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Dietary vitamin E and C intake is inversely associated with the severity of nonalcoholic fatty liver disease

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ABSTRACT

Background & aims: Although antioxidants have a protective potential in nonalcoholic fatty liver disease (NAFLD) and steatohepatitis (NASH), there is limited evidence regarding the role of dietary intake of antioxidants. The aim was to test the association between dietary vitamins E and C intake and NAFLD, NASH and fibrosis markers.

Methods: Cross-sectional study of a large cohort of subjects undergoing colonoscopy. The presence of NAFLD was evaluated by ultrasonography. The level of steatosis was defined using SteatoTest, moderate-severe NASH using new quantitative NashTest and borderline-significant fibrosis ≥ F1–F2 using FibroTest. Nutritional intake was measured by food frequency questionnaire (FFQ).

Results: Overall, 789 subjects were included (52.6% men, age 58.83 ± 6.58 years), 714 had reliable FibroMax. Adjusting for BMI, dietary and lifestyle factors, the upper tertile of vitamin E intake/1000 Kcal was associated with lower odds of NASH (OR = 0.64, 0.43–0.94, P = 0.024). There was an inverse association between reaching the recommended vitamin E intake and NASH (OR = 0.48, 0.30–0.77, P = 0.002). The upper tertile of vitamin C intake/1000 Kcal was associated with lower odds of NAFLD and NASH (OR = 0.68, 0.47–0.99, P = 0.045; OR = 0.57, 0.38–0.84, P = 0.004, respectively). Both vitamins were related with the level of steatosis according to SteatoTest.

Conclusion: Vitamin E and C intake may be protective from NAFLD-related liver damage.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is emerging as the most common chronic liver condition across the globe, with global prevalence in the general population estimated to be 25% [1]. NAFLD includes two pathologically distinct conditions with different prognoses: non-alcoholic fatty liver (NAFL) which is simple steatosis and non-alcoholic steatohepatitis (NASH) which can progress to liver fibrosis, cirrhosis, hepatocellular carcinoma (HCC), the need for liver transplant, and is associated with extrahepatic manifestations such as cardiovascular disease, leading to a large economic burden and poor health-related quality of life [1–3]. Thus, NASH treatment must be handled by a multidisciplinary team aimed also at improving behavioral risk factors [2,4]. However, there is little understanding regarding the contribution of environmental factors to the risk of developing a progressive course [1]. There is evidence that oxidative stress is involved in NAFLD pathogenesis and especially in the onset and progression NASH [4–8]. Patients with NASH have an increased oxidative stress, increased lipid peroxidation, and oxidative DNA damage associated with reduced antioxidant defense capacities and increased inflammatory activities [7,9], highlighting the possibility of ameliorating oxidative stress by dietary antioxidants as an effective strategy to prevent NASH.

Although the role of dietary macronutrients in NAFLD has been thoroughly studied, less is known about the role of micronutrients including antioxidants [10]. In addition, the efficacy of antioxidant supplement therapy in human NAFLD has not been sufficiently demonstrated [11]. Vitamin E and vitamin C are two major antiox-

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idants, fat soluble and water soluble, respectively. Vitamin E plays a role in anti-inflammatory activities, gene expression, cellular signaling and cell proliferation [12]. Vitamin E encompasses a group of 8 plant based molecules; the most abundant form is α-tocopherol [13]. Due to its ability to inhibit reactive oxygen species (ROS) production during the development of steatohepatitis, vitamin E has been investigated as a therapeutic agent in NAFLD and especially NASH [14]. In experimental studies, vitamin E supplement reduced oxidative stress [15], serum transaminase levels and hepatic steatosis in mice NASH model [16]. Among humans, vitamin E has been used as monotherapy or with other drugs to treat NAFLD or NASH and had a favorable effect in some of these studies in reduction of serum alanine aminotransferase (ALT) levels and NASH, but not with improvement in fibrosis [17–22]. However, while the recommended intake of vitamin E for adults is 15 mg/day (or 22.4 IU/day) [23], the supplements studied contained 400–1000 IU. To our best knowledge, there are no large studies that tested the potential protective role of dietary vitamin E in NAFLD and NASH.

The association between vitamin C dietary intake and NAFLD has been studied, but not extensively, and with conflicting results. Furthermore, most studies were among Asian populations with limited external validity to a Western diet [24–28]. Therefore, the aim of the current study was to test the association of dietary vitamins E and C intake and NAFLD in large cohort of subjects undergoing colonoscopy. We hypothesized that dietary intake of these antioxidants will have a protective association with NAFLD and NASH.

