

Total Homocysteine in Patients With Type 1 Diabetes

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OBJECTIVE — Our aim was to study the presence of moderate hyperhomocysteinemia, a risk factor for premature cardiovascular disease, its modifying vitamin factors (folates, vitamins B₁₂ and B₆), and lipid risk factors in juvenile type 1 diabetes.

RESEARCH DESIGN AND METHODS — A total of 91 patients with type 1 diabetes (46 girls and 45 boys) were studied, with ages ranging from 11 to 18 years, a duration of diabetes from 1 to 15 years, and in pubertal development (stages III, IV, V). In all patients, cholesterol, triglycerides, HDL and LDL cholesterol, lipoprotein(a), folates, cobalamin, vitamin B₆, and total homocysteine were determined by specific assays. Microalbuminuria, defined as a ratio of albumin/creatinine >3 mg/mmol creatinine, was analyzed in the first morning specimen.

RESULTS — Plasma total homocysteine (tHcy) concentrations were not different in the 91 diabetic children (median [range]) (11–15 years, 6.1 μmol/l [3.2–9.6]; 16–18 years, 7.3 μmol/l [3.9–12]) compared with the control group (11–15 years, 6.6 μmol/l [4.4–10.8]; 16–18 years, 8.1 μmol/l [4.6–11.3]). No significant differences were found in tHcy values in relation to the metabolic control of the disease as assessed by glycohemoglobin values, the duration of disease, alterations in fundus oculi, or presence of lymphocytic thyroiditis. A positive correlation was found between tHcy and plasma creatinine in type 1 diabetic patients that might be related with the increase in muscle mass. There was a negative correlation between tHcy and serum folate ($P < 0.001$) and vitamin B₁₂ ($P < 0.05$), but not with vitamin B₆ levels. No significant correlations were found between tHcy and the lipid parameters.

CONCLUSIONS — Hyperhomocysteinemia was not detected in adolescents with type 1 diabetes.

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Retrospective and prospective studies have demonstrated that hyperhomocysteinemia is a risk factor for premature cardiovascular disease (1,2) independent of other classic risk factors, such as smoking, hypercholesterolemia, arterial hypertension, and diabetes (3). In fact, diabetes is an important risk factor not only for premature atherosclerosis but also for its rapid progression, because the risk of cardiovascular and peripheral vascular disease is associated with the metabolic abnormalities involved in dia-

betes (4). Moderate hyperhomocysteinemia is common in the general population and has been linked with cardiovascular disease (5). The association of two risk factors for atherosclerosis, diabetes and hyperhomocysteinemia, strongly increases the risk of cardiovascular disease (6). Plasma total-homocysteine (tHcy) has been studied in adult type 1 (4,7–9) and type 2 (8,10–13) diabetic patients, and moderate hyperhomocysteinemia was generally found. From these studies, it was suggested that hyper-

homocysteinemia contributes to the accelerated atherosclerotic process in diabetes (11). Recent studies concluded that diabetic patients with the lowest age at onset and the poorest metabolic control are the most prone to developing a rapid increase in plasma tHcy (14). However, no data are available about young type 1 diabetic patients.

Our aim was to study the presence of moderate hyperhomocysteinemia and its modifying factors (folates and vitamins B₁₂ and B₆) in adolescents with type 1 diabetes. We studied the possible relationship between hyperhomocysteinemia in type 1 diabetes and the duration of the disease, the degree of metabolic control, the presence of microalbuminuria, alterations in fundus oculi, and chronic lymphocytic thyroiditis.

RESEARCH DESIGN AND

METHODS — We studied 91 juvenile type 1 diabetic patients in treatment with a combination of short-acting (Actrapid) and intermediate-acting insulin (NPH) (injected twice a day), with a supplement of Actrapid when capillary glycemia becomes ≥ 10 mmol/l. The age range of our subjects was 11–18 years; sex was 46 girls and 45 boys. Since tHcy varied with age but not with sex in childhood and adolescence (15), the patients were classified in two age-groups: 11–15 and 16–18 years (Table 1). The duration of diabetes ranged from 1 to 15 years. All patients were in pubertal development stages III, IV, or V. Patients were also grouped according to the evolution time of the disease (<5 years, $n = 42$; >5 years, $n = 49$). Complications of diabetes were taken into account, such as nonproliferative retinopathy, assessed by fluorescein angiography (microaneurysms, $n = 7$); orthopedic exploration (camptodactily versus limited joint mobility, $n = 11$); microalbuminuria, defined by a albumin/creatinine ratio >3 mg/mmol in the first morning urine; and association with thyroiditis ($n = 7$), diagnosed by enlarged thyroid gland and positive titers of thyroid antibodies (antithyroperoxidase antibodies >40 U/ml).

Reference values were derived from apparently normal children and adolescents (by history and analytical data) who came to the laboratory for analytical control

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Abbreviations: HPLC, high-performance liquid chromatography; tHcy, total homocysteine.

