Turkish Journal of Endocrinology and Metabolism, (2004) 3 : 107-111 **ORIGINAL ARTICLE**

Plasma Total Homocysteine Levels in Childhood Obesity

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The objective was to investigate total plasma homocyst(e)ine (tHcy) in childhood obesity and in a control group and the contribution of insulin to tHcy values in childhood obesity.

A total of 18 subjects with exogenous obesity and 20 subjects of an age- and sex-matched control group were recruited. Fasting samples for tHcy, vitamin B12, folate, glucose, insulin, lipid profile and creatinine were measured in both groups. Moreover, oral glucose tolerance test (OGTT) were performed in 18 obese subjects to better assess their insulin resistant state and the subjects were divided in two groups according to peak serum insulin concentrations during OGTT (> 150 µU/ml or hyperinsulinemic group and < 150 µU/ml or normoinsulinemic group).

Obese subjects had higher tHcy levels compared with controls (6.1 ± 1.8 vs. 4.6 ± 1.1 µmol/l; p= 0.005). There were significant differences in the body mass index, waist/hip ratio, and insulin levels between the groups but not in systolic blood pressure, diastolic blood pressure, lipid profile, vitamin B12, folate and creatinine levels. After making statistical corrections for risk factors, obese subjects had also higher tHcy levels than controls. Fasting tHcy negatively correlated with folate (r= -0.67, p= 0.003) in obese subjects. In a multivariate regression model for all subjects, the independent correlates for tHcy were obese state (p=0.001) and folate (p=0.002). In a multivariate regression model for tHcy in obese subjects, the independent correlate for tHcy was only folate (p< 0.001). tHcy levels were not significantly different between hyperinsulinemic and normoinsulinemic obese subjects.

These results suggest that higher tHcy levels are present in the childhood obesity and that tHcy could play a role in the higher risk of cardiovascular disease in obesity. Furthermore, our study demonstrates that obese state and folate but not insulin are a main correlate of tHcy in childhood obesity and suggests that decreased folate may contribute to impairment of tHcy metabolism in childhood obesity.

Key words: homocysteine, childhood obesity, cardiovascular disease

Introduction

Hyperhomocysteinemia is a well-established independent risk factor for atherosclerotic and thromboembolic vascular disease (1,2). Hereditary enzymatic deficiencies and nutritional deficiencies of folate, pyridoxine or cobalamin (B12), as well as chronic renal failure are associated with elevated blood tHcy and accelerated atherosclerosis (1,2). The mechanism of tHcy angiotoxicity seems to involve the nitric oxide system by inducing oxidant stress (3,4). Oxidative stress has been suggested to cause insulin resistance and may be a possible link with atherosclerosis (5). High fat intake has been shown to be linked with childhood obesity (6), which is associated with an unfavorable profile of risk factors for atherosclerosis such as hyperinsulinemia, hyperlipidemia, and elevated blood pressure (7). Currently, there is very little evidence dealing with possible relationship among tHcy levels, anthropometric data, and metabolic risk factors for atherosclerosis in childhood obesity. The aim of the present study was to investigate tHcy level in subjects with childhood exogenous obesity and controls, and a possible relationship among plasma tHcy levels, anthropometric data, blood pressure, folate and vitamin B_{12} levels, serum lipid parameters, and insulin.

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Methods

Subjects

We studied 18 patients (8 females and 10 males; age range 10-16; mean age 12.9 ± 2.2) with exogenous obesity and 20 healthy controls (10 females and 10 males; age range 10-16; mean age 13.5 ± 1.9). Patients with waist/hip ratio (WHR) of over 0.8 and their body mass index (BMI) of over 95th percentile were included into obesity criteria. The study was cross sectional in nature. None had intrauterine growth restriction according to their history. Also, none had clinical evidence of endocrine induced disorders such as hypothyroidism or Cushing's disease. Anthropometrical findings were assessed using the 1978 Turkish Standards (8). A standard mercury sphygmomanometer was used to measure blood pressure (BP) in all patients at rest. BMI was calculated as weight/height square meter (kilograms per meter^2). WHR was calculated as waist circumference (cm) divided by hip circumference (cm). All the control subjects were in good health and non obese. There was no evidence of any infections, and of immunologic, allergic or neoplastic disorders. The hospital ethics committee approved the study, and written informed consent forms were obtained from all subjects.

