

# **Dietary and other lifestyle correlates of serum folate concentrations in a healthy adult population in Crete, Greece: a cross-sectional study**

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# **Abstract**

## **Background**

Folate has emerged as a key nutrient to optimising health, and impaired folate status has been identified as risk factor for cardiovascular disease, various types of cancers, and neurocognitive disorders. The study aimed at examining the distribution and determinants of serum folate concentrations in a healthy adult population in Crete, Greece.

## **Methods**

A cross-sectional sample of 486 healthy adults (250 men, 236 women) aged  $39 \pm 14$  years, personnel of the University/University Hospital of Crete in Greece, was examined as part of a health prevention programme. Serum folate and vitamin B<sub>12</sub> concentrations were measured by a microbiological assay, and total homocysteine was determined fluorometrically and by high-pressure liquid chromatography. Lifestyle questionnaires were completed and nutrient intakes and food consumption was assessed by 24-h dietary recalls. Multivariate analyses were performed using SPSS v10.1.

## **Results**

The geometric mean (95% confidence interval [CI]) concentrations of serum folate were 15.6  $\mu\text{mol/l}$  (14.6-16.8) in men and 19.2  $\mu\text{mol/l}$  (17.9-20.7) in women ( $p < 0.001$ ). Inadequate folate levels ( $\leq 7 \text{ nmol/l}$ ) were present in 6.8% of men and 2.1% of women ( $p < 0.001$ ). Approximately 76% of men and 87% of women did not meet the reference dietary intake for folate (400  $\mu\text{g/day}$ ). Serum folate was inversely related to total homocysteine levels ( $p < 0.001$ ), and increased tobacco and coffee consumption

were associated with lower folate concentrations ( $p < 0.05$  for both). Dietary intakes of MUFA, fibre, calcium, potassium, folate, and vitamins A, E, C, B<sub>1</sub>, and B<sub>6</sub> significantly increased by increasing quartile of serum folate levels. Consumption of potatoes, legumes, fruits, and vegetables were favourably related to the serum folate status.

## **Conclusions**

Serum folate concentrations were associated with various demographic, lifestyle and dietary factors in healthy Cretan adults. Large-scale epidemiological studies should be conducted within the general Greek adult population to assess the prevalence of impaired folate status and further examine associations with dietary patterns and chronic disease risk. Considering the importance of folate in health maintenance, it is also important to increase the public's awareness on modifiable lifestyle patterns and especially diet and tobacco use, associated with improved folate status.

## Background

Dietary habits in a population may be a determining factor of health, and inadequate intakes of certain nutrients have been associated with increased risk for chronic disease, such as cardiovascular disease, diabetes, and cancer. More recently, folate has drawn attention as a key nutrient involved in health maintenance and chronic disease prevention [1]. Folate acts as a coenzyme in several single-carbon transfers leading to the biosynthesis of nucleic acids, certain neurotransmitters, phospholipids, and hormones. It is involved in homocysteine metabolism, donating a methyl group during homocysteine remethylation into methionine, and inadequate folate status has been characterized as a major cause of hyperhomocysteinaemia [2]. Moreover, folate deficiency is an established risk factor for the development of certain types of cancer, cardiovascular disease [3, 4], developmental defects (e.g. neural tube defects), and neurological or psychiatric disorders [5-7]. Although much of the adverse effects of impaired folate status is explained through its interplay with homocysteine levels, there is evidence suggesting direct health-beneficial effects of folate, including its favourable effects on vascular endothelial function and redox cellular status [1].

From a public health perspective, assessing the folate status in a population and examining its dietary or other correlates, is of great importance. This would allow the identification of groups at risk for folate deficiency that would mostly benefit from public health interventions to increase dietary folate intake.

Crete (Greece), once known for the low cardiovascular mortality among adult men in early 1950's, has now increasing rates of cardiovascular disease, a trend that appears to be related to dietary and lifestyle changes that have been taking place during the last decades [8, 9]. The traditional Cretan diet – a variant of the Mediterranean diet – has been gradually abandoned, and current Cretans consume higher amounts of saturated fat, meat, and cheese, and lower amounts of bread, fruits, vegetables, legumes, and fibre [9-11]. Among other consequences, such dietary changes are expected to result in decreased intake of dietary folate

and thus, impaired folate status. To date, however, no data are available with regard to the folate status of the adult population in Crete.

