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ORIGINAL ARTICLE

Nutritional determinants of plasma total homocysteine distribution in the Canary Islands

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Objective: We sought to define plasma homocysteine reference values in healthy individuals in the Canary Islands and to determine its relations to folate and vitamin B12 intakes and concentrations.
Design: Cross-sectional study.
Setting: Population-based representative sample of 557 participants, aged 18–65 years, from the Canary Islands Nutrition Survey (ENCA).
Subjects: All participants completed two 24-h dietary recalls and a general questionnaire collecting socio-demographic and health-related lifestyle information.
Interventions: Plasma homocysteine and serum vitamin B12 levels were measured by immunoassay, whereas folate levels through an automated ionic capturing method.
Results: Median plasma homocysteine was 11.9 μmol/l, higher in men (13.1 μmol/l) than in women (10.9 μmol/l) ($P<0.001$) and positively associated with age in both sexes ($P<0.001$). The prevalence of hyperhomocysteinaemia ($\geqslant 15$ μmol/l), 21.4%, was also greater in men (32.2%) than in women (13.4%). There were significant negative correlations between plasma homocysteine and serum ($r=-0.32$, $P<0.001$) and erythrocyte ($r=-0.26$, $P<0.001$) folate, as well as serum vitamin B12 ($r=-0.28$, $P<0.001$) concentrations. When divided in quartiles of vitamin intakes or concentrations, men with the lowest vitamin B12 and folate serum values had significantly higher plasma homocysteine concentrations than those in the other three quartiles. In women, hyperhomocysteinaemia was higher in the lowest quartiles of folate intake and serum and erythrocyte folate concentrations.
Conclusions: These data provide further evidence that hyperhomocysteinaemia is a sensitive marker of inadequate folate and vitamin B12 status, allowing for the identification of those with greatest need for nutritional interventions.
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Keywords: homocysteine; reference ranges; folate; vitamin B12

Introduction

Homocysteine is an amino acid solely derived from dietary methionine. Folate and vitamin B12 affect its metabolism (Wilcken and Wilcken, 2001). Consumption or low blood levels in these vitamins, as well as certain dietary habits and genetic background, may raise plasma homocysteine levels (Verhoef *et al.*, 1996; Mann *et al.*, 1999; Stolzenberg-Solomon *et al.*, 1999). Its metabolism may also be influenced by lifestyle factors, such as smoking (Nygård *et al.*, 1998; Kato *et al.*, 1999; Rasmussen *et al.*, 2000; de Bree *et al.*, 2001; Jacques *et al.*, 2001), alcohol and coffee consumption (Nygård *et al.*, 1998; Stolzenberg-Solomon *et al.*, 1999; Rasmussen *et al.*, 2000; de Bree *et al.*, 2001; Jacques *et al.*, 2001; Mayer *et al.*, 2001; Mennen *et al.*, 2002) as well as physical activity (de Bree *et al.*, 2001; Mennen *et al.*, 2002).

Hyperhomocysteinaemia has been associated with cardiovascular diseases (Eikelboom *et al.*, 1999; Graham, 1999; Brattström and Wilcken, 2000; Wald *et al.*, 2002) and folate

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supplements may reduce plasma levels and vascular risk (Chait *et al*., 1999; Selhub *et al*., 2000; Quinlivan *et al*., 2002).

Canary Island residents present the highest age-adjusted mortality rate for cardiovascular diseases in all of Spain. The prevalence of classic risk factors in our community has been reported, but homocysteine has never been considered in this population. In this paper, we attempt to set plasma homocysteine reference values and assess their relation to intake and blood concentration of certain vitamins.

Materials and methods

The Nutritional Survey of the Canary Islands was carried out between 1996 and 1998 to ascertain the nutritional status of this population (Serra-Majem *et al*., 2000). In the first stage, 1747 individuals, ranging in age from 6 to 75 years, participated in a dietary survey, including anthropometric parameters and socio-demographic data, personal and family history and lifestyles. Of those interviewed, 44.8% (782 subjects) took part in a second biochemical stage: haematologic and lipid levels and concentrations of minerals and vitamins. Plasma homocysteine was studied in 557 individuals (236 men and 321 women), excluding those aged under 18 years (180). Another 45 subjects were excluded owing to technical problems (insufficient blood sample, storage or transport difficulties in certain analyses).

