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Nutritional therapy for peripheral arterial disease: a double-blind, placebo-controlled, randomized trial of HeartBar®

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Abstract: We investigated the clinical effects of a food bar enriched with L-arginine and a combination of other nutrients known to enhance the activity of endothelium-derived nitric oxide (EDNO) in individuals with claudication from atherosclerotic peripheral arterial disease. The study was a 2-week, double-blind, placebo-controlled trial of subjects randomized to three groups (two active bars, one active and one placebo bar, and two placebo bars per day) followed by an 8-week open-label period. Subjects ($n = 41$) were outpatient volunteers with intermittent claudication. Pain-free and total walking distances were measured by variable-grade, treadmill exercise testing. Quality of life was assessed using the Medical Outcome Survey (SF-36). After 2 weeks of treatment, the pain-free walking distance increased 66% while the total walking distance increased 23% in the group taking two active bars/day. The general and emotional/social functioning components of the SF-36 also improved. These effects were not observed in the one active bar/day and placebo groups. The effects were maintained after 10 weeks and, in addition, an improvement in walking distance was observed in the group taking one active bar. These findings reveal that use of a nutrient bar designed to enhance EDNO activity improves pain-free and total walking distance as well as quality of life in individuals with intermittent claudication.

Key words: antioxidant; endothelium; intermittent claudication; L-arginine; nitric oxide

Introduction

Atherosclerotic cardiovascular disease represents one of the most common causes of morbidity and mortality in the western hemisphere.[1] Atherosclerosis can manifest itself in the forms of coronary, cerebral and/or peripheral arterial disease (PAD). Clinically, patients with PAD experience symptoms of intermittent claudication (muscle aching and cramping during walking secondary to ischemia in the calf, thigh or buttock). More severe disease may cause pain at rest or even ulceration. Currently, medical therapies for the treatment of PAD are only modestly effective in relieving symptoms. Clearly, new approaches to the treatment of PAD with greater efficacy, less toxicity and with an ability to alter the course of the disease are desirable.

A new therapeutic strategy that may be effective in this group of individuals is to enhance endothelial vasodilator function. The endothelium elaborates several vasodilators, including prostacyclin, endothelium-derived hyperpolarizing factor, adrenomedullin, and endothelium-derived nitric oxide (EDNO). Of these, EDNO is the most potent.[2] EDNO is an endogenous messenger molecule that plays a critical role in the regulation of vascular resistance, as well as in the vasodilation of conduit vessels as they accommodate to increases in blood flow. EDNO relaxes vascular smooth muscle cells and inhibits platelet and monocyte interactions with the vessel wall, and suppresses the proliferation of vascular smooth muscle. Impairment of EDNO activity limits blood flow through diseased coronary or peripheral vessels.[3]

The precursor for EDNO is the semi-essential amino acid L-arginine.[4] L-arginine is converted to nitric oxide and citrulline by endothelial nitric oxide synthase (eNOS). In patients with hypercholesterolemia and/or atherosclerosis, the impairment of EDNO synthesis can be reversed by administration of L-arginine.[5–18] Likewise, in patients with coronary and peripheral arterial diseases, administration of L-arginine enhances EDNO synthesis, improves endothelium-dependent vasodilation and ameliorates the symptoms of angina and intermittent claudication.[19–21]

However, the beneficial effects of L-arginine have been observed with intravenous administration or high doses (6–21 g/day) of oral supplementation. Although these high doses of L-arginine were well tolerated by subjects in these studies, the use of this amount of L-arginine in pills or capsules may not be practical. Indeed, at the lowest dose that has been shown to be effective, 12 capsules (500 mg each) daily would be required. Accordingly, we developed and evaluated a convenient and palatable nutrient bar enriched with L-arginine, as well as with antioxidant vitamins in a soy protein and oat fiber base. We have previously demonstrated that use of the bar restores flow-mediated vasodilation in individuals with hypercholesterolemia (unpublished observation). In that study, hypercholesterolemic individuals were found to have impaired flow-mediated vasodilation of the brachial artery. Two or three bars per day for 2 weeks reversed this impairment. We hypothesized that restoration of endothelial function would

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reduce symptoms in individuals with peripheral arterial disease. To test this hypothesis, we performed a randomized, double-blind, placebo-controlled trial of this nutrient bar in outpatients with intermittent claudication.

Methods

Study design

The study was a randomized, double-blind, placebo-controlled trial of dietary supplementation with either two active nutrient bars (2 Bar Group), one active and one placebo bar (1 Bar Group) or two placebo bars (Placebo Group) per day. All subjects signed an informed consent before undergoing screening procedures. At the screening visit, a medical history and physical exam were performed. Baseline venous blood samples were taken for determination of serum lipid, chemistry, and hematologic values. In the screening period, treadmill testing was performed and an SF-36 questionnaire was administered to establish baseline values. Following baseline treadmill exercise testing, eligible subjects were randomized to one of the three treatment groups. Subjects were instructed to take the bars with or between meals separated by at least 4 h. At the end of the 2-week period subjects returned after an overnight fast for venous blood sampling and SF-36 testing. On a separate occasion, subjects returned for follow-up treadmill testing 12–16 h after consuming the last study bar. Subjects were then invited to participate in a 2-month open-label period. Participants in the open-label period were generally assigned to the same groups they were in during the randomization period except that requests to cross over at the start of the period were granted. The study was approved by an independent review board for protection of human subjects.

