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 ophthaldehyde (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 ± 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.