In this study, we report on the folate status of a group of apparently healthy Cretan adults, personnel of the University of Crete and the University Hospital of Heraklion (Crete), which were examined as part of a health prevention programme by the Preventive Medicine and Nutrition Clinic. We describe the distribution of serum folate concentrations by age and sex, and investigate associations with nutrient intakes, food consumption, and lifestyle habits, namely tobacco, coffee, and alcohol consumption. Our findings document certain patterns of dietary intake and lifestyle habits associated with folate levels, with potential implications for the development of future health intervention studies.

## **Methods**

### **Study sample**

The study was conducted as part of a health prevention programme undertaken by the Preventive Medicine and Nutrition Clinic at the University of Crete School of Medicine. The personnel of University Hospital of Heraklion (Crete, Greece) and the University of Crete School of Medicine were asked to participate in the health assessment survey by written announcements. Subjects were excluded if they had history of any chronic disease such as cancer, diabetes mellitus, hypertension, liver or coronary heart disease. The study sample consisted of 381 (out of 2445) employees (184 men, 197 women, aged 20-72 years) and 105 (out of 155) (66 men, 39 women, aged 20-40 years) practicing medical students.

### **Laboratory measurements**

Early morning venous blood samples were drawn for biochemical screening tests, following a 12-hour overnight fast. The blood samples (10 ml) were transferred to the Nutritional Research Laboratory of the University of Crete in tanks containing ice packs so as to maintain

a temperature of 3-4°C. Blood was centrifuged and 1.5 ml aliquots were pipetted into plastic Eppendorf tubes. One aliquot was used for blood analysis of triacylglycerol (TG), total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) measurements [12] on the same day of collection, while the other was stored (at -80°C) for determination of serum folate, vitamin B<sub>12</sub>, and total homocysteine. Serum samples were sent in dry ice to Trinity College of Dublin (Ireland), and serum folate and vitamin B<sub>12</sub> concentrations were measured by a 96-well plate microbiological assay [13, 14]. Serum total homocysteine concentrations were determined fluorometrically and by high-pressure liquid chromatography (HPLC) [15].

### **Health assessment questionnaires**

Purpose designed questionnaires were administered to ascertain biographical data, lifestyle behaviours on topics including cigarette smoking and medical history [12]. To validate the results, multiple crosschecked questions on the same topic were addressed to the participants. Smokers were classified as those who stated smoking more than one cigarette per day for at least three consecutive months. Ex-smokers were defined as those who had not been smoking for the last six consecutive months, and non-smokers as those who did not fall in any of the two previous groups.

### **Dietary survey**

Dieticians administered a 24h dietary recall to all participants. Detailed descriptions of all foods, beverages and supplements consumed during the 24h period before the interview, including the quantity, cooking method and brand names were recorded. Dietary records were collected from 383 participants (197 men, 186 women). Food quantities were assessed by the use of household measures and colour food-model photographs. The 24h dietary recall has been previously validated in relation to fat intake based on measurements with adipose tissue aspiration and fatty acid composition analysis [8]. Nutrient contents were analysed according to the food database developed at the Department of Social Medicine of the University of Crete in 1998 [11]. The database includes about 500 foods, both single and composite. The

macro- and micro-nutrient composition of about 20 foods has been chemically determined at Wageningen Agricultural University. The fatty acid content of 105 fat-containing foods was determined at the TNO Nutrition and Food Research Institute, The Netherlands, during 1997. For the remainder of fat-containing foods, the fatty acid analyses were drawn from the analyses available within the European “trans fatty acid research project” database (TTDB, version 1.2) developed at the TNO Nutrition and Food Research Institute between 1995 and 1997. For the foods whose composition was not chemically determined, values from the US Department of Agriculture database v11.1 were used. Recipe calculations were used for composite Greek foods with the ingredients being weighed prior to and also following cooking in each case.

### **Statistical analyses**

The distribution of serum folate was markedly skewed toward high values and was corrected by log transformation. The geometric means and their 95% confidence intervals (CI) are presented. Variables not normally distributed were also log-transformed. We tested for statistically significant differences between men and women using one-way analysis of variance (ANOVA). The chi-squared ( $\chi^2$ ) test was used for categorical variables. Serum folate concentrations were considered sub-optimal when  $\leq 7$  nmol/l [16]. To estimate mean total homocysteine and vitamin B<sub>12</sub> concentrations across levels of serum folate, age and sex-specific quartiles were calculated. We determined the age and gender-adjusted geometric mean folate concentrations within categories of tobacco and alcohol consumption with analysis of covariance (ANCOVA). Linear contrasts were applied to test for trends across groups with  $\geq 3$  groups.

