

## Dietary L-Arginine Supplementation Normalizes Platelet Aggregation in Hypercholesterolemic Humans

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**Objectives.** The present study was designed to test the hypothesis that long-term dietary supplementation with the nitric oxide precursor L-arginine would enhance vascular or platelet-derived nitric oxide activity, or both, and thereby inhibit platelet reactivity in hypercholesterolemic humans.

**Background.** We have shown that reduced vascular activity of nitric oxide in hypercholesterolemic rabbits can be restored by L-arginine supplementation. The improvement in nitric oxide activity is associated with an inhibition of platelet aggregation *ex vivo*. This effect is most likely due to increased elaboration of endothelium- or platelet-derived nitric oxide, or both, because the inhibition of platelet reactivity was associated with elevation of intraplatelet cyclic guanosine monophosphate and was reversed by the nitric oxide synthase antagonist *N*-methyl-arginine.

**Methods.** In a double-blinded, randomized, placebo-controlled trial, hypercholesterolemic patients were assigned to L-arginine hydrochloride, 8.4 g/day orally, or placebo for 2 weeks. Platelet-rich plasma was obtained for aggregometry induced by collagen (1 to 10  $\mu\text{g/ml}$ ) at four points: baseline, after 2 weeks of treatment, after a 2-week washout and after a long-term washout of 16 weeks on average. Aggregation was quantified by light transmittance and expressed as a percent transmittance observed with platelet-poor plasma.

**Results.** Compared with normocholesterolemic control subjects, platelets from hypercholesterolemic subjects stimulated with 5  $\mu\text{g/ml}$  of collagen showed increased aggregability (68.6% in

hypercholesterolemic patients vs. 54.5% in normocholesterolemic control subjects,  $p \leq 0.02$ ). After 2 weeks of treatment with L-arginine (but not placebo), platelet reactivity was modestly reduced; this effect persisted for 2 weeks after discontinuation of arginine (52.6% in arginine-treated patients vs. 65.1% in normocholesterolemic control subjects,  $p = 0.07$ ). After 18 weeks (i.e., 16 weeks after discontinuing arginine treatment), the platelets of hypercholesterolemic patients once again became hyperaggregable, and the extent of platelet aggregation was significantly increased compared with the 4-week point (73.6% after vs. 52.6% during arginine treatment,  $p < 0.01$ ). No significant change in platelet reactivity was seen in placebo-treated hypercholesterolemic patients throughout the study. L-Arginine treatment was well tolerated without side effects.

**Conclusions.** This double-blinded, placebo-controlled study demonstrates that dietary supplementation with L-arginine can modestly attenuate the increased platelet reactivity seen in hypercholesterolemic patients. The data are consistent with our previous studies in hypercholesterolemic animals, demonstrating that L-arginine restores endogenous nitric oxide activity and inhibits platelet aggregation. Enhancement of endogenous nitric oxide activity is a potential novel therapeutic strategy worthy of further study.

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Platelet adherence and aggregation are augmented in hypercholesterolemic animals and humans (1-4). Platelets derived from individuals with type IIa hyperlipidemia manifest en-

hanced sensitivity to agonists of aggregation *ex vivo* (4). The mechanism of this alteration in platelet reactivity is not well defined, although changes in platelet membrane fluidity (5), arachidonic acid metabolism (5,6) or calcium flux (2), or a combination of these, may contribute.

Hypercholesterolemia may also exert its effects by altering the activity of endogenous regulators of platelet reactivity such as prostacyclin and nitric oxide. Endothelium-derived nitric oxide and prostacyclin act synergistically to inhibit platelet adherence and aggregation (7,8). In the normal microcirculation, endothelium-derived nitric oxide acts on circulating platelets to reduce their tendency to aggregate (9,10). However, in hypercholesterolemic animals and humans, vascular nitric oxide activity is reduced (11-18). This vascular dysfunction may explain the observation that platelets become activated as they

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**Abbreviations and Acronyms**

cGMP = cyclic guanosine monophosphate  
LDL = low density lipoprotein

traverse the coronary circulation of patients with coronary artery disease (19-21). This platelet activation can be reversed by the administration of nitric oxide donors (20,21). We and other investigators have previously demonstrated that the attenuation of vascular nitric oxide activity can be reversed in hypercholesterolemic states by administration of the nitric oxide precursor L-arginine (22-27). In hypercholesterolemic rabbits, arginine administration enhances vascular nitric oxide activity and inhibits platelet reactivity (28). Finally, L-arginine is also known to directly inhibit platelet reactivity, probably by its metabolism to nitric oxide by platelet-derived nitric oxide synthase (29).