2. Methods

2.1. Study design and population

This is a cross sectional study among 40–70 years old subjects who underwent screening colonoscopy at the Department of Gastroenterology and Hepatology in the Tel-Aviv Medical Center, and agreed to participate in a metabolic and hepatic screening study between the years 2010 and 2015 (previously described in detail Ref. [29]). Exclusion criteria included: presence of HBsAg or anti-HCV antibodies, fatty liver suspected to be secondary to hepatotoxic drugs and excessive alcohol consumption (≥30 g/day in men or ≥20 g/day in women) [30]. In addition, subjects who reported an unreasonable caloric intake were excluded; below or above the acceptable range for men 800–4000 Kcal/day and for women 500–3500 Kcal/day [31].

The study was approved by the Tel-Aviv medical center IRB committee and all participants signed an informed consent.

2.2. Data collection and definition of hepatic and metabolic variables

Study participants were invited for a single day visit, in which they underwent fasting blood tests, liver ultrasound, a face-to face interview using a structured questionnaire, assembled by the Israeli Ministry of Health and used in national surveys [32], including demographic details, health status, alcohol consumption, smoking and exercise habits. In addition, they completed food frequency questionnaire (FFQ). To avoid report bias, the participants were informed of their abdominal ultrasonography (AUS) and blood tests results only after completing the questionnaires. Fatty liver was diagnosed by AUS using standardized criteria as previously described [33].

Presumed NASH and fibrosis were evaluated non-invasively by FibroMax which includes FibroTest, new quantitative NashTest, and SteatoTest, (BioPredictive, Paris, France), and has been validated extensively [34,35]. The FibroTest includes serum α2-macroglobulin, apolipoprotein-A1, haptoglobin, total bilirubin, and γ-glutamyl transpeptidase adjusted for age and gender. The SteatoTest includes the same components plus alanine aminotransferase, body mass index (BMI), serum cholesterol, triglycerides and fasting glucose. Compared to reference NashTest, the new quantitative NashTest 2 includes the components of the SteatoTest without glucose and BMI [35]. The procedures were those recommended by BioPredictive, including exclusion of non-reliable results [36]

The SteatoTest was treated as a continuous variable to describe the level of steatosis. The presence of moderate-severe NASH was defined as a result of ≥0.5 [35]. The presence of at least borderline-significant fibrosis ≥ F1–F2 was defined as a result of ≥0.31 [37].

Type-2 diabetes was defined as fasting glucose ≥126 mg/dl and/or HbA1C ≥ 6.5% and/or use of diabetic medications.

2.3. Nutritional and lifestyle variables evaluation and definitions

The semi-quantitative FFQ, which was assembled by the Food and Nutrition Administration, Ministry of Health and tailored to the Israeli population, is composed of 117 food items with specified serving sizes. For each food item, participants indicated their average frequency of consumption over the past year. The nutrient components were obtained from the Israeli National Nutrient Database (BINAT), Ministry of Health. Vitamins intake was calculated as mg/day and analyzed in two ways: per 1000 kcal/day to adjust for caloric intake, with a cutoff of the upper tertile of the population's consumption or with a cutoff of the Dietary Reference Intake (DRI) recommended daily intake; ≥15 mg/d vitamin E and ≥75 mg/d for women or ≥90 mg/d for men vitamin C [23].

The physical activity questionnaire included weekly frequency and duration of each training session to calculate weekly hours of the following activities: outdoor walking (intended to improve fitness), indoor walking on treadmill, running, biking, swimming, aerobic fitness (at class or stepper), ball games, dancing, body shaping and body balance.

2.4. Statistical analysis

Statistical analyses were performed using SPSS version 23 (IBM-SPSS Armonk, NY) or SAS version 9.4 (SAS Institute, Cary, NC, USA) software. Continuous variables are presented as means ± SD. To test differences in continuous variables between two groups, the independent samples t-test was performed. Associations between nominal variables were performed with the Pearson Chi-Square test. A multivariate logistic regression analysis was performed to test the adjusted association between vitamin E or C consumption and NAFLD, NASH or fibrosis adjusting for potential confounders (relevant variables which were different between the levels of vitamins consumption as depicted in Table 1). A linear regression analysis was performed to test the adjusted association between vitamins consumption and steatosis. Odds ratio (OR) and 95% confidence interval (CI) are presented. P value of <0.05 was considered statistically significant for all analyses.

3. Results

3.1. Description of the study population and comparison between subjects with high (by upper tertile) and low vitamin E or C intake (Table 1)

Out of 970 subjects who participated in the study, 789 were eligible as previously described [29]. NAFLD was diagnosed by AUS in 38.7% (n = 305) and type 2 diabetes in 14.8% (n = 117). Reliable FibroMax test was obtained from 714 subjects (7 had unreliable test and 68 had no serum sample), and 712 had the whole tests needed for the SteatoTest. In this subsample, 51.7% were men, mean age was 58.80 ± 6.59 years and mean BMI was 28.55 ± 5.40 Kg/m^2.