Nutrient intakes were calculated across age and sex-specific quartiles of serum folate, after adjusting for tobacco use, body mass index, and energy intake. Pearson’s partial correlation coefficients ( $r$ ) were used to evaluate linear associations between nutrient intakes and serum folate after controlling for the aforementioned covariates. Finally, individuals were categorized according to levels of consumption of various foods (none, below or above

median consumption) and levels of serum folate (below or above 1<sup>st</sup> quartile). The *Mantel-Haenszel* chi-squared test was then used to assess linear associations between food consumption and serum folate. All the *p* values reported are two-tailed and statistical significance was defined as *p* < 0.05. Statistical analyses were performed using the Statistical Package for Social Sciences (SPSS for Windows 11.5.0, SPSS, Inc.).

## Results

The mean ± standard deviation (SD) age was 39 ± 14 years for men and 39 ± 13 years for women (**Table 1**). Approximately 86% of the subjects lived in urban areas and current smokers were the 38% of men and 31% of women (*p* > 0.05).

The distribution of serum folate concentrations and dietary folate intakes is presented for all subjects by gender and age in **Table 2**. The geometric mean of serum folate was 15.6 nmol/l (95% CI, 14.6-16.8 nmol/l) in men and 19.2 nmol (17.9-20.7 nmol/l) in women (*p* < 0.001). In both men and women age was positively associated with serum folate. The prevalence of sub-optimal serum folate (≤7 nmol/l) was 6.8% in men and 2.1% in women (*p* < 0.001). Men had higher mean intake of dietary folate than women (294 vs. 247 µg/d, *p* = 0.003). However, the energy-adjusted intake of folate was higher in women than men (161 vs. 144 µg/1000 kcal/d, *p* > 0.05). Eighty-seven percent of women and 76% of men (*p* = 0.013) consumed folate <400 µg/day. Dietary intake of folate increased by age, especially in men (*p* for trend < 0.01).

Associations between age and sex-specific quartiles of serum folate and mean levels of total homocysteine and vitamin B<sub>12</sub> are presented in **Table 3**. Serum folate showed an inverse linear association with total homocysteine (*p* for trend < 0.001), and subjects at the highest folate quartile had 18.5% lower mean total homocysteine concentrations compared to those at the lowest quartile. No significant association was observed between serum folate and vitamin B<sub>12</sub> levels.

The relations between serum folate and the use of tobacco, coffee, and alcohol are shown in **Table 4**. The age- and gender-adjusted geometric mean serum folate was  $\approx 2$  nmol/l lower in current smokers than in non- or ex-smokers ( $p < 0.05$ ). Similarly, serum folate levels were inversely related to the quantity of tobacco ( $p$  for trend  $< 0.05$ ), and individuals who smoked  $\geq 20$  cigs/day had 21.2% lower mean folate concentrations compared to those who smoked  $< 10$  cigs/day. However, these associations disappeared following adjustment for intake of dietary folate. Increasing coffee intake was also related to decreased serum folate ( $p$  for trend  $< 0.05$ ), and subjects consuming  $> 200$  g/day had 14.8% lower mean serum folate levels compared to non-consumers. Similarly, this association disappeared following adjustment for intake of dietary folate and vitamin C. No significant relationship was observed between alcohol intake and serum folate.

The associations between nutrient intakes and serum folate are shown in **Table 5**. Intake of mono-unsaturated fatty acids (MUFA) – both as raw quantity and as percentage of energy intake – was positively associated with increasing quartile of serum folate. Intakes of dietary fibre ( $r = 0.19$ ), calcium ( $r = 0.14$ ), and potassium ( $r = 0.10$ ) were also positively related to serum folate after controlling for possible effects of age, gender, smoking status, and energy intake. Serum folate concentrations demonstrated favourable associations with dietary intakes of several vitamins, namely folate ( $r = 0.13$ ), vitamins A ( $r = 0.23$ ), E ( $r = 0.14$ ), C ( $r = 0.21$ ), B<sub>1</sub> ( $r = 0.13$ ), and B<sub>6</sub> ( $r = 0.13$ ).

