

Effect of *Lactobacillus plantarum* 299v on cardiovascular disease risk factors in smokers¹⁻³

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ABSTRACT

Background: The short-chain fatty acids formed in the human colon by the bacterial fermentation of fiber may have an antiinflammatory effect, may reduce insulin production, and may improve lipid metabolism. We previously showed in hypercholesterolemic patients that supplementation with the probiotic bacteria *Lactobacillus plantarum* 299v significantly lowers concentrations of LDL cholesterol and fibrinogen.

Objective: We determined the influence of a functional food product containing *L. plantarum* 299v on lipid profiles, inflammatory markers, and monocyte function in heavy smokers.

Design: Thirty-six healthy volunteers (18 women and 18 men) aged 35–45 y participated in a controlled, randomized, double-blind trial. The experimental group drank 400 mL/d of a rose-hip drink containing *L. plantarum* 299v (5×10^7 colony-forming units/mL); the control group consumed the same volume of product without bacteria. The experiment lasted 6 wk and entailed no changes in lifestyle.

Results: Significant decreases in systolic blood pressure ($P < 0.000$), leptin ($P < 0.000$), and fibrinogen ($P < 0.001$) were recorded in the experimental group. No such changes were observed in the control group. Decreases in F₂-isoprostanes (37%) and interleukin 6 (42%) were also noted in the experimental group in comparison with baseline. Monocytes isolated from subjects treated with *L. plantarum* showed significantly reduced adhesion ($P < 0.001$) to native and stimulated human umbilical vein endothelial cells.

Conclusion: *L. plantarum* administration leads to a reduction in cardiovascular disease risk factors and could be useful as a protective agent in the primary prevention of atherosclerosis in smokers. *Am J Clin Nutr* 2002;76:1249–55.

KEY WORDS Probiotic bacteria, *Lactobacillus plantarum*, systolic blood pressure, leptin, fibrinogen, oxidative stress, cardiovascular disease, smokers

INTRODUCTION

Cigarette smoking, because it creates a predisposition to premature atherosclerosis, occupies a prominent position among the risk factors for ischemic heart disease (1, 2). Tobacco smoke contains substances that trigger the generation of free radicals, fueling chronic oxidative stress and inflammation (3, 4). This process stimulates the production of acute phase proteins and cytokines, exemplified by elevated concentrations of fibrinogen, C-reactive protein, tumor necrosis factor, and interleukin 6 (IL-6) in chronic smokers (5). Further along this chain of events is the direct activation of

monocytes-macrophages and T cells (6, 7). The increased adhesiveness of these cells to vascular walls is a key event in the early stage of atherosclerosis (8). Additionally, destabilization of the atheromatous plaque, which may lead to myocardial infarction, has been attributed to the action of activated macrophages (9). Smokers have lower concentrations of HDL cholesterol, and their LDL is more susceptible to oxidative modification, producing particles with atherogenic properties (10).

The multifarious influence of tobacco smoke on atherogenesis explains the difficulties encountered in developing fully satisfactory therapies to reduce oxidative stress and chronic inflammation in current smokers. Functional foods containing acidophilic bacteria have emerged as a useful modality for the prevention and treatment of some infectious diseases (11, 12). It seems worthwhile, then, to investigate the potential effect of functional foods on some of the risk factors of atherosclerosis, especially because the role of acidophilic bacteria in reducing cholesterol concentrations has already been suggested (13).

We showed previously in patients with moderately elevated cholesterol concentrations that supplementation of the diet with ProViva (Probi AB, Lund, Sweden), a functional food product containing fruit juice, fermented oat, and *Lactobacillus plantarum* 299 v, significantly lowers concentrations of LDL cholesterol and fibrinogen (14). These bacteria settle in the large intestine, where they are responsible for the fermentation of dietary fiber. The end products of this process are short-chain fatty acids, chiefly acetic, propionic, and butyric acids. Only acetic and propionic acids are absorbed into the blood, pass into the liver, and enter the metabolic pathways (15). It has been postulated that short-chain fatty acids, mainly propionic acid, improve glucose tolerance and inhibit cholesterol synthesis in the liver, presumably by inhibiting the rise in the serum concentration of free fatty acids and by improving insulin sensitivity (16–18). We also recently showed that ibuprofen, a nonsteroidal antiinflammatory drug derived from

