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Homocysteine concentrations in overweight children and adolescents

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SUMMARY

OBJECTIVE: The aim of this study was to describe homocysteine concentrations in overweight and obese children and adolescents and relate them to blood pressure levels, renal function, and insulin resistance.

METHODS: This is a cross-sectional and observational study with 64 overweight children and adolescents (mean age: 11.6±3.5 years) in outpatient follow-up. The following parameters were evaluated: body mass index z-score, waist-to-height circumference ratio, pubertal stage, blood pressure, serum homocysteine, glycemia, insulin, lipid profile, renal function, high-sensitivity C-reactive protein, microalbuminuria, and creatinuria. Statistical analysis: analysis of variance and logistic regression (dependent variable: homocysteine) ($p<0.05$).

RESULTS: The mean body mass index z-score was 2.9±1.1. The mean homocysteine concentrations were 8.6±2.2 μmol/L (10th and 90th percentiles: 6.6 and 11.2 μmol/L, respectively), with no difference when compared with children with severe obesity and obesity/overweight ($p=0.431$). High values of waist-to-height ratio (93.8%), systolic blood pressure (18.8%), diastolic blood pressure (12.5%), glycemia (4.7%), low-density lipoprotein cholesterol (31.1%), triglycerides (35.9%), non-high-density lipoprotein cholesterol (34.4%), and microalbuminuria (21.9%) were obtained. The mean glomerular filtration rate was 122.9±24.6 mL/min/1.73 m^2. Homocysteine concentrations were not associated with any of the studied variables ($R^2=0.095$).

CONCLUSION: Homocysteine concentrations in overweight children and adolescents (mean 8.6±2.2 μmol/L) were not associated with body mass index z-score, blood pressure, renal function, and insulin resistance.

KEYWORDS: Homocysteine. Pediatric obesity. Biomarkers. Heart disease risk factors.

INTRODUCTION

Homocysteine (Hcy) is an amino acid containing sulfhydryl that forms during methionine metabolism. The interest in Hcy as a causal risk factor for cardiovascular diseases (CVDs) in childhood began with the observation that more than 50% of children with homocystinuria of genetic origin die prematurely from vascular diseases[1].

A meta-analysis evaluating the dose-response effect suggested that Hcy concentrations are linear and positively associated with all-cause mortality risk. This risk has increased by 33.6% for each 5 μmol/L increase in Hcy concentrations in adults (risk ratio: 1.336, 95%CI 1.254–1.422, $p<0.001$)[2].

Obesity seems to relate to high Hcy concentrations and represents a risk for the development of CVD, considering that overweight individuals experience events in earlier ages, live with events for longer periods, and have a shorter life expectancy compared to individuals with a normal body mass index (BMI)[3].

A recent meta-analysis involving 14 studies with adults found significantly higher Hcy concentrations in obese individuals compared to healthy controls, regarding their eating habits, insulin resistance (IR), and drug use[4].

In the pediatric age group, systematic review and meta-analysis, including studies published from 1999 to 2017, showed that these were predominantly cross-sectional and mainly evaluated adolescents. In the meta-analysis (n=6) and cross-sectional studies (n=3), the authors identified that high Hcy concentrations correlated weekly and directly with excess weight in children and adolescents (odds ratio [OR]: 1.08; 95%CI 1.04–1.11)[5].

Considering the participation of high Hcy concentrations and excess weight in the risk for the development of CVD and the lack of studies in the pediatric age group, especially in our country, this study aimed to describe the Hcy concentrations in overweight children and adolescents and to verify an association with blood pressure (BP), renal function, and IR.

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METHODS

Through a cross-sectional and observational study, 64 overweight and obese children and adolescents aged 5–19 years were followed up at the Nutrition Outpatient Clinic of the Department of Pediatrics of the Centro Universitário FMABC Santo André, Brazil.

Of the 80 children eligible for the study who regularly attended the outpatient clinic from August 2014 to May 2015, 64 (80%) were included.

Children and adolescents with obesity secondary to diseases of genetic or hormonal cause and carriers of other chronic diseases, birth weight of less than 2,500 g, and under medications that could interfere with renal function, lipid profile, and glucose tolerance were included.

A questionnaire containing questions related to obesity and associated morbidities, socioeconomic status, and morbid personal and family history of CVDs was applied.

Pubertal staging was classified according to the one proposed by Marshall and Tanner.

Anthropometric assessment was performed according to protocols standardized by the World Health Organization (WHO)[6]. Weight and height measurements were expressed as body mass index Z-score (zBMI), calculated using the WHO AnthroPlus software. For the anthropometric classification, the cutoff points recommended by the WHO were adopted[7]. The waist-to-height ratio (WHtR) was classified as altered when the value was equal to or greater than 0.5[8].