We further assessed food consumption by the study participants according to their serum folate status (**Table 6**). Increased consumption of potatoes, legumes, and vegetables were associated with serum folate concentrations  $\geq 1^{\text{st}}$  quartile (age and gender-specific). Fifty-three percent of subjects with serum folate  $\geq 1^{\text{st}}$  quartile consumed fruits and/or vegetables  $\geq 360$  g/day, compared to 34% of those with serum folate  $< 1^{\text{st}}$  quartile ( $p < 0.001$ ). These associations were most apparent within the sub-sample of current smokers, wherein intake of fruits was also favourably related to serum folate ( $p = 0.009$ ). On the other hand, increased intake of cereals and meat (especially red meat) was inversely related to serum folate status.

## Discussion

Folate has recently emerged as a key nutrient to optimising health, and inadequate folate status has been identified as risk factor for cardiovascular disease, various types of cancers, and neurocognitive disorders [1, 17]. The present study described the distribution of serum folate concentrations in a healthy adult population in Crete, Greece and examined associations with dietary intakes and lifestyle habits, namely tobacco, coffee, and alcohol consumption. The relationship between serum folate and vitamin B<sub>12</sub> and total homocysteine was also examined.

The results of the study should be viewed in light of some limitations. The present data used a cross-sectional design that implies that no conclusions can be drawn on the causal effect of the determinants of serum folate levels. The dietary survey was performed using one single 24-h dietary recall and may thus be subject to both systematic and random bias, one source being the lack of inclusion of weekends. The regular use of vitamin supplements was not recorded and this precluded further analyses of the serum folate status. Also, the participation rate was relatively low (<20%) and selection bias cannot be excluded. Lastly, the unique sample of Hospital/Medical School personnel is both strength (as it decreases potential for confounding) and limitation (as the sample is not representative of the general Greek adult population) of the survey.

Despite these limitations, there are several findings in this study that are worth to be discussed. In this sample of apparently healthy Cretan adults, sub-optimal folate levels were found in 6.8% of men and 2.1% of women, using serum concentrations  $\leq 7$  nmol/l as a cut-off value [16]. Depending on the criteria used, the proportion of subjects with impaired folate status in other studies ranges 0-79% [18-21]. Differences in serum folate concentrations and cut-off values may be due to analytical methods, race-ethnicity differences and genetic backgrounds [1, 22]. The mean daily intake of folate by the study subjects (294  $\mu\text{g}/\text{d}$  in men, 247  $\mu\text{g}/\text{d}$  in women) also stands between lower figures that have been reported by Plannells *et al.* [19] for Spanish adults (205  $\mu\text{g}/\text{d}$  in men, 197  $\mu\text{g}/\text{d}$  in women) and higher ones that Ford *et*

*al.* [21] and Rasmussen *et al.* [23] have described for US men (343 µg/d) and Dutch women (283 µg/d in the 20-35-year-old group), respectively. Notably, more than 75% of the study participants did not meet the RDI for folate (400 µg/day). Similarly to the results of other studies [19, 20], men had higher mean intakes of dietary folate than women, but when expressed by energy intake, women had higher intake of folate than men.. This might – at least in part – explain the higher serum folate concentrations in women, although other factors (e.g. hormonal, bioavailability of dietary folate etc.) may be responsible for this discrepancy.

In both men and women, subject age was positively related to serum folate levels. One possible explanation for this association is the higher intake of folate and folate-rich foods (especially fruits and vegetables) with increasing age among adults (data not shown). A similar trend has been reported in other studies and is generally attributed to the preference for more “healthy” or “traditional” diets among older adults [19, 20, 24]. With regard to the adult population in Crete, recent data indicate the gradual abandonment of the traditional Cretan diet in favour of more “westernised” diets, with consumption of higher amounts of saturated fat, meat, and cheese, and lower amounts of fruits, vegetables, legumes, and fibre [9-11].

In agreement with several other studies [25-29], serum folate was inversely related to total homocysteine concentrations. Hyperhomocysteinaemia is a well-established risk factor for atherothrombotic disease, Alzheimer’s disease and other neuropsychiatric disorders, osteoporosis and hip fractures [30, 31]. This overlap of medical disorders that are linked both to increased homocysteine and inadequate folate status has led to the suggestion that the disease-promoting effects of low folate are mediated through increased homocysteine levels [1, 4, 7, 29, 32]. Yet, recent data indicate that folate may act independently of homocysteine, especially through its direct effects on vascular endothelium and cellular redox status [1].

