Insulin Is an Independent Correlate of Plasma Homocysteine Levels in Obese Children and Adolescents

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OBJECTIVE — The aim of the study was to investigate whether anthropometric and metabolic risk factors for coronary heart disease (CHD) contribute to the variation in homocysteine levels in obese children and adolescents.

RESEARCH DESIGN AND METHODS — A total of 84 children and adolescents were assessed for fasting total homocysteine, methyltetrahydrofolate reductase polymorphism (C677T mutation), folate and vitamin B₁₂ status, and anthropometric and metabolic risk factors for CHD.

RESULTS — No significant sex differences were found for all available anthropometric and metabolic characteristics except for homocysteine, which was significantly higher in boys than in girls (7.1 vs. 6.3 μmol/l; P < 0.05). After adjustment for age and sex, homocysteine correlated significantly with BMI, fat mass, percentage of fat mass, and insulin and showed an inverse correlation with folate levels. Homocysteine did not correlate with vitamin B₁₂ total cholesterol; LDL, HDL, and VLDL; triglycerides; and glucose. BMI and fat mass correlated significantly with insulin and showed a significant inverse correlation with folate. We found no association between homocysteine and the C677T mutation. In multiple regression analyses, insulin was found to be the main correlate of homocysteine.

CONCLUSIONS — Our study demonstrates for the first time that insulin is a main correlate of homocysteine in obese children and adolescents and suggests that fat mass–associated hyperinsulinemia may contribute to impairment of homocysteine metabolism in childhood obesity.

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Homocysteine is derived from the metabolic conversion of the essential amino acid methionine. In the remethylation pathway of homocysteine to methionine, vitamin B₁₂ and folate act as cofactors (1). One of the essential enzymes in the remethylation process is methyltetrahydrofolate reductase (MTHFR) (2). Mild-to–moderate hyperhomocysteinemia has been shown to be associated with arterial thrombosis in several case-control and cross-sectional studies (3,4). The mechanisms by which hyperhomocysteinemia may predispose to arterial thrombosis are not entirely clear but consist of endothelial cell damage (5), inhibition of fibrinolysis (6), activation of the coagulation cascade (7), impaired generation of nitric oxide and prostacyclin (8,9), and enhanced collagen production by smooth muscle cells (10). Major determinants of plasma homocysteine levels are folate, vitamin B₁₂, and B₆ intake, renal function, and to a lesser extent cigarette smoking, arterial hypertension, hypercholesterolemia, physical exercise, coffee consumption, and alcohol consumption (11). In addition, individuals homozygous for the thermolabile form of MTHFR also show higher levels of homocysteine, mainly in the presence of low folate (12). One further dietary determinant of plasma homocysteine level may be fat intake because fat intake is associated with higher homocysteine levels in healthy men, probably because of a lower intake of essential vitamins (13). High fat intake has been shown to be linked with childhood obesity (14,15), which is associated with an unfavorable profile of risk factors for coronary heart disease (CHD) such as hyperinsulinemia, hyperlipidemia, and elevated blood pressure (16,17).

Currently no data are available that deal with a possible relationship among homocysteine levels, body composition, and metabolic risk factors for CHD in childhood obesity. The aim of the present study was to investigate a possible relationship among plasma homocysteine levels, the MTHFR polymorphism, body composition, blood pressure, folate and vitamin B₁₂ levels, serum lipid parameters, and insulin.

RESEARCH DESIGN AND METHODS

Participants

The study involved 84 consecutive white obese (BMI >85th percentile for age and sex) (18) children and adolescents (46 boys, 38 girls) 4.4–17.6 years of age (median 11.9 years) who attended the outpatient clinic of the children's hospital for a basic obesity checkup. Parents were asked to present their children after an overnight fast. None of the children was on any weight management program. At admission, a medical history and physical examination were performed to ensure that the participants were healthy. All children and adolescents had normal liver and renal function as assessed by standard clinical chemistry analyses. No participants were taking multivitamin preparations or medications known to affect lipid metabolism. Resting blood pressure was measured in the sitting position after a 15-min rest.
BMI (kg/m²) 28.8 ± 5.1 29.3 ± 4.8 0.61
Age (years) 10.8 ± 3.1 11.4 ± 2.6 0.37

46 38 —

Table 1—Characteristics of the study population (n = 84)

