Endothelial Function in Healthy Younger and Older Hyperhomocysteinemic Subjects

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OBJECTIVES: To compare endothelium-dependent vasomotor response in healthy younger and older subjects without classic cardiovascular risk factors, with high and normal fasting homocysteine (tHcy) levels.

DESIGN: We compared endothelium-dependent vasodilatation, using ultrasound, in healthy younger (aged 18–40) and older (≥70) people with normal (<13 μmol/L) and high (>15 μmol/L) tHcy levels. Exclusion criteria were smoking, personal history of cardiovascular disease, hypertension, chronic diseases, vitamin intake, obesity, abnormal serum lipids levels, and creatinine higher than 130 μmol/L.

SETTING: Research laboratory.

MEASUREMENTS: In addition to tHcy levels, serum folate and vitamin B_{12} levels were measured.

RESULTS: We studied 17 younger and 12 older hyperhomocysteinemic subjects and respective aged-matched normohocysteinemic subjects. Endothelium-dependent vasodilatation was lower in the hyperhomocysteinemic older people ($P < .01$) than in all younger subjects and in normohomocysteinemic older people. Serum vitamin B_{12} levels were higher in younger and older normal controls. Folic acid levels were higher in younger controls and in both older groups.

CONCLUSIONS: This study shows an effect of high circulating tHcy on vascular reactivity in older people. Because serum levels of tHcy are associated with nutritional status of vitamin B_{12} and folic acid, prospective studies are necessary to demonstrate the effects of a long-term nutritional supplementation with vitamins on vascular function and global cardiovascular risk. **J Am Geriatr Soc 50:1019–1023, 2002.**

Key words: hyperhomocysteinemia; endothelial function

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Hyperhomocysteinemia is considered an independent risk factor for cardiovascular disease. Epidemiological and prospective studies link plasma homocysteine levels to coronary artery, peripheral vascular, and cerebrovascular atherothrombotic disease,[1–3] but prospective observational studies are not consistent in demonstrating this association.[4]

Homocysteine is a sulfur-containing amino acid derived from the demethylation of dietary methionine. Genetic and nutritional factors determine plasma levels. Enzymatic defects involved in homocysteine metabolism are common. Levels of vitamins such as folic acid, B_6, and B_{12} are inversely related to homocysteine levels. These vitamins are necessary for the metabolic pathways of remethylation and trans-sulphuration.[5]

The mechanism by which homocysteine might cause vascular damage is unclear. Experimental evidence suggests that homocysteine facilitates oxidative arterial injury, decreases nitric oxide (NO) release, increases protein homocysteinylation, damages the vascular endothelium, impairs endothelium-dependent vasomotor regulation, augments the proliferation of vascular smooth muscle, or alters coagulation properties of the blood.[6,7]

Endothelial dysfunction is one of the earliest phenomena observed in the process of atherogenesis.[8] All classical cardiovascular risk factors are associated with a decrease in endothelium-dependent vasodilatation.[9] Impairment of endothelial function has been observed in monkeys and humans after dietary-induced homocysteine increase.[10,11] In addition, younger healthy subjects free of clinical atherosclerosis or cardiovascular risk factors who have normal baseline flow-mediated brachial artery reactivity experience a dose-response reduction in their flow-mediated brachial artery reactivity after acute hyperhomocysteinemia induced by a high oral L-methionine load.[12] Woo et al. reported that endothelium-dependent dilatation was lower in Chinese hyperhomocysteinemic subjects, aged 40 to 70,[13] but other authors have not observed endothelial dysfunction in healthy younger subjects with spontaneous or induced hyperhomocysteinemia. Nevertheless, age has an influence on endothelium-dependent dilatation, which is independent from the presence of classic cardiovascular risk factors.

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As described above, not all studies are consistent concerning the cardiovascular consequences of hyperhomocysteinemia. In this study we compared the endothelium-dependent vasomotor response in healthy younger and older volunteers, without classic cardiovascular risk factors, with high and normal basal homocysteine serum levels.