Another finding was the inverse relationship between tobacco consumption and serum folate concentrations. Smokers had significantly lower serum folate levels than non-smokers, and serum folate concentrations decreased by increasing consumption of cigarettes. A negative association between smoking and serum folate has been reported in other studies

[19, 25, 26, 29, 33], and is generally attributed to the different nutritional status of smokers. More specifically, smokers tend to consume lower amounts of several vitamins, fruits and vegetables, resulting in decreased intake of dietary folate [12, 34-36]. Similarly, in our study, the association between smoking and serum folate abolished its statistical significance after controlling for dietary folate intake. It should be noted, however, that smoking may have more direct anti-folate effects, producing local vitamin deficiency in individual tissues as demonstrated by Piyathilake *et al.* [37].

In the present study, serum folate concentrations were inversely related to coffee consumption. Such a relationship has only been shown indirectly, in studies examining serum total homocysteine levels in association with coffee intake. In some of these studies, the positive association between coffee consumption and homocysteine was lost after accounting for serum folate and/or dietary folate intake, indicating a negative association between coffee intake and folate status [26-29]. As in the case of tobacco consumption, the association between serum folate and coffee was confounded by differences in dietary intake of certain nutrients, namely folate and vitamin C.

The results from the dietary survey demonstrated significant associations between serum folate and intakes of several important nutrients, including MUFA, fibre, calcium, potassium, folate, and vitamins A, E, C, B<sub>1</sub>, and B<sub>6</sub>. A relationship between dietary folate intake and serum folate concentrations has been reported in some [18, 19, 23] but not all [20] studies. The favourable associations between serum folate and dietary micronutrients was reflected by differences in consumption of various foods. Individuals with higher serum folate levels, had higher intakes of potatoes, fruits, vegetables, and legumes, which are all considered major sources of folate [19, 20]. Conversely, higher intakes of cereals and meat products was related to decreased serum folate concentrations (below the 1<sup>st</sup> quartile). These findings are in accordance with those reported by both cross-sectional [38-42] and diet-intervention studies [43, 44], which suggest a positive association between folate status and a dietary pattern characterized by high consumption of fruits, vegetables, legumes and low consumption of refined cereals and meat. It should be noted however, that the lower intakes of cereals and

meat among the study subjects with lower serum folate levels might be simply due to substitution by other foods, namely fruits, vegetables, and legumes. Finally, an alternative explanation for the associations between serum folate levels and nutrients and foods, might be that serum folate serves as an overall nutritional biomarker and indicator of diet and health quality [45, 46].

## **Conclusions**

Conclusively, in this cross-sectional study of healthy Cretan adults we found various demographic, lifestyle and dietary factors associated with serum folate concentrations. Considering the importance of folate in health maintenance and prevention of chronic disease, it is important to increase the public's awareness on modifiable lifestyle habits and especially diet, associated with improved folate status. In view of the high percentage of adults with inadequate intake of dietary folate, our results also emphasize the need to undertake large-scale epidemiological studies within the general Greek adult population, in order to assess the prevalence of impaired folate status, and further examine associations between dietary patterns, serum folate, and chronic disease risk.

## **Competing interests**

None.

## **Authors' contributions**

CH performed blood sampling, collected data, and reviewed the manuscript. GB collected data and drafted the manuscript. ML collected data and carried out the statistical analysis. In JS' laboratory biochemical measurement of serum folate, vitamin B<sub>12</sub> and homocysteine was

performed. JS also reviewed the manuscript. AK conceived of the study, participated in its design, and helped to draft the manuscript. All authors read and approved the final manuscript.

