relative abundance of bacterial genera

The bar graphs show the changes in relative abundance of bacterial genera significantly affected by vitamin C supplementation. Indicated *p*-values were obtained by estimating time-by-group interactions based on DESeq2. Data are presented as mean and SEM.

3.6.4. Functional analysis of gut microbiota

Next, the functional contents of the gut microbiota were inferred based on the 16S rRNA gene sequence data and a reference genome database. The interaction plots in **Figure 14** show the changes between week 0 and week four in the relative abundance of the following six microbial metabolic pathways in each group: polyamine biosynthesis I; polyamine biosynthesis II; arginine and polyamine biosynthesis; L-tyrosine biosynthesis; L-phenylalanine biosynthesis; and Entner-Doudoroff pathway. At week 0, there were no significant differences between the groups in the predicted relative abundance of the six pathways (Figure 14). However, vitamin C supplementation significantly reduced the relative abundance of microbial polyamine biosynthesis pathways compared to placebo supplementation (p = 0.06, p = 0.007 and p = 0.03) (**Figure 15**A). In addition, bacterial aromatic amino acid biosynthesis was significantly decreased in the vitamin C group compared to the placebo group (both p = 0.01) (Figure 15B). For the Entner-Doudoroff pathway, known that certain Gram-negative bacteria use for glucose catabolism under aerobic conditions, vitamin C supplementation significantly decreased the predicted relative abundance compared to placebo supplementation (p = 0.03) (Figure 15C).

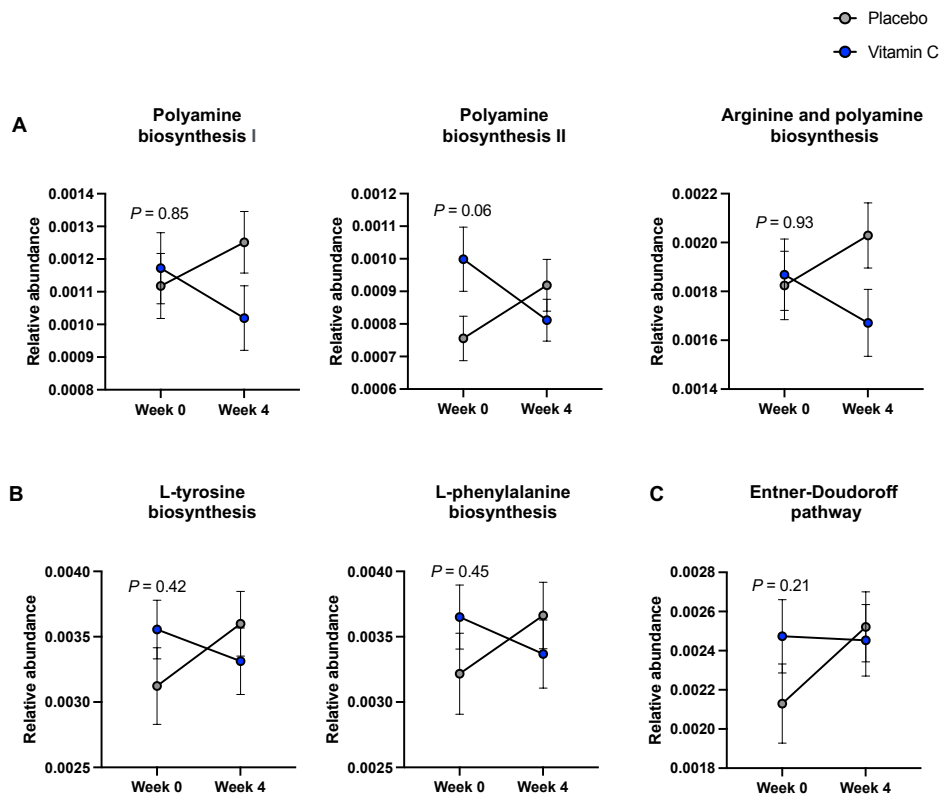

Figure 14. **Changes in the predicted relative abundance of microbial metabolic pathways**

Functional analysis based on the 16S rRNA gene sequence data and a reference genome database predicted the relative abundance of bacterial metabolic pathways. Interaction plots show changes in the predicted relative abundance of following bacterial pathways with significant time-by-group interactions based on a linear mixed-effect modeling: (A) polyamine biosynthesis, (B) aromatic amino acid biosynthesis, and (C) Entner-Doudoroff pathway. Indicated *p*-values were obtained by comparing the baseline measures between the vitamin C and placebo groups using a Mann-Whitney *U* test. Data are presented as mean and SEM.

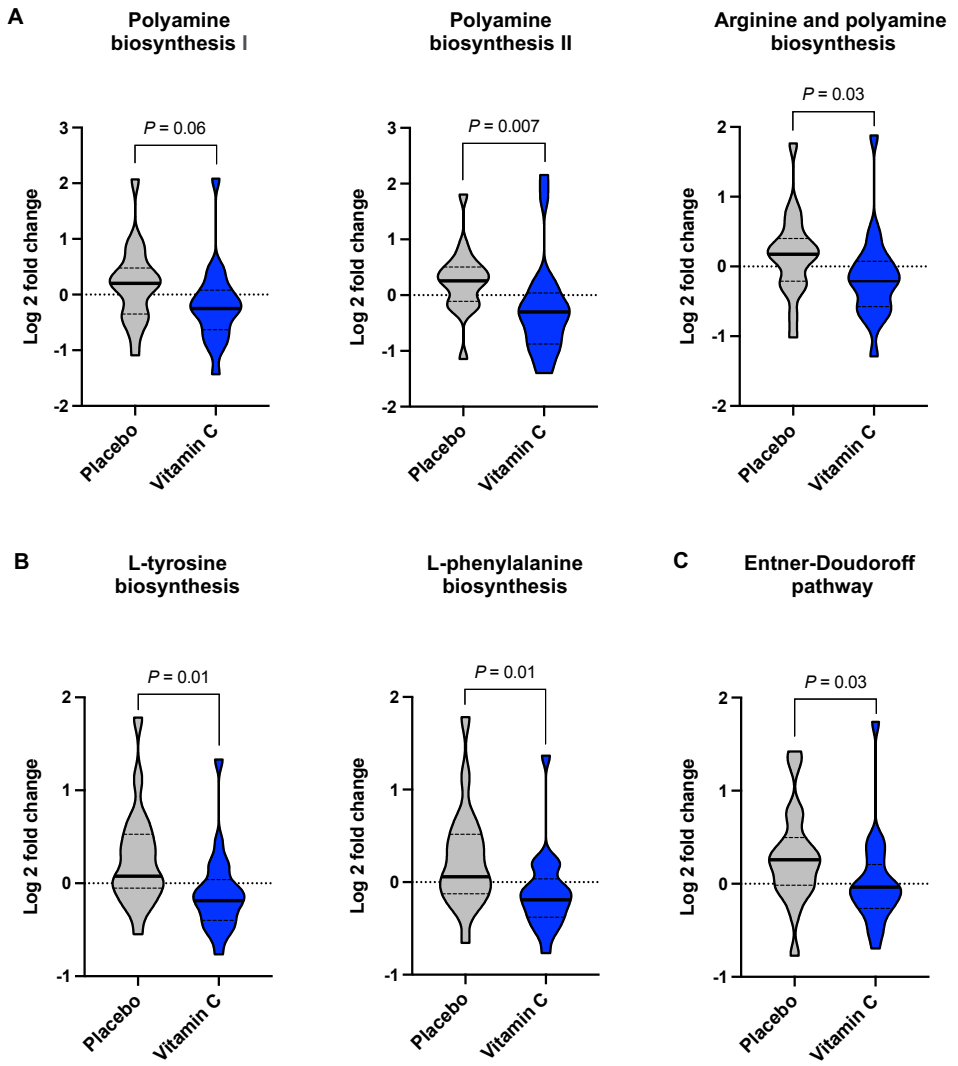

Figure 15. Effects of vitamin C supplementation on the predicted relative abundance of microbial metabolic pathways

Functional analysis based on the 16S rRNA gene sequence data and a reference genome database predicted the relative abundance of bacterial metabolic pathways. Indicated *p*-values were obtained by estimating time-by-group interactions in the relative abundance of (A) polyamine biosynthesis, (B) aromatic amino acid biosynthesis, and (C) Entner-Doudoroff pathway using a linear mixed-effect

modeling. The width of the violin plot is proportional to the density of the values. The lowest and highest dashed lines inside the plot represent the first and third quartiles, respectively. The median is shown as a solid line inside the plot.

3.6.5. Gut microbial-derived molecule and metabolite

In order to verify the results of the functional analysis of gut microbiota, the concentrations of LBP and spermidine were measured in participants' sera.