Table 1
Comparison between subjects with high (by upper tertile) and low vitamins E or C intake (Mean ± SD, unless otherwise stated).

Variable (units, normal range)	Vitamin E consumption (mg/1000 Kcal)			Vitamin C consumption (mg/1000 Kcal)		
	<5.45 (n = 526)	≥5.45 (n = 263)	P value	<91.40 (n = 526)	≥91.40 (n = 263)	P value
Age (years)	58.78 ± 6.41	58.93 ± 6.90	0.763	58.78 ± 6.53	58.94 ± 6.68	0.736
Gender (men %)	54.60	48.70	0.118	54.80	48.30	0.087
BMI (kg/m^2, 20–25)	28.72 ± 5.40	28.17 ± 5.48	0.183	28.65 ± 5.58	28.33 ± 5.12	0.443
Abdominal obesity[a]	64.00	57.60	0.083	63.40	58.80	0.206
Glucose (mg/dl, <100)	92.15 ± 23.88	86.98 ± 16.32	**<0.001**	90.73 ± 22.58	89.82 ± 20.12	0.580
HOMA-IR (score)	3.12 ± 4.34	2.71 ± 2.17	0.145	2.94 ± 2.50	3.07 ± 5.49	0.656
HbA1C (%)	5.92 ± 0.83	5.79 ± 0.60	**0.011**	5.90 ± 0.79	5.83 ± 0.71	0.268
Type-2 diabetes (%)	18.30	8.00	**<0.001**	16.00	12.50	0.202
Triglycerides (mg/dl)	121.10 ± 72.30	105.79 ± 53.15	**0.001**	117.36 ± 67.49	113.25 ± 65.68	0.416
Total cholesterol (mg/dl)	182.36 ± 35.45	180.09 ± 35.81	0.400	180.75 ± 34.94	183.29 ± 36.79	0.345
ALT (U/L, 5–39)	27.18 ± 15.04	23.60 ± 12.01	**0.001**	26.49 ± 14.65	25.00 ± 13.21	0.164
AST (U/L, 7–40)	25.70 ± 9.02	23.10 ± 7.16	**<0.001**	25.20 ± 9.08	24.08 ± 7.25	0.082
GGT (U/L, 6–28)	30.03 ± 31.36	24.72 ± 16.96	**0.002**	29.85 ± 29.99	25.08 ± 21.36	**0.011**
SteatoTest (score)	0.39 ± 0.20	0.34 ± 0.19	**0.002**	0.38 ± 0.20	0.35 ± 0.18	**0.045**
CRP)mg/l, <5) n= 766	3.75 ± 5.55	3.70 ± 5.91	0.911	4.02 ± 6.29	3.16 ± 4.16	**0.024**
Low ferritin (<14 ng/ml)	5.90	6.20	0.857	5.20	7.60	0.193
Dietary intake and lifestyle habits						
Energy (Kcal/day)	1925.2 ± 681.2	2227.7 ± 693.5	**<0.001**	2006.2 ± 698.2	2065. 7 ± 702.2	0.261
Cholesterol (mg/day)	338.33 ± 184.20	328.60 ± 210.72	0.505	351.06 ± 193.72	303.14 ± 189.00	**0.001**
Fat (% daily Kcal)	36.44 ± 6.28	36.07 ± 7.32	0.493	37.87 ± 6.41	33.21 ± 5.99	**<0.001**
Saturated fat (% daily Kcal)	13.04 ± 3.72	11.06 ± 3.22	**<0.001**	13.20 ± 3.69	10.74 ± 3.07	**<0.001**
Fibers (gr/day)	22.13 ± 10.62	25.41 ± 13.41	**<0.001**	21.44 ± 10.37	26.78 ± 13.36	**<0.001**
[b]Red and/or processed meat (portions/day)	0.72 ± 1.02	0.48 ± 0.78	**<0.001**	0.70 ± 0.99	0.52 ± 0.85	**0.007**
Coffee (all types cups/day)	3.36 ± 3.57	2.51 ± 2.30	**<0.001**	3.39 ± 3.45	2.45 ± 2.63	**<0.001**
Physical activity (hours/week)	2.29 ± 3.34	1.94 ± 2.62	0.144	2.15 ± 3.26	2.22 ± 2.83	0.755
Smoking (current %)	15.20	20.20	0.080	16.30	17.90	0.591
[c]Pack years	13.54 ± 21.76	14.85 ± 22.29	0.428	14.18 ± 21.92	13.57 ± 21.99	0.712
Alcohol consumption (portions/ week)	1.92 ± 3.19	1.34 ± 2.52	**0.006**	1.74 ± 3.09	1.69 ± 2.81	0.817
[d]Supplement use (% users)	7.80	4.20	0.054	6.70	6.50	0.919

Bold text indicates significant association.