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## Tables

**Table 1 - Demographics and other characteristics of the study sample**

	Men (N=250)	Women (N=236)	
	Mean (SD) or %		p value <sup>1</sup>
Age (years)	39 (14)	39 (13)	— <sup>2</sup>
District of residence (% urban)	86.4	85.6	—
Tobacco use (% current use)	37.7	31.0	—
BMI (kg/m <sup>2</sup> )	26.8 (3.5)	26.1 (5.8)	<0.001
Systolic blood pressure (mmHg)	129.4 (16.7)	124.2 (20.3)	<0.001
Diastolic blood pressure (mmHg)	84.3 (10.0)	79.2 (10.9)	<0.001
Total cholesterol (mg/dL)	224.0 (50.3)	221.7 (54.0)	—
Triglycerides (mg/dL)	128.2 (83.3)	99.9 (56.9)	<0.001
HDL-cholesterol (mg/dL)	45.3 (9.8)	56.5 (12.2)	<0.001
Energy intake (kCal/day) <sup>3</sup>	2133 (793)	1605 (629)	<0.001
Total fat (% energy)	39.7 (9.8)	40.2 (11.1)	—
SFA (% energy)	11.5 (5.0)	11.1 (4.9)	—
Dietary cholesterol (g/day)	213.7 (157.4)	153.6 (130.9)	<0.001
Dietary fibre (g/day)	22.1 (11.3)	18.7 (11.0)	0.001

<sup>1</sup> Analysis of variance (ANOVA) or chi-squared test.

<sup>2</sup> Not statistically significant.

<sup>3</sup> N=197 for men and N=186 for women.

**Table 2 - Serum folate concentrations and dietary folate intake in healthy**

**Cretan adults <sup>1</sup>**

		Serum folate (nmol/l) <sup>2</sup>	% ≤7 nmol/l		Folate intake		% <400 µg/d
					(µg/d) <sup>3</sup>	(µg/1000kcal/d)	
Men							
20-34y	(n=107)	13.8 (12.5-15.3)	7.5	(n=103)	248 (213-283)	119 (106-133)	83.5
35-50y	(n=80)	16.0 (14.2-18.0)	6.3	(n=72)	351 (297-406)	174 (149-198)	69.4
>50y	(n=63)	18.6 (15.8-21.9) <sup>4</sup>	6.3	(n=20)	325 (242-407) <sup>5</sup>	166 (130-201) <sup>6</sup>	65.0
Total	(n=250)	15.6 (14.6-16.8)	6.8	(n=195)	294 (265-323)	144 (132-157)	76.4
Women							
20-34y	(n=101)	17.4 (15.8-19.2)	3.0	(n=96)	247 (207-288)	159 (134-184)	86.5
35-50y	(n=79)	19.4 (17.2-22.0)	1.3	(n=72)	242 (198-287)	155 (133-177)	88.9
>50y	(n=56)	22.8 (19.3-26.9) <sup>7</sup>	1.8	(n=17)	266 (160-372)	192 (129-255)	76.5
Total	(n=236)	19.2 (17.9-20.7)	2.1	(n=185)	247 (219-275)	161 (144-177)	86.5

<sup>1</sup> Data are presented as geometric mean (95% confidence interval).

<sup>2</sup> Men had significantly lower serum folate concentrations than women ( $p < 0.001$ ).

<sup>3</sup> Men had significantly higher intake of dietary folate (µg/d) than women ( $p = 0.003$ ).

<sup>4</sup>  $p = 0.001$  (trend by age).

<sup>5</sup>  $p = 0.003$  (trend by age).

<sup>6</sup>  $p < 0.001$  (trend by age).

<sup>7</sup>  $p = 0.008$  (trend by age).

**Table 3 - Association between serum total homocysteine and vitamin B<sub>12</sub> concentrations and quartiles (age and sex-specific) of serum folate in healthy Cretan adults**

	Serum folate quartiles (age & sex-specific)				
	Q1	Q2	Q3	Q4	
	Geometric mean				<i>P</i> for trend <sup>1</sup>
Serum total homocysteine (μmol/L)	12.4	10.3	10.2	10.1	<0.001
Serum vitamin B <sub>12</sub> (pmol/L)	307.5	305.2	310.4	286.1	— <sup>2</sup>

<sup>1</sup> ANOVA (linear contrasts).

<sup>2</sup> Not statistically significant.

**Table 4 - Association between serum folate concentrations and consumption of tobacco, coffee, and alcohol in healthy Cretan adults**

	Geometric mean (SEM <sup>1</sup> )	95% confidence interval
Tobacco consumption		
Non/ex-smokers (n=314) <sup>2</sup>	18.0 (1.0)	16.9-19.1
Current smokers (n=165)	16.0 (1.0)	14.7-17.4
<10 cigs/day (n=52) <sup>3</sup>	17.7 (1.1)	15.3-20.5
11-19 cigs/day (n=36)	15.0 (1.1)	12.6-17.9
≥20 cigs/day (n=76)	14.6 (1.1)	12.9-16.5
Coffee consumption <sup>4</sup>		
None (n=102)	18.2 (1.1)	16.4-20.2
≤200 g/day (n=195)	16.9 (1.0)	15.7-18.2
>200 g/day (n=86)	15.5 (1.1)	13.8-17.3
Alcohol consumption		
None (n=293)	17.0 (1.0)	16.0-18.1
≤180 g/day (n=43)	16.0 (1.1)	13.6-18.9
>180 g/day (n=47)	16.8 (1.1)	14.4-19.7

<sup>1</sup> Standard error of the mean.