The concentrations of serum LBP that binds to LPS, a molecule found in the outer membrane of Gram-negative bacteria, were determined considering the observation that vitamin C supplementation had decreased the predicted relative abundance of the Entner-Doudoroff pathway mostly discovered in Gram-negative bacteria. As shown in **Figure 16**A, there was a significant direct correlation between changes in serum LBP concentrations and changes in the relative abundance of the microbial Entner-Doudoroff pathway ($r = 0.521$; $p < 0.001$). At week 0, there was no significant difference between the placebo and vitamin C groups in baseline measures of serum LBP (Figure 16B). However, the placebo group showed a significant increase in LBP concentrations at week four compared to week 0 ($p = 0.009$), whereas the vitamin C group did not show a significant change in LBP concentrations (Figure 16B). Consequently, there was a significant time-by-group interaction in the concentration change of serum LBP ($p = 0.006$) (Figure 16C).

The concentration of spermidine, one of the most common bacteria-producing polyamines, was measured in the serum samples based on the observation that vitamin C supplementation was predicted to decrease the relative abundance of microbial polyamine biosynthesis. As presented in **Figure 17**A**,** there was a significant direct correlation between changes in serum spermidine concentrations and changes in the relative abundance of microbial arginine and polyamine biosynthesis ($r = 0.459$; $p < 0.01$), which indicated that intestinal

microbiota-derived polyamines were absorbed in the systemic circulation. At week 0, there was no significant difference between the placebo and vitamin C groups in baseline measures of serum spermidine (Figure 17B). However, during the intervention period, two groups showed opposite patterns of change in spermidine concentrations: the placebo group showed a great increase at week four compared to week 0 ($p = 0.05$), whereas the vitamin C group showed a trend of decrease ($p = 0.07$) (Figure 17B). Consequently, there was a significant time-by-group interaction in the concentration change of serum spermidine ($p = 0.014$) (Figure 17C).

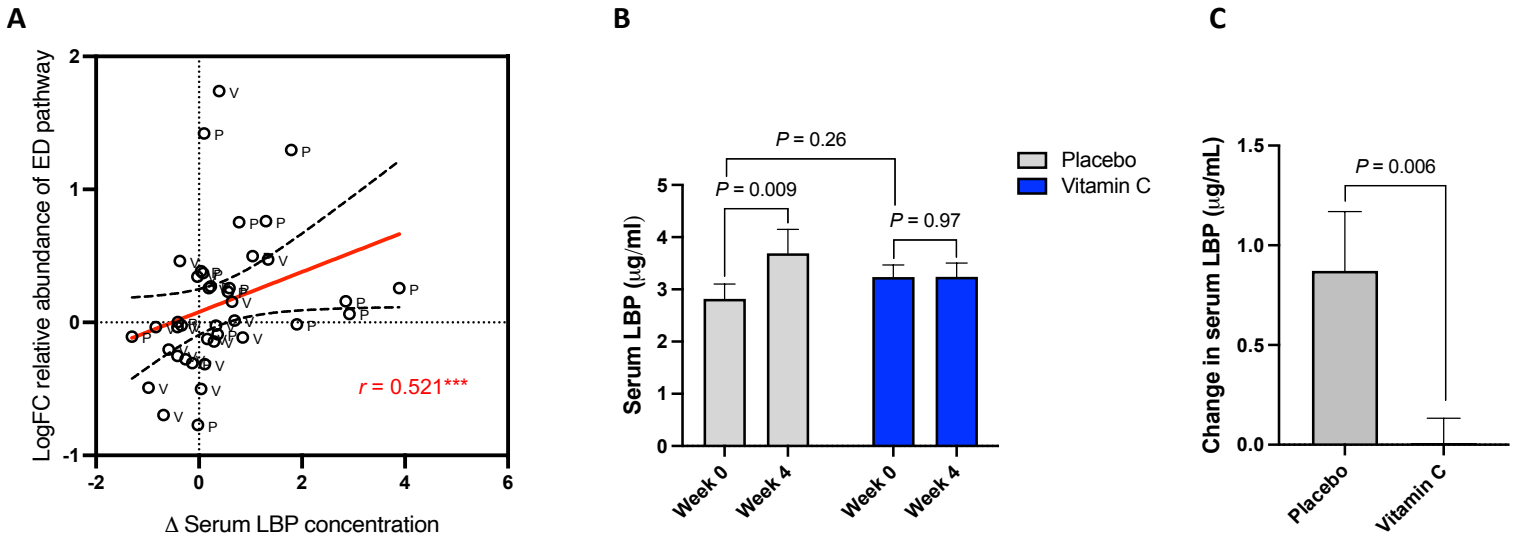

Figure 16. **Effects of vitamin C supplementation on serum concentrations of LBP**

(A) The correlation coefficient between changes (week 4 – week 0) in serum LBP concentrations and fold changes in the predicted relative abundance of the ED pathway was estimated using Spearman correlation analysis (***$p < 0.001$). Each circle represents the individual's measure (V, vitamin C group; P, placebo group). (B) Between-group difference in the week 0 measures was tested using an unpaired *t*-test. Comparison between the week 0 and week 4 measures within each group was performed using a paired *t*-test. Data are presented as mean

and SEM. (C) *P*-value was obtained by estimating a time-by-group interaction in the serum LBP change (week 4 – week 0) using a linear mixed-effect modeling. Data are presented as mean and SEM. LBP, lipopolysaccharide-binding protein; ED pathway, Entner-Doudoroff pathway; FC, fold change.

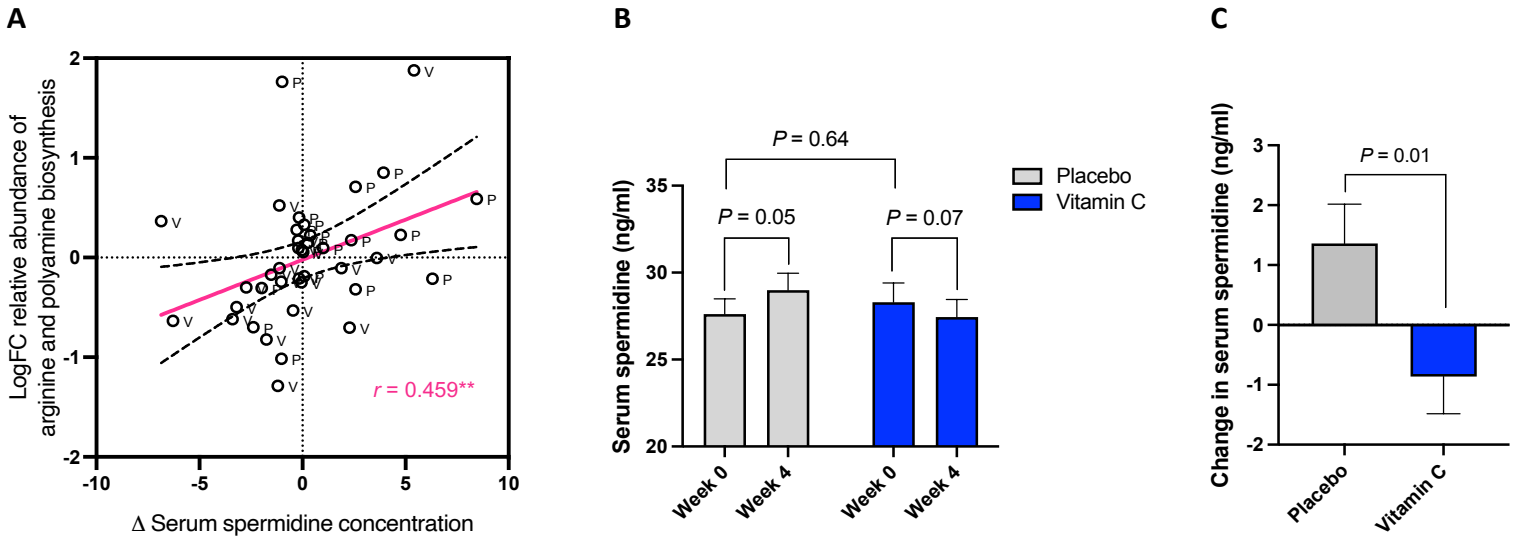

Figure 17. **Effects of vitamin C supplementation on serum concentrations of spermidine**

(A) The correlation coefficient between changes (week 4 – week 0) in serum spermidine concentrations and fold changes in the predicted relative abundance of the polyamine biosynthesis pathway was estimated using Spearman correlation analysis (**$p < 0.01$). Each circle represents the individual's measure (V, vitamin C group; P, placebo group). (B) Between-group difference in the week 0 measures was tested using an unpaired *t*-test. Comparison between the week 0 and week 4 measures within each group was performed using a paired *t*-test. Data

are presented as mean and SEM. (C) *P*-value was obtained by estimating a time-by-group interaction in the serum spermidine change (week 4 – week 0) using a linear mixed-effect modeling. Data are presented as mean and SEM. FC, fold change.