[a] Abdominal obesity: waist circumference ≥102 cm for men or ≥88 cm for women.

[b] Red and/or processed meat includes: beef steak or roast, beef internal organs, fried beef patties, lamb and pork, hamburger, salami, pastrami, sausages, processed schnitzel and canned meat.

[c] Pack years calculated among ever smokers, never smokers were considered as zero.

[d] Supplement use: vitamin E and/or vitamin C and/or multivitamin.

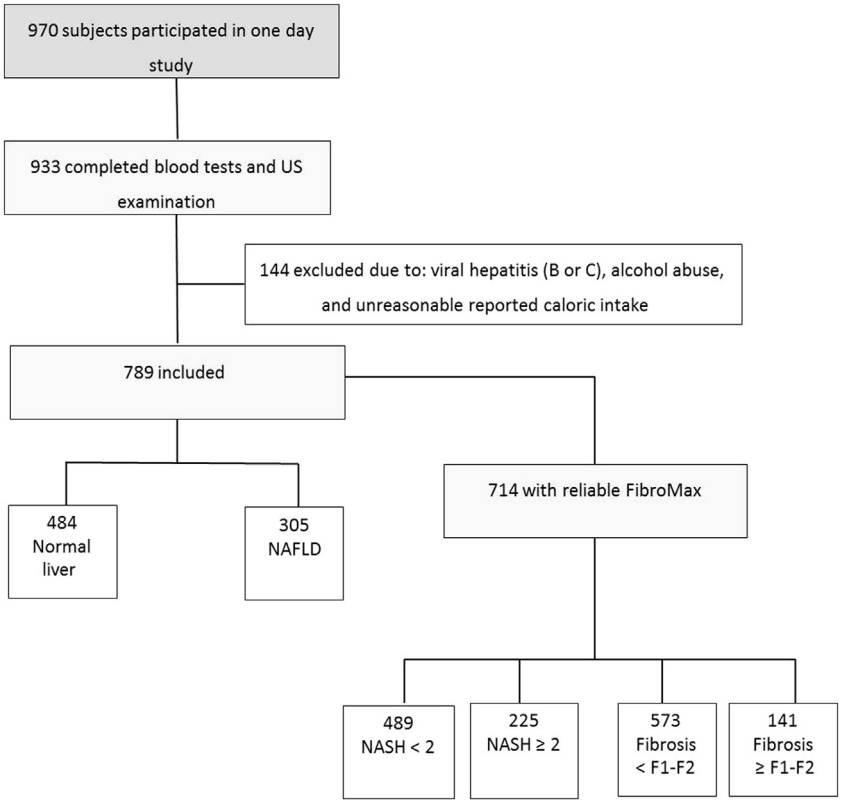

Fig. 1. Flow-chart of the study sample.

