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Research Article

Association of Intake of Dietary Vitamin C and Vegetables with Non-Alcoholic Fatty Liver Disease (NAFLD) In Middle-Aged Japanese Men

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Abstract

Non-alcoholic fatty liver disease (NAFLD) has become one of the most common causes of liver disease world-wide and has been recognized as a major health burden. The aim of the present study was to determine the associations of plasma Vitamin C (VitC) levels and intake of dietary VitC and vegetables with ultrasound-diagnosed non-alcoholic fatty liver disease (NAFLD) in Japanese men. The plasma VitC levels and the intake of dietary VitC and vegetables decreased with the severity of fatty liver. After adjustment for the age, body mass index (BMI), physical activity, and smoking status, higher intake of dietary VitC and vegetables was significantly associated with a lower prevalence of NAFLD. The odds ratio (OR) for the highest quartile (Q4) versus the lowest quartile (Q1) of the intake of dietary VitC was 0.27 (P=0.045) and the OR for Q4 versus Q1 of vegetable intake was 0.29 (P=0.035). These results suggest that increases in the intake of dietary VitC and vegetables prevent NAFLD independently of BMI.

Keywords: Dietary Vitamin C Intake; NAFLD; Plasma Vitamin C; Vegetable Intake

Abbreviations: BMI: Body Mass Index

BP: Blood Pressure

NAFLD: Non-Alcoholic Fatty Liver Disease

OR: Odds Ratio

ROS: Reactive Oxygen Species

VitC: Vitamin C

VitE: Vitamin E

WC: Waist Circumference

Introduction

Non-alcoholic Fatty Liver Disease (NAFLD) is a spectrum of chronic liver conditions that are characterized by relatively benign hepatic steatosis to Non-Alcoholic Steatohepatitis (NASH), severe cirrhosis and fibrosis. NAFLD has become one of the most common causes of liver disease world-wide and has been recognized as a major health burden. Currently, there is still no specific treatment for NAFLD except lifestyle modification involving exercise and dietary adjustment. NAFLD is strongly linked to obesity, with a reported prevalence as high as 80% in obese patients and only16% in individuals with a normal BMI and without metabolic risk factors [1,2]. Obesity is a state of chronic low-grade inflammation [3]. Oxidative stress is considered to cause the metabolic disturbances in obesity. In agreement with a putative role of oxida-

tive stress in the etiology of NAFLD, the antioxidant nutrient, Vitamin C (VitC), but not Vitamin E (VitE), has been reported to reduce oxidative stress and inhibit the development of experimental liver steatosis in rats [4]. VitC is a water-soluble, chain-breaking antioxidant that scavenges essentially all physiologically relevant free radicals [5]. VitC deficiency is defined as plasma levels below 23 μM [6]. The deficiency of VitC potentially enhances susceptibility to oxidative stress and disease [7]. VitC status inversely correlates with BMI and is significantly decreased in obese compared with lean individuals [8-11]. Thus, the association of NAFLD with obesity and oxidative stress suggests an important role of VitC in NAFLD progression. Some studies showed correlations of NAFLD progression with dietary VitC intake, while others did not [12]. Furthermore, dietary VitC intake is not necessarily correlated with plasma VitC levels [13]. The results of the studies examining VitC plasma levels in NAFLD [14-16] are inconclusive and do not allow a definitive conclusion regarding plasma VitC levels as well as VitC intake in NAFLD [12]. Thus, there is a need for further study to elucidate the effects of plasma VitC levels and dietary intakes of VitC on NAFLD. In this study, to clarify the role of VitC in the development and progression of NAFLD, we investigated the association of the plasma VitC levels and intake of dietary VitC and vegetables with NAFLD in Japanese men.

Subjects and Methods

Subjects

The subjects were 198 Japanese men who participated in a health examination conducted in 2007, 2008, 2009, 2011, and 2013 at Nara Health Promotion Center. All participants who had present and past medical treatment and medication use, evidence of other liver diseases (viral hepatitis B or C, primary biliary cirrhosis, sclerosing cholangitis, autoimmune hepatitis hemochromatosis, or drug-induced liver disease), or more than 20 grams per day of alcohol consumption were excluded. The study was designed in accordance with the principles of the Declaration of Helsinki of the World Medical Association and approved by the Ethics Committee of Nara Women's University, Japan. At the time of enrolment, written informed consent was obtained from each participant.