Subjects

Subjects were recruited by radio, newspaper and internet advertisements from the San Francisco Bay area between February and May 1998. Eligible subjects were adults with intermittent claudication (pain, numbness or severe fatigue involving muscles of one or both lower extremities reproducibly provoked by walking and relieved by rest) due to atherosclerotic peripheral arterial disease in a steady clinical state for at least 6 months with no change in symptoms for the previous 3 months. The presence of PAD was verified by a Doppler-measured resting ankle/brachial index (ABI) of $\leqslant 0.90$, which dropped at least 25% following exercise. As a condition for selection, subjects were required to be able to walk on the treadmill for at least 1 min and the variability between two consecutive (of a maximum of three) baseline treadmill tests was required to be $\leqslant 25\%$. Subjects were excluded if they had: any disease process, other than peripheral arterial disease, limiting treadmill exercise capacity; evidence of arterial disease of a non-atherosclerotic nature; undergone major surgery in the previous 3 months; a leg amputation above the ankle; suffered a myocardial infarction in the previous 3 months; type I diabetes; uncontrolled hypertension (>180 systolic or >100 diastolic); a current malignancy; significantly impaired renal or hepatic function; or if they were currently enrolled in another clinical trial. Subjects were required to discontinue certain products affecting peripheral vessels specifically pentoxifylline, carnitine, prostacyclin analogs or L-arginine 28 days prior to acquiring baseline data. Subjects were otherwise asked to continue their regular medications, diet and lifestyle habits and were free to follow any other medical care regimens.

Study product

The active bar is an L-arginine-enriched nutrient bar (HeartBar®, Cooke Pharma, Belmont, CA, USA). Each 50-g active bar contains 3.3 g of L-arginine as well as antioxidant vitamins and minerals, folic acid and B complex vitamins in a soy protein and oat fiber base. Each placebo bar is similar to the active bar with respect to weight and flavor as well as caloric, carbohydrate, protein, fiber and fat content but is not supplemented with L-arginine. The protein source of the placebo bar is whey (L-arginine-poor) rather than soy (L-arginine-rich). A subset in the placebo group received bars with vitamins and minerals and the rest in the placebo group received bars devoid of vitamins and minerals. Because there were no differences in the effects of the placebo bars with or without vitamins, these groups are consolidated for the statistical analysis. The components of the active and placebo bars are listed in Table 1.

Treadmill testing

Treadmill testing was performed using the Gardner variable-grade, constant-speed treadmill protocol.[22] In brief, the treadmill speed is set at a constant 2.0 miles per hour. The slope of the treadmill begins at 0° and increases 2° every 2 min. This protocol increases the work rate through time such that each incremental increase in walking distance is associated with a greater incremental increase in work. An advantage of this protocol is that the results are more reproducible than continuous-load protocols.[23] A disadvantage is that this protocol compresses improvements in walking distances into smaller increments compared with continuous-load protocols. Although any given patient achieves a greater absolute distance using the variable-grade protocol, the change in distance following an intervention is less by virtue of the fact that a greater work rate is required to perform beyond the original distance. Thus, improvements in walking distance are compressed into shorter distances compared with constant-grade testing such that even small-percentage improvements in treadmill performance can be interpreted as clinically significant.[23]

The primary objective of the treadmill testing was to determine the pain-free walking distance; a secondary endpoint was the measure of total walking distance. For determination of the pain-free walking distance, subjects were asked to identify the onset of any pain, cramp, numbness or extreme fatigue in any lower-extremity muscle. For determination of total walking distance, subjects were asked to identify the point at which they would normally stop and rest due to claudication symptoms. Analyses of serum lipid, chemistry and hematology were performed by Unilab (San Jose, CA, USA).

Quality of life

Quality of life was measured by means of the Medical Outcomes Scales Health Survey (SF-36).[24] The SF-36 is a general health questionnaire which has been validated in large populations with a variety of diseases. The questionnaire outcomes include physical function, social function, the

Table 1 Composition of active (HeartBar®) and placebo bars.

	HeartBar®		Placebo with vitamins		Placebo without vitamins	
Size (g)	50		50		50	
Calories (kcal)	180		170		170	
Ingredients	Amount	(% DV)[a]	Amount	(% DV)[a]	Amount	(% DV)[a]
Total fat	3 g		1.5 g		1.5 g	
saturated	0 g		0 g		0 g	
polyunsaturated	1.5 g		0 g		0 g	
monounsaturated	0.5 g		1 g		1 g	
Cholesterol	0 mg		0 mg		0 mg	
Total carbohydrate	25 g		30 g		30 g	
dietary fiber	3 g		2 g		2 g	
sugars	15 g		13 g		13 g	
sugar alcohol	5 g		10 g		10 g	
Protein	14 g		13 g		13 g	
L-arginine	3.3 g		0 g		0 g	
Vitamin C	250 mg	420	250 mg	420	0 mg	0
Vitamin E	200 IU	670	200 IU	670	1 IU	2
Niacin	25 mg	130	25 mg	130	0 mg	0
Vitamin B6	2 mg	100	2 mg	100	0 mg	0
Vitamin B12	4.8 μg	80	4.8 μg	80	0.4 μg	6
Folate	200 μg	50	200 μg	50	16 μg	4

[a]Per cent daily values (% DV) are based on a 2000 calorie diet.

subjects' perception of their general health, well-being, and satisfaction with treatment. Each of the areas of functioning is scored separately on a scale of 0–100%.[25] Composite scores were derived from General Health, Bodily Pain and Vitality scores (General), Physical Functioning and Role-Physical (Physical), and Mental Health, Role-Emotional, and Social Functioning (Emotional/Social).