3.6.6. Relationship between gut microbiota and mental vitality

To identify whether mental vitality levels were associated with gut microbiota profiles, Spearman correlation analysis was performed between the changes in bacterial genera or predicted functional pathways and the changes in self-reported mental vitality or experimentally measured cognitive performance. *Bacillaceae*, which was increased by vitamin C supplementation, directly correlated with subjective attention while inversely correlated with fatigue (**Figure 18**). *Anaerotruncus*, increased in the vitamin C group, directly correlated with subjective attention, work dedication, and comprehensive work engagement scores (Figure 18). On the other hand, *Desulfovibrio*, reduced by vitamin C supplementation, was inversely correlated with cognitive performance in the Stroop test (Figure 18). In addition, changes in the predicted microbial metabolic functions also showed multiple correlations with mental vitality measures: bacterial polyamine and aromatic amino acid biosynthesis, which were decreased in the vitamin C group, inversely correlated with work absorption and dedication; and bacterial Entner-Doudoroff pathway and polyamine biosynthesis, reduced by vitamin C supplementation, inversely correlated with cognitive performance in the Stroop test (Figure 18). These findings suggest that vitamin C-induced alteration in the gut microbiota profiles is intimately related to mental vitality improvement.

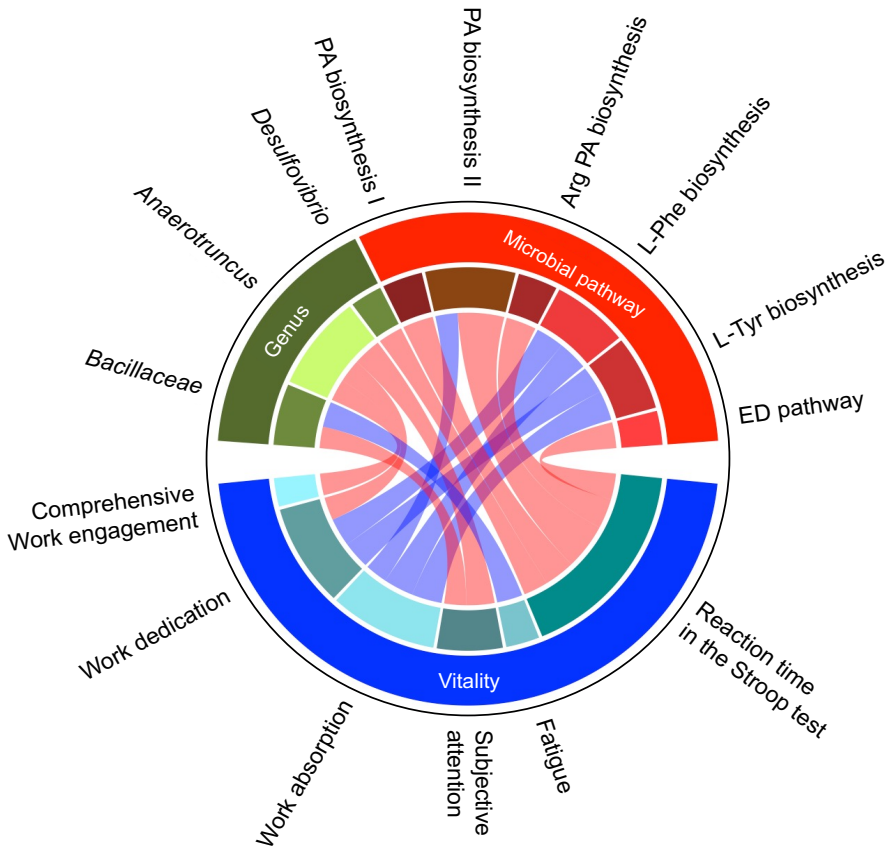

Figure 18. Relationship between gut microbiota profiles and mental vitality indices

The circos plot depicts a correlation network between changes in gut microbiota profiles and mental vitality indices. Changes in gut microbiota profiles include specific genera and predicted microbial metabolic functions with significant time-by-group interactions ($p < 0.05$; DESeq2 or linear mixed-effect modeling). Mental vitality indices include work engagement (work dedication and absorption), subjective attention, fatigue, and reaction time in the Stroop color-word test. The levels of work engagement, subjective attention, and fatigue were measured using validated self-reported questionnaires. In the Stroop color-word test, the words

were colored in one of four different colors of ink (blue, red, yellow, and green), and each word was not colored in the respective color (e.g., the word BLUE was not colored in blue ink). Participants were asked to match the corresponding color of the word as quickly and accurately as possible. Reaction time in the Stroop test refers to the average time to complete the Stroop task: shorter reaction times indicate better cognitive performance. The connecting lines in the center indicate direct (red) or inverse (blue) correlations. The thickness of the connecting lines is proportional to the absolute value of the Spearman coefficient. The correlation network only shows the statistically significant Spearman coefficients ($p < 0.05$). Arg, arginine; PA, polyamine; ED pathway, Entner-Doudoroff pathway; L-Phe, L-phenylalanine; L-Tyr, L-tyrosine.

3.7. Cytokines

3.7.1. Changes in serum concentrations of cytokines

Based on the observation that vitamin C supplementation reduced circulating microbial-derived LPS, measurement of systemic inflammatory cytokines was performed. Thus, following proinflammatory cytokines were simultaneously measured in sera: IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13 and TNF-α. Of the 10 cytokines, the measured values of IL-1β, IL-2, IL-4, IL-12p70, and IL-13 were below the lower quality limit (LLOQ) in most samples; therefore, those five cytokines were excluded from all subsequent analyses.

At week 0, there were no significant differences between the placebo and vitamin C groups in baseline measures of IL-6, IL-8, IL-10 and IFN-γ (**Figure 19**). However, the placebo group showed a higher baseline concentration of TNF-α compared to the vitamin C group ($p < 0.001$) (Figure 19). The placebo group presented significant increases in serum concentrations of IL-6, IL-10 and IFN-γ at week four compared to week 0 (all $p < 0.05$) (Figure 19). On the other hand, the vitamin C group showed significant decreases at week four in IL-8 and TNF-α concentrations compared to baseline measures (all $p < 0.05$) (Figure 19). Analyzing of time-by-group interaction for the concentration change from baseline, the decrease in serum IL-8 was slightly larger in the vitamin group than in the placebo group ($p = 0.08$) (**Figure 20**). Moreover, vitamin C supplementation greatly decreased the concentrations of IL-6, IL-10 and TNF-α compared to placebo supplementation (all $p < 0.05$) (Figure 20).

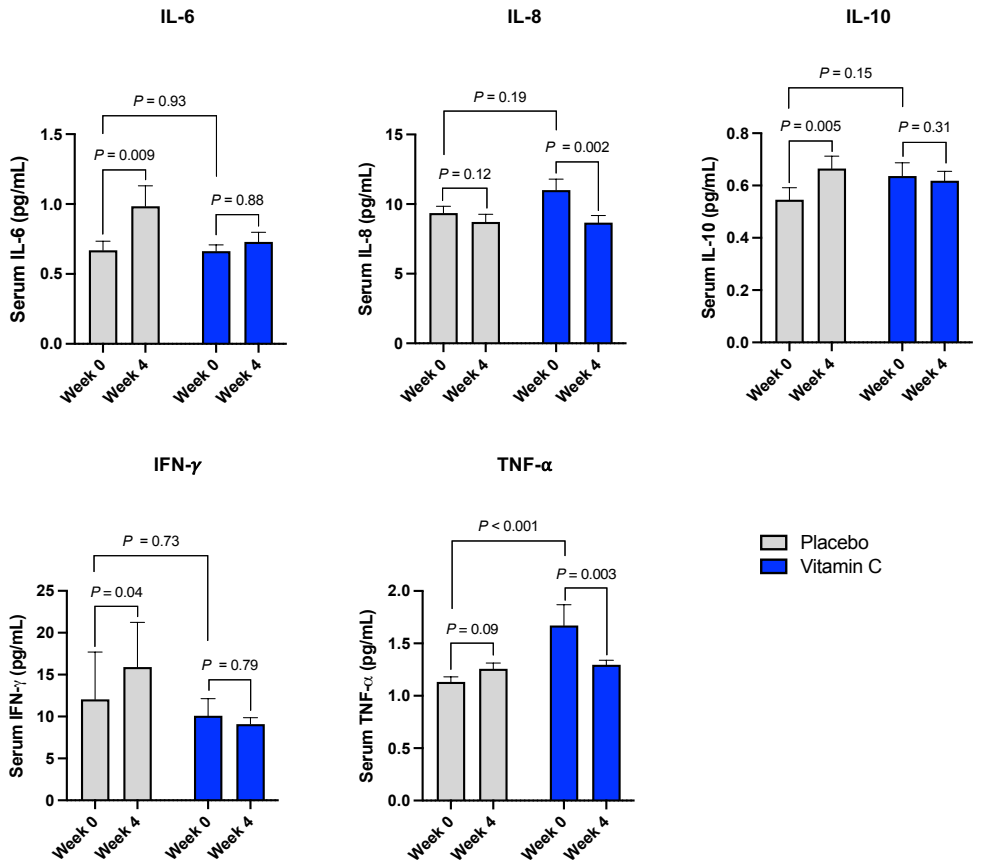

Figure 19. Changes in serum concentrations of cytokines

The bar graphs show the concentrations of serum IL-6, IL-8, IL-10, IFN-γ and TNF-α. Comparison the week 0 measures between the vitamin C and placebo groups was performed using a Mann-Whitney *U* test. Comparison between the week 0 and week 4 measures within each group was performed using a Wilcoxon's signed rank test. Data are presented as mean and SEM. IL, interleukin; IFN, interferon; TNF, tumor necrosis factor.

