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ORIGINAL ARTICLE

Interactions between lifestyle and *MTHFR* polymorphisms on homocysteine concentrations in young adults belonging to the 1982 Pelotas Birth Cohort

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BACKGROUND/OBJECTIVES: Homocysteine (Hcy) is a key intermediate in methionine metabolism. A high plasma concentration of Hcy is an independent risk factor for cardiovascular diseases among other determinants. In this study, we aimed to investigate the interactions between methylenetetrahydrofolate reductase enzyme gene *(MTHFR)* polymorphisms and lifestyle variables (smoking, alcohol intake and physical activity) on Hcy concentrations in a young Brazilian population.
SUBJECTS/METHODS: The study population comprised 3803 individuals from the Pelotas Birth Cohort, aged 22–23 years. Allelic discrimination assays and chemiluminescence immunoassays were performed for genotyping and serum Hcy measurements, respectively. Linear regression models were used to explore the effect of gene–lifestyle interactions on Hcy concentrations.
RESULTS: Men carrying the *MTHFR* 677TT genotype, who were also smokers and drinkers (⩾15 g of alcohol per day), had the highest concentration of Hcy (*P*-value for the interaction < 0.001 for smoking and 0.002 for alcohol intake). In contrast, high folate concentrations attenuated the effects of the *MTHFR C*677T genotype on serum Hcy concentrations (*P*-value for interaction < 0.001). Also, among males, blood folate concentration was the only lifestyle variable able to modify the influence of *MTHFR* A1298C genotypes on Hcy concentrations (*P*-value for the interaction < 0.001). There was no strong evidence of an interaction between the *MTHFR* genotypes and the lifestyle variables in women.
CONCLUSIONS: In summary, our study demonstrates a sex difference in Hcy concentrations among Brazilian young adults regarding *MTHFR* C677T–lifestyle interactions that are worsened under conditions of low blood folate. Identification of potentially modifiable factors related to an increase in homocysteine in young adults, especially in those who are genetically susceptible, is important to prevent negative health consequences in the future.

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INTRODUCTION

Homocysteine (Hcy) is a sulfur-containing amino acid produced by the conversion of methionine, an essential amino acid present in foods and regularly consumed in the diet. Hcy acts as a key intermediate in one-carbon metabolism being metabolized through two vitamin B-dependent pathways, which are controlled by three key enzymes—methylenetetrahydrofolate reductase (MTHFR), methionine synthase and cystathionine beta-synthase[1]

A high blood concentration of Hcy has been recognized as an independent risk factor for cardiovascular diseases in observational studies,[2–4] and is also associated with several other disorders such as neural tube defects and non-syndromic oral clefts.[5,6] Although the benefits from homocysteine lowering interventions for cardiovascular diseases have not been confirmed,[7] it is plausible that the positive effect is exclusively related to stroke risk.[8] The inconsistency related to hyperhomocysteinemia as a cause of cardiovascular diseases could result from the interactions between nutrients, metabolic and genetic factors.[9,10]

Hcy concentrations depend on age, sex and lifestyle variables, such as smoking, alcohol intake, physical activity and nutritional status, especially related to folate and vitamin B deficiencies.[2,4,11,12] As recently discussed by Reilly *et al*,[13] on the basis of a meta-analysis of randomized trials, a reduction in Hcy concentrations by approximately 25% was demonstrated after supplementation with folic acid, the synthetic form of folate. In addition, single-nucleotide polymorphisms (SNPs) that have an impact on Hcy concentrations are described.[14] The *MTHFR* C677T (rs1801133), corresponding to a C to T substitution at nucleotide 677 in exon 4 of the *MTHFR* gene, causes an alanine to valine (Ala222Val) change, producing a thermolabile form of the enzyme with reduced activity. Therefore, the homozygous form of the variant allele, *MTHFR* 677TT genotype, is associated with higher blood concentrations of Hcy than carriers of the 677CC or the 677CT genotypes. The influence of the *MTHFR* C677T polymorphism was clearly confirmed in genome-wide association studies.[15,16] A second polymorphism of the same gene is located in the exon 7, the *MTHFR* A1298C (rs1801131). This polymorphism results in a glutamine to alanine (Glu429Ala) change, which is also associated with decreased enzyme activity but has a weaker effect compared with the *MTHFR* C677T polymorphism. Furthermore, a recent meta-analysis reported that the *MTHFR* C677T polymorphism increased the risk of ischemic stroke in adults, especially in

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regions with low dietary folate consumption.[8] Taking into account that ischemic stroke comprises almost 90% of cerebrovascular diseases and is the third cause of mortality following cardiac diseases and cancer,[17] this finding highlights the necessity of lowering Hcy by improving patients' nutritional status.

