The influence of one-carbon metabolism gene polymorphisms and gene-environment interactions on homocysteine, vitamin B12, folate and lipids in a Brazilian adolescent population

Original Article

The Influence of One-carbon Metabolism Gene Polymorphisms and Gene–environment Interactions on Homocysteine, Vitamin B12, Folate and Lipids in a Brazilian Adolescent Population

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Abstract

Background: Several single-nucleotide polymorphisms (SNPs) have been associated with the metabolism of Vitamin B12, folic acid, homocysteine and lipids. However, the interaction between SNPs involved in the one-carbon metabolism pathway and macronutrient intake on cardiovascular risk factors in the Brazilian population has not yet been investigated. Hence, the present study examined whether the association of ten SNPs involved in the one-carbon metabolism pathway with Vitamin B12, folic acid, homocysteine and lipid levels is modified by dietary factors and physical activity in adolescents with cardiovascular risk. **Materials and Methods:** A total of 113 adolescents (10–19 years old), from a public school in the city of Goiânia, Goiás, Brazil, underwent anthropometric, biochemical and food consumption evaluations and genetic tests. **Results:** After adjusting for potential confounders, SNPs rs4633 (catechol-O-methyltransferase, *COMT*), rs602662 (fucosyltransferase 2, *FUT2*) and rs1801394 (5-methyltetrahydrofolate-homocysteine methyltransferase reductase) showed significant associations with folic acid ($P = 0.042$), Vitamin B12 ($P = 0.009$) and oxidised low-density lipoprotein (ox-LDL) ($P = 0.041$) concentrations, respectively. The *COMT* SNP rs4680 showed a significant interaction with carbohydrate intake on ox-LDL concentrations ($P_{interaction} = 0.005$). In addition, the *FUT2* SNP rs602662 showed a significant interaction with protein intake on homocysteine concentrations ($P_{interaction} = 0.007$). However, after correction for multiple testing, none of these associations and interactions were statistically significant. **Conclusions:** For the first time, we provide evidence for the interactions between *COMT* SNP rs4680 and carbohydrate intake on ox-LDL levels and the *FUT2* SNP rs602662 and protein intake on homocysteine concentrations. However, replication of our results in a larger sample size is required to confirm our findings.

Keywords: Brazilian adolescents, carbohydrate, cardiovascular disease, hyperhomocysteinaemia, nutrigenetics, oxidised low-density lipoprotein

Introduction

Cardiovascular disease (CVD) has remained the leading cause of mortality in Brazil since the latter part of the 1960s.[1,2] Although effective tobacco control policies and access to improved healthcare have led to drastic improvements in cardiovascular health, an upwards trend in unhealthy eating habits and physical inactivity has been observed in the Brazilian population.[2] Smoking, obesity, hypertension, hyperlipidaemia and insulin resistance have long been recognised as major risk factors for CVDs;[3] however, the aetiology of CVD is not yet fully understood.[4] There has recently been renewed interest in the relationship between elevated homocysteine levels and the development of CVD.[5]

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Epidemiological studies have shown that hyperhomocysteinaemia is a well-known independent risk factor for atherosclerotic vascular disease and hypercoagulability states.[5] It is known to mediate adverse effects on vascular endothelium and smooth muscle cells.[6] In addition, hyperhomocysteinaemia reduces high-density lipoprotein (HDL) synthesis[7] and enhances the synthesis of lipoprotein A.[8] Some studies have indicated that up to 25% of coronary events may be attributed to the increase in homocysteine levels,[9] which have been shown to inversely correlate with B-complex vitamins, such as folate and Vitamin B12. Although B vitamins have a role in reducing blood homocysteine concentrations, the effect of these vitamins on cardiovascular function remains unclear.[10] A few studies have indicated that high folate and Vitamin B12 status are associated with a reduced risk of coronary heart disease.[11,12] Therefore, maintaining the concentrations of homocysteine, Vitamin B12, folate and lipids within the body is of grave importance.

The one-carbon metabolism pathway is a network of biochemical reactions involved in the transfer of single-carbon units (CH3 or methyl group), controlled by different enzymes and nutritional cofactors.[13] Cells require one-carbon units for nucleotide synthesis and methylation reactions. At present, common variants in genes of the one-carbon metabolism pathway have been reported to influence the concentrations of homocysteine, folate, Vitamin B12 and lipids.[14] A few studies have examined whether the association between genetic variants involved in the one-carbon metabolism pathway and homocysteine concentrations is modified by lifestyle factors such as diet.[15,16] However, no studies, to date, have examined the interaction between one-carbon metabolism-related genes and lifestyle factors on Vitamin B12, folate and lipid concentrations. Hence, seven genes involved in the one-carbon metabolism were selected for our study (betaine-homocysteine S-methyltransferase [*BHMT*], catechol-O-methyltransferase [*COMT*], fucosyltransferase 2 [*FUT2*], methylenetetrahydrofolate reductase [*MTHFR*], 5-methyltetrahydrofolate-homocysteine methyltransferase or methionine synthase [*MTR*], 5-methyltetrahydrofolate-homocysteine methyltransferase reductase or methionine synthase reductase [*MTRR*] and transcobalamin 2 [TCN2]).

The aims of the present study were to determine whether ten single-nucleotide polymorphisms (SNPs) from seven selected candidate genes related to the one-carbon metabolism cycle were associated with Vitamin B12, homocysteine, folic acid and lipid-related outcomes and whether these associations were modified by environmental factors (diet and physical activity). Interaction and association analyses were carried out in 113 adolescents with cardiovascular risk factors from the city of Goiânia, Goiás, Brazil.

Materials and Methods

Study participants

This cross-sectional study was conducted in a public school in the city of Goiânia, Goiás, Brazil, between March and May 2014. A total of 454 students were initially enrolled into the study, and 201 students were found to be eligible for participation. After screening through lifestyle, socioeconomic and clinical history, only 113 adolescents (aged 10–19 years) were selected to answer a food frequency record and provided a blood sample for biochemical and DNA analyses. Full details of the methodology have been explained previously.[17] Table 1 shows the characteristics of the study participants.

Participants were selected on the basis that they were overweight/obese and/or were previously diagnosed with dyslipidaemia, but not with CVD.[17] The presence of dyslipidaemia was identified by the use of specific medications or when the interviewees reported having hypercholesterolaemia or hypertriglyceridemia, previously diagnosed by a physician. Individuals were not included in the study if they were previously diagnosed with CVD, they used lipid-lowering drugs and they were supplemented with folic acid, cobalamin and/or pyridoxine and/or nutritional treatment.