Measurement

The participants underwent routine health examinations, anthropometric and blood pressure (BP) measurements, and the collection of fasting blood samples. A face-to-face interview was conducted by trained interviewers using questionnaires about the medical condition, medication use, lifestyle of physical activity (more than 30 min of exercise or 1 hour of walking per day), current smoking, and alcohol drinking (≥20 grams of ethanol per day), and a previously validated food frequency questionnaire [17].

Ultrasonography of The Liver

Diagnosis of NAFLD in this study was based on sonographic evidence of a fatty liver and negative test results of HBsAg and anti-HCV antibody. Abdominal ultrasonography was performed by clinical hepato-gastroenterologists using Ultrasound GE LOGIQ7 (General Electric Co., CT, USA). The results of ultrasonography were divided into mild, moderate, and severe stages according to the criteria described by Saverymuttu et al. [18].

Anthropometry

The Body Mass Index (BMI) was calculated as the body weight (kilogram) divided by the square of the height (meter). The Waist Circumference (WC) was measured using an anthropometric measuring tape at a horizontal plane midway between the lowest rib and iliac crest. Blood pressure (BP) was measured in triplicate with a validated semi-automatic sphygmomanometer.

Laboratory Measurements

All serum and plasma samples were obtained in a fasting state. The biochemical parameters such as Alanine Transaminase Activity (ALT), Aspartate Transaminase Activity (AST), Triacylglycerol (TG), total cholesterol, LDL-cholesterol, HDL-cholesterol, fasting blood glucose, fasting insulin, HbA1c, adiponectin, VitC, and VitE were measured by Osaka Kessei Research Laboratories Inc. (Osaka, Japan). The insulin resistance index was calculated using Homeostasis Model Assessment (HOMA-IR) (HOMA-IR=fasting insulin (μU/millilitre) × fasting glucose (milligram/decilitre)/405).

Statistical analysis

Statistical analyses were performed using SPSS software version 23.0 (IBM Inc., Armonk, NY, USA). All statistical tests were 2-sided and $P<0.05$ was considered significant. The distribution of continuous variables was examined for normality by Shapiro-Wilk's test. Continuous and categorical data are expressed as the mean ± Standard Deviation (SD) and as a proportion (%), respectively. Differences between groups was examined by the Jonckheere-Terpstra test or the Chi-square test. Spearman's correlation coefficients were calculated to assess possible relationships between variables. Logistic regression analysis calculating the odds ratios (OR) and 95% Confidence Intervals (CI) was performed to examine the associations between NAFLD across quartiles of variables considering the lowest quartile as the reference.

Results

Subjects characteristics

The subjects participating in this study were 36 to 77 years old (those in their thirties, forties, fifties, sixties, and seventies were

8, 71, 98, 18, and 3 men, respectively). Of the total population, 63.6 and 54.5 % subjects were with NAFLD and overweight/obese (BMI ≥ 25), respectively. The subjects were divided into 4 groups based on the results of ultrasonography: normal (n=72), mild-(n=40), moderate-(n=63), and severe-NAFLD (n=23). Their characteristics are shown in (Table 1). As the severity of fatty liver increased, there were higher BMI, WC, BP, AST, ALT, TG, fasting glucose, insulin, HbA1c, and HOMA-IR and lower levels of HDL-cholesterol, adiponectin, and VitC. No significant trend was found in age, life style factors, total cholesterol, LDL-cholesterol, or VitE among the 4 groups. The presence of metabolic disturbance among 4 groups is shown in (Table 2). There were significant increased proportions of subjects with overweight/obese, abdominal obesity, insulin resistance, abnormality for ALT or AST, and deficiency of VitC as the severity of fatty liver increased, although no significant difference was observed in hypertension, dyslipidaemia, or diabetes. The proportions of subjects with overweight/obese and deficiency of VitC were 83 and 22% in moderate NAFLD group, and 96 and 91 % in severe NAFLD group, respectively. These results suggested that more than 90% of the subjects with severe NAFLD were obese and VitC deficient.