METHODS

Subjects

Seventeen hyperhomocysteinemic (homocysteine (tHcy) >15 μmol/L) male volunteers aged 18 to 40 and 12 older subjects with a mean age ± standard deviation (SD) of 73.5 ± 5 (6 men and 6 women) were recruited from a preventive health evaluation. Each hyperhomocysteinemic subject was paired with a healthy volunteer with normal homocysteine levels (tHcy <13 μmol/L) of the same age, body mass index (BMI), and physical activity. Exclusion criteria for entry to the study were smoking, personal history of cardiovascular or renal disease, hypertension, diabetes mellitus, vitamin ingestion, BMI greater than 30 kg/m^2, blood pressure greater than 140/90 mm/hg, low-density lipoprotein cholesterol (LDL-C) greater than 4.65 mmol/L, triacylglycerol greater than 2.29 mmol/L, fasting glucose greater than 6.1 mmol/L, or creatinine greater than 130 μmol/L.

All subjects gave informed and written consent. The local ethics committee approved the study.

Laboratory Procedures

After an overnight fast, 20 mL of venous blood was drawn, to measure total proteins, creatinine, total cholesterol, LDL-C, high-density lipoprotein cholesterol (HDL-C), triacylglycerol, homocysteine, folic acid, and vitamin B_{12} levels. The blood samples were immediately centrifuged and frozen at −20°C until assayed.

Glucose, creatinine, total cholesterol, HDL-C, and triacylglycerol were measured using routine laboratory automated methods, using Abbott kits (Abbott Laboratories, Abbott Park, IL). Folic acid and vitamin B_{12} were measured using an ion capture technique using Abbott kits (IMx system for folate and B_{12}). Serum homocysteine was measured using Abbott kits (Abbott IMx for homocysteine). These procedures are based on the fluorescence polarization immunoassay technology.

Arterial Reactivity Studies

An ultrasound method described by Sorensen et al.[14] was used for the measurement of endothelium-dependent and independent arterial dilatation. Brachial arterial diameter was measured using a 7.0 Mhz linear array transducer in a standard Advanced Technology Laboratories HDI 3000 system (Philips Medical Systems, Best, The Netherlands). Each study was performed after 3 hours fasting and 15 minutes of supine rest. A pneumatic cuff was placed around the forearm, 2 cm distal of the middle arm, inflated to a pressure of 300 mmHg for 5 minutes, and then deflated. Arterial end-diastolic internal diameter was measured at baseline and 60 and 90 seconds after cuff deflation. The brachial artery was scanned again after a 15-minute rest and 3 minutes after sublingual nitroglycerin, to evaluate endothelium-independent vasodilatation. The maximum increase in end-diastolic brachial artery diameter from baseline was used as the measure of dilatation.

All the ultrasound procedures were recorded on videotape for later measurement. The vessel diameter was measured by two independent observers blinded to clinical and laboratory details of the subjects.

Statistical Analysis

Statistical analysis was performed using Statistica for Windows version 4.5 (StatSoft Inc., Tulsa, OK). Descriptive data are expressed as mean ± SD. Comparisons between study groups were made using one-way analysis of variance (ANOVA). Kruskal-Wallis ANOVA median test was performed to compare the effect of hyperhomocysteinemia on vascular reactivity between groups. Simple correlations between variables were calculated using Pearson's correlation coefficient.

RESULTS

Two hundred fifty healthy people were screened during 12 months. We recruited 17 younger and 12 older hyperhomocysteinemic subjects, eligible according to the inclusion criteria, without classic cardiovascular risk factors and nonsmokers.

Younger and older hyperhomocysteinemic subjects and their respective controls were well matched for age, BMI, and biochemical parameters as shown in Tables 1 and 2. Serum LDL-C, total cholesterol, and triacylglycerol were similar in the four groups, but HDL-C in both older groups was higher than in the younger groups ($P < .05$).

There were no differences in BMI, blood pressure, and biochemical parameters between older men and women.