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TABLE 1
Ingredients of the test and placebo products¹

Ingredient	Test product	Placebo product
<i>Lactobacillus plantarum</i> 299v (CFU/mL)	5 × 10 ⁷	—
Rosehip powder (g/100 mL)	2.7	2.8
Sucrose (g/100 mL)	9.5	10
Stabilizers: modified starch and carrageenan (g/100 mL)	0.56	0.59
Citric acid (g/100 mL)	0.14	0.15
Ascorbic acid (g/100 mL)	0.033	0.035
Oat flour (g/100 mL)	0.75	—
Malted barley flour (g/100 mL)	0.04	—

¹CFU, colony-forming units.

propionic acid, significantly reduces IL-6 and fibrinogen and increases HDL-cholesterol concentrations in smokers (19).

The aim of the present study was to document the influence of *L. plantarum* 299v on the concentrations of lipids and inflammatory markers in smokers. We additionally measured plasma concentrations of F₂-isoprostanes and the generation of oxygen free radicals by mononuclear cells.

SUBJECTS AND METHODS

Subjects

We examined 36 healthy subjects (18 women, 18 men) aged 42.3 ± 3.9 y who were current smokers (lifetime dose > 5 pack-years) with moderately elevated fibrinogen concentrations (> 3.0 g/L). Persons who had taken antibiotics, fiber, or vitamin supplements during the 6-wk period before the experiment were excluded. A controlled double-blind study with placebo was designed, and the participants were randomly divided into 2 groups. Group A (*n* = 18) received the test product and group B (*n* = 18) received a control product (placebo). Each participant consumed 400 mL/d of the test product containing *L. plantarum* 299v or the control product without bacteria for 6 wk. The participants agreed to take the product as a dietary supplement and to not change their regular diets or physical activity in any respect. The present study was approved by the Human Medical Ethics Committee at the Pomeranian Academy of Medicine, Szczecin, Poland.

The test product containing *L. plantarum* 299v is marketed in Sweden and Finland under the brand name ProViva and was developed by Probi AB (20). The ingredients of the test and control products are shown in **Table 1**. Both products were manufactured by the dairy company Skånemejerier (Lunnarp, Sweden) and were packed in identical 1-L packages (Tetra Rex; EloPak, Stabekk, Norway). The products were stored in a refrigerator before use. The level of lactobacilli was constant throughout the shelf-life of the product.

Identification of *L. plantarum* 299v in feces

L. plantarum 299v (DSM 9843) bacteria were isolated and identified from fecal samples at the end of the experiment. Colonies of *L. plantarum* 299v were picked and identified by the randomly amplified polymorphic DNA method (21).

Peripheral blood mononuclear cells

Twenty-five microliters heparin-containing blood, diluted 1:1 with phosphate-buffered saline (PBS), was layered over 15 mL

Ficol-Paque (Pharmacia, Uppsala, Sweden) and centrifuged (400 × *g* for 40 min at 22 °C). The mixed mononuclear cell band was removed by aspiration, washed twice with PBS, divided into 3 aliquots, and centrifuged (3000 × *g*, 10 min, 20 °C). One aliquot was resuspended in RPMI-1640 media containing 20% autologous serum, 100 U penicillin/mL, 100 μg streptomycin/mL, and 20 mmol HEPES/L. The cell suspension was plated at a concentration of 0.5 × 10⁶ monocytes per 16-mm multiwell dish and incubated for 2 h at 37 °C under humidified 5% CO₂ in room air to allow the monocytes to adhere to the dish. The nonadherent cells were removed by washing twice with medium. The adherent cells were used to determine the production of intracellular reactive oxygen species. The second aliquot was suspended in medium 199 with 20 mmol HEPES/L, counted, and used in the adhesion assay. The mononuclear cell preparation consisted of ≈30% monocytes and 70% lymphocytes.

