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Total plasma homocysteine: influence of some common physiological variables

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Summary. The purpose of this study was to investigate H(e) concentration in plasma from 80 healthy donors in relation to age (6 newborns are also included), sex, daily variation (9, 11 a.m.; 2, 6, 12 p.m.) and a period of 5 subsequent months. A significant correlation ($r = 0.63$, $p < 0.001$) was observed between plasma H(e) and age and a statistical difference ($p < 0.05$) was found between female and male. No circadian rhythm or significant variations over 5 months were found.

Keywords: Amino acids – Homocysteine – Plasma – Reference values – Circadian variations

Introduction

Occlusive cardiovascular diseases are among the most frequent causes of mortality and morbidity in the Western world (Clarke, 1991). In addition to conventional risk factors such as cholesterol, smoking habits, hypertension and ECG abnormalities, also hereditary factors, affecting metabolism of the arterial wall, take part in the development of the arteriosclerotic process (Nora, 1980). In the last decade, in an attempt to clarify the contribution of these generally underestimated genetic and environmental variables, attention has been focused on a possible role of homocysteine (HCYS) metabolism in the pathogenesis of atherosclerosis.

Greatly elevated total homocyst(e)ine [H(e)] plasma levels ($>200\ \mu M$) have been found in subjects with homocystinuria, an inherited metabolic disease. Among its clinical features, severe venous and arterial thrombotic episodes and precocious arteriosclerosis are the most life-threatening complications (Ueland, 1989).

More recently mild hyperhomocysteinemia has been observed also in young patients with arterial thrombosis (Wilcken, 1989) or coronary artery disease (Genest, 1990). Based on these findings it has been hypothesized that a even a

moderate increase of the plasma H(e) levels is linked to the development of premature occlusive vascular disease (Brattstrom, 1989).

To establish a diagnostic criterion able to identify in a normal population subjects having predisposition for occlusive vascular pathologies, the establishment of reference values for plasma H(e) is of importance. Thus we have determined H(e) plasma levels in healthy subjects taking into consideration sex, age, and diurnal and longitudinal variations.

Material and methods

The study groups

Control group: healthy male (n = 40) and female (n = 40) blood donors (age ranged from 3 to 80 years) and 6 newborns. All donors were in good nutritional state with a normal serum protein profile (6–8 g/dl). They had blood counts, coagulation screening tests (PT and PTT), blood glucose and hepatic and renal biochemical parameters in the physiological range.

Subjects with known metabolic and vascular disorders involving variations of homocyst(e)inemia (Horowitz, 1981), were not included. Factors or particular therapies known to disturb methionine metabolism (Miller, 1972; Ueland, 1989), were excluded. When H(e) was over 15 μM, serum vitamin B12 and folate levels were determined to exclude nutritional deficiencies.

Investigation procedure

To determine age- and sex-related reference values, H(e) levels in plasma were assayed in control subjects of both sexes subdivided in 4 groups: A) 3–20 yr B) 21–40 yr C) 41–60 yr D) 61–80 yr. Each group was composed of 20 probands. In addition, 6 newborns were also investigated.

The biological variability of plasma H(e) levels was investigated in 6 healthy subjects with blood samples collected every month over 5 months.

Diurnal variations of H(e) plasma concentration was investigated in 6 healthy subjects (20–40 yr) by sampling blood at 9, 11 a.m., 2, 6, and 12 p.m. All subjects, after a breakfast meal at 9.15, had lunch between 1 and 2 p.m., and dinner between 9 and 10 p.m. A standard diet was followed and probands pursued their normal activities before and during the protocol.

Methods

Venous blood, drawn after an overnight fast, was collected into sodium citrate (0.129 M, v/v 9:1) vacutainer tubes. The plasma was separated immediately by centrifugation (1200 g for 15 min) and stored at $-20°C$ until analysis.

After reduction with sodium borohydride, the samples were derivatized with ophthaldialdehyde (OPA) and injected into C18 HPLC column as already described by Fermo et al. (1992). Plasma H(e) levels found with this method were in good agreement with H(e) concentrations obtained applying different other analytical techniques including GC-MS and amino acids analyser (Fermo, 1992).

Statistical analysis

Results are expressed as the mean $\pm$ standard deviation (SD). Non-paired Student's test was used to assess the significance of differences between mean values. Linear regression analysis was used for correlations between different variables.

Results

The mean value of plasma H(e) in 80 healthy subjects was 12.7 ± 4.50 μM.

The data reported in Table 1 show that H(e) plasma concentrations increased significantly with age. A very low H(e) mean concentration was observed in the 6 newborns (6.5 ± 1.2 μM), increasing to 9.6 ± 2.9 μM at 3–20 years, to 11.2 ± 2.3 μM at 21–40 years and to 18.0 ± 5.26 μM at 61–80 years. Regression analysis of H(e) plasma concentration versus age showed a significant correlation ($r = 0.63$, $p < 0.001$). An explanation for the increase of H(e) throughout life is not readily apparent; Armstrong and Stave (1973) have already described this relationship for cysteine which is the end product of the transsulfuration pathway where HCYS is an important intermediate (Ueland, 1989). In our study a steep increase of H(e) concentration was observed in the younger groups. Regression analysis applied to this restricted age-group (3–20 years) gave a slope of 0.36, much higher than the one obtained for all subjects ($a = 0.14$, $p < 0.001$)). Presumably, the lower plasma H(e) levels found in younger children reflect greater uptake of amino acids from extracellular fluids by the tissues during growth (Armstrong, 1973; Gregory, 1986). Particularly the marked reduced concentration of methionine, the most important HCYS precursor, found in cord blood (Nicolaidu, 1962), could explain the lower H(e) levels observed in neonatal group.