This randomized, double-blinded, placebo-controlled trial was performed to determine whether oral arginine supplementation could similarly correct the abnormality of platelet reactivity in hypercholesterolemic humans.

## Methods

**Subjects.** Normocholesterolemic volunteers (13 men, 1 woman) and hypercholesterolemic patients (17 men, 6 women) were studied. Before recruitment in the study, a careful medical history was obtained and a physical examination was performed, as well as laboratory studies to exclude individuals with hematologic, renal or hepatic dysfunction and clinically evident atherosclerosis. Hypercholesterolemia was defined as a total plasma cholesterol level >240 mg/dl and a low density lipoprotein (LDL) cholesterol level >160 mg/dl. None of the individuals had historic evidence of atherosclerosis (symptoms of angina, claudication or cerebrovascular ischemia), nor did they have clinical evidence of arterial occlusive disease (decreased pulses, asymmetric blood pressure or bruits). In addition, no subject had hypertension, diabetes mellitus or congestive heart failure. None of the subjects were taking diuretic agents, vasoactive medications, nonsteroidal anti-inflammatory medications, aspirin or ticlopidine. This study was approved by the Stanford University Administrative Panel on Human Subjects in Medical Research, and each subject gave written informed consent before entry into the study.

**Study design.** Hypercholesterolemic patients were assigned in a double-blind, randomized (2:1) fashion to receive dietary supplementation with either L-arginine (n = 15) or placebo (n = 8) over a period of 2 weeks. Capsules contained either 700 mg of L-arginine hydrochloride or placebo (Avacil, an inert filler). Patients were instructed to take three capsules four times daily for a total dose of 8.4 g of L-arginine hydrochloride per day or placebo. The average American consumes ~5.6 g of arginine daily (30). Therefore, in this study, arginine intake was ~150% of normal intake. Previous

studies in our laboratory have shown that this dose of L-arginine results in a 60% increase in plasma arginine levels in hypercholesterolemic patients (Theilmeier G, Zalpour C, Ma A, et al. Unpublished data.). The experimental studies described below were carried out at four different points: at baseline (before starting L-arginine/placebo supplementation), after 2 weeks of supplementation, after 4 weeks (i.e., 2 weeks after discontinuation of supplementation) and on average 16 weeks after discontinuation. At each visit, the patients were asked about any side effects related to the intake of the study drug.

**Experimental methods.** *Blood sampling.* Blood was drawn from each subject in the postabsorptive state. Alcohol, caffeine and tobacco consumption were prohibited 12 h before venipuncture. After resting quietly for at least 20 min, peripheral venous blood was collected using an 18-gauge needle and releasing the tourniquet before slowly drawing blood back into the syringe to minimize trauma. The portion of blood designated for platelet aggregometry was anticoagulated with sodium citrate (final concentration 0.38%, pH 7.4) and processed immediately.

Subjects were studied in experimental sets of three to five individuals. Each set consisted of one or two normocholesterolemic and two to four hypercholesterolemic individuals. In each experimental set, venipuncture and experimental studies were carried out in parallel to minimize differences between groups due to variations in time or technique.

*Hematologic and biochemical studies.* Venous blood was processed for plasma lipid analysis (total cholesterol, high density lipoprotein and triglyceride), as determined by enzymatic colorimetric methods. Low density lipoprotein cholesterol was calculated using the Friedewald equation. Plasma arginine values were determined by high pressure liquid chromatography. A number of standard biochemical variables were assessed, including measures of hepatic function (aspartate transaminase, alanine transaminase, alkaline phosphatase and albumin), renal function (blood urea nitrogen, creatinine), hematologic function (hemoglobin, complete blood count, differential white blood count, platelet count) and glucose. These studies were performed in the Stanford University Hospital Laboratory using standard clinical laboratory methods.

