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Effect of Short-Term Vitamin (Folic Acid, Vitamins B₆ and B₁₂) Administration on Endothelial Dysfunction Induced by Post-Methionine Load Hyperhomocysteinemia

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Hyperhomocysteinemia, either fasting or after an oral methionine load, may impair endothelial dysfunction.^{1–4} Treatment with vitamins (folic acid, B₁₂ or B₆) has been shown to reduce plasma homocysteine levels,⁵ but it is not clear to what extent such treatment may normalize endothelial dysfunction. The aim of this study was to evaluate the effect of short-term vitamin administration on ameliorating endothelial dysfunction induced by post-methionine load hyperhomocysteinemia.

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We recruited 16 volunteers (2 men, 14 women; mean age 46 ± 4 years, range 41 to 55) from the hospital staff and community. Subjects were included only if they were clinically well and had no family history of premature vascular disease, systemic hypertension, diabetes mellitus, hyperlipidemia, and smoking. No subjects were taking regular medications. We obtained written informed consent from all subjects and the study was approved by our institutional committee on ethical practice.

After an overnight fast (10 to 14 hours), venous blood samples were drawn from all volunteers to measure the concentrations of plasma homocysteine. Supine blood pressure was measured for all subjects and 10 minutes later all persons had a noninvasive ultrasound study of the brachial artery to evaluate endothelial function. After the ultrasound study, an

oral methionine loading test (0.1 g/kg body weight of L-methionine) with L-methionine mixed in orange juice was given and blood samples for homocysteine were again obtained 4 hours later. During the test, only low-methionine nutrients were allowed. The ultrasound study was performed again 4 hours after methionine load. On a separate occasion, we repeated the above procedure with co-administration of folic acid (5 mg) to examine the acute effect of folic acid on methionine-induced endothelial dysfunction. After the baseline evaluation, subjects received folic acid 5 mg, vitamin B₆ 100 mg (Johnson Chemical Pharmaceutical Works Ltd., Taiwan) and vitamin B₁₂ 0.5 mg (Shiteh Organic Pharmaceutical Co., Ltd., Taiwan) daily for the following 5 weeks.⁶ After vitamin administration, homocysteine measurements and ultrasound study were performed again.

Venous blood samples were sampled into tubes containing ethylenediaminetetraacetic acid. Samples were centrifuged within 30 minutes at 2,000 rpm for 10 minutes. The plasma was then separated and stored at –70°C until analysis. Total homocysteine concentrations were measured by fluorescence polarization immunoassay with the Abbott IMx analyzer (Abbott Laboratories, Dallas, Texas) as described by Shipchandler et al.⁷

Ultrasound measurements were performed according to the method described by Celermajer et al.⁸ Endothelium-dependent flow-mediated vasodilation in response to reactive hyperemia and endothelium-independent nitroglycerin-induced vasodilation were evaluated in the right brachial artery. Arterial diameter was measured at rest, during reactive hyperemia, again at rest (after vessel recovery), and after administration of 0.6 mg sublingual nitroglycerin, using a

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high-resolution ultrasound machine (Advanced Technology Laboratories [Bothell, Washington] 3000 system) that was equipped with an L10-5 linear array transducer. The condition of reactive hyperemia was induced by inflation of a pneumatic cuff on the upper arm to suprasystolic pressure, followed by cuff deflation after 4.5 minutes. Arterial diameter was measured from 1 media-adventitia interface to the other for ≥ 3 times at baseline and every 30 seconds following reactive hyperemia and after administration of nitroglycerin. The maximum diameter was taken as the average of the 3 consecutive maximum diameter measurements following hyperemia and nitroglycerin, respectively. Flow-mediated vasodilation (FMD) and nitroglycerin-induced vasodilation (NMD) were then calculated as the percent change in diameter compared with baseline.

Data are expressed as mean $\pm$ SD. Student's paired *t* test was used to examine the changes of homocysteine, vessel diameter, FMD, and NMD from baseline to 4 hours after methionine load, and the differences between pre- and post-vitamin administration. Multiple linear regression analysis was constructed to evaluate the relation between the changes of homocysteine levels and FMD values after vitamin administration, adjusted by sex and age. A *p* value <0.05 was considered significant. All statistical analyses were performed by the SAS system (SAS Institute, Inc., Cary, North Carolina).

Before vitamin administration, the mean plasma homocysteine level without co-administration of folic acid increased from 7.0 ± 1.6 $\mu\text{mol/L}$ at baseline (range 5.3 to 10.6) to 22.7 ± 3.8 $\mu\text{mol/L}$ at 4 hours after methionine load (range 17.2 to 30.2) ($p < 0.001$). With co-administration of folic acid, the mean homocysteine levels at baseline (7.1 ± 1.7 $\mu\text{mol/L}$) and 4 hours (22.3 ± 3.7 $\mu\text{mol/L}$) were similar to those without co-administration of folic acid ($p = \text{NS}$). After vitamin administration, the mean plasma homocysteine level at baseline decreased to 5.2 ± 1.1 $\mu\text{mol/L}$ (range 4.1 to 7.9) with a 26% reduction compared with that before administration ($p < 0.001$). Post-vitamin plasma homocysteine at 4 hours after methionine load was reduced to 17.0 ± 2.1 $\mu\text{mol/L}$ (range 14.0 to 21.8) with 25% lower than that before vitamin administration ($p < 0.001$) (Table I).