	Normal	**NAFLD**			
		Mild	**Moderate**	**Severe**	**P**
Age (y)	51.6 ± 7.1	54.2 ± 6.3	50.7 ± 7.1	51.1 ± 8.4	0.389
Life style factors					
Physical activity (%yes)	35.6	40.5	32.2	40.0	0.847
Current smoking (%yes)	16.9	29.7	20.3	13.3	0.415
Anthropometry indicators					
BMI (kilogram/m^2)	23.6 ± 2.3	25.3 ± 1.9	26.9 ± 2.4	29.4 ± 3.4	<0.001
WC (centimetre)	85.8 ± 6.8	89.7 ± 5.2	93.2 ± 6.9	98.2 ± 7.3	<0.001
SBP (mmHg)	124.6 ± 12.9	126.6 ± 14.1	132.7 ± 13.5	133.4 ± 12.1	<0.001
DBP (mmHg)	78.7 ± 9.0	81.9 ± 9.8	83.5 ± 10.8	86.3 ± 8.9	0.001
Biochemical parameters					
AST (IU/Litre)	20.2 ± 5.1	21.2 ± 5.2	29.8 ± 15.1	33.3 ± 10.9	<0.001
ALT (IU/ Litre)	20.9 ± 8.8	24.7 ± 10.5	45.9 ± 28.2	60.7 ± 26.3	<0.001
TG (milligram/decilitre)	130.5 ± 123.0	150.3 ± 74.9	155.3 ± 83.0	140.1 ± 74.5	0.020
Total cholesterol (milligram/ decilitre)	212.8 ± 31.1	201.5 ± 28.0	213.5 ± 31.5	201.2 ± 31.9	0.549
HDL-cholesterol (milligram / decilitre)	55.3 ± 14.1	48.9 ± 9.1	49.6 ± 9.6	45.7 ± 8.4	<0.001
LDL-cholesterol (milligram / decilitre)	132.1 ± 28.9	127.6 ± 24.6	137.5 ± 30.7	133.1 ± 30.8	0.326
Glucose (milligram / decilitre)	98.9 ± 13.8	99.1 ± 8.7	105.0 ± 11.9	110.5 ± 15.4	<0.001
Insulin (μU/millilitre)	6.3 ± 3.2	8.1 ± 3.1	12.0 ± 7.8	16.4 ± 9.1	<0.001

HbA1c (%)	5.2	±	0.4	5.2	±	0.3	5.4	±	0.4	5.6	±	0.4	<0.001
HOMA-IR	1.55	±	0.92	1.99	±	0.80	3.16	±	2.23	4.60	±	2.91	<0.001
Adiponectin (μ gram/millilitre)	8.1	±	2.9	6.1	±	2.5	5.6	±	2.6	4.4	±	1.1	<0.001
Vitamin C (μmols/Litre)	45.7	±	17.4	41.2	±	13.6	38.2	±	18.6	14.3	±	4.6	<0.001
Vitamin E (milligram/decilitre)	1.14	±	0.48	0.96	±	0.33	1.16	±	0.40	0.92	±	0.28	0.719
Data are expressed as the mean ± standard deviation or proportion (%).													

Table 1: Characteristics of Subjects.

	Normal	NAFLD			
		Mild	Moderate	Severe	P
Overweight/obese	26.4	62.5	82.5	95.7	<0.001
Abdominal obesity	55.6	82.5	88.9	100.0	<0.001
Hypertension	15.3	25.0	27.0	30.4	0.281
Dyslipidemia	56.9	52.5	69.8	65.2	0.266
Diabetes	4.2	2.5	9.5	17.4	0.090
Insulin Resistance	11.3	27.5	60.3	69.6	<0.001
Abnormal ALT or AST value	2.8	7.5	41.3	82.6	<0.001
VitC deficiency	12.5	12.5	21.7	90.9	<0.001
Data are expressed as yes%.					
Overweight/Obese: BMI >=25, Abdominal Obesity: WC>=85centimetre, Hypertension: SBP>=140 or DBP>=90, Dyslipidemia: TG>=150, LDL-Cholesterol>=140, or HDL-Cholesterol<40milligram /deciliter, Diabetes: Glucose>=126milligram/deciliter or HbA1c>=6.5, Insulin Resistance: HOMA-IR>=2.5, Abnormal Value of ALT or AST: ALT>40 or AST>37, VitC Deficiency: VitC< 23μM. P, Chai-square Test.					

Table 2: Presence of Metabolic Disturbance.

Dietary intake

Intake of energy, nutrients, and food groups is shown in (Table 3). A significant decreased trend was only shown in the intake of dietary VitC and vegetables by Jonckheere-Terpstra test. The intake of vegetables in NAFLD group (mild + moderate + severe) was significantly different from that in the normal (the mean intake of vegetables in normal, 210.3 gram and NAFLD group, 165.9 gram; p=0.008 by Mann-Whitney's test).