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

Table 1—Plasma tHcy and other analytical data in patients with juvenile type 1 diabetes compared with reference values

	Diabetic patients	Reference values
n	91	90
Age (years)	11–18	11–18
Glucose (mmol/l)	10.2 (2–21.3)	3.3–6.4
Creatinine (mmol/l)	85.3 (11–122)	31–101
Cholesterol (mmol/l)	4.66 (2.9–7.0)	2.47–6.2
Triglycerides (mmol/l)	0.72 (0.3–5.9)	0.56–2.3
HDL cholesterol (mmol/l)	1.35 (0.59–2.33)	>0.9
LDL cholesterol (mmol/l)	2.93 (1.46–4.72)	<3.36
Glycohemoglobin (%)	9.2 (5.2–14.5)	4.2–5.9
Folate (nmol/l)	19.7 (6.3–45)	>5.0
Cobalamin (pmol/l)	397.5 (158–1097)	155–679
Vitamin B ₆ (µg/l)	11.5 (4.2–33.5)	3.6–18
tHcy (µmol/l)		
11–15 years	6.1 (3.2–9.6)	4.4–10.8
16–18 years	7.3 (3.9–12)	4.6–11.3
Albuminuria (mg/mmol creatinine)	<3.0	<3.0

Data are n, range, median (range), or median.

of minor surgical interventions. Their age range was 11–18 years and their sex distribution was 47 girls and 43 boys. No family antecedents of cardiovascular disease were present.

Samples from the patients and the normal population for reference values were collected following the Helsinki Declaration of 1975, as revised in 1983, and informed consent was obtained from the families for the study.

Methods

Venous blood samples were collected after a 12-h fasting period from the antecubital vein to analyze a number of components. Blood glucose was measured by a glucose oxidase method, serum and urine creatinine by a kinetic Jaffé reaction, and cholesterol and triglycerides by an enzymatic method (AU510; Merck, Darmstadt, Germany). HDL cholesterol was measured enzymatically after precipitation of apolipoprotein B-containing lipoproteins with magnesium dextran sulphate; LDL cholesterol was calculated with the Friedewald formula; glycohemoglobin by high-performance liquid chromatography (HPLC) (Auto A1c HA-8110; Menarini, Firenze, Italy); folates and cobalamin by radioassay using purified intrinsic factor and purified folate binding protein (Simultrac; Becton Dickinson, Orangeburg, NY) with a Co/Gamma Counter (LKB Wallac 1272; Cambridge, U.K.); vitamin B₆ by HPLC (Integral 4000; Perkin Elmer, Beaconsfield, U.K.) with flu-

orescence detection (LC 240, Chromsystem Kit; Chromsystems Instruments & Chemicals GmbH, Martinsreid, Germany); total homocysteine by HPLC with fluorescence detection of the SBDF derivatives

(Sigma Chemicals, St. Louis, MO) (15); urinary albumin by nefelometry (Behring BNA Nefelometer; Behring, Marburg, Germany). Urinary albumin and creatinine concentration were measured in the first morning specimen.

Statistics

Since tHcy values did not follow a Gaussian distribution, results are presented as median and range. All statistical tests were two-tailed, and a 5% level of significance was used to evaluate differences. Nonparametric Mann-Whitney U test was applied in the case of two independent samples and Spearman's rank correlation coefficient test was used to test for monivariate relationships between different variables.

RESULTS — Plasma total homocysteine concentrations were not different between our patients with diabetes—aged 11–15 years, 6.1 (3.2–9.6) µmol/l, median (range); aged 16–18 years, 7.3 (3.9–12) µmol/l—and our control group—aged 11–15 years, 6.6 (4.4–10.8) µmol/l; 16–18 years, 8.1 (4.6–11.3) µmol/l (Mann-Whitney U test) (Fig. 1). No significant differences were found between tHcy values in relation to

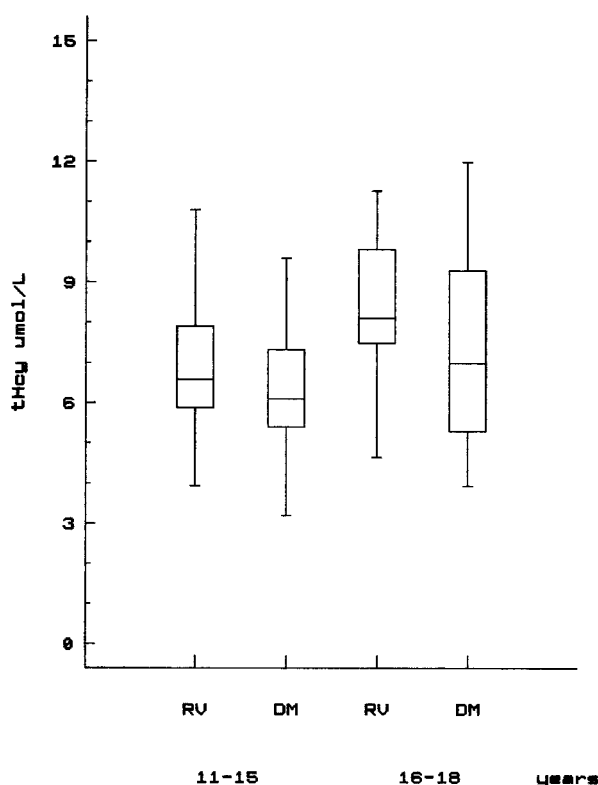


Figure 1—Multiple box-plot of plasma tHcy in juvenile type 1 diabetes (DM) versus reference values (RV).