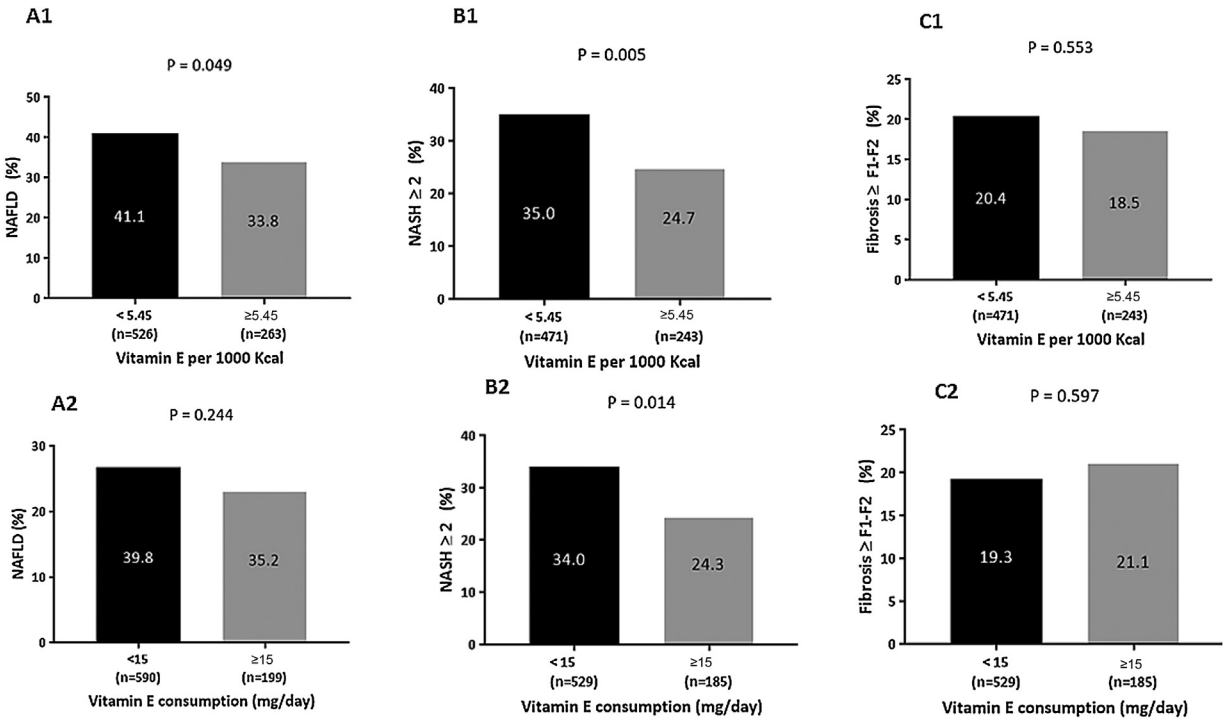

Fig. 2. Univariate association of vitamin E intake, per 1000 Kcal (upper tertile) or according to recommended intake, with NAFLD (A1&2), NASH ≥ 2 (B1&2) or Fibrosis ≥ F1–F2 (C1&2). Recommended vitamin E intake is above 15 mg/day.

Presumed NASH was observed in 31.5% (n = 225) similar to previous publication [35], and presumed borderline-significant fibrosis (≥F1–F2) in 19.7% (n = 141) (Fig. 1).

Among the total sample, 73.6% (n = 581) reached the recommended daily intake of vitamin C and only 25.2% (n = 199) reached the recommended daily intake of vitamin E. Only 6.6% (n = 52) of the subjects used vitamin supplements (vitamin E and/or vitamin C and/or multivitamin), with no significant differences across the levels of dietary vitamins intake. Subjects at the upper tertile of vitamin E consumption (per 1000 Kcal) had a better metabolic profile and a lower SteatoTest. In addition, they tended to consume more fibers and less saturated fatty acids (SFA) (%total calories), alcohol, coffee and red and/or processed meat. In contrast, there were no differences between high and low vitamin C consumers in metabolic measures, but those at the upper tertile of vitamin C consumption had lower C reactive protein (CRP) and SteatoTest levels. Subjects at the upper tertile of vitamin C consumption also consumed more fibers and less SFA, red and/or processed meat, coffee and cholesterol. No significant difference in physical activity performance was observed for both vitamins (Table 1).

3.2. *Univariate analysis of the association between vitamin E or C intake and NAFLD and presumed NAFLD-related liver damage (Figs. 2 & 3)*

The prevalence of NAFLD and NASH was higher among subjects who consumed less vitamin E per 1000 Kcal (Fig. 2A-1 & 2B-1). In terms of reaching the recommended vitamin E intake, the prevalence of presumed NASH was significantly lower among subjects who reached the appropriate intake (Fig. 2B-2). The prevalence of NAFLD and presumed NASH was higher among subjects who consumed less vitamin C per 1000 Kcal (Fig. 3A-1 & 3B-1). No differences were seen across the categories of vitamin C recommended intake. None of the vitamins was related with presumed fibrosis (Figs. 2C-1 & 2C-2 and 3C-1 and 3C-2).

3.3. *Multivariate analysis of the association between vitamin E or C intake and NAFLD and presumed NAFLD-related liver damage (Tables 2–4)*

In a multivariate analysis, the upper tertile of vitamin E intake per 1000 Kcal was associated with lower odds of NASH (Table 2, fully adjusted model C). There was no association with NAFLD or presumed fibrosis.

Similarly, there was a significant inverse association between reaching the recommended vitamin E intake and NASH adjusting for all potential confounders (Table 2, fully adjusted model C). The association with NAFLD was significant only in the model that was not adjusted for type-2 diabetes (Table 2, model B). There was no association with fibrosis.

In a multivariate linear regression, there was a significant inverse association between both upper tertile of vitamin E intake per 1000 Kcal and reaching the recommended vitamin E intake and the level of steatosis according to the SteatoTest adjusting for all potential confounders (Table 4).

The upper tertile of vitamin C intake per 1000 Kcal was associated with significantly lower odds of NAFLD and NASH (Table 3, fully adjusted model B). In addition, it was inversely associated with the level of steatosis according to the SteatoTest in the multivariate linear regression (Table 4). There was no association with fibrosis. There was no significant association between reaching the recommended vitamin C intake and any of the outcomes.

Since coffee intake was lower among high vitamin E or C consumers, we further adjusted for coffee intake, but it had no effect on the results (model not shown). For both vitamins and in all analyses, adjustment for abdominal obesity instead of BMI did not change the results (models not shown).

