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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]
Epidemiological studies have shown that hyperhomocysteinaemia is a well-known independent risk factor for atherosclerotic vascular disease and hypercoagulability states. It is known to mediate adverse effects on vascular endothelium and smooth muscle cells. In addition, hyperhomocysteinaemia reduces high-density lipoprotein (HDL) synthesis and enhances the synthesis of lipoprotein A. Some studies have indicated that up to 25% of coronary events may be attributed to the increase in homocysteine levels, 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. A few studies have indicated that high folate and Vitamin B12 status are associated with a reduced risk of coronary heart disease. 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. 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. 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. 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], catecho-l-O-methyltransferase [COMT], fucosyltransferase 2 [FUT2], methyltetrahydrofolate 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. The characteristics of the study participants are shown in Table 1.

Participants were selected on the basis that they were overweight/obese and/or were previously diagnosed with dyslipidaemia, but not with CVD. 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. 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. 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 95th 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. Plasma ox-LDL levels were measured...
SNPs [1-4] contribute to hyperhomocysteinaemia,[5] it is unknown whether BHMT homocysteine. While variations in the gene may
MTRR rs1801394, [14] Previous studies indicate that the SNPs
MTR the SNPs rs3797546 and rs492842 alter homocysteine levels.

The following ten common SNPs involved in the one-carbon metabolism pathway were selected based on the published reports: [16,22-26] rs1801131 and rs1801133 (677C>T) and rs492842 of BHMT and rs602662 of FUT2. rs1801131 [1-4] are associated with folate concentrations. Furthermore, genome-wide significant associations with serum B12 have been reported for the SNPs TCN2 rs1801198[8] 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® platform (Life Technologies, Foster City, CA, USA) with validated TaqMan Assay. 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]

**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/m²)          | 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±8.92 | 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

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,[12] 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,[10] 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[8] 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]
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 correction (adjusted P value for association was <0.00071). For all other tests, a P value of 0.05 was considered significant.

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 = 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 = 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 = 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 = 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 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

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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) |      |                     |                       |                   |                                               |                                               |                        |                                               |
| AA            | 0.31 | 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) |      |                     |                       |                   |                                               |                                               |                        |                                               |
| CC            | 0.30 | 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) |      |                     |                       |                   |                                               |                                               |                        |                                               |
| AA            | 0.16 | 523.97±221.74        | 7.22±3.29             | 11.03±3.31        | 46.64±12.73                                   | 90.42±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) |      |                     |                       |                   |                                               |                                               |                        |                                               |
| AA            | 0.37 | 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) |      |                     |                       |                   |                                               |                                               |                        |                                               |
| GG            | 0.26 | 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) |      |                     |                       |                   |                                               |                                               |                        |                                               |
| GG            | 0.45 | 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) |      |                     |                       |                   |                                               |                                               |                        |                                               |
| CC            | 0.37 | 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) |      |                     |                       |                   |                                               |                                               |                        |                                               |

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            | 0.97 | 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           | 0.50 | 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) |      |                     |                       |                  |                                               |                                               |                     |                                                  |
| BHMT gene (rs4680) |      |                     |                       |                  |                                               |                                               |                     |                                                  |
| TT            | 0.50 | 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            | 0.26 | 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            | 0.25 | 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             |      |                     |                       |                  |                                               |                                               |                     |                                                  |
| FUT2 gene (rs4633) |      |                     |                       |                  |                                               |                                               |                     |                                                  |
| GG            | 0.25 | 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            | 0.26 | 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            | 0.49 | 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             |      |                     |                       |                  |                                               |                                               |                     |                                                  |

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 reductase, MTRR: 5-methyltetrahydrofolate-homocysteine methyltransferase, 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 (Pinteraction > 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

| SNP Interaction | Vitamin B12 Interaction | Homocysteine Interaction |
|-----------------|--------------------------|--------------------------|
| **P values**    |                          |                          |
| Interaction     |                          |                          |
| rs1801131*      |                          |                          |
| fat energy intake | 0.685                   |                          |
| Interaction     |                          |                          |
| rs1801133*      |                          |                          |
| fat energy intake | 0.429                   |                          |
| Interaction     |                          |                          |
| rs1805087*      |                          |                          |
| fat energy intake | 0.368                   |                          |
| Interaction     |                          |                          |
| rs1801394*      |                          |                          |
| fat energy intake | 0.733                   |                          |
| Interaction     |                          |                          |
| rs1801198*      |                          |                          |
| fat energy intake | 0.789                   |                          |
| Interaction     |                          |                          |
| rs4680*         |                          |                          |
| fat energy intake | 0.662                   |                          |
| Interaction     |                          |                          |
| rs4633*         |                          |                          |
| fat energy intake | 0.455                   |                          |
| Interaction     |                          |                          |
| rs3797546*      |                          |                          |
| fat energy intake | 0.353                   |                          |
| Interaction     |                          |                          |
| rs492842*       |                          |                          |
| fat energy intake | 0.034                   |                          |
| Interaction     |                          |                          |
| rs602662*       |                          |                          |
| fat energy intake | 0.087                   |                          |

**Contd...**
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. [15,36] 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

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**Table 4: Contd...**

| Interaction between SNP | Interaction between SNP | Interaction between SNP |
|------------------------|------------------------|------------------------|
| rs492842* fat energy intake | rs492842* protein energy intake | 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 homocysteine

| Interaction between SNP | Interaction between SNP | Interaction between SNP |
|------------------------|------------------------|------------------------|
| rs1801131* fat energy intake | rs1801131* protein energy intake | 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.
Table 5: Interaction between single-nucleotide polymorphisms and dietary factors on lipid traits

| Interaction between SNPs and dietary factors on HDL-C | 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 |
| 0.964 | 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 |

Contd...
Table 5: Contd...

**Interaction between SNPs and dietary factors on LDL-C**

| Interaction | Value1 | Value2 | Value3 |
|-------------|--------|--------|--------|
| Interaction between SNP rs602662* fat energy intake | 0.380 | 0.392 | 0.402 |
| Interaction between SNP rs602662* protein energy intake | 0.399 | 0.462 | 0.610 |

**Interaction between SNPs and dietary factors on triglycerides**

| Interaction | Value1 | Value2 | Value3 |
|-------------|--------|--------|--------|
| Interaction between SNP rs1801131* fat energy intake | 0.070 | 0.792 | 0.394 |
| Interaction between SNP rs1801131* protein energy intake | 0.938 | 0.798 | 0.266 |
| Interaction between SNP rs1801131* carbohydrate energy intake | 0.648 | 0.362 | 0.245 |

**Interaction between SNPs and dietary factors on LDL-ox**

| Interaction | Value1 | Value2 | Value3 |
|-------------|--------|--------|--------|
| Interaction between SNP rs1801131* fat energy intake | 0.161 | 0.962 | 0.582 |
| Interaction between SNP rs1801131* protein energy intake | 0.399 | 0.972 | 0.908 |
| Interaction between SNP rs1801131* carbohydrate energy intake | 0.493 | 0.126 | 0.784 |

*Contd...*
Table 5: Contd...

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

*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 hyperhomocysteinemia 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    | Additive  | 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.