Statistical analysis

The primary variable analyzed for efficacy was the pain-free walking distance. Additional efficacy outcomes included total walking distance and SF-36 scores. The data for walking distances were analyzed in terms of differences from baseline (both absolute and per cent). Data are presented as mean ± standard error of the mean (SEM). Comparisons between groups were made by analysis of variance (ANOVA). Non-parametric data was analyzed after raw data was log-transformed. Differences that were found to be significant by ANOVA were analyzed further using Fisher's Least Significant Difference. Comparisons before and after treatment were made using two-tailed, paired Student's *t*-test. Except for a priori comparisons, a Bonferroni correction was used for multiple comparisons.[26]

Results

Demographics

Of 156 individuals screened, 41 met the inclusion/exclusion criteria and were randomized. The methods of recruitment employed resulted in a large number of responses from individuals with undiagnosed intermittent leg pain and, therefore, a large number of ineligible subjects, most failing the ABI with and without exercise requirements. Subjects were randomized to the 2 Bar Group ($n = 12$), 1 Bar Group ($n = 15$) or Placebo Group ($n = 14$). The three groups were similar with respect to demographics, baseline serum lipid values and baseline walking distances, except for the following differences. The 1 Bar Group had a higher prevalence of diagnosed coronary artery disease ($p < 0.01$ by chi square), a higher prevalence in the use of lipid-lowering treatments ($p < 0.05$ by chi square) and a higher prevalence of those claiming to adhere to a low-fat diet ($p < 0.02$ by chi square). Additionally, the 2 Bar Group had higher total serum cholesterol and LDL levels as well as a higher cholesterol/HDL ratio ($p < 0.02$ by ANOVA, see Table 5). The characteristics of the subjects in each group are given in Table 2. Two subjects in the 1 Bar Group discontinued the study (one subject claimed two bars per day were too filling, the other subject cited a disagreeable taste of either the placebo or the active bar). Thirty-nine subjects completed the study with a compliance of greater than 75%.

Evaluation of efficacy

There were no statistical differences in walking distances before therapy. The 1 Bar Group nonetheless had higher mean baseline walking distances (Table 3) because of the walking ability of one subject (Figures 1a and 2a). The mean baseline pain-free walking distance of the other 12 compliant members of that group was 139 ± 22 m. Following 2 weeks of intervention, the pain-free walking distance in the 2 Bar Group was significantly greater than the Placebo and 1 Bar Groups (66% increase or 100 ± 45 m additional distance, $p < 0.05$ vs Placebo and 1 Bar Groups, and $p < 0.05$ vs baseline distance by paired Student's *t*-test; Table 3, Figure 1b). This same group demonstrated a 23% increase (77 ± 31 m additional distance) in total walking distance ($p = 0.05$ vs placebo by ANOVA and vs baseline by paired Student's *t*-test; Table 3 and Figure 2b). Efficacy was also examined by analyzing the number of responders (defined by a 25% or greater improvement in pain-free walking distance). After 2 weeks, the 2 Bar Group

Table 2 Baseline demographic and treadmill data of subjects.

	Placebo	1 Bar Group	2 Bar Group	*p*-value
n	14	15	12	
Age (years)	70.6 ± 2	68.2 ± 2	66.3 ± 3	NS
Weight (lb)	173 ± 8	185 ± 8	180 ± 12	NS
Male (*n*)	9	14	8	NS
Caucasian (*n*)	14	15	12	NS
Diabetics (*n*)	4	2	2	NS
With hypercholesterolemia (*n*)	4	9	2	NS
With hypertension (*n*)	2	3	3	NS
Current smokers (*n*)	4	2	0	NS
Ever smokers (*n*)	13	14	12	NS
Alcoholic drinks/day (*n*)	1.0 ± 0.3	1.1 ± 0.3	1.1 ± 0.4	NS
With CAD (*n*)	5	11	2	0.009
With erectile dysfunction (*n*)	5	4	3	NS
On pentoxyfylline (*n*)	2	0	0	id
Hx LE Sx (*n*)	1	2	2	NS
On antilipid Rx (*n*)	4	9	2	0.05
On CCB (*n*)	1	4	1	NS
On ACE inhibitor (*n*)	3	6	2	NS
On ASA (*n*)	6	8	3	NS
On beta-blocker (*n*)	3	1	1	NS
On low-fat diet (*n*)	1	7	1	0.02
On exercise program (*n*)	4	6	7	NS

Continuous data analyzed by ANOVA, dichotomous data analyzed by chi square.
CAD, coronary artery disease; Hx LE Sx, history of angioplasty or bypass of a lower extremity artery; Rx, treatment; CCB, calcium channel blocker; ACE, angiotensin converting enzyme inhibitor; ASA, aspirin.
NS, not significant by chi square; id, insufficient data.

Table 3 Treadmill data of subjects following 2 weeks of intervention.

	Placebo	1 Bar Group	2 Bar Group	*p*-value
n	13	14	12	
Pain-free walking distance (m)				
Before	116 ± 17	206 ± 72	159 ± 34	NS
After	135 ± 22	221 ± 69	260 ± 67*	NS
Change in pain-free walking distance (m)	19 ± 11 (18%)	14 ± 18 (21%)	100 ± 45 *f* (66%)	0.05
Total walking distance (m)				
Before	267 ± 120	226 ± 75	197 ± 45	NS
After	236 ± 41	339 ± 83	327 ± 69*	NS
Change in total walking distance (m)	8 ± 12 (4%)	32 ± 23 (17%)	77 ± 31 *f* (23%)	0.05

Values in mean ± SEM.
p-value, between-group significance by ANOVA; NS, not significant.
f, $p < 0.05$ by Fisher's Least Significant Difference test; $*p < 0.05$ vs before intervention by paired Student's *t*-test.

and 1 Bar Group combined had a significantly greater number of responders than the Placebo Group (2 Bar Group: 7 of 12 (58%); 1 Bar Group: 6 of 15 (40%); and Placebo Group: 2 of 13 (15%); $p > 0.05$ by chi square). A similar result was demonstrated for total walking distance – defined by a 20% or greater improvement (2 Bar Group: 5 of 12 (42%); 1 Bar Group: 5 of 15 (33%); and Placebo Group; 1 of the 13 (8%); $p > 0.05$ by chi square).

Quality of life

The 2 Bar Group was observed to have a significant improvement in the perceived general, emotional and social function portions of the SF-36. The composite general health score improved 10% ($p = 0.01$ by ANOVA, $p < 0.05$ by paired Student's *t*-test; Table 4 and Figure 3). The emotional/social health composite score improved 6% ($p = 0.02$ by ANOVA). There were no such effects in the Placebo or 1 Bar Groups.