Analytical methods

In all obese subjects, a standard 2-h oral glucose tolerance tests (OGTT, since 8 a.m.) were performed after 3 days on a high-carbohydrate diet (300 g. daily) and an overnight fast. Blood was sampled 0, 30, 60, 90 and 120 min. after oral glucose intake for measurement of glucose and immunoreactive insulin (9). For calculation of mean serum insulin (MSI) during OGTT, the area under the insulin curve (AUIC) was calculated under the trapezoidal rule. Obese subjects were divided into two groups according to peak serum insulin concentrations during OGTT (hyperinsulinemic group, as judged by peak serum insulin concentrations higher than 150 μU/ml (10) and < 150 μU/ml or normoinsulinemic group).

tHcy concentrations were determined in EDTA plasma. Samples were separated from the cells, and matched samples were spiked with tHcy and than assayed by IMMULITE 2000 Analyzer. Results were expressed μmol/L. The normal range was 2-50 μmol/l. Glucose levels were determined by the glucose oxidase method (11).

Plasma insulin, folate and plasma vitamin B_{12} were measured by competitive immunoassay and IMMULITE 2000 Analyzer, and the normal range for folate was 3-17 ng/mL, and for vitamin B_{12} was 193-982 pg/mL. Serum creatinine and lipid profiles [total cholesterol (TC), triglycerides (TG), high-density lipoprotein-cholesterol (HDL-C) and low-density lipoprotein-cholesterol (LDL-C) levels) were measured by an automated enzymatic method using Olympus 2700 Analyzer.

Statistical methods

Data were expressed as mean ± SD. The Kolmogorov–Smirnov test was applied separately for the obese group and the controls to check the normality of the variables. Differences between data were studied using the Student's t test. A simple correlation analysis was processed by Pearson's method to assess the relationship between tHcy levels and other parameters in both groups. Multiple linear regression analysis was performed in forward stepwise selection to identify independent factors affecting tHcy and to estimate the final predictor of its variability. Statistical significance was taken as $p < 0.05$. All statistical analysis was performed using the Statistical Package for Social Sciences (SPSS/Windows version 11·0, SPSS inc., Chicago, IL, USA).

Results

The difference between obese patients and controls regarding sex and age were not significant ($p>0.05$). Clinical and laboratory characteristics of obese patients and controls are presented in Table 1. Obese subjects had higher tHcy levels compared with controls (6.1 ± 1.8 vs. 4.6 ± 1.1 μmol/l; P= 0.005). There were significant differences in body mass index, waist/hip ratio, and insulin levels between groups but not in systolic BP, diastolic BP, glucose, lipid profile, vitamin B_{12}, folate and creeatinine levels (Table 1). After making statistical corrections for risk factors, obese subjects had also higher tHcy levels than controls. Fasting tHcy negatively correlated with folate (r= -0.67, p= 0.003) in obese subjects, but not in controls. No correlations were found between tHcy and other parameters in both groups (Table 2). In a multivariate regression model for all subjects, the independent correlates for tHcy were obese state (p=0.001) and folate (p=0.002) with the total variance explained as 39

%. In a multivariate regression model for the tHcy in obese subjects, the independent correlate for tHcy was only folate (p< 0.001) with the total variance explained as 45 %.

Table 1. Clinical and laboratory characteristics of the study population.

	Obese subjects (n=18)	Controls (n=20)	P
Age (years)	12.9 ± 2.2	13.5 ± 1.9	NS
Systolic BP (mmHg)	105.8 ± 12.2	103.2 ± 6.7	NS
Diastolic BP (mmHg)	70 ± 9.3	69.5 ± 4.5	NS
Body mass index	29 ± 3.3	17.4 ± 1.9	< 0.001
Waist/hip ratio	0.86 ± 0.07	0.74 ± 0.04	< 0.001
Fasting glucose (mg/dl)	99.8 ± 13.2	95.1 ± 7.1	NS
Fasting insulin (μU/ml)	22.7 ± 10.9	11.2 ± 3.5	< 0.001
Total cholesterol (mg/dl)	167.2 ± 28.8	167.6 ± 27.1	NS
Tryglyceride (mg/dl)	162.1 ± 79.6	159.6 ± 32.8	NS
HDL-cholesterol (mg/dl)	34.5 ± 9.2	38.9 ± 2.4	NS
LDL-cholesterol (mg/dl)	102.8 ± 27.1	108 ± 15.1	NS
Creatinine (mg/dl)	0.5 ± 0.04	0.6 ± 0.03	NS
B12 (pg/ml)	271.5 ± 90.89	230.7 ± 49.7	NS
Folate (ng/ml)	9.62 ± 3.08	9.2 ± 4.3	NS
tHcy (μmol/l)	6.1 ± 1.8	4.6 ± 1.1	0.005