Endothelial cell isolation and culture

Human umbilical vein endothelial cells (HUVECs) were obtained from umbilical cords by collagenase digestion as described by Jaffe (22). In brief, veins from umbilical cords were perfused with PBS to remove blood cells, filled with 0.1% collagenase (type Ia), and left for 10 min at 37 °C. The suspended HUVECs were supplemented with fetal bovine serum (FBS), centrifuged (3000 × *g*, 10 min, 20 °C), and cultured at 37 °C under humidified 5% CO₂ in room air in gelatin-coated 25-cm² flasks and 6-well or 24-well tissue culture plates filled with medium 199. The medium also contained 100 U penicillin/mL, 100 μg streptomycin/mL, 2.5 μg fungizone/mL, 2 mmol glutamine/L, 20 mmol HEPES/L, 20% FBS, and 50 μg endothelial cell growth supplement/mL. The medium was replaced every 2 d until the cells reached confluence (3–5 d). HUVEC purity was confirmed by a “cobblestone” morphology typical of quiescent endothelial cells and by factor VIII staining.

Adhesion assay

Second-pass HUVECs were cultured in gelatin-coated 24-well plates. When confluent monolayers were formed, the medium was changed to medium 199 containing 10% FBS, 2 mmol glutamine/L, and 20 mmol HEPES/L without endothelial cell growth supplement; tumor necrosis factor (100 U/mL) was added to some of the wells 12 h before the experiment. The HUVECs were then washed with PBS and cocultured for 30 min with peripheral blood mononuclear cells (PBMCs) suspended in medium 199 with 20 mmol HEPES/L to a density of 1.5–2.0 × 10⁶/mL (0.5 mL per well). The PBMC suspension was withdrawn and the wells were washed twice with PBS to remove nonadherent cells. The endothelial cells with adherent mononuclear cells were detached by mild trypsinization. The initial suspensions and the suspensions from each well were counted 3 times. The cells detached from each well, consisting of endothelial cells, monocytes, and lymphocytes, were treated for 30 min at 4 °C with saturating amounts of fluorescein-conjugated anti-CD45 and phycoerythrin-conjugated anti-CD14 monoclonal antibodies. The cells were then washed with fluorescence-activated cell sorting (FACS) buffer, fixed in 1% paraformaldehyde, and analyzed (10 000 cells per sample) by FACS (Becton Dickinson, San Jose, CA). The proportion of monocytes in the suspension was established by measuring fluorescence I (FL I expression of CD45) and fluorescence II (FL II expression of CD14). The absolute number of monocytes adhering to endothelial cells was calculated in relation to the total number of cells obtained after trypsinization. The



TABLE 2

Clinical characteristics of smokers before and after treatment with *Lactobacillus plantarum* 299v or placebo¹

Variable	Pretreatment	Posttreatment	Two-factor ANOVA		
			Treatment	Time	Interaction
Age (y)					
<i>L. plantarum</i>	42.3 ± 2.8	—	—	—	—
Placebo	41.8 ± 2.8	—			
BMI (kg/m ²)					
<i>L. plantarum</i>	24.8 ± 4.8	25.2 ± 4.8	0.360	0.005	0.135
Placebo	25.8 ± 3.7	25.9 ± 3.8			
SBP (mm Hg)					
<i>L. plantarum</i>	134 ± 20	121 ± 16 ²	0.402	0.001	0.001
Placebo	128 ± 18	126 ± 16			
DBP (mm Hg)					
<i>L. plantarum</i>	89 ± 13	84 ± 16	0.754	0.025	0.732
Placebo	89 ± 17	85 ± 15			

¹ $\bar{x} \pm SD$; $n = 7$ F, 11 M (*L. plantarum*) and 11 F, 7 M (placebo). SBP, systolic blood pressure; DBP, diastolic blood pressure. *P* values were calculated by using a two-factor repeated-measures ANOVA followed by Tukey's test.

²Significantly different from pretreatment, $P < 0.001$.

results are expressed as the percentage of monocytes added. The intraassay error with this technique was low; the CV was <5 %.