*Preparation of platelet-rich plasma, platelet-poor plasma and aggregometry.* Platelet-rich plasma was obtained by centrifuging freshly obtained citrated venous blood at 250g for 10 min at 23°C and aspirating the supernatant, carefully avoiding contamination with red blood cells. Platelet-poor plasma was obtained by centrifuging platelet-rich plasma at 16,000g for 10 min. Platelet-rich plasma was adjusted to 300,000 platelets/ $\mu$ l by dilution with platelet-poor plasma, according to cell counts obtained with a Coulter Counter ZM (Coulter Electronics Ltd., Luton, Bedfordshire, England, UK). Platelet aggregation in 400- $\mu$ l aliquots of platelet-rich plasma was assessed according to Born and Cross (31) using a Chrono-Log dual-channel aggregometer (Chrono-Log Corp.) with continuous stirring at 800 rpm. Changes in light transmission occur-

**Table 1.** Biochemical and Hematologic Values Before, During and After Treatment With Study Drug

	Creatinine (mg/dl)	BUN (mg/dl)	Glucose (mg/dl)	SGOT (mg/dl)	SGPT (mg/dl)	Alkaline Phosphatase (mg/dl)	WBC (1,000/ $\mu$ l)	Hematocrit (%)	Platelets (1,000/ $\mu$ l)
NC									
Baseline	1.06 $\pm$ 0.03	15.2 $\pm$ 2.1	92.5 $\pm$ 6.3	51.6 $\pm$ 18.0	77.6 $\pm$ 36.8	63.3 $\pm$ 5.0	5.8 $\pm$ 0.3	44.9 $\pm$ 0.8*	231.4 $\pm$ 14.9
2 weeks	1.05 $\pm$ 0.03	14.8 $\pm$ 1.0	89.5 $\pm$ 4.1	51.7 $\pm$ 16.8	59.5 $\pm$ 19.1	66.2 $\pm$ 5.3	5.8 $\pm$ 0.5	44.2 $\pm$ 0.9	248.8 $\pm$ 9.9
4 weeks	1.03 $\pm$ 0.03	14.6 $\pm$ 1.2	96.5 $\pm$ 7.6	53.6 $\pm$ 21.6	63.1 $\pm$ 21.7	68.0 $\pm$ 5.7	5.9 $\pm$ 0.5	45.0 $\pm$ 1.1	215.0 $\pm$ 15.6
HC <sub>P</sub>									
Baseline	1.00 $\pm$ 0.05	15.1 $\pm$ 2.1	89.0 $\pm$ 4.5	34.4 $\pm$ 3.0	34.2 $\pm$ 5.0	79.3 $\pm$ 3.0	5.5 $\pm$ 0.3	41.0 $\pm$ 1.1	239.8 $\pm$ 39.5
2 weeks	1.10 $\pm$ 0.05	17.8 $\pm$ 2.1	81.0 $\pm$ 10.6	36.4 $\pm$ 4.4	41.8 $\pm$ 7.4	78.4 $\pm$ 3.2	5.4 $\pm$ 0.7	41.4 $\pm$ 1.0	202.5 $\pm$ 21.5
4 weeks	0.98 $\pm$ 0.05	16.2 $\pm$ 1.6	92.2 $\pm$ 1.7	30.7 $\pm$ 2.0	31.6 $\pm$ 2.1	77.1 $\pm$ 4.4	5.4 $\pm$ 0.5	42.0 $\pm$ 1.0	209.3 $\pm$ 16.2
HC <sub>A</sub>									
Baseline	1.08 $\pm$ 0.03	15.1 $\pm$ 1.6	95.2 $\pm$ 3.2	36.9 $\pm$ 2.9	41.8 $\pm$ 3.8	82.3 $\pm$ 4.8	5.4 $\pm$ 0.3	41.8 $\pm$ 0.8	205.0 $\pm$ 17.8
2 weeks	1.08 $\pm$ 0.04	18.7 $\pm$ 1.7	98.4 $\pm$ 2.9	38.4 $\pm$ 4.6	53.6 $\pm$ 13.6	81.7 $\pm$ 5.4	5.5 $\pm$ 0.4	41.9 $\pm$ 0.7	210.3 $\pm$ 9.1
4 weeks	1.08 $\pm$ 0.05	17.3 $\pm$ 2.3	96.1 $\pm$ 3.7	37.7 $\pm$ 4.8	41.4 $\pm$ 5.0	80.0 $\pm$ 3.1	5.8 $\pm$ 0.5	42.2 $\pm$ 0.9	222.0 $\pm$ 11.7