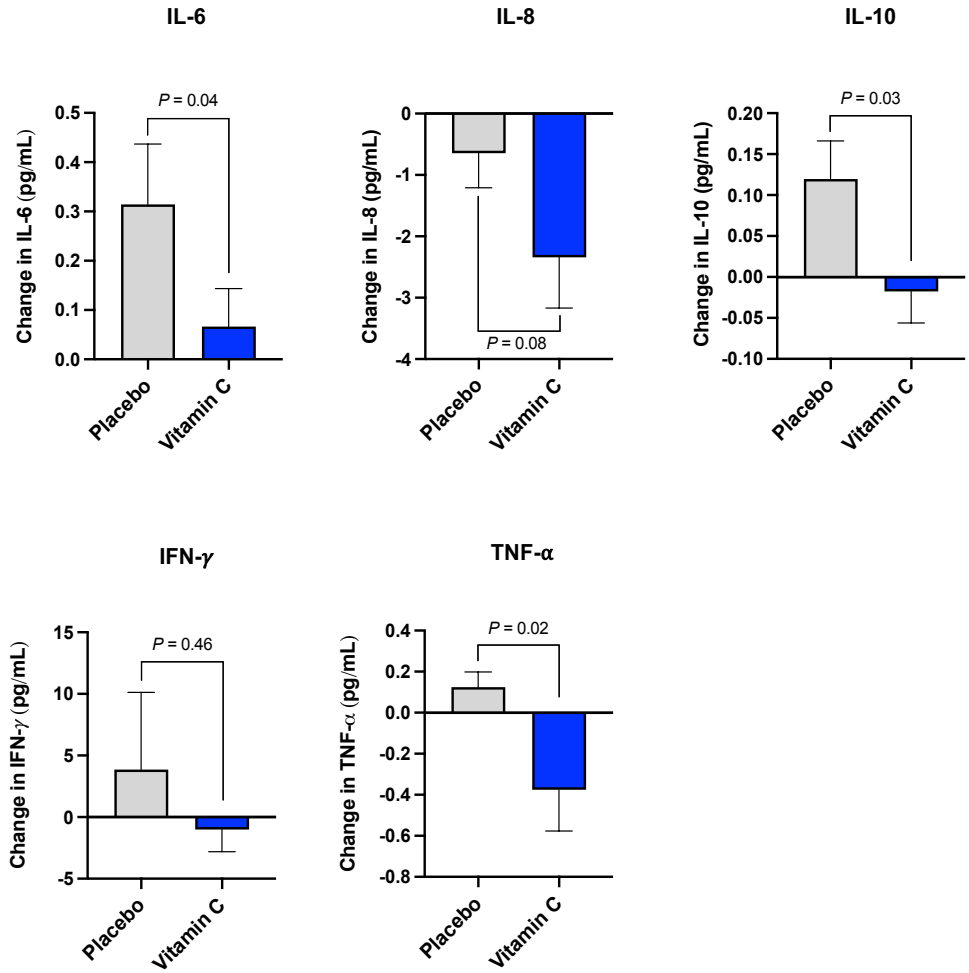

Figure 20. **Effects of vitamin C supplementation on serum concentrations of cytokines**

The bar graphs show concentration changes (week 4 – week 0) in serum IL-6, IL-8, IL-10, IFN-γ and TNF-α. Indicated *p*-values were obtained by estimating time-by-group interactions in the concentration change using a linear mixed-effect modeling. Data are presented as mean and SEM. IL, interleukin; IFN, interferon; TNF, tumor necrosis factor.

3.7.2. Relationship between changes in cytokine levels and cognitive performance

Next, correlation analysis was performed to determine whether each change from baseline in serum concentrations of IL-6, IL-8, IL-10, IFN-γ and TNF-α correlated with reaction time in the Stroop test conducted at week four. As a result, the placebo group showed significant direct correlations between changes in serum IL-8 and IL-10 and reaction time in the Stroop test (IL-8, $r = 0.47$ and $p = 0.04$; IL-10, $r = 0.45$ and $p = 0.05$) (**Figure 21**). On the other hand, in the vitamin C group, there was no significant direct correlation between cognitive performance and changes in cytokine concentrations (Figure 21). These findings indicate that poor cognitive performance was associated with increased concentrations of serum cytokines. Further, the decrease in systemic cytokine levels driven by vitamin C supplementation is intimately linked with better performance in the cognitive test.

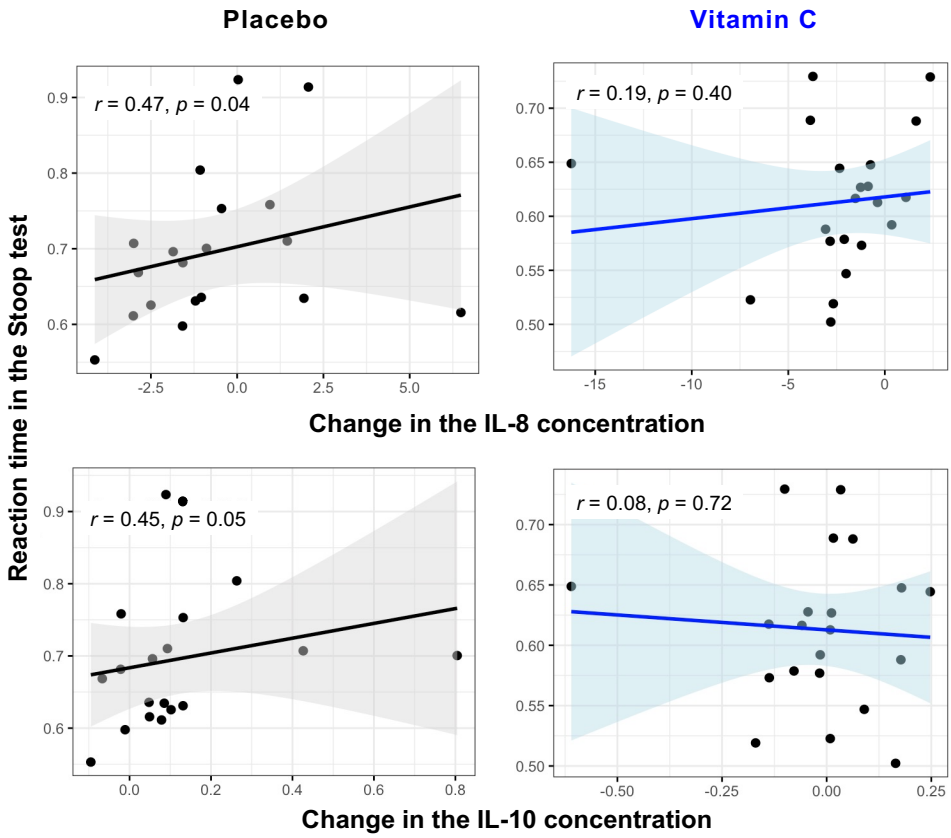

Figure 21. Relationship between changes in cytokine levels and cognitive performance

The correlation coefficient between changes (week 4 – week 0) in serum IL-8 or IL-10 and cognitive performance in the Stroop color-word test was estimated using Spearman correlation analysis. The Stroop color-word test was performed to measure cognitive functions such as attention, cognitive flexibility, and information processing speed. In the Stroop color-word test, the words were colored in one of four different colors of ink (blue, red, yellow, and green), and each word was not colored in the respective color (e.g., the word BLUE was not colored in blue ink). Participants were asked to match the corresponding color of the word as quickly and accurately as possible. Shorter reaction times indicate

better Stroop task performance. Each dot represents an individual measure. Shadows enclosing the lines represent the 95% confidence interval. IL, interleukin.

3.8. Neurotransmitters

3.8.1. Changes in serum concentrations of neurotransmitters

Serum concentrations of neurotransmitters were measured to determine whether any changes in neurotransmitter profiles had mediated the vitamin C-induced improvement in mental vitality. Following neurotransmitters were measured in sera using UHPLC-MS: L-DOPA, dopamine, norepinephrine, epinephrine, and serotonin. Of the five neurotransmitters, dopamine and epinephrine were not detected in most samples; therefore, these two neurotransmitters were excluded from all subsequent analyses.

For serum concentrations of L-DOPA, norepinephrine, and serotonin, there were no significant differences between the placebo and vitamin C groups in baseline measures (**Figure 22**). During the intervention period, the placebo group showed a decreasing trend in the L-DOPA concentration, whereas the vitamin C group showed an increasing trend, but there was no significant time-by-group interaction in L-DOPA concentrations (**Figure 23**A) For norepinephrine and serotonin, there were also no significant time-by-group interactions in concentration changes (Figure 23B and 23C).

