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Plasma total homocysteine in Brazilian overweight and non-overweight adolescents: a case-control study

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Abstract

Objective: To test the hypothesis that overweight adolescents have higher plasma total homocysteine (tHcy) levels than non-overweight adolescents and to explore the association between plasma tHcy levels with folate, vitamin B12 and some risk factors for CVD in both groups.

Methods: A case-control study conducted with 239 adolescents aged 15-19 years in the city of São Paulo, Brazil; 86 overweight and 153 non-overweight frequency matched by age, gender, pubertal and socioeconomic status. tHcy, folate, vitamin B12, lipid profile, glucose, insulin and insulin resistance were measured.

Results: No significant differences were found in tHcy, folate and vitamin B12 levels between overweight and non-overweight groups. The geometric means of tHcy were elevated in both groups (overweight: 11.8 µmol/L; non-overweight: 11.6 µmol/L) higher for boys than for girls (P < 0.001). Folate deficiency was identified in 68.6% of total studied population. Triacylglycerol, LDL cholesterol, insulin resistance were higher and HDL cholesterol was lower in overweight than non-overweight adolescents. In the multiple linear regression model, in overweight group, tHcy was independently associated with age (P = 0.041), sex (P = 0.004) and folate (P = 0.022) and in non-overweight group, with age (P = 0.049), sex (P < 0.001), folate (P = 0.018) and vitamin B12 (P = 0.030).

Conclusions: Obesity was not a determinant factor of tHcy levels. Age, sex and folate were independent determinants of plasma tHcy levels. The high prevalence of
Homocysteinuria has been observed in children with hyperhomocysteinemia. This observation led to the hypothesis that homocysteine may contribute to the development of atherosclerosis based upon pathological examinations of autopsy material from children with hyperhomocysteinemia. Folate, vitamin B12 and vitamin B6 are essential cofactors in these pathways. Plasma homocysteine (tHcy) levels are controlled by interplay of genetic and nutritional factors. Individuals with a nutritional deficiency that leads to low blood levels of folate, vitamin B12, or vitamin B6 are at risk of hyperhomocysteinemia.

McCully and Wilson proposed the homocysteine theory of atherosclerosis based upon pathological examinations of autopsy material from children with hyperhomocysteinemia. This observation led to the hypothesis that homocysteine may contribute to the development of atherosclerosis. Results from about 80 clinical and epidemiological studies have shown that a moderate elevation of tHcy levels can be associated with an increased risk of cardiovascular disease.

Obese adolescents present a high risk for developing dyslipidemias, hyperinsulinism and insulin resistance. A few studies have investigated plasma tHcy levels in overweight adolescents. Determining tHcy, folate, vitamin B12, lipid profile, insulin resistance and their relations with overweight and obesity in adolescence is relevant for the adoption of preventive measures with the objective of correcting deficiencies, improving quality of life, reducing risk of chronic diseases, especially cardiovascular diseases, and consequently increasing life expectancy. Thus, our purpose was to test the hypothesis that overweight adolescents have higher plasma tHcy levels than non-overweight adolescents and to explore the association between plasma tHcy concentration with folate, vitamin B12, lipid profile, glucose, insulin and insulin resistance in both groups.

Subjects and methods

The case-control study was carried out in the city of Sao Paulo, state of Sao Paulo, Southeastern Brazil, from August to December 2002. For allocation into overweight and non-overweight groups, a team of trained nutritionists and pediatricians weighed and measured 1,420 adolescents born between January 01, 1982 and December 31, 1987, representing 98.68% of all students enrolled in one public high-school of Sao Paulo. Sixteen (1.11%) youngsters refused to be evaluated and three (0.21%) were not found after at least three attempts. The adolescents were measured during their physical education classes and body mass indexes (BMI) were calculated as weight (Kg)/height2 (m). Of the 263 eligible adolescents, participants in every phase of the study, 9.12% (n = 24) were excluded (4 - hypothyroidism; 6 - vitamin supplementation; and 6 - use of medication that could alter the tHcy results). The sample then comprised 239 adolescents; 86 identified as overweight (BMI ≥ 85th) and 153 as non-overweight (5th ≤ BMI < 85th) frequency matched by age, sex, socioeconomic status and pubertal stage 4 or 5 according to Tanner. Data on birth, personal history (e.g., history of chronic diseases), familial cardiovascular disease (coronary artery disease, stroke, or peripheral vascular disease in the family, including parents, grandparents, uncles and aunts) and use of medication were collected using a standardized and pre-tested questionnaire applied by trained nutritionists and pediatricians to both overweight and non-overweight adolescents. Adolescents suffering from severe illness with clinical and laboratory confirmation of the diagnosis...
Biochemical Analysis