The present study was approved by the Federal University of Goiás (addendum in protocol number 422.329, 07/10/2013). Informed consent was obtained from all the study participants, and participants were allowed to leave the study at will and opt out from any of the procedures. Written informed consent was obtained from the study participants whose age was above 18 years and, for those whose age was <18 years, consent was obtained from their parents or guardians. All clinical investigations were conducted according to the principles expressed in the Declaration of Helsinki.

Anthropometric and biochemical measurements

Details of anthropometric measurements have been described previously.[17] In brief, at baseline, all participants were measured for weight, height and waist circumference using standard study protocols. Body mass index (BMI) was estimated as weight (in kg) divided by the square of body height (m). BMI was classified according to the WHO (2007) classification for BMI/age according to sex.[18] Individuals below the 15th percentile were considered below normal weight, those between the 15th and 85th percentiles were classified as normal weight, those who fit between the 85th and 97th percentiles were classified as overweight and those above the 97th percentile were considered obese.

For the determination of biochemical parameters, blood samples (12 ml) were collected by peripheral venous puncture in the morning, after a 12-h fast. The blood samples were used to measure homocysteine, Vitamin B12, folic acid, lipid profile (including oxidised low-density lipoprotein [ox-LDL]) concentrations and for DNA extraction. Vitamin B12 and homocysteine concentrations were analysed using a chemiluminescence method. HDL-cholesterol (HDL-C) was determined after precipitation of the LDL and very-LDL (VLDL) fractions. The Friedewald formula was applied to obtain the measurement of LDL and VLDL cholesterol.[19] Plasma ox-LDL levels were measured

Table 1: The characteristics of the study participants stratified by sex

	All (n=113)	Boys (n=47)	Girls (n=66)	P*
Age (years)	13.87±2.37	13.32±2.35	14.26±2.32	0.037
Height (m)	1.62±0.11	1.63±0.14	1.61±0.08	0.205
Weight (kg)	63.53±17.59	66.36±20.13	61.51±15.37	0.169
BMI (kg/m^2)	24.01±4.92	24.33±5.07	23.79±4.84	0.567
Vitamin B12 (pg/ml)	519.80±232.15	534.62±252.96	509.24±217.50	0.569
Homocysteine (µmol/l)	7.04±2.99	7.90±2.91	6.42±2.92	0.009
Folic acid (ng/ml)	11.02±3.27	10.78±3.62	11.20±3.01	0.519
TAG (mg/dl)	94.05±54.16	99.00±59.00	90.53±50.61	0.415
Total cholesterol (mg/dl)	155.42±26.34	150.47±24.51	158.95±27.20	0.091
HDL (mg/dl)	46.29±11.79	43.60±11.47	48.21±11.72	0.040
LDL (mg/dl)	90.28±21.00	87.04±18.19	92.59±22.64	0.167
VLDL (mg/dl)	18.85±10.82	19.83±11.75	18.15±10.15	0.419
Oxidised-LDL (U/L)	6.42±13.69	5.92±11.47	6.77±15.12	0.749
Total energy (kcal/day)	2521.63±585.84	3010.08±594.92	2173.79±213.40	<0.0001
Carbohydrate intake (energy %)	47.70±20.59	40.86±19.56	52.56±20.05	0.003
Fat intake (energy %)	25.36±13.22	22.84±11.58	27.16±14.09	0.087
Protein intake (energy %)	16.99±8.38	14.56±6.94	18.72±8.92	0.006

*P values showing the differences in mean values between boys and girls. Data presented as mean±SD. P values were calculated by using Independent t-test. BMI: Body mass index, LDL: Low-density lipoprotein, HDL: High-density lipoprotein, VLDL: Very low-density lipoprotein, TAG: Triacylglycerol

using commercially available sandwich enzyme-linked immunosorbent assay (Mercodia AB, Uppsala, Sweden).[20]

Assessment of dietary intake and physical activity

The study participants undertook a food consumption record by trained research staff. This method was used to collect participant's usual food intake, highlighting household measures and portion sizes. All information provided by the participants was double checked for accuracy. Energy and nutrient intake from the recorded data were calculated based on the Avanutri® software (Avanutri Informática Ltda, Rio de Janeiro, Brazil), with emphasis on lipids, Vitamin B12 and folic acid. Wherever appropriate, nutrient intake values were adjusted to energy by the nutrient (energy adjusted) residual method.[21]

The Global Physical Activity Questionnaire, short form, was used to assess physical activity. Individuals were divided into physically active and inactive individuals.

Single-nucleotide polymorphism selection and genotyping

The following ten common SNPs involved in the one-carbon metabolism pathway were selected based on the published reports:[16,22-26] rs1801133 (677C>T) and rs1801131 (1298A>C) of *MTHFR*; rs1805087 (2756A>G) of *MTR*; rs1801394 (66A>G) of *MTRR*; rs1801198 (776G>C) of *TCN2*; rs4680 (158G>A) and rs4633 of *COMT*; rs3797546 and rs492842 of *BHMT* and rs602662 of *FUT2*. *COMT* rs4633,[13] *MTHFR* rs1801133 and *MTHFR* rs1801131 SNPs[1-4] are essential variants known to influence circulating homocysteine. While variations in the *BHMT* gene may contribute to hyperhomocysteinaemia,[5] it is unknown whether the SNPs rs3797546 and rs492842 alter homocysteine levels. Previous studies indicate that the SNPs *MTR* rs1805087, *MTRR* rs1801394,[14] *MTHFR* rs1801133 and *MTHFR* rs1801131[1-4] are associated with folate concentrations. Furthermore, genome-wide significant associations with serum B12 have been reported for the SNPs *TCN2* rs1801198[6] and *FUT2* rs602662.[4,7] The most commonly studied, *MTHFR* SNP rs1801133, has shown associations with total cholesterol, HDL-C and LDL-cholesterol (LDL-C).[8,9] Finally, the *COMT* rs4680 SNP was found to be associated with triacylglycerol (TAG),[10,11] total cholesterol and LDL-C levels.[12]

DNA was then extracted from peripheral leucocytes in the blood, using a commercial kit (Roche™ Diagnostics GmbH, Mannheim, Germany) following the manufacturer's guidelines accordingly. The purity and concentration of the DNA samples were assessed using a NanoDrop® ND-1000 spectrophotometer (Thermo Scientific, Wilmington, NC, USA). The ten SNPs involved in the one-carbon metabolism were genotyped using real-time polymerase chain reaction using the QuantStudio™ OpenArray TaqMan™ platform (Life Technologies, Foster City, CA, USA) with personalised cards for 12K Flex system QuantStudio® (Life Technologies) with validated TaqMan Assay. The frequency of each SNP in this study sample was in agreement with the Hardy–Weinberg equilibrium (P > 0.05) [Table 2]. All analyses were performed by an experienced laboratory technician who was blinded to the details of the study participants.

