

ORIGINAL COMMUNICATION

Folate intake, plasma folate and homocysteine status in a random Finnish population

G Alfthan^{1*}, MS Laurinen², LM Valsta², T Pastinen^{3†} and A Aro¹

¹Department of Health and Functional Capacity, National Public Health Institute, Mannerheimintie, Helsinki, Finland;

²Department of Epidemiology and Health Promotion, National Public Health Institute, Mannerheimintie, Helsinki, Finland; and

³Department of Molecular Medicine, National Public Health Institute, Mannerheimintie, Helsinki, Finland

Objective: To assess the folate status of Finnish adults using plasma folate and homocysteine as biomarkers and to evaluate dietary and supplementary folate intakes.

Materials and methods: Plasma folate, vitamin B₁₂ and total homocysteine (tHcy) were determined in a random sample of 643 subjects aged 25–74 y living in the Helsinki area. The methylenetetrahydrofolate reductase (MTHFR)-genotypes were analyzed from a subsample (*n* = 394). Dietary intake data by 24 h recall and use of vitamin supplements were collected.

Results: Plasma folate was normal (≥ 5 nmol/l) in 99% of subjects and optimal (≥ 8 nmol/l) in terms of a minimum tHcy in 90%. Mean plasma folate of non-supplement users was 13.7 and 12.9 nmol/l and tHcy 11.3 and 9.2 μ mol/l for men and women, respectively. Elevated tHcy (> 14 μ mol/l) was found in 11% of subjects. Homozygote frequency for MTHFR genotype TT was 5.0% and their plasma tHcy was 14.8 μ mol/l compared to the mean of the other subjects, 10.5 μ mol/l, *P* < 0.05. The mean dietary folate intake was 241 μ g/day (29 μ g/MJ of energy) for men and 205 μ g/day (33 μ g/MJ) for women, respectively. The main dietary sources of folate were vegetables 12%, wholemeal ryebread 11%, fruits 10%, and potato 10%. Regular supplement users (*n* = 97) received on average 207 μ g folic acid per day from supplements.

Conclusions: The folate status of Finnish adults seems to be adequate according to energy adjusted folate intake, plasma folate and homocysteine. The MTHFR homozygote frequency was low compared to other countries. Regular use of supplementary folic acid less than 300 μ g increased plasma folate, but supplemental folic acid over 300 μ g was required to lower tHcy values significantly.

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Keywords: folate status; dietary intake; homocysteine; MTHFR; vitamin B₁₂

Introduction

It is well documented that prophylactic supplementation with folic acid dramatically reduces the incidence of birth complications and neural tube defects (NTD: Scholl & Johnson, 2000; Czeizel & Dudas, 1992). Apart from clear deficiency, an adequate folate intake may be important as a possible protective factor for cardiovascular diseases by itself (Rimm *et al*, 1998; Voutilainen *et al*, 2001) or indirectly via its homocysteine lowering effect (Homocysteine Lowering Trialists' Collaboration, 1998). The dietary recommen-

dations for folate are currently in a turmoil, with widely different values between countries ranging from 300 μ g to over 400 μ g/day (Sandström & Gibney, 2001). Accurate assessment of folate intake is relatively difficult due to analytical problems with food folate levels, large variation within food sources, stability during processing and storage (Vahteristo *et al*, 1998). A complementary way to find out the folate status is to use blood folate as a biomarker of intake. Blood folate reflects folate intake, although different factors affect absorption and bioavailability, causing variations in tissue levels (Gregory, 1997). The most frequently observed genetic cause of mildly elevated plasma homocysteine levels is a common single nucleotide polymorphism (SNP) C677T in the gene for methylenetetrahydrofolate reductase (MTHFR; Frosst *et al*, 1995), converting alanine to valine. Homozygosity for the T-allele has a population frequency of 5–15%. Homozygosity *per se* is not considered

*Correspondence: G Alfthan, National Public Health Institute, Mannerheimintie, 166, Helsinki, FIN-00300, Finland.
E-mail: georg.alfthan@ktl.fi

†Montreal Genome Centre, Montreal, Quebec, Canada.

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to be a risk factor for cardiovascular disease (CVD), although it results in elevated plasma homocysteine levels (Brattström *et al*, 1998).

The aim of this study was to assess the dietary intake of folate using updated food folate data in view of new folate recommendations and relate this to folate and homocysteine status in a random population sample.