Blood samples were collected by peripheral venous puncture, in the morning, after a 12-hour fast, in order to determine tHcy, folate, vitamin B12, HDL cholesterol, LDL cholesterol, triacylglycerol, glucose and insulin levels. Blood samples for the measurement of tHcy were collected in tubes containing EDTA. Plasma was isolated by centrifugation (3,000 X g at 4ºC for 20 min) and immediately stored at -80º for two months until analyzed. The plasma tHcy levels were measured by high performance liquid chromatography (HPLC). Hyperhomocysteinemia was defined as a tHcy concentration > 15 µmol/L. Serum levels of folate were measured using Ion Capture Technology (AxSYM System-ABBOTT Laboratories, Illinois, USA) and vitamin B12 were measured by MEIA-Microparticle Enzyme Immunoassay Technology (AxSYM System-ABBOTT Laboratories, Illinois, USA). The normality range considered for serum folic acid was 5.0-16.0 ng/mL and for vitamin B12 was 200-1,000 pg/mL. HDL cholesterol and triacylglycerol levels were measured by using colorimetric method (VITROS SYSTEMS CHEMISTRY 750 XRC-Ortho-Clinical Diagnostics, Inc-Johnson & Johnson Company, New York, USA). LDL cholesterol levels were calculated with the Friedewald formula when triacylglycerol was lower than 400 mg/dL. Glucose was detected by enzymatic method utilizing hexokinase and glucose-6-phosphate dihydrogenase enzymes (Advia Chemistry System 1650-Bayer). Insulin was measured by two-site immunoenzymometric assay (TOSOH-TOSOH CORPORATION, Tokyo, Japan). The insulin resistance was determined by homeostasis model assessment for insulin resistance (HOMA-IR), calculating the product of fasting plasma insulin (µU/mL) and fasting plasma glucose (mmol/L) divided by 22.5.

Statistical Analysis

The continuous variables that were not normally distributed according to Shapiro-Wilk test were log-transformed (tHcy, folate, vitamin B12, HDL cholesterol, LDL cholesterol, triacylglycerol, insulin and HOMA-IR) and their values were presented as geometric mean and 95% confidence interval. The continuous variables normally distributed were expressed as mean and standard deviation. Comparisons between overweight and non-overweight groups and between males and females were assessed by Student’s t-test.

Correlations between the tHcy and all other continuous variables were calculated using Pearson’s correlation coefficient in overweight and non-overweight groups. In order to observe the association between tHcy and all studied variables (age, sex, folate, vitamin B12, HDL cholesterol, LDL cholesterol, triacylglycerol, glucose, insulin and HOMA-IR) a simple linear regression model was used and the significant variables (P < 0.05) were included in the multiple linear regression model. Statistical tests were considered significant when P < 0.05. The statistical analyses were completed using the software Stata 8.0 (Stata Corp., College Station, TX, USA).

Results

The clinical and biochemical characteristics of the overweight and non-overweight adolescents are described in Table I. Some data are expressed as mean and standard deviation and others as geometric mean and 95% confidence interval. Plasma tHcy levels were high in both overweight and non-overweight groups. According to Student’s t-test there was no significant difference in tHcy levels between the overweight and non-overweight groups even when distributed according to sex. Elevated levels of tHcy appeared in both groups independently of age and sex. The correlations of tHcy with age and sex were significant (P < 0.05; r = 0.28 for age and r = 0.29 for sex). The analysis of homocysteine in overweight and non-overweight adolescents

