The association of plasma homocysteine levels with serum leptin and apolipoprotein B levels in childhood obesity

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BACKGROUND: An elevated plasma total homocysteine (tHcy) level has recently been established as an independent risk factor for thrombosis and vascular disease. However, the relationship between hyperhomocysteinemia and cardiovascular disease and obesity remains controversial. The aim of the study was to investigate a possible relationship between plasma tHcy levels and measures of childhood obesity.

SUBJECTS AND METHODS: Forty children and adolescents with exogenous obesity and 20 non-obese subjects in an age-and sex-matched control group were investigated. Fasting samples were collected for plasma tHcy, serum insulin, leptin, vitamin B12, folate, creatinine and lipid parameters. Anthropometric characteristics and body compositions were assessed in both groups.

RESULTS: The obese patients had significantly higher tHcy levels than the non-obese controls (14.3±11.8 μmol/L vs 8.7±5.9 μmol/L; \( P=0.017 \)). In both groups, plasma tHcy was positively related to serum leptin, but serum apolipoprotein B (apo B) levels were positively related to plasma tHcy levels only in obese patients.

CONCLUSIONS: Our study demonstrates for the first time that leptin and apo B are main correlates of tHcy in obese children and adolescents and suggests that hyperleptinemia and increased apo B may contribute to impairment of tHcy metabolism in childhood obesity.

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of direct peripheral actions. These include angiogenesis, hematopoeisis, lipid and carbohydrate metabolism and effects on the reproductive, cardiovascular and immune systems; these effects are reviewed elsewhere. In obese humans, an increased fat mass correlates with increased plasma leptin levels and is also associated with the development of insulin resistance. Currently, no data are available that deal with a possible relationship among tHcy levels, insulin levels, leptin levels, body composition, and metabolic risk factors for CVD in childhood obesity. Therefore, the aim of the present study was to investigate a possible relationship between plasma tHcy levels and measures of childhood obesity.

Subjects and Methods
Subjects consisted of 40 obese (BMI>95th percentile for age and sex) children and adolescents, including 23 boys and 17 girls, and 20 age- and sex-matched non-obese children and adolescents, including 11 boys and 9 girls. The age of subjects ranged from 7 to 17 years in both the obese and non-obese control groups. Developmental stage was not formally assessed, although 18 subjects in the obese group and 9 subjects in non-obese group were Tanner stage II and bigger. At admission, a medical history and physical examination were performed to ensure that the subjects were healthy. All children and adolescents had normal liver and renal function as assessed by standard clinical chemistry analyses.

Before entering a course of treatment, they were instructed to visit the clinic in the morning, after an overnight fast. Blood was then drawn and, at the same time, anthropometric measurements were made, including body weight, height, blood pressure, waist girths, hip girth, and triceps and subscapular skinfold thicknesses. Waist-hip ratio (WHR) was calculated by division of the two parameters. Skinfold thickness was measured by calipers at the triceps and subscapular region. Fat mass and lean body mass were calculated by DEXA (dual energy x-ray absorptiometry). A fan beam X-ray bone densitometer (Hologic QDR-4500A; Hologic Inc.) with human software version 9.8 was used. Resting blood pressure was measured in the sitting position after a 15-minute rest using a mercury sphygmomanometer and a cuff appropriately sized for the arm size of the subject. Informed consent was obtained from each child and a legal guardian.