Food consumption was assessed using two 24-h recalls on non-consecutive days. Conversion of foods into energy and nutrients was carried out using the Composition Tables of Spanish Foods (Mataix *et al*., 1998), supported by French tables (Favier *et al*., 1995). Folate, vitamin B12 and energy intakes were adjusted to reduce intra-individual variability (Liu *et al*., 1978).

Subjects also answered a questionnaire including information on demographic variables (age, gender and marital, educational and socio-economic status), smoking, alcohol consumption and physical activity. Categories for classifying individuals by smoking status were based on the World Health Organization criteria, which define a smoker as anyone currently smoking at the time of the interview, either on an occasional or regular basis. As for alcohol consumption, a moderate drinker was defined in men as those who daily consumed up to 40 g of alcohol, and in women those whose intake ranged from 1 to 25 g/day (Catarino, 1992), being those over these levels deemed as excessive drinkers. Light physical activity was defined on the basis of walking or non-intense exercise four times a week minimum, and moderate in persons practicing active sports or running on a regular basis. Beyond these limits, subjects were classified as sedentary or vigorously active (Ministerio de Sanidad y Consumo, 1997).

Participants were weighed and measured without shoes and outer clothing, using portable electronic bathroom scales and Kawe height scales, with categories defined on the basis of tertiles of body mass index (BMI) distribution.

Blood samples were obtained in the morning after subjects had fasted for 12 h and had been in a reclining position for at least 15 min. Samples for determining erythrocyte folate were transported immediately to the Haematology Unit of the Hospital Universitario Insular of Gran Canaria. Samples for assessing serum folate and vitamin B12 and plasma homocysteine levels were centrifuged in equipment refrigerated at 4°C at 3000 r.p.m. for 15 min. Test tubes were placed in grids and supernatant separated with single use Pasteur pipettes. Serum and erythrocyte folate were analysed through an automated ionic capturing method, whereas serum vitamin B12 was analysed via the micro-particle enzyme immune assay method, both with Abbott AXSYM equipment, also in the Haematology Unit.

Aliquot portions for determining plasma homocysteine were frozen at −80°C and sent for biochemical testing at the University of Barcelona's Clinical Hospital, with polarized fluorescence immunoassay in an AXSYM (Abbot) analyser. Bio-Rad quality controls were applied and the inter-assay variation coefficient was less than 6.3%.

Quantitative variables were reported for the mean and typical deviation or median and percentiles. The qualitative variables were given in percentages. Kolmogorov–Smirnov test for normality was calculated for plasma homocysteine with the Z statistic. To explain differences in mean values of plasma homocysteine per quartiles of the serum concentrations of vitamins, a general linear model was adjusted for sex and age. Mean equality contrast between different groups was analysed using the variance analysis. Statistical significance was set at 0.05, and confidence intervals at 95%. The SPSS-PC programme (SPSS, Chicago, IL, USA) was used to carry out statistical analysis.

Results

Table 1 describes median overall and gender-specific plasma homocysteine, serum and erythrocyte folate and vitamin B12 values, as well as median daily intake of these vitamins. For plasma homocysteine, concentrations ranged between 5.5 and 109.5 μmol/l, with a median of 11.9 μmol/l, significantly greater in men (13.1 μmol/l) than in women (10.9 μmol/l) ($P<0.001$).

Folate intake was also significantly higher in men (161.6 μg/day) than in women (141.9 μg/day), but we did not find major differences between sexes for serum folate, erythrocyte folate and serum vitamin B12 levels, or in vitamin B12 intake. There was a significant negative correlation between plasma homocysteine levels and serum folate ($r=-0.32$, $P<0.001$), erythrocyte folate ($r=-0.26$, $P<0.001$) and serum vitamin B12 concentrations ($r=-0.28$, $P<0.001$), but neither with folate nor vitamin B12 intakes.

