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***MAT1A* variants modulate the effect of dietary fatty acids on plasma homocysteine concentrations**

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Abstract

Background and Aim—Dietary n-3 polyunsaturated fatty acids (PUFA) are associated with decreased plasma homocysteine (Hcy), an important biomarker for cardiovascular disease. The S-adenosylmethionine synthetase type-1 (*MAT1A*), an essential enzyme in the conversion of methionine to S-adenosylmethionine, plays a key role in homocysteine metabolism. This study investigated the interaction between dietary fatty acids and *MAT1A* genotypes on plasma Hcy concentrations among Boston Puerto Ricans.

Methods and Design—Plasma Hcy and *MAT1A* genotypes were determined in 994 subjects of the Boston Puerto Rican Health Study. Dietary fatty acid intakes were assessed by interviews using a questionnaire adapted from the NCI/Block food frequency form.

Result—In the cross-sectional analysis, genetic variant *MAT1A* 3U1510 displayed a significant interaction with dietary n-3:n-6 PUFA ratio in determining plasma Hcy (*p*-value for interaction = 0.025). 3U1510G homozygotes had significantly lower plasma Hcy concentration than major allele homozygotes and heterozygotes (AA+AG) (*p*-value for trend = 0.019) when the n-3:n-6 ratio was >0.09. Two other *MAT1A* variants, d18777 and i15752, also showed significant interactions with different constituents of dietary fat influencing Hcy concentrations. Furthermore, haplotypes consisting of three variants displayed a strong interaction with n3:n6 ratio influencing Hcy concentrations.

Conclusions—Our results suggest that *MAT1A* genotypes appear to modulate effects of dietary fat on plasma Hcy.

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Author's contributions to manuscript

TH, YL, and JS carried out the studies, analyzed data and drafted the manuscript; CL, LP, YL, CS and JC participated in manuscript preparation; and CL, KT, DL and JO participated in the project design. All authors read and approved the final manuscript.

Keywords

methionine adenosyltransferase; dietary fatty acids; plasma homocysteine

Introduction

Elevated plasma homocysteine (Hcy), a thiol-containing amino acid byproduct of methionine metabolism [1], has been demonstrated to be an independent risk factor for cardiovascular disease (CVD) [1]. In addition to pathophysiological conditions, including menopause, renal disease, and hypothyroidism [2], the etiology of hyperhomocysteinemia (HHcy) is known to be multifactorial, including genetic and environmental factors, such as diet and lifestyle [2–3]. The genetic causes of elevated plasma Hcy include rare inborn errors of Hcy metabolism, such as cystathionine beta-synthase (CBS) and methylenetetrahydrofolate reductase (*MTHFR*) [4]. Recently, studies of polymorphisms from the critical genes involved in Hcy metabolic pathways demonstrated that *MTHFR* 677C>T [5], *MTHFR* 1298A>C [6], *MTRR* 66A>G [7] and *MTR* 2756A>G [8] were associated with elevated plasma Hcy concentration. For environmental factors, lifestyle and diet play an important role in Hcy metabolism. Two-thirds of HHcy subjects in an elderly US population were associated with low plasma/serum concentrations of one or more of B group vitamins [9]. Smoking, drinking alcohol and physical activity have also been associated with elevated plasma Hcy [10].

Importantly, n-3 polyunsaturated fatty acids (n-3 PUFA), which have a protective effect on the cardiovascular system [11], were shown to improve Hcy metabolism [12]. Previously, we reported that plasma Hcy was significantly negatively correlated with the plasma/platelet phospholipids (PL) n-3 PUFA and n-3:n-6 PUFA ratio [12–13]. Subsequent intervention studies have demonstrated that n-3 PUFA supplementation decreases plasma Hcy [14]. However, the results from studies evaluating the relationship between fatty acids and plasma Hcy are inconclusive [15]. Whether genetic variation may account for such inconsistent results is unknown. The relationship between n-3 PUFA and plasma Hcy is not yet fully understood. Methionine adenosyltransferase (MAT), an essential enzyme in methionine metabolism, catalyzes the conversion of methionine to S-adenosylmethionine (SAM). SAM is subsequently converted to S-adenosyl homocysteine and then homocysteine in separate reactions [16]. We previously demonstrated that *MAT1A* variants were associated with stroke and hypertension [16]. Therefore, we hypothesize that *MAT1A* variants, single nucleotide polymorphisms (SNPs), modulate the effect of dietary fatty acids on plasma Hcy.