Before vitamin administration, the mean FMD value without co-administration of folic acid decreased from $14.3 \pm 3.3\%$ at baseline to $8.6 \pm 3.6\%$ at 4 hours after methionine load ($p < 0.001$). With co-administration of folic acid, the mean FMD values at baseline ($14.1 \pm 3.2\%$) and 4 hours ($8.8 \pm 3.8\%$) were similar to those without co-administration of folic acid ($p = \text{NS}$). After vitamin administration, the mean FMD values between baseline ($14.1 \pm 2.6\%$, $p = \text{NS}$ vs pre-vitamin baseline) and 4 hours after methionine load ($13.8 \pm 2.9\%$, $p < 0.001$ vs pre-vitamin methionine load) were not significantly different. Vessel size and NMD were not significantly different either between baseline and 4 hours or between pre- and post-vitamin administration (Table I). To further investigate the relation between homocys-

TABLE I Pre- and Post-vitamin Homocysteine and Ultrasound Measurements at Baseline and Four Hours After Methionine Load in All 16 Subjects

	Before Vitamin	After Vitamin	<i>p</i> Value*
Homocysteine ($\mu\text{mol/L}$)			
Baseline	7.0 ± 1.6	5.2 ± 1.1	<0.001
Methionine load	22.7 ± 3.8	17.0 ± 2.1	<0.001
<i>p</i> Value†	<0.001	<0.001	
Vessel size (mm)			
Baseline	3.3 ± 0.5	3.3 ± 0.5	0.27
Methionine load	3.3 ± 0.5	3.4 ± 0.5	0.37
<i>p</i> Value†	0.14	0.10	
FMD (%)			
Baseline	14.3 ± 3.3	14.1 ± 2.6	0.63
Methionine load	8.6 ± 3.6	13.8 ± 2.9	<0.001
<i>p</i> Value†	<0.001	0.4	
NMD (%)			
Baseline	19.8 ± 4.5	20.1 ± 4.6	0.55
Methionine load	19.2 ± 4.6	20.2 ± 4.6	0.26
<i>p</i> Value†	0.18	0.54	

*Before vitamin versus after vitamin.
†Baseline versus methionine load.
Data are expressed as mean $\pm$ SD.

teine concentrations and FMD after vitamin administration, we arbitrarily divided the subjects into 2 groups according to the homocysteine value of 15 $\mu\text{mol/L}$, which is the upper limit of normal range of the general population.⁹ Nine subjects with homocysteine levels ≤ 15 $\mu\text{mol/L}$ (mean 14.5 ± 1.5 $\mu\text{mol/L}$) had normal FMD after methionine load ($14.3 \pm 2.7\%$) compared with that at baseline ($14.1 \pm 2.4\%$, $p = \text{NS}$). The other 7 subjects had a higher mean homocysteine level (18.8 ± 2.4 $\mu\text{mol/L}$, $p < 0.01$); their post-methionine load FMD ($12.2 \pm 3.6\%$) was improved compared with that before vitamin administration ($7.2 \pm 2.9\%$, $p < 0.01$), but was not as normal as their post-vitamin baseline ($14.0 \pm 4.2\%$, $p < 0.05$). After vitamin administration, significant correlation was found between the changes of post-methionine load plasma homocysteine levels and FMD values ($r = -0.59$, $p < 0.05$) (Figure 1).

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This study shows that short-term vitamin administration significantly reduces homocysteine levels and thus ameliorates endothelial dysfunction induced by post-methionine load hyperhomocysteinemia. Hyperhomocysteinemia, either fasting or post-methionine load, is an independent risk factor for atherosclerosis.^{10,11} Endothelial dysfunction appears to be an early manifestation of atherosclerosis.¹² Homocysteine, a sulfur-containing amino acid that is formed during methionine metabolism, is known to produce endothelial cell injury in both experimental cellular and animal studies.^{13–15} Although the precise mechanisms are not fully understood, homocysteine may decrease the bioavailability of nitric oxide by increasing its degradation via abnormal interactions between nitric oxide and the free thiol moiety of homocysteine,¹⁵ and generating homocysteine-related oxygen free radicals via autoxidation with consequent catabolism of nitric oxide.^{16,17} In humans, impairment of FMD has been

FIGURE 1. Correlation between the post-vitamin changes of post-methionine load homocysteine and flow-mediated vasodilation.

recently found in healthy adults with marked fasting hyperhomocysteinemia.^{1,2} More recently, impaired FMD has been demonstrated in healthy adults with mild to moderate hyperhomocysteinemia induced by an oral methionine load.^{3,4} This finding is informative because mild to moderate hyperhomocysteinemia (15 to 30 $\mu\text{mol/L}$) is relatively common in the general population (normal range 5 to 15 $\mu\text{mol/L}$)^{9,18} due to inherited enzyme variants, vitamins (folic acid, B₁₂ or B₆) deficiency, or in association with disease states such as renal failure.¹⁹ In this study, co-administration of folic acid (5 mg) did not immediately improve FMD. This finding is different from that reported by Usui et al,²⁰ who found that co-administration of folic acid (20 mg) may improve methionine-induced endothelial dysfunction via amelioration of oxidative stress. The reason for this discrepancy may result from the different amounts of folic acid administered; the amounts of folic acid needed to alter homocysteine concentration may be less than those needed to directly protect endothelial functional responses.

In summary, short-term vitamin administration effectively reduces post-methionine load homocysteine levels and thereby ameliorates endothelium-dependent flow-mediated vasodilation.

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