Table I

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Overweight</th>
<th>Non-overweight</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>41/45</td>
<td>70/83</td>
<td></td>
</tr>
<tr>
<td>Age (Years)</td>
<td>16.0 ± 1.0</td>
<td>16.3 ± 1.0</td>
<td>0.079</td>
</tr>
<tr>
<td>BMI†</td>
<td>29.6 ± 2.9</td>
<td>20.4 ± 2.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>tHcy (µmol/L)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>11.8 (10.9-12.8)</td>
<td>11.6 (11.0-12.2)</td>
<td>0.696</td>
</tr>
<tr>
<td>Female</td>
<td>13.5 (11.9-15.4)</td>
<td>13.1 (12.1-14.2)</td>
<td>0.662</td>
</tr>
<tr>
<td>Folate (ng/mL)</td>
<td>4.3 (3.9-4.7)</td>
<td>4.1 (3.9-4.4)</td>
<td>0.495</td>
</tr>
<tr>
<td>Vitamin B12 (µg/mL)</td>
<td>411.0 (373.4-452.2)</td>
<td>451.5 (422.0-483.1)</td>
<td>0.106</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>43.5 (41.2-45.8)</td>
<td>50.2 (48.2-52.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>LDL cholesterol (mg/dL)</td>
<td>98.4 (93.3-103.8)</td>
<td>90.6 (86.9-94.5)</td>
<td>0.018</td>
</tr>
<tr>
<td>Triacylglycerol (mg/dL)</td>
<td>90.3 (81.1-100.2)</td>
<td>73.3 (69.5-77.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>83.6 (±6.4)</td>
<td>82.3 (±6.4)</td>
<td>0.138</td>
</tr>
<tr>
<td>Insulin (µU/mL)</td>
<td>11.7 (10.3-13.1)</td>
<td>6.4 (5.9-6.8)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>HOMA-IR†</td>
<td>2.4 (2.1-2.7)</td>
<td>1.3 (1.2-1.4)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Means or geometric means of the overweight and non-overweight adolescents were compared by Student’s t-test.
* Mean ± SD.
† Geometric mean; 95% CIs in parentheses.
‡ Significant sex difference: § P = 0.001; ¶ P < 0.001 (Student’s t-test).
† Mean ± SD.
** Significant geometric mean difference: 95% CIs in parentheses.
†† Significant age difference: * P = 0.001; ** P < 0.001 (Student’s t-test).
BMI, body mass index; tHcy, total homocysteine; HDL-c, high-density lipoprotein; LDL, low density lipoprotein; HOMA-IR, Homeostasis model assessment for insulin resistance; Fasting plasma insulin (µU/mL) x fasting plasma glucose (mmol/L)/22.5.
the nutritional condition. We observed hyperhomocysteinemia (> 15 µmol/L) in 46 adolescents (19.2%) from the total sample. In both groups, plasma tHcy levels exhibited higher mean values in males than in females (P < 0.001).

Likewise, there was no significant difference between overweight and non-overweight adolescents in relation to folate and vitamin B₁₂ levels. Considering the adolescents as a whole, the deficiencies of folate and vitamin B₁₂ were identified in 68.6% (n = 164) and 2.5% (n = 6), respectively.

Overweight adolescents presented geometric means significantly higher for LDL cholesterol, triacylglycerol, insulin and HOMA-IR than non-overweight adolescents. The HDL cholesterol geometric mean was lower in overweight than in non-overweight individuals. There was no significant difference in the glucose mean between the groups.

Applying Pearson correlation coefficient with tHcy as dependent variable and the clinical and laboratorial variables as independent, we observed a negative correlation with folate (r = -0.2928, P = 0.0062) and positive with age (r = 0.2534, P = 0.0186) in the overweight group. In the non-overweight group there was a negative correlation with folate (r = -0.1798, P = 0.0262) and vitamin B₁₂ (r = -0.1959, P = 0.0152) and a positive correlation with age (r = 0.1599, P = 0.0483). There was no correlation between tHcy and HDL cholesterol, LDL cholesterol, triacylglycerol, glucose, insulin and HOMA-IR in either the overweight or non-overweight groups.

The association between tHcy and folate in overweight and non-overweight adolescents obtained in the linear regression model is shown in figure 1.