4. Discussion

The current study analyzed the association of dietary vitamin E intake with NAFLD and presumed NASH according to validated

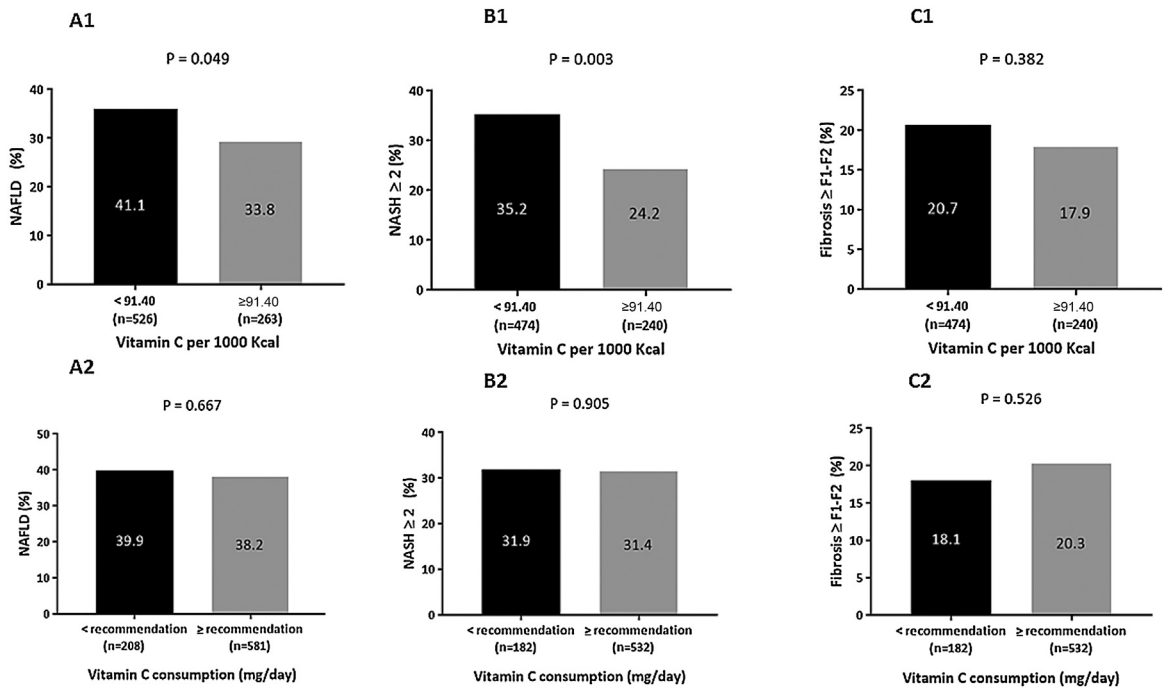

Fig. 3. Univariate association of vitamin C intake, per 1000 Kcal (upper tertile) or according to recommended intake, with NAFLD (A1&2), NASH ≥ 2 (B1&2) or Fibrosis ≥ F1–F2 (C1&2). Recommended vitamin C intake is above 75 mg/day for women or above 90 mg/day for men.

Table 2
Multivariate association of vitamin E intake and NAFLD and presumed related liver damage.

	NAFLD (n cases = 305) OR (95% CI) P	NASH ≥ 2 (n cases = 225)	Fibrosis ≥ F1–F2 (n cases = 141)
Vitamin E per 1000 Kcal (upper tertile)			
Model[a]			
<5.45	1 (ref)	1 (ref)	1 (ref)
≥5.45	0.75 (0.53–1.07) 0.109	0.57 (0.39–0.82) **0.003**	0.91 (0.59–1.41) 0.678
Model[b]			
<5.45	1 (ref)	1 (ref)	1 (ref)
≥5.45	0.70 (0.49–1.02) 0.063	0.60 (0.41–0.89) **0.011**	0.96 (0.60–1.52) 0.856
Model[c]			
<5.45	1 (ref)	1 (ref)	1 (ref)
≥5.45	0.81 (0.55–1.18) 0.266	0.64 (0.43–0.94) **0.024**	1.03 (0.64–1.66) 0.894
Vitamin E recommendation (mg/day)			
Model[a]			
<15	1 (ref)	1 (ref)	1 (ref)
≥15	0.70 (0.46–1.06) 0.090	0.45 (0.29–0.71) **<0.001**	0.84 (0.51–1.38) 0.485
Model[b]			
<15	1 (ref)	1 (ref)	1 (ref)
≥15	0.61 (0.39–0.95) **0.029**	0.46 (0.29–0.73) **0.001**	0.74 (0.43–1.27) 0.274
Model[c]			
<15	1 (ref)	1 (ref)	1 (ref)
≥15	0.70 (0.44–1.10) 0.121	0.48 (0.30–0.77) **0.002**	0.79 (0.45–1.38) 0.407