Reactive oxygen species production

Cellular oxidation was measured on the basis of the reactive oxygen species-mediated (hydrogen peroxide) conversion of non-fluorescent 2',7'-dichlorofluorescein (DCFH), loaded into cells as 2',7'-dichlorofluorescein diacetate into fluorescent DCF, reflecting enhanced oxidative stress (23). Briefly, freshly isolated PBMCs were resuspended in PBS and incubated with 20 μ M DCFH/L, with or without phorbol myristate acetate (100 ng/mL), for 30 min in the dark. The fluorescence intensity of the DCF fluorophore formed by the peroxide oxidation of its nonfluorescent precursor was detected with a cytofluorimetric assay (FACScan; Becton Dickinson). Monocytes were gated on the basis of forward scatter and side scatter, and the results are expressed as mean fluorescence intensity.

F₂-isoprostane

Plasma F₂-isoprostane was measured as described by Morrow and Roberts (24). F₂-isoprostane was extracted with C₁₈ and isolated by filtration with silica minicolumns. The compounds were then converted to pentafluorobenzyl ester trimethylsilyl ether derivatives and analyzed by a gas chromatography-mass spectrometry assay.

Biochemical assays

After the subjects had fasted overnight, blood samples were taken by venipuncture into tubes containing disodium EDTA (1.5 mg/mL), and the plasma was separated in a cold centrifuge (500 \times g for 15 min at 4 °C). Homocysteine was measured in frozen samples (-70 °C) by HPLC with a fluorescence detector, according to the method of Araki and Sako (25), with test kits from Bio-Rad (Munich, Germany). Fibrinogen was measured according to Clauss with test kits from bioMerieux (Lyon, France). Lipoprotein(a) was measured by electroimmunodiffusion with test kits from ImmunoAg (Wien, Austria). The concentration of triacylglycerols and total cholesterol was determined by using enzymatic tests (CHOD-PAP and GPO-PAP; Boehringer Mannheim, Mannheim, Germany). HDL cholesterol was measured after precipitation of lipoproteins containing apolipoprotein B with

phosphotungstic acid in the presence of Mg²⁺, and LDL cholesterol after precipitation with polyvinyl sulfate (kits from Boehringer Mannheim).

IL-6 was measured in serum with an enzyme-linked immunosorbent assay kit (Boehringer Mannheim). The assay is based on the quantitative sandwich enzyme-immunoassay principle, with the use of 2 monoclonal antibodies from mouse directed against 2 different epitopes of IL-6. Insulin concentrations were measured by using a Microparticle enzyme immunoassay system from Abbott Laboratories (Tokyo), and leptin by using a radioimmunoassay kit from Linco Research (St Charles, MO).

Statistical analysis

The results are presented as means \pm SDs. Differences between groups were assessed by using a two-factor repeated-measures analysis of variance followed by Tukey's test. All calculations were made with the STATSOFT statistical package (version 5.1 PC; StatSoft Polska, Warsaw)

The results obtained for lipoprotein(a) were compared by using the nonparametric Wilcoxon's test for paired values. Two-sided *P* values < 0.05 were considered to be significant.

RESULTS

Study population

No significant differences in body mass index, blood pressure, and the serum lipid profile were present at baseline between the experimental and control groups.

L. plantarum and placebo tolerance

Both the test and the control products were well accepted by the smokers, and no adverse events were reported. After daily supplementation for 6 wk, significant amounts of *L. plantarum* 299v were found in fecal samples from 70% of the subjects in the experimental group.

Clinical effects

Daily supplementation of the diet with *L. plantarum* for 6 wk resulted in a significant reduction in systolic blood pressure ($P < 0.001$), averaging 13 mm Hg (Table 2). The decrease was

TABLE 3
Effects of *Lactobacillus plantarum* 299v or placebo on biochemical variables in smokers¹

