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Hyperhomocysteinemia correlates with insulin resistance and low-grade systemic inflammation in obese prepubertal children

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Abstract

Obesity is an independent risk factor for the development of cardiovascular disease frequently associated with hypertension, dyslipemia, diabetes, and insulin resistance. Higher homocysteine (Hcy) levels are observed in the hyperinsulinemic obese adults and suggest that Hcy could play a role in the higher risk of cardiovascular disease in obesity. We analyzed total Hcy levels in obese prepubertal children and their possible association with both metabolic syndrome and various inflammatory biomarkers and leptin. We studied 43 obese children (aged 6-9 years) and an equal number of nonobese children, paired by age and sex. The obese subjects presented significantly elevated values for insulin ($P = .003$), C-reactive protein ($P = .033$), and leptin ($P < .001$). No significant differences were found in Hcy levels between the obese and nonobese children. However, Hcy concentration was significantly higher in the hyperinsulinemic obese children than in the normoinsulinemic group ($P = .002$). Using multivariant regression analysis, in the obese group, corrected for age and sex, the homeostasis model assessment for insulin resistance (P partial $= .001$) and leptin (P partial $= .02$) are independent predictive factors for Hcy. In the control group, corrected for age and sex, the homeostasis model assessment for insulin resistance (P partial $= .005$) and leptin (P partial $= .031$) also are independent predictive factor for Hcy. Increased plasma Hcy, particularly in hyperinsulinemic obese children, may be causally involved in the pathogenesis of atherosclerosis and/or cardiovascular disease, both of which are common in obesity.

1. Introduction

Obesity is a chronic pathology with high morbidity-mortality rates, frequently associated with various metabolic disorders defined as metabolic syndrome [1-4]. The syndrome is associated with insulin resistance and systemic low-grade inflammatory state [5].

Hyperhomocysteinemia is now regarded as an independent risk factor for atherothrombotic and thromboembolic vascular disease [6-9]. Elevated plasma homocysteine (Hcy) levels occur in a large proportion of patients with coronary artery disease [10].

The exact mechanism by which higher Hcy levels may translate into increased coronary heart diseases and/or thrombotic risk remains speculative. Several plausible mechanisms for Hcy-induced atherosclerosis have been proposed, including endothelial dysfunction [11], impaired flow-mediated vasodilation [12], increased proliferation of vascular smooth muscle cells [13], enhanced coagulability [14], and inflammatory effects [15].

The mechanism of Hcy angiotoxicity seems to involve the nitric oxide system by inducing oxidant stress [16-18]. Oxidative stress has been suggested to cause insulin resistance and may be its possible link with atherosclerosis [19,20]. Insulin resistance has been found to be associated with elevated plasma total Hcy levels [21]. Higher Hcy levels are observed in the hyperinsulinemic obese subjects [22].

Plasma Hcy concentrations appear to be regulated by acute hyperinsulinemia in nondiabetic patients, although not

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in type 2 diabetic subjects [23], suggesting that insulin resistance may contribute to the development of hyperhomocysteinemia and cardiovascular disease. We do not yet know the exact relationship between these risk factors.

As has been reported, inflammation may be one of the possible mechanisms by which increased levels of Hcy develop atherosclerosis [15].

Previous studies support a possible regulatory role of insulin in the metabolism of Hcy and indicate that hyperhomocysteinemia is an integral component of this rat model of metabolic syndrome [24]. High concentrations of Hcy should also be added in the human metabolic syndrome.

Obese children report resistance to insulin (within the context of metabolic syndrome) [3,25], low-grade systemic inflammation, and increased leptin levels [26,27], all of which are a risk for the development of cardiovascular disease.

In this work, we aimed to study total Hcy levels in prepubertal obese children and their possible association with both metabolic syndrome and various inflammatory biomarkers and leptin.

2. Materials and methods

2.1. Subjects

A case-control study was carried out in obese children of both sexes. One group was composed of 43 obese children (body mass index [BMI] over 90th percentile in growth curves for the study population [28]), and another with the same number of nonobese children (BMI minor 85th percentile) paired by age and sex (aged 6-9 years) as the control group. All subjects were at Tanner stage 1.