Open-label period

Thirty-five of the 39 subjects enrolled in the open-label period. Twenty subjects took two bars/day, eight subjects took one bar/day, and seven subjects in the Placebo Group agreed to take no bars during this additional 8-week period but to otherwise adhere to the requirements of the study.

Following the open-label period, the improvement in pain-free walking distance (+73 ± 27 m or 54% over baseline) in the 2 Bar Group remained significant ($p < 0.01$ vs baseline by paired Student's *t*-test). Furthermore, by 10 weeks, the 1 Bar Group demonstrated a significant

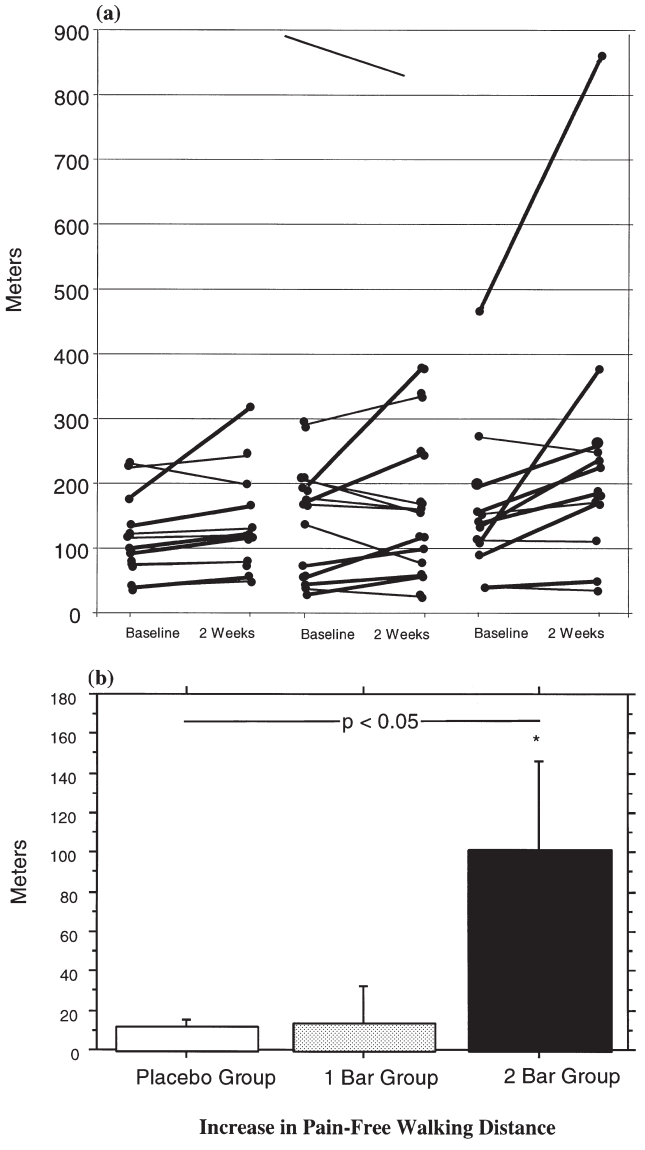

Figure 1 Pain-free walking distance following 2 weeks of intervention. (a) Individual values; (b) mean changes from baseline. Values in mean ± SEM, $*p < 0.05$ vs baseline data.

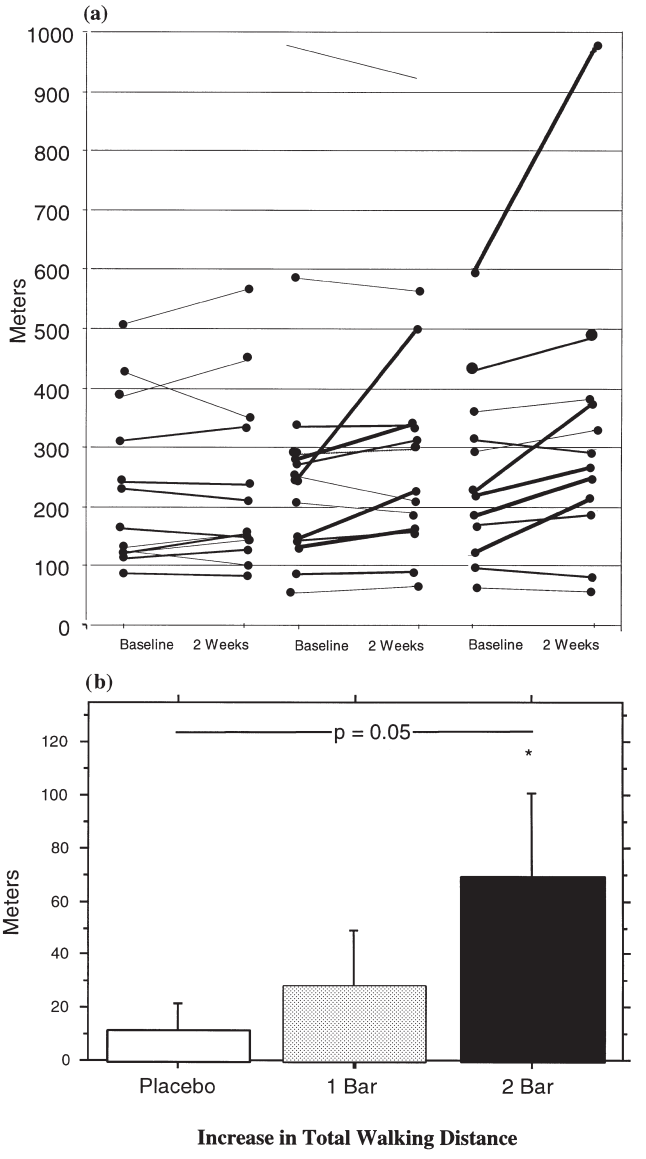

Figure 2 Total walking distance following 2 weeks of intervention. (a) Individual values; (b) mean changes from baseline. Values in mean ± SEM, $*p < 0.05$ vs baseline data.

improvement over baseline ($p < 0.05$ by paired Student's *t*-test) demonstrating a 74 ± 28 m increase in pain-free walking distance. The 63% mean increase in pain-free walking distance observed in this group is the result of non-parametric data secondary to an unusually large increase in walking distance in one subject. This data was analyzed by log transformation of the raw data and, by this method, shown not to be significantly different between groups by ANOVA. No change (+20 ± 27 m or 21%, NS) in pain-free walking was observed in the No Bar Group. Total walking distance improved further (+78 ± 37 m) in the 2 Bar Group, resulting in a 31% increase over baseline ($p < 0.05$ by paired Student's *t*-test). No significant changes in total walking distance were observed in the 1 Bar and No Bar Groups after 10 weeks (–6 ± 39, NS).