Table 2—Plasma tHcy in patients with juvenile type 1 diabetes in relation to duration of disease and the presence of complications

	n	tHcy ($\mu\text{mol/l}$)
Duration of diabetes		
≤ 5 years	42	6.71 \pm 2.07
> 5 years	49	6.68 \pm 1.81
Metabolic control		
Glycohemoglobin $> 7\%$	70	6.55 \pm 1.79
Glycohemoglobin $\leq 7\%$	21	7.31 \pm 1.83
Complications		
Microaneurysms	7	6.84 \pm 2.16
Camptodactily versus limited joint mobility	11	6.42 \pm 1.98
Thyroiditis	7	7.10 \pm 1.3

Data are n or means \pm SD.

the evolution time of the disease, metabolic control, or the presence of microaneurysms in fundus oculi or of lymphocytic thyroiditis (Table 2). Values for microalbuminuria, expressed as albumin/creatinine ratio, were < 3 mg/mmol in all patients (Tables 1 and 2).

A positive correlation was found between total homocysteine and plasma creatinine ($r = 0.43$, $P < 0.0001$). There was a negative correlation between tHcy and serum folate ($r = -0.33$, $P < 0.001$) and vitamin B₁₂ ($r = -0.23$, $P < 0.05$), while no significant correlation was found between plasma tHcy and vitamin B₆ levels. There were no significant correlations between tHcy and the lipid parameters studied.

CONCLUSIONS — Plasma tHcy values are determined by genetic and nutritional factors. Deficient activities of the enzymes of the trans-sulfuration and remethylation pathways, as well as a common mutation (C677T) causing a thermolabile variant of the enzyme methylenetetrahydrofolate reductase, may cause hyperhomocysteinemia. Moreover, three vitamins involved in homocysteine metabolism as coenzymes (vitamins B₁₂ and B₆) and substrate (folate) are important determinants of plasma tHcy, especially folate (16). Therefore, determination of these modifying factors is needed in evaluating the presence of hyperhomocysteinemia and its possible etiology. In our patients, vitamin levels were within the normal range, suggesting that no nutritional problems affecting vitamin levels had altered tHcy values despite the diet followed for their diabetes.

Considering tHcy studies in diabetic patients, hyperhomocysteinemia seems to be associated with the clinical manifesta-

tions of macroangiopathy (10) and proliferative retinopathy (9), while other researchers found high tHcy levels only in patients with clinical signs of diabetic nephropathy (11). They found no association between plasma tHcy and different degrees of retinopathy, so through further studies they concluded that tHcy does not play a major role in the progression of diabetic retinopathy (14). In another study (8), only diabetic patients with macroangiopathy and nephropathy (and even those with only proteinuria or microalbuminuria) showed hyperhomocysteinemia, but not diabetic patients without renal insufficiency. Only one author found low tHcy concentrations in adult patients with type 1 diabetes and microalbuminuria, perhaps by comparing them with a nonhomogeneous control sample (7). Our study showed no differences in tHcy levels between patients and reference values. None of our patients had developed microalbuminuria, and folate levels were high in all of them as an index of good nutritional status. No association was found between tHcy and other risk factors for cardiovascular disease. Only creatinine values showed an association with tHcy concentrations. This finding might reflect the increase of creatinine synthesis during puberty, secondary to the development of muscle mass. Other authors concluded that neither type 1 diabetes nor diabetic retinopathy per se is associated with hyperhomocysteinemia, which would only depend on impaired renal function (4,17). Homocysteine metabolism is especially important in renal parenchyma (18) and is altered in early stages of impaired renal function, depending on individual genetic and nutritional factors (17). In our patients, no alteration in renal function was

observed, so this possibility seems an unlikely explanation.

The special interest of the study of plasma tHcy in diabetes lies in the possibility of easily correcting hyperhomocysteinemia by supplementation with low folate doses (2,14). Although tHcy values seem still to be normal in diabetic adolescents, they may become higher in adults in association with renal impairment and low blood folate levels (14), especially in people carrying the C677T mutation (13). The correlation of tHcy values with folate found in our patients and in most other studies (10,14) confirms the relationship between the two parameters. People with diabetes may require additional folate intake to avoid the combined risk factors for atherosclerosis that strongly increase the risk for cardiovascular disease (6). The search for hyperhomocysteinemia in adult diabetic patients and the possibility of folate supplementation seems therefore very useful, as has also been suggested by other authors (19).

In conclusion, hyperhomocysteinemia was not detected in juvenile type 1 diabetes, at least in the age range studied, and seems to have no predictive value per se for cardiovascular disease.

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