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, methylenetetrahydrofolate reductase polymorphism (C677T mutation), folate and vitamin B_{12} 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 B_{12}; 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 hyperinsulinism 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 B_{12} and folate act as cofactors (1). One of the essential enzymes in the remethylation process is methylenetetrahydrofolate 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 B_{12} and B_6 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 B_{12} 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

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Abbreviations: CHD, coronary heart disease; MTHFR, methylenetetrahydrofolate reductase.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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

	Boys	Girls	*P*
n	46	38	—
Age (years)	10.8 ± 3.1	11.4 ± 2.6	0.37
BMI (kg/m^2)	28.8 ± 5.1	29.3 ± 4.8	0.61
Fat mass (kg)	31.1 ± 13.8	31.64 ± 11.3	0.84
Percentage fat mass	44.7 ± 7.3	45.2 ± 6.3	0.79
Blood pressure (mmHg)			
Systolic	126.3 ± 14.6	123.8 ± 11.2	0.39
Diastolic	64.8 ± 9.8	63.6 ± 12.3	0.64
Triglycerides (mmol/l)	1.3 ± 0.8	1.2 ± 0.7	0.68
Total cholesterol (mmol/l)	4.4 ± 0.7	4.5 ± 0.7	0.52
HDL cholesterol (mmol/l)	1.12 ± 0.28	1.16 ± 0.25	0.53
LDL cholesterol (mmol/l)	2.43 ± 0.69	2.38 ± 0.62	0.72
VLDL (mg/dl)	121 ± 88	115 ± 77	0.71
Glucose (mmol/l)	5.16 ± 0.66	5.12 ± 0.38	0.63
Insulin (pmol/l)	152 ± 132	126 ± 77	0.27
B_{12} (pmol/l)	266 ± 101	316 ± 120	0.05
Folate (nmol/l)	18.9 ± 7.6	21.5 ± 3	0.14
Homocysteine (μmol/l)	7.1 ± 1.7	6.2 ± 1.4	0.02
Creatinine (μmol/l)	63.1 ± 5.1	70.3 ± 6.3	0.01

Data are *n* or means ± SD.

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. The study protocol was approved by the Investigation Review Board of the University of Graz, Graz, Austria.

Analytical methods

Venous blood samples were taken after an overnight fast between 7:30 and 9:30 A.M. Total homocysteine was determined by high-performance liquid chromatography with fluorometric detection (19). To determine the C677T mutation of the MTHFR gene (2), blood cells were frozen at −20°C. The amplification reaction mixture was subjected to 30 cycles of amplification at 94°C for 30 s, 62°C for 30 s, and 72°C for 30 s. The polymerase chain reaction products were precipitated with ethanol and were digested overnight. DNA fragments were analyzed by 3% agarose gel electrophoresis. Vitamin B_{12} and folate were determined using radioimmunoassays. Total cholesterol, triglyceride, and glucose levels were assessed by automated enzymatic methods (Hitachi 937; Roche). HDL and VLDL were determined after precipitation of the lipoproteins by dextran sulfate. LDL cholesterol was calculated using the Friedewald formula. Insulin was measured by means of an insulin-specific radioimmunoassay (Linco).

Measurement of body composition

Measurement of body composition was performed by means of bioelectrical impedance (Bioelectrical Impedence Analyzer Akern-RJL 101/S) with an applied current of 0.8 mA at a fixed frequency of 50 kHz. Measurements were performed in the fasting state after the children had been resting in the supine position for 10 min. Fat-free mass was estimated by the resistance index, height and age of the subjects were determined using the equations of Schaefer et al. (20), and fat mass was estimated as the difference between body mass and calculated fat-free mass.

Statistical analysis

Data that were not normally distributed were $\log_{10}$ transformed (VLDL, triglycerides, glucose, and insulin). An unpaired *t* test and analysis of variance were used to compare parameters between groups where appropriate. Correlations between variables of interest were calculated using Pearson's correlation coefficient, and partial correlation was performed to adjust for the influence of confounding variables. The significance of variables was tested by multiple regression analysis based on the results of the bivariate correlations. The independence of variables to contribute to the variance of the dependent variable was tested by means of stepwise multiple regression models. The significance level of *P* values was set at 5%. Calculations were performed using SPSS for Windows (SPSS, Chicago) and WinStat 3.1 (Kalmia).

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 B_{12} ($r = -0.32$, $P < 0.005$). 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-

Table 2—*Correlations among homocysteine, indexes of obesity, insulin, folate, creatinine, and systolic blood pressure after adjusting for age and sex*

	Homocysteine			
	Adjusted for age		Adjusted for sex	
	r	*P*	*r*	*P*
BMI	0.32	<0.005	0.45	<0.0001
Fat mass	0.27	<0.01	0.4	<0.0005
Percentage fat mass	0.23	<0.05	0.27	<0.01
Insulin	0.38	<0.001	0.53	<0.0001
Folate	−0.31	<0.01	−0.39	<0.001
Creatinine	0.16	0.15	0.4	<0.001
Systolic blood pressure	0.2	0.07	0.32	<0.005

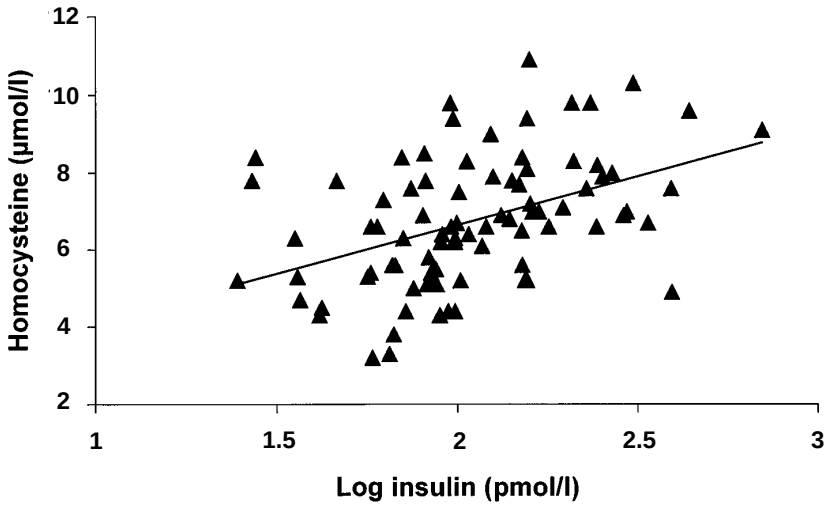

Figure 1—*Correlation between log insulin and homocysteine.*

sure ($r = 0.32$, $P < 0.005$) correlated significantly with homocysteine but not after adjustment for age. Homocysteine did not correlate with vitamin B_{12}; total, LDL, HDL, and VLDL; 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 3—*Multiple regression analysis with homocysteine as the dependent variable*

	Multiple regression model			Stepwise regression model		
Dependent variable	Independent variables	β ± 95% CI	*P*	Independent variables	β ± 95% CI	*P*
Homocysteine	Sex	−0.66 ± 0.72	0.07	Log_{10} insulin	2.48 ± 1.37	0.00056
	Age	0.009 ± 0.15	0.90	Folate	−0.12 ± 0.10	0.022
	BMI	0.058 ± 0.126	0.36			
	Log_{10} insulin	1.96 ± 1.75	0.028			
	Folate	−0.087 ± 0.106	0.106			

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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