\*p  $\leq$  0.02 compared with corresponding values in hypercholesterolemic placebo and hypercholesterolemic arginine groups. Values expressed as mean value  $\pm$  SEM. BUN = blood urea nitrogen; HC<sub>A</sub> = hypercholesterolemic patients (arginine group); HC<sub>P</sub> = hypercholesterolemic patients (placebo group); NC = normocholesterolemic control subjects; SGOT = serum glutamic oxaloacetic transaminase; SGPT = serum glutamic pyruvic transaminase; WBC = white blood cell count.

ring during platelet aggregation were recorded continuously, and results are presented as the increase in light transmission 4 min after addition of the agonist, with platelet-poor plasma as a reference (=100%). Aggregation was induced with collagen (final concentrations 1-, 2.5-, 5- and 10- $\mu$ g/ml platelet-rich plasma), obtained from Chrono-Log and freshly prepared from a stock solution on each study day. Results are expressed as mean value  $\pm$  SEM. Multigroup comparisons of all variables were carried out using one-way analysis of variance with post hoc analysis using the Fisher protected least significant difference test.

## Results

**Biochemistry and hematology.** L-Arginine was well tolerated without objective evidence of side effects by patient reports or physical examination. However, one patient complained of slight nausea while taking L-arginine. Plasma arginine levels were not different between hypercholesterolemic and normocholesterolemic individuals (74  $\pm$  8 vs. 80  $\pm$  6  $\mu$ mol, respectively, p = 0.51). Administration of L-arginine for 2 weeks increased plasma L-arginine levels by 55% (from 74  $\pm$  8 to 117  $\pm$  12  $\mu$ mol, p < 0.01). Two weeks after cessation of L-arginine therapy, plasma arginine levels returned to baseline values (74  $\pm$  8 vs. 79  $\pm$  7  $\mu$ mol [values at baseline vs. those at 4 weeks], p = 0.65). Plasma arginine levels throughout the study did not change in normocholesterolemic subjects. There were no differences between the normocholesterolemic (n = 14) and hypercholesterolemic (n = 23) groups in measures of hematologic, hepatic and renal function and glucose at baseline, except for a slightly lower hematocrit within the normal range, of no clinical significance in hypercholesterolemic patients (Table 1). Hypercholesterolemic patients had significantly higher total serum cholesterol and LDL cholesterol levels compared with normal control subjects (p  $\leq$  0.0002) (Table 2). High density lipoprotein cholesterol and triglyceride

values were not different between the groups (Table 2). Oral administration of L-arginine did not result in any changes in lipid profile (Table 2), biochemical or hematologic variables (Table 1).

**Platelet aggregation.** At baseline, platelet-rich plasma from hypercholesterolemic patients had a tendency toward increased aggregability in response to several concentrations of collagen (Table 3). This difference reached statistical significance at a collagen concentration of 5  $\mu$ g/ml (p  $\leq$  0.02) (Fig. 1A, Table 3). After 2 weeks of L-arginine administration, aggregation in response to 5  $\mu$ g/ml of collagen was modestly attenuated. At this time point, no significant difference was found in the extent of aggregation of platelets derived from arginine-treated hypercholesterolemic patients and normocholesterolemic volunteers (in response to 5  $\mu$ g/ml of collagen, 65.6% in arginine-treated patients vs. 61.3% in normocholesterolemic control subjects, p = 0.57) (Fig. 1A, Table 3). In contrast, there was no change in the aggregability of platelets derived from hypercholesterolemic patients receiving placebo. After 4 weeks (i.e., 2 weeks after discontinuation of L-arginine treatment), the effect of L-arginine therapy on platelet aggregation persisted: platelet aggregation in response to 5  $\mu$ g/ml of collagen was modestly reduced in patients previously treated with L-arginine compared with patients receiving placebo (52.6% in arginine-treated patients vs. 72.7% in placebo-treated hypercholesterolemic subjects, p  $\leq$  0.03) (Fig. 1B, Table 3). Indeed, even compared with platelets derived from normal control subjects, those from arginine-treated patients tended to be less aggregable (52.6% in arginine-treated patients vs. 65.1% in normocholesterolemic control subjects, p = 0.08) (Fig. 1A, Table 3). Compared with pretreatment values, platelet aggregation in response to 5  $\mu$ g/ml of collagen was reduced in patients who had received supplemental arginine (52.6% in arginine-treated patients at 4 weeks vs. 70.9% in arginine-treated patients at baseline, p  $\leq$  0.01) (Fig. 1, Table 3). The response to the administration of arginine was quite

