nutrición clínica y Dietética Hospitalaria

Artículo Original

Nutr. clín. diet. hosp. 2017; 37(1):106-116
DOI: 10.12873/371ribasdefarias

Serum homocysteine and cysteine levels and associated factors in children and adolescents

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Recibido: 25/mayo/2016. Aceptado: 10/octubre/2016.

RESUMO

Introdução: Estudos têm identificado níveis séricos elevados de homocisteína (Hcy) e cisteína (Cys) como fatores de risco para doenças cardiovasculares, importante causa de morte em todo o mundo. Fatores associados com elevados níveis desse marcadores bioquímicos em adultos parecem bem conhecidos, no entanto na faixa etária pediátrica, dados sobre essas associações são escassos.

Objetivo: identificar os fatores associados às diferentes concentrações séricas de homocisteína (hcy) e cisteína (cys) em crianças e adolescentes. Métodos: trata-se de um estudo transversal incluindo 483 indivíduos de 7 a 15 anos, ambos os sexos, de Mutuípe, Bahia. Níveis séricos de Hcy e Cys foram as variáveis desfecho, e condições sóciodemográficas, clínicas, bioquímicas e de estilo de vida foram incluídas como variáveis independentes. Utilizou-se a regressão logística politômica para avaliar a associação entre diferentes níveis de hcy e cys e variáveis de interesse, usando a razão de prevalência (RP) como medida de associação.

Resultados: elevados níveis séricos de Hcy associaram-se ao sexo masculino (RP=3.74; p<0.01), idade ≥12 anos (RP=2.56; p<0.01), sobrepeso (RP=2.32; p=0.02), pressão arterial aumentada (RP=1.97; p<0.01), baixos níveis de HDL-c (RP=1.21; p=0.03), hipertrigliceridemia (RP=1.62; p=0.03) e baixo consumo de alimentos protetores (RP=1.46; p=0.02). Para a Cys, seus níveis aumentados associaram-se à idade ≥12 anos (RP=2.1; p=0.03), sobrepeso (RP=2.52; p=0.03); pressão arterial aumentada (PR=1.28; p=0.03), baixos níveis de HDL-c (RP=1.15; p=0.01), hipertrigliceridemia (RP=1.41; p=0.02) e baixo consumo de alimentos protetores (RP=1.46; p=0.01).

Conclusões: idade ≥12 anos, sobrepeso, pressão arterial aumentada, baixos valores de HDL-c, hipertrigliceridemia e baixo consumo de alimentos protetores associaram-se aos níveis séricos elevados tanto de Hcy, quanto de Cys, com exceção do sexo masculino, que se associou apenas à hiperhomocisteinemia. Considerando que esses fatores têm apresentado ocorrência crescente na infância e adolescência, a prevenção e controle de níveis elevados de Hcy e Cys deve ser adotada, prevenindo doenças crônicas não transmissíveis nesse estágio da vida.

PALAVRAS CHAVES

Homocisteína, cisteína, fatores associados, crianças, adolescentes

ABSTRACT

Introduction: Studies have identified high serum homocysteine (Hcy) and cysteine (Cys) levels as risk factor for cardiovascular diseases, important cause of death in all world. The factors associated with high levels of these biochemistry markers in adults are well known; however, data are sparse on these associations in the pediatric age group.

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Objective: the objective was to identify factors associated with different concentrations of serum Hcy and Cys in children and adolescents.

Methods: a cross-sectional study with 483 individuals of 7-15 years of age of both sexes, from a municipality of Bahia. Serum Hcy and Cys levels were considered outcome variables, with exposure being evaluated according to sociodemographic, clinical, biochemical and lifestyle variables. Polytomous logistic regression was used to evaluate the association between exposure and outcome.

Results: high serum Hcy levels were associated with being male (PR=3.74; p<0.01), age ≥12 years (PR=2.56; p<0.01), being overweight (PR=2.32; p=0.02), high blood pressure (PR=1.97; p<0.01), low HDL-c levels (PR=1.21; p=0.03), high triglyceride levels (PR=1.62; p=0.03) and poor intake of foods that protect against hyperhomocysteinemia (PR=1.46; p=0.02). High serum Cys levels were associated with age ≥12 years (PR=2.1; p=0.03), being overweight (PR=2.52; p=0.03); high blood pressure (PR=1.28; p=0.03), low HDL-c levels (PR=1.15; p=0.01), high triglyceride levels (PR=1.41; p=0.02) and poor intake of foods that protect against hypercysteinemia (PR=1.46; p=0.01).