Plasma homocysteine differences by sex remained significant in all age groups, with both men and women presenting a marked increase with age ($P<0.001$, Table 2): average

Table 1 Plasma homocysteine and vitamin levels, median (P_{25}–P_{75})

	Men (n = *236*)[a]	*Women* (n = *321*)[a]	P-*value*[b]	*Total* (n = *557*)[a]
Plasma homocysteine (μmol/l)	13.1 (11.2–15.7)	10.9 (9.5–13.2)	<0.001	11.9 (10.2–14.3)
Folate intake (μg/day)	161.6 (136.3–184.3)	141.9 (126.4–164.2)	<0.001	148.6 (129.3–172.2)
Vitamin B12 intake (μg/day)	9.5 (6.9–14.0)	9.4 (6.7–12.2)	NS	9.5 (6.8–13.1)
Serum folate (ng/ml)	8.1 (6.1–9.9)	7.9 (6.2–9.9)	NS	8.0 (6.2–9.9)
Erythrocyte folate (ng/ml)	200.8 (161.5–250.8)	197.0 (166.0–244.4)	NS	198.2 (165.4–247.0)
Serum vitamin B12 (pg/ml)	438.9 (328.9–588.9)	452.3 (325.6–583.4)	NS	446.2 (326.4–584.7)

Abbreviation: NS, not significant.
[a]Except for erythrocyte folate: $n = 232$ (men), $n = 311$ (women) and $n = 543$ (total).
[b]Mann–Whitney test.

Table 2 Plasma homocysteine concentration and hyperhomocysteinaemia prevalence by age groups and sexes

Sex/age groups	*Plasma homocysteine* (μmol/l)				*Hyperhomocysteinaemia* (⩾15 μmol/l)	
	P_5	P_{50}	P_{95}	P-*value*[a]	%	P-*value*[b]
Men				<0.001		<0.001
18–25 years ($n = 26$)	8.5	12.3	39.5		26.9	
25–45 years ($n = 88$)	8.6	12.2	24.8		17.0	
45–65 years ($n = 88$)	8.2	13.6	24.5		38.6	
65–75 years ($n = 34$)	12.1	15.3	25.9		58.8	
Total ($n = 236$)	8.6	13.1	24.9		32.2	
Women				<0.001		0.020
18–25 years ($n = 36$)	7.5	10.4	21.3		13.9	
25–45 years ($n = 132$)	6.8	10.5	18.2		10.6	
45–65 years ($n = 116$)	7.6	11.3	18.5		11.2	
65–75 years ($n = 37$)	9.9	13.2	22.8		29.7	
Total ($n = 321$)	7.5	10.9	19.1		13.4	
Both sexes				<0.001		<0.001
18–25 years ($n = 62$)	7.7	10.9	22.6		19.4	
25–45 years ($n = 220$)	7.4	11.1	18.3		13.2	
45–55 years ($n = 204$)	7.7	12.0	20.5		23.0	
65–75 years ($n = 71$)	10.3	14.2	24.1		43.7	
Total ($n = 557$)	7.8	11.9	20.5		21.4	

[a]Kruskal–Wallis test.
[b]χ^2-test.

increase for both sexes between 18 and 25 and 65 and 75 years was approximately 25%.

Overall hyperhomocysteinaemia (⩾15 μmol/l) prevalence was 21.4%, with 13.4% in women and 32.2% in men. For both sexes, the 65–75 age groups presented the highest prevalence: almost 60% of men and 30% of women above 15 μmol/l (Table 2). There was no case of severe hyperhomocysteinaemia (>30 μmol/l) in women, although it was observed in six men under 65 years.

When categorizing on the basis of BMI, smoking habits, alcohol intake, physical activity, hypertension and cholesterol levels, we did not detect significant differences in mean plasma homocysteine figures nor in hyperhomocysteinaemia (⩾15 μmol/l) prevalence in men and women (Table 3). A weak association was found between vitamin B12 intake and alcohol consumption (Spearman's $\rho = 0.145$).

Table 4 shows the association of plasma homocysteine with folate and vitamin B12 intakes, and its relationship with blood vitamin levels. Concurrent significant differences by sex were only present based on the serum folate quartiles ($P < 0.001$). In men, the adjusted mean of plasma homocysteine is 49% greater in the lowest quartile than in the highest and 25% greater in women.