**Table 2.** Lipid Profiles Before, During and After Treatment With Study Drug

	Total Cholesterol (mg/dl)	LDL Cholesterol (mg/dl)	HDL Cholesterol (mg/dl)	Triglycerides (mg/dl)
NC				
Baseline	172.1 ± 9.6*	100.3 ± 8.5*	51.3 ± 3.2	102.3 ± 15.6
2 weeks	162.2 ± 8.3*	95.5 ± 7.0*	47.5 ± 2.5	97.5 ± 14.5
4 weeks	163.9 ± 8.7*	96.0 ± 7.5*	46.4 ± 2.6	107 ± 7.3
HC <sub>P</sub>				
Baseline	255.2 ± 10.1	181.1 ± 7.9	56.4 ± 5.9	139.9 ± 21.6
2 weeks	256.0 ± 8.6	182.9 ± 7.4	47.3 ± 5.9	110.3 ± 18.2
4 weeks	251.5 ± 9.2	182.5 ± 8.0	48.2 ± 2.7	105.5 ± 13.4
HC <sub>A</sub>				
Baseline	279.3 ± 22.7	189.1 ± 19.6	46.5 ± 4.2	178.1 ± 77.8
2 weeks	267.7 ± 22.6	175.7 ± 8.4	43.7 ± 3.5	153.4 ± 40.6
4 weeks	260.0 ± 18.6	165.6 ± 10.0	42.9 ± 3.1	192.0 ± 81.8

\*p ≤ 0.0002 compared with corresponding values in hypercholesterolemic placebo and hypercholesterolemic arginine groups. Values expressed as mean value ± SEM. HDL = high density lipoprotein; LDL = low density lipoprotein; HC<sub>A</sub> = hypercholesterolemic patients (arginine group); HC<sub>P</sub> = hypercholesterolemic patients (placebo group); NC = normocholesterolemic control subjects.

heterogeneous; 3 of the 13 subjects receiving arginine manifested striking reductions in percent aggregation to 5 μg/ml of collagen, whereas the effect of arginine on the platelet aggregation in the remaining subjects was modest. In contrast, in placebo-treated patients and normocholesterolemic control subjects, no significant difference in platelet aggregation was observed compared with baseline values (Fig. 1, Table 3). After an average of 18 weeks (i.e., 16 weeks after discontinuation of dietary supplementation), a significant increase in the aggregability of platelets from patients previously treated with arginine was seen (73.6% in arginine-treated patients at 18 weeks vs. 52.6% in arginine-treated patients at 4 weeks, p <

0.01; Table 3). In contrast, no significant change was observed in platelets from normal control subjects (68.8% in normocholesterolemic control subjects at 18 weeks vs. 65.1% in normocholesterolemic control subjects at 4 weeks (p = 0.32) (Table 3).