Conclusions: age >12 years, being overweight, high blood pressure, low HDL-c levels, high triglyceride levels and poor intake of protective foods are common factors found in individuals with increased serum Hcy and Cys levels. Being male was associated with high serum Hcy levels alone. Considering that these factors are already present early in life, measures should be adopted to prevent and control high Hcy and Cys levels, promoting health and preventing chronic non-communicable diseases at this stage of life.

KEY WORDS

Homocysteine, cysteine, associated factors, children, adolescents

ABBREVIATIONS

FFQ: food frequency questionnaire.

HPLC: high performance liquid chromatography.

HCY: homocysteine.

CYS: cysteine.

INTRODUCTION

Studies have identified high serum homocysteine levels as a risk factor for cardiovascular disease[1-3], an important cause of death in all world[4]. The mechanism underlying cardiovascular disease involves the proliferation of vascular smooth muscle, and endothelial dysfunction and damage[5].

A meta-analysis of prospective studies involving populations with no diagnosis of cardiovascular disease at baseline found that for every 5 μmol/L increase in homocysteine levels there was a 20% increased risk of cardiac events irrespective of the traditional risk factors for these diseases[3].

A recent study conducted with 13,247 individuals from two US cohorts reported that adding homocysteine levels to the traditional Framingham risk score to predict cardiovascular events significantly improved prediction, particularly in individuals for whom the risk of cardiovascular disease was intermediate[6]. This finding reinforced data from other studies on the association between homocysteine and cardiac disease.

Between 5 and 10% of the adult population worldwide have high homocysteine levels[7]. Data for other population groups are sparse. In Brazil, hyperhomocysteinemia was identified in 19.4% of children and adolescents in the city of São Paulo, with a higher prevalence being found in boys[8].

Although the chemical structure and metabolic behavior of serum cysteine and homocysteine are similar, a potential association between cysteine and cardiovascular events has received little attention from investigators[9]. Nevertheless, the few data available suggest similar associations between both biochemical parameters and cardiovascular disease[9-11].

Despite the lack of an established cut-off point for cysteine in children and adolescents, a study conducted in São Paulo, in which the classification of the 90th percentile of the sample was used, reported high serum cysteine levels in 9.6% of boys and 9.0% of girls[12].

Although the factors associated with high serum homocysteine and cysteine levels in the adult population are already known, few studies have evaluated these associations in the pediatric age group.

OBJECTIVE

The objective of the present study was to identify the factors associated with high serum homocysteine and cysteine levels in children and adolescents.

METHODS

Sample and study design

This was a cross-sectional study including schoolchildren from Mutuípe-Bahia, Brazil. Bood samples were collected from 540 children. Thirty-seven of these children could not be traced, while in an additional 15 cases, the serum was insufficient for measurement and 5 were lost during homocysteine and cysteine analysis. Therefore, this study consisted of 483 boys and girls of 7 to 15 years of age, selected at random from a list of elementary schoolchildren registered with the Mutuípe Municipal Education Department in 2006.

Consequently, this study sample has a power of 93% and 94% to identify a 10% prevalence rate of high serum levels of homocysteine and cysteine, respectively, taking as a refer-

ence the prevalence rates of hyperhomocysteinemia and of high serum cysteine levels reported for children in São Paulo[12].

Exclusion Criteria

The exclusion criteria consisted of actors that could affect homocysteine or cysteine levels such as the use of medication (anticonvulsants, diuretics, thiazides and corticoids) and the presence of conditions such as diabetes mellitus, chronic renal failure, liver disease or hyperthyroidism[13]. Pregnancy, breastfeeding and any physical handicaps that would prevent an anthropometric evaluation from taking place also constituted exclusion criteria. Nevertheless, none of the children had any of these conditions.

METHODS

Sociodemographic and lifestyle-related data

The sociodemographic data consisted of the number of rooms in the house and the number of individuals living in it, the principal form of lighting and the occupation of the head of the family. These data were used to calculate a socioeconomic index. Data were collected on water supply to the home, the source of drinking water and how garbage and household waste are disposed, with these data being used to calculate the environmental index. The answers to these variables were awarded scores that ranged from 0 to 4, with 0 representing the poorest conditions and 4 the best.

Maternal education was evaluated separately, given that this factor is also associated with the cultural and dietary aspects of the society in which the individual lives.

