

# The association of dietary antioxidants intake with the risk of cardiovascular disease: Tehran Lipid and Glucose Study

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## Research

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# Abstract

**Aim:** This study investigated the association between daily consumption of dietary vitamins A, E, C and zinc and the incidence of cardiovascular disease (CVD).

**Methods:** Eligible adults (n=5102) were selected from among participants of the Tehran Lipid and Glucose Study with an average follow-up of 5.3 years. Dietary intakes were assessed using a valid and reliable semi-quantitative food frequency questionnaire. Anthropometrics and biochemical variables were evaluated at baseline and follow-up examinations. Multivariate Cox proportional hazard regression models were used to estimate the development of CVD in relation to total intakes of vitamins A, E, C and zinc.

**Results:** This study was conducted on 2253 men and 2849 women, aged  $47.0 \pm 11.6$  and  $45.6 \pm 10.5$  years, respectively. Main source of dietary vitamins A, E, C and zinc was fruits, vegetables and legumes in our study. Risk of CVD decreased from quartiles 1 to 4 for vitamin E intake (HR (95% CI): 1.00, 0.91, 0.77, 0.57, P trend =0.03). The association between risk of CVD and the quartiles of vitamin A, vitamin C and zinc intake was not significant.

**Conclusion :** Our study suggests an inverse association between vitamin E intake and the risk of CVD, results emphasizing the potential protective role of fruits and vegetables in the prevention of CVD.

## Introduction

Cardiovascular disease (CVD), the leading cause of death worldwide, is the collective term/compound name for disorders afflicting the blood vessels and heart; an estimated 17.9 million people died from CVDs in 2016 (1). It has already been shown that inflammation and enhanced oxidative stress has been involved as a fundamental etiology in the onset and progression of CVD (2). Chronic inflammatory conditions attenuate blood levels of antioxidants because of continuous generation of elevated levels of reactive oxygen species (ROS); adequate antioxidant intakes are also suggested to beneficially interfere with CVD by quenching ROS (3).

Antioxidant vitamins and minerals such as vitamins A, E, C and zinc may slow the development and progression of CVD (4, 5). Observational epidemiological studies suggest that higher dietary intakes of vitamin E were associated with lower risk of CVD (6). Although, these findings laid the basis for additional, more powerful clinical research in this area.

Vitamin C is a major water-soluble antioxidant in plasma, and observational studies have shown inverse associations of dietary vitamin C with CVD outcomes (7, 8). Although, in several large randomized controlled trials, the benefits for vitamin C in CVD prevention were not confirmed. Despite the antioxidant potential of vitamin A being first determined in 1932 (9), there is limited data about the association between vitamin A and CVD (10). Zinc is an essential trace metal with antioxidant and anti-inflammatory activities; insufficient intake of zinc has persistently been reported in CVD patients (11) and analysis of

hair shows that patients with CVD have lower levels of zinc (12), a recent review of cohort studies on CVDs and zinc status reported no association between CVD events and zinc intake (13); therefore, further investigations and additional evidence from observational studies are required.

Previous studies have reported inverse associations between high consumption of antioxidant-rich foods such as vegetable and fruit intakes and risk of CVD (14, 15). Vegetables and fruits are a principal dietary source of vitamins E, A, C and zinc for the Iranians (16); however, whether this protective effect is caused by antioxidant vitamins or mineral remains unclear. Clinical and observational studies in United States and Europe have shown associations of antioxidant vitamins with decreased risk of death from CVD (8, 17, 18); however, the evidence from Asian populations is limited (19), which is why this study aimed to prospectively evaluate the association between dietary antioxidant intakes (vitamins A, E, C and zinc) and CVD in a group of Tehranian adults

## Methods

### Study population:

Subjects of this cohort study were selected from participants of the Tehran Lipid and Glucose Study (TLGS), a population-based prospective study performed to determine the risk factors for non-communicable diseases in a sample of residents from District 13 of Tehran, the capital of Iran (20). The first examination survey was performed from 1999 to 2001 on 15,005 individuals aged  $\geq 3$  years, using the multistage stratified cluster random sampling technique, and follow-up examinations were conducted every 3 years; 2002–2005 (survey 2), 2005–2008 (survey 3), 2008–2011 (survey 4), and 2012–2015 (survey 5) to identify recently developed diseases.

Of 14712 individuals participating in baseline (surveys 3 and 4) of our study, 9057 subjects were randomly selected for dietary assessment based on age- and sex-stratified random sampling; of these, 5531 people who aged  $\geq 30$  years in baseline and had completed data were included and followed until 2014. Of these participants, we excluded Subjects with under- or over-report of energy intake ( $< 800$  or  $\geq 4200$  kcal/day) ( $n=227$ ) and also subjects with history of CVD ( $n=414$ ) at baseline. Finally, after excluding participants missing any follow up data ( $n=15$ ), 5102 subjects remained and entered the analysis (Figure 1).

All participants signed a written informed consent form before taking part in this investigation. The study was implemented based on the Declaration of Helsinki and the study protocol was accepted by the ethics committee of the Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran. All methods were performed in line with their relevant guidelines and regulations.

### 2.2. Dietary intake measurements

Dietary assessment was used by a valid and reliable 168-item semi-quantitative food frequency questionnaire (FFQ); trained interviewers collected information on usual dietary intake, through face-to-

face private interviews. The consumption frequency of each food item on a daily, weekly, or monthly basis was converted to daily intakes, and portion sizes were then converted grams using household measures. (20). Dietary intakes of vitamins E, C, A, , and zinc were calculated and considered as grams per week.

### **Physical activity**

Physical activity level was assessed using the Persian-translated modifiable activity questionnaire (MAQ) with high reliability and moderate validity (21). Data on the time and frequency of light, moderate, high, and very hard intensity activities were obtained according to the list of common activities of daily life over the past year and these activity data were transformed into metabolic equivalent-hours/week (Met/h/week) (22).