Statistical analysis

Statistical analyses were carried out using the SPSS software (version 22; SPSS Inc., Chicago, IL, USA). Data distribution was verified by the Shapiro–Wilk test. Individuals with BMI of ≥25 kg/m^2 were categorised as obese and those with a BMI of <25 kg/m^2 were classified as non-obese. Descriptive statistics for continuous variables were shown

Table 2: Genotype distribution of single-nucleotide polymorphisms involved in the one carbon-metabolism pathway

Gene symbol	SNP rs number	Major allele/ minor allele	Common homozygotes (%)	Heterozygotes (%)	Rare homozygotes (%)	Minor allele frequency	HWE (*P*)
MTHFR	rs1801131	A/C	56 (49.9)	43 (38.1)	13 (11.5)	0.31	0.29
MTHFR	rs1801133	C/T	55 (48.7)	41 (36.3)	12 (10.6)	0.30	0.31
MTR	rs1805087	A/G	77 (68.1)	32 (28.3)	2 (1.8)	0.16	0.52
MTRR	rs1801394	A/G	45 (39.8)	49 (43.4)	16 (14.2)	0.37	0.66
TCN2	rs1801198	G/C	60 (53.1)	33 (29.2)	10 (8.8)	0.26	0.10
COMT	rs4680	G/A	35 (31)	48 (42.5)	24 (21.2)	0.45	0.33
COMT	rs4633	C/T	44 (38.9)	51 (45.1)	16 (14.2)	0.37	0.84
BHMT	rs3797546	T/C	67 (59.3)	27 (23.9)	7 (6.2)	0.20	0.08
BHMT	rs492842	T/C	35 (31)	43 (38.1)	27 (23.9)	0.46	0.07
FUT2	rs602662	G/A	34 (30.1)	52 (46.0)	24 (21.2)	0.45	0.62

MTHFR: Methylene tetrahydrofolate reductase, *MTR*: 5-methyltetrahydrofolate-homocysteine methyltransferase, *MTRR*: 5-methyltetrahydrofolate-homocysteine methyltransferase reductase, *TCN2*: Transcobalamin 2, *COMT*: Catechol-O-methyltransferase, *BHMT*: Betaine-homocysteine methyltransferase, *FUT2*: Fucosyltransferase 2, HWE: Hardy–Weinberg equilibrium, SNPs: Single-nucleotide polymorphisms

as means and standard deviation (SD). The mean differences between continuous variables and the genotypes were analysed by the independent sample *t*-test.

Linear regression was used to examine the association of the SNPs involved in the one-carbon metabolism pathway with Vitamin B12, folic acid, homocysteine and lipid concentrations (TAG, HDL-C, LDL-C and ox-LDL). The interaction between the SNPs and dietary factors on determining Vitamin B12, folic acid, homocysteine and lipid concentrations was determined by including the interaction term (SNP * diet) in the general linear regression models. Models were adjusted for age, sex, BMI and total energy intake, wherever appropriate. The dominant model was applied only for those SNPs which had a frequency of rare homozygotes ≤19%. Correction for multiple testing was applied using Bonferroni correction (adjusted *P* value for association was <0.00071 [10 SNPs *7 outcomes (B12, folic acid, homocysteine, TAG, HDL-C, LDL-C and ox-LDL concentrations) = 70 tests]) and for interaction <0.00018 (10 SNPs *7 outcomes [B12, folic acid, homocysteine, TAG, HDL-C, LDL-C and ox-LDL concentrations] *4 lifestyle factors = 280 tests). All data were expressed as mean ± SD.

Power calculation

Given that there were no previously reported effect sizes, we were unable to perform a power calculation. However, based on the effect sizes that were observed for the associations, we performed a retrospective power calculation using the Quanto software, version 1.2.4 (May 2009, Department of Preventive Medicine, Keck School of Medicine, University of Southern California, 2001 N Soto Street, Los Angeles, CA 90032). Power calculations were carried out in the form of least detectable effects based on the assumption of significance levels and powers of 5% and 80%, respectively. At 80% power, the minimum detectable effects ranged from beta 7.5 U/L (ox-LDL) for a SNP with minor allele frequency (MAF) of 15% to beta 8.5 U/L for a SNP with MAF 50% for a sample size of 113 individuals.

Results

Characteristics of the participants

The clinical characteristics of the studied population are shown in Table 1. The sample consisted of 47 boys and 66 girls. The mean age ± SD of the student group was 13.32 ± 2.35 years for boys and 14.26 ± 2.32 years for girls. When the metabolite means were categorised by sex, plasma homocysteine and HDL concentrations were found to show significant differences between boys and girls ($P = 0.009$ and $P = 0.040$, respectively). In the study population, dietary intake of carbohydrate (energy %) and protein intake (energy %) were higher in girls than boys ($P = 0.003$ and $P = 0.006$, respectively), while there was no significant difference observed ($P = 0.087$) in dietary intake of fat (energy %) between girls and boys [Table 1].

Association between single-nucleotide polymorphisms and Vitamin B12, folic acid, homocysteine and lipid traits

When analysing associations between ten SNPs involved in genes related to the one-carbon metabolism cycle and biochemical indexes, we found that *COMT* rs4633 was significantly associated with folic acid ($P_{association} = 0.042$). Folic acid was significantly lower in CC common homozygous individuals (10.25 ± 2.99 ng/ml) than in pooled TT and CC individuals (11.67 ± 3.29 ng/ml) ($P_{association} = 0.042$) [Table 3]. Furthermore, homozygosity for the G allele at the *FUT2* rs602662 SNP was significantly associated with lower Vitamin B12 concentrations compared with the wild-type group where Vitamin B12 concentrations were 24.27% lower in GG individuals than in AA individuals ($P_{association} = 0.009$) [Table 3]. In addition to these findings, the minor allele (G) of the *MTRR* rs1801394 SNP was significantly associated with elevated ox-LDL levels ($P_{association} = 0.041$) [Table 3]. After Bonferroni correction, none of the results were considered statistically significant ($P > 0.00071$).