In the obese group, a division was made between normoinsulinemic children and hyperinsulinemic children (basal insulin > the mean ± 2 SD of the control group, cutoff point 11.34 μU/mL).

All parents submitted written consent, and the study was authorized by the ethical investigation committee of our hospital.

Children with primary hyperlipidemia, hypertension, diabetes, or glucose intolerance and secondary obesity were excluded from the study. Any child receiving pharmacological treatment was also excluded. All children had similar lifestyles, with no significant physical training program, and all the children are of Spanish origin.

2.2. Blood sampling and analysis

Blood samples were collected after 12 hours of fasting from a vein in the antecubital fossa. All collections were made between 8:00 and 9:00 AM. Whole blood specimens were collected in different tubes to obtain serum and plasma. The samples were separated in aliquots and frozen immediately at −45°C until determination could be performed.

The Hcy concentration was determined for all the children, as well as C-reactive protein (CRP), interleukin 6 (IL-6), leptin, folate, vitamin B_{12}, and different variables related to metabolic syndrome (insulin, lipids, blood pressure, hydrocarbonate metabolism, hemostasis).

Glucose, cholesterol, and triglycerides were determined in a random access analyzer (Axon, Bayer Diagnostics, Tarrytown, NY) with Bayer Diagnostics reactives. Homeostasis model assessment for insulin resistance (HOMA-IR) was used to detect the degree of insulin resistance. The resistance can be assessed from the fasting glucose and insulin concentrations by the formula: (HOMA-IR) = [insulin (mU/L) × glucose (mmol/L)]/22.5. High-density lipoprotein cholesterol (HDL-C) was determined after precipitation of chylomicrons, very low-density lipoproteins, and low-density lipoproteins, with phosphotungstic acid and magnesium ions.

The Hcy assay is based on the fluorescence polarization immunoassay technology (AxSYM System Homocysteine, Abbott Laboratories, Chicago, IL) and was performed on the AxSYM automatic analyzer (Abbott Laboratories). Folate and vitamin B_{12} levels were measured by an Access2-Immunoassay System (Beckman Coulter, Fullerton, CA).

Insulin was quantified by a microparticle immunoassay (IMx System Insulin, Abbott Laboratories) in an IMx automatic analyzer (Abbott Laboratories). C-reactive protein was determined by nephelometry (CRP reagent, Behringwerke, Marburg, Germany) in a Dade Behring Analyzer II Nephelometer (Marburg, Germany).

Antigenic immunoassay methods were used for the quantification of plasminogen activator inhibitor 1 (PAI-1) (Asserachrom PAI-1, Diagnostica Stago, Asnieres-sur-Seine, France), IL-6 (Quantikine human IL-6, RD Systems, Wiesbaden-Nordenstadt, Germany), and leptin (Quantikine human leptin, RD Systems) carried out in a microtiter plate analyzer (Biokit, Pharmacia, Barcelona, Spain). The fibrinogen was measured by quantitative assay using thrombin in an automatic analyzer (Electra 1600, Ortho Clinical Diagnostics, Madrid, Spain).

2.3. Anthropometric measurements

Weight was measured to the nearest 0.1 kg and height to the nearest 0.1 cm. BMI was calculated as the weight in kilograms divided by the square of height in meters. Waist circumferences were measured at the level of the umbilicus, and hip circumferences at the level of greater trochanters and pubic symphysis to the nearest 0.1 cm.

2.4. Statistical analysis

Statistical assessment was conducted using Microstat (Ecosoft, Indianapolis, IN) or GraphPad InStat (GraphPad Software, San Diego, CA). Abnormal values (outliers) were excluded. An observation was considered an outlier if its residual was more than 3 SEs of estimate (SD about the regression line) ($Sy \cdot x$). Results were expressed as mean ± SEM, with a 95% confidence interval (95% CI). The distribution of each variable was tested for departure from gaussian distribution, and variance equality was

controlled by Snedecor F test. The mean values of the groups were compared using Student unpaired t test. Statistical significance was set at $P < .05$.