Although not statistically significant, plasma homocysteine level was inversely correlated with folate and vitamin B12 intakes in men. The largest differences in plasma homocysteine concentration among men were found in the extreme quartiles of serum vitamin B12 (−6.14 pg/ml,

Table 3 Plasma homocysteine concentration and hyperhomocysteinaemia prevalence distributions by cardiovascular risk factors

	Men					*Women*				
	Plasma homocysteine (μmol/l)			*Hyperhomocysteinaemia (⩾15 μmol/l)*		*Plasma homocysteine (μmol/l)*			*Hyperhomocysteinaemia (⩾15 μmol/l)*	
	n	*Mean*[a] *(95% CI)*	P-*value*[b]	*%*	P-*value*[c]	n	*Mean*[a] *(95% CI)*	P-*value*[b]	*%*	P-*value*[c]
BMI (kg/m²)			0.956		0.211			0.427		0.139
<24	69	14.90 (12.86–16.93)		24.6		107	11.73 (11.09–12.38)		10.3	
24–28	83	14.48 (12.71–16.25)		33.7		96	11.36 (10.72–12.01)		10.4	
>28	76	14.64 (12.76–16.52)		38.2		103	11.96 (11.31–12.60)		18.4	
Cigarettes/day			0.679		0.693			0.662		0.921
0	147	14.85 (13.54–16.15)		32.7		239	11.65 (11.23–12.07)		13.8	
1–10	23	15.43 (12.13–18.73)		39.1		40	11.66 (10.61–12.70)		10.0	
10–20	23	14.67 (11.36–17.98)		34.8		21	11.52 (10.12–12.93)		14.3	
>20	43	13.27 (10.84–15.71)		25.6		20	12.58 (11.15–14.01)		15.0	
Alcohol (g/day)			0.859		0.387			0.057		0.835
Non-drinker	55	14.48 (12.35–16.62)		36.4		206	11.45 (11.01–11.90)		14.1	
Moderate drinker[d]	124	14.82 (13.40–16.24)		28.2		102	11.95 (11.32–12.58)		11.8	
Excessive drinker[e]	57	14.23 (12.14–16.33)		36.8		13	13.54 (11.76–15.32)		15.4	
Physical activity			0.578		0.256			0.226		0.259
Sedentary	136	15.10 (13.75–16.46)		34.6		220	11.86 (11.43–12.29)		15.9	
Light	61	13.89 (11.86–15.91)		36.1		78	11.09 (10.37–11.81)		7.7	
Moderate	29	13.30 (10.26–16.34)		17.2		17	12.50 (10.94–14.05)		11.8	
Vigorous	5	16.15 (8.79–23.51)		20.0		4	11.62 (8.41–14.82)		0.0	
Hypertension			0.072		0.001			0.880		0.088
Yes	33	17.11 (14.26–19.96)		57.6		52	11.79 (10.87–12.72)		21.2	
No	166	14.24 (13.00–15.47)		28.9		254	11.72 (11.31–12.12)		12.2	
LDL cholesterol (mg/dl)			0.063		0.069			0.464		0.463
<160	156	15.34 (14.09–16.60)		36.5		219	11.73 (11.29–12.16)		11.9	
⩾160	77	13.26 (11.46–15.05)		24.7		94	11.42 (10.74–12.10)		14.9	
HDL cholesterol (mg/dl) (♂/♀)			0.641		0.974			0.746		0.755
<35/42	72	14.23 (12.36–16.09)		32.4		62	11.55 (10.74–12.36)		14.5	
⩾35/42	164	14.76 (13.53–15.99)		32.2		253	11.79 (11.30–12.10)		13.0	

Abbreviations: BMI, body mass index; CI, confidence interval; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
[a]Adjusted for age and folate intake.
[b]F-test.
[c]χ^2-test.
[d]⩽40 g/day men and ⩽25 g/day women.
[e]>40 g/day men and >25 g/day women.