### **Blood pressure and anthropometric measurements**

Systolic and diastolic blood pressure were measured twice (with a 30 sec interval in between) in a sitting position after 15 min of rest.

The body weight was measured to the nearest 100 g, using a digital scale (Seca 707), while subjects were minimally clothed and barefoot. Height was measured to the 0.5 cm by a tape measure, in standing position without shoes and with shoulders in normal alignment. Waist circumference (WC) was measured with a non-flexible tape meter without any pressure to body surface at the level of the umbilicus and was taken at the end of a normal expiration, over light clothing. Measurements were recorded to the nearest 0.1 cm.

### **Laboratory assays**

Blood samples were drawn into vacutainer tubes between 7:00 to 9:00 a.m., after a 12–14 hour overnight fast from subjects, who were in sitting position and were centrifuged within 30 to 45 min of collection. All biochemical analyses were performed using a Selectra 2 auto-analyzer at the TLGS research laboratory on the day of blood collection. Fasting blood glucose (FBS) concentration was measured on the day of blood collection by the enzymatic colorimetric method with the glucose oxidase technique. The standard 2-h post-challenge blood glucose test was performed using oral administration of 82.5 g glucose monohydrate solution (equivalent to 75 g anhydrous glucose) for all individuals who were not on glucose-lowering drugs.

HDL-C concentration was assessed after precipitation of the apolipoprotein B-containing lipoproteins with phosphotungstic acid. Total cholesterol (TC) and TG were measured using the enzymatic colorimetric method. For the TC assay, cholesteryl ester hydrolase was used to convert cholesteryl ester to cholesterol, which was then oxidized by cholesterol oxidase to cholesterol-4-en-3-one and H<sub>2</sub>O<sub>2</sub>. For the TG assay, TG was broken down to glycerol and free fatty acids using lipoprotein lipase and glycerol was then phosphorylated to glycerol phosphate by glycerokinase; glycerol phosphate was converted to

dihydroxyacetone phosphate and H<sub>2</sub>O<sub>2</sub> by glycerol phosphate oxidase. The Friedewald equation ( $LDL-C = TC - HDL-C - TG/5$ ) was used to calculate LDL-C concentrations in samples with TG (23).

## Definitions

Details of CVD outcome data have been described elsewhere (24). Coronary heart disease (CHD) events included cases of definite myocardial infarction (diagnostic electrocardiographic [ECG] results and biomarkers), probable myocardial infarction (positive ECG findings plus cardiac symptoms or signs plus missing biomarkers or positive ECG findings plus equivocal biomarkers), proven CHD by angiography, unstable angina pectoris (new cardiac symptoms or changing symptom patterns and positive ECG findings with normal biomarkers), and CHD death. CVD was defined as stroke (a new neurological deficit that lasted more than 24 h), CHD events, or CVD death (fatal stroke or fatal CHD). CVD risk score was measured according to the sex specific 'general CVD' algorithms were derived that age, systolic BP, treatment for hypertension, total cholesterol, HDL-C, type 2 diabetes status, and smoking (25). Hypertension was defined as SBP $\geq$ 140 or DBP $\geq$ 90 mmHg, or receiving antihypertensive drug treatment (26).

## Statistical analyses

Statistical analyses were carried out using the Statistical Package for Social Sciences (version 21.0; SPSS). A two-tailed P value <0.05 was used to determine statistical significance. We used a Chi-square test for qualitative variables and the Student's t test for quantitative variables to compare the characteristics between men and women. In case of non-normal nutritional and biochemical variables (triglyceride concentration), log-transformed values were used for statistical analysis. The hazards ratio (HR) and 95% confidence interval of incident CVD were assessed using multivariable Cox proportional hazard regression models. Person-years of follow-up was calculated for each individual from the date of inclusion to the date of diagnosis of CVD, death, or end of the follow-up, whichever came first. Survival time for censored individuals was calculated as the interval between the first and last observation dates. Event date was considered as the middle-time between the date of follow up visit at which the events were diagnosed for the first time, and the most recent follow up visit preceding the diagnosis. The incidence of CVD during the follow up period were considered as dichotomous variables (yes/no) in the models. Vitamin E, vitamin C, vitamin A and zinc intakes were categorized into quartiles, given the first quartile as the reference. The median of each quartile was used as a continuous variable to assess the overall trends of HRs across quartiles of dietary vitamins A, E, C and zinc intakes in the Cox proportional hazard regression models. The proportional hazard assumption of multivariate Cox models were assessed using Schoenfeld's global test of residuals.

The confounders were selected based on literature; and each confounder was included in the uni-variable Cox regression model. A two-tailed P value <0.20 was used for determining inclusion in the model. The Cox regression models were adjusted for several potential confounders; the analyses were adjusted for age, sex, CVD risk score(continuous), family history of CVD, physical activity (continuous), total energy intake, fiber (gr/1000 Kcal) and total fat (percentage of energy) intakes; in models for estimating HR.

## Results

Mean age at baseline was  $47.0 \pm 11.6$  and  $45.6 \pm 10.5$  years in men and women, respectively. Table 1 shows the baseline characteristics of men and women. Men were older, had worse smoking habits, greater levels of physical activity, lower BMI and higher WC than women; in addition compared to women, men had higher values of SBP, DBP, total cholesterol, TG/HDL-ratio and FPG except for 2 h- plasma glucose. Men consumed greater amounts of energy and carbohydrate intake than women and intakes of total fat, SFA, MUFA, PUFA, fiber, vitamin A and vitamin C were higher in women, compared to the men.