Physical activity level was evaluated using a structured questionnaire on the frequency with which physical activity was performed, excluding the once-a-week exercise that is part of the school curriculum. Another lifestyle-related variable included in the study was alcohol consumption.

Family history of a chronic non-communicable disease

The student and/or his/her parent or guardian provided information on any chronic, non-communicable diseases in the family and, if present, the student's relationship to that individual was recorded.

Anthropometric data: weight, height and waist circumference

Anthropometric status was evaluated according to body mass index for age (BMI/age) and sex, and by excess abdominal fat. Weight and height were measured as defined by Lohman[14], and waist circumference were measured according to the World Health Organization (WHO)[15].

The recommendations of the WHO for individuals of 5-19 years of age were used to classify BMI/age[16]. Considering that there is no consensus on the cut-off point of waist circumference for the diagnosis of excess abdominal fat in children and adolescents, the value of the 90th percentile of the study sample was adopted as recommended by Freedman *et al.*[17].

Arterial blood pressure

Blood pressure was measured and classified in accordance with the VI Brazilian Guidelines on Arterial Hypertension and the levels were classified according to height, age and sex, adopting the 90th percentile as the cut-off point[18].

Dietary Intake

Dietary intake was evaluated using a food frequency questionnaire (FFQ), which was adapted from an instrument that had previously been used in children and adolescents in the same town[19].

The data obtained were treated in accordance with a score methodology[20]. The overall dietary intake of each food item was converted into scores by multiplying the weekly frequency of intake by 4 (the number of weeks in a month) and dividing it by 30 (the number of days in a month), thus arriving at a mean daily intake score, which were then classified into tertiles.

After these scores were calculated, two dietary groups were constructed:

a) Group of food items that increase the risk of high serum homocysteine and cysteine levels: food items poor in complex B vitamins, as not whole grains, pasta, cookies, candies, pastry, fries, vegetable oils.

b) Group of food items that protect against high serum homocysteine and cysteine levels, including food items that are sources of fiber, complex B vitamins and minerals (meats, fish, dark green vegetables, whole and enriched grain products, legumes and citrus fruits).

Biochemical tests

Blood samples were collected in the morning after at least 12 hours of fasting. A 10-ml sample was collected by venous puncture into a sterile, disposable Vacutainer (BD®) with no anticoagulant. Immediately after collection, the blood was centrifuged at 3000 rpm for 5 minutes. Aliquots of serum were stored in previously demineralized Eppendorf tubes at -20oC. Total cholesterol (TC), HDL-c, triglyceride and glucose levels were measured using an enzymatic method. LDL cholesterol (LDL-c) levels were calculated according to the Friedewald equation[21].

Serum homocysteine and cysteine levels were measured at the Inborn Errors of Metabolism Laboratory of the Federal

University of São Paulo by high performance liquid chromatography (HPLC) with isocratic elution and fluorometric detection, considered the gold-standard methodology for this procedure[22]. Briefly, the measurement process involved the following steps: reduction (to reduce and release the protein-bound thiols), precipitation of the proteins, and derivatization, according to the methodology proposed by Pfeiffer, Huff and Gunter (1999)[23].

Classification of the biochemical variables

Glucose: Glucose levels were classified according to the criteria established by the Brazilian Diabetes Society (glycemia ≥ 126mg/dl)[24].

Lipid profile: The cut-off points suggested by Jolliffe and Janssen (2006)[25] for adolescents of 12-19 years of age, which take into account the variations in lipid profile according to age and sex, were adopted. For schoolchildren under 12 years of age, the recommendation of the National Health and Nutrition Examination Survey (NHANES)) was used to classify HDL-c, while the definitions proposed by the National Cholesterol Education Program (NCEP) were used to classify total cholesterol, LDL-c and triglycerides. These cut-off points were adopted based on the results of studies showing that the use of these classification resulted in the highest accuracy for classifying the risk of adolescents developing dyslipidemia as adults[26,27].

Identification of variables

The outcome variables consisted of serum homocysteine (Hcy) and cysteine (Cys) levels, which were classified into quintiles. The covariables included were age, sex, maternal education, environmental index, socioeconomic index, blood pressure, glucose, total cholesterol, LDL-c, HDL-c, triglycerides, BMI/age, practice of physical activity, family history of chronic non-communicable diseases, intake of foods that protect against hyperhomocysteinemia and hypercysteinemia, intake of foods that increase the risk of hyperhomocysteinemia and hypercysteinemia and alcohol consumption.