Venous blood samples were taken between 7:30 and 9:30 A.M after an overnight fast. The tHcy concentration in plasma was determined by high-performance liquid chromatography with fluorometric detection. The sample, internal standard and phosphate buffered saline (PBS, pH 7.4) were mixed. Tris-2-carboxy-ethylphosphine (TCEP) in water-soluble was added to reduce the thiols and mixed sulfides. After incubation at room temperature for 30 minutes, trichloroacetic acid was added to precipitate proteins. Next, the centrifuged supernatant was mixed with NaOH, EDTA and SBD-F (ammonium-7-fluoro- benzo-2-oxa-1.3-diazole-4-sulfonate) in borate buffer. Samples were incubated at 60°C in a water bath for 1 hour, to form fluorescent derivates. After cooling at 4°C, 10 μL of the samples were injected to HPLC. HPLC was carried out on a solvent delivery system and a fluorescence detector; 0.1 mol/L acetic acid-acetate buffer, pH 5.5 containing 30 mL/L methanol was used to elute the constituents as mobile phase. Flow rate was 0.7 mL/minute. L-homocysteine calibrators (5-100 μmol/L) were prepared in PBS, pH 7.4 and in pooled EDTA plasma. The tHcy peaks separated with analytic HPLC column were provided in 3.3 minutes. The tHcy concentrations were calculated according to peak levels. Variation co-efficient was 3.5%. Serum folate and vitamin B12 were measured by Simultrac-SNB Radioassay kit vitamin B12 (57Co)/Folate (125). Serum glucose, serum lipids [total cholesterol (TC), triglyceride, high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol] and serum lipoprotein [Apolipoprotein A (apo A), Apolipoprotein B (apo B) and Lipoprotein a] concentrations were assayed on a Konelab 60i autoanalyzer (Thermo Electron Corp, www.thermo.com). Serum concentrations of insulin were measured by Coat-A-Count Insulin from DPC (Diagnostic Products Corporation, Los Angeles, CA). Obese subjects with fasting insulin levels >20 μU/ml were considered hyperinsulinemic. Serum concentrations of leptin were measured with commercially available IRMA kits (Human Leptin Irma DSL-23100).

Data were expressed as mean ±SD. Differences between data were studied using Student’s t-test. Correlations were assessed using Pearson’s correlation coefficient. The significance of variables was tested by multiple regression analysis based on the results of the bivariate correlations. In the regression model we verified tHcy as a dependent variable and included age, BMI, total fat mass, leptin, apo B, insulin, folate values as independent variables. Statistical significance was taken as P<0.05. Data were analyzed using SPSS for Windows (version 11.0, SPSS).
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Results
In the obese group (n=40), all anthropometric parameters were higher than in the non-obese control group (n=20) except for height (Table 1). Systolic and diastolic blood pressure, total fat mass and lean body mass were significantly higher in obese subjects than non-obese subjects. There were no differences between males and females.

The obese subjects had significantly higher total homocysteine levels than the non-obese controls (Table 2). The mean leptin and insulin concentrations were also significantly higher, and the mean serum TC, triglyceride and serum apo B values were significantly higher in the obese group compared with those in the control group. All obese subjects were hyperinsulinemic according to fasting insulin levels.

Among several parameters analyzed, only leptin and apo B correlated significantly with total homocysteine in obese subjects (Table 3). Total homocysteine concentrations correlated positively with leptin and apo B concentrations in the obese group (Figure 1). Total homocysteine concentrations correlated positively only with leptin in non-obese subjects. None of the other anthropometric and laboratory parameters were correlated with total homocysteine concentration in either group.

In the multiple regression analyses, the contribution of the independent variables leptin, apo B, total body fat, insulin, age, BMI and folate to the variance in total homocysteine was investigated. In stepwise multiple regression analysis, only leptin contributed to the variance in total homocysteine (R=0.55, β=0.42, P=0.006).

Discussion
Homocysteine is a nonprotein-forming, thiol-containing amino acid formed by demethylation of methionine. It is metabolized by remethylation to methionine or by transulfuration to cysteine. An elevated plasma total homocysteine level has recently been established as an independent risk factor for thrombosis and vascular disease. However, the relationship between hyperhomocysteinemia and CVD remains controversial. Although some prospective studies have confirmed that total homocysteine is an independent risk factor for CVD, other studies have not found such a relationship. Data are relatively sparse on variations in total homocysteine levels among obese subjects at increased risk for type 2 diabetes or CVD. Our study demonstrated an unfavorable relationship among fasting plasma total homocysteine levels, leptin levels and apo B levels in obese children and adolescents.