A significant positive age-related correlation was also found when the male and female groups were considered separately ($r = 0.70$, $n = 40$ and $r = 0.69$, $n = 40$ for male and female, respectively).

The distribution of values for plasma H(e) was also influenced by sex. The data reported in Table 1 show that H(e) plasma concentrations were significantly higher in males than in females ($p < 0.05$). The higher H(e) levels found in men confirm results already reported in literature (Malinow, 1989). These findings might represent one of the explanations of the protection against cardiovascular pathologies showed by women in comparison with men of same age (Boers, 1983). This hypothesis is also supported by the striking high H(e) plasma levels found by us in men in the highest age-group (Table 1), where a sex difference, similarly to the male 21–40 years old, was also noticed ($p < 0.01$).

Table 1. Age and sex related reference values for plasma H(e)

Sex	N	Total	Newborns	3–20 yr	21–40 yr	41–60 yr	61–80 yr
		(n = 40)	(n = 6)				
Female + Male	20	12.7 ± 4.5	6.5 ± 1.2	9.6 ± 2.9	11.2 ± 2.3	11.8 ± 1.8	18.0 ± 5.3[e]
Male	10	14.0 ± 5.4[e]	—	10 ± 3.5	12.7 ± 1.7[a,d]	12.5 ± 2.0	21.3 ± 5.3[c,d]
Female	10	11.4 ± 3.2	—	9.3 ± 2.2	9.7 ± 1.8	11.2 ± 1.3[b]	15.1 ± 3.2[e]

Values are given as μmol/L (mean ± SD)
[a] $p < 0.05$ vs.3–20 yr
[b] $p < 0.05$ vs all other groups
[c] $p < 0.005$ vs all other groups
[d] $p < 0.01$ vs female
[e] $p < 0.05$ vs female

The increase of H(e) concentration observed in women of 41–60 years of age (11.2 ± 1.3), could be explained by an hormonal effect. It has been reported elsewhere that H(e) levels in postmenopausal women are considerably higher than in the premenopausal (Blom, 1988).

The H(e) levels in relation to age and sex are presented in Fig. 1.

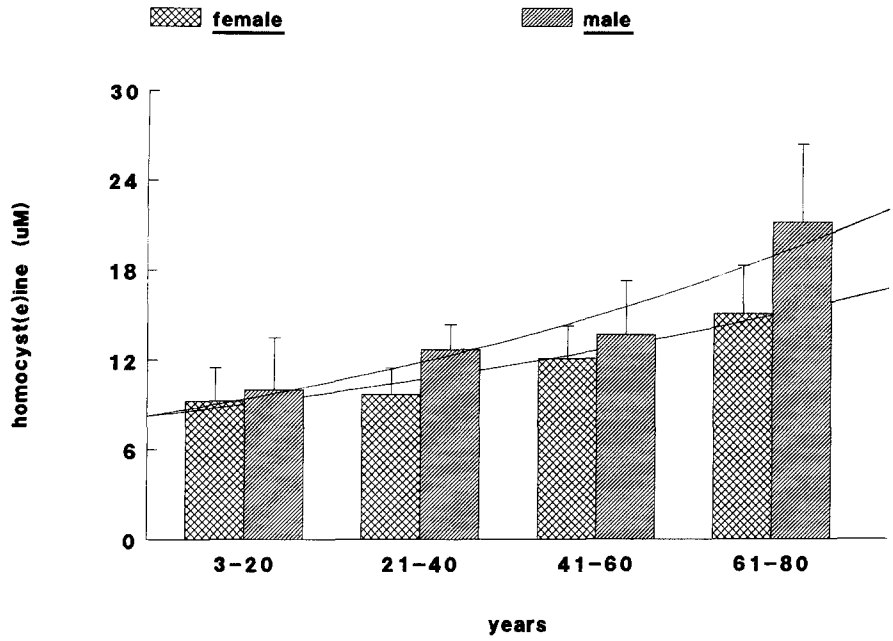

Fig. 1. Influence of age and sex on H(e) plasma levels. The bars represent the mean (±SD) of H(e) plasma concentration in relation to sex stratified by age-groups. Trends of H(e) concentrations in male and female classes with regard to age are represented by the upper and lower curve lines, respectively

No significant longitudinal or diurnal changes, in relation to food intake, of the mean of H(e) plasma levels were observed. There was a slight but not significant decrease of H(e) levels between 11 a.m. and 6 p.m. This modest change could be explained, as is already indicated in literature (Scriver, 1973), by the powerful homeostasis of plasma amino acid levels in human.

In conclusion, the establishment of age reference value for H(e) should be helpful in identifying those subjects with abnormalities of methionine metabolism and who are at higher risk of developing vascular occlusive disease.

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