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Vitamin C Preserves Endothelial Function in Patients with Coronary Heart Disease after a High-Fat Meal

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Summary

Background: It has been suggested that an oxidative mechanism is involved with the impaired endothelium-dependent vasodilatation that occurs after a high-fat meal.

Hypothesis: The study was undertaken to evaluate the effect of a single oral dose of vitamin C (2 g) on postprandially impaired endothelium-dependent vasodilatation in patients with coronary heart disease (CHD).

Methods: This study included 74 patients with CHD and 50 subjects without CHD with risk factors. The two groups were divided into two subgroups that did or did not receive 2 g of vitamin C (CHD/VitC and CHD/control, n = 37; non-CHD/VitC and non-CHD/control, n = 25) after a high-fat meal (800 calories, 50 g fat). Serum levels of triglyceride, total cholesterol, low-density lipoprotein cholesterol, and high-density lipoprotein cholesterol in the fasting state and at 2, 4, 5, and 7 h after the high-fat meal were measured. Endothelial function was assessed in the brachial artery by high-resolution ultrasound at baseline and at 4 h postprandially.

Results: The postprandial serum triglyceride concentration increased significantly at 2–5 h after the high-fat meal in all groups. The fasting flow-mediated dilatation (FMD) ($p < 0.02$) and nitroglycerin-induced dilatation (NID) ($p < 0.05$) of patients with CHD were impaired compared with those of non-CHD subjects. Postprandial FMD was significantly aggravated in the non-CHD/control group ($p < 0.01$) and the CHD/control group ($p < 0.001$), but the postprandial FMD in patients and subjects taking vitamin C showed no significant change, although the CHD/VitC group had a mild tendency toward improvement ($p = 0.064$) and non-CHD/VitC group had a mild tendency toward aggravation ($p = 0.852$). The change of NID after a high-fat meal did not reach statistical significance in the four groups. The decrement of postprandial FMD correlated positively with the increment of 2-h serum triglyceride concentration in the patients without vitamin C ($n = 62$, $r = 0.545$, $p < 0.001$).

Conclusions: The postprandial state after a high-fat meal is critical in atherogenesis, as it induces endothelial dysfunction through an oxidative stress mechanism. Vitamin C treatment has a promising benefit for patients with CHD.

Key words: coronary heart disease, high-fat meal, endothelial dysfunction, oxidative stress

Introduction

A large body of evidence supports the view that elevated plasma triglyceride is an important cardiovascular risk factor. Postprandial triglyceride-rich lipoproteins (TRLs) seem to be more atherogenic.[1] It has been reported that endothelial dysfunction exists in the postprandial state after a high-fat meal[2] for healthy subjects, and remnant lipoproteins are known to be responsible for postprandial endothelial dysfunction,[3] but the underlying mechanisms remain elusive.

A close association between decreased plasma antioxidant vitamins and impaired endothelial function has been found in patients with CHD and in subjects with risk factors.[4,5] Vitamin C (ascorbic acid) has a sustained beneficial effect on endothelial function in long-term treatment of patients with CHD.[6] Recently, it has been demonstrated that oral intake of vitamin E can improve endothelium-dependent vasorelaxation in patients with high remnant lipoprotein levels,[3] suggesting that in-

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creased oxidative stress may at least partly contribute to the postprandial state.

Humans are in a postprandial state, which may be associated with high oxidative stress in the vessels, most of the time during the day. Previously, some researchers demonstrated a beneficial effect of a combination of vitamins C and E on postprandial endothelial dysfunction in healthy subjects.[7] A high-fat diet is a more important risk factor for patients with CHD than for healthy subjects. In this study, we chose patients with CHD as main subjects to explore the change of endothelial function after a high-fat meal and the effect of a single oral administration of vitamin C on postprandial endothelial function.