Interaction between single-nucleotide polymorphisms and Vitamin B12, folic acid and homocysteine

An interaction was observed between the *BHMT* SNP rs492842 and dietary fat intake on Vitamin B12 levels ($P = 0.034$). In addition, further interactions were found between the

Table 3: Association between single-nucleotide polymorphisms involved in the one-carbon metabolism pathway and Vitamin B12, homocysteine, folic acid and lipid traits

SNPs	MAF	Vitamin B12 (pg/ml)	Homocysteine (μmol/l)	Folic acid (ng/ml)	High-density lipoprotein cholesterol (mmol/l)	Low-density lipoprotein cholesterol (mmol/l)	Triglycerides (mmol/l)	Oxidised-low density lipoprotein cholesterol (U/L)
MTHFR gene (rs1801131)	0.31							
AA		524.23±223.49	6.90±2.96	11.26±3.34	47.18±12.84	87.55±21.54	95.05±62.54	7.90±18.65
A/C		520.21±241.74	7.19±3.07	10.87±3.20	45.68±10.60	92.54±20.21	92.73±45.32	5.07±5.67
Dominant model (AA vs. AC + CC) *P*		0.916	0.895	0.682	0.708	0.180	0.739	0.209
MTHFR gene (rs1801133)	0.30							
CC		562.36±238.63	6.80±3.00	11.51±3.18	46.04±10.33	90.67±22.87	86.33±46.07	4.28±5.21
C/T		491.42±224.74	7.37±3.00	10.75±3.37	46.25±13.14	89.08±19.51	100.26±61.36	8.95±19.12
Dominant model (CC vs. CT + TT) *P*		0.058	0.100	0.158	0.468	0.519	0.300	0.106
MTR gene (rs1805087)	0.16							
AA		523.97±221.74	7.22±3.29	11.03±3.31	46.64±12.73	89.42±20.73	92.83±58.11	6.70±14.34
A/G		518.59±518.59	6.77±2.21	11.08±3.27	45.82±9.78	91.71±22.29	95.44±46.26	6.01±12.72
Dominant model (AA vs. AG + GG) *P*		0.815	0.301	0.919	0.886	0.517	0.893	0.836
MTRR gene (rs1801394)	0.37							
AA		560.53±249.40	6.61±2.58	10.96±3.42	46.29±9.67	90.78±23.02	93.98±62.30	3.13±3.81
A/G		494.11±218.14	7.35±3.27	11.14±3.20	46.38±13.09	89.60±19.91	93.28±48.75	8.95±17.46
Dominant model (AA vs. AG + GG) *P*		0.265	0.394	0.508	0.827	0.814	0.958	0.041
TCN2 gene (rs1801198)	0.26							
GG		523.88±221.89	6.99±2.60	11.34±3.26	46.93±12.74	90.70±22.25	91.88±50.19	5.99±13.23
G/C		530.67±263.13	6.94±3.16	10.67±3.14	44.95±9.83	90.09±19.48	98.53±61.99	7.82±15.69
Dominant model (GG vs. GC + CC) *P*		0.982	0.751	0.231	0.213	0.776	0.463	0.497
COMT gene (rs4680)	0.45							
GG		495.54±234.10	6.98±3.20	10.02±2.81	44.00±10.67	86.71±20.17	102.14±65.53	5.14±6.30
GA		546.98±249.81	6.88±2.32	11.56±3.24	45.58±11.29	87.71±22.27	95.27±57.18	7.07±17.47
AA		515.50±181.22	7.08±3.94	11.59±3.61	49.21±13.83	96.83±17.83	81.13±27.52	7.20±14.86
Additive model *P*		0.825	0.843	0.094	0.258	0.186	0.376	0.658
COMT gene (rs4633)	0.37							
CC		511.77±248.82	6.98±3.28	10.25±2.99	45.05±10.87	87.91±19.92	99.84±61.18	4.99±6.00
C/T		532.07±221.84	7.00±2.77	11.67±3.29	47.25±12.36	90.99±21.46	89.36±49.63	7.54±17.11
Dominant model (CC vs. CT + TT) *P*		0.907	0.740	0.042	0.524	0.337	0.364	0.146
BHMT gene (rs3797546)	0.20							

Contd...

Table 3: Contd...

SNPs	MAF	Vitamin B12 (pg/ml)	Homocysteine (μmol/l)	Folic acid (ng/ml)	High-density lipoprotein cholesterol (mmol/l)	Low-density lipoprotein cholesterol (mmol/l)	Triglycerides (mmol/l)	Oxidised-low density lipoprotein cholesterol (U/L)
TT		536.31±233.97	7.04±3.18	11.51±3.33	46.21±11.33	89.57±21.17	94.55±56.16	6.68±15.25
T/C		523.41±224.50	6.53±2.03	10.54±2.90	46.59±12.48	92.18±21.76	99.29±54.96	6.74±12.72
Dominant model (TT vs. TC + CC) *P*		0.971	0.151	0.231	0.461	0.495	0.811	0.791
BHMT gene (rs492842)	0.46							
TT		554.89±254.53	6.65±2.66	11.92±3.21	47.57±11.81	85.91±24.16	90.83±43.39	6.47±12.79
TC		492.35±196.18	7.64±3.30	10.14±3.16	45.77±10.46	93.84±17.92	97.05±55.94	8.74±18.36
CC		469.70±201.74	6.91±3.14	11.27±3.35	44.26±11.06	89.37±21.64	99.07±68.56	3.34±4.95
Additive model *P*		0.293	0.602	0.095	0.872	0.179	0.915	0.220
FUT2 gene (rs602662)	0.45							
GG		471.41±193.77	6.93±3.35	11.47±3.31	44.68±10.01	84.50±20.31	90.12±54.27	8.21±13.26
GA		494.73±220.75	7.18±2.46	10.69±3.19	47.21±13.38	90.52±20.81	93.40±54.93	4.99±13.69
AA		622.50±268.60	6.84±3.64	11.15±3.40	46.29±10.37	97.79±21.98	104.17±56.13	7.42±15.24
Additive model *P*		0.009	0.677	0.620	0.622	0.063	0.664	0.374