In the present study, we conducted a population-based evaluation to investigate the combined contributions of *MAT1A* genotype and dietary fatty acids to HHcy in the Boston Puerto Rican population. This population has experienced severe health disparity, including high rates of hypertension, diabetes, obesity, and CVD [16,17]. We examined the effects of *MAT1A* variants and dietary fatty acids on plasma Hcy concentration and assessed their potential interactions in modifying plasma Hcy.

Methods

Study design and subjects

This study was conducted within the ongoing Boston Puerto Rican Health Study (BPRHS) as described previously [17]. The analysis included 994 subjects who participated in the BPRHS study and had complete data on dietary intake, anthropometry, biochemical parameters, and *MAT1A* genotype. Interviews were conducted in volunteers' homes to collect demographic and anthropometric data, and detailed data were collected on dietary

intake using a questionnaire previously adapted from the NCI/Block food frequency form and validated for this population [18]. Physical activity was estimated as a physical activity score, based on the Paffenbarger questionnaire [19]. Smoking status was described in three categories: current, former, and never smoking. Alcohol consumption was defined as current drinkers and nondrinkers.

Fasting blood samples were collected the morning following health interviews in the volunteer's home [17]. Approval for the BPRHS was obtained from the Institutional Review Boards of the Tufts Medical Center and Tufts University.

Population admixture

Population admixture was calculated using STRUCTURE 2.2 based on 100 SNPs selected as ancestry informative markers specifically for Puerto Rican populations [16]. Using the estimated admixture of each subject, we adjusted for population admixture for all genotype-associated analyses.

MAT1A SNP selection and genetic analysis

A panel of eight SNPs mapping in/near the *MAT1A* gene were selected for genotyping based primarily on linkage disequilibrium analysis of HapMap data for the CEU population and the characteristics of these eight SNPs have been described [16]. Results of TAGGER [20] run with the parameters of pair-wise option, CEU population, $r^2 > 0.80$, minor allele frequency > 0.00 , placed most SNPs into one of eleven blocks. Each of the SNPs chosen for genotyping falls into a different LD block within *MAT1A*, and these blocks overall span approximately 10 kbp to either side of the gene. DNA was isolated from blood samples using QIAamp DNA Blood Mini kits according to the manufacture's instructions (Qiagen, Valenc, CA). Eight SNPs were genotyped using the TaqMan SNP genotyping system (Applied Biosystems, Foster City, CA).

Linkage disequilibrium and haplotype analysis

Pair-wise linkage disequilibria among SNPs were estimated as correlation coefficients (r^2) using the haploview program. For haplotype analysis, the global association between haplotypes and plasma Hcy, and estimated haplotype frequencies we used the R software (haplo.stats package).

Measurement of anthropometric and plasma biochemical parameters

Anthropometric variables, including height and weight were measured with standard techniques. Body mass index (BMI) was calculated as weight (kg)/height (m)². Fasting blood samples were collected by venipuncture in all participants. Total plasma Hcy was measured using HPLC with fluorescence detection [21] with coefficient of variation of 6%. Plasma pyridoxal phosphate (PLP) was determined using the radio-enzymatic method of Camp, et al. [22] with coefficient of variation of 7%. Plasma folate and vitamin B₁₂ were measured using Immulite chemiluminescent kits according to manufacturer's instructions (Diagnostic Products Corporation/Siemens, Los Angeles, CA) with coefficient of variation of 4% and 5% respectively. Plasma creatinine was assessed using a modified 'Jaffe' method [23] with coefficient of variation of 2%.