Methods

Study Population

The study included 74 normocholesterolemic patients with CHD (40 had a history of myocardial infarction; 34 had confirmed angiographical evidence of obstructive atherosclerosis in a coronary artery) and 50 subjects without CHD (characterized by no CHD history, 24-h ambulatory monitoring electrocardiography without evidence of myocardial ischemia, and no abnormality of cardiac structure and function by echocardiogram) but with a history of smoking and/or hypertension, who were admitted to our hospital between October 1998 and June 2000 for treatment or diagnostic evaluation. Of these subjects (100 men and 24 women; mean age 57.3 ± 7.6 years), 64 had essential hypertension and 54 were smokers. The mean body mass index (BMI) for all subjects was 24.2 ± 3.1 (kg/m^2), mean level of fasting glucose was 5.03 ± 0.79 mmol/l, mean systolic blood pressure was 125 ± 19 mmHg, and mean diastolic blood pressure was 81 ± 11 mmHg. All subjects had fasting serum triglyceride concentration < 3.0 mmol/l and total cholesterol concentration 4.0–5.0 mmol/l. Their dietary habits, constituents, and quantity, and their daily activity were investigated by interviews using the Nutrition and Health Questionnaire.

Subjects with diabetes, thyroid diseases, liver and kidney disease, malignancy, chronic consuming diseases, dyspepsia, and malabsorption were excluded. No subject took oral hypoglycemic or hypolipidemic agents, insulin, or estrogen. All subjects refrained from taking beta blocker and diuretics for 1 week, and nitrates, intravenous infusion, smoking, drinking, and a fat-rich diet for 24 h before the high-fat meal.

The research protocol was approved by the Ethics Committee of Hunan Medical University. All subjects gave fully informed consent before study entry.

Oral High-Fat Tolerance Test and Administration of Vitamin C

All subjects were in attendance at 7–8 A.M. after a 12-h overnight fast. The oral high-fat tolerance test was undertaken as described by Vogel *et al.*,[2] and modified by a nutritionist according to Chinese dietary habits. The high-fat meal consisted of 800 calories with 50 grams of fat (5 g of saturated fat), 345 mg of cholesterol, 28 g of protein, and 60 g of carbohydrates. All subjects finished the high-fat meal in 15 min. Both patients with CHD and subjects without CHD were divided into two subgroups that did or did not receive 2 g of vitamin C (CHD/VitC and CHD/control, n = 37; non-CHD/VitC and non-CHD/control, n = 25) after the high-fat meal. Blood samples were taken at baseline and at postprandial 2, 4, 5, and 7 h. During the 7-h test subjects were allowed to drink only water and were not allowed to smoke, drink wine, or eat any food. Only slow walking was permitted. Administration of routine oral drugs and intravenous infusion were prohibited for subjects until the last sample was collected.

Brachial Artery Vasodilatation

According to the research of Vogel *et al.*,[2] the most obvious impairment of endothelial function occurred at 4 h after a high-fat meal. Therefore, in this study the flow-mediated dilation was measured in our department before and at 4 h after the high-fat meal at the same location of the brachial artery with a previously described noninvasive technique.[8, 9] All imaging was performed by a single, highly skilled sonographer who was unaware of the study. Briefly, all subjects rested in the supine position for at least 10 min before the measurement in a temperature-controlled room. Brachial artery diameter was imaged using 7.5 mHz linear array transducer ultrasound system in the subject's right arm. The brachial artery was imaged at a location 3–7 cm above the anticubital crease. The brachial artery diameter of baseline (D_0), reactive hyperemia (D_1), and sublingual nitroglycerin (D_2) were recorded. First, the baseline brachial artery diameter (D_0) was measured. A blood pressure cuff was inflated on the proximal portion of the arm to 250–300 mmHg; by deflating the cuff after 5 min of inflation, flow was increased through the brachial artery. The brachial artery was scanned continuously 30 s before and 90 s after cuff deflation. The brachial artery diameter of reactive hyperemia (D_1) was recorded. The flow-mediated vasodilatation (FMD, $[D_1 - D_0]/D_0 \times 100\%$) was used as a measure of endothelial-dependent vasodilatation. The brachial artery was then allowed to return to baseline level. Then, 0.5 mg of nitroglycerin was given sublingually and the brachial artery (D_2) was imaged after 4 min. The nitroglycerin-induced vasodilatation (NID, $[D_2 - D_0]/D_0 \times 100\%$) was used as a measure of endothelial-independent vasodilatation. Arterial blood flow was measured as Doppler flow velocity multiplied by the cross-sectional area (π r2).