The detection limit for CRP was 0.175 mg/L, and in the statistical evaluation, all values of less than 0.175 mg/L were treated as 0.10 mg/L.

Correlation between variables was evaluated using Pearson correlation coefficient and regression analysis. Multivariant regression analysis was performed using the stepwise method. For each variable, potential confounding factors ($.05 < P < .2$) were evaluated by an analysis of raw and adjusted regression coefficients.

3. Results

Table 1 shows the anthropometric data and selected biochemical parameters of the obese and control groups. The median age was 8.01 (obese) and 8.20 years (control), with a range of 6 to 9 years.

Nonsignificant differences were found in Hcy levels between obese and nonobese children (95% CI, 6.38-7.14 vs 6.11-6.68 μmol/L) (Table 1). However, mean plasma Hcy concentration was significantly higher in the hyperinsulinemic obese children than in the normoinsulinemic group (95% CI, 6.67-8.94 vs 6.07-6.76 μmol/L) (Table 2).

Folate (13.85 ± 0.59 nmol/L obese vs 12.93 ± 0.72 nmol/L control; $P = .326$) and vitamin B_{12} levels (586 ± 24 pg/mL obese vs 547 ± 28 control pg/mL; $P = .293$) were similar in both groups.

The mean values (obese vs control) for insulin (95% CI, 7.17-9.37 vs 5.31-6.92 μU/mL), HOMA-IR (95% CI, 1.52-2.03 vs 1.34-1.48), CRP (95% CI, 1.25-2.02 vs 0.45-1.44 mg/L), and leptin (95% CI, 13.07-18.70 vs 3.51-5.89 ng/mL) were significantly higher in the obese children (Table 1).

Table 1
Descriptive statistics and selected biochemical parameters of the study groups

	Control (n = 43)	Obese (n = 43)	P
Male/female	17/26	17/26	
Age (y)	8.12 ± 0.12	7.83 ± 0.15	.135
BMI (kg/m^2)	16.95 ± 0.22	22.33 ± 0.29	<.001
WHR	0.844 ± 0.006	0.850 ± 0.001	.327
Glucose (mmol/L)	4.74 ± 0.084	4.83 ± 0.047	.353
Insulin (μU/mL)	6.12 ± 0.41	8.27 ± 0.56	.003
HOMA-IR	1.309 ± 0.088	1.778 ± 0.131	.004
Cholesterol (mg/dL)	164.2 ± 3.85	172.3 ± 3.77	.137
Triglycerides (mg/dL)	50.70 ± 2.11	65.39 ± 4.27	.003
HDL-C (mg/dL)	55.87 ± 1.88	49.99 ± 1.36	.013
PAI-1 (ng/mL)	19.16 ± 2.81	31.71 ± 4.58	.022
Fibrinogen (g/L)	2.33 ± 0.058	2.84 ± 0.081	<.001
CRP (mg/L)	0.947 ± 0.25	1.635 ± 0.194	.033
IL-6 (pg/mL)	1.68 ± 0.358	2.24 ± 0.335	.256
Leptin (ng/mL)	4.71 ± 0.61	15.89 ± 1.43	<.001
Hcy (μmol/L)	6.40 ± 0.18	6.82 ± 0.15	.082

Values are mean ± SEM. WHR indicates waist-to-hip ratio.

Table 2
Descriptive statistics and selected biochemical parameters of the obese group

	Hyperinsulinemic (n = 14)	Normoinsulinemic (n = 29)	P
Male/female	5/9	12/17	
Age (y)	7.92 ± 0.25	7.76 ± 0.18	.606
BMI (kg/m^2)	22.83 ± 0.34	22.26 ± 0.38	.347
Glucose (mmol/L)	4.91 ± 0.069	4.81 ± 0.059	.312
Insulin (μU/mL)	12.49 ± 0.56	6.48 ± 0.47	<.001
HOMA-IR	2.73 ± 0.144	1.40 ± 0.110	<.001
Cholesterol (mg/dL)	171.71 ± 7.7	173.58 ± 4.32	.821
Triglycerides (mg/dL)	83.21 ± 7.35	59.31 ± 3.68	.002
HDL-C (mg/dL)	48.12 ± 2.44	49.91 ± 1.68	.548
PAI-1 (ng/mL)	39.62 ± 5.62	29.93 ± 3.89	.163
Fibrinogen (g/L)	2.84 ± 0.14	2.75 ± 0.09	.590
CRP (mg/L)	1.94 ± 0.28	1.52 ± 0.27	.336
IL-6 (pg/mL)	2.97 ± 0.65	1.95 ± 0.39	.163
Leptin (ng/mL)	21.76 ± 2.64	12.68 ± 1.21	<.001
Hcy (μmol/L)	7.81 ± 0.52	6.41 ± 0.17	.002