<sup>2</sup> Non/ex-smokers had significantly higher serum folate concentrations than current smokers ( $p=0.029$ , ANCOVA controlling for age and gender)

<sup>3</sup>  $p=0.047$ , linear trend by level of tobacco consumption (ANCOVA controlling for age and gender)

<sup>4</sup>  $p=0.035$ , linear trend by level of coffee consumption (ANCOVA controlling for age and gender)

**Table 5 - Mean nutrient intakes in relation to serum folate quartiles (age and sex-specific) in healthy Cretan adults**

	Serum folate quartiles (age & sex-specific)				$r^1$
	Q1	Q2	Q3	Q4	
Energy (kcal)	1699.7	1768.8	1594.6	1646.7	-0.03
Carbohydrates (g)	179.1	184.7	192.7	185.2	0.03
% energy	44.5	45.3	47.7	45.4	0.00
Protein (g)	67.8	60.0	62.4	61.3	-0.05
% energy	14.5	13.3	13.2	14.0	-0.06
Total fat (g)	72.6	72.5	70.8	72.3	0.01
% energy	41.1	40.9	39.5	40.2	0.00
SFA (g)	19.3	19.6	19.4	18.3	-0.04
% energy	11.7	11.6	11.7	11.0	-0.05
PUFA (g)	8.8	9.2	8.8	8.7	0.01
% energy	5.4	5.5	5.3	5.2	0.00
MUFA (g)	30.1	33.0	31.6	33.7	0.11 <sup>2</sup>
% energy	18.4	19.6	18.9	20.6	0.13 <sup>2</sup>
Trans FA (g)	1.4	1.3	1.5	1.1	-0.07
n-6 FA (g)	8.8	8.0	8.3	7.4	-0.04
n-3 FA (g)	0.9	0.7	0.8	0.6	-0.04
Cholesterol (mg)	192.4	170.2	206.1	166.3	-0.04
Fibre (mg)	13.5	16.3	18.1	18.0	0.19 <sup>3</sup>
Calcium (mg)	549.5	622.9	648.3	697.1	0.14 <sup>3</sup>
Iron (mg)	10.6	10.5	11.6	11.2	0.08
Magnesium (mg)	233.7	243.5	238.8	258.7	0.08
Phosphorous (mg)	928.6	946.2	917.2	946.0	-0.02
Sodium (mg)	1506.9	1583.2	1677.5	1492.1	0.00
Potassium (mg)	2289.0	2190.7	2184.8	2565.3	0.10 <sup>2</sup>
Folate ( $\mu\text{g}$ )	187.8	211.1	220.9	232.5	0.13 <sup>2</sup>
Niacin (mg)	13.3	13.1	14.0	12.7	-0.01
Vitamin A ( $\mu\text{g}$ )	538.1	546.6	768.3	1010.7	0.23 <sup>4</sup>
Vitamin E (mg)	6.1	7.0	7.0	7.6	0.14 <sup>3</sup>
Vitamin C (mg)	75.5	80.9	93.4	133.4	0.21 <sup>4</sup>
Vitamin B <sub>1</sub> (mg)	1.2	1.3	1.4	1.4	0.13 <sup>2</sup>
Vitamin B <sub>2</sub> (mg)	1.2	1.3	1.4	1.4	0.06
Vitamin B <sub>6</sub> (mg)	1.3	1.3	1.3	1.5	0.13 <sup>2</sup>
Vitamin B <sub>12</sub> ( $\mu\text{g}$ )	4.0	3.1	4.0	3.0	-0.03

<sup>1</sup> Coefficient for the correlation between nutrient intakes and serum folate concentrations (all log transformed) controlling for age, gender, body mass index, tobacco use, and energy intake.

<sup>2</sup>  $p < 0.05$ .

<sup>3</sup>  $p < 0.01$ .