The multiple linear regression model showed that tHcy in the overweight group remained independently associated with age, sex and folate and that in the non-overweight group the tHcy remained independently associated with age, sex, folate and vitamin B₁₂ (tables II and III).

**Discussion**

Our results showed that there was no significant difference in plasma tHcy levels between overweight

| Table II | Variables associated with plasma tHcy levels in overweight adolescents* |
|-----------------|-----------------------------------------------|-----------------|
| β coefficient | 95% CI | P    |
| Age (Years)    | 0.032  | (0.001; 0.063) | 0.041 |
| Sex†           | -0.095 | (-0.159; -0.031) | 0.004 |
| Folate         | -0.219 | (-0.406; -0.032) | 0.022 |
| Vitamin B₁₂    | -0.090 | (-0.255; 0.074) | 0.278 |

* Values for plasma tHcy levels, folate and vitamin B₁₂ were log₁₀-transformed before analysis. β coefficients and P values were determined by multiple linear regression analyses and adjusted for all variables listed. β coefficients are the log₁₀ of tHcy per unit change in the independent variable (age, folate and vitamin B₁₂).† Females in relation to males. n = 86.

| Table III | Variables associated with plasma tHcy levels in non-overweight adolescents* |
|-----------------|-----------------------------------------------|-----------------|
| β coefficient | 95% CI | P    |
| Age (Years)    | 0.021  | (0.000; 0.042) | 0.049 |
| Sex†           | -0.089 | (-0.132; -0.047) | <0.001 |
| Folate         | -0.156 | (-0.286; -0.026) | 0.018 |
| Vitamin B₁₂    | -0.127 | (-0.243; 0.012) | 0.030 |

* Values for plasma tHcy levels, folate and vitamin B₁₂ were log₁₀-transformed before analysis. β coefficients and P values were determined by multiple linear regression analyses and adjusted for all variables listed. β coefficients are the log₁₀ of tHcy per unit change in the independent variable (age, folate and vitamin B₁₂). † Females in relation to males. n = 153.
and non-overweight adolescents. Similarly, other studies with children and adults have not found an association between tHcy and BMI\(^{25-28}\). On the other hand, Tungtrongchitr and cols.\(^7\) observed higher plasma tHcy levels in the overweight group of adults when compared with non-overweight group, although the case group also presented lower serum folate levels when compared to the control group.

Apart from nutritional condition, the results of tHcy levels in this study presented high values when compared to other studies with adolescents in the same age range. Four studies that assessed plasma tHcy levels in adolescents found lower values than our study. De Laet and cols.\(^{20}\) observed a geometric mean of 9.78 \(\mu\)mol/L in boys and 8.33 \(\mu\)mol/L in girls, aged 15-19 years. The tHcy levels assessed by National Health and Nutrition Examination Survey (NHANES III), based on a sample of 295 boys and 345 girls aged 16-19 years, found geometric mean values of 8.7 \(\mu\)mol/L for boys and 7.2 \(\mu\)mol/L for girls combining race and ethnic groups\(^8\). In Bogalusa Heart Study\(^2\), a geometric mean of 6.3 \(\mu\)mol/L (5.9-6.7 \(\mu\)mol/L) was verified in individuals aged 15-17 years. Bates and cols.\(^{24}\), found geometric mean of 8.5 \(\mu\)mol/L for boys and 7.8 \(\mu\)mol/L for girls aged 15-18 years.

Another factor to be considered refers to the prevalence of hyperhomocysteinemia in the studied population. Hyperhomocysteinemia (tHcy > 15 \(\mu\)mol/L) was detected in 18.6% (n = 16) overweight adolescents and 19.6% (n = 30) non-overweight adolescents. A normal plasma tHcy concentration is approximately 10 \(\mu\)mol/L, varying from 5 to 15 \(\mu\)mol/L. Values above 15 \(\mu\)mol/L are considered to be hyperhomocysteinemia\(^9\). The prevalence of hyperhomocysteinemia is estimated at 5% in general population and 13-47% among patients with symptoms of atherosclerotic vascular diseases\(^{10, 11}\). Hyperhomocysteinemia has been recognized as an important independent risk factor for cardiovascular disease\(^{12-14}\). McCully\(^{30}\), states that the ideal plasma concentration of this aminoacid should be below 10 \(\mu\)mol/L. In patients with coronary artery disease, Nystad and cols.\(^3\) estimated that the mortality ratio for an increase of 5 \(\mu\)mol/L in the tHcy concentration was 1.6 between 10 and 15 \(\mu\)mol/L and 2.5 between 15 and 20 \(\mu\)mol/L. Results of meta-analysis, concluded that a 5 \(\mu\)mol/L tHcy increment elevates CVD risk by as much as cholesterol increases of 20 mg/dL\(^{15}\).