NS: not significant, tHcy: plasma total homocysteine

Table 2. Correlations between tHcy and other parameters in both groups.

	Obese subjects (n=18)		Controls (n=20)	
	r	p	r	P
Age (years)	-0.03	NS	0.05	NS
Systolic BP (mmHg)	0.20	NS	-0.001	NS
Diastolic BP (mmHg)	0.17	NS	-0.1	NS
BMI	0.04	NS	-0.1	NS
Waist/hip ratio	-0.35	NS	-0.12	NS
Fasting glucose (mg/dl)	-0.06	NS	0.19	NS
Fasting insulin (μU/ml)	-0.06	NS	0.4	NS
Total cholesterol (mg/dl)	0.001	NS	0.09	NS
Tryglyceride (mg/dl)	-0.27	NS	-0.01	NS
HDL-cholesterol (mg/dl)	0.02	NS	-0.07	NS
LDL-cholesterol (mg/dl)	0.22	NS	0.38	NS
Creatinine (mg/dl)	-.025	NS	-0.27	NS
B12 (pg/ml)	-0.33	NS	-0.29	NS
Folate (ng/ml)	-0.67	0.003	-0,4	NS

All obese subjects had normal glucose tolerance (12), but 7 of them were hyperinsulinemic. tHcy levels were not significantly difference between hyperinsulinemic and normoinsulinemic obese subjects (Table 3).

Table 3. Clinical and laboratory characteristics of the hyperinsulinemic and normoinsulinemic obese subjects (peak serum insulin concentrations > 150 μU/ml or hyperinsulinemic group and < 150 μU/ml or normoinsulinemic group during OGTT)

	Hyperinsulinemic (n=8)	Normoinsulinemic (n=10)	P
Age (years)	0.5 ± 0.04	0.6 ± 0.03	NS
Systolic BP (mmHg)	102.8 ± 12.5	108 ± 12.2	NS
Diastolic BP (mmHg)	68.5 ± 10.6	71 ± 8.7	NS
BMI	29.3 ± 3.6	28.8 ± 3.3	NS
Waist/hip ratio	0.87 ± 0.05	0.86 ± 0.09	NS
Mean AUIC (μU/ml)	4004.7 ± 939.1	1279.6 ± 470.5	<0.001
Total cholesterol (mg/dl)	172.1 ± 31	163.9 ± 28.3	NS
Tryglyceride (mg/dl)	191.7 ± 86.8	141.4 ± 71.3	NS
HDL-cholesterol (mg/dl)	30 ± 9.6	37.8 ± 7.8	NS
LDL-cholesterol (mg/dl)	110.1 ± 36.9	97.8 ± 18	NS
Creatinine (mg/dl)	0.6 ± 0.05	0.6 ± 0.04	NS
B12 (pg/ml)	247.1 ± 53.6	288.6 ± 109.3	NS
Folate (ng/ml)	9.7 ± 4	9.5 ± 2.4	NS
tHcy (μmol/l)	5.7 ± 1.8	6.4 ± 1.8	NS

NS: not significant, tHcy: plasma total homocysteine, AUGC: the area under the glucose curve, AUIC: the area under the insulin curve

Discussion

Childhood obesity is associated with an increased cardiovascular risk in later life. Besides dyslipidaemia and disturbances in the hemostatic system (13), plasma tHcy concentrations have also been shown to be linked to indexes of childhood obesity and hyperinsulinism (14). Hyperinsulinemia and hyperhomocysteinemia are now other well accepted risk factors for atherosclerosis (15,16).