Bold text indicates significant association.
Model[a] adjusted for: age, gender, energy intake (in the analysis by vitamin E recommended intake) and BMI; Model[b] additionally adjusted for: physical activity, SFA intake (% of total Kcal), smoking status, daily alcohol portions, fibers, cholesterol, red and/or processed meat intake. Model[c] additionally adjusted for type-2 diabetes.

tests. Generally, we have shown that adequate vitamin E intake (>15 mg/day) according to the DRI [23] is independently associated with lower odds of presumed NASH. An association with NAFLD by AUS or level of steatosis according to the SteatoTest was also noted but it was less robust. Similarly to vitamin E, the upper tertile of vitamin C per 1000 Kcal was independently inversely associated with NAFLD by AUS or level of steatosis according to the SteatoTest, and presumed NASH, but not with fibrosis. However, unlike vitamin E, there was no association with daily recommended vitamin C intake. While vitamin E in a relatively low dose, even lower than the recommended intake, had a protective association with presumed NASH, it required a high dose of vitamin C (about ≥180 mg/day in an average consumption of 2000 kcal/d), twice the recommendation, to show a protective association.

However, an adequate consumption of dietary vitamin E is not trivial, as in our study population, only 25.2% reached the recommended dietary intake of vitamin E. Even lower proportion was demonstrated among American population (the National Health and Nutrition Examination Survey; NHANES) (1999–2000), in which only 5% of men and 4% of women met vitamin E recommended intake [38]. Since the best sources of vitamin E are nuts, seeds, plant oils, green leafy vegetables and fortified cereals [39], the differences of intake may be due to the typical American diet

Table 3
Multivariate association of vitamin C intake and NAFLD and presumed related liver damage.

	NAFLD (n cases = 305) OR (95% CI) P	NASH ≥ 2 (n cases = 225)	Fibrosis ≥ F1-F2 (n cases = 141)
Vitamin C per 1000 Kcal (upper tertile)			
Model[a]			
<91.40	1 (ref)	1 (ref)	1 (ref)
≥91.40	0.74 (0.52–1.04) 0.083	0.55 (0.38–0.80) **0.002**	0.88 (0.57–1.37) 0.577
Model[b]			
<91.40	1 (ref)	1 (ref)	1 (ref)
≥91.40	0.68 (0.47–0.99) **0.045**	0.57 (0.38–0.84) **0.004**	0.94 (0.58–1.51) 0.793
Vitamin C recommendation (mg/day)			
Model[a]			
<75 women; 90 men	1 (ref)	1 (ref)	1 (ref)
≥75 women; 90 men	0.91 (0.61–1.35) 0.629	0.89 (0.59–1.34) 0.572	1.26 (0.75–2.10) 0.380
Model[b]			
<75 women; 90 men	1 (ref)	1 (ref)	1 (ref)
≥75 women; 90 men	0.91 (0.60–1.39) 0.674	0.92 (0.60–1.43) 0.717	1.52 (0.88–2.62) 0.135

Bold text indicates significant association.
Model[a] adjusted for: age, gender, energy intake (in the analysis by vitamin C recommended intake) and BMI; Model[b] additionally adjusted for: physical activity, SFA intake (% total Kcal), smoking status, daily alcohol portions, fibers, cholesterol, red and/or processed meat intake.

Table 4
Multivariate linear regression for the association of vitamin E and C intake and SteatoTest level.

Vitamin E per 1000 Kcal (upper tertile) ≥5.45[a]	Vitamin E recommendation ≥15 (mg/day)[a]	Vitamin C per 1000 Kcal (upper tertile) ≥91.40	Vitamin C recommendation ≥75 women; 90 men (mg/day)
Non-standardized Coefficient B (Standard Error for B)			
P			
−0.04 ± 0.01	−0.03 ± 0.01	−0.03 ± 0.01	−0.01 ± 0.01
0.006	0.025	0.013	0.520

All models adjusted for: age, gender, energy intake (in the analysis by vitamin E or C recommended intake), BMI, physical activity, SFA intake (% of total Kcal), smoking status, daily alcohol portions, fibers, cholesterol and red and/or processed meat intake.
[a] Further adjusted for type-2 diabetes.

that is rich in meat and cheese and poor in vegetables and plant oils [40]. As expected, in the current study, the main sources of vitamin E were vegetables & fruits and plant oils (27% and 22% of total vitamin E intake, respectively). This implies that higher vitamin E intake is an indicator for a better diet. To control for that, we adjusted the association for caloric intake as well as for major dietary components relevant for NAFLD; saturated fat, fibers, cholesterol [41] and red and/or processed meat consumption that has been previously demonstrated to be associated with NAFLD [29]. In addition, we adjusted for other lifestyle habits that accompanied a higher intake of vitamin E, and the association with NAFLD-features persisted. However, residual confounding should be taken into consideration in any observational study.