Values are given as mean±SD. *P* values for differences between genotypes were obtained using linear regression model adjusted for age, sex and BMI. Adjusted *P* value after correction for multiple testing was 0.00071. MAF: Minor allele frequency, *MTHFR*: Methylene tetrahydrofolate reductase, *MTR*: 5-methyltetrahydrofolate-homocysteine methyltransferase, *MTRR*: 5-methyltetrahydrofolate-homocysteine methyltransferase reductase, *TCN2*: Transcobalamin 2, COMT: Catechol-O-methyltransferase, *BHMT*: Betaine-homocysteine methyltransferase, *FUT2*: Fucosyltransferase 2, SNPs: Single-nucleotide polymorphisms, SD: Standard deviation

FUT2 SNP rs602662 with dietary protein intake ($P = 0.007$) and carbohydrate intake ($P = 0.031$) on homocysteine concentrations [Table 4]. We found that rare AA homozygotes of the *FUT2* SNP rs602662 had higher homocysteine levels (mean ± standard error [SE]: 8.038 ± 0.896 μmol/L) compared to the GG allele carriers (mean ± SE: 5.857 ± 1.039 μmol/L) among those in the highest tertile of protein intake (mean ± SE: 148.618 ± 5.777 g/day); however, the difference in the means of homocysteine concentrations between the genotype groups in the highest tertile of protein intake was not statistically significant ($P = 0.227$), which could be because of the small sample size.

Interaction between single-nucleotide polymorphisms and dietary factors on lipid concentrations

Interactions were observed between the *COMT* SNPs (rs4680 and rs4633) and dietary carbohydrate intake on HDL-C concentrations ($P = 0.011$ and $P = 0.036$, respectively). Furthermore, an interaction was found between the *COMT* SNP (rs4680) and dietary carbohydrate intake on ox-LDL concentrations ($P = 0.005$) [Table 5]. However, none of the interactions between the SNPs and dietary intake on lipid outcomes reached statistical significance after correction for multiple testing.

Gene–physical activity interactions on Vitamin B12, folic acid, homocysteine and lipid profile

No statistically significant interactions were observed after correction for multiple testing between the ten SNPs and physical activity on Vitamin B12, folic acid, homocysteine and lipid traits ($P_{interaction} > 0.00018$) [Supplementary Table 1].

Discussion

To our knowledge, this is the first genetic epidemiological study to investigate the interactions between SNPs involved in the one-carbon metabolism pathway and environmental/lifestyle factors on Vitamin B12, folic acid, homocysteine and lipid levels (HDL-C, LDL, TAG and ox-LDL) in the Brazilian adolescent population. Our study provides evidence for novel interactions between SNP rs4680 (*COMT* gene) and carbohydrate intake on ox-LDL levels and the SNP rs602662 (*FUT2* gene) and protein intake on homocysteine concentrations in Brazilian adolescents. Given that ox-LDL and hyperhomocysteinaemia are well-known independent risk factors for atherosclerotic vascular disease,[5,27] our findings have significant public health implications.

Genes involved in one-carbon metabolism are of particular interest because of their role in CVDs.[28] From the ten SNPs which were investigated in this study, association of the SNP rs4633 at the *COMT* gene with folic acid concentrations ($P = 0.042$), the SNP rs602662 at the *FUT2* gene with Vitamin B12 levels ($P = 0.009$) and finally the SNP rs1801394 at the *MTRR* gene with ox-LDL concentrations ($P = 0.041$) was observed. Even though the findings were not significant after Bonferroni correction, the

Table 4: Interaction between single-nucleotide polymorphisms and dietary factors on Vitamin B12, homocysteine and folic acid traits

***P* values for the interaction between SNPs and dietary factors on Vitamin B12**		
Interaction between SNP rs1801131* fat energy intake	Interaction between SNP rs1801131* protein energy intake	Interaction between SNP rs1801131* carbohydrate energy intake
0.685	0.095	0.074
Interaction between SNP rs1801133* fat energy intake	Interaction between SNP rs1801133* protein energy intake	Interaction between SNP rs1801133* carbohydrate energy intake
0.429	0.067	0.115
Interaction between SNP rs1805087* fat energy intake	Interaction between SNP rs1805087* protein energy intake	Interaction between SNP rs1805087* carbohydrate energy intake
0.368	0.539	0.206
Interaction between SNP rs1801394* fat energy intake	Interaction between SNP rs1801394* protein energy intake	Interaction between SNP rs1801394* carbohydrate energy intake
0.733	0.070	0.743
Interaction between SNP rs1801198* fat energy intake	Interaction between SNP rs1801198* protein energy intake	Interaction between SNP rs1801198* carbohydrate energy intake
0.789	0.109	0.631
Interaction between SNP rs4680* fat energy intake	Interaction between SNP rs4680* protein energy intake	Interaction between SNP rs4680* carbohydrate energy intake
0.662	0.265	0.559
Interaction between SNP rs4633* fat energy intake	Interaction between SNP rs4633* protein energy intake	Interaction between SNP rs4633* carbohydrate energy intake
0.455	0.490	0.799
Interaction between SNP rs3797546* fat energy intake	Interaction between SNP rs3797546* protein energy intake	Interaction between SNP rs3797546* carbohydrate energy intake
0.353	0.979	0.281
Interaction between SNP rs492842* fat energy intake	Interaction between SNP rs492842* protein energy intake	Interaction between SNP rs492842* carbohydrate energy intake
0.034	0.678	0.331
Interaction between SNP rs602662* fat energy intake	Interaction between SNP rs602662* protein energy intake	Interaction between SNP rs602662* carbohydrate energy intake
0.087	0.144	0.533
***P* values for the interaction between SNPs and dietary factors on homocysteine**		
Interaction between SNP rs1801131* fat energy intake	Interaction between SNP rs1801131* protein energy intake	Interaction between SNP rs1801131* carbohydrate energy intake
0.806	0.803	0.625
Interaction between SNP rs1801133* fat energy intake	Interaction between SNP rs1801133* protein energy intake	Interaction between SNP rs1801133* carbohydrate energy intake
0.975	0.621	0.433
Interaction between SNP rs1805087* fat energy intake	Interaction between SNP rs1805087* protein energy intake	Interaction between SNP rs1805087* carbohydrate energy intake
0.123	0.922	0.389
Interaction between SNP rs1801394* fat energy intake	Interaction between SNP rs1801394* protein energy intake	Interaction between SNP rs1801394* carbohydrate energy intake
0.252	0.645	0.456
Interaction between SNP rs1801198* fat energy intake	Interaction between SNP rs1801198* protein energy intake	Interaction between SNP rs1801198* carbohydrate energy intake
0.869	0.212	0.341
Interaction between SNP rs4680* fat energy intake	Interaction between SNP rs4680* protein energy intake	Interaction between SNP rs4680* carbohydrate energy intake
0.062	0.189	0.054
Interaction between SNP rs4633* fat energy intake	Interaction between SNP rs4633* protein energy intake	Interaction between SNP rs4633* carbohydrate energy intake
0.596	0.359	0.133
Interaction between SNP rs3797546* fat energy intake	Interaction between SNP rs3797546* protein energy intake	Interaction between SNP rs3797546* carbohydrate energy intake
0.713	0.614	0.209