	Per day	Normal			NAFLD									
					Mild			Moderate			Severe			P
Energy	kilocalorie	2151	±	426	2170	±	462	2215	±	405	2123	±	581	0.931
Protein	gram	68.6	±	14.6	67.8	±	14.0	69.9	±	13.6	69.4	±	19.3	0.561
Fat	gram	62.1	±	19.4	65.2	±	20.6	66.4	±	16.1	59.9	±	25.4	0.637

Carbohydrate	gram	311.0	±	72.9	305.1	±	75.4	316.9	±	69.7	313.8	±	84.5	0.663
Na	milligram	3535	±	1326	3626	±	1120	3687	±	1203	2909	±	810	0.303
K	milligram	2275	±	590	2132	±	421	2262	±	620	2034	±	612	0.152
Ca	milligram	543	±	229	515	±	135	512	±	177	460	±	224	0.167
Mg	milligram	245	±	62	233	±	46	244	±	60	221	±	60	0.465
Fe	milligram	7.2	±	3.1	6.7	±	1.6	6.9	±	1.4	6.7	±	2.1	0.642
Zn	milligram	8.9	±	2.0	8.6	±	1.9	8.8	±	2.1	8.5	±	2.4	0.725
Vitamin A	μg	519	±	206	458	±	135	458	±	147	443	±	180	0.056
Vitamin D	μg	8.1	±	5.9	7.2	±	3.0	7.0	±	3.3	8.3	±	5.6	0.800
Vitamin E	milligram	6.5	±	1.9	6.2	±	1.5	6.5	±	1.6	6.1	±	2.2	0.528
Vitamin K	μg	187	±	69	175	±	58	176	±	62	171	±	107	0.076
Vitamin B1	milligram	0.95	±	0.34	0.90	±	0.27	0.92	±	0.21	0.99	±	0.40	0.754
Vitamin B2	milligram	1.07	±	0.33	1.04	±	0.26	1.04	±	0.26	1.09	±	0.37	0.657
Vitamin B6	milligram	1.14	±	0.30	1.06	±	0.23	1.08	±	0.26	1.03	±	0.33	0.112
Vitamin B12	μg	7.14	±	3.83	0.94	±	2.71	6.64	±	2.64	7.42	±	3.83	0.697
Vitamin C	milligram	93.8	±	46.9	82.2	±	30.9	82.7	±	47.4	76.1	±	30.8	0.033
cholesterol	milligram	318	±	120	314	±	118	340	±	107	341	±	114	0.130
Fiber	gram	12.7	±	3.6	11.7	±	2.7	11.8	±	2.92	11.2	±	3.42	0.058
Food group														
Cereals	gram	536.0	±	153.6	517.4	±	176.0	540.1	±	150.0	583.2	±	159.0	0.217
Fats and oils	gram	15.1	±	8.6	14.9	±	7.8	15.4	±	8.9	12.2	±	4.7	0.571
Legumes	gram	45.5	±	31.7	44.8	±	28.7	45.1	±	26.0	37.4	±	41.4	0.380
Fruits	gram	93.7	±	89.5	95.8	±	73.6	73.9	±	79.5	70.9	±	78.8	0.058
Vegetables	gram	210.3	±	108.5	165.4	±	67.2	174.0	±	89.0	165.9	±	92.7	0.012
Fish and shellfish	gram	66.8	±	41.1	65.0	±	29.5	66.2	±	30.3	76.3	±	56.1	0.388
Meat and poultry	gram	75.0	±	49.9	82.6	±	59.1	86.7	±	45.5	82.3	±	55.2	0.091
Eggs	gram	33.2	±	23.1	31.9	±	19.7	35.9	±	21.0	37.6	±	29.2	0.351
Milk and dairy products	gram	146.3	±	117.1	144.9	±	96.5	135.2	±	126.0	106.5	±	130.1	0.071
Data are expressed as the mean ± standard deviation. P, Jonckheere-Terpstra test.														

Table 3: Intake of Energy, Nutrients, and Food groups.

Relation Between Plasma VitC Level and Clinical Markers and Dietary Intake

The relationships between plasma VitC level and clinical markers and intake of dietary VitC and vegetables are shown in (Table 4). The levels of plasma VitC significantly correlated with BMI, WC, BP, ALT, insulin, HOMA-IR, and adiponectin, while no correlation was observed in the intake of VitC or vegetables. Neither intake of dietary VitC nor vegetables was correlated with these clinical factors including the plasma VitC levels. However, a significant correlation between intake of VitC and vegetables was observed (r=0.507, p<0.001).

