HOMOCYSTEINE AND ITS NUTRITIONAL DETERMINANTS IN TWO ETHNIC GROUPS OF SLOVAKIA

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SUMMARY

Determinants of total homocysteine involve demographic (age, sex, ethnic origin), genetic (enzymatic defects of metabolic homocysteine pathways) and acquired factors (deficiency of B-group vitamins, state of health, lifestyle).

Plasma levels of total homocysteine and serum levels of vitamin B_{12}, folic acid, vitamin B_6 were measured in adult apparently healthy Romany minority subjects (n=119) and compared with those levels in apparently healthy subjects of majority Slovak population (n=146).

Mean homocysteine level was similar in both groups (9.92 μmol/l in the Romany vs. 9.61 μmol/l in majority group). Hyperhomocysteinemia was only observed in 3% of both ethnic probands. Vitamin B_{12} level was equal (301 μmol/l in the Romany and 311 μmol/l in majority group). Deficient levels were found in 4% of the Romany subjects and 9% of the majority subjects. Folic acid level was significantly lower in Romany group (11.3 nmol/l vs. 14.8 nmol/l) with deficiency in 42% of the Romany subjects vs. 28% in the majority subjects. This finding is a consequence of lower consumption of fruit, vegetables, pulses and whole grain products in the Romany group. Vitamin B_6 deficiency was found in 68% of the Romany subjects and 40% of majority subjects. This vitamin is homocysteine determinant under excessive methionine intake (overnutrition with predominance of animal protein intake). As was demonstrated in a dietetic questionnaires, the Romany subjects are more frequent consumers of meat and eggs. This nutrition regime should indicate an increased homocysteine level under vitamin B_6 deficiency.

The results of normal homocysteine levels in the Romany population under condition of higher folic acid and vitamin B_6 deficiencies, smoking and higher alcohol consumption may suggest a more effective homocysteine metabolism in relation to different ethnic origin.

Key words: homocysteine, folic acid, vitamin B_{12}, vitamin B_6, Romany population, Slovak majority population

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INTRODUCTION

The sulfur-containing amino acid homocysteine (Hcy) is a lipid independent risk factor for atherosclerosis. Each 5 μmol/l increment in total fasting Hcy concentration was shown to be associated with a 60–80% higher risk of coronary artery disease, a 50% higher risk of cerebrovascular disease and a 6-fold higher risk of peripheral vascular disease (1). Complex of demographic (age, sex, ethnic origin), genetic (enzyme deficiency) and acquired (health state, lifestyle, nutrition) factors affect the Hcy homeostasis (2). The hyperhomocysteinemia can be determined by mainly the B-group vitamin deficiencies as well as by smoking, excessive consumption of alcohol and coffee, lack of exercise and by renal disease, organ transplants, hypothyreoidism (2, 3). Folic acid and vitamin B_{12} are inevitable in Hcy reduction by remethylation cycle (renovation of methionine need). Transsulfuration pathway of Hcy degradation (Hcy conversion into cysteine) prevails under condition of excessive methionine intake (above 3 g, overnutrition with significant predominance of animal food sources) (3, 4).

Hcy levels and values of nutritional markers of Hcy metabolism (5) were measured in the ethnic Romany minority and compared with the Slovak majority population.

SUBJECTS AND METHODS

Groups of volunteers consisted of 119 apparently healthy adult Romany subjects and 146 persons of the majority population from Southern Slovakia (Zlate Klasy district). Southern Slovakia is a region with a relatively high density of the Romany population. Both groups were selected randomly, with only conditions being age within 19–60 years and the same number of probands in every age decade. Individuals with diagnosed cardiovascular and oncological diseases, diabetes, renal disease and thyroid gland disease were excluded. The study was conducted in co-operation with a district general practitioner. Characteristic of groups is presented in Table 1.

Blood sample was drawn in the standard way. EDTA (ethylenediaminetetraacetic acid) was used as an anticoagulant. Total Hcy in plasma was assessed by the HPLC method (high performance liquid chromatography) with fluorescence detection and SBD-F (7-fluorobenzofurazane-4-sulfonic acid) as a derivation agent (6). Vitamin B_{12} and folate in serum were measured using Elecsys Immunoassay tests (Boehringer, Germany). Vitamin B_6 (pyridoxine + pyridoxal-phosphate) in serum was detected by the HPLC method (Chromsystems test, Germany).