## Discussion

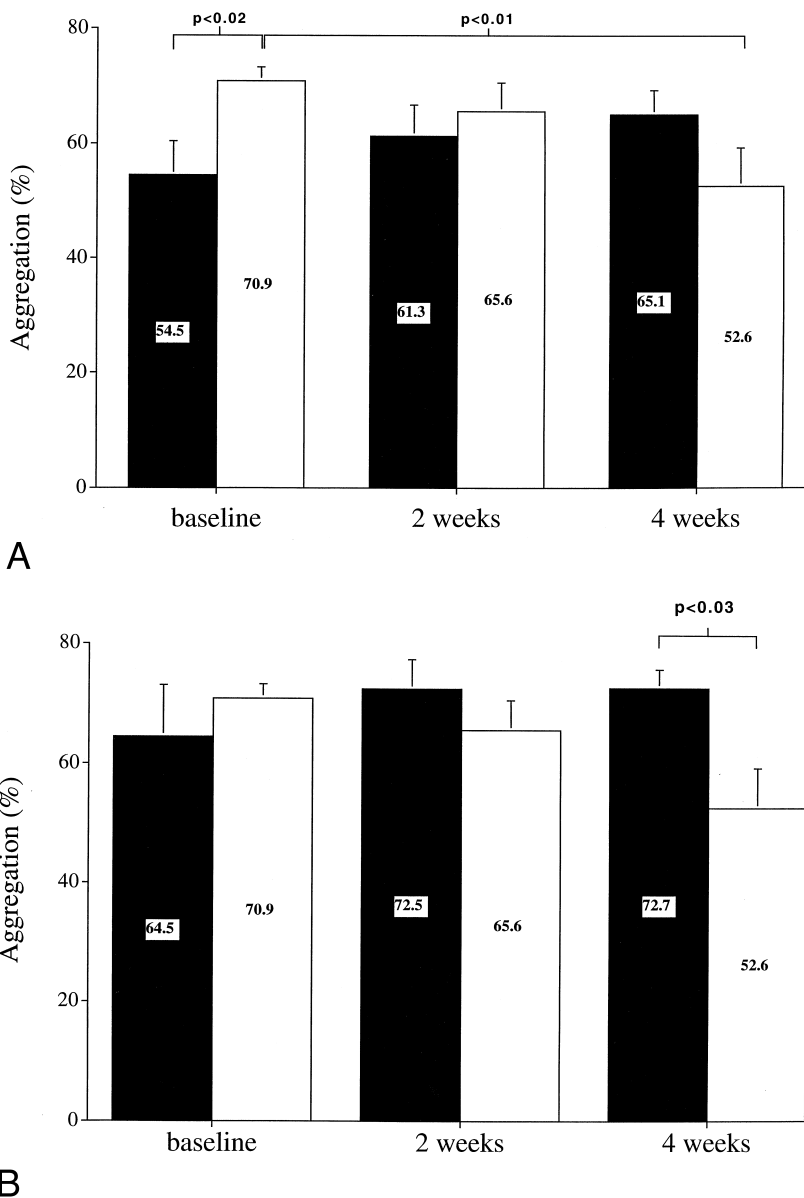
**Major findings.** The salient findings of this investigation are that: 1) platelet reactivity is increased in hypercholesterolemic patients compared with normocholesterolemic control subjects; 2) L-arginine supplementation modestly attenuates platelet aggregation, although the magnitude of this effect

**Table 3.** Platelet Aggregation in Response to Increasing Concentrations of Collagen

	Collagen (μg/ml)			
	1	2.5	5	10
Baseline				
NC	13.7 ± 6.6	31.5 ± 7.6	54.5 ± 5.9	65.9 ± 4.5
HC <sub>A</sub>	19.4 ± 6.0	47.7 ± 6.2	70.9 ± 2.4*	70.6 ± 5.0
HC <sub>P</sub>	14.7 ± 9.9	34.7 ± 10.3	64.5 ± 8.6	72.6 ± 5.8
HC	17.8 ± 5.1	43.2 ± 5.4	68.6 ± 3.3*	71.3 ± 3.8
2 weeks				
NC	4.6 ± 4.6	37.7 ± 8.7	61.3 ± 5.4	67.7 ± 4.1
HC <sub>A</sub>	13.2 ± 5.5	47.9 ± 7.0	65.6 ± 5.0	70.4 ± 2.1
HC <sub>P</sub>	27.3 ± 10.3	54.4 ± 6.7	72.5 ± 4.9	76.0 ± 4.6
4 weeks				
NC	16.5 ± 7.1	45.4 ± 7.5	65.1 ± 4.2	70.9 ± 2.9
HC <sub>A</sub>	15.8 ± 5.6	40.9 ± 7.5	52.6 ± 6.7†	68.0 ± 3.4
HC <sub>P</sub>	21.9 ± 12.6	53.9 ± 6.5	72.7 ± 3.1‡	76.4 ± 3.7
18 weeks				
NC	38.3 ± 12.0	67.5 ± 9.5	68.8 ± 6.4	73.7 ± 3.5
HC <sub>A</sub>	42.4 ± 8.5	64.9 ± 3.3	73.6 ± 4.3	69.7 ± 4.5

\*p ≤ 0.02 compared with baseline values of normocholesterolemic control subjects. †p ≤ 0.01 compared with baseline or 18-week values of hypercholesterolemic patients in arginine group. ‡p ≤ 0.03 compared with 4-week values of hypercholesterolemic patients in arginine group. Data are expressed as mean value ± SEM. HC = all hypercholesterolemic patients; HC<sub>A</sub> = hypercholesterolemic patients (arginine group); HC<sub>P</sub> = hypercholesterolemic patients (placebo group); NC = normocholesterolemic control subjects.