Dietary Assessment

Dietary intake was assessed using a questionnaire (FFQ) that was designed for and validated in this population [18]. Dietary data were linked to the Minnesota Nutrient Data system (1999, version 25) for nutrient analysis. Intakes of fatty acids were expressed as percentages of total energy intake and were included in analyses as both continuous and categorical

variables. Intakes were classified into two groups according to the median intake of the population, to construct categorical variables.

Statistical analyses

Data analyses were performed using SAS for Windows, version 9.1 (SAS Institute). All continuous and dependent variables were examined for normal distribution. Dependent variables with abnormal distribution were BOX-COX-transformed to achieve normality before fitting statistical models [16]. Men and women were analyzed together to ensure adequate statistical power. Chi-square tests were conducted to examine whether the genotype frequencies of the selected SNPs were in Hardy-Weinberg equilibrium. Relationships between dietary fatty acids, *MAT1A* genotypes and plasma Hcy were evaluated using linear regression models. To examine interactions between dietary fatty acid intake and genotype on Hcy, the population medians for total saturated fatty acids (SFA), monounsaturated fatty acid (MUFA), and total PUFA, n-3 PUFA, and n-6 PUFA intakes were used as cutoffs to dichotomize these variables. Then interactions between dietary fatty acids (as dichotomized variables) and *MAT1A* genotypes/haplotypes were tested in a general linear model while controlling the main effects of fatty acid and genotypes, and adjustment for potential confounding factors (age, sex, physical activity, alcohol use, smoking, population admixture, plasma folate, vitamin B₆, vitamin B₁₂, and genotypes of *MTHFR* 1298 A>C and *MTHFR* 677 C>T). All results were expressed as mean \pm SD. Differences between groups were considered to be statistically significant at p -value <0.05 .

Results

Information about demographic, biochemical, and dietary intake data are provided in Table 1. Men and women had similar mean ages. No significant differences were observed across sex for vitamins B₆, and B₁₂. However, mean body mass index (BMI, kg/m²) was significantly higher in women than in men, whereas smoking and alcohol consumption were more prevalent in men than in women. Plasma Hcy concentration for all subjects ranged from 3.90-30.4 μ mol/L. Men had significantly higher mean Hcy. Genotype frequencies of eight *MAT1A* SNPs did not deviate from Hardy-Weinberg equilibrium (data not shown).

Intake of n-6 PUFA was significantly positively associated with plasma Hcy ($p=0.025$), in a dose independent manner (Table 2). In addition, we found that the ratio of dietary n-3:n-6 PUFA was significantly negatively associated with plasma Hcy ($p=0.048$, Table 2), also in a dose independent manner. However, we did not find significant associations between other dietary fatty acids and plasma Hcy concentration.

To examine the interaction between fatty acids and *MAT1A* genotypes, subjects were divided into two groups, based on the median of dietary fatty acid (Table 3). We observed a significant interaction between dietary total MUFA as a categorical variable and *MAT1A* i15752 for plasma Hcy ($p=0.030$). When dietary MUFA intake was low (<11.4 % energy/d), plasma Hcy was lower in carriers of the A allele compared to GG subjects ($p=0.033$). Consistent with the results for the categorical variable, dietary total MUFA, as a continuous variable, appears to interact with *MAT1A* i15752 by influencing plasma Hcy ($p=0.032$, Fig. 1A). With greater MUFA intake, plasma Hcy was higher in carriers of the A allele. We also observed that dietary total fat, as a categorized variable, interacted significantly with *MAT1A* i15752 with respect to plasma Hcy ($p=0.015$). When total fat intake was low (<31.3 % energy/d), A carriers had significantly lower plasma Hcy than G homozygotes ($p=0.047$), while there was no significant difference between A carriers and non-carriers ($p=0.158$) when total fat intake was high (Table 3).