No study has demonstrated a relationship between total homocysteine and leptin levels in obesity. Leptin

Table 1. Anthropometric parameters for both groups.

|                     | Obese n=40 | Control n=20 | P  |
|---------------------|------------|--------------|----|
| Age (years)         | 11.4±2.5   | 11.6±2.6     | 0.81|
| Height (cm)         | 147.1±15.4 | 141.5±15.2   | 0.18|
| Weight (kg)         | 60.2±16.2  | 37.3±11.1    | 0.001|
| BMI (kg/m²)         | 27.4±4.3   | 18.2±2.2     | 0.001|
| WHR                 | 0.89±0.06  | 0.83±0.05    | 0.001|
| Triceps SFT (cm)    | 27.4±5.5   | 10.8±4.1     | 0.001|
| Subscapular SFT (cm)| 29.7±7.7   | 6.7±2.2      | 0.001|
| Sistolic BP (mmHg)  | 116.1±16.8 | 104.5±12.6   | 0.004|
| Diastolic BP (mmHg) | 72.8±13.9  | 65.7±12.0    | 0.047|
| Total fat mass (kg) | 22.7±6.5   | 8.4±2.9      | 0.001|
| Lean body mass (kg) | 36.9±19.6  | 28.7±8.9     | 0.003|

BMI: Body mass index, WHR: Waist-hip ratio
Data are means (±SD), P<0.05.

Table 2. Laboratory data for both groups.

|                     | Obese n=40 | Control n=20 | P  |
|---------------------|------------|--------------|----|
| tHcy (mol/L)        | 14.3±11.8  | 8.7±5.9      | 0.017|
| Vitamin B12 (pg/ml) | 602.3±283.8| 592.2±314.5  | 0.90 |
| Folate (ng/ml)      | 10.7±5.1   | 10.8±8.5     | 0.95 |
| Leptin (ng/ml)      | 27.5±16.0  | 6.8±8.3      | 0.001|
| Glucose (mg/dl)     | 98.7±16.4  | 92.1±13.7    | 0.06 |
| Insulin (μIU/ml)    | 45.3±32.1  | 14.5±5.3     | 0.001|
| Creatinine (mg/dl)  | 0.57±0.13  | 0.57±0.14    | 0.90 |
| Tryglyceride (mg/dl)| 174.7±91.1 | 101.0±34.5   | 0.001|
| Total cholesterol (mg/dl) | 176.2±25.8 | 153.0±35.0 | 0.02 |
| LDL-cholesterol (mg/dl) | 97.6±32.8 | 84.3±35.8 | 0.17 |
| HDL-cholesterol (mg/dl) | 43.7±13.8 | 48.4±13.2 | 0.21 |
| Apolipoprotein A (mg/dl) | 138.3±21.4 | 136.8±20.6 | 0.93 |
| Apolipoprotein B (mg/dl) | 93.3±23.3 | 78.7±16.2 | 0.007|
| Lipoprotein a (mg/dl) | 29.5±33.9 | 36.6±36.4 | 0.46 |

tHcy: plasma total homocysteine, HDL: high-density lipoprotein, LDL: low-density lipoprotein
Data are means (±SD), P<0.05.
Table 3. Correlations among tHcy levels and measures of obesity in obese children and adolescents.

|                   | Homocysteine |    | P    |
|-------------------|--------------|----|------|
| Age (years)       | 0.10         | 0.53|
| BMI (kg/m²)       | 0.021        | 0.17|
| Systolic BP (mmHg)| 0.007        | 0.96|
| Total fat mass (kg)| 0.12      | 0.46|
| Lean body mass (kg)| 0.04        | 0.80|
| Leptin (ng/ml)    | 0.42         | 0.006|
| Insulin (μIU/ml)  | 0.23         | 0.14|
| Folate (ng/ml)    | 0.06         | 0.67|
| Creatinine (mg/dl)| 0.13         | 0.41|
| Tryglyceride (mg/dl)| 0.28     | 0.07|
| Total cholesterol (mg/dl)| 0.08   | 0.62|
| Apolipoprotein A (mg/dl)| 0.18   | 0.25|
| Apolipoprotein B (mg/dl)| 0.34   | 0.027|
| Lipoprotein a (mg/dl)| 0.30   | 0.05|