Statistical Analysis

Means and standard deviations were used to describe the continuous variables. Levels of Hcy and Cys were compared in relation to the exposure variables using Student's t-test for equal or unequal variances. The variables *Hcy* and *Cys* were submitted to logarithmic transformation, since their distribution was not normal.

To identify the factors associated with serum Hcy and Cys levels, polytomous logistic regression analysis was adopted, appropriate for outcome variables with more than two categories[28,29]. The prevalence ratio (PR) was adopted as the estimator[28].

A polytomous regression model was constructed for each outcome variable (serum Hcy and Cys). Initially, a univariate analysis was performed to select candidate variables for the multivariate model. For this, a significance level of $p < 0.20$ was adopted. Those variables that remained in the model at a statistical significance level of $p<0.05$ were included in the final model.

The goodness-of-fit of the polytomous logistic regression model was assessed for each individual model using the 1st quintile of Hcy and Cys as the reference level. For this test, non-significant p-values were adopted as indicators of the goodness-of-fit of the model[28].

The analyses were performed using the Stata/IC statistical software package for Mac, version 12.0 (StataCorp, College Station, TX, USA).

Ethical Aspects

The study protocol was submitted to the internal review board of the School of Nutrition, Federal University of Bahia, and approved under reference number 03/06.

The child's participation in the study required authorization from his/her parents and/or guardians. After being duly informed with respect to the study objectives and agreeing to the inclusion of the child in the study, the parents and/or guardians signed an informed consent form.

RESULTS

Serum Hcy levels varied according to sex, with a mean of 7.1 ± 1.88 μmol/L for the girls and 7.7 ± 1.88 μmol/L for the boys. The same did not apply to Cys levels. Furthermore, mean Hcy levels increased with age in both boys and girls, with mean levels being higher in the boys than in the girls at all ages (Table 1). In the case of Cys, an increase in mean values was found from 10 years of age onwards, following the same trend irrespective of sex.

The results showed in Table 2 identified that in the case of girls under 10 years of age with low HDL-c levels, serum Hcy levels were higher than those found in girls of the same age with normal levels of this lipoprotein. No associations were found between mean Hcy levels and any of the other variables in the girls, irrespective of age.

With respect to the boys, mean Hcy levels were higher in those who were overweight compared to those of normal weight at all the ages investigated. It was also found that in the boys of 12 years of age or more mean Hcy levels were higher in those with an above-normal waist circumference, low HDL-c levels and high blood pressure. In those under 10 years of age mean Hcy levels were higher when serum triglyceride levels were also high. No associations were found between mean Hcy levels and any of the other variables in boys (Table 2).

Table 1. Mean serum homocysteine and cysteine levels according to sex and age. Mutuípe, Bahia, Brazil, 2006.

Age	Serum homocysteine levels (Mean/SD;µmol/L)			
	Girls	Boys	Total	p-value*
- <10 years	6.6 / 1.43	7.4 / 1.82	6.9 / 1.63	<0.01
- 10-12 years	7.0 / 1.76g	7.5 / 1.44	7.2 / 1.64	0.02
- >12 years	7.5 / 2.12	8.0 / 2.16	7.7 / 2.15	0.02
Total	7.1 / 1.88	7.7 / 1.88	7.3 / 1.90	<0.01
Age	**Serum cysteine levels (Mean / SD; µmol/L)**			
	Girls	**Boys**	**Total**	**p-value****
- <10 years	396.4 / 62.9	393.1 / 62.7	395.1 / 62.6	> 0.38
- 10-12 years	402.7 / 69.8	414.3 / 59.9	407.8 / 65.6	0.03
- >12 years	406.5 / 66.3	419.5 / 65.9	409.3 / 65.9	0.03
Total	406.8 / 66.7	410.4 / 63.8	408.3 / 65.5	> 0.05

N=483.
* t-test for comparison of means with unequal variance.
** t-test for comparison of means with equal variance.

Considering results showed in Table 3, in girls under 10 years of age, higher mean Cys levels were found in the overweight girls. For those over 12 years of age, mean Cys levels were higher in those with a larger waist circumference and with high blood pressure. No associations were found between mean Cys levels and any of the other variables in girls. No associations were found between Cys levels according to age and sex, and total cholesterol, LDL-c or glucose levels.