We observed that SNP d18777 displayed significant interactions with dietary total fat in association with plasma Hcy concentration ($p < 0.0001$). Concentrations of plasma Hcy in participants carrying the d18777A allele, the stroke-associated allele [16], were significantly lower than G homozygotes ($p = 0.001$) when consuming lower ($< 31.3\%$ energy/d) total fat (Table 3). On the other hand, those carriers (of d18777A) with higher total fat intake ($> 31.3\%$ energy/d) had a significantly higher Hcy ($p = 0.006$). We also observed interactions between intakes of SFA ($p = 0.022$), MUFA ($p = 0.001$), total PUFA ($p = 0.035$) and d18777 genotype for plasma Hcy concentration (Table 3). Consistent results were observed in that dietary MUFA as a continuous variable significantly interacted with d18777 in relation to plasma Hcy ($p = 0.002$, Fig. 1B). With higher MUFA, the level of plasma Hcy was higher in carriers of the A allele. However, we did not observe interactions between other fatty acids (n-3 PUFA, n-6 PUFA and n-3:n-6) and *MATIA* genotypes (d18777, i15752) for Hcy (data not shown). Further examination whether *MATIA* 3U1510 modulated the effect of dietary fatty acid intake on Hcy revealed a significant interaction between the ratio of dietary n-3:n-6 and 3U1510 for plasma Hcy ($p = 0.025$) (Suppl. Fig. 1). When dietary n-3:n-6 ratio was low (< 0.09), there was no significant difference between the genotypes in plasma Hcy ($p = 0.996$), but when dietary n-3:n-6 ratio intake was high (> 0.09), homozygotes for the G allele had significantly lower plasma Hcy than did A carriers ($p = 0.019$).

To examine the combined effect of *MATIA* genetic variants on plasma Hcy, we performed haplotype analysis based on three *MATIA* variants (i15172, 3U1510, and d18777). For these three SNPs, we observed four haplotypes: GGG, GAG, AGA, GGA, with frequencies ranging from 0.09 to 0.38, accounting for 87% of all haplotypes in the population (Supplementary Table 1). *MATIA* haplotypes displayed a significant global association with plasma Hcy ($p = 0.003$). A strong interaction between AGA haplotype and dietary n-3:n-6 ratio on plasma Hcy was observed (Table 4, $p = 0.002$), whereas other haplotypes did not display significant interactions with dietary n-3:n-6 ratio for plasma Hcy (Table 4). We also found a significant interaction between dietary MUFA and haplotype AGA for plasma Hcy ($p = 0.027$, Supplementary Fig. 2). When daily intake of total MUFA was high ($\geq 11.35\%$ energy/d), those carrying the AGA haplotype had significantly higher plasma Hcy than non-carriers ($p = 0.031$, Supplementary Fig. 2). We did not find an interaction between other fatty acids and *MATIA* haplotypes for plasma Hcy (data not shown).

Discussion

In the present study we report that dietary n-6 PUFA and n-3:n-6 PUFA ratio were significantly associated with plasma Hcy concentration, although no difference in plasma Hcy based on *MATIA* genotype was detected. We also identified interactions of dietary n-3:n-6 ratio and MUFA with *MATIA* variants (SNP or haplotypes) in relation to plasma Hcy concentration. While these interactions have not been reported previously, several other studies provide evidence to support our findings (below).