Table 4 Plasma homocysteine concentration and hyperhomocysteinaemia prevalence distributions per quartiles of vitamins intakes and blood levels

Vitamin quartiles[a]	*Men*					*Women*				
	Plasma homocysteine (μmol/l)			*Hyperhomocysteinaemia (⩾15 μmol/l)*		*Plasma homocysteine (μmol/l)*			*Hyperhomocysteinaemia (⩾15 μmol/l)*	
	n	*Mean (95% CI)*	P-*value*[b]	%	P-*value*[c]	n	*Mean (95% CI)*	P-*value*[b]	%	P-*value*[c]
Folate intake (μg/day)[d]			0.431		0.005			0.002		0.026
Q1 (<129.3)	45	14.55 (12.49–17.21)		37.8		94	12.56 (11.90–13.23)		19.1	
Q2 (129.3–148.6)	46	15.60 (13.27–17.92)		28.3		93	11.53 (10.87–12.19)		15.1	
Q3 (148.6–172.2)	61	15.21 (13.16–17.25)		47.5		79	11.77 (11.06–12.49)		12.7	
Q4 (>172.2)	84	13.47 (11.75–15.19)		20.2		55	10.39 (9.52–11.26)		1.8	
Vitamin B12 intake (μg/day)[d]			0.265		0.679			0.145		0.606
Q1 (<6.8)	57	16.38 (14.28–18.48)		36.8		82	11.95 (11.24–12.65)		14.6	
Q2 (6.8–9.5)	59	14.32 (12.26–16.38)		30.5		81	10.98 (10.27–11.68)		9.9	
Q3 (9.5–13.1)	48	13.50 (11.22–15.78)		35.4		91	11.98 (11.32–12.65)		16.5	
Q4 (>13.1)	72	14.15 (12.30–16.01)		27.8		67	11.88 (11.10–12.65)		11.9	
Serum folate (ng/ml)[e]			<0.001		<0.001			<0.001		<0.001
Q1 (<6.2)	68	17.91 (16.04–19.78)		50.0		81	13.21 (12.52–13.90)		27.2	
Q2 (6.2–8.0)	48	13.93 (11.76–16.10)		33.3		89	11.60 (10.97–12.23)		11.2	
Q3 (8.0–9.9)	64	13.71 (11.82–15.61)		25.0		78	11.19 (10.52–11.86)		9.1	
Q4 (>9.9)	56	12.05 (9.93–14.17)		17.9		73	10.53 (9.78–11.28)		5.5	
Erythrocyte folate (ng/ml)[e]			0.882		0.009			0.014		0.005
Q1 (<165.4)	61	14.14 (12.07–16.21)		47.5		75	12.70 (11.98–13.42)		24.0	
Q2 (165.4–198.2)	53	15.24 (13.21–17.27)		34.0		83	11.58 (10.93–12.24)		15.7	
Q3 (198.2–247.0)	57	14.38 (12.38–16.37)		24.6		79	11.39 (10.72–12.05)		8.9	
Q4 (>247.0)	61	14.69 (12.69–16.69)		21.3		74	10.98 (10.23–11.73)		5.4	
Serum vitamin B12 (pg/ml)[e]			<0.001		<0.001			0.089		0.047
Q1 (<326.4)	58	18.57 (16.61–20.53)		53.4		81	12.15 (11.49–12.82)		22.2	
Q2 (326.4–446.1)	64	14.50 (12.66–16.34)		35.9		76	11.80 (11.12–12.49)		12.0	
Q3 (446.1–584.6)	53	12.73 (10.67–14.79)		20.8		86	11.72 (11.07–12.37)		11.6	
Q4 (>584.6)	61	12.43 (10.53–14.33)		18.0		78	10.94 (10.26–11.62)		7.7	

Abbreviations: CI, confidence interval.
[a]Quartiles for the entire population.
[b]F-test.
[c]χ^2-test.
[d]Adjusted for age and folate or vitamin B12 intake.
[e]Adjusted for age, folate and vitamin B12 intakes and (folate or vitamin B12) serum levels.

$P=0.001$) and serum folate (-5.86 ng/ml, $P<0.001$). No association was found between plasma homocysteine and erythrocyte folate in men.