Endothelium-dependent vasodilatation was significantly lower in the hyperhomocysteinemic older group than in all younger subjects or in normohomocysteinemic older people. There were no differences in endothelium-dependent vasodilatation between younger hyperhomocysteinemic and normohomocysteinemic subjects, or between normohomocysteinemic older and younger subjects as shown in Figure 1. Endothelium-independent vasodilatation was similar in all groups (Figure 2). Gender did not influence endothelium-dependent and -independent vasodilatation in older people. There was no association between serum lipid levels and vascular reactivity in any of the study groups.

Plasma vitamin B_{12} levels were higher in normohomocysteinemic controls than in hyperhomocysteinemic subjects ($P < .05$). Plasma folate levels were higher in both older groups ($P < .05$), and younger hyperhomocysteinemic subjects had the lowest plasma folate levels ($P < .05$) (Table 2). In younger people, inverse correlations between homocysteine, B_{12}, and folate levels were observed ($r = -0.60$ and $r = -0.40$, respectively). In older subjects we observed an inverse correlation only between homocysteine and vitamin B_{12} levels ($r = -0.44$), as shown in Figure 3.

DISCUSSION

In this study, we found that hyperhomocysteinemia is associated with a decrease in endothelium-dependent vasodilatation in older but not younger healthy people. These

Table 1. Demographic Characteristics of Younger and Older Hyperhomocysteinemic Subjects and Their Controls

	Younger Subjects		Older Subjects	
	tHcy ≥15 μmol/L n = 17	tHcy <13 μmol/L n = 17	tHcy ≥15 μmol/L n = 12	tHcy <13 μmol/L n = 12
Characteristic	mean ± standard deviation			
Age*, years	28.4 ± 5.3	30.1 ± 6.1	73.5 ± 5.0	73.1 ± 4.7
Body mass index, kg/m^2	24 ± 3	24 ± 1	25.5 ± 3.7	25.5 ± 3.7
Systolic blood pressure, mmHg	122.2 ± 8.8	118.8 ± 8.9	126.6 ± 14	130.8 ± 7.9
Diastolic blood pressure, mmHg	78.6 ± 4.7	74.7 ± 6.0	80.0 ± 6.3	81.7 ± 7.5

*Analysis of variance $P < .001$.
tHcy = homocysteine.

data are important, considering the controversial information from the literature. Cross-sectional, case-control, and epidemiological studies suggest that hyperhomocysteinemic individuals are at higher risk for developing cardiovascular disease, but not all prospective studies have shown a predictive ability of plasma homocysteine for cardiovascular disease. These conflicting results could be due to age differences between studies or the lack of consideration of other dependent variables such as smoking, obesity, hypertension, hypercholesterolemia, or chronic diseases. In the present study, we matched our patients by age, BMI, and physical activity. Also, we excluded those subjects with classical cardiovascular risk factors or chronic diseases to avoid the possible confounding effect of such parameters. Our results are in accordance with those of Chao et al., who observed that methionine-induced mild hyperhomocysteinemia impairs endothelium-dependent vasodilatation in older but not younger healthy adults.[15] Also, Tawakol et al., in a study performed in older people, concluded that hyperhomocysteinemia impaired vascular reactivity.[16] Moreover, Hanratty et al. did not observe alterations in acetylcholine-mediated endothelium-dependent vasodilatation in younger healthy hyperhomocysteinemic (<30) volunteers.[17]

Thus, hyperhomocysteinemia is an age-related risk factor for cardiovascular disease that could be related to several physiological changes associated with the aging process. In animal and human models, the endothelium-dependent vasodilatation declines with aging, probably due to a decrease in NO production[15,18] together with an increase in the vasoconstrictor response to endothelin-1.[19] Human umbilical endothelial cells in culture, exposed for a short period to high concentrations of homocysteine, produce and release NO, S-nitrosothiol, and S-nitrosohomocysteine, all potent vasodilator substances,[20] but continuous exposure to homocysteine decreases NO production.[21] Consequently, vascular reactivity impairment in hyperhomocysteinemic older people could be explained by

Table 2. Laboratory Features of Younger and Older Hyperhomocysteinemic Subjects and Their Controls