The repeatability of the technique has been established previously.[8]

Lipid Measurement

All blood samples were centrifuged at 4˚C. Serum total cholesterol (TC) and triglyceride (TG) levels were measured using enzymatic methods by a specialist who was blinded to the study. The high-density lipoprotein cholesterol (HDL-C) level was enzymatically determined after precipitation of

TABLE I Clinical characteristics of the study population

	CHD/VitC (n = 37)	CHD/control (n = 37)	Non-CHD/VitC (n = 25)	Non-CHD/control (n = 25)
Age (years)	57.2 ± 9.1	57.8 ± 8.3	56.8 ± 6.8	57.0 ± 7.2
Gender (M/F)	30/7	30/7	20/5	20/5
BMI (kg/m^2)	24.2 ± 2.3	24.1 ± 2.4	24.0 ± 3.5	24.3 ± 2.7
Hypertension (%)	48.6	45.7	56.0	60.0
SBP (mmHg)	125 ± 30	127 ± 16	124 ± 15	123 ± 16
DBP (mmHg)	82 ± 10	80 ± 11	81 ± 13	80 ± 11
Smoker (%)	45.9	43.2	44.0	40
FBS (mmol/l)	4.9 ± 0.7	5.2 ± 0.9	5.1 ± 0.6	4.9 ± 0.8
MI/AD	20/17	20/17	\	\

Abbreviations: CHD/VitC = patients with coronary heart disease taking vitamin C, CHD/control = patients with coronary heart disease not taking vitamin C, non-CHD/VitC = patients without coronary heart disease taking vitamin C, non-CHD/control = patients without coronary heart disease not taking vitamin C, BMI = body mass index, SBP = systolic blood pressure, DBP = diastolic blood pressure, FBS = fasting blood glucose, MI = subjects with myocardial infarction, AD = subjects with angiographically diagnosed coronary heart disease.

apolipoprotein B-containing lipoproteins using dextran sulphate/magnesium chloride. Low-density lipoprotein cholesterol (LDL-C) concentration was computed using the Friedewald formula: LDL-C = TC − HDL-C − (TG/2.2), given TG < 4.5 mmol/l.

Statistical Analysis

All values were presented as the mean ± standard deviation (SD). Log transformation was made for distribution-dependent analyses. Differences between the means intra- and intergroup were analyzed by *t*-test or one-way analysis of variance. To compare the percent incidence (smoker, etc.) or actual numbers (proportion of the genders), chi-square tests were used. Coefficients of correlation (*r*) were calculated by the Pearson correlation analysis. Data were analyzed with the Statistical Package for Social Services (version 7.5) (SPSS, Inc., Chicago, Ill., USA). Statistical significance was assumed at a two-tailed value of $p < 0.05$.

Results

Table I shows the clinical characteristics of the four groups. There were no significant differences in age, gender, BMI, systolic or diastolic blood pressure, smoking habit, hypertensive proportion, and fasting blood sugar level. The proportion of patients with myocardial infarction and angiographically diagnosed CHD was similar between Groups CHD/VitC and CHD/control.