Materials and methods

Subjects

The National FINRISK Study 1997 survey is a randomized population study for the survey of risk factors for chronic diseases in adult Finns (Vartiainen *et al*, 2000). The study comprised 7200 men and women aged 25–64 y from five areas and 1258 men and women aged 65–74 y from two of these areas, 72 and 84% of those invited, respectively. From among these, a random sample of 40% by sex and age were allocated to the dietary survey including a 24 h dietary recall (FINDIET Study Group, 1998). There were 643 subjects from the Helsinki area for whom dietary data and plasma values were complete. This sample represented 95% of those who participated. Data for 19 subjects were not included for various reasons, eg pregnancy and vitamin B₁₂ injections.

Food consumption and nutrient intake calculation

Food consumption was assessed by 24 h recall by trained interviewers with a portable computer (FINDIET Study Group, 1998). The program 'NUTTI' was designed at the Unit of Nutrition for collecting data on quality, quantity and preparation of foods and beverages in a standardized way. In this study the intake of folates from natural folates from foods and folic acid supplements were calculated separately. The use of folic acid supplements was evaluated by a structured questionnaire that was filled at home. Dietary intake was calculated from the FINDIET 1997 food consumption dataset by the nutrient intake calculation software, applying the data from the FINELI[®] food composition database (FINDIET Study Group, 1998; Ovaskainen, 2001). Supplement users were classified on basis of regular folic acid-containing supplement use and the received dose was estimated from the content of folic acid of the preparations. Estimation of folate losses from food preparation was done according to published data (Bergström, 1994) and expert advice (L Vahteristo, personal communication). A loss of 30% was used in calculations for both cooked and baked meat, fish, egg, vegetables, cereal, milk products (pasteurized) and vegetables.

Collection of blood

Venous blood samples were taken into EDTA-vacuum tubes after a 4 h fast between 11 am and 6 pm and plasma aliquots

were stored at –70°C. A whole blood sample for DNA analyses was stored at –20°C. Due to breakdown of a freezer only 394 samples out of 643 were available.

Chemical analyses

Plasma total homocysteine (tHcy) was determined by a modification of the high-pressure liquid chromatographic method described by Ubbink *et al* (1991). The mobile phase was modified to consist of 0.37 M acetate and 0.5% methanol, pH 4.15. The peak heights were calibrated using a secondary serum standard. The precision between series ($n=13$) for an in-house serum pool was 5.6% at the level of 7.1 µmol/l. The accuracy was verified by participating in an interlaboratory quality control scheme in which the mean annual bias was –2.2% for 12 sera ranging from 9.4 to 83 µmol/l (Möller *et al*, 1999).

Folate and vitamin B₁₂ were determined using the Simultrac–SNB dual radioassay for both folate and vitamin B₁₂ (Becton-Dickinson). The precision between series ($n=8$) for three reference sera was less than 9%. The lower limit of the reference range was for folate 5.0 nmol/l and for vitamin B₁₂ 200 pmol/l.

DNA analysis

The DNA ($n=394$) was extracted from EDTA whole blood by a standard method (Bell *et al*, 1981). A nested PCR reaction was performed to amplify the genomic fragment flanking the common 677C to T transition of the MTHFR gene. For the first round of PCR the following conditions were employed: forward PCR primer had a 5' biotin and its sequence was GGA GAA GGT GTC TGC GGG A; the reverse primer had the sequence AAG CTG CGT GAT GAT GAA AT. The amplifications were carried out using 20 ng of DNA, 0.6 µM primers, 0.2 mM dNTPs and 0.5 U of AmpliTaq Gold DNA polymerase (Perkin Elmer, Branchburg, NJ, USA) in 15 µl of DNA polymerase buffer supplied with the enzyme. After initial activation of the polymerase at 95°C for 11 min, the thermocycling parameters were: 95°C for 30 s, 57°C for 30 s, and 72°C for 30 s for 35 cycles, followed by final extension at 72°C for 6 min. The nested PCR reaction was carried out using 0.1 µl of the first-round PCR product as a template, and the reverse primer was replaced by primer GCT GCG TGA TGA TGA AAT CG. All primers were synthesized by Interactiva Biotechnologie GmbH (Ulm, Germany). The nested reaction was carried out in 50 µl volume using the same concentrations of reagents and cycling parameters as described above. The PCR product was then genotyped by solid-phase minisequencing essentially as previously described (Syvänen *et al*, 1993) using the nested PCR primer as the detection primer. All the genotyping reactions were carried out in duplicate. The genotyping results were in Hardy–Weinberg equilibrium as analyzed by the Arlequin 1.1 software.