Based on cardioprotective effects ascribed to n-3 PUFA and vascular damage attributed to plasma Hcy [3], a growing number of recent studies have investigated the relationship between n-3 PUFA and Hcy metabolism. In the present study, we demonstrated that a high dietary n-3:n-6 ratio was significantly associated with lower plasma Hcy, which is consistent with our previous report showing that plasma Hcy concentration was significantly negatively correlated with plasma phospholipid (PL) concentration (mg/100 mL) of total n-3 ($r = -0.270$, $p = 0.002$) and with the n-3:n-6 ratio ($r = -0.265$, $p = 0.002$) [12]. In sex-, age- and BMI-controlled partial correlations, the plasma Hcy concentration was significantly negatively correlated with platelet PL 22:6 n-3 and with n-3:n-6 ratio ($p < 0.01$), and positively correlated with 22:4 n-6 ($p < 0.05$) [13]. These results suggest that increased consumption of dietary n-3 PUFA may be associated with lower plasma Hcy. A subsequent

intervention study supported this hypothesis by demonstrating that n-3 PUFA decreased plasma Hcy concentrations in participants with diabetes and dyslipidemia who were treated with a statin-fibrate combination [24]. Furthermore, plasma Hcy was significantly decreased in acute myocardial infarction survivors after one year of n-3 PUFA treatment [14]. In a third study, consumption of n-3 PUFA supplements (3 g/day) over a two-month period decreased Hcy in participants with diabetes with no change in fasting blood sugar, malondialdehyde, or C-reactive protein [25].

Collectively, earlier studies provide strong support for n-3 PUFA in regulating plasma Hcy concentration, but results have not been entirely consistent [15]. Some intervention studies have observed lower plasma Hcy after n-3 PUFA supplementation [14,24], while other studies did not show beneficial effects of n-3 PUFA on plasma Hcy [15]. We hypothesize that variation in genes which encode critical enzymes in the metabolism of methionine may underlie these inconsistent results, and that intake of fatty acids may modulate Hcy through its regulation of enzyme activity and gene expression. MAT catalyzes the formation of S-adenosylmethionine from methionine and ATP. Defects in *MAT1A* which partially inactivate MAT activity [26] are one cause of hypermethioninemia [26] and this may, therefore be expressed differentially through *MAT1A* genotypes by dietary fatty acid interactions. Dietary factors may also alter MAT function, as demonstrated in our earlier animal study which showed that n-3 PUFA up-regulated *MAT* gene expression and enzyme activity [27]. While we did not observe *MAT1A* 3U1510 genotype-associated differences in plasma Hcy in the current study, we did show that *MAT1A* 3U1510 significantly interacted with the dietary n-3:n-6 ratio in modulating plasma Hcy. Further, *MAT1A* haplotypes displayed strong interaction with n-3:n-6 ratio - carriers of the risk haplotype (AGA) tend to have higher plasma Hcy when the ratio is low compared to non-carriers. These results support our hypothesis that *MAT1A* genotype may modulate the regulatory effect of n-3 PUFA or n-6 PUFA on Hcy metabolism. Moreover, we observed that *MAT1A* i15752 interacted with total fat and total MUFA, and that *MAT1A* d18777 interacted with total fat, SFA, MUFA and total PUFA in modulating plasma Hcy. Although total fat, SFA, MUFA and total PUFA were not associated with altered Hcy metabolism independent of genotype, these dietary fat intakes displayed significant interaction with *MAT1A* i15752 and d18777 genotypes on plasma Hcy concentrations.

There are, however, some limitations to the present study. First, the sample size is small, which limits statistical power. Second, we calculated the dietary fatty acids using a questionnaire previously adapted from the NCI/Block food frequency form for this population. Compared with plasma fatty acids, dietary fatty acids may have some limitation. Lastly, it is debatable that elevated Hcy could be a consequence of vascular disease, instead of a cause. Higher n-3 PUFA intake may reduce vascular pathology and thus reduce plasma Hcy concentration. Therefore, the mechanisms by which n-3 PUFA regulates plasma Hcy level remains to be illustrated.