Compared with 50 subjects without CHD, 74 patients with CHD had impaired fasting FMD (6.33 ± 0.79 vs. 3.61 ± 0.59%, $p < 0.02$) and NID (23.97 ± 1.85 vs. 19.11 ± 1.46%, $p < 0.05$). There was no difference in baseline diameter and baseline blood flow before and after the meal among the four groups (Table II). The postprandial FMD was significantly aggravated in the non-CHD/control group (6.20 ± 0.89 vs. 2.98 ± 0.56%, $p < 0.01$) and the CHD/control group (3.59 ± 0.63 vs. 0.58 ± 0.40%, $p < 0.001$). The impairment of FMD was more serious in the patients with CHD (80.84%) than in the subjects with no CHD (51.94%, $p < 0.05$). The postprandial FMD of patients and subjects taking vitamin C showed no significant change, although the CHD/VitC group had a mild tendency toward improvement (3.63 ± 0.59 vs. 4.51 ± 0.33%, $p = 0.064$) and the non-CHD/VitC group had mild tendency toward aggravation (6.45 ± 3.99 vs. 5.67 ± 2.35%, $p = 0.852$). The change of NID after the high-fat meal did not reach statistical significance in the four groups (Fig. 1).

The changes of postprandial serum triglyceride, TC, HDL-C, and LDL-C concentrations in the four groups are shown in Figure 2. The postprandial serum triglyceride concentration

TABLE II Baseline artery diameter and baseline blood flow before and 4 h after a high-fat meal

	BAD (mm)		BBF (ml/min)	
	Preprandial	Postprandial	Preprandial	Postprandial
CHD/control	3.75 ± 0.43	3.83 ± 0.46	82.98 ± 36.29	82.66 ± 48.92
CHD/VitC	3.72 ± 0.63	3.70 ± 0.61	83.07 ± 30.73	83.44 ± 31.62
Non-CHD/control	3.74 ± 0.56	3.76 ± 0.59	98.10 ± 42.42	96.26 ± 57.93
Non-CHD/VitC	3.72 ± 0.48	3.73 ± 0.49	99.73 ± 24.81	98.78 ± 16.63

Abbreviations: BAD = baseline artery diameter, BBF = baseline blood flow. Other abbreviations as in Table I.

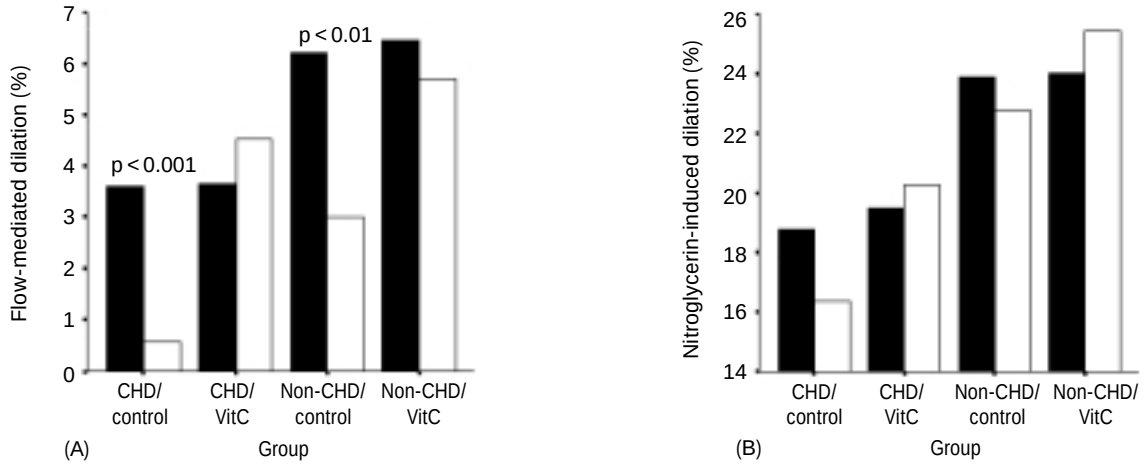

FIG. 1 Postprandial flow-mediated dilation (FMD) was significantly aggravated in the non-CHD/control and CHD/control groups. Postprandial FMD of patients (CHD/VitC) and subjects (non-CHD/VitC) taking vitamin C showed no significant change (A). The change of nitroglycerin-induced dilation (NID) after a high-fat meal did not reach statistical significance in the four groups (B). ■ = baseline, □ = postprandial.