Statistical methods

Differences in class variables were tested by the χ^2 -test and continuous variables by *t*-test, analysis of variance and Spearman correlations for associations between plasma variables using the SAS program for VAX computers, version 6.0. A mixed model for measurement error was used in testing the differences in nutrient intakes on the basis of 24 h recall. For determination of the cut-off value for plasma folate we calculated the cumulative mean tHcy in descending order of plasma folate, first in steps of 100 subjects until the lowest 100 plasma folate, then in steps of 10 subjects. Using analysis of variance and subsequently *t*-test we compared the deciles with centiles.

Results

Plasma status

The mean plasma folate concentration was similar in both men (*n*=318) and women (*n*=325), 14.6 and 14.3 nmol/l, respectively (Table 1). The mean plasma folate concentration of men aged 55–64 was higher compared both to the younger and the older age groups (Figure 1), but no age relationship was found for women. The prevalence of a low plasma folate (< 5 nmol/l) was < 1%. Values exceeding the upper reference range (25 nmol/l) were found in 23% of supplement users and only in 4% of nonusers.

The mean plasma vitamin B₁₂ concentration was 369 and 371 pmol/l for men and women, respectively, and there was no difference between age groups. The prevalence of a low plasma vitamin B₁₂ (< 200 pmol/l) in both sexes was 4.7% (18 women and 12 men).

The plasma mean \pm s.d. tHcy was 11.3 \pm 3.6 μ mol/l for men and 9.2 \pm 2.8 μ mol/l for women, *P* < 0.0001. Plasma tHcy increased in women from the age group 35–44 onwards, but for men only the oldest age group had a higher mean compared to the younger (Figure 1), *P* < 0.001. Mild hyperhomocysteinemia (> 14 μ mol/l) was found in 17% of men and in 6% of women. The role of folic acid-containing vitamin supplements on plasma folate and tHcy was assessed separately (Table 1). The mean plasma folate concentration was significantly higher in both men and women who regularly took folic acid-containing multi-

vitamin supplements compared with nonusers. Also plasma vitamin B₁₂ was significantly higher in vitamin B₁₂ supplement users compared with nonusers.

The supplement users were further divided into two groups according to estimated daily dose of folic acid,

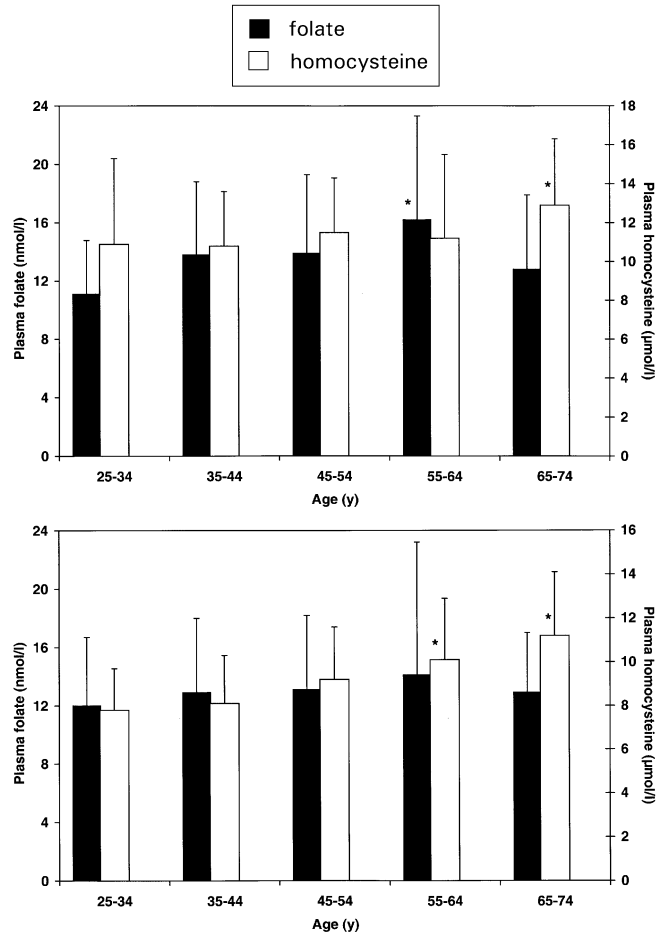


Figure 1 (a) Mean plasma folate and total homocysteine by 10 y age group in men. * Male plasma folate, *P* < 0.001 vs youngest and oldest, #male plasma tHcy, *P* < 0.05 vs 25–44 age group. (b) Mean plasma folate and total homocysteine by 10 y age group in women. * Female plasma tHcy, *P* < 0.001 vs 25–54 age group.