Evaluation of safety

There were no differences between the placebo and treatment groups in parameters of safety. There were no significant changes in values with respect to serum chemistry, and hematologic or lipid levels. The lipid panel before and after intervention is given in Table 5. No serious adverse events were reported during the 10-week intervention. Minor adverse events included dry mouth, which was reported by two subjects in the 2 Bar Group. One subject in the Placebo Group reported a change in bowel habits.

Discussion

The important findings in this study are:

1. Nutritional support with two active bars/day results in a 66% improvement in pain-free walking and a 23% improvement in total walking distance as measured by treadmill testing after a 2-week period of dietary intervention. These improvements were maintained at 10 weeks of intervention.

Table 4 SF-36 data of subjects following 2 weeks of intervention.

	Placebo	1 Bar Group	2 Bar Group	*p*-value
n	13	14	12	
Composite General				
Before	59 ± 5	59 ± 4	55 ± 6	NS
After	58 ± 4	63 ± 4	65 ± 6 *f*∗	0.01
Composite Physical				
Before	58 ± 7	62 ± 6	59 ± 9	NS
After	66 ± 8	65 ± 6	62 ± 8	NS
Composite Emotional/Social				
Before	71 ± 3	73 ± 2	60 ± 5	NS
After	68 ± 3	75 ± 1	66 ± 5 *f*	0.02

Values in mean ± SEM.
p-value, between-group significance by ANOVA; NS, not significant.
f, $p < 0.05$ by Fisher's Least Significant Difference test; $*p < 0.05$ vs before intervention by paired Student's *t*-test.

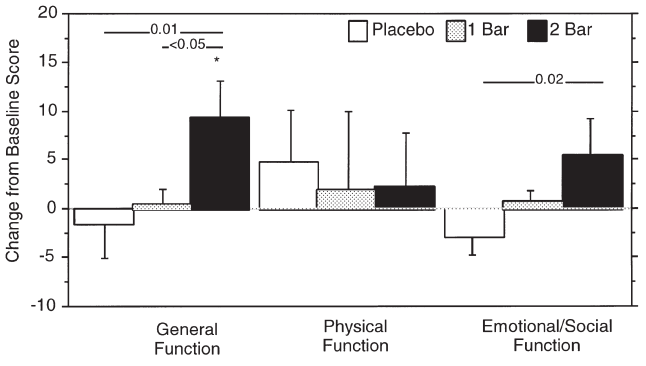

Figure 3 Change in SF-36 General, Physical, and Emotional/Social composite scores following 2 weeks of intervention. Values in mean ± SEM, $*p < 0.05$ vs baseline data.

2. This same intervention results in a significant improvement in perceived general, emotional and social health as measured by the SF-36 Health Status Questionnaire.

The results of this study compare favorably with the results of other interventions in claudicants, particularly considering that a graded-workload protocol was used. The treadmill protocol used in this study utilizes an increasing treadmill slope over time, which results in an increasing work-rate over time. The pain-free walking distance increased by 66% after 2 weeks, which is an absolute increase of 48% over placebo. However, the increase in metabolic equivalents (METS) achieved is estimated to be greater than 62% over placebo (based on calculations in healthy individuals walking the same distance[27]). Using a similar graded-workload treadmill protocol, a recent clinical study of cilostazol reported an estimated treatment effect of 20% improvement over placebo in pain-free walking distance after 16 weeks of intervention.[28] Earlier trials using other interventions typically have used protocols that do not accelerate MET values over time and therefore the percentage increase in distance is identical to the percentage increase in work. For example, a double-blind, placebo-controlled trial with pentoxifylline reported an absolute increase in pain-free walking distance of 30% over placebo after 16 weeks and 56% after 24 weeks of therapy.[29] In that study the same percentage increases would be expected for MET values due to the treadmill protocol used.

Other therapies have used continuous work protocols as well. Verapamil, which may also be useful in the treatment of intermittent claudication, increased pain-free walking by 29% over placebo using a metronome-controlled, constant step rate on a level surface.[30] Studies using intravenous prostacyclin analogs (6-h infusions daily for 5 days) have reported no more that a 19% increase in pain-free walking distance over placebo at 8 weeks.[31] Likewise, prostaglandin E_1, when given intravenously for 5 days per week for 4 weeks and then twice weekly for 4 additional weeks, resulted in a 37% increase in walking distance over the placebo group.[32] Finally, Brevetti and colleagues have performed a number of studies examining the clinical effect of propionyl L-carnitine in patients with intermittent claudication. In the largest of these studies, these investigators found no difference in walking distances from placebo over the course of the study until 180 days of intervention. At that time, subjects taking 1–2 g/day of propionyl L-carnitine demonstrated a 24% increase in pain-free walking over placebo.[33]

In addition to effects on walking distances, we observed an effect on the General and Emotional/Social Functioning composite scores. In contrast, there was no perceived benefit in composite Physical Functioning scores. This is in contrast with the findings of studies of other therapies for peripheral arterial disease. One explanation may be that the major benefit of this therapy was increased pain-free walking distances with less of an effect on total walking distances. The General Functioning scales elicit perceived improvements in the magnitude of pain and how much pain interferes with activities of daily living, while the Physical Functioning scales elicit perceived improvements in the ability to walk certain distances. The improvement in Emotional/Social scores, which elicit perceived improvements in energy, extent of socialization and accomplishments, may be understood in the context of some specific responses. Such responses include a perception of increased energy, the ability to return to the care of a spouse, and an increased sense of enjoyment of such things as a game of golf.