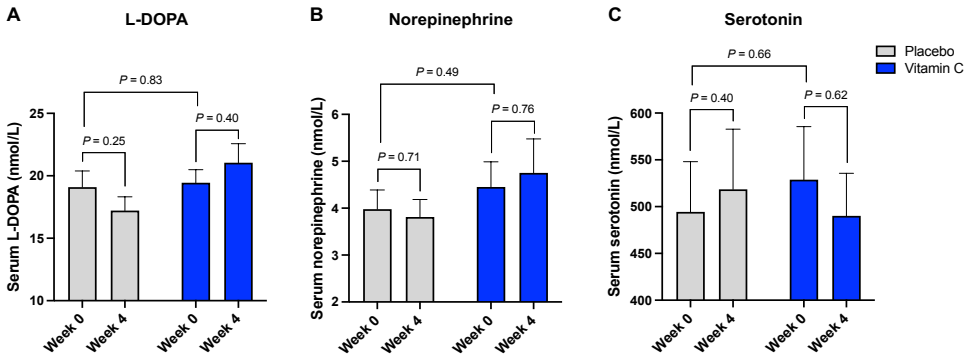

Figure 22. Changes in serum concentrations of neurotransmitters

The bar graphs show the concentrations of serum (A) L-DOPA, (B) norepinephrine, and (C) serotonin. Comparison the week 0 measures between the vitamin C and placebo groups was performed using a Mann-Whitney *U* test. Comparison between the week 0 and week 4 measures within each group was performed using a Wilcoxon's signed rank test. Data are presented as mean and SEM.

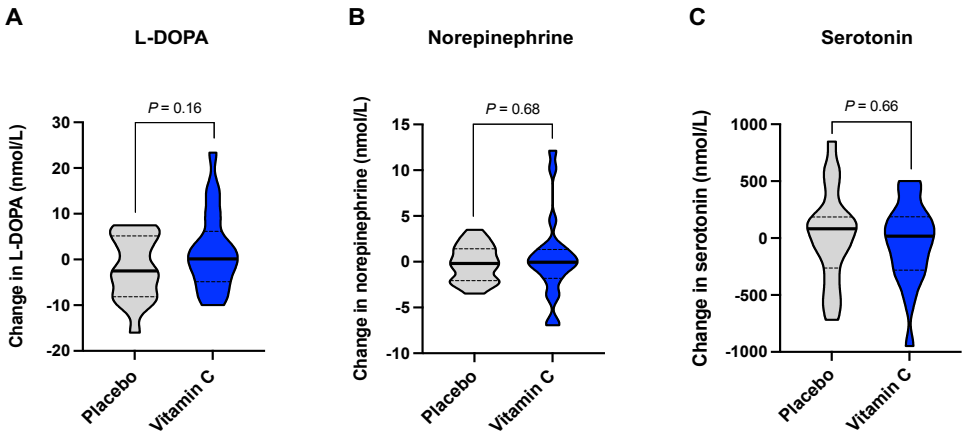

Figure 23. Effects of vitamin C supplementation on serum concentrations of neurotransmitters

Concentration changes (week 4 – week 0) in serum (A) L-DOPA, (B) norepinephrine, and (C) serotonin are shown. Indicated *p*-values were obtained by estimating time-by-group interactions using a linear mixed-effect modeling. The width of the violin plot is proportional to the density of the values. The lowest and highest dashed lines inside the plot represent the first and third quartiles, respectively. The median is shown as a solid line inside the plot.

3.8.2. Relationship between changes in neurotransmitter levels and work engagement

Next, correlation analysis was performed to determine whether each change from baseline in serum concentrations of L-DOPA, norepinephrine, and serotonin correlated with changes in work engagement indices. There were no significant correlations between work engagement indices and norepinephrine or serotonin concentrations in both the placebo or vitamin c groups (**Figure 24**). On the other hand, an increase in L-DOPA concentrations directly correlated with an increase in work engagement only in the vitamin C group: in particular, robust positive correlations were observed in work dedication, absorption, and comprehensive engagement (all $p < 0.05$) (Figure 24). These findings suggest that the enhancement of attention and motivation toward work or study, induced by vitamin C supplementation, is related to the normal functioning of dopamine, which is synthesized from L-DOPA in the brain.

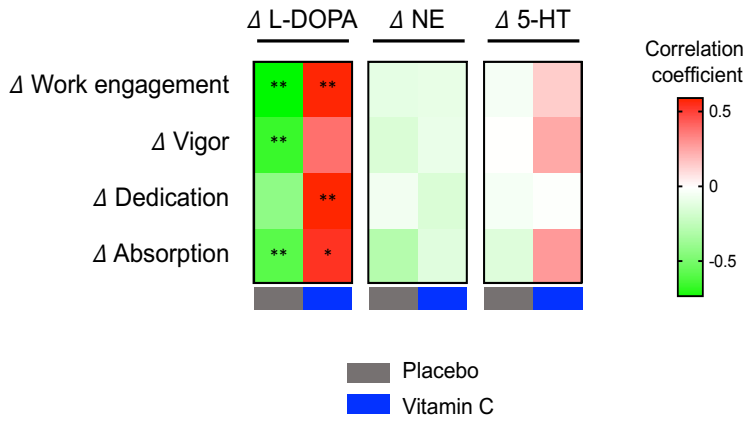

Figure 24. Relationship between changes in neurotransmitter levels and work engagement

The heatmap depicts the correlation coefficients between changes (week 4 – week 0) in serum concentrations of neurotransmitters (L-DOPA, norepinephrine, and serotonin) and changes in self-reported work engagement. Work engagement, defined as a positive and fulfilling state of mind toward work or study, consists of three dimensions: vigor, dedication, and absorption. The comprehensive work engagement score was calculated as the sum of work vigor, dedication, and absorption scores. Correlation coefficients are indicated by color keys (green, inversely correlated; red, directly correlated), and the strength of color is proportional to the absolute value of the coefficient (*$p < 0.05$ and **$p < 0.01$; Pearson's or Spearman correlation analysis). NE, norepinephrine; 5-HT, serotonin.

4. Discussion

A randomized, double-blind, placebo-controlled trial was conducted to determine the effect of vitamin C on neuropsychological functioning and to investigate how vitamin C improves mental vitality via the gut-microbiota-brain axis. A four-week intervention trial was performed in healthy young subjects with below-saturation levels of serum vitamin C. Daily supplementation with 1000 mg of vitamin C increased subjective attention more than placebo supplementation. Notably, vitamin C group showed a significant increase in self-rated attention levels compared to the placebo group. In addition, vitamin C supplementation significantly increased motivation and concentration on work or study more than placebo supplementation, contributing to longer attention spans during cognitive tasks. Along with this, the abundance of LPS-producing gut bacteria, which induce a signaling cascade of proinflammatory cytokine release, was significantly reduced in the vitamin C group and was associated with changes in mental vitality indices. These findings provide insight into the beneficial effects of vitamin C on mental vitality mediated by gut microbial changes.

Today, the prevalence of scurvy, a vitamin C deficiency disease, is rare worldwide (Ceglie *et al.* 2019). Therefore, clinical evidence is urgently needed to support that maintaining adequate vitamin C status is essential for optimal health outcomes, not just preventing scurvy. However, few human studies have investigated the clinical significance of optimal vitamin C status. In addition, previous clinical trials reporting health effects of vitamin C supplementation have some critical limitations and are therefore of low quality to provide conclusive evidence. In this regard, Lykkesfeldt and Poulsen pointed out that most available

vitamin C supplementation studies did not use vitamin C as a single agent and the population vitamin C status at entry was not clear, leaving the reliability of the study findings controversial (Lykkesfeldt and Poulsen 2010). In addition, it was noted that endpoint markers for vitamin C status, such as plasma vitamin C concentrations, were not determined in most clinical trials. The current intervention study, designed to overcome these limitations, provides a notable perspective on the role of vitamin C in mental function. In particular, this study reported the effects of vitamin C supplementation on various psychological measures such as subjective attention, work engagement, and self-regulation ability, which had not been previously reported. Work engagement indicates one's interest and enthusiasm in work and job and is associated with favorable emotional, physical, and cognitive outcomes (Bakker, Albrecht, and Leiter 2011). Those with higher work engagement are reported to be energetic, proud, and optimistic and effectively immerse themselves in work. Current findings suggest that improved vitamin C status increases individuals' willingness to put more effort into work activities and intellectual commitment. In addition, vitamin C supplementation increased participants' self-rating of attention more than placebo supplementation, which may have contributed to promoting feelings of focus and engagement during the task (Schaufeli and Bakker 2004). Motivation is one of the critical psychological domains that determine mental energy. It is associated with success and achievement at school or the workplace (Muraven 2010). In addition, participants supplemented with vitamin C experienced an increase in self-regulatory resource levels compared to their baseline states, whereas the placebo group did not. In a mouse model genetically modified to be incapable of synthesizing ascorbic acid, vitamin C-deficient mice exhibited hedonistic eating despite no energy deficit

(Fiona E. Harrison 2013), suggesting that inadequate vitamin C status could lead to a loss of ability to control one's behavior and emotions. Motivation and self-regulation contribute to goal-directed behaviors by helping one overcome difficulties and take on challenges (Tangney, Baumeister, and Boone 2004). In the present study, optimal vitamin C status helped subjects maintain concentration for long periods, even in states of high cognitive fatigue. These findings indicate that vitamin C enhances mental resilience under mental stress, leading to goal pursuit and accomplishment. Although there is little evidence of the effect of optimal vitamin C status on attentional abilities, a previous cross-sectional study reporting serum vitamin C concentrations directly correlated with performance on the Stroop test partially supports the current findings (Travica *et al.* 2019). BDNF, an essential regulator of neuronal survival and synaptic plasticity, is a reliable biomarker for changes in cognitive domains (Lima Giacobbo *et al.* 2019). *In vitro* experiments and animal studies have shown that vitamin C induces the expression and production of BDNF (Grant, Barber, and Griffiths 2005; Delrobaei *et al.* 2019). However, there was no change in serum BDNF concentrations in the current vitamin C supplementation group. These results indicate that vitamin C-induced superior attention might have been attributed to increased mental vitality rather than cognitive enhancement. Current findings support that optimal vitamin C status could energize one’s daily life by promoting non-cognitive factors, such as motivational strategies and self-regulatory resources. However, further clinical studies in general populations with varied demographic factors are needed to support these findings.