Values are mean ± SEM.

PAI-1 and fibrinogen levels were significantly higher in the obese group (Table 1).

The obese group was divided into hyperinsulinemic and normoinsulinemic children, showing increased levels of Hcy, leptin, and triglycerides in hyperinsulinemic obese children (Table 2).

3.1. Hcy and metabolic syndrome

With regard to the metabolic syndrome components, the univariant correlation analysis is summarized in Table 3. In the single linear correlation, for the obese group, plasma Hcy concentration was positively correlated with insulin, HOMA-IR, and leptin, but not with lipid profile (Fig. 1).

Using multivariant regression analysis, in the obese group, HOMA-IR (P partial = 0.001) and leptin (P partial = 0.02), corrected for age and sex, are independent predictive factor for Hcy.

Table 3
Coefficients of simple correlation (r) between Hcy and different variables of the obese and control groups (n = 43)

	Hcy			
	Obese		Control	
	r	P	r	P
BMI	0.2682	.082	0.0494	.772
WHR	0.2477	.121	0.0922	.587
Insulin	0.5484	<0.001	0.4151	.010
HOMA-IR	0.4380	.006	0.3917	.017
Cholesterol	0.2324	.144	0.0781	.655
Triglycerides	0.0589	.714	0.1642	.297
HDL-C	−0.1177	.464	−0.1550	.361
PAI-1	0.1681	.294	0.0487	.788
Fibrinogen	0.1096	.495	0.2528	.114
CRP	0.3337	.042	0.1589	.355
IL-6	0.3707	.018	0.0342	.843
Leptin	0.3764	.013	0.3721	.025

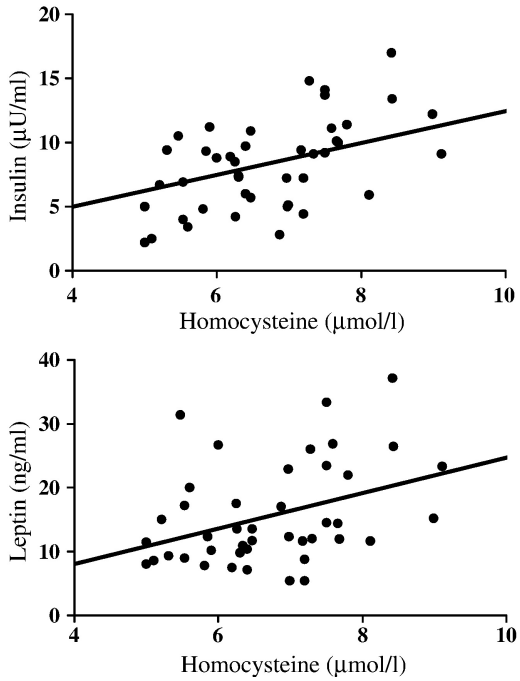

Fig. 1. Serum Hcy concentrations as a function of insulin (r = 0.5484, P < .001) and leptin (r = 0.3764, P = .013) in obese children.

In the control group, Hcy also correlated with insulin, HOMA-IR, and leptin (Table 3). In this group, corrected for age and sex, HOMA-IR (P partial = .005) and leptin (P partial = .031) are independent predictive factors for Hcy.

3.2. Hcy and inflammatory biomarkers

In the obese group, plasma Hcy concentration correlates positively with IL-6 and C-reactive protein (Table 3), but not in the control group.