Table 1 Mean (s.d.) plasma folate, vitamin B₁₂ and total homocysteine (tHcy) concentrations in men and women by vitamin supplement use

Supplement use ^a	Men			Women		
	No	Yes		No	Yes	
	<i>n</i> = 318	87%	13%	<i>n</i> = 325	83%	17%
Age	50	50	53	50	50	51
P-folate (nmol/l)	14.6 (6.6)	13.7 (5.7)	20.9 (8.6) ^c	14.3 (8.6)	13.0 (6.3)	20.5 (14.1) ^c
P-tHcy (μmol/l)	11.3 (3.6)	11.5 (3.6)	10.3 (3.2)	9.2 (2.8) ^b	9.3 (2.8)	8.6 (2.9)
P-vitamin B ₁₂ (pmol/l)	371 (132)	362 (125)	436 (160) ^c	391 (164)	383 (166)	428 (149) ^c

^aFolic acid-containing supplements; ^b*P* < 0.05 compared with men. ^c*P* < 0.05 compared with supplement nonusers.

dichotomized according to the recommended intake of dietary folate in Finland, 300 µg. In subjects obtaining on average < 300 µg/day folic acid from supplements, the mean plasma folate was significantly higher compared with supplement nonusers ($P < 0.001$) and even higher, 2.6-fold, in those who received > 300 µg/day (Figure 2).

Plasma mean tHcy concentration was not affected by a regular supplementation with < 300 µg folic acid per day, but was significantly lower in those subjects who received > 300 µg folic acid per day (mean 513 µg, range 360–1560 µg).

There was a statistically significant inverse relationship between plasma folate and tHcy, $r = -0.23$, $P < 0.0001$ and plasma vitamin B₁₂ and tHcy, $r = -0.16$, $P < 0.0001$ and a positive correlation between plasma folate and vitamin B₁₂, $r = 0.19$, $P < 0.0001$.

By stepwise analysis we found a cut-off value for plasma folate concentration using plasma tHcy as a functional marker of folate status. The cut-off value was 8.0 nmol/l, below which plasma tHcy levels tended to increase. The mean ± s.d. tHcy was significantly higher ($P < 0.01$) in the five deciles with the lowest plasma folate compared with those with higher plasma folate (12.8 ± 5.12 vs 9.99 ± 2.89 µmol/l). This approach increased the fraction of both men and women having an inadequate folate status from 1 to 10.5%. Adequate refers here to persons with minimum plasma tHcy, and does not take into consideration NTD risk.

The frequency of the C677T mutant allele was 0.232 in the subjects for whom genotyping was available ($n = 394$). There were 5.0% ($n = 20$) homozygotes for the TT genotype and 36.3% heterozygotes for the CT genotype. The mean plasma tHcy was 40% higher in the homozygotes compared with the other genotypes ($P < 0.05$), who had similar means, 10.5 µmol/l. The genotype accounted for 6.7% of the variance in plasma tHcy. The subjects who were genotyped were divided into two groups according to their median energy

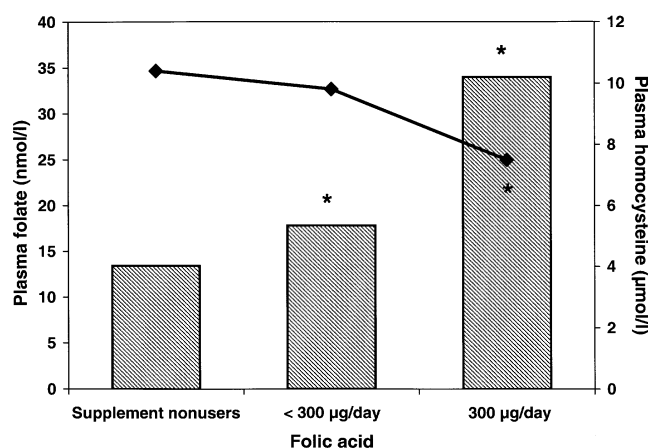


Figure 2 Mean plasma folate and total homocysteine in folic acid supplement nonusers and in users taking less or more than 300 µg folic acid daily.