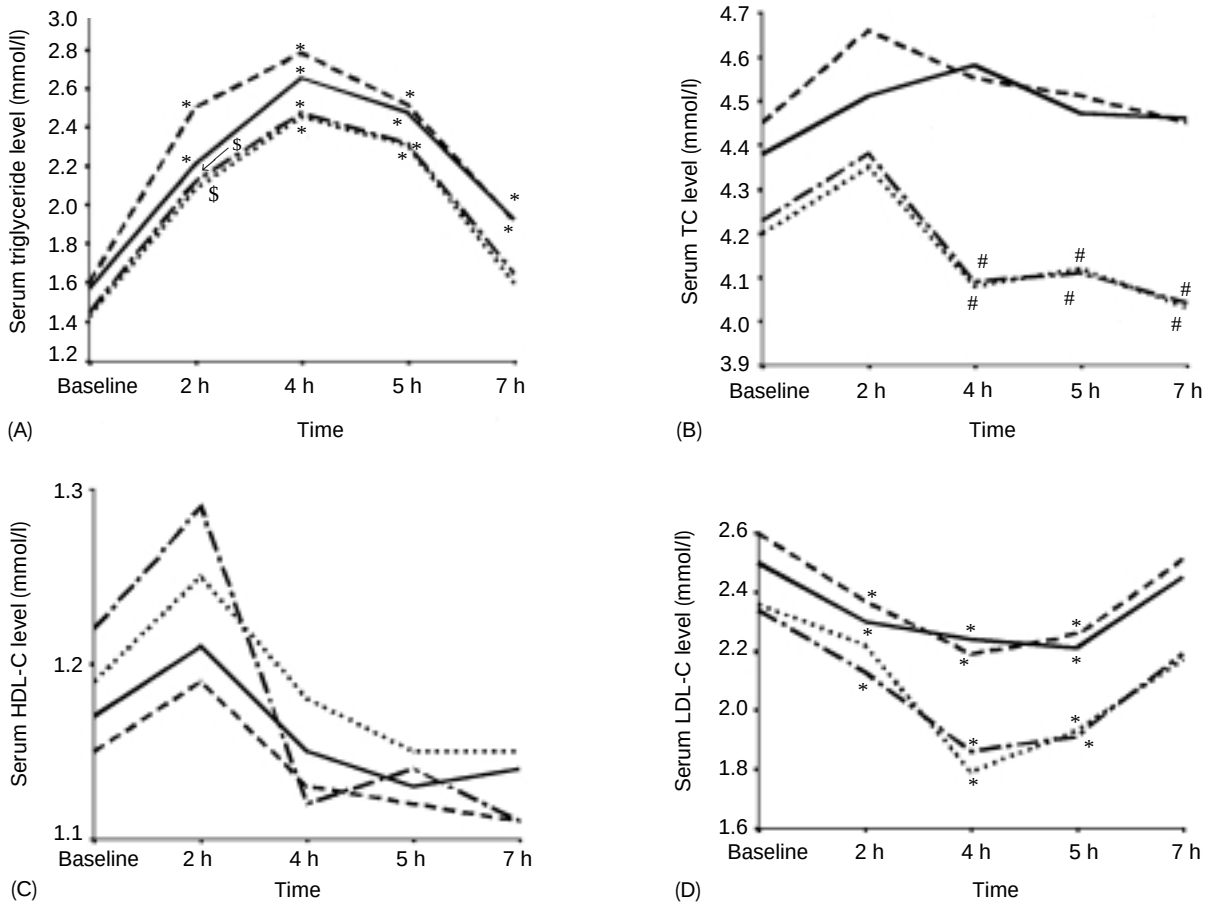

FIG. 2 Postprandial serum triglyceride concentration increased significantly 2, 4, and 5 h after the high-fat meal in all groups. The two CHD subgroups had significantly higher serum triglyceride concentration even at 7 h (A). The changes of postprandial serum total cholesterol (TC) and high-density lipoprotein cholesterol (HDL-C) concentrations did not reach statistical significance compared with their baseline levels except that the patients with CHD had higher TC levels than the subjects without CHD at 4, 5, and 7 h (B and C). The postprandial serum low-density lipoprotein cholesterol (LDL-C) concentration decreased significantly at 2–5 h after the high-fat meal (D). —— = Group CHD/VitC, – – – = CHD/control, ••••• = Group non-CHD/VitC, –•–• = non-CHD/control. * $p < 0.05$ vs. baseline. \$ $p < 0.05$ vs. baseline and Group CHD/control. # $p < 0.05$ vs. Group CHD/VitC and CHD/control.

increased significantly at 2–5 h after the high-fat meal in all groups. The changes of postprandial serum triglyceride, TC, HDL-C, and LDL-C concentrations were similar between the two subgroups of patients with and the two subgroups without CHD. There was no significant difference in most of postprandial triglyceride levels among the four groups, except that the two CHD subgroups had higher triglyceride levels at 2 h than the two non-CHD subgroups ($p < 0.05$).