<table>
<thead>
<tr>
<th></th>
<th>Boys</th>
<th>Girls</th>
<th>P</th>
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<tbody>
<tr>
<td>n</td>
<td>46</td>
<td>38</td>
<td>—</td>
</tr>
<tr>
<td>Age (years)</td>
<td>10.8 ± 3.1</td>
<td>11.4 ± 2.6</td>
<td>0.37</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>28.8 ± 5.1</td>
<td>29.3 ± 4.8</td>
<td>0.61</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>31.1 ± 13.8</td>
<td>31.64 ± 11.3</td>
<td>0.84</td>
</tr>
<tr>
<td>Percentage fat mass</td>
<td>44.7 ± 7.3</td>
<td>45.2 ± 6.3</td>
<td>0.79</td>
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</table>

Blood pressure (mmHg)
- Systolic
  - Boys: 126.3 ± 14.6
  - Girls: 123 ± 11.2
  - P = 0.39
- Diastolic
  - Boys: 64.8 ± 9.8
  - Girls: 63.6 ± 12.3
  - P = 0.64

Triglycerides (mmol/l)
- Boys: 1.3 ± 0.8
- Girls: 1.2 ± 0.7
- P = 0.68

Total cholesterol (mmol/l)
- Boys: 4.4 ± 0.7
- Girls: 4.5 ± 0.7
- P = 0.52

HDL cholesterol (mmol/l)
- Boys: 1.12 ± 0.28
- Girls: 1.16 ± 0.25
- P = 0.53

LDL cholesterol (mmol/l)
- Boys: 2.43 ± 0.69
- Girls: 2.38 ± 0.62
- P = 0.72

VLDL (mg/dl)
- Boys: 121 ± 88
- Girls: 115 ± 77
- P = 0.71

Glucose (mmol/l)
- Boys: 5.16 ± 0.66
- Girls: 5.12 ± 0.38
- P = 0.63

Insulin (pmol/l)
- Boys: 152 ± 132
- Girls: 126 ± 77
- P = 0.27

B12 (pmol/l)
- Boys: 266 ± 101
- Girls: 316 ± 120
- P = 0.05

Folate (nmol/l)
- Boys: 18.9 ± 7.6
- Girls: 21.5 ± 3
- P = 0.14

Creatinine (µmol/l)
- Boys: 63.1 ± 5.1
- Girls: 70.3 ± 6.3
- P = 0.01

Data are n or means ± SD.

RESULTS — The entire study consisted of 84 subjects (46 boys, 38 girls). BMI was >85th percentile for age and sex in all individuals and >95th percentile in 77 individuals. No significant sex differences were found for all available anthropometric and metabolic characteristics except for homocysteine, which was significantly higher in boys than in girls (P < 0.05). Creatinine was significantly higher in girls than in boys (P = 0.01). The main clinical characteristics and biologic parameters are given in Table 1.

Log insulin (r = 0.53, P < 0.001), homocysteine (r = 0.33, P < 0.005), and creatinine (r = 0.6, P < 0.001) increased in an age-dependent manner. Negative correlations were found between age and folate (r = -0.41, P < 0.001) and vitamin B12 (r = -0.32, P < 0.005). Table 2 shows age- and sex-adjusted correlations between homocysteine and determined parameters. The correlation between log insulin and homocysteine is shown in Fig. 1. Creatinine (r = 0.28, P < 0.01) and systolic blood pres-
Homocysteine in childhood obesity

![Graph: Correlation between log insulin and homocysteine.]

Figure 1— Correlation between log insulin and homocysteine.

Homocysteine correlated with vitamin B12; total, LDL, HDL, and triglycerides; and glucose. A total of 11% of our patients were homozygous for the MTHFR C677T mutation, and 42% were heterozygous. We found no association between the C677T genotype and plasma homocysteine levels.

In multiple regression analyses, the contribution of the independent variables sex, age, BMI, log insulin, and folate to the variance in homocysteine was investigated (Table 3). In the stepwise regression model, only log insulin and folate contributed to the variance in homocysteine (adjusted \( R^2 = 0.3, P < 0.0001 \)).