Contd...

Table 4: Contd...

***P* values for the interaction between SNPs and dietary factors on homocysteine**		
Interaction between SNP rs492842* fat energy intake	Interaction between SNP rs492842* protein energy intake	Interaction between SNP rs492842* carbohydrate energy intake
0.232	0.227	0.606
Interaction between SNP rs602662* fat energy intake	Interaction between SNP rs602662* protein energy intake	Interaction between SNP rs602662* carbohydrate energy intake
0.334	0.007	0.031
***P* values for the interaction between SNPs and dietary factors on folic acid**		
Interaction between SNP rs1801131 * fat energy intake	Interaction between SNP rs1801131* protein energy intake	Interaction between SNP rs1801131* carbohydrate energy intake
0.378	0.642	0.774
Interaction between SNP rs1801133* fat energy intake	Interaction between SNP rs1801133* protein energy intake	Interaction between SNP rs1801133* carbohydrate energy intake
0.595	0.587	0.722
Interaction between SNP rs1805087* fat energy intake	Interaction between SNP rs1805087* protein energy intake	Interaction between SNP rs1805087* carbohydrate energy intake
0.834	0.887	0.498
Interaction between SNP rs1801394* fat energy intake	Interaction between SNP rs1801394* protein energy intake	Interaction between SNP rs1801394* carbohydrate energy intake
0.641	0.826	0.327
Interaction between SNP rs1801198* fat energy intake	Interaction between SNP rs1801198* protein energy intake	Interaction between SNP rs1801198* carbohydrate energy intake
0.845	0.759	0.547
Interaction between SNP rs4680* fat energy intake	Interaction between SNP rs4680* protein energy intake	Interaction between SNP rs4680* carbohydrate energy intake
0.610	0.495	0.228
Interaction between SNP rs4633* fat energy intake	Interaction between SNP rs4633* protein energy intake	Interaction between SNP rs4633* carbohydrate energy intake
0.721	0.248	0.050
Interaction between SNP rs3797546* fat energy intake	Interaction between SNP rs3797546* protein energy intake	Interaction between SNP rs3797546* carbohydrate energy intake
0.188	0.394	0.754
Interaction between SNP rs492842* fat energy intake	Interaction between SNP rs492842* protein energy intake	Interaction between SNP rs492842* carbohydrate energy intake
0.084	0.971	0.447
Interaction between SNP rs602662* fat energy intake	Interaction between SNP rs602662* protein energy intake	Interaction between SNP rs602662* carbohydrate energy intake
0.521	0.775	0.115

P values were obtained by using a general linear model adjusted for age, sex and BMI. SNPs: Single-nucleotide polymorphisms, BMI: Body mass index

association between the *FUT2* SNP rs602662 and Vitamin B12 concentrations is in accordance with previous studies.[14,22,29-33] Since the current sample size is relatively small, further studies utilising a larger sample size are required to confirm the observed associations.

To date, only one study has shown a gene–diet interaction on ox-LDL concentrations in a population from the Attica region in Greece.[34] In this study, there was an interaction of the *MTHFR* SNP rs1801133 with the Mediterranean diet on ox-LDL concentrations. A high adherence to the Mediterranean diet was found to be associated with decreased ox-LDL concentrations in T allele carriers of SNP rs1801133.[34] Further to this, many studies have reported that *MTHFR* variants (C677T and A1298C) are linked to higher homocysteine levels when folate consumption is low.[35,36] In the present study, we identified significant gene–diet interactions between SNP rs4680 at the *COMT* gene and carbohydrate intake on ox-LDL concentrations and the SNP rs602662 at the *FUT2* gene and protein intake on homocysteine concentrations. However, further stratification of participants based on their consumption of low-, medium- and high-dietary carbohydrate/protein did not show a statistically significant association between the SNP and the outcome in any of the tertiles, which could account for the small sample size. This is the first study to provide evidence for gene–diet interactions at the *COMT* and *FUT2* gene loci, on ox-LDL and homocysteine concentrations, respectively, and hence, we do not have any previous studies to compare our findings.

Total carbohydrate intake has increased considerably in Brazil in the last few decades.[37] Data from two population-based surveys conducted in women over 35 years of age from Rio de Janeiro reported that the carbohydrate intake has increased from 352 g (95% confidence interval [CI]: 325–382) in 1995 to 437 g (95% CI: 415–458) in 2005.[37] Interestingly, our study in this Brazilian adolescent population has identified

Table 5: Interaction between single-nucleotide polymorphisms and dietary factors on lipid traits