Systemic BP was measured at the time of the interview, according to the recommendation of the Task Force, 2004. BP values were classified according to sex, age, and height percentiles and were considered inadequate when above the 90th percentile[9]. BP measurements were performed by a pediatrician using calibrated equipment and periodically reviewed.

The examinations were performed at the Clinical Analysis Laboratory of the Centro Universitário FMABC. A sample of 10 mL of blood was obtained by peripheral venipuncture, after 12-h fast, to determine total cholesterol (TC), low-density lipoprotein cholesterol (LDL-c), high-density lipoprotein cholesterol (HDL-c), non-HDL cholesterol (NHDL = TC - HDL), and triglycerides (TG) (colorimetric method); blood glucose (colorimetric method) and insulin (immunoenzymatic method), from which Homeostasis Model Assessment for insulin resistance (HOMA-IR) was calculated; urea and creatinine (colorimetric method); and high-sensitivity C-reactive protein (hs-CRP immunoenzymatic method). For lipid profile classification, the cutoff points recommended by the American Academy of Pediatrics were adopted[10].

Hcy analysis was performed by chemiluminescence method, with reference values between 5 and 15 µmol/L. The enzyme immunoassay was performed using automatic chemiluminescence equipment, model Immulite 2000. The chemiluminescence method showed results comparable to those obtained by high-performance liquid chromatography in school-age children[11].

Plasma creatinine was used to calculate the estimated creatinine clearance or estimated glomerular filtration rate (eGFR) according to Schwartz's equation: GFR (mL/min/1.73 m²) = 0.43 × height (cm)/plasma creatinine (mg/dL)[12].

Isolated urine sample (first in the morning, 20 mL) was collected for the calculation of albuminuria and creatinuria. Microalbuminuria (MA) was defined as the albumin/creatinine ratio with values between ≥30 and <300 mg/g[13].

For statistical analysis, the SPSS (IBM®) software version 24.0 was used. Categorical variables were presented as absolute and percentage values. The continuous ones were evaluated for their normality. The parametric variables were presented in the form of mean±standard deviation and the nonparametric variables in the form of median (minimum; maximum). The nonparametric variables (MA, hs-CRP, HOMA-IR, and insulin) underwent logarithmic transformation, and for the analyses, the analysis of variance test was used for comparison. Logistic regression was performed (dependent variable: Hcy). The significance level was set at 5%.

The research protocol was approved by the Ethics Committee of Centro Universitário FMABC (n 1,080,802).

RESULTS

Table 1 describes the general characteristics of overweight and obese children and adolescents included in the study. The mean age was 11.6±3.5 years. Most children and adolescents had obesity and severe obesity of 81.3% and the increased WHtR was observed in 93.8%. High systolic and diastolic BP (above the 90th percentile) occurred in 18.8 and 12.5%, respectively.

Table 2 describes the means and medians of BP values, anthropometric indicators, and laboratory variables. The mean zBMI was 2.9±1.1 with Hcy concentrations of 8.6±2.2 µmol/L (10th and 90th percentiles: 6.6 and 11.2 µmol/L, respectively).

The mean GFR was 122.9±24.7 mL/min/1.73 m² (minimum and maximum: 78.9 and 192.1 mL/min/1.73 m², respectively). Four (6.2%) patients had a GFR below 90 mL/min/1.73 m². Of these, three were adolescents, all obese, and one had an associated MA. There was no significant correlation between zBMI, insulin, and HOMA-IR with MA values or GFR.

There was no statistically significant difference when comparing the Hcy concentrations in overweight, obese, and severely obese children (Figure 1) (p=0.431). Table 3 shows that Hcy concentrations were also not associated with any of the studied variables (R^2=0.095) in this group of overweight children and adolescents.

DISCUSSION

This study showed mean concentrations of Hcy of 8.6±2.2 µmol/L in overweight children and adolescents. There was no association of the Hcy concentrations with BP, renal function, or IR.

One study described the distribution of total Hcy among a representative sample of American children and adolescents (n=2027, ages between 4 and 19 years) and tested the differences between sex, age, and race-ethnicity categories. The geometric mean concentrations of Hcy adjusted for age were 6.2 and 5.8 µmol/L in non-Hispanic Caucasian boys and girls, 6.4 and 6.1 µmol/L in non-Hispanic African-American boys and girls, and 6.4 and 5.5 µmol/L in Mexican-American boys and girls, respectively[14]. The values found in the American study were lower than those observed in our study.

High concentrations of Hcy have been associated with increased risk for the cardiovascular, cerebrovascular, and thromboembolic diseases in adults. Values of 10 µmol/L or smaller are probably safe for adult individuals, but values of 11 µmol/L or above may suggest the need for intervention. There are no indications of values related to negative outcomes for the pediatric age group[15].