**Figure 1.** Changes over time in platelet reactivity in response to 5  $\mu\text{g/ml}$  of collagen in hypercholesterolemic patients on L-arginine supplementation ( $\text{HC}_A$ ,  $n = 15$ ) or placebo ( $\text{HC}_P$ ,  $n = 8$ ) and normocholesterolemic control subjects ( $\text{NC}$ ,  $n = 14$ ). Data are presented as mean value  $\pm$  SD. **A**, Comparison between normal control subjects ( $\text{NC}$ , **solid bars**) and patients in the L-arginine group ( $\text{HC}_A$ , **open bars**). At baseline, platelets from hypercholesterolemic patients in the arginine group showed significantly higher aggregability than those of normal control subjects. No significant change was noted in control subjects throughout the study. By contrast, significantly reduced platelet aggregation was seen in L-arginine-treated patients at 4 weeks compared with baseline. **B**, Comparison between hypercholesterolemic patients in the placebo ( $\text{HC}_P$ , **solid bars**) and L-arginine groups ( $\text{HC}_A$ , **open bars**). At baseline no significant difference was seen between the groups. No significant change was noted in patients receiving placebo throughout the study. By contrast, significantly reduced platelet aggregation was seen in L-arginine-treated patients at 4 weeks compared with baseline. At 4 weeks, platelets from arginine-treated patients were significantly less reactive than platelets from placebo-treated patients.



varies significantly among individual patients; 3) the inhibitory effect of L-arginine on platelet aggregation persists for at least 2 weeks after discontinuing arginine supplementation; and 4) L-arginine supplementation for this short course was not complicated by side effects, nor associated with any significant changes in biochemical or hematologic variables other than an elevation in plasma L-arginine levels.

**Exogenous arginine enhances nitric oxide synthesis.** Based on our previous studies in hypercholesterolemic animals and humans, we postulate that the antiplatelet effect of L-arginine is due to its metabolism to nitric oxide. We and other investigators have shown that endothelial vasodilator function is depressed in hypercholesterolemic animals and humans (11-18,32,33). This defect is due to an increased degradation and/or reduced synthesis of endothelium-derived nitric oxide (22-27,34-36). The abnormality can be corrected by adminis-

tration of L-arginine (22-27). This amino acid is the substrate for nitric oxide synthase, which metabolizes L-arginine to L-citrulline and nitric oxide. In hypercholesterolemic humans and animals, administration of L-arginine restores endothelium-dependent vasodilation; this is not a physiochemical effect of arginine because administration of D-arginine, which is not metabolized by nitric oxide synthase, does not improve endothelium-dependent vasodilation (22,23,26). Furthermore, we have directly measured the release of nitric oxide from the rabbit thoracic aorta using chemiluminescence (36). The vessels from hypercholesterolemic animals receiving supplemental arginine for 2 weeks generate significantly more nitric oxide *ex vivo*, whereas vessels from animals fed L-nitroarginine (an antagonist of nitric oxide synthase) make significantly less nitric oxide.

We and other investigators have extended these studies to

humans. Intravenous administration of L-arginine to hypercholesterolemic humans enhances nitric oxide-dependent vasodilation in the human forearm and coronary circulation (26,27). More recently, we have shown that the endothelial vasodilator dysfunction described in cardiac transplant recipients may also be reversed by administration of L-arginine (37). In summary, there is abundant evidence that, in hypercholesterolemia and atherosclerosis, L-arginine can restore vascular nitric oxide activity.

**Nitric oxide inhibits platelet aggregation.** Nitric oxide is a known inhibitor of platelet adherence and aggregation. Exogenous nitric oxide donors, such as nitroglycerin, inhibit platelet reactivity (38–42). These agents stimulate soluble guanylate cyclase to increase intracellular levels of cyclic guanosine monophosphate (cGMP), the putative second messenger of nitric oxide. This leads to phosphorylation of cGMP-dependent phosphoproteins (such as vasodilator-stimulated phosphoprotein), which are thought to modulate platelet reactivity, perhaps by reducing intracellular calcium levels (10,43). Endogenous nitric oxide also regulates platelet responsiveness (29,44,45). Evidence from experimental models indicates that both platelet- and endothelium-derived nitric oxide are involved in this regulation. Human platelets contain constitutive and inducible forms of nitric oxide synthase (46) and incubation of platelets with arginine *ex vivo* enhances their elaboration of nitric oxide (29). This platelet-derived nitric oxide increases intraplatelet cGMP and inhibits platelet aggregation.