The pattern for the women tested was quite different. There was a statistically significant inverse relationship between plasma homocysteine level and folate intake ($P<0.01$), although no such association was found with vitamin B12 intake. Erythrocyte folate was also inversely related to plasma homocysteine, as was serum folate, although the former more moderately ($P<0.05$). Finally, serum vitamin B12 was not associated.

Frequencies of hyperhomocysteinaemia ($\geqslant 15\,\mu$mol/l) as quartiles of intake or blood levels of folate and vitamin B12 are also included in Table 4. Hyperhomocysteinaemia was always significantly greater in men than in women. Differences in hyperhomocysteinaemia frequencies in the vitamin quartiles were significant, except in the case of vitamin B12 intake.

Men were three times more likely to develop hyperhomocysteinaemia in the lowest quartiles of serum folate and vitamin B12, and almost twice in the case of erythrocyte folate and folate intake than in the highest quartiles of each.

In women, the risks were even greater: 10 times more in the lowest quartile of folate intake compared to the highest, and 5, 4.4 and 3 times different between the highest and the lowest quartiles of serum and erythrocyte folate and serum vitamin B12, respectively.

Discussion

In this study, we found that 21.4% of Canarians presented plasma homocysteine levels greater than $15\,\mu$mol/l. This percentage is much higher than in the US population (Selhub *et al*., 1999), in which a lower cutoff point was applied ($>11.4\,\mu$mol/l in men and $>10.4\,\mu$mol/l in women), and in the Finnish population (Alfthan *et al*., 2003) with a $14\,\mu$mol/l cutoff point.

In this latter study, mean plasma homocysteine concentration (11.3 and $9.2\,\mu$mol/l for men and women, aged 25–74 years) was lower than in our population for both sexes, and the same occurs in the French population (Mennen *et al*., 2002), for subjects aged 35–60 years (10.82 and $8.74\,\mu$mol/l for men and women). On the contrary, there was hardly any difference compared with a random sample of the Dutch population, 20–65 years of age (14.6 and $13.1\,\mu$mol/l for men and women) (de Bree *et al*., 2001).

Within Spain, the Canary Islands presented mean plasma homocysteine values greater than those found in a Basque sample group, 20–80 years (men $9.53\,\mu$mol/l; women $7.79\,\mu$mol/l) (Pijoán-Zubizarreta *et al*., 2001).

Previous values for our community have been even higher (Rodríguez-Esparragón *et al*., 2003): in 235 hypertensive subjects and 223 normotensive controls, mean plasma homocysteine concentrations were $16.2\pm6.5\,\mu$mol/l in men and $12.3\pm4.0\,\mu$mol/l in women, albeit their mean age was approximately 12 years greater than those of our population sample. Our results merit more studies on the causal role that hyperhomocysteinaemia may play in the raised morbidity and mortality attributable to heart disease in the Canary Islands, including its relationship with vitamin B6, serum creatinine, liver function and genetic factors.

As has been widely reported in other communities (Nygård *et al*., 1998; de Bree *et al*., 2001; Jacques *et al*., 2001; Mennen *et al*., 2002; Ganji and Kafai, 2003), we found greater plasma homocysteine levels in men, exceeding that of women by 20%. The difference in all age groups was approximately $2\,\mu$mol/l. Given the role of folate and vitamin B12 in homocysteine metabolism, it is worth pointing out that we did not find gender disparities in plasma concentrations of these vitamins nor in vitamin B12 intake, but they were detected in folate intake. However, such differences in homocysteinaemia for both sexes persist even after adjusted for these variables. Thus, probably genetic factors must be involved.

The increasing levels of plasma homocysteine with age have also been described previously (Nygård *et al*., 1998; de Bree *et al*., 2001; Jacques *et al*., 2001; Ganji and Kafai, 2003). Our results indicate that levels for the 65–75 age group surpassed those of subjects 18–25 by 30%. It has been reported that older people consume less homocysteine-related vitamins and their renal re-absorption capacity for these vitamins is also reduced (Stampfer and Willett, 1993; Bostom *et al*., 1995). However, this was not our case, as vitamin consumption in our population did not vary with age (Serra-Majem *et al*., 2000).