To our best knowledge, this is the first large study that refers to dietary vitamin E consumption in relation of NAFLD and NASH among adults. However, our findings are in agreement with cross-sectional registry based study among children with liver histology, indicating that vitamin E dietary consumption was insufficient compared to the recommended daily allowance and that low consumption of vitamin E was associated with steatosis but not with NASH [42]. In addition, our results are in agreement with clinical trials, which have shown a therapeutic effect of high dose vitamin E supplement in NASH, not fibrosis and conflicting results for steatosis [17,18]. These studies did not include diabetic patients. In a recently published non-randomized but propensity-score-adjusted study [43], a 800 IU daily vitamin E treatment for ≥2 years was associated with decreased risk of death or transplant and hepatic decompensation after a median follow-up of 5.6 years in patients with severe fibrosis and cirrhosis. These benefits were evident in patients with and without diabetes. To our best knowledge, only one RCT has examined the effects of 400 IU daily vitamin E vs. vitamin E + pioglitazone vs placebo in diabetic patients with NASH. Vitamin E treatment alone led to a reduction in steatosis but not necroinflammation following 18 months treatment [44].

Noteworthy, that high dose vitamin E supplement raises some concerns regarding increased risk for mortality [45], hemorrhagic stroke [46] and prostate cancer [47]. Therefore, a potential protective effect of a lower dose provided by diet is interesting.

Our findings about the protective association of vitamin C dietary intake with NAFLD are in accordance with previous cross-sectional studies [24,25,28]. No randomized clinical trials (RCTs) of vitamin C as a single therapy were performed, but Harrison et al. have demonstrated that six months of 1000 mg/d of vitamin C supplement combined with 1000 IU of vitamin E improved liver fibrosis [48].

From a mechanistic point of view, experimental studies in animal models have partially explained the clinical effects of vitamin E supplementation. In one study using the methionine-choline deficient diet model, vitamin E supplement reduced oxidative stress, hepatocyte apoptosis and necroinflammation [15]. In a further study in the same experimental model, vitamin E was also shown to ameliorate fibrosis [16]. However, this experimental model is far from the features of human NASH. Interestingly, immunohistochemical analysis of pre- and post-treatment biopsies from the PIVENS trial demonstrated that treatment response with vitamin E is associated with inhibition of the Hedgehog signaling [49]. To our knowledge, no study has been conducted on the effects of vitamin C in an animal NASH model. A previous study demonstrated that mice drinking red orange juice while feeding a high fat diet are protected from metabolic dysfunction and fatty liver [50]. However, orange juice contains hundreds of bioactive molecules.

This study has several limitations to consider. First, the cross-sectional design of the study does not allow causal inference. Second, dietary habits were self-reported and thus prone to a report bias. However, since the participants and the research team were

both blinded to the AUS and FibroMax results, it is a non-differential repot bias and therefore may have only weakened the observed associations. Third, the diagnosis of NAFLD and presumed NASH or fibrosis were determined by AUS and FibroMax markers, respectively, and not by liver histology, which cannot be performed in a sample of apparently healthy volunteers. However, AUS is the most accepted and common screening method for NAFLD in the general population [51]. The FibroTest validity has been demonstrated in several studies and biomarkers of fibrosis are considered as reasonably acceptable non-invasive procedures, but as for the reference NashTest, although validated, non-invasive tests are still considered to be insufficient for the diagnosis of NASH [30]. Noteworthy, that the high prevalence of presumed NASH in this study is similar to previous publication [35], and may be reasonable in a population older than 50 and metabolically altered (average weight indicated overweight and prevalence of type-2 diabetes was relatively high). Other limitations also include lack of data that may be considered for future studies; the amount of steatosis by AUS or other quantitative imaging measures [52], markers of oxidative stress and questions on reproductive status which can be considered as an intervening variable [53].

The strengths of the study include its large sample size and meticulous assessment of dietary intake as well as other lifestyle parameters that could be controlled for. Furthermore, the association with liver fat was measured by two unrelated methods (AUS and biomarkers) adding to the robustness to the results. Therefore, this study expends the knowledge on an understudied question; while nutritional guidelines for NAFLD refer to moderate weight loss and adherence to Mediterranean diet [30], there are no recommendations for dietary micronutrients intake. Most studies that tested the role of micronutrients, especially vitamin E, used megadose supplements which neither can be reached by regular dietary intake nor can reflect the role of dietary micronutrients in NAFLD.

In conclusion, dietary vitamin E and C intake may be protective from NAFLD and mainly NASH. Prospective studies with liver histology are needed to confirm these findings.

Conflict of interest

None declared.

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