This study aimed to assess the interactions between *MTHFR* polymorphisms and lifestyle variables, such as smoking, alcohol intake, physical activity and blood folate, on blood homocysteine concentrations, among young Brazilian adults.

MATERIALS AND METHODS

Study population

This is a cross-sectional analysis based on the 1982 Pelotas Birth Cohort that included all births in the urban area of Pelotas, a city in Southern Brazil, in 1982. In this year, all maternity hospitals in the city were visited daily, and 99.2% of the births were identified. Those liveborns whose families lived in the urban area of the city were evaluated, and their mothers were interviewed ($n = 5914$). These subjects have been followed up on several occasions. Further details of the study methodology have been described elsewhere.[18] In 2004–2005, 4297 members of the cohort (mean age: 22.8 years, range: 21.9–23.7 years) were evaluated, who, added to the 282 members known to have died, represented a follow-up rate of 77.4%. The subjects (4297) answered a questionnaire, and 3831 individuals donated a venous blood sample. Owing to an insufficient volume of blood and obtaining a DNA sample being a priority in the 22–23-year follow-up, serum samples were obtained from only 3826 individuals.

Ethical issues

All phases of the 1982 Pelotas Birth Cohort Study (registration number 4.06.01.087) were approved by the Research Ethics Committee of the Federal University of Pelotas, which is affiliated with the Brazilian Federal Medical Council (ethical permission number 029/2003). Written informed consent was obtained from participating subjects during the 2004–2005 visit.

Biological and lifestyle variables

The serum Hcy concentrations were determined in 3821 samples by a chemiluminescence immunoassay using Immulite 1000, as described by the manufacturer (Siemens, Erlanger, Germany). The within-assay and inter-assay precision were 9.1 and 10.8%, respectively. A random sampling method was performed by selecting a subset of 2569 individuals for measuring the serum folate concentration using an Elecsys-2010 immunoassay analyzer (Roche-Hitachi, Tokyo, Japan). The within-assay and inter-assay precision were 2.5 and 3.6%, respectively. Owing to technical failures in laboratory measurements, missing data were observed in five samples of Hcy and six samples of folate, respectively.

The biological and lifestyle variables used in the present study were as follows: sex (male, female); self-reported skin color (white, black, brown or other); smoking (smokers were those individuals who reported smoking at least one cigarette every day in the last week); alcohol intake (reported by the number of drinks consumed per day: none (0 g/day), one drink (0.01–14.9 g/day), two drinks (15.0–29.9 g/day) or more than two drinks ($\geqslant$ 30.0 g/day)); and physical activity (leisure time physical activity was assessed by the long version of the International Physical Activity Questionnaire and calculated by adding the time spent walking and in other moderate physical activity and the time spent in vigorous activities (the latter was multiplied by two)) as active individuals ($\geqslant$150 min/week) or less active individuals ($<$ 150 min/week)).[19] Blood folate concentrations were divided into tertiles (tertile 1: 2.32–7.01; tertile 2: 7.02–9.51; tertile 3: 9.52–21.0 ng/ml) and reported as low (L), medium (M) and high (H) blood folate levels, respectively. All variables described above were obtained from the 1982 Pelotas Birth Cohort database: follow-up 22–23 years.

Genotyping

DNA extraction was performed on peripheral whole-blood leukocytes using the salting-out method based on Miller's protocol[20] from 3831 genomic DNA samples. The *MTHFR* C677T (rs1801133) and *MTHFR* A1298C (rs1801131) polymorphisms were genotyped using TaqMan pre-designed SNP Genotyping Assays, 'C_12028833_20' and 'C_850486_20', respectively, using a 7500 Fast Real-Time PCR System (Applied Biosystems-Life Technologies, Foster City, CA, USA). The reactions were performed in a total volume of 6 µl, as follows: 3 µl of Taqman PCR Master Mix (Applied Biosystems), 0.3 µl of assay mix, 2.2 µl of DNase/RNase-free water and 0.5 µl of DNA (20 ng). The standard reaction conditions were an initial denaturation step at 95°C for 10 min, followed by 40 cycles of denaturation at 94 °C for 15 s and annealing and extension at 60 °C for 1 min each. The genotyping repeatability was evaluated in 5% of randomly selected DNA samples, and we observed $>$99.9% of genotyping concordance for both polymorphisms. Missing data were related to lack of DNA amplification (*MTHFR* C677T, $n = 17$ and *MTHFR* A1298C, $n = 10$).