Table 1  
Baseline characteristics of adult participants of the Tehran Lipid and Glucose Study

	Total sample	Men	Women	P
	N = 5102	N = 2253	N = 2849	
Baseline age (years)	46.2 ± 11.1*	47.0 ± 11.6	45.6 ± 10.5	< 0.001
Current smokers (%)	19.1	34.8	6.7	< 0.001
Physical activity (MET/min/week)	524 ± 793	588 ± 844	473 ± 687	< 0.001
BMI (Kg/m <sup>2</sup> )	28.1 ± 4.5	27.1 ± 4.0	28.8 ± 4.8	< 0.001
Waist circumference (cm)	93.8 ± 11.0	96.5 ± 10.3	91.8 ± 11.4	< 0.001
SBP (mmHg)	114 ± 16.7	118 ± 15.9	112 ± 17.1	< 0.001
DBP (mmHg)	76.3 ± 10.2	78.7 ± 10.3	74.4 ± 10.2	< 0.001
Total cholesterol (mg/dl)	219 ± 121	234 ± 138	207 ± 117	< 0.001
LDL (mg/dl)	118 ± 31	118 ± 31	118 ± 32	0.51
TG/HDL-ratio	3.8 ± 3.0	4.6 ± 3.7	3.1 ± 2.4	< 0.001
FPG (mg/dl)	98.1 ± 26.6	99.3 ± 26.2	97.2 ± 27.0	0.007
2 h- plasma glucose (mg/dl)	108 ± 43.1	106 ± 44.5	110 ± 40.3	0.004
Energy intake (kcal/day)	2314 ± 714	2416 ± 728	2230 ± 695	< 0.001
Carbohydrate (% of energy)	58.5 ± 8.3	59.9 ± 6.4	57.5 ± 9.5	< 0.001
Protein (% of energy)	14.6 ± 5.2	14.4 ± 2.7	14.7 ± 8.1	0.19
Total fat (% of energy)	30.4 ± 10.3	28.7 ± 6.0	31.8 ± 17.6	< 0.001
SFA (% of energy)	10.1 ± 11.1	9.5 ± 2.7	10.6 ± 16.9	0.003
MUFA (% of energy)	10.4 ± 11.3	9.7 ± 2.6	10.9 ± 16.9	< 0.001
PUFA (% of energy)	6.3 ± 12.1	5.8 ± 1.9	6.7 ± 16.9	< 0.001
Fiber (g/1000 kcal)	9.7 ± 3.3	9.0 ± 2.8	10.2 ± 3.8	< 0.001
Vitamin E (mg/day)	12.1 ± 5.3	11.2 ± 5.0	12.8 ± 44.5	0.08

MET: Metabolic Equivalent, BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, MUFA: Mono-unsaturated fatty acids. PUFA: Poly-unsaturated fatty acids; SFA, Saturated fat

\*values are mean ± SD unless otherwise listed

P-values derived through Student's t-test and chi-square test for quantitative and qualitative variables, respectively

	Total sample	Men	Women	P
Vitamin C (mg/day)	170 ± 123	160 ± 108	178 ± 130	< 0.001
Vitamin A (µg/day)	600 ± 371	565 ± 350	629 ± 399	< 0.001
Zinc (mg/day)	13.7 ± 26.3	14.0 ± 12.4	13.4 ± 45.1	0.58
<b>Food consumption</b>	<b>Vitamin E (mg)</b>	<b>Vitamin C (mg)</b>	<b>Vitamin A (µg)</b>	<b>Zinc (mg)</b>
<b>Fruits</b>				
Q1: 0.70 ± 0.27 <sup>b</sup>	9.7 ± 4.7	79.7 ± 44.1	421 ± 256	11.3 ± 8.1
Q2: 1.60 ± 0.27	10.8 ± 10.8	123 ± 36.4	534 ± 320	12.9 ± 10.3
Q3: 2.69 ± 0.38	14.0 ± 66.2	178 ± 89.0	625 ± 351	15.3 ± 66.5
Q4: 5.59 ± 2.55	14.1 ± 5.0	300 ± 137	822 ± 446	15.1 ± 14.3
P for trend <sup>c</sup>	< 0.001	< 0.001	< 0.001	0.01
<b>Vegetables</b>				
Q1: 1.89 ± 0.53	9.1 ± 4.5	101 ± 83.4	357 ± 212	10.5 ± 8.7
Q2: 3.28 ± 0.36	11.0 ± 4.5	145 ± 94.4	492 ± 213	12.2 ± 7.9
Q3: 4.57 ± 0.39	12.3 ± 5.3	183 ± 92.5	632 ± 278	13.4 ± 10.3
Q4: 7.66 ± 2.82	16.1 ± 66.2	251 ± 142	920 ± 484	18.5 ± 67.3
P for trend	< 0.001	< 0.001	< 0.001	< 0.001
<b>Legumes</b>				
Q1: 0.02 ± 0.01	11.6 ± 5.3	157 ± 126	514 ± 341	11.6 ± 6.7
Q2: 0.07 ± 0.01	11.8 ± 5.6	172 ± 122	598 ± 389	13.0 ± 10.6
Q3: 0.15 ± 0.02	11.5 ± 4.8	167 ± 107	613 ± 360	13.7 ± 12.6
Q4: 0.35 ± 0.18	13.6 ± 8.2	183 ± 132	677 ± 407	16.4 ± 67.0
P for trend	< 0.001	< 0.001	< 0.001	< 0.001

MET: Metabolic Equivalent, BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, MUFA: Mono-unsaturated fatty acids. PUFA: Poly-unsaturated fatty acids; SFA, Saturated fat

\*values are mean ± SD unless otherwise listed

P-values derived through Student's t-test and chi-square test for quantitative and qualitative variables, respectively