In summary, the present study confirms earlier studies reporting the relationship between PUFA intake and plasma Hcy. Our results further suggest that interactions between *MAT1A* variants and dietary fatty acids modulate plasma Hcy concentration. In light of strong evidence that elevated plasma Hcy is an important risk factor for cardiovascular disease, understanding the role of dietary factors in the potential amelioration of genetically based risk of hyperhomocysteinemia is critical. Confirmation of these interactions will require further investigation in additional populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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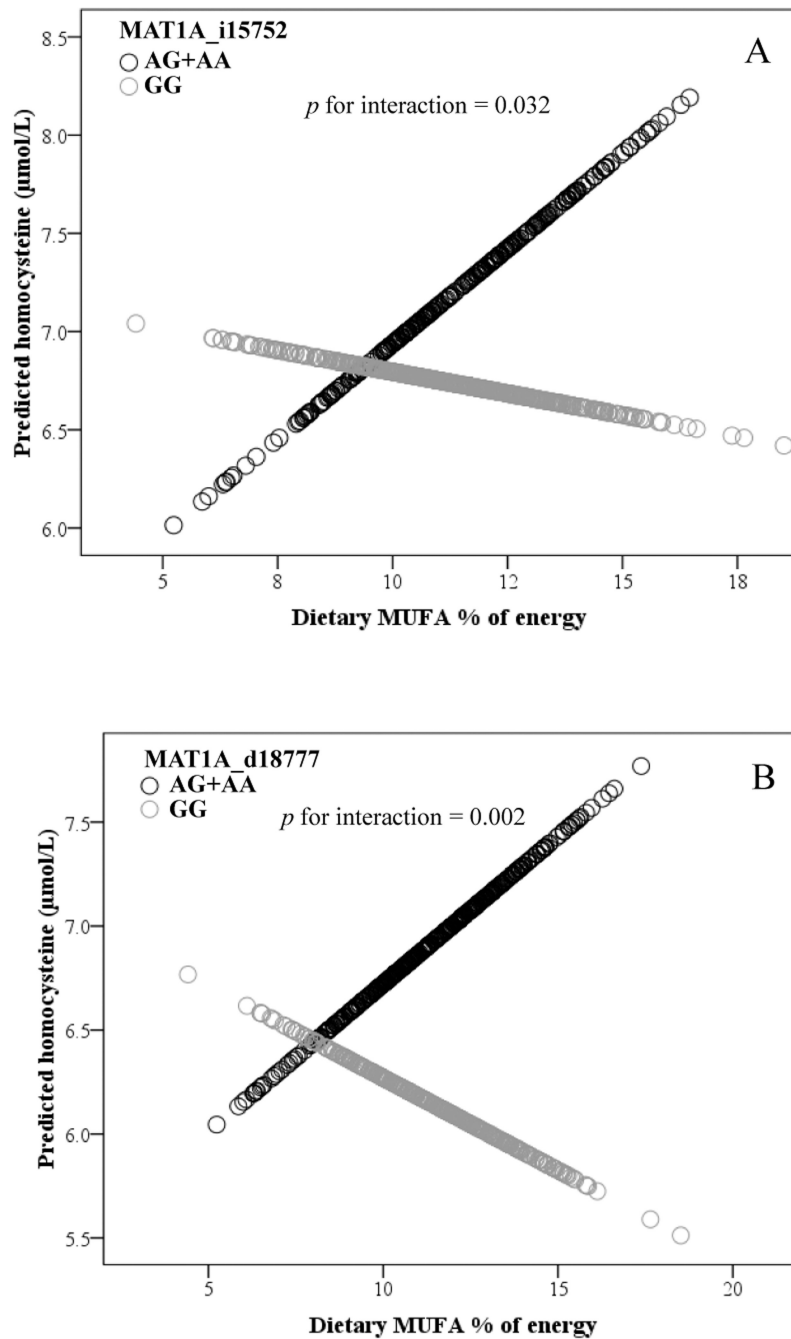


Figure 1.