*Statistical difference vs nonusers.

adjusted folate intake (32.5 MJ/day). Among those having a folate intake over the median, the mean plasma tHcy concentration was 9.9 µmol/l in both the CC and CT genotypes, but significantly higher in the TT homozygotes, 12.0 µmol/l ($P < 0.05$; Figure 3). In the below median folate intake group, the mean tHcy concentration was 11.0 µmol/l in both the CC and CT groups, but much higher in the TT homozygotes, 17.6 µmol/l ($P < 0.001$). In this group (low folate, TT genotype), concomitantly with the high tHcy, the mean plasma folate concentration was significantly lower than in the other genotypes ($P < 0.05$). Plasma folate did not differ between genotypes in those receiving above the median folate.

Dietary intake

Folates. The mean total intake of folates (diet + supplements) was 245 µg/day for men and 209 µg/day for women ($P < 0.05$) taking into account the loss due to food preparation (9–11% on average) Table 2. However, when the intake from the diet was adjusted for energy, the intake was higher in women than in men ($P < 0.05$). The mean folate intake in µg/day was 20% higher among the youngest women compared with older women ($P < 0.028$), but the energy-adjusted intake was similar as was also the proportion of folic acid from supplements to total folate intake was similar in both men and women and nearly doubled the average intake in those who took supplements regularly.

Table 3, shows the relationship between plasma folate, vitamin B₁₂, their intake and tHcy and smoking by plasma folate tertiles for supplement nonusers. Neither in men nor in women did the estimated folate intake predict the plasma folate level.

The main specific folate sources for men were wholemeal ryebread (11.6%), vegetables (10.4%) and potatoes (10%). For women they were vegetables (14.5%), fruit (10%), and wholemeal ryebread (9.8%). Among the largest sources of

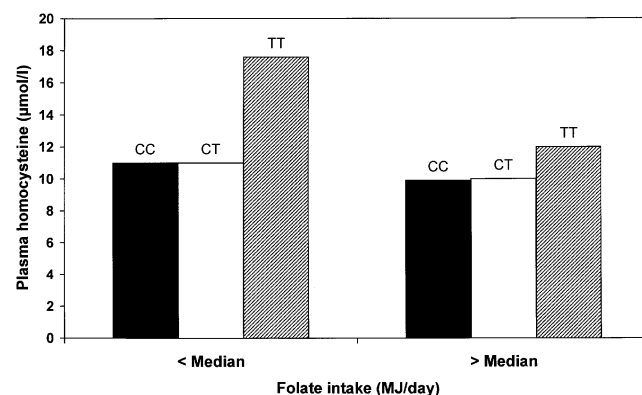


Figure 3 Mean plasma total homocysteine by MTHFR genotype according to median total folate intake. < Median: TT vs CC and CT, $P < 0.001$; > median: TT vs CC, $P < 0.05$.

Table 2 The mean intake of folate and vitamin B₁₂ from the diet and supplements of men and women living in the Helsinki area

Supplement use ^a	Men			Women		
		No	Yes		No	Yes
	n = 318	87%	13%	n = 325	83%	17%
<i>Intake</i>						
From diet folate (µg/day)	275	270	296	230 ^b	226 ^b	243 ^b
including loss	245	241	262	209 ^b	205 ^b	223 ^b
Folate (µg/MJ/day)	33	32	34	37 ^b	37 ^b	38 ^b
including loss	29	29	30	33 ^b	33 ^b	35
Vitamin B ₁₂ (µg/MJ/day)	1.0	1.0	1.2	0.9	0.9	0.8
<i>From supplements</i>						
Folic acid (µg/day)			175 (142)			207 (224)
Vitamin B ₁₂ (µg/day)			24.2 (77.2)			34.4 (72.6)

^aFolic acid-containing multivitamin supplements. ^bP < 0.05 difference between men and women.

folate for both sexes was 'other foods', which contributed 14.4% of the intake for men and 12.8% for women and included brewer's yeast, contained in most breads. The contribution of fruit juices was similar in both men and women, 4%.