The association between high concentrations of Hcy and excess weight seems related to the dysfunction of the adipose tissue with inhibition of lipolysis by activating the protein kinase influenced by adenosine monophosphate[16]. Evidences pointing to an association between high concentrations of Hcy and overweight in the pediatric age group are still insufficient, as described in a recently published meta-analysis. After the combination of studies in meta-analysis (n=6), there was a

Table 1. General characteristics of the studied sample of overweight children and adolescents.

Variable		N=64
Sex	Male	32 (50%)
Age	<10 years	24 (37.5%)
Pubertal stage	Prepubescent	29 (45.3%)
	Pubescent 2 and 3	24 (37.5%)
	Pubescent 4 and 5	11 (17.2%)
BMI Z-score	Severe obesity	24 (37.5%)
	Obesity	28 (43.8%)
	Overweight	12 (18.8%)
WHtR	≥0.5	60 (93.8%)
Systolic blood pressure	>90th percentile	12 (18.8%)
Diastolic blood pressure	>90th percentile	8 (12.5%)
Mean arterial pressure	>90th percentile	14 (21.9%)
Family history	Obesity	24 (37.5%)
	Systemic arterial hypertension	32 (50%)
	Diabetes	11 (17.2%)
	Dyslipidemia	11 (17.2%)
	Cardiovascular disease	25 (39.1%)
Fasting glucose	>100 mg/dL	3 (4.7%)
Total cholesterol	>200 mg/dL	18 (28.1%)
LDL-c	>130 mg/dL	20 (31.1%)
HDL-c	<45 mg/dL	17 (26.6%)
Triglycerides	>100 mg/dL	23 (35.9%)
Non-HDL-c	>145 mg/dL	22 (34.4%)
Microalbuminuria	>30 mg/g	14 (21.9%)

BMI Z-score: body mass index z-score; WHtR: waist-to-height circumference ratio; LDL-c: low-density lipoprotein cholesterol; HDL-c: high-density lipoprotein cholesterol.

Table 2. Characteristics of mean or median blood pressure values, anthropometric indicators, and laboratory variables evaluated in overweight children and adolescents (n=64).

Variable		Mean±SD or median (min-max)
Systolic blood pressure	mmHg	110.7±15.3
Diastolic blood pressure	mmHg	69.6±12.6
Body mass index	Z-score	2.9±1.1
Homocysteine	µmol/L	8.6±2.2
Microalbuminuria	mg/g	9.4 (0.7–300.7)
Creatinine clearance	mL/min/1.73 m²	122.9±24.6
hs-CRP	mg/dL	4.1 (0.3–38.3)
Total cholesterol	mg/dL	183.9±32.3
HDL-c	mg/dL	48.9±13.8
LDL-c	mg/dL	116.2±25.9
Triglycerides	mg/dL	100.7±56.3
Non-HDL-cholesterol	mg/dL	134.9±31.8
Glycemia	mg/dL	86.3±8.9
Insulin	µU/mL	8.7 (2.0–37.3)
HOMA-IR		1.8 (0.4–10.0)

hs-CRP: high-sensitivity C-reactive protein; LDL-c: low-density lipoprotein cholesterol; HDL-c: high-density lipoprotein cholesterol; Non-HDL-c: non-HDL cholesterol (total cholesterol - HDL-c); HOMA-IR: Homeostasis Model Assessment of insulin resistance.

weak and direct correlation between Hcy concentrations and BMI by age only in cross-sectional studies (n=3) and a direct but nonstatistically significant correlation in cohort studies (n=3). The authors emphasized that the majority of the studies were conducted with adolescents and indicates the necessity of developing future longitudinal studies to better identify the associations[5].

Moreover, it is a consensus that the concentrations may vary with age, sex, and pubertal staging in both healthy and obese children[16].

Brasileiro et al. did not find any differences between the total concentrations of Hcy of Brazilian adolescents with overweight/obesity and healthy adolescents[16]. The mean Hcy concentration in the study was 11.8 µmol/L, higher than what we observed (8.6 µmol/L), and folate deficiency was found in 68.6% of the sample. Some hypotheses can be suggested for this difference: the study was conducted exclusively with adolescents (mean age 16 years) and the present study with children and adolescents, and the data collection of the cited study was performed when the mandatory fortifications of corn and wheat flour with a minimum of 4.2 mg of iron and 150 µg of folic acid for every 100 g of flour were not yet put into force in Brazil, starting in 2004[17]. In countries such as the United States and Canada, it was found that the mandatory fortification of folic acid foods led to a decrease in total Hcy concentrations in all age groups, more expressive in individuals with higher pre-fortification values[18].