<sup>4</sup>  $p < 0.001$ .

**Table 6 - Association between foods consumption and serum folate status in healthy Cretan adults**

	Total sample (n=383)			Non/ex-smokers (n=242)			Current smokers (n=137)		
	<P25 <sup>1</sup>	≥P25	<i>p</i> <sup>2</sup>	<P25	≥P25	<i>p</i>	<P25	≥P25	<i>p</i>
Serum folate concentration									
Bread									
None	11.8%	10.3%		13.1%	11.6%		11.6%	7.4%	
<90g	44.1%	43.4%		45.9%	42.0%		44.2%	45.7%	
≥90g	44.1%	46.3%	0.451	41.0%	46.4%	0.491	44.2%	46.8%	0.565
Cereals									
None	37.3%	45.5%		37.7%	45.3%		32.6%	47.9%	
<90g	25.4%	26.7%		29.5%	25.4%		25.6%	26.6%	
≥90g	37.3%	27.8%	0.045	32.8%	29.3%	0.377	41.9%	25.5%	0.044
Potatoes									
None	76.5%	66.2%		80.3%	69.6%		76.7%	56.4%	
<122g	13.7%	14.9%		11.5%	12.7%		14.0%	20.2%	
≥122g	9.8%	18.9%	0.008	8.2%	17.7%	0.067	9.3%	23.4%	0.018
Legumes									
None	85.3%	77.1%		83.6%	80.1%		90.7%	71.3%	
<300g	6.9%	7.8%		8.2%	5.5%		2.3%	12.8%	
≥300g	7.8%	14.9%	0.042	8.2%	14.4%	0.344	7.0%	16.0%	0.029
Vegetables									
None	22.6%	12.1%		13.1%	12.2%		30.2%	11.7%	
<190g	43.1%	41.6%		44.3%	38.7%		46.5%	46.8%	
≥190g	34.3%	46.3%	0.006	42.6%	49.2%	0.463	23.3%	41.5%	0.005
Fruits									
None	43.1%	33.5%		36.1%	28.7%		60.5%	39.4%	
<261g	30.4%	33.5%		27.9%	35.9%		27.9%	30.9%	
≥261g	26.5%	33.0%	0.425	36.1%	35.4%	0.582	11.6%	29.8%	0.009
Fruits and/or vegetables									
None	10.8%	2.8%		4.9%	2.8%		18.6%	2.1%	
<360g	54.9%	44.1%		47.5%	40.9%		60.5%	53.2%	
≥360g	34.3%	53.1%	<0.001	47.5%	56.4%	0.188	20.9%	44.7%	<0.001
Meat									
None	43.1%	59.4%		44.3%	65.2%		39.5%	50.0%	
<100g	22.5%	22.1%		26.2%	19.9%		18.6%	25.5%	
≥100g	34.3%	18.5%	0.002	29.5%	14.9%	0.022	41.9%	24.5%	0.079
Red meat									
None	50.0%	68.7%		49.2%	74.0%		46.5%	61.7%	
<90g	18.6%	16.0%		24.6%	13.8%		18.6%	16.0%	
≥90g	31.4%	15.3%	<0.001	26.2%	12.2%	<0.001	34.9%	22.3%	0.081
Eggs									
None	84.3%	82.2%		88.5%	81.2%		74.4%	86.2%	
<50g	7.8%	7.5%		8.2%	7.2%		9.3%	7.4%	
≥50g	7.8%	10.3%	0.153	3.3%	11.6%	0.089	16.3%	6.4%	0.060
Dairies									
None	17.6%	24.9%		21.3%	27.1%		11.6%	21.3%	
<130g	45.1%	34.2%		44.3%	29.3%		41.9%	44.7%	
≥130g	37.3%	40.9%	0.864	34.4%	43.6%	0.771	46.5%	34.0%	0.098
Fish-sea food									
None	75.5%	65.8%		70.5%	65.7%		81.4%	64.9%	
<85g	9.8%	15.7%		6.6%	15.5%		9.3%	19.1%	
≥85g	14.7%	18.5%	0.226	23.0%	18.8%	0.961	9.3%	16.0%	0.083

<sup>1</sup> <25<sup>th</sup> percentile (1<sup>st</sup> quartile, age & sex-specific) of serum folate concentrations.

<sup>2</sup> The *Mantel-Haenszel* chi-squared test for linear associations.