Notably, only individuals supplemented with vitamin C had a direct correlation between elevated L-DOPA concentration and increased work

motivation. However, such a positive correlation was not discovered in those who received a placebo supplement. Dopaminergic signaling is deeply concerned with both emotional arousal and successful executive control (Puglisi-Allegra and Ventura 2012; Dang *et al.* 2016; Logue and Gould 2014). Since L-DOPA is a precursor of dopamine, it is plausible that there was any interference with normal dopamine functioning in the placebo group (Cools 2006). Several *in vivo* studies strongly suggest that vitamin C is directly involved in dopamine synthesis, synaptic release and uptake (L. Lee *et al.* 2001; Wagner, Jarvis, and Carelli 1985; Rebec and Pierce 1994). For example, previous animal studies have reported that vitamin C deficiency induces abnormalities in the dopaminergic system, manifested by decreased social behavior (Fiona E. Harrison 2013; Ward *et al.* 2013). The current results indicate that optimal vitamin C status promoted motivational function by increasing the availability of L-DOPA, not affecting its concentration. High circulating vitamin C, which saturates vitamin C levels in most tissues, including the brain, might have contributed to L-DOPA conversion or synaptic dopamine transmission (Rebec and Pierce 1994; Myken *et al*. 2022). In addition, the disturbance of dopaminergic action observed in the current placebo group might have been partly due to increased circulating polyamines derived from the gut microbiota. The gut microbiome may contribute to some extent to the polyamine pool in the human body (Tofalo, Cocchi, and Suzzi 2019). In particular, agmatine, a kind of bacterial polyamine, is known to cross the blood-brain barrier and serve as a precursor of putrescine in neurons (Reis and Regunathan 2000; Sánchez-Jiménez *et al.* 2013). There has been reported that intraperitoneal or intracerebral injection of spermidine significantly affected the polyamine metabolism and levels in the mouse hypothalamus and disrupted blood-brain barrier integrity, which

supports the possibility that gut bacteria-derived spermidine may exert a significant effect on the brain function (D. Jiang *et al.* 2021; Glantz *et al.* 1996). There has been no report of the physiological role of increased polyamine in a healthy population. However, in schizophrenia, which is associated with dopamine system dysfunction, high levels of polyamines are frequently observed in the blood and brain of patients (Turecki 2013; Baroli *et al.* 2020; Fiori and Turecki 2008). In neurons, S-adenosyl methionine (SAM) is essential for adenosylmethionine decarboxylase 1, a rate-limiting enzyme of polyamine biosynthesis, to convert putrescine to spermidine (Turecki 2013). Once decarboxylated, SAM is irreversibly committed to the polyamine synthesis pathways. Since SAM is also involved in various pathways that regulate amine pools in the brain, including dopamine, it has been hypothesized that high levels of cerebral polyamines in psychiatric disorders may disrupt the dopamine availability (Baroli *et al.* 2020; Sánchez-Jiménez *et al.* 2013). Moreover, excess polyamine levels in the brain can be detrimental to the normal function of neurotransmitters since cytosolic polyamines in the neurons are responsible for modulating neuronal excitability (Limon *et al.* 2016). Even small changes in polyamine concentrations can significantly modulate excitatory synaptic inputs and neuronal activity (Fiori and Turecki 2008). Accordingly, it has been proposed that excess cytosolic polyamines in psychiatric disorders may induce a polyamine efflux to the extracellular space, thereby making excitatory receptors more sensitive (Limon *et al.* 2016). Polyamine-driven hyperexcitation could lead to neuronal remodeling, resulting in abnormalities and hypofunction of neurotransmitters. However, there have been no reports on whether increased exogenous polyamines affect brain function in a healthy population, so further studies are needed to understand the detailed action.

Several genera were differentially abundant between the vitamin C and placebo groups. Above all, vitamin C significantly reduced the abundance of *Desulfovibrio* that produces LPS, a pathogen-associated molecular pattern. *Desulfovibrio*, a noxious genus for some cases, has been associated with the incidence and severity of a variety of inflammatory diseases, including ulcerative colitis, colorectal cancer, systemic sclerosis, and irritable bowel syndrome (Rowan *et al.* 2010). In addition, vitamin C reduced the abundance of Gram-negative bacteria that use the Entner-Doudoroff pathway, such as *Pseudomonas*, *Escherichia coli*, and *Azotobacter*, also known to produce LPS (King *et al.* 2009; Olins and Warner 1967; Conway 1992; Raetz and Whitfield 2002). It is well established that LPS induces the release of proinflammatory cytokines by binding to a receptor complex consisting of a cluster of differentiation 14, toll-like receptor 4, and myeloid differentiation factor 2 on the cell surface of monocytes, dendritic cells, and macrophages (Poltorak *et al.* 1998). A large body of evidence from preclinical experiments has shown the direct causal relationship between LPS-induced neuroinflammation and consequent cognitive impairment (Catorce and Gevorkian 2016). Translocation of gut bacterial components into the circulation leads to a high chance for peripheral cytokines to cross the blood-brain barrier and activate microglia in the brain, inducing neuroinflammation and neuronal damage (Kacimi, Giffard, and Yenari 2011). Stimulated microglia accumulate inflammatory factors and neurotoxic reactive astroglia in specific brain regions crucial in cognitive function (Singh, Jiang, and Gupta 2007; Harry 2013; S. Lee, Kim, *et al.* 2011; S. Lee *et al.* 2012). In humans, sustained stimulation by endotoxins, such as LPS, has been suggested as a risk factor for low-grade systemic inflammation that interferes with healthy mental functioning (Cai *et al.*

2017; Demircan *et al.* 2016; Gialluisi *et al.* 2020; Dyer *et al.* 2020). In particular, low-grade inflammation has been shown to induce a decrease in striatal dopamine levels (Treadway, Cooper, and Miller 2019). This is because the energy availability of the dopamine system is recalibrated to compensate for the increased metabolic demands of chronic low-grade inflammation, impairing attention and motivation and reducing effort expenditure for reward (Straub 2017). In a mouse model intentionally injected with LPS, intracerebroventricular injection of vitamin C alleviated LPS-induced activation of microglia and production of proinflammatory cytokines (X.Y. Zhang *et al.* 2018). Thus, the improved mental vitality in the current study might have been attributed to both colonic and systemically absorbed vitamin C. Colonic vitamin C induces gut bacterial balance, reducing the likelihood of microbial-derived proinflammatory agents being absorbed into the body. On the other hand, absorbed vitamin C travels through the circulatory system to the brain, where it may exert a more direct anti-inflammatory effect. These results indicate that sufficient amounts of vitamin C, exceeding the vitamin C-absorption capacity in the small intestine, induces the symbiosis of the colonic microbiome by inhibiting the flourish of endotoxin-producing bacteria in the gut and allows cerebral vitamin C levels to be saturated, promoting healthy psychological functioning.

This study has several limitations. First, this study used a 2-day dietary record to determine whether there were differences in diet between the vitamin C and placebo groups. However, dietary records are limited in measuring participants' usual dietary intake unless several independent days are observed. Second, some participants had serum vitamin C concentrations higher than 50 μmol/L at week 0 despite having values below 50 μmol/L at screening. Considering that circulating

vitamin C levels represent a relatively recent intake of vitamin C, this may be because their usual dietary intakes were not maintained during the run-in period despite sufficient instructions from the investigators. Third, the current sample size was not large enough to determine whether vitamin C supplementation had different effects on mental function depending on the participants' general characteristics, such as sex and genetic variation at the SLC23A1 gene locus. Therefore, further studies involving a larger number of samples are required to detect different health outcomes of vitamin C supplementation according to intrinsic factors of individuals. Another limitation is that it was difficult to determine the detailed physiological actions of vitamin C in the brain due to ethical concerns in human studies. To overcome this, advanced technique, such as functional magnetic resonance imaging, is needed in future clinical studies to understand brain activity and neurological mechanisms induced by vitamin C. In addition, dose-response assessment is necessary to provide a broad understanding of the efficacy of vitamin C administration.