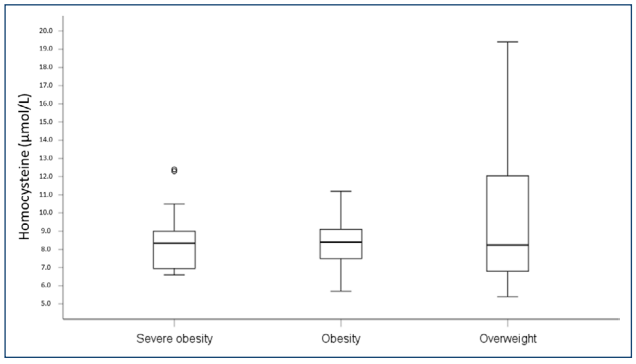

Figure 1. Homocysteine concentrations in overweight, obese, and severely obese children.

A cross-sectional and controlled study conducted in Turkey showed significantly higher concentrations of Hcy in obese children compared to controls without excess weight. Contrary to what was observed in our study, the authors have verified a significant correlation between the total concentrations of Hcy and age, BMI, TG, and HDL-c in the obese group. There was no association with the HOMA-IR[19]. The increased concentrations of insulin promote inhibition of hepatic cystathionine-B-synthase activity with consequent increase in circulating Hcy concentrations[20].

In this study, there was no association between Hcy concentrations and renal function evaluated based on MA and creatinine clearance. Current data suggest that a healthy kidney plays an important role in the clearance and metabolism of Hcy, as with other amino acids. Hcy concentrations increase as renal function declines and progresses to advanced renal disease, with the vast majority of dialysis patients presenting mild-to-moderate hyperhomocysteinemia. The values of GFR estimated from serum creatinine or calculated creatinine clearance are consistently and inversely correlated with plasma Hcy levels[21].

We have not found any association between Hcy concentrations and BP. Although higher plasma Hcy concentrations

Table 3. Association of studied variables with homocysteine concentrations in overweight children and adolescents (n=64).

Variable		B	95%CI		p-value
Age	Years	-0.005	-0.020	0.010	0.513
Systolic BP	mmHg	0.019	-0.043	0.082	0.532
Diastolic BP	mmHg	0.018	-0.054	0.090	0.625
WHtR	cm/cm	0.131	-7.505	7.767	0.973
hs-CRP (log)	mg/dL	-0.330	-1.277	0.616	0.487
HOMA-IR (log)		-0.389	-2.372	1.594	0.696
Microalbuminuria (log)	mg/g	-0.503	-1.536	0.530	0.333
Creatinine clearance	mL/min/1.73 m²	0.001	-0.023	0.025	0.937
BMI Z-score		-0.065	-0.623	0.493	0.815
Non-HDL-cholesterol	mg/dL	-0.005	-0.024	0.014	0.595

Logistic regression dependent variable: homocysteine (µmol/L). R^2=0.095.
BMI Z-score: body mass index z-score; BP: blood pressure; WHtR: waist-to-height circumference ratio; hs-CRP: high-sensitivity C-reactive protein; HOMA-IR: Homeostasis Model Assessment of insulin resistance.

have been associated with high BP in cross-sectional studies with adults[22,23], a cohort study entitled the Framingham Heart Study showed an association only in the unadjusted model; the multivariate analysis did not show a causal relationship[24].

This study presents some limitations such as the cross-sectional design that does not allow establishing a cause-effect relationship, the reduced sample size, and the absence of a healthy control group without excess weight.

CONCLUSION

The presence of complications/comorbidities was observed in about 30% of those with dyslipidemias, 20% with increased BP levels, and 22% with MA >30 mg/g. The mean total Hcy concentration was 8.6±2.2 µmol/L (10th and 90th percentiles: 6.6 and 11.2 µmol/L, respectively). There was no association between Hcy concentrations and zBMI, BP, renal function, or IR. In this study, the values that are higher than those described in other studies in the literature in countries that practice mandatory folic acid fortification of foods remind us the importance of monitoring Hcy concentrations in overweight individuals in the pediatric age group.

DECLARATION

All authors declare to be responsible for the content made available for publication.

ETHICAL ASPECTS

The study was approved by the Research Ethics Committee of the FMABC University Center, opinion number: 1080802. Families received information, risks, and benefits from the study. Literate children received a TALE and their guardians a TCLE.

AUTHORS' CONTRIBUTIONS

JDGS: Data curation, Formal Analysis, Writing – original draft. **FISS:** Conceptualization, Methodology, Validation, Writing – review & editing. **JCPF:** Data curation, Methodology, Project administration, Writing – original draft. **LSS:** Formal Analysis, Methodology. **ADVG:** Conceptualization, Methodology, Validation. **ROSS:** Conceptualization, Methodology, Visualization, Writing – review & editing.

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