The study with multivariant regression analysis, in the obese group, corrected for age and sex, showed that plasma IL-6 and CRP are not independent predictive factors for Hcy.

4. Discussion

In this work, we describe increased levels of Hcy in hyperinsulinemic obese children of a very young age; these levels are significantly associated not only with insulin concentration, but also with variables of inflammation and leptin.

The presence of hyperinsulinism and insulin resistance has been described in obese children [3,29]. Some of the disorders related to metabolic syndrome have been described in children [2,3,25]. Low-grade systemic inflammation and increased leptin levels are reported in very young obese children [27].

Hyperinsulinemia and hyperhomocysteinemia are now also accepted risk factors for atherosclerosis [6,30]. The exact mechanism by which higher Hcy levels may translate into increased risk for coronary heart disease remains speculative.

In our study, we describe increased total Hcy levels in obese children with hyperinsulinemia (>11.34 μU/mL) compared with obese children with normal insulin levels (<11.34 μU/mL), with similar levels of folate and vitamin B_{12}. The association between insulin resistance and Hcy has been proposed in healthy, obese, and nonobese subjects [21,31,32], as well as in hyperinsulinemic obese adults [22].

It has been suggested that increased levels of Hcy may be an additional component of metabolic syndrome [24]. In prepubertal obese children, we have found a correlation between Hcy and some of the components of metabolic syndrome, although only insulin appears to be an independent predictive factor for Hcy.

In the obese and nonobese children, we have found that HOMA-IR is an independent predictive factor for Hcy, suggesting that insulin is a determinant of Hcy concentration in both physiological and pathological situations in very young children.

Obesity is frequently associated with high plasma leptin concentrations and leptin resistance. The concentration of leptin in serum is correlated with insulin resistance and metabolic syndrome [33,34], and it has been shown as an independent risk factor for coronary heart disease [35]. Our group showed an independent association between insulin and leptin in prepubertal obese children [27].

In the present work, we found plasma leptin concentration significantly higher in the hyperinsulinemic obese children when compared with the obese normoinsulinemic group. As what occurs with insulin, in the obese and nonobese children, we found that leptin levels are an independent predictive factor for Hcy.

Chronically high levels of insulin, characteristic of metabolic syndrome, associated with increases of leptin (insulin and leptin have been reported as increased in the prepubertal obese children) [3,27], may play a role in the increase of Hcy, which we find in the obese hyperinsulinemic subjects. At the same time, increased levels of Hcy may facilitate the onset of atherosclerosis by means of various mechanisms. The present study does not allow for conclusions to be drawn as to whether increased plasma Hcy levels is the cause or result of insulin and leptin resistance. However, we can affirm that Hcy, insulin, and leptin are described as altered from very early ages in obese children and may play a part in the development of the metabolic complications associated with obesity.

Inflammation is one of the possible mechanisms by which high levels of Hcy could facilitate the onset of atherosclerosis. The elevated CRP concentrations in obese subjects might be explained by the expression of the cytokine IL-6 in adipose tissue [36] and its release into the circulation [37]. IL-6 is a proinflammatory cytokine that stimulates the production of CRP in the liver [38]. For some authors, Hcy affects the expression of the same inflammatory factors [15,39].

At the same time, these proinflammatory cytokines, given off by adipose tissue, can lead to an increase in the secretion of PAI-1 [40]. In addition, fibrinogen has been associated with inflammatory reaction. Both parameters can generate a situation favoring atherothrombosis, with increased coagulation and decreased fibrinolysis. In our results, both PAI-1 and fibrinogen are increased in the obese children.

In the obese children we studied, an increase was seen in some indicators of inflammation, such as CRP. In the obese group, plasma Hcy concentration correlates positively with IL-6 and CRP. These results suggest the atherogenic effects of Hcy, at least, in part from its inflammatory effects.

In conclusion, we have found in very young obese hyperinsulinemic children increased levels of Hcy, which are significantly associated not only with insulin resistance, but also with variables of inflammation and leptin.

The increased level of plasma Hcy, particularly in hyperinsulinemic obese children, may be causally involved in the pathogenesis of atherosclerosis and/or cardiovascular diseases, which are so common in obesity.

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