Table 1. *Group characteristic, homocysteine, vitamin B_{12}, folic acid, vitamin B_6 levels and incidence of negative values*

	Majority population	Romany population
n (m+w)	146 (56+90)	119 (48+71)
Age span (y)	19–60	19–60
Average age (y)	36.2±0.9	35.2±1.0
BMI ($kg.m^{-2}$)	25.0±0.4	28.7±0.6 *
Homocysteine (μmol/l)	9.61±0.24	9.92±0.34
<10	62%	63%
10–15	35%	34%
>15	3%	3%
Vitamin B_{12} (μmol/l)	311±8	301±10
<179	9%	4%
Folic acid (nmol/l)	14.8±0.6	11.3±0.5 *
<9.5	28%	42%
Vitamin B_6 (μg/l)	3.56±0.14	2.59±0.11 *
<3	40%	68%

Results are expressed as mean±SEM *p<0.001

Table 2. *Smoking, education and nutrition data*

	Majority population	Romany population
Smokers	34%	60%
Daily alcohol consumers	7%	13%
Education		
elementary	5%	69%
apprenticeship	22%	25%
secondary	57%	6%
university	16%	0%
Consumption frequency		
Plant food		
Whole grain products		
daily and every other day	69%	53%
never or rarely	9%	26%
Vegetables		
daily and every other day	12%	12%
never or rarely	20%	40%
Fruit		
daily or every other day	61%	51%
never or rarely	0%	4%
Soy		
1–2 times monthly	23%	11%
never or rarely	71%	83%
Other pulses		
1–2 times monthly	48%	22%
never or rarely	10%	23%
Animal food		
daily and every other day		
Meat	12%	39%
Dairy products	34%	36%
Eggs	10%	20%

In addition to anthropometric characteristics (body mass index BMI = weight/height^2) and biochemical examination, lifestyle indicators including food habit were assessed from questionnaires focused on frequency of consumption of selected food items (daily or every other day, 1–2 time weekly, 1–2 time monthly, never or almost never) or frequency of other lifestyle factors. The survey was carried out in spring. The χ^2 test and regression analysis were used for final statistical evaluation.

RESULTS AND DISCUSSION

Mean Hcy plasma concentration in Romany group is similar to Hcy value of the majority group – nonsignificantly changed (Table 1) with equal finding of normal values (below 10 μmol/l), other levels 10–15 μmol/l (continual concentration with substantial risk) and over 15 μmol/l – hyperhomocysteinemia (7). The frequency of hyperhomocysteinemia corresponds with published data for the general population (8). Hcy values significantly correlate with folic acid values ($r = –0.22$, $p<0.01$) and with vitamin B_{12} values ($r = –0.17$, $p<0.01$) in the combined group of the majority plus minority subjects. An inverse relation of Hcy values to vitamin B_6 values was nonsignificant ($r = –0.02$, $p>0.05$). Hyperhomocysteinemia was found in 5 majority persons and 4 persons from the Romany group. Five subjects with hyperhomocysteinemia suffered from folic acid deficiency, two hyperhomocysteinemic subjects were deficient in folic acid and vitamin B_{12}. Two probands with risk Hcy values had the vitamin values in normal range. Further factors of increased Hcy (smoking, alcohol – Table 2) can be suggested at normal vitamin conditions. No vitamin B_6 deficiency was observed in the hyperhomocysteinemic subjects.

Mean vitamin B_{12} level is equal in both ethnic investigated groups (see Table 1) with low frequency of deficient values, only 4% in the Romany and 9% in the majority population. A lower number of vitamin B_{12} deficient persons in the Romany group is a consequence of higher frequency of animal food consumption – meat and eggs (Table 2). Vitamin B_{12} is only contained in animal food.