Vitamin B₁₂. The mean energy adjusted intake of vitamin B₁₂ was 1.0 µg/MJ for men and 0.9 µg/MJ for women. The mean intake from supplements was 24.2 µg/day for men (13% users) and 34.4 µg/day for women (19% users; Table 2).

Discussion

There is much controversy concerning the role of plasma total homocysteine as a risk factor for CVD; whether hyperhomocysteinemia is a causal factor or the consequence of the

disease (Christen *et al*, 2000; Knekt *et al*, 2001a, b). Homocysteine may be a marker of endothelial dysfunction (Woo *et al*, 1997) and thrombotic events (Ridker *et al*, 1997). The concept of homocysteine being a functional marker of sub-optimal folate status has gained popularity (Jacob *et al*, 1994, Brouwer *et al*, 1998). This means that by measuring plasma tHcy one can distinguish between functional folate deficiency vs a low folate intake (Jacob *et al*, 1994). In our population we estimated that the cut-off value for plasma folate concentration was 8.0 nmol/l, which would be necessary to maintain a 'normal' plasma homocysteine level. In that case, the prevalence of low plasma folate in our population would increase from 1% to 10.5%. In a similar approach Lewis *et al* (1992) suggested a cut-off value of 15 nmol/l. Brouwer *et al* (1998) established a cut-off value of 10 nmol/l using also an oral methionine tolerance test and folic acid supplementation, resulting in 31% being folate deficient. Depending on the criteria, the proportion of people with a suboptimal folate status in other recent studies has varied between 0 and 79% (Brussaard *et al*, 1997; Selhub *et al*, 1999; Rasmussen *et al*, 2000; Brouwer *et al*, 1998; Quinn & Basou 1996). The absolute cut-off value varies due to analytical methods, populations and criteria and is probably best defined as a certain percentile of a defined population.

A matter of debate lately in Finland has been the need to fortify foods with folic acid in order to prevent NTD. In Finland, foods are not enriched with folic acid. The birth prevalence of NTDs in Finland in the 1990s has varied between 20 and 30 per 60 000 births (Ritvanen, 1996). Fortification of enriched grain products with folic acid in US since 1996 has resulted in a substantial decrease in the prevalence of low plasma folate concentrations as well as a decrease in the prevalence of high tHcy concentrations (Jacques *et al*, 1999). During this time the birth prevalence of NTDs decreased by 19% (Honein *et al*, 2001). The effectiveness of preconceptual folic acid is considered poor in populations having a low incidence of NTD (Mills *et al*, 1992). In Finland the incidence is low as is also the frequency

Table 3 Mean (s.d.) plasma folate, vitamin B₁₂ and total homocysteine (tHcy) and the intake of folate and vitamin B₁₂ by plasma folate tertiles in supplement nonusers

	Plasma folate tertiles			P
	Low	Middle	High	
<i>Male (n = 277)</i>				
Age	47	50	53	*
Smoking (%)	38	32	39	NS
P-folate (nmol/l)	8.6 (1.8)	12.7 (1.1)	19.7 (5.4)	
P-vitamin B ₁₂ (pmol/l)	337 (123)	364 (104)	382 (142)	*
P-tHcy (µmol/l)	13.1 (4.8)	10.9 (2.6)	10.4 (2.5)	***
Folate intake (µg/day) ^a	250	227	250	NS
<i>Female (n = 269)</i>				
Age	48	51	50	NS
Smoking (%)	33	28	29	NS
P-folate (nmol/l)	8.0 (1.3)	11.6 (1.2)	19.1 (7.0)	
P-vitamin B ₁₂ (pmol/l)	355 (128)	380 (195)	411 (165)	NS
P-tHcy (µmol/l)	9.8 (3.1)	9.8 (2.7)	8.4 (2.2)	***
Folate intake (µg/day) ^a	204	198	211	NS

^aLosses (9–11%) due to food preparation taken into account.

of persons with the MTHFR TT-genotype who would benefit the most (in terms of tHcy lowering) from an increased intake of folates, as we have shown. An expert panel has thus recommended that 400 µg folic acid be taken periconceptually by low-risk women and 4000 µg by high-risk women (Ritvanen, 1996).