	Younger Subjects		Older Subjects	
	tHcy ≥15 μmol/L n = 17	tHcy <13 μmol/L n = 17	tHcy ≥15 μmol/L n = 12	tHcy <13 μmol/L n = 12
Characteristic	mean ± standard deviation			
tHcy, μmol/L*	24.8 ± 10.0‡	9.6 ± 1.8†	20.0 ± 5.5‡	9.8 ± 1.5†
Total proteins, g/L	76 ± 5	77 ± 3	75 ± 6	75 ± 4
Albumin, g/L	48 ± 3	47 ± 2	43 ± 5	43 ± 4
Creatinine, μmol/L	79.6 ± 26.5	76.9 ± 26.5	87.5 ± 8.8	72.5 ± 8.8
Total cholesterol, mmol/L	4.89 ± 0.93	4.55 ± 0.92	5.59 ± 0.72	5.74 ± 0.57
LDL-cholesterol, mmol/L	3.0 ± 0.82	3.25 ± 0.93	3.41 ± 0.65	3.64 ± 0.48
HDL-cholesterol,* mmol/L	1.09 ± 0.21†	1.09 ± 0.26	1.57 ± 0.49‡	1.66 ± 0.65‡
Triglycerides, mmol/L	1.28 ± 0.36	1.01 ± 0.41	1.44 ± 0.28	1.20 ± 0.39
Vitamin B_{12}, pmol/L*	170 ± 57†	270 ± 76‡	160 ± 110†	310 ± 190‡
Folic acid, nmol/L*	11 ± 8†	16 ± 4§	33 ± 7‡	34 ± 7‡

*Analysis of variance $P < .001$.
Scheffé post-hoc comparison of means † = significantly different from ‡ and §; § = significantly different from ‡, $P < .05$.
tHcy = homocysteine; LDL = low-density lipoprotein; HDL = high-density lipoprotein.

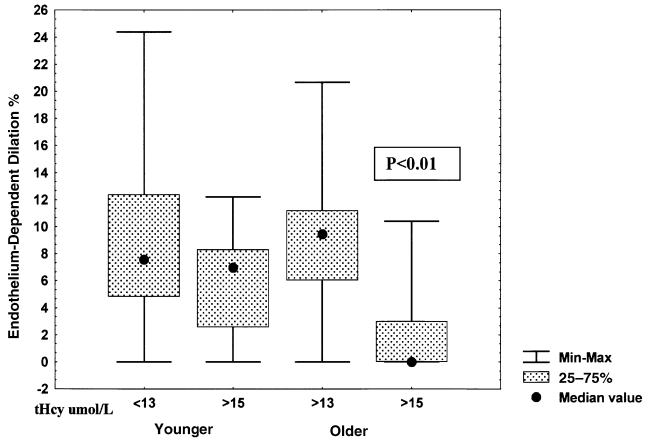

Figure 1. Effect of hyperhomocysteinemia on endothelium-dependent vasodilatation in younger and older subjects. Kruskal Wallis analysis of variance median test, $P < .01$. tHcy = homocysteine.

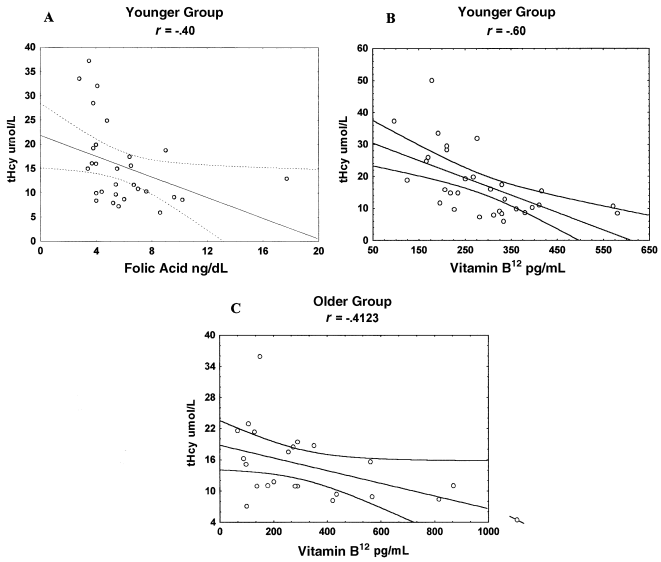

Figure 3. A. Correlation between serum homocysteine levels and folic acid in younger subjects. B. Correlation between serum homocysteine and vitamin B_{12} in younger subjects. C. Correlation between serum homocysteine and vitamin B_{12} in older subjects. tHcy = homocysteine.

an alteration of NO production attributed to long-term exposure to elevated levels of homocysteine or to an additive effect of aging and hyperhomocysteinemia on the NO pathway.