Statistical analysis

Statistical analyses were performed using Stata version 12.1 (Stata Corporation, College Station, TX, USA). The Hardy–Weinberg Equilibrium was tested for each SNP by the χ^2 test. Associations between the genotype frequencies and the studied lifestyle variables were estimated using crude and skin color-adjusted multinomial regression models. Homocysteine was log transformed, owing to its positively skewed distribution, and standardized (s.d. units). The mean differences in serum Hcy concentrations according to genotype and lifestyle variables were estimated using linear regression. Linear regression models were also used to explore the effect of SNP–environment interactions on serum Hcy concentrations. Subjects with missing data for homocysteine concentration or genotype (*MTHFR* C677T or *MTHFR* A1298C) were excluded from all analyses. Statistical significance was defined as $P < 0.05$.

RESULTS

A total of 3803 individuals (1905 males and 1898 females) were included in the analyses, except for analyses involving blood folate, which was conducted in a resulting subset of 2551 individuals (1230 males and 1321 females) (Figure 1). The serum Hcy concentrations according to the biological, lifestyle and genetic variables are summarized in Table 1. The mean serum Hcy concentrations were found within the normal range for 22–23-year-old individuals, and were higher in men than in women (9.5 (9.4, 9.6) vs 7.4 (7.2, 7.5), respectively $P < 0.001$). No difference according to self-reported skin color was observed in Hcy concentrations between males ($P = 0.438$) and females ($P = 0.406$). Smoking was related to higher Hcy concentrations in both sexes, males ($P = 0.003$) and females ($P = 0.001$). In contrast, alcohol intake was not associated with Hcy concentrations in males ($P = 0.135$) or in females ($P = 0.105$). Physical activity was positively associated with Hcy only in women, where more active women showed higher Hcy concentrations ($P = 0.018$). Blood

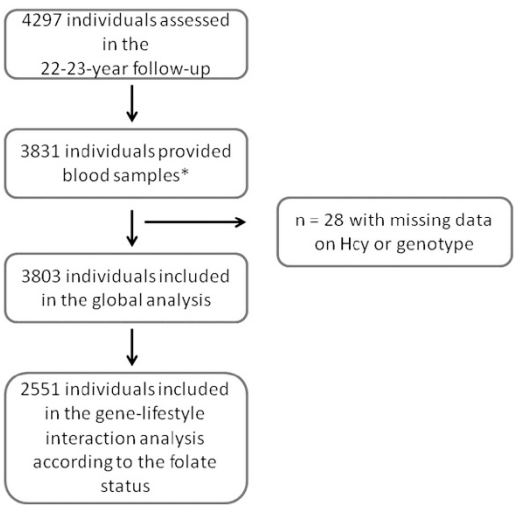

Figure 1. Flow diagram showing number of samples included in the present study (*2563 out of 3831 also had information on blood folate).

Table 1. Mean (95% CI) serum homocysteine (μmol/l) concentrations according to sex, lifestyle variables and *MTHFR* genotypes in the 1982 Pelotas Birth Cohort (RS, Brazil)

Variables	*Males*			*Females*		
	n	*Mean (95% CI)*	P[a]	n	*Mean (95% CI)*	P[a]
All	1905	9.5 (9.4, 9.6)		1898	7.4 (7.2, 7.5)	
Skin color			0.438			0.406
White	1419	9.4 (9.2, 9.6)		1427	7.4 (7.3, 7.5)	
Black	299	9.7 (9.3, 10.1)		311	7.5 (7.3, 7.8)	
Brown	113	9.9 (9.2, 10.6)		91	7.1 (6.6, 7.6)	
Other	74	9.5 (8.7, 10.4)		69	7.6 (7.0, 8.1)	
Smoking			0.003			0.001
No	1374	9.3 (9.1, 9.5)		1449	7.3 (7.2, 7.4)	
Yes	531	9.9 (9.6, 10.2)		449	7.7 (7.5, 7.9)	
Alcohol intake (g/day)			0.135			0.105
0	470	9.6 (9.2, 9.9)		767	7.3 (7.1, 7.4)	
0.01–14.9	944	9.3 (9.1, 9.6)		975	7.5 (7.3, 7.6)	
⩾15	491	9.8 (9.4, 10.1)		156	7.5 (7.1, 7.8)	
Physical activity[b]			0.518			0.018
Active	963	9.5 (9.2, 9.7)		371	7.6 (7.4, 7.9)	
Less active	942	9.6 (9.3, 9.8)		1527	7.3 (7.2, 7.4)	
Blood folate[c,d]			< 0.001			< 0.001
Low	383	10.7 (10.3, 11.1)		469	7.7 (7.5, 7.9)	
Medium	417	8.9 (8.6, 9.3)		432	6.9 (6.7, 7.1)	
High	430	8.5 (8.2, 8.9)		420	6.7 (6.5, 6.9)	
MTHFR C677T			< 0.001			< 0.001
CC	946	8.8 (8.6, 9.1)		881	7.1 (7.0, 7.3)	
CT	790	9.3 (9.1, 9.6)		837	7.4 (7.3, 7.6)	
TT	169	14.2 (13.6, 14.7)		180	8.6 (8.3, 9.0)	
MTHFR A1298C			< 0.001			0.141
AA	1012	9.9 (9.7, 10.1)		1047	7.5 (7.3, 7.6)	
AC	753	9.1 (8.8, 9.4)		710	7.3 (7.2, 7.5)	
CC	140	8.9 (8.3, 9.6)		141	7.1 (6.7, 7.5)	