Conflicting data have been published on the association of tHcy and obesity. A weak correlation between BMI and tHcy was found in children (17), whereas BMI was positively associated with tHcy in adults only when the levels attained by methionine load were considered (18). In the Hordaland Homocysteine Study, BMI was a strong determinant of total cysteine but not related to tHcy (19). The several features of the metabolic syndrome, simultaneously present in the same individuals, probably act as confounding factors in correlation studies.

We found that increased tHcy and nsulin levels are present in obese subjects compared with healthy

controls. A negative relationship was also found between fasting plasma tHcy and folate. A possible role of nutritional deficiencies of folate and B_{12} should be ruled out, since similar plasma levels of these vitamines were found in both groups. The increased tHcy levels remained significant even after correction for other factors including plasma vitamin levels. In the present study, no significant correlation was found between tHcy and anthropometric data such as BMI and WHR in childhood obesity. However, obesity was a significant predictor of plasma tHcy in the present study.

Obese subjects tend to be insulin resistance. In the present study, we were not able to find a significant difference in tHcy between hyperinsulinemic and normoinsulinemic obese subjects. Moreover, there was no significant relationship between tHcy and insulin levels in both groups. According to our findings, other possible sources of elevated homocysteinemia, such as genetic variation of methyllenetetrahydrofolate reductase activity or functionnal vitamin B6 deficiency (20) have not been assessed in the present study.The association between insulin resistance and tHcy has recently been proposed in healthy, non-obese subjects (21) and adult hyperinsulinemic obese subjects (22).

Gallistl et al. have demonstrated a linear relationship between plasma tHcy and log transformed plasma insulin in obese children and adolescents (14). However, in that study plasma insulin was also inversely related to plasma folate, suggesting possible subclinical folate deficiency in obese children. Studies have documented an inverse relationship between folate intake and fat intake in the diet of children (23) and adults (24). Thus, it is important to study a folate replete population in order to determine the role of other possible regulators of plasma tHcy, such as insulin. It is possible that fortification of food with folate will eliminate subclinical folate deficiency in most people and decrease plasma tHcy in the general population (25). In our study, we were not able to demonstrate a relationship tHcy and insulin in both normoinsulinemic obese subjects and hyperinsulinemic obese subjects. It is possible that other factors regulating plasma tHcy (such as other hormones) will then be easily appreciated. In this context, it is remarkable that in our study there was a relationship between plasma tHcy and folate, suggesting that the population was folate deplete and that folate intake can lower plasma tHcy in obese subjects. We also observed a statistically significant relationship between obese state and tHcy, which has not been previously described. Further researches are needed to confirm this relationship and elucidate its mechanism and implications.

Another possible explanation for differences between studies is the presence of hypertension which may have independently elevated plasma tHcy. Sheu et al. demonstrated plasma tHcy was significantly higher in subjects with hypertension compared with those with a normal blood pressure (26). Furthermore, in that study, plasma tHcy correlated significantly with insulin secretion in response to an oral glucose tolerance test. Similarly, in the Framingham Offspring Study, plasma tHcy was higher in subjects who had hyperinsulinemia along with hypertension and/or microalbuminuria compared with those with hyperinsulinemia alone (27). In the current study, none of our subjects had hypertension and the mean age of our patients were lower than theirs. It is possible that plasma tHcy rises only late in hyper-insulinemic obese subjects after the onset of hypertension, hyperlipidemia and endothelial dysfunction.

The increase in tHcy levels may not be a general complication of the insulin resistance syndrome, since healthy volunteers with altered insulin-mediated glucose disposal have recently been shown to have no increased tHcy levels (28) as did in our study. That is, in hyperinsulinemic obese subjects especially in childhood, there seems not to be an association with increased tHcy levels, which may contribute to the development of cardiovascular complications which are so common in obesity. Our results found in this study should be interpreted with caution because of its method limitations (cross-sectional design, heterogeneity of study population, and small number of studied patients).

In conclusion, our study suggested that obese state and folate but not insulin are a main correlate of tHcy in childhood obesity and that decreased folate may contribute to impairment of tHcy metabolism in childhood obesity. This relationship may need to be considered when evaluating the role of plasma tHcy as a risk factor in patients with childhood obesity.

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