Although the numbers of subjects in the groups were

Table 5 Serum lipid levels before and after 2 weeks of intervention.

	Placebo	1 Bar Group	2 Bar Group	*p*-value
n	13	14	12	
Total cholesterol (mg/dl)				
Before	199 ± 9	209 ± 10	239 ± 11 *f*	0.02
After	201 ± 10	207 ± 8	229 ± 13	NS
HDL (mg/dl)				
Before	52 ± 5	54 ± 5	48 ± 5	NS
After	51 ± 6	50 ± 5	44 ± 5	NS
LDL (mg/dl)				
Before	114 ± 9	113 ± 8	147 ± 10 *f*	0.02
After	119 ± 9	117 ± 7	149 ± 14	NS
VLDL (mg/dl)				
Before	34 ± 3	37 ± 5	46 ± 5	NS
After	31 ± 3	34 ± 4	41 ± 4	NS
Triglycerides (mg/dl)				
Before	187 ± 22	204 ± 28	221 ± 25	NS
After	183 ± 30	202 ± 37	213 ± 26	NS
Chol/HDL ratio				
Before	4.2 ± 0.4	4.2 ± 0.3	5.7 ± 0.5	0.01
After	4.4 ± 0.4	4.4 ± 0.4	5.7 ± 0.5	NS

Values in mean ± SEM.
p-value, between-group significance by ANOVA; NS, not significant.
f, $p < 0.05$ by Fisher's Least Significant Difference test.

small, the lack of effect on walking distances in the subset of individuals taking the placebo with antioxidant vitamins suggests that the L-arginine in the bar has much to do with the efficacy of the bar. The beneficial effect of supplemental L-arginine is likely due to its conversion to nitric oxide. Administration of L-arginine improves endothelial vasodilator function in hypercholesterolemic rabbits, in association with increases in vascular nitric oxide elaboration.[12,16–18] In patients with atherosclerotic peripheral arterial disease, administration of L-arginine improves limb blood flow in association with increased urinary nitrogen oxides and cGMP (which reflects increased nitric oxide synthesis).[19,34,35] Böger and colleagues observed in patients with critical limb ischemia (severe claudication) that acute intravenous administration of L-arginine increased vascular nitric oxide production (as measured by urinary nitrate excretion) by 130% and blood flow to the legs by 42%.[20] The effect of L-arginine on limb blood flow was comparable with intravenous prostaglandin E_1 (which is currently the best available medical therapy in Europe for this condition).

L-arginine appears to be rate limiting for NO synthesis in patients with hypercholesterolemia and/or atherosclerosis; this may be due to the increased levels of asymmetric dimethylarginine (ADMA) observed in these individuals.[36] ADMA is a circulating competitive antagonist of NO synthase that is elevated in individuals with atherosclerosis as well as in those with risk factors for atherosclerosis (hypercholesterolemia, hypertension, diabetes mellitus, renal failure, homocysteinemia, tobacco use and increased age).[37–44] Its elevation in these disorders may explain in part the endothelial impairment observed with these conditions. The effects of ADMA in hypercholesterolemia can be reversed by exogenous oral L-arginine, consistent with the action of ADMA as a competitive inhibitor of NO synthase.[45,46]

In addition to L-arginine, several other components were added to the bar to enhance its effect. As mentioned above, the antioxidant vitamins C and E improve endothelial vasodilator function, most likely by preserving EDNO bioactivity.[47,48] EDNO is a labile molecule, with a brief half-life that is shortened still further by oxygen-derived free radicals.[49] The phytoestrogens in the soy protein base of this food bar also have antioxidant activity that improve vasodilation in humans.[50,51] In addition, it is possible that phytoestrogens may have a favorable effect on endothelial expression of the enzyme NO synthase.[52] The gene encoding endothelial NO synthase contains estrogen-responsive elements in its promoter region, which increases its transcription, leading to increased endothelial levels of NO synthase.[53] Phytoestrogens may also have a favorable effect on the lipid profile, as does the oat fiber found in the nutrient bar.[54] The action of these components (with a contribution from the small amount of niacin in the product) may explain the beneficial effect of the bar on the lipid profile. Finally, the product also contains folate, B6 and B12, which are known to reduce homocysteine levels.[55,56] Homocysteine, which is known to accelerate atherogenesis, is highly prevalent in individuals with atherosclerosis.

To summarize, a 2-week intervention using two L-arginine-enriched nutritional bars per day resulted in a significant improvement in pain-free and total walking distances as well as in perceived general health and emotional and social functioning. These improvements were observed after 8 additional weeks of open-label use. There were no

serious adverse events reported and few minor adverse events. HeartBar may be useful in the dietary management of peripheral arterial disease.