A. Interaction between dietary MUFA and *MAT1A* i15752 on plasma homocysteine. Adjusted for age, sex, BMI, population admixture, smoking, alcohol use, dietary energy, plasma folate, plasma vitamin B₁₂ and plasma vitamin B₆, and genotypes of *MTHFR* 1298 A>C and *MTHFR* 677 C>T. Sample size: GG (n=360); AG+AA (n=532)

B. Interaction between dietary MUFA and *MAT1A* d18777 on plasma homocysteine: Adjusted for age, sex, BMI, population admixture, smoking, alcohol consumption, dietary energy, plasma folate, plasma vitamin B₁₂ and plasma vitamin B₆, and genotypes of *MTHFR* 1298 A>C and *MTHFR* 677 C>T. Sample size: GG (n=430); AG+AA(n=390).

Table 1

Demographic, anthropometric, biochemical data in the Boston Puerto Rican Health Study population

Characteristics of Participants	Men	Women
	n=292	n=702
Age, y	57.7 ± 7.6	57.9 ± 7.2
BMI, kg/m ²	29.7 ± 5.1	33.1 ± 7.0*
Smoker, n (%)	80 (31.12)	126 (19.84)*
Drinker, n (%)	132 (51.36)	219 (34.48)*
Alcohol, g/d	9.22 ± 30.43	1.55 ± 6.55*
Energy intake, kcal/d	2696 ± 1321	2175 ± 1115*
Total fat, % of energy	31.9 ± 5.4	30.8 ± 5.2*
Total SFA, % of energy	9.8 ± 2.5	9.3 ± 2.2
Total MUFA, % of energy	11.7 ± 2.1	11.2 ± 2.1
Total PUFA, % of energy	7.8 ± 1.7	7.6 ± 1.8
n-3 PUFA, % of energy	0.67 ± 0.16	0.68 ± 0.17
n-6 PUFA, % of energy	7.16 ± 1.61	6.93 ± 1.73
n-3:n-6 ratio	0.09 ± 0.02	0.10 ± 0.02
Plasma Folate, ng/mL	17.7 ± 8.7	20.1 ± 9.4*
Plasma Vitamin B ₁₂ , pg/mL	527 ± 276	550 ± 284
Plasma Vitamin B ₆ , nmol/L	61.4 ± 60.3	59.2 ± 63.3
Plasma Hcy, μmol/L	10.7 ± 6.2	8.8 ± 4.2*

Values are Mean ± SD or n (%).

* Significantly different from men ($p < 0.01$).

SFA: saturated fatty acid, MUFA: monounsaturated fatty acid, PUFA: polyunsaturated fatty acid

Table 2

Association between dietary fatty acids and plasma homocysteine by quartile of dietary fatty acids.

% of energy	plasma homocysteine level [†] (μmol/L)				p-value (trend)
	Q ₁	Q ₂	Q ₃	Q ₄	
Total Fat	9.30 ± 3.73	9.14 ± 3.89	8.84 ± 3.20	9.64 ± 4.59	0.183
Total SFA	9.44 ± 3.92	9.38 ± 3.92	8.87 ± 4.00	9.22 ± 3.69	0.404
Total MUFA	8.91 ± 3.41	9.46 ± 4.15	9.18 ± 3.66	9.38 ± 4.27	0.444
Total PUFA	9.01 ± 3.44	9.56 ± 4.21	8.84 ± 3.72	9.51 ± 4.11	0.127
n-3 PUFA	9.31 ± 3.84	9.18 ± 3.97	9.65 ± 4.33	8.74 ± 3.27	0.094
n-6 PUFA	8.94 ± 3.30	9.45 ± 4.13	8.77 ± 3.43	9.77 ± 4.52	0.025
n-3:n-6 ratio	9.65 ± 4.11	9.41 ± 4.51	9.12 ± 3.94	8.74 ± 2.77	0.048

The association was tested using general linear models.

All the data was presented in mean ± SD.

[†] Adjusted for age, sex, BMI, smoking, drinking, population admixture, diabetes, dietary energy, plasma vitamin B₁₂ and plasma vitamin B₆, *MTHFR* 1298 A>C, *MTHFR* 677 C>T.