Endothelium-derived nitric oxide also inhibits platelet adherence and aggregation (44,45). Platelets incubated with cultured endothelial cells exhibit increased cGMP and a reduced rate and extent of aggregation. These effects are due to nitric oxide because they are blocked by methylene blue, which prevents the activation by nitric oxide of soluble guanylate cyclase (44). Indeed, endothelium-derived nitric oxide appears to “condition” platelets as they course through the vasculature; after one circuit through the coronary circulation (of the isolated guinea pig heart), platelet cGMP is increased, and *ex vivo* platelet aggregation is inhibited (9,10). This effect is markedly enhanced by coadministration of acetylcholine, which stimulates the elaboration of endothelium-derived nitric oxide, but does not directly affect platelets. In an experimental model of balloon angioplasty, platelets adhere to the denuded surface of the canine coronary artery; intravenous infusions of arginine inhibit the adherence of platelets *in vivo*. This effect is blocked by antagonists of nitric oxide synthase (45), indicating that the effect of arginine is due to its metabolism to nitric oxide. Oxidized LDL has been shown to decrease L-arginine uptake and nitric oxide synthase expression in human platelets (47), providing a putative mechanism for the increased activity of platelets in hypercholesterolemia.

**Antiplatelet effect of L-arginine.** Because nitric oxide is such a potent modulator of platelet reactivity, the reduced activity of nitric oxide in hypercholesterolemic states may further contribute to the observed enhancement of platelet aggregability. Therefore, administration of L-arginine may be expected to decrease platelet reactivity by restoring endothelium-derived nitric oxide activity as well as by providing additional

substrate for nitric oxide synthase in platelets. We have tested this hypothesis in hypercholesterolemic animals and have demonstrated that L-arginine administration is associated with an inhibition of platelet reactivity in hypercholesterolemic rabbits.

In this animal model, 10 weeks of dietary arginine supplementation inhibited platelet aggregation (28). This effect of dietary arginine was likely due to its conversion to nitric oxide, because the effect was not mimicked by dietary methionine, and was reversed by exposure of the platelets to the nitric oxide synthase inhibitor *N*-methyl-arginine. Moreover, the effect of arginine administration was associated with elevations of intraplatelet cGMP and reductions in intraplatelet calcium levels (28). Similar findings have been reported after short-term administration of high dose arginine (21 g/day) in healthy young men (48). Although the number of subjects we studied was small, the results are concordant with earlier observations. In the present study, the antiaggregatory effect of dietary arginine persisted for at least 2 weeks after its cessation. The exact mechanism of arginine's effect and its persistence remain unknown. Possible explanations include that arginine may be stored in cells (i.e., endothelial cells or bone marrow stem cells) and continue to be used after discontinuation of treatment, sustaining nitric oxide activity and inhibiting platelet function. Alternatively, arginine administration may be affecting platelet reactivity by a mechanism independent of nitric oxide; for example, L-arginine is known to be a secretagogue of insulin and growth hormone.

Platelet-vessel wall interactions and release of platelet-derived growth factors contribute to the development of atherosclerosis (49). Inhibition of platelet reactivity may slow the progression of disease or alter the response to vascular injury. The antiatherogenic effect of fish oil supplements is believed to be due to the antiplatelet effect of eicosapentaenoic fatty acids in marine lipids (50). The resistance to atherosclerosis in swine with von Willebrand's disease is also attributed to reduced platelet-vessel wall interactions (51). Similarly, the ability of supplemental L-arginine to reduce intimal lesion formation in hypercholesterolemic animals (52) may be explained in part by its ability to inhibit platelet aggregation.

**Conclusions.** We have demonstrated that L-arginine supplementation attenuates platelet aggregation in hypercholesterolemic patients. The intervention was tolerated well, without significant side effects. Future studies need to be performed to determine if these antiplatelet effects of L-arginine will reduce vascular thrombosis and the progression of vascular disease in humans.

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