	Total sample	Men	Women	P
Whole grains				
Q1: 0.10 ± 0.07	11.2 ± 5.2	149 ± 118	511 ± 344	11.3 ± 8.3
Q2: 0.44 ± 0.13	11.6 ± 5.5	167 ± 107	599 ± 386	12.5 ± 8.7
Q3: 1.31 ± 0.43	13.5 ± 66.2	175 ± 131	622 ± 385	14.1 ± 13.7
Q4: 5.01 ± 3.79	12.5 ± 4.8	189 ± 123	669 ± 384	16.7 ± 66.9
P for trend	0.25	< 0.001	< 0.001	0.002
Refined grains				
Q1: 4.90 ± 1.34	10.6 ± 5.7	173 ± 126	614 ± 432	11.0 ± 8.1
Q2: 7.12 ± 0.72	11.3 ± 5.1	168 ± 111	591 ± 347	12.3 ± 10.9
Q3: 10.0 ± 0.95	12.0 ± 5.0	173 ± 118	607 ± 378	14.0 ± 12.8
Q4: 16.6 ± 5.29	14.6 ± 66.2	166 ± 129	590 ± 356	17.4 ± 66.7
P for trend	0.02	0.007	0.004	< 0.001
Dairy products				
Q1: 0.92 ± 0.30	12.3 ± 66.3	137 ± 119	449 ± 325	12.5 ± 67.4
Q2: 1.69 ± 0.18	11.2 ± 4.6	161 ± 108	544 ± 332	12.2 ± 7.8
Q3: 2.35 ± 0.22	11.9 ± 5.0	181 ± 125	624 ± 344	13.6 ± 9.4
Q4: 3.71 ± 1.07	13.1 ± 5.5	201 ± 123	785 ± 326	16.2 ± 11.0
P for trend	0.19	< 0.001	< 0.001	< 0.001
MET: Metabolic Equivalent, BMI: body mass index, SBP: systolic blood pressure, DBP: diastolic blood pressure, MUFA: Mono-unsaturated fatty acids. PUFA: Poly-unsaturated fatty acids; SFA, Saturated fat				
*values are mean ± SD unless otherwise listed				
P-values derived through Student's t-test and chi-square test for quantitative and qualitative variables, respectively				

Association between vitamin E, vitamin C, vitamin A and zinc and quartiles of food groups are presented in Table 2. A higher consumption of fruit, vegetable, legumes, refined grains, fish and poultry was associated with higher concentrations of vitamin E. Vitamin C intake was positively associated with all food groups, except for refined grains and red meat that showed negative association with vitamin C intake. There was a positive association between vitamin A and quartiles of all food groups except for red meat. Compared to those in the lower quartiles, zinc intakes were significantly higher among individuals

in the upper quartiles of fruit, vegetable, legumes, whole grains, refined grains, dairy, fish and poultry intake.

Table 2  
Antioxidants intakes with respect to quartiles of food groups' consumption

Food consumption	Vitamin E (mg)	Vitamin C (mg)	Vitamin A (µg)	Zinc (mg)
Red meat				
Q1: 0.07 ± 0.03	12.0 ± 5.4	181 ± 140	612 ± 404	13.3 ± 9.2
Q2: 0.16 ± 0.02	11.7 ± 4.9	172 ± 120	602 ± 349	13.4 ± 12.0
Q3: 0.27 ± 0.04	13.4 ± 66.2	168 ± 122	595 ± 344	14.3 ± 66.3
Q4: 0.60 ± 0.32	11.5 ± 5.6	159 ± 98.6	593 ± 416	13.8 ± 13.8
P for trend	0.70	< 0.001	0.12	0.32
Fish and poultry				
Q1: 0.41 ± 0.15	10.7 ± 4.9	148 ± 116	499 ± 346	11.3 ± 8.3
Q2: 0.84 ± 0.13	11.2 ± 4.9	161 ± 112	554 ± 318	12.5 ± 8.7
Q3: 1.32 ± 0.16	12.1 ± 5.0	174 ± 112	627 ± 386	14.1 ± 13.7
Q4: 2.92 ± 1.87	14.6 ± 66.3	197 ± 137	722 ± 498	16.7 ± 66.9
P for trend	0.001	< 0.001	< 0.001	< 0.001
Sugar-sweetened soft drinks (ml/d)				
Q1: 0.76 ± 0.95	13.1 ± 66.1	170 ± 131	595 ± 382	14.2 ± 66.4
Q2: 8.29 ± 2.23	11.1 ± 4.6	159 ± 117	560 ± 376	12.7 ± 11.2
Q3: 24.3 ± 7.59	11.7 ± 4.8	174 ± 122	622 ± 380	14.1 ± 14.4
Q4: 109 ± 101	12.6 ± 5.7	176 ± 113	626 ± 377	13.8 ± 7.6
P for trend	0.55	< 0.001	< 0.001	0.68
Nuts				
Q1: 0.02 ± 0.01	11.2 ± 5.1	145 ± 109	537 ± 379	12.5 ± 108
Q2: 0.08 ± 0.01	13.6 ± 66.2	169 ± 135	596 ± 364	15.0 ± 66.7

<sup>a</sup>Values are serving/day, with the exception of sugar-sweetened soft drinks, Tea and coffee

<sup>b</sup>Data are mean ± SD unless otherwise listed

<sup>c</sup>P for trend were with linear regression using the mean of each quartile as a continuous variable for each food group