A positive correlation was observed between the decrement of postprandial FMD and the increment of 2-h serum TG concentration in subjects without vitamin C (Group CHD/control and non-CHD/control: $r = 0.545$, $p < 0.001$, $n = 62$) (Fig. 3).

Discussion

This study demonstrates that endothelial dysfunction exists in the patients with CHD who have significant aggravation of endothelial function after a high-fat meal. The postprandial hypertriglyceridemia was closely related to the aggravation, which could be blocked by pretreatment of 2 g vitamin C. These findings support the opinion that for patients with CHD the postprandial endothelial dysfunction can be attributed to increased triglyceride-rich lipoproteins (TRLs) and/or oxidative stress in the circulation.

The TRLs have a close relationship to endothelial dysfunction. The contrived hypertriglyceridemia through infusion of triglyceride emulsion resulted in the decrement of FMD in young, healthy men without risk factors of CHD.[10] In a previous study, Vogel *et al.*[2] reported that in 10 healthy subjects FMD was reduced 52% (21 ± 5 vs. 10 ± 3%) at 4 h after a high-fat meal. In the present investigation, a similar reduction (6.20 ± 0.89 vs. 2.98 ± 0.56%) was observed in 25 subjects without CHD but with risk factors. The two studies showed

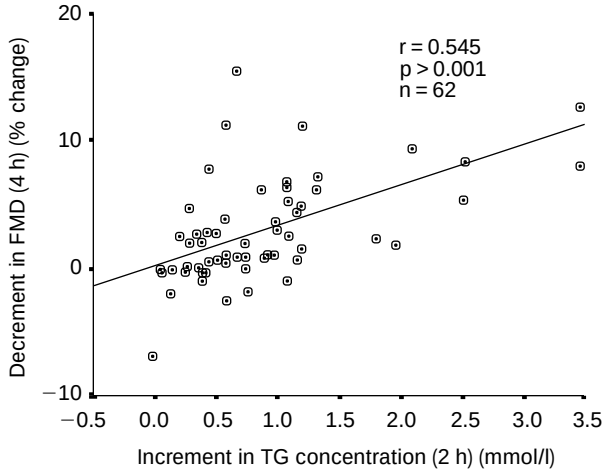

FIG. 3 Correlation between the decrement of flow-mediated dilation (FMD) at 4 h and the increment of postprandial serum triglyceride (TG) levels at 2 h after a high-fat meal in patients not taking vitamin C.