	Plasma VitC	
	r	p
BMI	-0.379	<0.001
WC	-0.327	0.002
SBP	-0.224	0.034
DBP	-0.224	0.034
ALT	-0.323	0.002
Insulin	-0.357	0.001
HOMA-IR	-0.366	<0.001
Adiponectin	0.304	0.007
Intake of dietary VitC	0.157	0.143
Intake of vegetables	0.113	0.289
r, correlation coefficient		

Table 4: Correlation of plasma VitC level with clinical markers and the intake of VitC and vegetables.

Association Between NAFLD and BMI, Plasma VitC Levels, and Intake of Dietary VitC and Vegetables

To determine the associations between the prevalence of NAFLD (mild+moderate+severe) across quartiles of BMI, plasma VitC levels, intake of dietary VitC and vegetables, considering the lowest quartile as the reference, logistic regression analysis was performed. The OR for NAFLD by quartiles of the variables is shown in (Table 5). A higher BMI was associated with a higher prevalence of NAFLD. The analysis indicated that the increase in median levels of BMI (from 22.6 to 29.1) increased the OR for NAFLD to 27.8-times. Conversely, a higher quartile of the levels of plasma VitC and intake of vegetables was associated with a decreased OR for NAFLD in a crude regression model. The increase in median levels of plasma VitC from 13.6 to 35.6, 45.9, and 61.7 μM decreased the OR to 0.38, 0.32, and 0.17, respectively. The increase in median intake of vegetable from 96 to 272 gram decreased the OR to 0.37. Following adjustment for age, BMI, physical activity and smoking, a higher quartile of intake of dietary VitC and vegetables associated with a decreased prevalence of NAFLD, although the significant association disappeared in plasma VitC level. The adjusted model demonstrated that the increase in the median intake of dietary VitC (from 48 to 127 milligram) and vegetables (from 96 to 272 gram) decreased the OR for NAFLD to 0.27 and 0.29, respectively.

	Quartiles of variable								P
	Q1 (Lowest)		Q2		Q3		Q4 (Highest)		
BMI									
Median (kilogram/meter^2)	22.6		24.7		26.3		29.1		
N of NAFLD (%)	24.4		59.1		80.0		90.0		
Unadjusted OR (95%CI)	1	(reference)	4.47	(1.88-10.62)	12.33	(4.77-31.92)	27.75	(8.96-85.93)	<0.001
Adjusted OR (95%CI)	1	(reference)	4.75	(1.83-12.32)	10.73	(3.90-29.55)	37.36	(9.36-149.13)	<0.001
plasma Vitamin C									
Median (μmols/Liter)	13.6		35.6		45.9		61.7		
N of NAFLD (%)	77.3		56.5		52.2		36.4		
Unadjusted OR (95%CI)	1	(reference)	0.38	(0.11-1.39)	0.32	(0.09-1.17)	0.17	(0.05-0.63)	0.008
Adjusted OR (95%CI)	1	(reference)	1.13	(0.23-5.59)	1.62	(0.31-8.55)	0.81	(0.15-4.42)	0.842
Intake of Vitamin C									

Median (milligram/day)	48		67		91		127		
N of NAFLD (%)	75.5		58.3		66.6		55.1		
Unadjusted OR (95%CI)	1	(reference)	0.45	(0.19- 1.08)	0.65	(0.27- 1.55)	0.40	(0.17-0.94)	0.093
Adjusted OR (95%CI)	1	(reference)	0.54	(0.17- 1.77)	0.53	(0.16- 1.78)	0.27	(0.08- 0.93)	0.045
Intake of Vegetables									
Median (gram/day)	96		147		195		272		
N of NAFLD (%)	72.0		66.7		66.7		49.0		
Unadjusted OR (95%CI)	1	(reference)	0.78	(0.33-1.84)	0.78	(0.33-1.82)	0.37	(0.16-0.86)	0.019
Adjusted OR (95%CI)	1	(reference)	0.70	(0.23-2.20)	0.74	(0.23-2.33)	0.29	(0.09-0.91)	0.035
NAFLD represents NAFLD group (mild + moderate + severe). Adjusted for age, BMI (excluded in the case of BMI), physical activity, and smoking.									

Table 5: Odds ratio (95% CI) for NAFLD by quartile of variables.