**CONCLUSIONS** — Evidence has accumulated implicating insulin resistance as a major factor in the pathogenesis of type 2 diabetes and related vascular disturbances (21), and a study suggested that homocysteine is a risk factor for coronary arteriosclerosis in type 2 diabetic patients (22). Our study demonstrates an unfavorable relationship among fasting plasma homocysteine levels, insulin, folate, and body composition in obese children and adolescents. We found that insulin contributed independently and significantly to the variance in plasma homocysteine. The coexistence of severe insulin resistance and hyperinsulinemia has even been demonstrated in preadolescent obese children (23), whereby hyperinsulinemia is considered secondary to the defects in insulin action (24) but has also been implicated in the development and maintenance of excess obesity (25).

Conflicting data exist that deal with a possible regulation of the homocysteine metabolism by insulin that probably depends on renal function. Patients with type 1 diabetes who have normal creatinine levels have decreased homocysteine levels (26), whereas hyperhomocysteinemia is common in nephropathic diabetic patients (27). On the other hand, plasma homocysteine concentrations are decreased by acute hyperinsulinemia in nondiabetic subjects, whereas insulin has no effect on homocysteine levels in type 2 diabetic subjects (28). In addition, insulin resistance has been demonstrated to be associated with elevated plasma homocysteine levels in nonobese subjects (29). In agreement with the assumption that hyperinsulinemia contributes to elevated homocysteine levels, Jacobs et al. (30) demonstrated an increased activity of transsulfuration enzymes and consecutive decreased homocysteine levels in rats with streptozotocin-induced diabetes. This effect was reversible after insulin treatment.

In our study, population insulin levels were strongly related to body fat mass, which reflects the fact that hyperinsulinemia and insulin resistance are strongly correlated with obesity (31). Weight gain of ~20% has shown a corresponding 50% increase in fasting insulin levels (32). Body fat percentage of children varies according to their diet composition, and obese children have a higher fat intake than nonobese children (14,15). BMI was significantly correlated with homocysteine levels and was an independent predictor of homocysteine levels in the multiple regression model when anthropometric parameters exclusively entered the equation (data not shown).

As in studies performed in adults, folate correlated inversely with homocysteine and contributed independently and significantly to the variance in homocysteine in stepwise multiple regression analysis. Folate itself was inversely related to fat mass and BMI, probably because of reduced intake of vegetables in favor of fat, which has been demonstrated in adults (13). This assumption agrees with the observation that lower fat intake in children is associated with increased intake of folate (33).

That insulin was the main independent correlate explaining the variation in homocysteine levels in multiple regression analyses is intriguing. One report suggested that the genes for the transsulfuration enzymes cystathionine β-synthase and cystathionine γ-lyase could be regulated by insulin and/or counterregulatory hormones (30). On the other hand, the relationship could also be indirect because insulin levels reflect indexes of obesity (BMI and fat mass) that show a significant correlation with homocysteine levels. Because plasma homocysteine concentrations are not related to differences in insulin-mediated glucose disposal in healthy adults (34),

<table>
<thead>
<tr>
<th>Homocysteine</th>
<th>Multiple regression model</th>
<th>Stepwise regression model</th>
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<tr>
<td>( \beta \pm 95% \text{ CI} )</td>
<td>( P )</td>
<td>( \beta \pm 95% \text{ CI} )</td>
</tr>
<tr>
<td>Sex</td>
<td>(-0.66 \pm 0.72)</td>
<td>0.07</td>
</tr>
<tr>
<td>Age</td>
<td>0.009 ± 0.15</td>
<td>0.90</td>
</tr>
<tr>
<td>BMI</td>
<td>0.058 ± 0.126</td>
<td>0.36</td>
</tr>
<tr>
<td>Log₁₀ insulin</td>
<td>1.96 ± 1.75</td>
<td>0.028</td>
</tr>
<tr>
<td>Folate</td>
<td>(-0.087 \pm 0.106)</td>
<td>0.106</td>
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Intercept 4.66; adjusted \( R^2 = 0.30; P < 0.0001 \).
which is partially inconsistent with our results, biologic mechanisms that would explain the relationship of insulin and homocysteine are worth exploring.

In conclusion, our study suggests that insulin is an independent correlate of plasma homocysteine levels in obese children and adolescents. Because hyperhomocysteinemia has been demonstrated to be a risk factor for ischemic stroke in children (35), hyperinsulinemia combined with low dietary intake of folate may thus contribute further to the cardiovascular risk profile observed in obese children (36,37). Whether dietary intervention and weight loss could improve homocysteine levels in childhood obesity should be investigated.

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References