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References

1 US Bureau of the Census. Statistical Abstract of the United States. Springfield, VA: National Technical Information Service: 1990.
2 Moncada S, Palmer RMJ, Higgs EA. Nitric oxide: physiology, pathophysiology, and pharmacology. *Pharmacol Rev* 1991; **43**: 109–42.
3 Cooke JP, Tsao PS. Endothelial alterations in atherosclerosis: the role of nitric oxide. In: Webb D, Vallance P eds. *Endothelial function in hypertension*. Heidelberg: Springer-Verlag, 1997: 29–38.
4 Palmer RMJ, Ferrige AG, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. *Nature* 1988; **333**: 664–66.
5 Drexler H, Zeiher AM, Meinzer K, Just H. Correction of endothelial dysfunction in coronary microcirculation of hypercholesterolaemic patients by L-arginine. *Lancet* 1991; **338**: 1546–50.
6 Imaizumi T, Hirooka Y, Masaki H et al. Effects of L-arginine on forearm vessels and responses to acetylcholine. *Hypertension* 1992; **20**: 511–17.
7 Clarkson P, Adams MR, Powe AJ et al. Oral L-arginine improves endothelium-dependent dilation in hypercholesterolemic young adults. *J Clin Invest* 1996; **97**: 1989–94.
8 Tsao PS, McEvoy LM, Drexler H, Butcher EC, Cooke JP. Enhanced endothelial adhesiveness in hypercholesterolemia is attenuated by L-arginine. *Circulation* 1994; **89**: 2176–82.
9 Wang BY, Singer AH, Tsao PS, Drexler H, Kosek J, Cooke JP. Dietary arginine prevents atherogenesis in the coronary artery of the hypercholesterolemic rabbit. *J Am Coll Cardiol* 1994; **23**: 452–58.
10 Dubois-Rande JL, Zelinsky R, Roudot F et al. Effects of infusion of L-arginine into the left anterior descending coronary artery on acetylcholine-induced vasoconstriction of human atheromatous coronary arteries. *Am J Cardiol* 1992; **70**: 1269–75.
11 Wolf A, Zalpour C, Theilmeier G et al. Dietary L-arginine supplementation normalizes platelet aggregation in hypercholesterolemic humans. *J Am Coll Cardiol* 1997; **29**: 479–85.
12 Jeremy RW, McCarron H, Sullivan D. Effects of dietary L-arginine on atherosclerosis and endothelium-dependent vasodilatation in the hypercholesterolemic rabbit. Response according to treatment duration, anatomic site, and sex. *Circulation* 1996; **94**: 498–506.
13 Creager MA, Gallagher SJ, Girerd XJ, Coleman SM, Dzau VJ, Cooke JP. L-arginine improves endothelium-dependent vasodilation in hypercholesterolemic humans. *J Clin Invest* 1992; **90**: 1248–53.
14 Wang BY, Candipan RC, Arjomandi M, Hsiun PT, Tsao PS, Cooke JP. Arginine restores nitric oxide activity and inhibits monocyte accumulation after vascular injury in hypercholesterolemic rabbits. *J Am Coll Cardiol* 1996; **28**: 1573–79.
15 Tsao PS, Theilmeier G, Singer AH, Leung LL, Cooke JP. L-arginine attenuates platelet reactivity in hypercholesterolemic rabbits. *Arterioscler Thromb* 1994; **14**: 1529–33.
16 Randall MD, Ujiie H, Griffith TM. L-arginine reverses the impairment of nitric oxide-dependent collateral perfusion in dietary-induced hypercholesterolaemia in the rabbit. *Clin Sci (Colch)* 1994; **87**: 53–59.
17 Girerd XJ, Hirsch AT, Cooke JP, Dzau VJ, Creager MA. L-arginine augments endothelium-dependent vasodilation in cholesterol-fed rabbits. *Circ Res* 1990; **67**: 1301–308.
18 Cooke JP, Dzau J, Creager A. Endothelial dysfunction in hypercholesterolemia is corrected by L-arginine. *Basic Res Cardiol* 1991; **86** (suppl 2): 173–81.
19 Bode-Böger SM, Böger RH, Thiele W, Creutzig A, Frolich JC. L-arginine infusion therapy restores vascular NO production and improves the symptoms of intermittent claudication in patients with peripheral arterial disease. *Circulation* 1997; **96** (8, suppl I): I-419.
20 Bode-Böger SM, Böger RH, Alfke H et al L-arginine induces nitric oxide-dependent vasodilation in patients with critical limb ischemia. A randomized, controlled study. *Circulation* 1996; **93**: 85–90.
21 Lerman A, Burnett JC Jr, Higano ST, McKinley LJ, Holmes DR Jr. Long-term L-arginine supplementation improves small-vessel coronary endothelial function in humans. *Circulation* 1998; **97**: 2123–28.
22 Gardner AW, Skinner JS, Cantwell BW, Smith LK. Progressive vs single-stage treadmill tests for evaluation of claudication. *Med Sci Sports Exerc* 1991; **23**: 402–408.
23 Hiatt WR, Hirsch AT, Regensteiner JG, Brass EP. Clinical trials for claudication. Assessment of exercise performance, functional status, and clinical end points. Vascular Clinical Trialists. *Circulation* 1995; **92**: 614–21.
24 Ware JE Jr, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. *Med Care* 1992; **30**: 473–83.
25 Ware JJ. *SF-36 Health Survey, manual and interpretation guide.* Boston: Nimrod Press, 1993.
26 Zar JH. *Biostatistical analysis*, second edition. Englewood Cliffs, NJ: Prentice-Hall, 1984.
27 Kenney WL ed. *ACSM's guidelines for exercise testing and prescription*, fifth edition. Baltimore: William & Wilkins, 1995.
28 Money SR, Herd JA, Isaacsohn JL et al. Effect of cilostazol on walking distances in patients with intermittent claudication caused by peripheral vascular disease. *J Vasc Surg* 1998; **27**: 267–74.
29 Reich T, Gillings D. Effects of pentoxifylline on severe intermittent claudication. *Angiology* 1987; **38**: 651–56.
30 Bagger JP, Helligsoe P, Randsbaek F, Kimose HH, Jensen BS. Effect of verapamil in intermittent claudication. A randomized, double-blind, placebo-controlled, cross-over study after individual dose-response assessment. *Circulation* 1997; **95**: 411–14.
31 Virgolini I, Fitscha P, O'Grady J, Barth H, Sinzinger H. Effects of taprostene, a chemically stable prostacyclin analogue, in patients with ischaemic peripheral vascular disease: a placebo controlled double-blind trial. *Prostaglandins Leukot Essent Fatty Acids* 1989; **38**: 31–35.
32 Diehm C, Balzer K, Bisler H et al. Efficacy of a new prostaglandin E1 regimen in outpatients with severe intermittent claudication: results of a multicenter placebo-controlled double-blind trial. *J Vasc Surg* 1997; **25**: 537–44.
33 Brevetti G, Perna S, Sabba C, Martone VD, Condorelli M. Propionyl-L-carnitine in intermittent claudication: double-blind, placebo-controlled, dose titration, multicenter study. *J Am Coll Cardiol* 1995; **26**: 1411–16.
34 Gryglewski RJ, Grodzinska L, Kostka-Trabka E et al. Treatment with L-arginine is likely to stimulate generation of nitric oxide in patients with peripheral arterial obstructive disease. *Wien Klin Wochenschr* 1996; **108**: 111–16.
35 Slawinski M, Grodzinska L, Kostka-Trabka E, Bieron K, Goszcz A, Gryglewski RJ. L-arginine – substrate for NO synthesis – its beneficial effects in therapy of patients with peripheral arterial disease: comparison with placebo-preliminary results. *Acta Physiol Hung* 1996; **84**: 457–58.
36 Bode-Böger SM, Böger RH, Kienke S, Junker W, Frolich JC. Elevated L-arginine/dimethylarginine ratio contributes to enhanced systemic NO production by dietary L-arginine in hypercholesterolemic rabbits. *Biochem Biophys Res Commun* 1996; **219**: 598–603.
37 Lentz SR, Sobey CG, Piegors DJ et al. Vascular dysfunction in monkeys with diet-induced hyperhomocyst(e)inemia. *J Clin Invest* 1996; **98**: 24–29.
38 MacAllister RJ, Fickling SA, Whitley GS, Vallance P. Metabolism of methylarginines by human vasculature; implications for the regulation of nitric oxide synthesis. *Br J Pharmacol* 1994; **112**: 43–48.
39 Vallance P, Leone A, Calver A, Collier J, Moncada S. Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. *Lancet* 1992; **339**: 572–75.
40 Faraci FM, Brian JE Jr, Heistad DD. Response of cerebral blood ves-