In the case of boys over 12 years of age, mean serum cysteine levels were higher in those with lower HDL-c levels and high triglyceride levels. No statistically significant associations were found with any of the other variables (Table 3).

Table 4 shows the results of the polytomous logistic regression models. With the exception of being male, which was found to be a risk factor for high serum Hcy levels in the different quintiles of distribution, the other risk factors evaluated were only associated with the highest category of Hcy level (the 5th quintile: >8.6 µmol).

According to these results, a 3.74 times higher prevalence of serum Hcy levels > 8.6 µmol/L was found in boys compared with the prevalence in the girls. The prevalence of high serum Hcy levels was 2.56 times greater in children over 12 years of age compared to the children less than 12 years of age. In overweight individuals, the prevalence of hyperhomocysteinemia was 2.32 times greater compared to individuals of normal weight. Additionally, in children and adolescents with high blood pressure, high triglyceride levels, low HDL-c levels and whose intake of protective foods was poor, there was a 97%, 62%, 21% and 46% greater prevalence of hyperhomocysteinemia, respectively, compared to the prevalence rates in individuals without these risk factors (Table 4).

With respect to serum Cys levels, an association was found with the exposure factors only in the 5th quintile of distribution of this variable (>463.4 µmol). Therefore, the prevalence of high Cys levels was 2.1 times greater in schoolchildren over 12 years of age compared to younger children, while the prevalence of hypercysteinemia was 2.52 times greater in overweight children compared to those of normal weight. In addition, it was found that individuals with high blood pressure, hypertriglyceridemia, low HDL-c levels and poor intake of protective foods had prevalence rates of hypercysteinemia that were 28%, 41%, 15% and 46% higher, respectively, than those found in individuals without these risk factors (Table 4).

DISCUSSION

In the present study, being over 12 years of age, having a poor intake of protective foods, being overweight, having high blood pressure, low HDL-c levels and high triglyceride levels were common factors present in individuals with high serum Hcy and Cys levels. Being male was associated with high serum Hcy levels alone and as these levels increased from the 3rd to the 5th quintile of distribution, the prevalence ratio also increased, indicating a greater susceptibility to hyperhomocysteinemia in males.

Table 2. Mean serum levels of homocysteine by sex and age, according to anthropometric status, lipid profile, glucose levels and blood pressure. Mutuípe, Bahia, Brazil, 2006.

	Serum levels of homocysteine (Mean/SD; µmol/L)					
	Girls			Boys		
	<10 years	10-12 years	>12 years	<10 years	10-12 years	>12 years
BMI/Age						
-Normal weight	6.55 /1.34	6.93/ 1.80	7.47/ 2.27	7.14/ 1.66*	7.53/ 1.50*	8.10/ 2.47*
- Overweight	6.96 / 2.00	7.45/ 1.70	7.99/ 1.59	8.67/ 2.33	8.75/ 1.84	9.15/ 2.03
WC						
- <P90	6.55/ 1.43	6.95/ 1.76	7.56/ 2.23	7.47/ 1.91	7.65/ 1.50	7.56/ 1.95*
- ≥P90	7.23/ 1.44	7.54/ 1.80	7.64/ 1.90	7.21/ 1.88	7.37/ 1.93	8.59/ 2.77
Total cholesterol						
- Within normal range	6.65/ 1.47	7.16/ 1.75	7.64/ 2.18	7.51/ 1.90	7.62/ 1.59	8.39/ 2.64
- High	6.45/ 1.33	6.70/ 1.91	6.76/ 1.61	7.19/ 1.95	7.55/ 1.42	7.42/ 3.79
LDL-c						
- Within normal range	6.56/ 1.42	7.17/ 1.77	7.64/ 2.17	7.49/ 1.93	7.50/ 1.36	7.42/ 2.64
- High	6.86/ 1.53	6.61/ 1.80	6.65/ 1.81	7.32/ 1.83	7.63/ 1.60	8.39/ 3.79
HDL-c						
- Within normal range	5.89/1.50**	6.69/ 1.23	7.16/ 1.37	7.16/ 1.50	7.40/ 1.01	7.96/2.53**
- Low	6.75/ 1.40	7.17/ 1.96	7.65/ 2.21	7.64/ 2.01	7.92/ 1.64	8.98/ 3.21
Triglycerides						
- Within normal range	6.41/ 1.38	7.05/ 1.77	7.52/ 2.45	7.08/1.60**	7.54/ 1.31	8.33/ 2.97
- High	6.77/ 1.47	7.10/ 1.81	7.65/ 1.74	8.43/ 2.28	7.71/ 1.89	8.41/ 2.13
Glucose						
- Within normal range	6.54/ 1.44	6.49/ 1.45	7.05/ 1.38	7.42/ 1.92	7.62/ 1.58	8.15/ 2.43
- High	6.60/ 1.40	7.11/ 1.80	7.62/ 2.20	8.33/ 0.53	7.51/ 1.47	8.39/ 2.68
Blood pressure						
- Within normal range	6.48/ 1.37	6.90/ 1.56	7.51/ 2.27	7.36/ 1.93	8.21/ 2.44	7.68/1.45*
- High	6.74/ 1.55	7.32/ 2.24	7.74/ 1.48	7.58/ 2.01	8.33/ 2.47	8.15/ 1.88