Despite some limitations, this study is the first to provide insight into the role of optimal vitamin C status in the improvement of mental vitality mediated through the gut-microbiota-brain axis in young adults. Sufficient vitamin C intake, which allows optimal levels of ascorbic acid, promotes gut microbiota balance and expected dopamine function, increasing enthusiasm and motivation for work and contributing to superior performance on cognitive tasks that require prolonged attention. Current findings suggest that optimal vitamin C status may help promote brain and gut health in young populations.

V. Summary and Conclusion

Study 1, a population-based cross-sectional study of healthy young adults, found that individuals with homozygotes for the rs6596473-minor allele were three times more likely to have suboptimal serum vitamin C concentrations of less than 50 μmol/L than those with homozygotes for the rs6596473-major allele. With regard to mental vitality and mood states, serum concentrations of vitamin C directly correlated with attention levels, whereas there was no significant correlation with levels of fatigue, stress, depression, and positive and negative affect.

In **Study 2**, a randomized, double-blind, placebo-controlled vitamin C supplementation trial was conducted in otherwise healthy young individuals with suboptimal serum vitamin C concentrations of less than 50 μmol/L. Daily supplementation with 1000 mg of vitamin C for 4 weeks increased vitamin C concentrations to an average of 88 μmol/L. Notably, vitamin C supplementation promoted subjective attention and participants' interest and enthusiasm for work compared to the placebo group. Moreover, participants supplemented with vitamin C were significantly faster and more accurate than those supplemented with placebo at responding to cognitive tasks requiring sustained attention under mental stress caused by a mental arithmetic test. The effects of vitamin C supplementation on gut-microbiota-brain function were examined to gain insight into the underlying mechanisms by which vitamin C promotes mental vitality. Vitamin C supplementation significantly increased the relative abundance of *Bacillaceae*, *Bifidobacterium*, and *Anaerotruncus* while decreased *Desulfovibrio* compared to placebo supplementation. Remarkably, changes in the abundance of *Bacillaceae* and *Anaerotruncus* were directly correlated with subjective attention, work engagement, and cognitive abilities. Conversely, changes in the abundance of *Desulfovibrio* were inversely correlated with cognitive abilities. In addition, the

analysis of functional abundances of gut microbiota showed that vitamin C supplementation reduced lipopolysaccharide (LPS) and polyamine-producing bacteria compared to the placebo, which was reflected in decreased serum concentrations of LPS-binding protein and spermidine. Furthermore, the abundance of bacterial polyamine biosynthesis and the Entner-Doudoroff pathway, which were decreased in the vitamin C group, inversely correlated with work absorption and cognitive abilities. Along with the decrease in circulating LPS levels, the vitamin C group showed decreased IL-6, IL-10, and TNF-α concentrations compared to the placebo. Notably, increases in IL-8 and IL-10 in the placebo group were responsible for poor performance on cognitive tasks. In contrast, vitamin C supplementation successfully inhibited these disruptions by reducing the release of proinflammatory cytokines. In addition, participants supplemented with vitamin C had a direct correlation between changes in L-DOPA concentrations and changes in work motivation, indicating a role for vitamin C in supporting expected dopamine function.

Taken together, vitamin C status is closely related to mental function. In particular, vitamin C promotes interest and enthusiasm for work and contributes to prolonged attention during cognitive tasks. The enhancement of mental vitality induced by sufficient vitamin C intake is attributed to normal dopamine function and the reduced abundance of LPS-producing gut bacteria associated with chronic inflammation. Current findings provide insight into the role of vitamin C in mental vitality using extensive data from a cross-sectional study and a randomized controlled trial. These findings suggest that maintaining optimal vitamin C status energizes a daily life, possibly contributing to mental well-being and preventing mental illness in young populations.

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국문초록

젊은 성인에서 비타민 C 가 정신적 활력에 미치는 영향 연구

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심 민 주

서론

비타민 C 는 인간이 스스로 합성할 수 없어 과일과 채소 등의 식품을 섭취하여 얻는 필수 영양소이다. 비타민 C 는 전자공여체와 보조인자로 기능함으로써 인체의 항산화 작용, 콜라겐과 카르니틴의 합성, 페닐알라닌과 티로신의 대사 등에 관여한다. 특히 뇌는 신체에서 매우 고농도의 비타민 C 를 보유하는 장기 중 하나로, 뇌에 존재하는 비타민 C 는 신경 세포를 발달시키고 산화 스트레스로부터 보호하며, 신경전달물질의 합성과 분비를 조절한다. 이는 비타민 C 가 뇌의 정상적인 기능을 유지하여 정신 건강을 이루는 데 매우 중요한 영양소라는 점을 시사한다. 또한, 신체의 비타민 C 가 결핍되면 극심한 무기력증이 나타나는 것으로 보아, 비타민 C 가 육체적 피로뿐만 아니라 정신적 활력에도 깊이 관여할 것으로 생각된다. 그러나 비타민 C 와 인간의 정신 건강 사이의 관련성은 아직 충분한 연구가 이루어지지 않았으며, 현재까지 이루어진 연구의 대부분은 노인이나 정신질환을 앓고 있는 환자군을 대상으로 수행되었다. 따라서 건강한 젊은

인구 집단에서 정신적 활력에 대한 비타민 C 의 역할을 규명할 수 있는 임상 연구가 필요하다.

연구 목적

본 연구를 통해 건강한 젊은 성인에서 비타민 C 상태와 정신적 활력 사이의 관계를 조사하고자 하였다. 따라서 첫째로, 단면연구(**연구 1**)를 실시하여 혈청 비타민 C 농도와 정신 활력 지표 사이의 상관성을 조사하였다. 둘째로, 비타민 C 보충 중재연구(**연구 2**)를 수행하여 비타민 C 와 정신 활력 사이의 인과성을 조사하고 비타민 C 가 정신 활력을 증진시키는 생리적 기전을 탐구하였다.

연구 내용 및 방법

연구 1 에서는 20~39 세의 건강한 성인 214 명을 대상으로 단면연구를 수행하였다. 먼저, 혈청 비타민 C 농도와 관련된 요인을 탐색하기 위해 흡연, 운동량, 알코올 섭취 빈도, 비타민 C 식이 섭취량 및 비타민 C 보충제 사용 여부와 같은 생활습관 요인을 조사하였다. 또한 나트륨 의존성 비타민 C 수송체 1(sodium-dependent vitamin C transporter 1)을 암호화하는 유전자 자리에 존재하는 단일염기다형성(single nucleotide polymorphism) rs6596473 의 유전자형을 분석하였다. 혈청에서의 비타민 C 농도는 고성능 액체 크로마토그래피를 사용하여 측정한 후, 혈청 농도가 50μM 미만인 것을 '불충분(suboptimal) 상태'로 판정하고 생활습관 요인 및 rs6596473 과의 상관성을 이항 로지스틱 분석으로 조사하였다. 연구대상자의 활력도와 기분 상태는 설문 검사를 이용하여 조사하였다.

활력도 지표로서 연구대상자가 느끼는 자신의 주의집중력과 피로감을 평가하였고, 기분 상태를 나타내는 지표로서 스트레스, 우울도, 긍정 및 부정 정서를 평가하였다. 선형 회귀 분석을 이용하여 혈청 비타민 C 농도와 활력 및 기분 상태 사이의 상관성을 조사하였다. **연구 2** 에서는 20~39 세의 젊은 성인을 대상으로 비타민 C 보충 중재연구를 수행하였다. 최근 한 달 이내에 비타민 C 보충제를 섭취한 이력이 없고, 건강하지만 혈청 비타민 C 농도가 50μM 미만인 자가 무작위 배정, 이중 맹검, 위약대조 중재연구에 참여하여 4 주 동안 하루 두 번씩 500mg 의 비타민 C(24 명) 혹은 플라시보(22 명)를 섭취하였다. 비타민 C 보충이 정신적 활력에 미치는 영향을 평가하기 위해 주관적 활력도, 인지 실험에서의 주의집중력, 혈청 내 뇌유래신경영양인자(brain-derived neurotrophic factor)의 농도 변화를 조사하였다. 주관적 활력도로서 피로도와 주의집중력, 직무와 공부에 대한 열의(활력, 헌신, 몰입), 자기조절력을 설문 검사를 이용하여 평가하였다. 연구대상자의 지속적 주의력(sustained attention)은 높은 난이도의 암산과제를 통해 인지적 피로감을 유발한 상태에서 스트룹 과제(Stroop task)에 정확하게 반응하는 속도를 측정하여 평가하였다. 뇌유래신경영양인자의 혈청 내 농도는 효소면역측정법을 이용하여 측정하였다. 비타민 C 보충이 기분 상태에 미치는 영향을 조사하기 위해 연구대상자의 스트레스, 우울도, 긍정 및 부정 정서, 불안도를 설문 검사로 평가하였다. 다음으로, 비타민 C 가 정신적 활력을 증진시키는 생리적 기전을 탐색하기 위해 중재 전후에 수집한 대변과 혈액 시료를 이용하여 장내 미생물총, 사이토카인 및 신경전달물질의 변화를 조사하였다. 16S rRNA 차세대 염기서열 분석법을 이용하여 장내 미생물 군집의 변화를

조사하고, 장 박테리아 시퀀스 데이터를 바탕으로 장내 미생물총의 기능적 특성을 분석하였다. 또한, 장내 미생물총의 기능 분석 결과를 근거로 하여, 스퍼미딘(spermidine)과 지질다당체결합단백질(lipopolysaccharide-binding protein)의 혈청 내 농도를 효소면역측정법으로 측정하였다. 혈청에 존재하는 사이토카인 중, IL-6, IL-8, IL-10, TNF-α, IFN-γ의 농도는 전기화학발광 다중 면역분석법으로 분석하였다. 신경전달물질 L-DOPA, 노르에피네프린, 세로토닌의 혈청 내 농도는 초고성능 액체 크로마토그래피 질량분석법을 이용하여 측정하였다.