[a]All *P*-values were obtained from linear regression models (*n* total = 3803). [b]Active individuals (⩾150 min/week); less active individuals (< 150 min/week). [c]Blood folate was available for a subsample of 1230 males and 1321 females (*n* total = 2551). [d]Blood folate was categorized as 'Low' (1° tertile = 2.32–7.01 ng/ml), 'Medium' (2° tertile = 7.02–9.51 ng/ml) and 'High' (3° tertile = 9.52–21.00 ng/ml).

folate was associated with Hcy concentrations in a dose-dependent manner in both sexes ($P < 0.001$). The *MTHFR* 677TT genotype increased serum Hcy concentrations by 60% in men and 20% in women compared with the CC genotype ($P < 0.001$). The other SNP of interest, *MTHFR* A1298C, resulted in a modest decrease of the Hcy concentrations: − 10% in men ($P < 0.001$) and − 5% in women ($P = 0.141$) for 1298CC vs 1298AA.

Both polymorphisms were in Hardy–Weinberg equilibrium (*MTHFR* C677T, $P = 0.64$; *MTHFR* A1298C, $P = 0.41$). The genotype frequencies in the whole population studied were 48% CC, 43% CT and 9% TT for *MTHFR* C677T, and 54% AA, 39% AC and 7%CC for *MTHFR* A1298C. These distributions were similar to previously published Brazilian population data.[21] Stratified analysis was used to assess the distribution of genotypes according to other variables. As expected, both polymorphisms were strongly associated with self-reported skin color ($P < 0.001$), and for that reason, subsequent analyses were adjusted for this variable. In general, adjusting for self-reported skin color did not substantially change the *P*-values. Blood folate was also strongly associated with polymorphism frequency, but only in men. No other variable (sex, smoking, alcohol intake and physical activity) was associated with the genotype distribution (Table 2).

In our study population, the *MTHFR* C677T polymorphism had a greater influence on serum Hcy concentrations than did the *MTHFR* A1298C polymorphism. We found an ~3.45 μmol/l increase in the blood Hcy concentrations of subjects carrying the 677TT genotype compared with those with the 677CC genotype. This difference was higher in men than in women (5.4 and 1.5 μmol/l, respectively).

In men, the *MTHFR* 677TT genotype combined with smoking or alcohol intake of 15 g or more per day was related to the highest concentrations of Hcy (*P*-value for the interaction < 0.001 for smoking and 0.002 for alcohol drinking). In contrast, high blood folate concentrations attenuated the effects of *MTHFR* C677T on blood Hcy concentrations (*P*-value for the interaction < 0.001). *MTHFR* A1298C interacted significantly only with blood folate (*P* for interaction < 0.001). However, men with high blood folate (9.52–21.0 ng/dl) had similar Hcy concentrations, regardless of the genotype (Table 3). In women, no strong evidence of SNP and environment interactions was found for smoking, alcohol intake or physical activity. However, women with the *MTHFR* 677TT genotype and low blood folate concentrations (2.32–7.01 ng/dl) had the highest serum Hcy concentrations (*P* for interaction = 0.052) (Table 4).

Table 2. Distribution of *MTHFR* C677T and A1298C genotypes according to gender and lifestyle variables in the 1982 Pelotas Birth Cohort (RS, Brazil)