N=483.
* p < 0.05t-test for comparison of means with equal variance.
** p < 0.05 t-test for comparison of means with unequal variance.

Table 3. Mean serum levels of cysteine by sex and age, according to anthropometric status, lipid profile, glucose levels and blood pressure. Mutuípe, Bahia, Brazil, 2006.

	Serum levels of cysteine (Mean / SD; µmol/L)					
	Girls			Boys		
	<10 years	10-12 years	>12 years	<10 years	10-12 years	>12 years
BMI/Age						
-Normal weight	392.7/61.2*	396.6/71.2	413.0/ 63.8	387.7/ 61.7	412.3/ 59.8	414.5/ 61.9
- Overweight	423.5/ 71.8	418.5/64.9	429.9/ 75.0	413.7/ 65.0	422.7/ 62.7	430.9/ 77.4
WC						
- <P90	395.2/ 61.2	417.9/ 65.0	396.6/70.3*	391.6/ 64.3	411.5/ 57.8	417.1/ 61.9
- ≥P90	412.0/ 87.7	411.8/ 71.3	426.6/ 64.4	417.4/ 8.03	430.0/ 72.0	423.1/ 79.4
Total cholesterol						
- Within normal range	393.3/ 64.8	400.8/ 70.0	420.4/ 66.6	396.7/ 63.4	414.6/ 60.6	420.5/ 65.2
- High	374.8/ 52.2	410.6/ 71.0	397.3/ 48.2	386.4/ 60.2	412.0/ 64.6	414.3/ 58.1
LDL-c						
- Within normal range	397.9/ 63.5	400.6/ 69.1	363.9/ 39.3	391.7/ 63.5	410.3/ 58.1	380.3/ 58.2
- High	386.9/ 61.1	412.3/ 74.8	420.2/ 66.3	398.0/ 62.2	435.5/ 68.5	420.5/ 65.2
HDL-c						
- Within normal range	398.1/ 56.6	389.9/ 72.3	411.9/ 68.7	388.3/ 62.1	404.1/ 66.0	417.8/58.2*
- Low	400.0/ 92.2	409.6/ 56.9	420.0/ 53.3	403.8/ 71.9	413.1/ 68.9	463.2/ 62.1
Triglycerides						
- Within normal range	399.8/ 62.4	402.0/ 72.8	412.5/ 64.9	389.5/ 57.6	392.9/ 48.4	404.1/59.9*
- High	381.2/ 65.3	405.0/ 64.5	417.7/ 67.0	416.4/ 91.7	420.1/ 71.5	440.5/ 57.1
Glucose						
- Within normal range	397.4/ 62.2	403.7/ 70.1	405.7/ 80.9	366.5/ 62.6	414.5/ 60.3	405.8/ 66.0
- High	377.1/ 83.8	387.8/ 70.5	417.5/ 65.3	394.1/ 82.8	412.7/ 61.3	419.8/ 67.8
Blood pressure						
- Within normal range	391.3/ 58.6	394.5/57.2	404.8/ 60.4*	393.9/ 66.3	414.8/ 64.2	421.4/ 63.8
- High	403.3/ 67.3	412.7/81.7	447.1/ 82.6	387.9/ 67.5	407.4/ 50.5	419.3/ 76.6

N=483

* p < 0.05t-test for comparison of means with equal variance.

Table 4. Factors associated with high serum homocysteine and cysteine levels in children and adolescents. Mutuípe, Bahia, Brazil,2006.