Our data indicate that women of fertile age had an adequate folate status (compared to older women) derived solely from food sources and that their plasma tHcy was low. In Finnish women, our folate intake estimate (205 µg/day) seems low compared with data for women from the US, 389 µg/day including supplements and fortified foods (Tucker *et al*, 1996) or 275 µg/day for subjects who did not use B vitamin supplements (Jacques *et al*, 1999), but it must be emphasized that our value is solely from unfortified foods alone, taking also into account losses due to food processing. Nevertheless, Rasmussen *et al*, (2000) report a similar value to ours for young (25–30 y) Danish women and Brussaard *et al* (1997) for 20–49 y old Dutch women. According to newly analyzed food folate data, Dutch women of fertile age, 16–50 y, were reported to have an even lower mean intake, 172 µg/day (Konings *et al*, 2001).

The effect on CVD risk mediated by lowering plasma homocysteine through folic acid food fortification seems small in Finland, because an association between serum homocysteine and CVD has not been found in any follow-up study on Finnish populations (Alfthan *et al*, 1994; Voutilainen, 2000; Knekt *et al*, 2001a, b). Furthermore, the common MTHFR SNP was not found to be associated with the risk of acute myocardial infarction (AMI) in our previous case–control study of Finnish AMI survivors (Pastinen *et al*, 1998). A significant lowering of plasma tHcy would, according to our results, require a substantial increase of food folate intake (or folic acid).

The mean energy adjusted intake of vitamin B₁₂ in both men and women was 4–5-fold with the recommendation, 0.2 µg/MJ/day. The high intake was reflected in the status as only <5% of the plasma levels were below the reference range.

The frequency of the homozygous variant genotype of MTHFR C677T (TT) mutation in this random population sample was 5.0%. This is in the lower range of published figures, 5.4–16.1% (Brattström *et al*, 1998; Motti *et al*, 1998). Our low value is comparable to data from other Finnish populations, 6.1% ($n=115$; Wirta *et al*, 1998) and 6.5% ($n=168$; Voutilainen *et al*, 2000.). Also the T allele frequency, 0.232, was low compared with worldwide published data, 0.052–0.487 (Rady *et al*, 1999).

It is well established that plasma homocysteine levels can be reduced by pharmacological doses (5 mg) of supplemental folic acid (Boushey *et al*, 1995). Recent intervention studies have shown that a similar reduction in plasma tHcy can be accomplished by far smaller supplements, 500 µg (den Heijer *et al*, 1998; Brouwer *et al*, 1999a, b). We found that in regular users of >300 µg supplementary folic acid per day (mean 513 µg) the mean plasma tHcy concentration was 28% lower

compared to non-users. Significant reductions (up to 18%) in plasma tHcy levels can be attained also by dietary means by increasing the consumption of vegetables, fruit and berries (Brouwer *et al*, 1999a, b; Appel *et al*, 2000; Riddell *et al*, 2000).

A relationship between folate intake (excluding folic acid supplements) and plasma folate concentrations has been reported in some populations (Jacques *et al*, 1999; Brussaard *et al*, 1997; Rasmussen *et al*, 2000), but not all (MRC Vitamin Study Research Group, 1991). Although our food folate data were up to date and losses due to food preparation were taken into account, we were unable to find an association between folate intake from unfortified food sources and plasma folate. This may be due in part to methodological bias, as food consumption was from a single previous day, which on an individual basis is not accurate enough for quantitative assessment of vitamin intake. On the other hand, fortified foods used in other countries (Tucker *et al*, 1996; Konings *et al*, 2001) may have contributed to the association found due to the better bioavailability of folic acid compared to dietary folates.

We found that vegetables, ryebread, fruit and potatoes contributed about 33% of the dietary folate. In Netherlands 33–36% of folates came from potatoes, vegetables and fruit and 18–20% from bread (Brussaard *et al*, 1997; Konings *et al*, 2001). If yeast had been estimated separately in Finland, our intake values would be quite similar.

Conclusions

The folate status of adults in Finland seemed acceptable, especially among women of fertile age. In terms of the functional marker of folate, total plasma homocysteine, 90% of the subjects had an optimal folate intake. In order to influence plasma homocysteine significantly, it would be necessary to double the present intake of folate to about 600 µg per day. Although the estimated mean folate intake was somewhat lower than the recommendation, fortification of foods with folic acid has not been considered necessary for the general public. Our data support this current view.

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