sels to an endogenous inhibitor of nitric oxide synthase. *Am J Physiol* 1995; **269** (5 Pt 2): H1522–27.
41 Böger RH, Bode-Böger SM, Szuba A, Tsao PS, Chan JR, Cooke JP. Asymmetric dimethylarginine: a new risk factor for endothelial dysfunction. *Circulation* 1997; **96**(8): I-32.
42 Böger RH, Bode-Böger SH, Tsao PS, Lin PS, Chan JR, Cooke JP. The endogenous NO synthase inhibitor asymmetric dimethylarginine (ADMA) exerts pro-atherogenic effects in cultured human endothelial cells. *Circulation* 1997; **96**(8): I-286.
43 Hamasaki H, Sato J, Masuda H et al. Effect of nicotine on the intimal hyperplasia after endothelial removal of the rabbit carotid artery. *Gen Pharmacol* 1997; **28**: 653–59.
44 Goonasekera CD, Rees DD, Woolard P, Frend A, Shah V, Dillon MJ. Nitric oxide synthase inhibitors and hypertension in children and adolescents. *J Hypertens* 1997; **15**: 901–909.
45 Böger RH, Bode-Böger SM. [Endothelial dysfunction in peripheral arterial occlusive disease: from basic research to clinical use]. *Vasa* 1997; **26**: 180–84.
46 Theilmeier G, Chan JR, Zalpour C et al. Adhesiveness of mononuclear cells in hypercholesterolemic humans is normalized by dietary L-arginine. *Arterioscler Thromb Vasc Biol* 1997; **17**: 3557–64.
47 Mosca L, Rubenfire M, Mandel C et al. Antioxidant nutrient supplementation reduces the susceptibility of low density lipoprotein to oxidation in patients with coronary artery disease. *J Am Coll Cardiol* 1997; **30**: 392–99.
48 Erl W, Weber C, Wardemann C, Weber PC. alpha-Tocopheryl succinate inhibits monocytic cell adhesion to endothelial cells by suppressing NF-kappa B mobilization. *Am J Physiol* 1997; **273** (2 Pt 2): H634–40.
49 White CR, Brock TA, Chang LY et al. Superoxide and peroxynitrite in atherosclerosis. *Proc Natl Acad Sci USA* 1994; **91**(3): 1044–48.
50 Wei H, Bowen R, Cai Q, Barnes S, Wang Y. Antioxidant and antipromotional effects of the soybean isoflavone genistein. *Proc Soc Exp Biol Med* 1995; **208**: 124–30.
51 Tikkanen MJ, Wahala K, Ojala S, Vihma V, Adlercreutz H. Effect of soybean phytoestrogen intake on low density lipoprotein oxidation resistance. *Proc Natl Acad Sci USA* 1998; **95**(6): 3106–10.
52 Knight DC, Eden JA. A review of the clinical effects of phytoestrogens. *Obstet Gynecol* 1996; **87** (5 Pt 2): 897–904.
53 Kleinert H, Wallerath T, Euchenhofer C, Ihrig-Biedert I, Li H, Forstermann U. Estrogens increase transcription of the human endothelial NO synthase gene: analysis of the transcription factors involved. *Hypertension* 1998; **31**: 582–88.
54 Anthony MS, Clarkson TB, Hughes CL Jr, Morgan TM, Burke GL. Soybean isoflavones improve cardiovascular risk factors without affecting the reproductive system of peripubertal rhesus monkeys. *J Nutr* 1996; **126**: 43–50.
55 Brattstrom LE, Israelsson B, Jeppsson JO, Hultberg BL. Folic acid – an innocuous means to reduce plasma homocysteine. *Scand J Clin Lab Invest* 1988; **48**: 215–21.
56 Van den Berg M, Boers GH, Franken DG et al. Hyperhomocysteinaemia and endothelial dysfunction in young patients with peripheral arterial occlusive disease. *Eur J Clin Invest* 1995; **25**: 176–81.