Variables	HCY* PR (95%CI); p-value†			
	2nd Quintile (5.8 - 6.7µmol/L)	3rd Quintile (6.7- 7.4µmol/L)	4th Quintile (7.4 - 8.6µmol/L)	5thQuintile (>8.6µmol/L)
Male	1.65 (0.82-2.32); 0.16	2.46 (1.24-2.88); 0.01	3.68(1.83-4.42);<0.01	3.74 (2.30-4.34);<0.01
Age>12 years	1.30 (0.67-1.71); 0.43	1.19 (0.61-1.72); 0.55	1.51 (0.76-2.01); 0.23	2.56 (1.97-3.14);<0.01
Low intake of protective foods	1.19 (0.82-1.36); 0.58	0.95 (0.50-1.42); 0.89	1.15 (0.791.42); 0.67	1.46 (1.23-1.72); 0.02
Overweight	1.33 (0.67-1.64); 0.40	1.47 (0.74-1.94); 0.26	1.73 (0.84-2.56); 0.13	2.32 (2.11-2.85); 0.02
High blood pressure	1.70 (0.65-2.42); 0.27	2.46 (0.99-3.12); 0.05	1.77 (0.76-1.99); 0.16	1.97 (1.46-2.20);<0.01
High TGŧ	1.15 (0.78-1.26); 0.67	0.94 (0.57-1.27); 0.86	1.09 (0.95-1.53); 0.79	1.62 (1.23-1.98); 0.03
Low HDL-c	0.86 (0.41-1.58); 0.69	0.67 (0.39-1.13); 0.42	0.94 (0.81-1.15); 0.59	1.21 (1.12-1.34); 0.03
Variables	**CYS§ PR (95% CI); p-value†**			
	2ndQuintile (352.6-383.3µmol/L)	3rdQuintile (383.3-416.2µmol/L)	4thQuintile (416.2-463.4µmol/L)	5thQuintile (>463.4µmol/L)
Age>12 years	0.89 (0.46-1.72); 0.73	1.37 (0.69-2.73); 0.36	1.10 (0.55-2.20); 0.77	2.10 (1.79-2.39); 0.03
Low intake of protective foods	1.73 (0.89-1.97); 0.33	1.51 (0.77-1.96); 0.79	1.91 (1.78-2.01); 0.06	1.46 (1.34-1.67); 0.01
Overweight	1.47 (0.59-2.65); 0.39	1.79 (0.74-2.31); 0.19	2.31 (0.97-2.51); 0.06	2.52 (1.60-2.95); 0.03
High blood pressure	1.03 (0.81-1.32); 0.76	1.19 (0.94-1.52); 0.13	1.22 (0.97-1.55); 0.08	1.28 (1.19-1.62); 0.03
High TGŧ	1.01 (0.51-1.94); 0.98	1.20 (0.62-2.32); 0.58	0.95 (0.47-1.91); 0.90	1.41 (1.19-1.88); 0.02
Low HDL-c	0.97 (0.94-1.01); 0.25	0.98 (0.94-1.01); 0.30	0.95 (0.92-1.09); 0.06	1.15 (1.05-1.25); 0.01

Sample size: 497.
The reference category for the equation was the 1stquintile(HCY<5.8µmol/L and CYS<352.6µmol/L).
*HCY = serum homocysteine.
†Polytomous logistic regression models; goodness-of-fit p-value not significant.
ŧTriglycerides.
§CYS = serum cysteine.

Other investigators have also reported higher Hcy levels in males, both in schoolchildren and in other age groups[30,31]. These differences may be explained by a greater efficacy in the remethylation and transmethylation phases in women, through mechanisms as yet unknown. These phases represent important steps in Hcy metabolism and the heightened efficacy of these steps in women may result in differences between males and females[32], thus explaining the association identified in the present study.

It is possible that the association between high serum Hcy and Cys levels and increasing age could be mediated by the poor intake of sources of micronutrients in adolescents, particularly the vitamin B complex, which is vital for Hcy and Cys metabolism. Indeed, vitamin B complex deficiency may inhibit some physiological steps in the metabolism of these amino acids. Recently, prevalence rates of a poor dietary intake of the vitamin B complex that ranged from 7.6% to 22.9% in males and from 9.5% to 34.2% in females were found for Brazilian adolescents of 13-19 years of age[33]. Therefore, these dietary patterns may also be common in the adolescents evaluated in the present study and may contribute towards explaining the association between high serum levels of Hcy and Cys and age.