Interaction between SNPs and dietary factors on HDL-C		
Interaction between SNP rs1801131 * fat energy intake	Interaction between SNP rs1801131* protein energy intake	Interaction between SNP rs1801131* carbohydrate energy intake
0.964	0.402	0.899
Interaction between SNP rs1801133* fat energy intake	Interaction between SNP rs1801133* protein energy intake	Interaction between SNP rs1801133* carbohydrate energy intake
0.393	0.471	0.994
Interaction between SNP rs1805087* fat energy intake	Interaction between SNP rs1805087* protein energy intake	Interaction between SNP rs1805087* carbohydrate energy intake
0.651	0.298	0.499
Interaction between SNP rs1801394* fat energy intake	Interaction between SNP rs1801394* protein energy intake	Interaction between SNP rs1801394* carbohydrate energy intake
0.896	0.712	0.676
Interaction between SNP rs1801198* fat energy intake	Interaction between SNP rs1801198* protein energy intake	Interaction between SNP rs1801198* carbohydrate energy intake
0.414	0.822	0.649
Interaction between SNP rs4680* fat energy intake	Interaction between SNP rs4680* protein energy intake	Interaction between SNP rs4680* carbohydrate energy intake
0.898	0.536	0.011
Interaction between SNP rs4633* fat energy intake	Interaction between SNP rs4633* protein energy intake	Interaction between SNP rs4633* carbohydrate energy intake
0.846	0.620	0.036
Interaction between SNP rs3797546* fat energy intake	Interaction between SNP rs3797546* protein energy intake	Interaction between SNP rs3797546* carbohydrate energy intake
0.274	0.162	0.555
Interaction between SNP rs492842 fat energy intake	Interaction between SNP rs492842* protein energy intake	Interaction between SNP rs492842* carbohydrate energy intake
0.604	0.960	0.513
Interaction between SNP rs602662* fat energy intake	Interaction between SNP rs602662* protein energy intake	Interaction between SNP rs602662* carbohydrate energy intake
0.650	0.123	0.813
Interaction between SNPs and dietary factors on LDL-C		
Interaction between SNP rs1801131 * fat energy intake	Interaction between SNP rs1801131* protein energy intake	Interaction between SNP rs1801131* carbohydrate energy intake
0.529	0.467	0.798
Interaction between SNP rs1801133* fat energy intake	Interaction between SNP rs1801133* protein energy intake	Interaction between SNP rs1801133* carbohydrate energy intake
0.640	0.656	0.737
Interaction between SNP rs1805087* fat energy intake	Interaction between SNP rs1805087* protein energy intake	Interaction between SNP rs1805087* carbohydrate energy intake
0.456	0.933	0.876
Interaction between SNP rs1801394* fat energy intake	Interaction between SNP rs1801394* protein energy intake	Interaction between SNP rs1801394* carbohydrate energy intake
0.487	0.384	0.222
Interaction between SNP rs1801198* fat energy intake	Interaction between SNP rs1801198* protein energy intake	Interaction between SNP rs1801198* carbohydrate energy intake
0.127	0.664	0.250
Interaction between SNP rs4680* fat energy intake	Interaction between SNP rs4680* protein energy intake	Interaction between SNP rs4680* carbohydrate energy intake
0.509	0.709	0.299
Interaction between SNP rs4633* fat energy intake	Interaction between SNP rs4633* protein energy intake	Interaction between SNP rs4633* carbohydrate energy intake
0.743	0.915	0.067
Interaction between SNP rs3797546* fat energy intake	Interaction between SNP rs3797546* protein energy intake	Interaction between SNP rs3797546* carbohydrate energy intake
0.594	0.097	0.306
Interaction between SNP rs492842* fat energy intake	Interaction between SNP rs492842* protein energy intake	Interaction between SNP rs492842* carbohydrate energy intake

Contd...

Table 5: Contd...

Interaction between SNPs and dietary factors on LDL-C		
0.380	0.392	0.402
Interaction between SNP rs602662* fat energy intake	Interaction between SNP rs602662* protein energy intake	Interaction between SNP rs602662* carbohydrate energy intake
0.399	0.462	0.610
Interaction between SNPs and dietary factors on triglycerides		
Interaction between SNP rs1801131 * fat energy intake	Interaction between SNP rs1801131* protein energy intake	Interaction between SNP rs1801131* carbohydrate energy intake
0.970	0.792	0.504
Interaction between SNP rs1801133* fat energy intake	Interaction between SNP rs1801133* protein energy intake	Interaction between SNP rs1801133* carbohydrate energy intake
0.938	0.798	0.266
Interaction between SNP rs1805087* fat energy intake	Interaction between SNP rs1805087* protein energy intake	Interaction between SNP rs1805087* carbohydrate energy intake
0.648	0.362	0.245
Interaction between SNP rs1801394* fat energy intake	Interaction between SNP rs1801394* protein energy intake	Interaction between SNP rs1801394* carbohydrate energy intake
0.176	0.285	0.857
Interaction between SNP rs1801198* fat energy intake	Interaction between SNP rs1801198* protein energy intake	Interaction between SNP rs1801198* carbohydrate energy intake
0.490	0.719	0.317
Interaction between SNP rs4680* fat energy intake	Interaction between SNP rs4680* protein energy intake	Interaction between SNP rs4680* carbohydrate energy intake
0.290	0.408	0.923
Interaction between SNP rs4633* fat energy intake	Interaction between SNP rs4633* protein energy intake	Interaction between SNP rs4633* carbohydrate energy intake
0.185	0.220	0.770
Interaction between SNP rs3797546* fat energy intake	Interaction between SNP rs3797546* protein energy intake	Interaction between SNP rs3797546* carbohydrate energy intake
0.127	0.741	0.457
Interaction between SNP rs492842* fat energy intake	Interaction between SNP rs492842* protein energy intake	Interaction between SNP rs492842* carbohydrate energy intake
0.237	0.216	0.989
Interaction between SNP rs602662* fat energy intake	Interaction between SNP rs602662* protein energy intake	Interaction between SNP rs602662* carbohydrate energy intake
0.360	0.082	0.817
Interaction between SNPs and dietary factors on LDL-ox		
Interaction between SNP rs1801131 * fat energy intake	Interaction between SNP rs1801131* protein energy intake	Interaction between SNP rs1801131* carbohydrate energy intake
0.161	0.962	0.582
Interaction between SNP rs1801133* fat energy intake	Interaction between SNP rs1801133* protein energy intake	Interaction between SNP rs1801133* carbohydrate energy intake
0.399	0.972	0.908
Interaction between SNP rs1805087* fat energy intake	Interaction between SNP rs1805087* protein energy intake	Interaction between SNP rs1805087* carbohydrate energy intake
0.493	0.126	0.784
Interaction between SNP rs1801394* fat energy intake	Interaction between SNP rs1801394* protein energy intake	Interaction between SNP rs1801394* carbohydrate energy intake
0.235	0.781	0.672
Interaction between SNP rs1801198* fat energy intake	Interaction between SNP rs1801198* protein energy intake	Interaction between SNP rs1801198* carbohydrate energy intake
0.832	0.100	0.489
Interaction between SNP rs4680* fat energy intake	Interaction between SNP rs4680* protein energy intake	Interaction between SNP rs4680* carbohydrate energy intake
0.353	0.348	0.005
Interaction between SNP rs4633* fat energy intake	Interaction between SNP rs4633* protein energy intake	Interaction between SNP rs4633* carbohydrate energy intake

Contd...