Variables	*MTHFR C677T,* n *(%)*					*MTHFR A1298C,* n *(%)*				
	CC	*CT*	*TT*	*Crude* P-value	*Adjusted* P-value[a]	*AA*	*AC*	*CC*	*Crude* P-value	*Adjusted* P-value[a]
Sex				0.14	0.13				0.40	0.39
Male	946 (49.7)	790 (41.5)	169 (8.9)			1012 (53.1)	753 (39.5)	140 (7.4)		
Female	881 (46.4)	837 (44.1)	180 (9.5)			1047 (55.2)	710 (37.4)	141 (7.4)		
Skin color				< 0.001					< 0.001	
White	1278 (44.9)	1286 (45.2)	282 (9.9)			1463 (0)	1156 (0)	227 (0)		
Black	370 (60.7)	204 (33.4)	36 (5.9)			395 (64.8)	182 (29.8)	33 (5.4)		
Brown	112 (54.9)	76 (37.3)	16 (7.8)			127 (62.3)	69 (33.8)	8 (3.9)		
Other	67 (46.9)	61 (42.7)	15 (10.5)			74 (51.8)	56 (39.2)	13 (9.1)		
Smoking				0.27	0.43				0.50	0.77
No	1340 (47.5)	1229 (43.5)	254 (9)			1513 (53.6)	1097 (38.9)	213 (7.6)		
Yes	487 (49.7)	398 (40.6)	95 (9.7)			546 (55.7)	366 (37.4)	68 (6.9)		
Alcohol intake (g/day)				0.56	0.55				0.82	0.88
0	616 (49.8)	505 (40.8)	116 (9.4)			675 (54.6)	465 (37.6)	97 (7.8)		
0.01–14.9	904 (47.1)	839 (43.7)	176 (9.2)			1027 (53.5)	752 (39.2)	140 (7.3)		
⩾ 15.0	307 (47.5)	283 (43.7)	57 (8.8)			357 (55.2)	246 (38)	44 (6.8)		
Physical activity[b]				0.23	0.31				0.07	0.05
Active	665 (49.9)	555 (41.6)	114 (8.6)			696 (52.2)	546 (40.9)	92 (6.9)		
Less active	1162 (47.1)	1072 (43.4)	235 (9.5)			1363 (55.2)	917 (37.1)	189 (7.7)		
Blood folate[c,d]				< 0.001	< 0.001				0.48	0.47
Low	370 (43.4)	351 (41.2)	131 (15.4)			468 (54.9)	313 (36.7)	71 (8.3)		
Medium	443 (52.2)	344 (40.5)	62 (7.3)			469 (55.2)	317 (37.3)	63 (7.4)		
High	444 (52.2)	368 (43.3)	38 (4.5)			451 (53.1)	342 (40.2)	57 (6.7)		

All crude and adjusted *P*-values were derived from multinomial regression models ($n = 3803$). [a]Adjusted for skin color. [b]Active individuals (⩾150 min/week); less active individuals (< 150 min/week). [c]Blood folate was available for a subsample of 1230 males and 1321 females (n total = 2551). [d]Blood folate was categorized as 'Low' (1° tertile = 2.32–7.01 ng/ml), 'Medium' (2° tertile = 7.02–9.51 ng/ml) and 'High' (3° tertile = 9.52–21.00 ml).

To further explore the *MTHFR* C677T–environment interactions in males, we stratified the SNP-smoking and SNP-alcohol intake analyses according to the blood folate tertiles (low, medium and high). The highest mean Hcy concentrations (20.9 µmol/l, 95% CI (19.1, 22.8)) were observed in *MTHFR* 677TT smokers with low blood folate (2.32, 7.01 ng/dl). Additionally, the mean Hcy concentrations in this same group were significantly higher than those in *MTHFR* 677TT smokers with high blood folate (11.4 µmol/l, 95% CI (8.2, 14.5)) and in *MTHFR* 677TT nonsmokers with low blood folate (16.3 µmol/l, 95% CI (15.2, 17.4)) (Figure 2a). A similar scenario was applied to alcohol intake. *MTHFR* 677TT men who reported drinking 15 g or more of alcohol per day and who had low blood folate showed the highest mean Hcy concentrations (18.8 µmol/l, 95% CI (17.2, 20.4)), which was significantly different from *MTHFR* 677TT men with high alcohol intake but high blood folate (12.8 µmol/l, 95% CI (9.2, 16.4)), and also from men with no or low alcohol intake and low blood folate (16.9 µmol/l, 95% CI (15.7, 18.1)) (Figure 2b).

DISCUSSION

The results suggest that the interaction between smoking status, blood folate and the *MTHFR* 677TT genotype affects the serum Hcy concentrations in young men from a Brazilian population. At low blood folate concentrations, the *MTHFR* 677TT male subjects, including both smokers and nonsmokers, have higher Hcy serum concentrations than those carrying the 677CT or 677CC genotypes, and this difference was even higher among smokers. Similar interaction results were observed for alcohol intake.

The difference in blood Hcy between sexes is well documented.[22,23] There is a gradual increase of Hcy that begins from 10 years of age, continuing through puberty, and becoming higher in men than in women, but being less pronounced later in life.[3,24,25] Several studies have attributed this difference to sex hormones and fat-free mass.[22,26] However, in an elderly population from the Framingham Study,[27] the Hcy sex difference was explained by the intake of vitamins B_{12} and B_6, although this was not corroborated by the Hordaland Homocysteine Study.[2] In fact, the Hcy concentrations in aging adults could be explained by different mechanisms, such as decrease in enzymes involved in Hcy metabolism, decreased renal function and vitamin B deficiencies.[28]

Our results regarding the influence of lifestyle on blood Hcy concentrations are in agreement with previous studies that demonstrated an association between Hcy concentrations with smoking and folate status.[2,4,29] On the other hand, as the association between alcohol intake and Hcy is probably influenced by the type of alcoholic beverage, quantity and frequency of consumption, we were not able to describe an association in our study. Alcohol and Hcy were reported to be associated in a J-shaped curve in some studies,[4,30] but not in all, highlighting how complex this association could be. With regard to physical activity, the reports are also controversial. A protective effect between physical activity and Hcy,[2,31] no effect[32,33] or even an adverse effect similar to that observed in our study has been reported in the literature.[4] It is possible that the higher Hcy concentrations found in active women could be due to an increase in muscle mass compared with that in less

active women. An additional well-designed study is needed to elucidate the real effect of physical activity on blood Hcy concentrations.