Poor intake of protective foods was positively associated with high Hcy and Cys levels in the present study. In this scenario of possible poor micronutrient intake, evaluated by the frequency, a deficiency of pyridoxine, vitamin B12 and folic acid may inhibit and/or compromise the metabolic pathways of the remethylation and transsulfuration of methionine, altering the serum levels of this amino acid[32].

Another important epidemiological finding of the present study concerns the association between high Hcy and Cys levels and overweight in schoolchildren. These results add to the finding of the obesogenic role of high Hcy and Cys levels, giving strength to the recently described theory of a metabolic role of these parameters in weight gain[34]. This theory is supported, principally, by results of experimental studies showing that high Cys levels stimulate the synthesis of fatty acids, promoting adiposity. These acids undergo auto-oxidation in the adipocytes, releasing hydrogen peroxide (H2O2), which acts as an inhibitor of lipolysis, stimulating lipid synthesis and resulting in the activation of lipogenesis. Furthermore, the inhibiting effect of high Hcy levels on lipolysis has been shown in *in vitro* and *in vivo* studies conducted by Wang *et al.* (2011)[35]. Those investigators reported that high Hcy levels inhibit lipolysis through a mechanism that involves activation of the AMP-activated protein kinase (AMPK), an important sensor of cellular energy level, which exerts an antilipolytic effect on the adipocytes, encouraging fat accumulation.

These results contribute to the body of evidence showing that, in this age group too, high Hcy and Cys levels are significantly associated with weight gain. When the association between these biochemical parameters and low HDL-c levels and high triglyceride levels is also taken into consideration, these findings become even more relevant.

In this respect, Gil-Prieto *et al.* (2009)[36] also identified an inverse association between serum HDL-c levels and Hcy values in adolescents. In a cross-sectional study, Anand *et al.* (2009)[37] identified an association between high Hcy, low HDL-c and high triglyceride levels. Few studies have been conducted to evaluate the association between Cys and the lipid profile of children and adolescents. In a cross-sectional study conducted with schoolchildren in a Brazilian town, Pereira da Silva *et al.* (2013)[12] found an association between Cys and HDL-c.

A possible mechanism underlying the association between Hcy, Cys and changes in HDL-c may involve the inhibition of apolipoprotein A-1 gene transcription, with a consequent reduction in the synthesis of HDL-c in the liver, leading to atherosclerosis[38]. Although studies have identified an association between Hcy and triglycerides, the mechanisms behind this association have yet to be clarified. On the other hand, considering that excess weight is associated with an undesirable lipid profile consisting of low HDL-c and high triglyceride levels, it is possible that the association between these lipids and Hcy and Cys occurs through the same metabolic pathways that, directly or indirectly, play a role in determining excess weight.

In the present study, high blood pressure was also associated with high serum Hcy and Cys levels. Some epidemiological studies have found a weak, albeit significant, association between Hcy levels and blood pressure in children and adolescents[30]. Although to the best of our knowledge no studies have been conducted on the association between Cys and blood pressure in the pediatric age group, data from the Hordaland Homocysteine Study for the adult population showed a strong association between blood pressure and high Cys levels[10].

With respect to this association, studies have shown that high serum Hcy and Cys levels are related to blood pressure due to the reaction of these elements with nitric oxide, forming nitrosothiol, reducing its bioavailability and resulting in damage to the endothelium-dependent vasodilation mediated by nitric oxide[39]. Experimental studies have also shown that hyperhomocysteinemia leads to an increase in the arterial stiffness caused by the destruction of elastin fibers, increasing collagen production and smooth muscle cell activity[40].

This study involves limitations that are inherent to its cross-sectional design in which no causal or temporal associations are established between the variables. Other limitations include the lack of adjustment of the models for vitamin B complex levels, serum creatinine and MTHFR 677C>T genotype, which globally contribute to the variation in Hcy in healthy populations. For this reason, further studies are necessary to confirm the hypotheses raised here.

CONCLUSIONS

Considering the methodology used, the robust statistical analysis and the evidence reported by other investigators it is possible to conclude that high Hcy and Cys levels in children and adolescents are associated with a set of factors that establish an important epidemiological scenario of morbidities from chronic non-communicable diseases, including overweight, high blood pressure, low HDL-c and high triglyceride levels. Considering that these factors are already present early in life, measures should be adopted to prevent and control high Hcy and Cys levels to promote health and prevent chronic non-communicable diseases at this stage of life.

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