that the increment in 2-h triglyceride levels correlated with postprandial endothelial dysfunction. In this study, the higher the 2-h triglyceride level in patients with CHD the more serious was endothelial dysfunction that took place postprandially (3.59 ± 0.63 vs. 0.58 ± 0.40%, 80.84%). The increased serum triglyceride level represents the accumulation of TRLs and their remnants in circulation. These findings support the finding that lipids in the increased TRL remnants directly contribute to the impairment of endothelial function.[3, 11, 12] It has been demonstrated that TRL remnants can influence vascular endothelial function by inducing a dysfunction of inhibitory G protein-dependent signal transduction, interfering with the receptor-activated L-arginine-nitric oxide (NO) pathway and inhibiting the response of the artery to the NO donor.[11, 12] The oxidized TRL remnants seem to exert more atherogenic effect on the endothelium.[12]

Several studies have previously confirmed that antioxidants can protect endothelial function. Gokce *et al.*[6] demonstrated that vitamin C has a beneficial effect on FMD at 2 h after a single dose (2 g) and 30 days after the long-term (500 mg/day) treatment in patients with CHD. Plotnick *et al.*[7] found that the combination of vitamins C (1 g) and E (800 IU) blocked postprandial endothelial dysfunction after a high-fat meal in healthy subjects. This study suggested that vitamin C had a more obvious beneficial effect on endothelial function in patients with CHD, although this benefit did not reach statistical significance. The fact that vitamin C did not influence the postprandial TRL metabolism indicated that more highly increased oxidative stress contributes to postprandial endothelial dysfunction in patients with CHD.

The precise mechanisms whereby oxidative stress is increased in the blood vessel remain unclear. In humans, hypertriglyceridemia has an even greater potential to invoke a higher oxidative burden than does hypercholesterolemia. Mon-ocytes and polymorphonuclear cells attached to the endothelium can release significantly more superoxide radicals when exposed to plasma from hypertriglyceridemic patients than when they are exposed to plasma from hypercholesteroemic patients or normolipidemic controls.[13, 14] In addition, postprandial hypertriglyceridemia can promote the formation of oxidized LDL particles that induce endothelial cells to produce excessive free radical superoxide anions.[15] Furthermore, the reduced serum paraoxonase activity in the postprandial period after a meal rich in fat[16] means increased oxidative stress and a weakening of the protection of HDL particles to endothelium.[17]

The major mechanism for the protective effect of vitamin C is mediated through inhibiting the oxidation of lipoproteins.[18, 19] Vitamin C also can protect NO from inactivation by superoxide anions and other reactive oxygen species, improve endothelium-dependent NO action by augmenting intracellular glutathione levels, and enhance NO synthesis in human endothelial cells.[20, 21] In this study, the reason for using 2 g of vitamin C is that this single oral dose can reach high enough concentration in the plasma (114 ± 11 μmol/l) and in the cytosol (1–2.5 mmol/l) to exert a protective effect on endothelial function.[18, 22, 23]

Study Limitations

The current study did not measure the level of plasma antibodies of oxidized LDL and serum paraoxonase activity, although we cannot exclude the possibility that postprandial oxidized LDL and weakened protection of HDL are responsible for the impairment of endothelium-dependent vasodilatation. A larger scale, strictly designed clinical trial would be helpful in interpreting the chronic effect of vitamin C on fat tolerance in patients with CHD.

Clinical Implications

Endothelial dysfunction changes constantly throughout the day and is not only related to meals and their fat content, but also to cigarette smoking and a variety of other stimuli. Compared with subjects without CHD, the patients with CHD have a quite limited reserve of endothelial function that is prone to further impairment. This study suggests that an oxidative stress mechanism is related to the impairment of endothelial function caused by a high-fat meal, and vitamin C treatment seems to promise benefit to patients with CHD.

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