The *MTHFR* C677T polymorphism detected a robust association between genotype and phenotype in our study, which was consistent with other reports.[14,34] It is noteworthy that the

Table 3. Mean (95% CI) serum homocysteine (Hcy) concentrations in males belonging to the 1982 Pelotas Birth Cohort (RS, Brazil) distributed according to *MTHFR* C677T and A1298C genotypes and lifestyle variables

Variables	*Hcy (μmol/l)*							
	MTHFR C677T				*MTHFR A1298C*			
	CC	*CT*	*TT*	P *for interaction*	*AA*	*AC*	*CC*	P *for interaction*
Smoking				< 0.001				0.237
No	8.8 (8.5, 9.1)	9.2 (8.9, 9.5)	13.3 (12.7, 14.0)		9.7 (9.4, 10.0)	9.0 (8.7, 9.3)	8.9 (8.2, 9.6)	
Yes	8.9 (8.4, 9.3)	9.7 (9.2, 10.1)	16.2 (15.3, 17.2)		10.4 (10.0, 10.8)	9.3 (8.8, 9.8)	9.2 (7.8, 10.5)	
Alcohol intake (g/day)				0.002				0.216
0	9.2 (8.8, 9.6)	9.3 (8.8, 9.9)	13.1 (12.0, 14.2)		9.8 (9.3, 10.3)	9.4 (8.8, 9.9)	9.2 (8.1, 10.4)	
0.01–14.9	8.6 (8.2, 8.9)	9.3 (8.9, 9.6)	13.9 (13.2, 14.6)		9.8 (9.4, 10.1)	8.9 (8.5, 9.3)	8.7 (7.9, 9.6)	
⩾15.0	8.9 (8.4, 9.3)	9.5 (9.0, 9.9)	15.9 (14.8, 16.9)		10.2 (9.8, 10.7)	9.2 (8.7, 9.8)	9.1 (7.7, 10.5)	
Physical activity[a]				0.962				0.275
Active	8.8 (8.4, 9.1)	9.3 (9.0, 9.7)	14.1 (13.4, 14.9)		9.9 (9.6, 10.3)	9.0 (8.6, 9.3)	8.7 (7.8, 9.7)	
Less active	8.9 (8.5, 9.2)	9.4 (9.0, 9.7)	14.3 (13.5, 15.0)		9.9 (9.5, 10.2)	9.2 (8.8, 9.6)	9.1 (8.3, 10.0)	
Blood folate[b,c]				< 0.001				< 0.001
Low	9.2 (8.7, 9.7)	9.8 (9.2, 10.4)	17.6 (16.6, 18.5)		11.8 (11.2, 12.4)	9.6 (9.0, 10.2)	8.9 (7.5, 10.2)	
Medium	8.4 (8.0, 8.9)	8.8 (8.3, 9.4)	12.6 (11.4, 13.8)		9.2 (8.6, 9.7)	8.7 (8.1, 9.3)	8.7 (7.3, 10.1)	
High	8.3 (7.8, 8.8)	8.7 (8.2, 9.2)	10.4 (8.6, 12.2)		8.6 (8.1, 9.1)	8.4 (7.8, 9.0)	8.7 (7.1, 10.3)	

Linear regression models adjusted for skin color were used to model the interaction between genetic variants and lifestyle variables and to predict adjusted means (and 95% CI) of Hcy concentration ($n = 3803$). [a]Active individuals (⩾150 min/week); less active individuals (< 150 min/week). [b]Blood folate was available for a subsample of 1230 males and 1321 females (n total = 2551). [c]Blood folate was categorized as 'Low' (1° tertile = 2.32–7.01 ng/ml), 'Medium' (2° tertile = 7.02–9.51 ng/ml) and 'High' (3° tertile = 9.52–21.00 ng/ml).