These results could also be attributed to an enhancement of oxidative stress caused by age and homocysteine. Oxidatively damaged deoxyribonucleic acid, proteins, and lipids accumulate with age and probably contribute to the process of aging.[22] In vitro, homocysteine generates cytotoxic substances such as superoxide anions and hydrogen peroxides, which can reduce or inactivate NO.[23] In humans, acute methionine-induced hyperhomocysteinemia increases plasma oxidation products,[24] and the same has been reported in hyperhomocysteinemic patients with peripheral vascular disease.[25] Usui[26] and Chambers[27] have reported that the reduction of superoxide anion production by folic acid and vitamin C prevents the decrease in flow-mediated vasodilatation after a methionine load, but other authors have not demonstrated that oxidability of LDL-C or lipoperoxidation assessed by thiobarbituric acid reactive substance or by cholesteryl ester hydroperoxide are greater in hyperhomocysteinemic or older subjects.[28,29] In this study, the healthy older controls had normal vascular reactivity, probably because they were a selected sample with normal laboratory parameters and without concomitant diseases, a state associated with a low risk of oxidative stress. Nevertheless the presence of a pro-oxidant substance, such as hyperhomocysteinemia, could alter vascular reactivity.

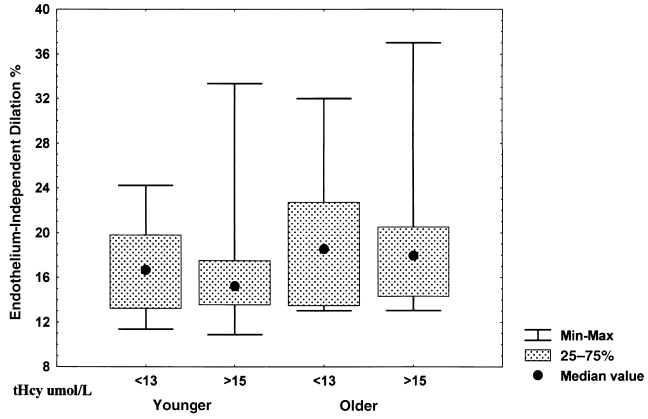

Figure 2. Effect of hyperhomocysteinemia on endothelium-independent vasodilatation in younger and older subjects. Kruskal Wallis analysis of variance median test, P = NS. tHcy = homocysteine.

The nutritional status of folate and vitamin B_{12} are determinants of total homocysteine concentration in the general population. Our group, like others, found an inverse relationship between these vitamins and homocysteine.[30–32] In contrast to the findings of other authors, in our sample, vitamin B_{12} was a more important determinant for high homocysteine levels than folic acid. We detected an association between homocysteine and folic acid only in younger people, whereas our older subjects had higher folate levels than younger subjects. We do not have a clear explanation for these findings. Hyperhomocysteinemia, and thus probably folic acid deficiency, has been associated with hypertension in older populations.[33] Also, smokers have lower folic acid levels and higher homocysteine levels.[34] Because we specifically excluded older subjects with hypertension and history of smoking, we might have inadvertently discarded patients with mild folic acid deficiency. Assay errors were unlikely, because all measurements were run at the same time.

In conclusion, hyperhomocysteinemia is an age-related risk factor for cardiovascular disease. Because serum levels of homocysteine are associated with nutritional status of vitamin B_{12} and folic acid, prospective studies are necessary to demonstrate the effects on vascular function of modifying homocysteine levels through long-term nutritional supplementation with vitamins.

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