Discussion

This study showed that the level of plasma VitC, and intake of dietary VitC and vegetables decreased as the severity of fatty liver increased. The lower plasma VitC levels was considered to be caused by the increase in oxidative stress and/or the decrease of the intake of dietary VitC and its main source, vegetables. The plasma VitC levels were found to inversely correlate with BMI and WC. In agreement with these results, epidemiological studies also showed that VitC status inversely correlated with BMI and significantly decreased in obese compared with lean individuals [8-11, 19]. The increase in BMI and WC resulted from fat accumulation. Fat-accumulated adipose tissue generates oxidative stress (Fat reactive oxygen species (ROS)) [20]. The association of oxidative stress with fat accumulation (obesity; increase in BMI and WC) is well known [21-23]. Indeed, patients with NAFLD showed the increase in oxidative stress as well as BMI [24,25]. Recently, the study using a transgenic mouse model has reported that augmented adipose oxidative stress (Fat ROS) inhibits healthy adipose expansion with ectopic lipid accumulation, such as fatty liver [20], indicating that there is no fat accumulation in the liver, if there is no ROS production. These suggest that NAFLD associates with ROS production which is prevented by VitC.

The VitC status (plasma VitC level) is a result of the balance between oxidative stress and the intake of VitC. The plasma VitC level did not correlate with the intake of VitC and its main source, vegetables, in this study. The intake of dietary VitC decreased as the severity of fatty liver increased. The intake of vegetables also decreased in NAFLD groups. These suggests that a lower level of plasma VitC in NAFLD observed in this study is caused by both the increased oxidative stress by the increased BMI and the decreased intake of dietary antioxidant nutrient, VitC and vegetables.

Logistic regression analysis demonstrated that higher quartiles of plasma VitC levels were associated with a lower OR for NAFLD. The 4.5-fold increase in the median of plasma VitC decreased OR for NAFLD to 0.17 in a crude model. However, the significant association disappeared in the adjusted model, suggesting that the plasma VitC level was dependent on BMI. The intakes of dietary VitC and vegetables were inversely associated with the prevalence of NAFLD. The increases in the median of the intake of VitC from 48 to 127 mg (about 2.6-fold increase) and vegetables from 96 to 272g (about 2.8-fold increase) decreased the OR to 0.27 and 0.29 in the adjusted model, respectively. These results suggest that the increase in the intake of VitC and vegetables prevents NAFLD independently of BMI. In agreement with our results, an inverse association of the intake of vegetables and dietary VitC with NAFLD was reported in the study using proton-magnetic resonance spectroscopy in China [26]. The inverse association between dietary VitC intake and NAFLD was also found in Chinese middle-aged and older adults [27]. However, plasma VitC and VitC intakes in Canadian patients with simple steatosis or NASH were reported to be similar to those in healthy controls [16]. This discrepancy may be explained by the difference between Western diet and Japanese or Chinese diet. Indeed, all Canadian participants of three groups in the study were described to follow a similar Western diet [16].

Vitamin E is a lipophilic molecule with antioxidant activity that prevents membrane damage by ROS. In this study, no significant difference between normal and NAFLD groups was observed

in the plasma VitE levels. The supplementation of VitE for patients with NASH resulted in clinical improvement [28, 29], but no beneficial effect was observed in other paper [30-33]. The daily supplementation of VitE was suggested to increase the risk of prostate cancer [21]. It is known that vitamin C functions in vivo to repair the membrane-bound oxidized vitamin E by the interaction at the membrane-cytosol interface [34], suggesting the decrease of VitE level may not occur if VitC status is sufficient to regenerate VitE.

Our study had a number of limitations. First, the ultrasonography has some limitations for diagnosis of NAFLD, although it is widely used as a first-line investigation for hepatic steatosis of subjects in a health examination as this study. It can accurately determine moderate and severe fatty infiltration, but the finding of a normal liver on ultrasonography cannot rule out mild fatty infiltration of the liver. It cannot distinguish between steatosis alone and NASH. The liver histology by biopsy is needed to determine the stage of NAFLD, although it is not possible in a health examination. Second, because the participants were limited to Japanese men, our findings may not be generalized to other populations. Third, because of the relatively small sample size, it is possible that associations between other nutrients and NAFLD might have been detectable in a larger population. Fourth, although we controlled for the confounding variables, we cannot rule out the possibility that unknown or unmeasured confounders account for the observed associations. Finally, the cross-sectional design of our investigation mandates that inferences about causality should be made with caution.

In conclusion, the plasma VitC levels and the intake of dietary VitC and vegetables decreased as the severity of fatty liver increased. The higher intake of dietary VitC and vegetables was associated with a lower prevalence of NAFLD independently of BMI. The dietary intake of VitC and vegetables may be important in the prevention of NAFLD.

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