Table 4. Mean (95% CI) serum homocysteine (Hcy) concentrations in females belonging to the 1982 Pelotas Birth Cohort (RS, Brazil) distributed according to *MTHFR* C677T and A1298C genotypes and lifestyle variables

Variables	*Hcy (μmol/l)*							
	MTHFR C677T				*MTHFR A1298C*			
	CC	*CT*	*TT*	P *for interaction*	*AA*	*AC*	*CC*	P *for interaction*
Smoking								
No	7.0 (6.8, 7.2)	7.3 (7.2, 7.5)	8.5 (8.1, 8.9)	0.589	7.4 (7.2, 7.6)	7.2 (7.0, 7.4)	6.8 (6.4, 7.3)	0.237
Yes	7.4 (7.1, 7.7)	7.7 (7.4, 8.1)	9.2 (8.5, 9.8)		7.7 (7.4, 8.0)	7.7 (7.3, 8.0)	7.8 (7.1, 8.5)	
Alcohol intake (g/day)								
0	7.0 (6.8, 7.3)	7.2 (7.0, 7.5)	8.4 (7.9, 8.9)	0.678	7.3 (7.1, 7.5)	7.2 (7.0, 7.5)	6.8 (6.2, 7.4)	0.481
0.01–14.9	7.2 (6.9, 7.4)	7.5 (7.3, 7.7)	9.0 (8.5, 9.5)		7.6 (7.4, 7.8)	7.4 (7.2, 7.7)	7.1 (6.6, 7.7)	
⩾15.0	7.1 (6.6, 7.6)	7.8 (7.2, 8.3)	7.6 (6.4, 8.8)		7.4 (6.9, 7.9)	7.4 (6.7, 8.0)	8.0 (6.8, 9.1)	
Physical activity[a]								
Active	7.2 (6.9, 7.6)	7.8 (7.4, 8.1)	9.0 (8.2, 9.8)	0.388	7.7 (7.4, 8.0)	7.6 (7.2, 8.0)	7.3 (6.3, 8.2)	0.958
Less active	7.1 (6.9, 7.2)	7.3 (7.2, 7.5)	8.6 (8.2, 8.9)		7.4 (7.2, 7.6)	7.3 (7.1, 7.5)	7.1 (6.6, 7.5)	
Blood folate[b,c]				0.052				0.456
Low	7.2 (6.9, 7.5)	7.6 (7.3, 7.9)	9.1 (8.6, 9.6)		7.8 (7.5, 8.0)	7.5 (7.2, 7.9)	7.6 (6.9, 8.3)	
Medium	6.8 (6.5, 7.1)	7.0 (6.7, 7.3)	7.3 (6.5, 8.1)		6.9 (6.7, 7.2)	6.9 (6.6, 7.3)	6.9 (6.2, 7.7)	
High	6.5 (6.2, 6.8)	6.8 (6.5, 7.1)	8.0 (7.1, 8.8)		6.9 (6.6, 7.2)	6.5 (6.2, 6.8)	6.3 (5.6, 7.1)	

Linear regression models adjusted for skin color were used to model the interaction between genetic variants and lifestyle variables and to predict adjusted means (and 95% CI) of Hcy concentration ($n = 3803$). [a]Active individuals (⩾150 min/week) or less active individuals (< 150 min/week). [b]Blood folate was available for a subsample of 1230 males and 1321 females (n total = 2551). [c]Blood folate was categorized as 'Low' (1° tertile = 2.32–7.01 ng/ml), 'Medium' (2° tertile = 7.02–9.51 ng/ml) and 'High' (3° tertile = 9.52–21.00 ng/ml).

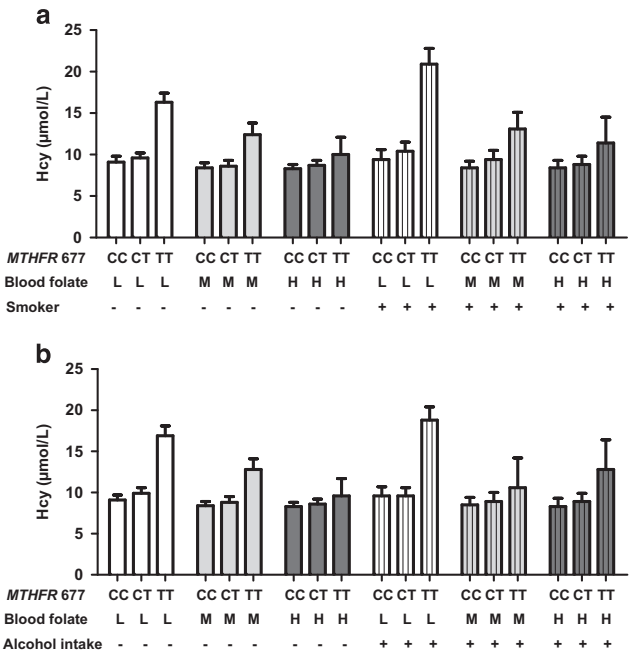

Figure 2. Mean (95% CI) concentrations of serum homocysteine (Hcy) in males of the 1982 Pelotas Birth Cohort (RS, Brazil) grouped according to their *MTHFR* C677T genotypes (CC, CT or TT), blood folate level (L: low (2.32–7.01 ng/ml); M: medium (7.02–9.51 ng/ml); H: high (9.52–21.00 ng/ml)) and smoking history ('–': non-smoker; '+': smoker) (**a**) or alcohol intake ('−': <1 drink/day; '+': $\geqslant 1$ drink/day) (**b**).