Hormonal influences have also been considered and the effect of menopause on plasma homocysteine concentration is worth noting (Morris *et al*., 2000; Rasmussen *et al*., 2000; Fernández-Miranda *et al*., 2001). Mean plasma homocysteine concentration in our menopausal population was $1\,\mu$mol/l higher than pre-menopausal women, once adjusted for age (12.3 vs $11.3\,\mu$mol/l).

We did not find lifestyle factors significantly associated with plasma homocysteine concentration, although the prevalence of hyperhomocysteinaemia is higher in those subjects with BMI greater than 28. Perhaps, the poor eating habits of overweight subjects, who consume less fruit and vegetables, may influence this association (Ortega *et al*., 1995; Haslam and James, 2005). Some studies in the literature find a slight relationship between these variables (Jacques *et al*., 2001; Koehler *et al*., 2001), although in some they disappear when adjusted for certain variables (Nygård *et al*., 1998) and in others there is none (Lussier-Cacan *et al*., 1996).

Although a positive correlation has been reported between plasma homocysteine and cigarette consumption (Nygård *et al*., 1998; Osganian *et al*., 1999; Rasmussen *et al*., 2000; de Bree *et al*., 2001; Jacques *et al*., 2001), or serum cotinine (Ganji and Kafai, 2003), it was not found in our gender-differentiated analysis, consistent with the findings of Saw *et al*. (2001) and Mennen *et al*. (2002). Lower serum folate,

erythrocyte folate and serum vitamin B12 levels in smokers, compared with those of non-smokers, may be the cause of their reduced plasma homocysteine concentration (Ganji and Kafai, 2003). Although in previous research (Henríquez *et al*., 2004) we found lower plasma levels of these vitamins among smokers, this was not the case with homocysteine.

We did not find any association between alcohol intake and plasma homocysteine, but other authors (de Bree *et al*., 2001; Jacques *et al*., 2001, Ganji and Kafai, 2003) have reported that liquor consumption, and to a lesser extent, beer and wine, is a predictor of plasma homocysteine levels. Moreover, B group vitamin and other nutritional deficiencies (Cravo and Camilo, 2000) have no role for confounding.

Regarding B group vitamins, the significant inverse association between folate intake and plasma homocysteinaemia has consistently been found, but not in the case of vitamin B12 intake (de Bree *et al*., 2001; Jacques *et al*., 2001; Saw *et al*., 2001). This occurs in our population, in which, furthermore, the relationship between folate intake and plasma homocysteine was not significant among men. Folate intake in the highest quartiles may not be enough to reduce plasma homocysteine, which occurs only in quantities greater than 200 μg/day (Jacob *et al*., 1994; Verhoef *et al*., 1996; Nygård *et al*., 1998; de Bree *et al*., 2001), more than 25 μg above values for our 75th percentile.

Clearly, nutritional supplementation with folate positively reduces plasma homocysteine levels (Brouwer *et al*., 1999; Jacques *et al*., 1999; Riddell *et al*., 2000; Rader, 2002; Quinlivan and Gregory, 2003). Low folate intake in the Canarian population may be explained by the low consumption of fruit and vegetables, in the one hand, and by the lack of pteroylglutamic acid-fortified food in our environment, on the other hand.

Serum folate, erythrocyte folate and serum vitamin B12 are good predictors of homocysteinaemia (Rasmussen *et al*., 2000; de Bree *et al*., 2001; Jacques *et al*., 2001; Saw *et al*., 2001; Ganji and Kafai, 2003), although often divergent when analysing by gender. Thus, Mennen *et al*. (2002) reported an inverse relationship between erythrocyte folate and plasma homocysteine only in men. However, we found this significant relationship among women. In the case of Mennen *et al*. (2002), serum folate was not measured, whereas this was our best predictor of plasma homocysteine in both sexes.

Finally, given homocysteine's importance as a cardiovascular risk factor, we would highlight that the great number of individuals with plasma homocysteine levels over 15 μmol/l is an argument to consider nutritional intervention, given that they tend to be those with lower concentrations of B group vitamins.

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