연구 결과

단면연구(**연구 1**)에서 수행한 이항 로지스틱 분석에 따르면 rs6596473 자리에 소수 염기(minor allele) 동형접합체를 가진 사람은 다수 염기(minor allele) 동형접합체를 가진 사람보다 혈청 비타민 C 농도가 50μM 미만으로 불충분할 가능성이 2.7 배 더 높았다(95% CI=1.058-7.304). 남성은 여성과 비교하여 혈청 비타민 C 농도가 불충분할 가능성이 4.6 배 더 높았다(95% CI=2.101-10.01). 이외에도 비타민 C 식이 섭취 수준이 가장 낮은 삼분위수에 속하는 자, 비타민 C 보충제를 사용하지 않는 자는 비타민 C 식이 섭취 수준이 가장 높은 삼분위수에 속하는 자, 비타민 C 보충제를 사용하는 자와 비교하여 혈청 비타민 C 농도가 불충분할 가능성이 각각 4.0 배(95% CI=1.773-8.832), 4.4 배(95% CI=2.099-9.405) 더 높았다. 선형 회귀 분석에서 혈청 비타민 C 농도와 활력도 사이의 상관성을 분석한 결과, 혈청 비타민 C 농도는 주의집중도와 유의한 양의 상관성을 보였다($\beta=0.161$, $p<0.05$). 또한 성별, 나이, BMI, 현재 흡연 상태, 알코올

섭취 유무, 운동량 수준을 통제하여도 혈청 비타민 C 농도가 높아질수록 주의집중도가 함께 높아지는 것을 관찰하였다(β=0.203, $p<0.01$). 중재연구(**연구 2**)에서 초기 혈청 비타민 C 농도가 50μM 미만으로 불충분했던 사람에게 하루 1,000mg 의 비타민 C 를 4 주간 보충한 결과, 혈청 비타민 C 농도가 초기 대비 106% 만큼 증가하였다($p<0.001$). 반면, 플라시보 섭취군의 혈청 비타민 C 농도는 초기 대비 30% 만큼 감소하였다($p<0.01$). 플라시보 섭취군은 주관적 주의집중도에서 유의한 변화를 보이지 않은 반면, 비타민 C 섭취군은 초기 대비 27% 만큼 증가하여($p<0.01$) 변화량에서 플라시보 섭취군과 유의한 차이를 보였다(1.9±2.7 vs. 0.3±2.5, $p<0.05$). 또한 플라시보 섭취군은 직무나 학업에 대한 몰입도에서 유의한 변화를 보이지 않았으나, 비타민 C 섭취군은 초기 대비 10% 만큼 증가하여($p<0.05$) 변화량에서 플라시보 섭취군과 유의한 차이를 나타냈다(2.2±4.0 vs. −0.5±4.1, $p<0.05$). 반면, 피로, 자기조절력, 뇌유래신경영양인자 농도, 기분 상태 지표에서는 비타민 C 보충의 유의한 효과가 관찰되지 않았다. 한편, 비타민 C 섭취군은 플라시보 섭취군보다 스트룹 과제에 정확하게 반응하는 데 더 짧은 시간(초)을 소요하며(82.3±9.4 vs. 89.1±12.1, $p<0.05$) 더 우수한 인지 과제 수행 능력을 보였다. 또한 중재가 종료된 후에 측정한 혈청 비타민 C 농도와 스트룹 과제에 반응하는 데 걸린 시간 사이에는 음의 상관 관계가 존재했다($r=-0.28$, $p=0.05$). 비타민 C 는 장 미생물총에도 유의미한 변화를 유도했는데, 비타민 C 섭취군은 플라시보 섭취군과 비교하여 *Bacillaceae*, *Bifidobacterium* 및 *Anaerotruncus* 의 상대적 풍부도가 증가한 반면, *Desulfovibrio* 의 상대적 풍부도는 감소하였다($p<0.05$). 또한,

Bacillaceae(r=0.30, p<0.05) 및 *Anaerotruncus*(r=0.35, p<0.05)의 풍부도 변화가 주관적 주의집중도 점수의 변화와 양의 상관성을 보였다. 반면, 스트룹 검사에서의 인지 수행 능력이 *Desulfovibrio* 의 풍부도 변화와 음의 상관성을 나타냈다(r=-0.31, p<0.05). 다음으로, 장 미생물총의 기능적 특징을 분석한 결과, 비타민 C 섭취는 플라시보 섭취와 비교하여 폴리아민 생합성 경로와 특정 그람 음성균이 사용하는 것으로 알려져 있는 Entner-Doudoroff(ED) 경로의 상대적 풍부도를 감소시켰다(p<0.05). 그 결과, 비타민 C 섭취는 플라시보 섭취와 비교하여 장내 박테리아가 생성하는 스퍼미딘의 혈청 농도(0.86±2.84 vs. 1.36±2.85ng/mL, p<0.05)와 그람 음성균의 세포 외벽에서 기인한 지질다당체에 결합하는 지질다당체결합단백질의 혈청 농도(0.00±0.59 vs. 0.87±1.30μg/mL, p<0.01)를 유의하게 감소시켰다. 또한, 박테리아의 폴리아민 생합성 경로(r=0.58, p<0.001) 및 ED 경로(r=0.33, p<0.05)의 풍부도 변화는 스트룹 과제에 반응하는 데 걸린 시간과 양의 상관관계를 나타냈다. 혈청 사이토카인의 경우, 플라시보 섭취군에서는 IL-6 와 IL-10 의 농도가 초기 대비 각각 46%(p<0.01), 22%(p<0.01) 만큼 증가하였지만, 비타민 C 섭취군은 두 지표에서 유의한 변화를 보이지 않아 변화량에서 플라시보 섭취군과 유의한 차이를 나타냈다(IL-6: 0.07±0.35 vs. 0.31±0.53, IL-10: -0.02±0.18 vs. 0.12±0.20, p<0.05). 또한, 비타민 C 섭취군에서는 TNF-α의 혈청 내 농도가 23% 만큼 감소한(p<0.01) 것에 반해, 플라시보 섭취군은 유의한 변화를 보이지 않아 TNF-α의 농도 변화에서 두 섭취군이 유의한 차이를 보였다(-0.38±0.92 vs. 0.12±0.32, p<0.05). 특히, 플라시보 섭취군에서는 IL-8 의 증가와 스트룹 검사에서의 수행 능력 사이에서 음의

상관성이 나타났는데($r=-0.47$, $p<0.05$), 비타민 C 섭취군에서는 이러한 상관성이 관찰되지 않았다. 한편, L-DOPA, 노르에피네프린 및 세로토닌의 혈청 농도 변화에서 두 섭취군은 유의미한 차이를 보이지 않았다. 하지만 비타민 C 섭취군만이 L-DOPA 농도의 변화와 직무 및 학업에 대한 열의도에서의 변화가 서로 강한 양의 상관관계를 보였다($r=0.58$, $p<0.001$).

결론

본 연구의 결과는 체내 비타민 C 상태와 정신 건강 사이의 밀접한 관련성을 보여준다. 특히, 건강하지만 체내 비타민 C 상태가 불충분한 젊은 성인에서 혈청 비타민 C 농도가 50μM 이상으로 충분히 높아지면, 비타민 C 가 도파민의 정상적인 기능을 도와 일에 대한 몰입과 동기부여를 촉진한다. 또한 비타민 C 는 만성 염증의 잠재적 위험이 되는 장내 미생물총을 억제함으로써 지속적 주의력과 같은 인지 수행 능력을 향상시킨다. 이러한 연구 결과는 비타민 C 가 장내 미생물총의 균형을 유도할 뿐만 아니라, 장-뇌 축을 통해 정신적 활력까지 증진시킬 수 있음을 나타낸다. 따라서 신체의 적절한 비타민 C 상태를 유도하는 영양 중재는 뇌 건강을 위협하는 생리적 요인을 차단하고 정신적 에너지를 증가시킴으로써 활력 있고 성공적인 일상을 이루는 데 도움을 줄 것으로 기대된다.

주요어: 비타민 C, 정신적 활력, 주의집중력, 동기 부여, 장내 미생물총, 장-뇌 축, 염증성 사이토카인, 단일염기다형성

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