influence of this polymorphism was clearly documented in a reliable genome-wide association study of homocysteine.[16]

In the present study, we observed that smoking, alcohol intake and blood folate each interact with the *MTHFR* C677T in determining Hcy concentrations in males only. Concerning the MTHFR C677T/smoking interaction, we confirmed an increase of Hcy concentrations as reported previously in males.[35] However, results showing the same interaction in both sexes, were also reported.[32,36] The 677TT genotype in male smokers showed the highest effect on Hcy that was largely evident when the lowest folate level was present. The interaction was also present in the highest folate status. Although our results are in accordance with some studies,[35,37,38] in others the genetic effect was reported to be reduced after abundant intake of folate.[39,40] Different biological mechanisms have been proposed to explain this interaction because smoking may reduce the availability of folate for the remethylation of Hcy to methionine, induce local effects in exposed cells, change plasma thiol redox or even inhibit enzymes involved in the Hcy metabolism, further reducing the low enzyme activity related to the *MTHFR* C677T polymorphism itself.[22]

A significant interaction between the *MTHFR* 677TT genotype, alcohol intake and folate level in young males, but not in females, was also found in our study. A previous intervention study showed a similar result; however, only women were investigated.[41] To understand the difference observed between sexes in our sample, it is important to note that alcohol users were less likely to be female (44%) than were nonusers (62%). Therefore, there was probably not sufficient statistical power to demonstrate an effect. On the other hand, another study reported no evidence of an interaction of the *MTHFR C*677T and alcohol intake on the inverse relation between folate and Hcy.[42] It is important to mention that alcohol may interfere with folate metabolism by reducing intestinal folate absorption[30] or inhibiting the methionine synthase enzyme,[43] which is involved in the methyl group transfer (5-MTHF) to homocysteine. Alcohol could strengthen the effect of the *MTHFR* TT genotype on blood Hcy concentration.

Taking into account the difference observed among the studies, it is important to note that the genetic influence on Hcy blood concentrations seems to decrease with aging and is also more pronounced in men than in women.[44] The *MTHFR* 677TT genotype was found to account for 1.3% of the variance in Hcy concentrations among 50-year-old women and 18.7% of the variance in Hcy concentrations among 35-year-old men. In fact, it was proposed that with aging, lifestyle may have a greater influence on blood Hcy concentrations than genetic determinants. In a large-scale genome-wide association study from the USA, including subjects aged 54–61 years, the *MTHFR* C677T polymorphism and environmental factors were described to account for 1 and 9% of the observed variance on blood Hcy concentrations, respectively.[45] All the considerations above could suggest that the difference related to gene–lifestyle interactions between sexes start as early as 22–23 years.

The strengths of this study include the large sample size and the young age of the target adult population. Population structure confounding was controlled for by adjusting for self-reported skin color based on the knowledge that the Brazilian population is formed by an extensive mixture from three different ancestral roots (Amerindians, Europeans and Africans).[46]

Some limitations of our study include the lack of funding to cover the cost of blood folate measurements of all participants. Also, the lack of measurements of vitamins should be mentioned, especially riboflavin, which has been described as an important nutrient that influences disease risk associated with the MTHFR C677T polymorphism.[13] Furthermore, we do not have information about supplement use in the 22–23-year follow-up. Finally, we did not analyze different types of alcoholic beverages. Inconsistent findings may exist because the effect of alcohol depends on the type of alcoholic beverage, as beer is a rich source of folate and vitamin B_6, whereas red wine and spirits contain negligible amounts of these vitamins.[47]

In conclusion, the present study demonstrates a strong interaction between the *MTHFR* 677TT genotype and lifestyle variables and an increase in the risk of elevated blood Hcy concentrations in young adult males. Therefore, differences exist between gene–lifestyle interactions according to the sex, occurring earlier in men than in women. The identification of potentially modifiable factors related to an increase of homocysteine in young adults, especially in those genetically susceptible, is important to prevent negative health consequences in the future.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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AUTHORS CONTRIBUTION

IOO and DPG designed the study; LPS, OMC and JWT carried out the laboratory analysis of the data; MCB and JVSM contributed to the statistical analysis; IOO and LPS wrote the paper; DPG, BLH and FKS contributed to the critical revision of the manuscript. All authors read and approved the final manuscript.

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