Food consumption	Vitamin E (mg)	Vitamin C (mg)	Vitamin A (µg)	Zinc (mg)
Q3: 0.17 ± 0.03	11.7 ± 11.7	177 ± 113	621 ± 373	13.4 ± 11.7
Q4: 0.55 ± 0.42	12.0 ± 5.6	188 ± 188	648 ± 393	13.8 ± 10.7
P for trend	0.96	< 0.001	< 0.001	0.33
Tea and coffee (ml/d)				
Q1: 200 ± 88.1	12.7 ± 66.4	156 ± 123	549 ± 359	14.5 ± 67.2
Q2: 379 ± 112	11.5 ± 4.8	169 ± 118	612 ± 398	13.1 ± 11.2
Q3: 679 ± 101	12.0 ± 4.6	175 ± 121	613 ± 342	13.5 ± 11.2
Q4: 1342 ± 741	12.5 ± 6.3	179 ± 121	628 ± 383	13.6 ± 7.8
P for trend	0.50	< 0.001	0.001	0.97
<sup>a</sup> Values are serving/day, with the exception of sugar-sweetened soft drinks, Tea and coffee				
<sup>b</sup> Data are mean ± SD unless otherwise listed				
<sup>c</sup> P for trend were with linear regression using the mean of each quartile as a continuous variable for each food group				

HRs (95% CI) of CVD for quartiles of dietary antioxidants (vitamins A, E, C and zinc) intakes are shown in Table 3. After adjustment for potential confounders, risk of CVD decreased from quartiles 1 to 4 for vitamin E intake (HR (95% CI): 1.00, 0.91, 0.77, 0.57,  $P_{\text{trend}}=0.03$ ). The associations between risk of CVD and quartiles of vitamins A, C and zinc intake were not significant.

Table 3  
Hazard Ratios (HR) and 95% CIs for cardiovascular diseases according to quartiles of dietary antioxidants intake.

Variables	Quartiles of intake				P <sub>trend</sub> <sup>a</sup>
	Q1	Q2	Q3	Q4	
Vitamin E intake					
Median, mg/d	6.8	9.5	12.4	16.8	
Cases, n	61	56	51	38	
Person-years, n	6711	6530	6593	6423	
Follow-up, y	5.3	5.2	5.2	5.1	
Incidence, %	9.0	8.5	7.7	5.9	
Crude	1.00 ref.	0.94(0.65– 1.35)	0.85(0.58– 1.23)	0.65(0.43– 0.98)	0.03
Model adjusted <sup>b</sup>	1.00 ref.	0.91(0.62– 1.64)	0.77(0.51– 1.18)	0.57(0.34– 0.97)	0.03
Vitamin C intake					
Median, mg/d	64.7	114.2	173.3	291.6	
Cases, n	55	52	63	46	
Person-years, n	6844	6643	6515	6256	
Follow-up, y	5.5	5.3	5.1	5.0	
Incidence, %	6.5	7.8	9.6	7.3	
Crude	1.00 ref.	1.19(0.80– 1.77)	1.47(1.00-2.16)	1.13(0.74– 1.70)	0.58
Model adjusted <sup>b</sup>	1.00 ref.	1.04(0.68– 1.59)	1.19(0.76– 1.85)	0.84(0.48– 1.48)	0.44
Vitamin A intake					
Median, µg/d	267	428	611	964	
Cases, n	53	60	41	52	

<sup>a</sup>Test for trend based on ordinal variable containing median value for each quartile.

<sup>b</sup>Adjusted for age, sex, CVD risk score, family history of CVD, physical activity, total energy intake, fiber and total fat intakes

	Quartiles of intake				
Person-years, n	7020	6020	6472	6145	
Follow-up, y	5.7	5.3	5.1	5.0	
Incidence, %	7.5	9.0	6.3	8.4	
Crude	1.00 ref.	1.21(0.83– 1.75)	0.84(0.56– 1.27)	1.13(0.77– 1.67)	0.81
Model adjusted <sup>b</sup>	1.00 ref.	1.16(0.78– 1.70)	0.82(0.52– 1.28)	1.00(0.61– 1.63)	0.74
Zinc					
Median intake (mg/d)	7.6	10.5	13.2	17.8	
Cases, n	51	61	41	53	
Person-years, n	6959	6728	6409	6161	
Follow-up, y	5.6	5.3	5.1	5.0	
Incidence, %	7.3	9.0	6.3	8.6	
Crude	1.00 ref.	1.24(0.85– 1.80)	0.88(0.58– 1.33)	1.16(0.81– 1.76)	0.62
Model adjusted <sup>b</sup>	1.00 ref.	1.20(0.80– 1.81)	0.81(0.49– 1.35)	1.09(0.58– 2.05)	0.97
<sup>a</sup> Test for trend based on ordinal variable containing median value for each quartile.					
<sup>b</sup> Adjusted for age, sex, CVD risk score, family history of CVD, physical activity, total energy intake, fiber and total fat intakes					

Kaplan-Meier cumulative survival curves for CVD according to quartiles of vitamins A, C, E and zinc intakes during follow-up years are shown in Figs. 2–5. Significant differences was found in the risk of CVD between quartiles of vitamin E intake.

## Discussion

The current investigation was a prospective cohort study, evaluating the association of dietary antioxidants (vitamins A, E, C and zinc) intakes with risk of CVD. Our results suggest that a higher intake of vitamin E, but not vitamins A, C and zinc intake, was inversely associated with incidence of CVD.

Simultaneously with this study, an association between dietary vitamin E intake and decreased risk of CVD has been found in observational epidemiologic studies (17, 26–28). Basic research suggests that most biological functions of vitamin E are a result of its antioxidant properties because of its ability to

inhibit oxidation of LDL-C and scavenge lipid radicals (29). By contrast, several large randomized controlled trials have failed to corroborate the benefits of vitamin E in CVD prevention (6), this inconsistency can be due to several factors based on timing of intervention, gene polymorphisms or inherent confounding, and pathophysiological conditions in study populations. Consequently, on interventional study, considering these aspects, supplemental doses of vitamin E were given to ameliorate the therapeutic strategies in the concept of personalized medicine value (30). Although, due to lack of scientific evidence, the American Heart Association does not recommend the use of vitamin E supplements to prevent CVD, they support the intake of foods abundant in antioxidant vitamins especially fruits and vegetables, which is the main source of vitamin E in our study (31).