Table 3
Interaction between *MAT1A* variants and dietary fatty acids on plasma homocysteine.

% of total energy	<i>MAT1A</i> i15752				<i>MAT1A</i> d18777				
	GG (n=360)	AG+AA (n=532)	p-value (trend)	p-value ¹ (interaction)	GG (n=430)	AG+AA (n=390)	p-value (trend)	p-value ¹ (interaction)	
Total Fat	≤ 31.31 ²	10.35 ± 0.27 ³	9.59 ± 0.34	0.047	0.015	10.73 ± 0.30	9.54 ± 0.29	0.001	<0.0001
	>31.31	9.23 ± 0.25	9.81 ± 0.34	0.158		8.89 ± 0.28	9.95 ± 0.28	0.006	
Total SFA	≤ 9.27	10.40 ± 0.27	9.82 ± 0.35	0.146	0.064	10.54 ± 0.30	9.85 ± 0.30	0.077	0.022
	>9.27	9.13 ± 0.25	9.58 ± 0.33	0.256		9.01 ± 0.28	9.54 ± 0.27	0.163	
Total MUFA	≤ 11.35	10.16 ± 0.26	9.47 ± 0.34	0.033	0.03	10.41 ± 0.30	9.51 ± 0.28	0.015	0.001
	>11.35	9.34 ± 0.26	9.87 ± 0.34	0.201		9.08 ± 0.28	9.89 ± 0.29	0.041	
Total PUFA	≤ 7.76	9.97 ± 0.25	9.53 ± 0.32	0.246	0.196	10.16 ± 0.29	9.53 ± 0.27	0.087	0.035
	>7.76	9.50 ± 0.26	9.70 ± 0.35	0.642		9.27 ± 0.29	9.75 ± 0.29	0.222	

¹ Adjusted for age, sex, BMI, population admixture, smoking, alcohol use, dietary energy, plasma folate, plasma vitamin B12, plasma vitamin B6, and genotypes of *MTHFR* 1298 A>C and *MTHFR* 677 C>T.

² Dichotomized values for fatty acids are adjusted for energy using the residuals method.

³ Data are expressed as mean ± SE.

Table 4

Interactions between *MAT1A* haplotypes and dietary n-3:n-6 ratio for plasma homocysteine

<i>MAT1A</i> haplotypes ¹	<i>MAT1A</i> haplotype			<i>p</i> -value (interaction) ⁴
	n-3:n-6 ratio	Carrier (n)	Non-Carrier (n)	
GGG	≤ 0.09 ²	9.93 ± 0.29 ³ (254)	9.89 ± 0.32 (187)	0.904
	>0.09	9.26 ± 0.22 (267)	9.23 ± 0.28 (175)	0.933
GAG	≤ 0.09	9.63 ± 0.44 (259)	9.98 ± 0.27 (182)	0.439
	>0.09	9.20 ± 0.42 (263)	9.47 ± 0.27 (179)	0.458
AGA	≤ 0.09	10.09 ± 0.36 (301)	9.67 ± 0.24 (141)	0.285
	>0.09	9.19 ± 0.37 (301)	9.47 ± 0.24 (140)	0.472
GGA	≤ 0.09	9.91 ± 0.51 (371)	9.80 ± 0.21 (70)	0.572
	>0.09	9.52 ± 0.53 (359)	9.33 ± 0.21 (83)	0.892

¹ *MAT1A* haplotypes were estimated based on three SNPs in the order: i15172, 3U1510, and d18777.

² Dichotomized values for n-3:n-6 ratio are adjusted for energy using the residuals method.

³ Data are expressed as mean ± SE.

⁴ Adjusted for age, sex, physical activity, drinking, smoking, population admixture, plasma folate, vitamin B₆, vitamin B₁₂, *MTHFR* 1298 A>C, *MTHFR* 677 C>T