Table 5: Contd...

Interaction between SNPs and dietary factors on LDL-ox		
0.217	0.372	0.984
Interaction between SNP rs3797546* fat energy intake	Interaction between SNP rs3797546* protein energy intake	Interaction between SNP rs3797546* carbohydrate energy intake
0.846	0.227	0.270
Interaction between SNP rs492842* fat energy intake	Interaction between SNP rs492842* protein energy intake	Interaction between SNP rs492842* carbohydrate energy intake
0.624	0.466	0.690
Interaction between SNP rs602662* fat energy intake	Interaction between SNP rs602662* protein energy intake	Interaction between SNP rs602662* carbohydrate energy intake
0.743	0.298	0.112

P values were obtained by using a general linear model adjusted for age, sex and BMI. SNPs: Single-nucleotide polymorphisms, HDL-C: High-density lipoprotein-cholesterol, LDL: Low-density lipoprotein-cholesterol, BMI: Body mass index, * refers to interaction and not any significant *P* value

an interaction between *COMT* SNP rs4680 and carbohydrate intake on ox-LDL concentrations. Despite our study being the first to report this gene–diet interaction, previous studies have shown that carbohydrate-restricted diets can promote weight loss and are associated with reduced CVD risk.[38] However, the exact mechanism by which the *COMT* SNP rs4680 interacts with dietary carbohydrate to influence ox-LDL concentrations is unknown, which requires further studies to understand the mechanism contributing to this association. Furthermore, in our study, we observed an interaction of the *FUT2* SNP rs1805087 with protein consumption on homocysteine levels. However, we have no previous studies to confirm and validate this novel finding. The findings in this article suggest that the inheritance of ox-LDL and homocysteine levels is complex, where several genes/polymorphisms are likely to contribute to the alteration of ox-LDL or homocysteine levels through gene–gene and gene–diet interactions. More in-depth research implementing animal studies, nutrigenomics and metabolomics are needed to clarify the effects of SNPs and carbohydrate on ox-LDL concentrations and protein on homocysteine concentrations, respectively.

One of the main limitations of our study is the small sample size. Given that there are no previously reported effect sizes for the *FUT2* and *COMT* SNP–diet interactions on blood homocysteine and ox-LDL concentrations, we were unable to calculate the statistical power of our study. Our retrospective power calculation showed that the minimum detectable effects for ox-LDL levels ranged from beta 7.5 U/L (ox-LDL) for a SNP with MAF of 15% to beta 8.5 U/L for a SNP with MAF 50%. Hence, if the actual effect sizes were lower than this, our study would be underpowered. However, our study did find significant associations and gene–diet interactions despite the small sample size, but the findings require a replication given that the significant *P* values did not reach the Bonferroni-corrected *P* value. Another limitation is that our study was of cross sectional design, and therefore, we were unable to examine the causal relationship between the SNP–diet interactions on blood homocysteine and ox-LDL concentrations. Therefore, randomised controlled trials with prospective genotyping are required to explore the causality using genetic markers. Given that our study relied on a usual food record, we cannot negate the possibility of misreporting and measurement error. On the other hand, the main strength of our study is that we examined the effect of ten SNPs on Vitamin B12, folic acid, homocysteine concentrations and lipid traits during adolescence, a critical period where lifestyle habits are usually followed through to adulthood. By studying this population, we were able to identify different genotypes of interest, which could be further investigated to improve the understanding of the role of these micronutrients in relation to the prevention of hyperhomocysteinaemia and increased ox-LDL concentrations. In addition, little is known about gene–diet interactions which influence ox-LDL concentrations, and thus our study adds to the limited body of research.

Conclusions

Our study shows an interaction between *COMT* SNP rs4680 and carbohydrate intake on ox-LDL levels among adolescents with cardiovascular risk factors. Furthermore, a borderline interaction was observed between *FUT2* SNP rs602662 and protein intake on homocysteine concentrations. After correction for multiple testing, none of the SNP–environment interactions on homocysteine, folate, Vitamin B12 or lipid concentrations were detected. Hence, our findings warrant confirmation in larger, well-characterised and well-powered prospective studies/randomised controlled trials, before any public health recommendations and personalised nutrition advice can be developed for the adolescent Brazilian population.

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Conflicts of interest

There are no conflicts of interest.

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Supplementary Table 1: *P* values for the interaction between single-nucleotide polymorphisms and physical activity levels on Vitamin B12, homocysteine, folic acid and lipid traits

Gene	rs number	Model	B12	Homocysteine	Folic acid	HDL	LDL	Triglycerides	LDL-ox
MTHFR	rs1801131	Dominant	0.758	0.640	0.034	0.570	0.764	0.139	0.496
MTHFR	rs1801133	Dominant	0.589	0.810	0.404	0.446	0.541	0.030	0.760
MTR	rs1805087	Dominant	0.607	0.560	0.940	0.440	0.890	0.345	0.134
MTRR	rs1801394	Dominant	0.106	0.325	0.238	0.956	0.915	0.701	0.563
TCN2	rs1801198	Dominant	0.414	0.321	0.941	0.517	0.726	0.186	0.887
COMT	rs4680	Additive	0.543	0.058	0.783	0.537	0.663	0.113	0.681
COMT	rs4633	Dominant	0.221	0.253	0.993	0.828	0.400	0.056	0.597
BHMT	rs3797546	Dominant	0.146	0.274	0.255	0.811	0.250	0.209	0.050
BHMT	rs492842	Additive	0.947	0.281	0.423	0.513	0.483	0.004	0.677
FUT2	rs602662	Additive	0.613	0.100	0.458	0.147	0.724	0.014	0.491

P values were obtained by using a general linear model adjusted for age, sex and BMI. HDL-C: High-density lipoprotein-cholesterol, LDL: Low-density lipoprotein-cholesterol, BMI: Body mass index