There is a lack of consensus on the anti-CVD attributes of vitamin C. A non-significant association between vitamin C intake and CVD has been found in a large Spanish cohort of university graduates (18). Contradictory to our results, observational studies on vitamin C and CVD risk demonstrate inverse associations between vitamin C and CVD outcomes, especially on heart failure (7) and hypertension (8). The contradiction in results can be due to differences in the definition of CVD. Vitamin C increases the nitric oxide bioactivity of the endothelium which causes decrease in blood pressure (32). Moreover, vitamin C reduces monocyte adhesion and inhibits LDL oxidation (33), which play an important role in decreasing the risk of atherosclerosis. In addition, vitamin C keeps atheroma plaques stable by preventing vascular smooth muscle cell apoptosis (34). In the current study, the low variability of vitamin C intake may explain the lack of significant results.

Consistent with our results, in a meta-analysis study (35), neither dietary nor supplemental vitamin A were associated with CVD risk. A large prospective study (10) indicates that among 4117 patients with stable angina pectoris in the upper tertile of serum vitamin A concentrations, serum apolipoprotein B (a predictor of CVD incidence) was associated with CVD risk, although, dietary intake of vitamin A did not correlate with serum concentrations, and it seems another mechanism other than intake of vitamin A, regulates the serum vitamin A concentrations (36), which could explain why we did not observe any association between dietary vitamin A and CVD risk.

In the present study, no significant results were found for dietary zinc intake, this result is consistent with systematic review of prospective cohort studies on dietary zinc intake or serum zinc levels and the incidence of CVD (13). However, high intake of dietary zinc was associated with a greater incidence of CVD in a large longitudinal study of Australian women (37). Then, more data from observational studies researching the mechanisms of zinc's action on the pathogenesis of CVD are required to provide a recommendation for dietary zinc in relation to the prevention of CVD.

Of the present study's major strengths, its prospective design facilitated the estimation of incident disease without much concern about reverse causality between nutrients and outcomes; vitamin and mineral intakes were estimated on the basis of all the foods in the FFQ and the evaluation of nutrient consumption from various food sources provided a new vision into the association between disease and nutrients. Among limitations, include as current data is based on food consumption report, while use of

multiple assessments of urine or circulating biomarkers of vitamins over time could be a more reliable approach. Considering the observational design of current research, we did not consider some of the confounders (e.g. supplements intake) which must be considered. Although, the percentage of daily supplement users was below 10%, and the relationship between dietary vitamin intakes and CVD did not seem to change after the justification of supplement users. Assessment of diet was conducted at baseline only, and changes in dietary habits were not taken during follow-up. Nevertheless, change in dietary habits is unlikely during 3 years.

## **Conclusion**

The qualitative assessment of this evidence suggests an inverse association between vitamin E intake and the risk of CVD, findings which emphasize the potentially protective role of fruits and vegetables in the prevention of CVD events.

## **Declarations**

### **Acknowledgement**

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### **Author contributions**

Conceptualization, P.M, Z.H and S.H.N; Formal analysis, Z.H, F.H.E and S.H.N; Methodology, P.M and F.H.E; Supervision, F.A; Writing original draft, P.M and Z.H; Writing review & editing, Z.H and F.H.E.

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### **Availability of data and materials**

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### **Ethics approval and consent to participate**

The study was implemented based on the Declaration of Helsinki and the study protocol was accepted by the ethics committee of the Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran. All methods were performed in line with their relevant guidelines and regulations.

### **Consent for publication**

Not applicable.

## Competing interests

The authors declare that there are no conflicts of interest.