In the Romany group, mean level of folic acid is significantly lower (see Table 1); frequency of folic acid deficiency is high (42% vs. 28% in the majority group). Vitamin deficient finding in the control majority group is higher than 16%, as it was found in previous studies (9). An actual investigation was performed in early spring period (March – April), and so the lower folic acid levels as well as the lower vitamin C levels (significant positive linear correlation between folic acid and vitamin C – $r=0.16$, $p<0.01$) are a consequence of seasonal effect. Folic acid values correspond with education scale (Fig.1). In the Romany group, a secondary education has 6% of probands and university education 0% of subjects (see Table 2). High folic acid deficiency in the Romany subjects can be connected with lower education level, which correlates with less nutrition knowledge. The Romany subjects consume fewer dark

grain products, vegetables, fruit, soy and other pulses (see Table 2). These food commodities are rich in folic acid.

Serum level of vitamin B_6 is significantly decreased in Romany group (see Table 1). Deficiency finding is high in both investigated ethnic groups (68% in the Romany and 40% in the majority group). A greater frequency of animal food consumption (see Table 2) may cause a predominance of transsulfuration Hcy metabolism pathway in part of the Romany persons [a higher body mass index (see Table 1), overnutrition, a possible increasing of methionine intake over 3 g]. This situation should indicate an increased Hcy levels in vitamin B_6 deficient subjects.

The higher incidence of folic acid or vitamin B_6 deficiencies in the Romany population made us assume a greater frequency of risk homocysteine levels (5, 9). The results of no higher finding of hyperhomocysteinemia in the Romany subjects suggest ethnic origin as an acceptable cause. There exists a genetic relation to Indians. Romany belong to the Indo-European race. The ethnography and anthropology locate their original home to the Central Northern India. Hughes and Ong (10) examine the hypothesis, that the higher rates of coronary heart disease in Indians (South Asians) compared with Malays and Chinese is partly attributable to differences in blood Hcy concentrations and related blood folate and vitamin B_{12} concentrations. While there were ethnic differences for plasma folate and vitamin B_{12} (in particular lower levels in Indians), there was no evidence that Hcy plays any part in the differential ethnic risk for atherosclerosis; mean plasma total Hcy levels did not show significant ethnic differences. This result is in agreement with our findings for the Romany population in Southern Slovakia as well as in Western Slovakia (11).

A difference in Hcy metabolism efficiency was also observed between ethnic groups in Africa or America (12, 13). Ubbink et al. (12) evaluate hyperhomocysteinemia in South African black population (a low coronary heart disease prevalence) with comparison to population prove to coronary heart disease (South African Whites). In black men, the plasma Hcy concentrations were significantly lower compared with Whites. Young Whites showed a methionine intolerance expressed as a high plasma Hcy concentration after an oral methionine load test. The results indicate that Blacks generally have a more effective Hcy metabolism after oral methionine loading. In spite of a lower folic acid concentration, the Hcy levels were significantly lower in Mexican American females than in non-Hispanic white and non-Hispanic black females (13).

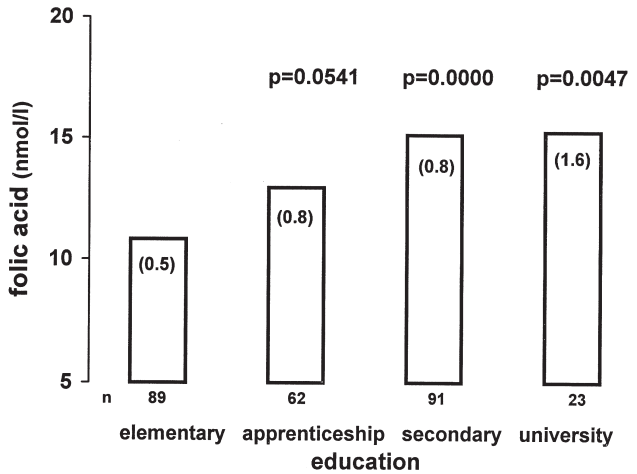

***Fig. 1**. Education dependence of folic acid levels in the combined group of majority plus minority populations (in brackets are SEM).*

Comparison of the literary facts with our results of normohomocysteinemia in the Romany population under condition of higher folic acid or vitamin B6 deficiencies and higher smoking and alcohol consumption brings an alternative conclusion of a more effective Hcy metabolism and perhaps a lower vitamin need for homocysteine homeostasis maintenance.

In conclusion, the higher risk for atherosclerosis in the Romany minority is caused by unfavourable lipid parameters and by hyperinsulinemia (14, 15). The results of this study document that hyperhomocysteinemia is not included into higher atherosclerosis risk.

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