BMI: Body mass index

In the present study, obese subjects had higher TC, triglyceride and apo B levels compared with non-obese subjects of similar age. However, we only found a significant positive correlation between tHcy and apo B levels. Apo B, which is a useful predictor of CVD in adults, was found to be useful in another study in children, particularly for detecting genetic disorders of lipid metabolism. However, no study has demonstrated a correlation between tHcy and apo B in the literature. In 482 patients already at high risk for atherosclerotic vascular disease by virtue of hyperlipidemia, 3.7% had high plasma tHcy. Plasma tHcy has been shown to be associated with a parental history of CVD in children with familial hypercholesterolemia. Elevated HDL cholesterol was well accepted as a protective factor against atherosclerosis. However, high HDL cholesterol is not necessarily protective in the setting of an elevated plasma tHcy.

According to our data, it is possible that the high levels of leptin observed in childhood obesity could contribute to its adverse effects on cardiovascular health by increasing tHcy levels.

In the present study, obese subjects had higher TC, triglyceride and apo B levels compared with non-obese subjects of similar age. However, we only found a significant positive correlation between tHcy and apo B levels. Apo B, which is a useful predictor of CVD in adults, was found to be useful in another study in children, particularly for detecting genetic disorders of lipid metabolism. However, no study has demonstrated a correlation between tHcy and apo B in the literature. In 482 patients already at high risk for atherosclerotic vascular disease by virtue of hyperlipidemia, 3.7% had high plasma tHcy. Plasma tHcy has been shown to be associated with a parental history of CVD in children with familial hypercholesterolemia. Elevated HDL cholesterol was well accepted as a protective factor against atherosclerosis. However, high HDL cholesterol is not necessarily protective in the setting of an elevated plasma tHcy.

Considerable in vitro data exist to suggest that tHcy may interact with elevated TC by increasing oxidation of LDL cholesterol. However, this area remains controversial. In one study, LDL cholesterol isolated from two patients with homocystinuria showed a similar degree of copper-catalyzed oxidation as LDL cholesterol from a group of healthy control subjects. According to our data, in the long term, childhood obesity associated with increased apo B may cause CVD. Thus, more studies are needed on the relationship of tHcy to lipids and lipoproteins in patients with childhood obesity.

Our study did not demonstrate an unfavorable relationship among fasting plasma tHcy levels, insulin, vitamin B12, folate, creatinine and body composition in obese children and adolescents. The few studies in which hyperinsulinemia in obese subjects has been assessed directly have shown positive associations with tHcy levels. Sanchez-Margalet et al. reported a significant positive correlation between fasting insulin and tHcy plasma levels. In another study, Gallistl et al. suggested that insulin is an independent correlate of plasma tHcy levels in obese children and adolescents. In that study, it was report-
ed that BMI was significantly correlated with tHcy levels and was an independent predictor of tHcy levels. In the present study, although there were elevated fasting insulin levels in obese subjects, insulin was not correlated with tHcy levels. Moreover, tHcy concentrations were not related to indexes of obesity (BMI and fat mass). In our study, serum creatinine did not alter associations between hyperinsulinemic obese subjects and tHcy levels, confirming findings in some but not all epidemiological studies. However, an important limitation of our study is that serum creatinine is probably not an adequately sensitive marker of true glomerular filtration rate among subjects with normal renal function. As in studies performed in adults, folate correlated inversely with tHcy and contributed independently and significantly to the variance in tHcy in stepwise multiple regression analysis, but we did not find a relationship between tHcy and folate or vitamin B12 levels in obese subjects, probably because of adequate intake of folate and vitamin B12.

Our analysis showed that leptin was the main independent correlate explaining the variation in tHcy levels in multiple regression analyses, but the present study does not permit conclusions about whether elevation of plasma tHcy is the cause or result of leptin resistance. Moreover, other possible sources of elevated tHcy, such as genetic variation of MTHFR activity or functional vitamin B6 deficiency have not been assessed in the present study. Nevertheless, in the present study, there seems to be an association with increased tHcy levels in obese subjects with hyperleptinemia and increased apo B levels, which may contribute to the development of the cardiovascular complications that are so common in obesity.

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