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## Figures

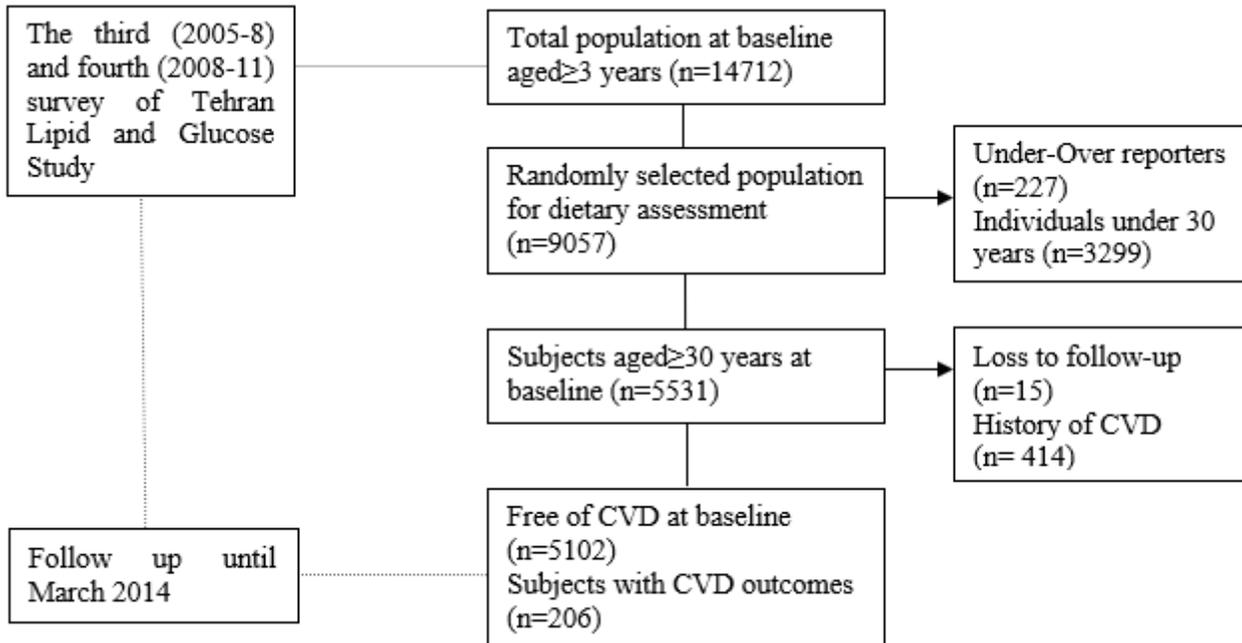


Figure 1

Study flowchart

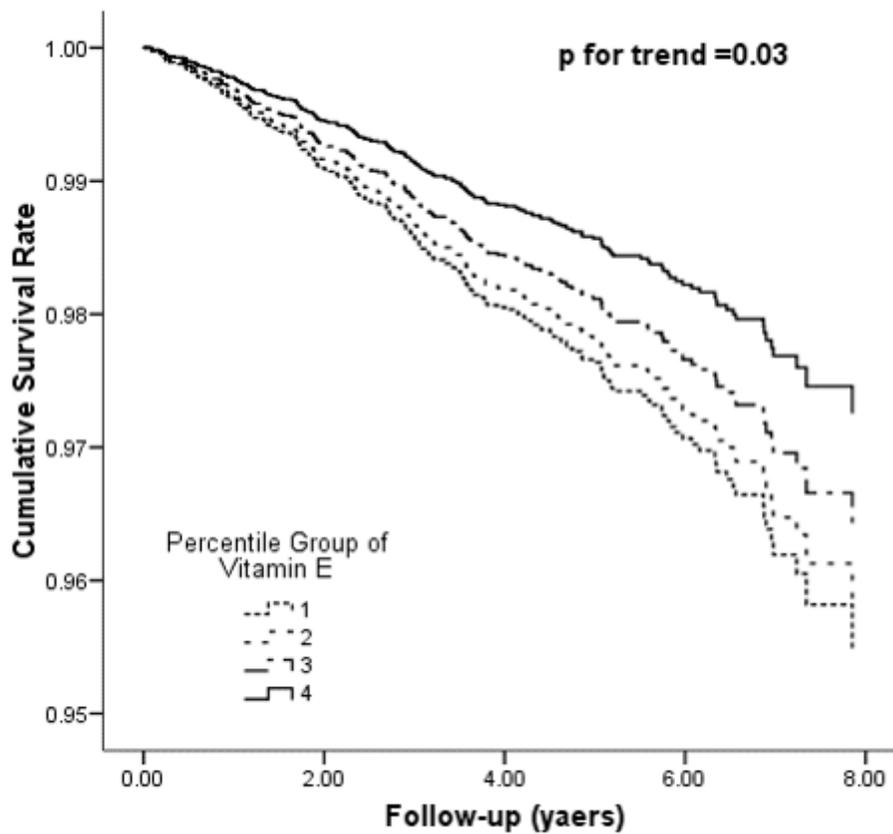
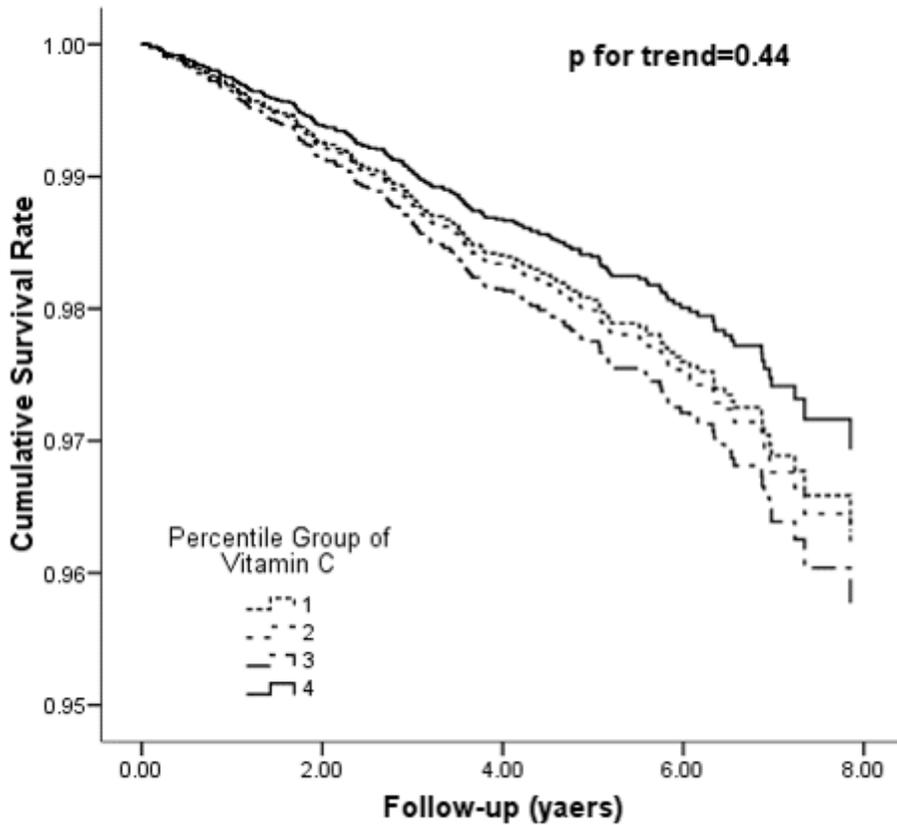