In our study we observed differences in tHcy levels between sexes, as these were higher in boys than in girls, in both overweight and non-overweight groups. This fact could be due to age > 15 years. Studies state that in non-pubertal children the tHcy levels are similar\(^{16, 20}\) and that after puberty, the levels are higher in boys than in girls\(^{21, 24, 27, 35}\). Men present higher tHcy levels than women, possibly due to stoichiometric formation of homocysteine in connection with creatine/creatinine synthesis that is proportional to muscular mass, generally bigger in men\(^{17}\). Another hypothesis perhaps is the hormonal protection in women\(^{18}\). These differences were also observed when the multiple linear regression model was applied.

Regarding age, this study demonstrated that despite the little range of age, the plasma tHcy levels increased with age in both groups, a finding also reported by other studies on adolescents aged over 15 years\(^{25-28}\)

We measured two cofactors involved in tHcy metabolism, folate and vitamin B\(_12\). Serum folate and vitamin B\(_12\) levels did not differ in overweight and non-overweight adolescents. There was a negative correlation between tHcy and folate in both overweight and non-overweight groups. There was a negative correlation of vitamin B\(_12\) with tHcy just in the non-overweight group. When the determinant factors of tHcy levels were analyzed applying the multiple linear regression model, we observed a significant negative association between tHcy and folate in both overweight and non-overweight groups. Several other studies have demonstrated an inverse association between tHcy and folate\(^{10, 13, 35}\). In children, tHcy was more closely correlated with folate and less with vitamin B\(_12\)\(^{10, 21}\). We observed that 66.3% (n = 57) of overweight and 69.9% (n = 105) of non-overweight adolescents, presented folate deficiency with serum values < 5.0 ng/mL. The high tHcy levels in the studied population might have been due to the great deficiency of folate we found. The increase of plasma tHcy levels is also considered a sensitive marker of folate deficiency\(^{20, 21}\). There is a good correlation between serum folate and vitamin B\(_12\) levels with their food intake\(^{8, 37}\). There is a good correlation with these vitamins or food fortification easily reduces the plasma tHcy levels\(^{39, 40}\). Several countries have already opted for food fortification with folate initially to reduce the risk of neural tube defects. In the United States and Canada, such food fortification with folate started at the end of the 90’s\(^{41, 42}\). The introduction of fortification with folate in U.S. has reduced the prevalence of hyperhomocysteinemia\(^4\). In Brazil, the legislation on food fortification with folate was recently approved but to-date has not been thoroughly implemented\(^43\).

We found significantly higher insulin levels and insulin resistance measured by HOMA-IR in overweight compared to non-overweight adolescents. Overweight individuals tend towards greater insulin resistance than non-overweight. Insulin resistance and hyperinsulinemia have been demonstrated in obese adolescents\(^{43, 44}\). However, when we assessed the relation between tHcy and insulin resistance we did not find any association, corroborating the findings of ot-
her studies. On the other hand, Gallistl and cols. reported an association between tHCy and plasma insulin in obese children and adolescents. However, in that study plasma insulin was also inversely related with plasma folate suggesting a subclinical deficiency in obese children.

Concluding our study, obesity did not influence the elevated tHCy levels, which were more associated with deficiency of folate. High tHCy levels may increase the risk for future development of CVD in this population, indicating the importance of preventive measures and educational programs regarding dietary habits and lifestyle. Genetic factors could also be further studied to identify other causes that may contribute to elevated tHCy levels.

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