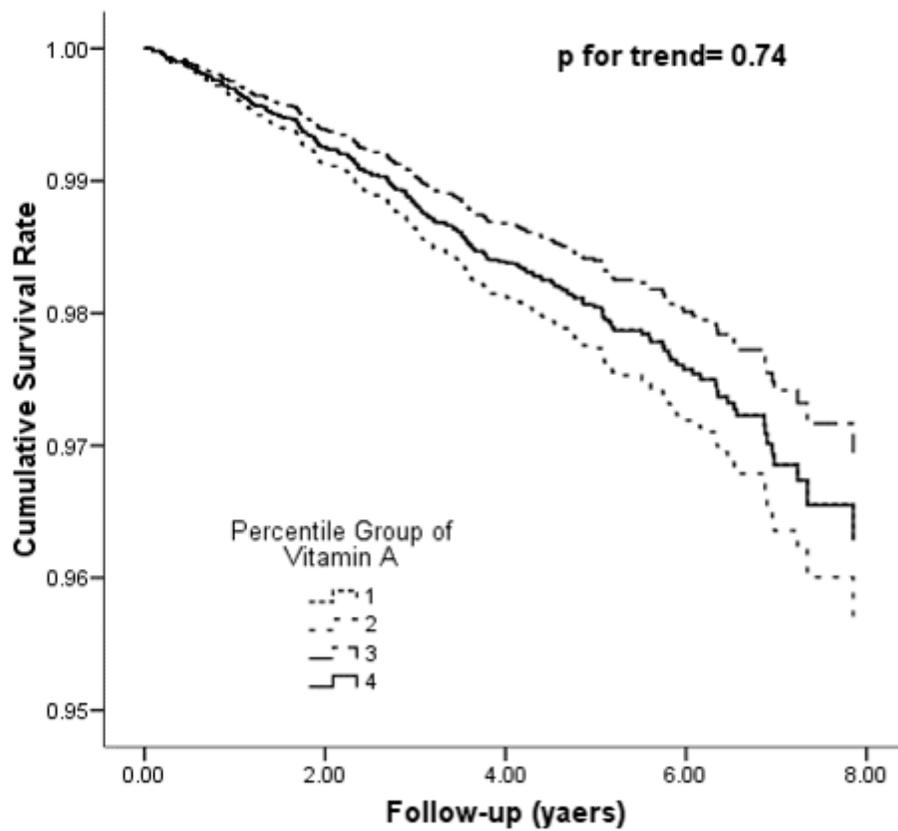
Figure 2

Multivariable-adjusted cumulative survival curves for incidence of CVD according to vitamin E categories. Multivariable-adjusted model included age, sex, CVD risk score, family history of CVD, physical activity, total energy intake, fiber and total fat intakes. Associations between risk of CVD and quartiles of vitamin E intake were significant.



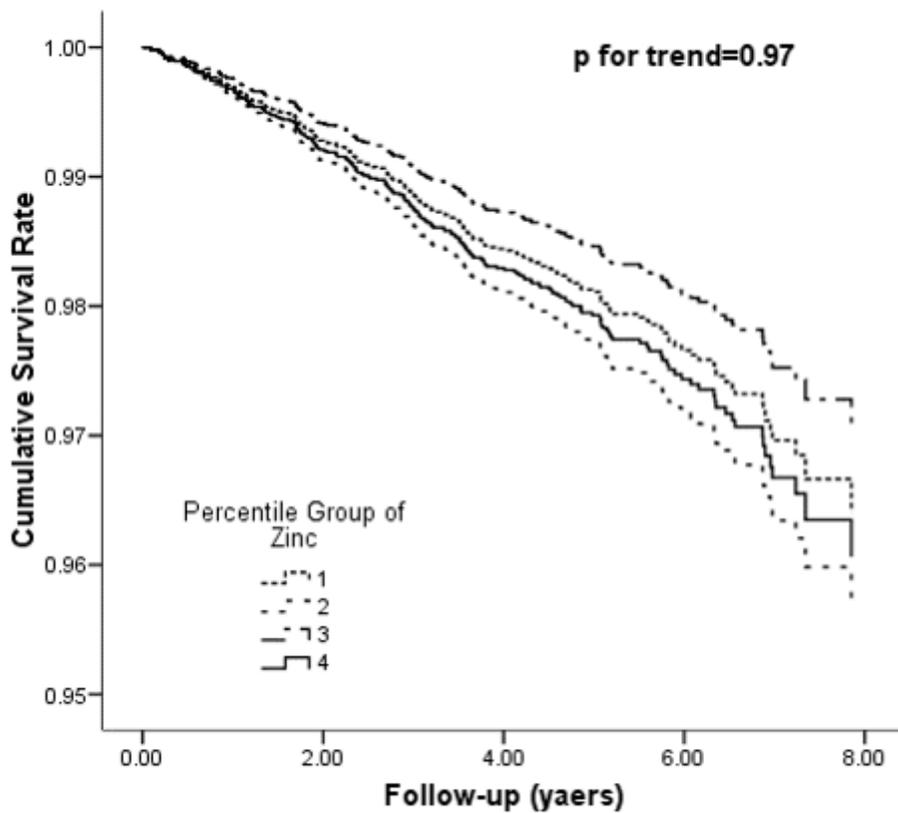
**Figure 3**

Multivariable-adjusted cumulative survival curves for incidence of CVD according to vitamin C categories. Multivariable-adjusted model included age, sex, CVD risk score, family history of CVD, physical activity, total energy intake, fiber and total fat intakes. Associations between risk of CVD and quartiles of vitamin C intake were not significant.



**Figure 4**

Multivariable-adjusted cumulative survival curves for CVD of diabetes according to vitamin A categories. Multivariable-adjusted model included age, sex, CVD risk score, family history of CVD, physical activity, total energy intake, fiber and total fat intakes. Associations between risk of CVD and quartiles of vitamin A intake were not significant.



**Figure 5**

Multivariable-adjusted cumulative survival curves for CVD of diabetes according to zinc categories. Multivariable-adjusted model included age, sex, CVD risk score, family history of CVD, physical activity, total energy intake, fiber and total fat intakes. Associations between risk of CVD and quartiles of zinc intake were not significant.