Variable	Pretreatment	Posttreatment	Two-factor ANOVA		
			Treatment	Time	Interaction
Triacylglycerols (mmol/L)					
<i>L. plantarum</i>	1.37 ± 0.43	1.46 ± 0.54	0.797	0.759	0.268
Placebo	1.40 ± 0.61	1.33 ± 0.65			
Cholesterol (mmol/L)					
<i>L. plantarum</i>	5.59 ± 0.88	5.51 ± 0.85	0.883	0.538	0.745
Placebo	5.51 ± 0.75	5.65 ± 1.04			
LDL cholesterol (mmol/L)					
<i>L. plantarum</i>	3.57 ± 0.96	3.15 ± 0.93	0.906	0.001	0.849
Placebo	3.52 ± 0.80	3.42 ± 0.88			
HDL cholesterol (mmol/L)					
<i>L. plantarum</i>	1.16 ± 0.21	1.29 ± 0.23	0.943	0.007	0.577
Placebo	1.19 ± 0.39	1.22 ± 0.34			
Fibrinogen (g/L)					
<i>L. plantarum</i>	3.80 ± 0.37	3.04 ± 0.33 ²	0.008	0.013	0.001
Placebo	3.68 ± 0.40	3.89 ± 0.48			
Lipoprotein(a) (g/L)					
<i>L. plantarum</i>	0.31 ± 0.28	0.30 ± 0.28	0.258	0.887	0.311
Placebo	0.20 ± 0.24	0.18 ± 0.21			
Homocysteine (μmol/L)					
<i>L. plantarum</i>	7.4 ± 2.7	7.5 ± 3.1	0.504	0.893	0.927
Placebo	8.1 ± 4.8	8.2 ± 7.5			
Glucose (mmol/L)					
<i>L. plantarum</i>	6.11 ± 1.22	6.11 ± 0.78	0.368	0.267	0.221
Placebo	5.66 ± 0.72	5.99 ± 0.83			
Insulin (μU/mL)					
<i>L. plantarum</i>	11.1 ± 5.7	7.9 ± 4.3	0.382	0.380	0.309
Placebo	8.3 ± 4.6	8.9 ± 5.4			
Leptin (μg/L)					
<i>L. plantarum</i>	13.0 ± 7.7	8.2 ± 4.6 ²	0.021	0.001	0.006
Placebo	16.8 ± 8.7	17.5 ± 7.8			

¹ $\bar{x} \pm SD$; $n = 18$. P values were calculated by using a two-factor repeated-measures ANOVA followed by Tukey's test.

²Significantly different from pretreatment, $P < 0.001$.

more evident in subjects with higher systolic blood pressure at baseline. This effect was not detected in the control group.

BMI increased slightly over time but did not differ significantly between the groups during the experiment. The final 24-h food intake record showed no significant differences in diet during the study.

Changes in lipids and other biochemical variables

We observed no significant changes in plasma concentrations of total cholesterol, triacylglycerol, and lipoprotein(a) after treatment in either group. However, LDL cholesterol tended to decrease (by 12%) and HDL cholesterol tended to increase (by 10%) in the experimental group (Table 3). We also found a significant reduction in leptin concentration (by 37%) and a nonsignificant decrease in insulin concentration (by 28%).

Effects on fibrinogen and interleukin 6

As shown in Table 3, fibrinogen concentrations decreased by 21% ($P < 0.001$) in the experimental group. By contrast, fibrinogen tended to increase in the control group. Because fibrinogen in hepatocytes is regulated by IL-6, we also examined plasma concentrations of this cytokine. A highly significant 41% decrease in plasma IL-6 concentrations was observed in the experimental group, whereas concentrations did not change significantly in the control group (Figure 1).

Effects on plasma F₂-isoprostanes

F₂-isoprostanes are sensitive biochemical markers of lipid peroxidation and oxidant stress. In chronic cigarette smokers, plasma concentrations of isoprostanes are higher than those in sex- and age-matched healthy nonsmokers (26). As shown in Figure 2, plasma F₂-isoprostane concentrations decreased significantly (by 31%) in the experimental group. No such effect was observed in the control group.

Reactive oxygen species production and monocyte adherence to resting and stimulated endothelial cells

To assess the metabolic activation of freshly isolated blood monocytes, we measured the production of reactive oxygen species by resting cells and by cells stimulated with phorbol myristate acetate in vitro. *L. plantarum* intake for 6 wk tended to slightly reduce reactive oxygen species production in both resting and stimulated monocytes (Figure 3; NS). No significant effect was seen in the cells isolated from subjects in the control group.

We previously showed that monocytes in smokers exist in an activated state, and the interactions between monocytes and endothelial cells do not depend solely on the level of expression of endothelial adhesion molecules, but also on the avidity of their ligand on monocytes, paralleling cell activation. Therefore, we developed a flow cytometry method to count the number of monocytes adhering to HUVECs (19). *L. plantarum*

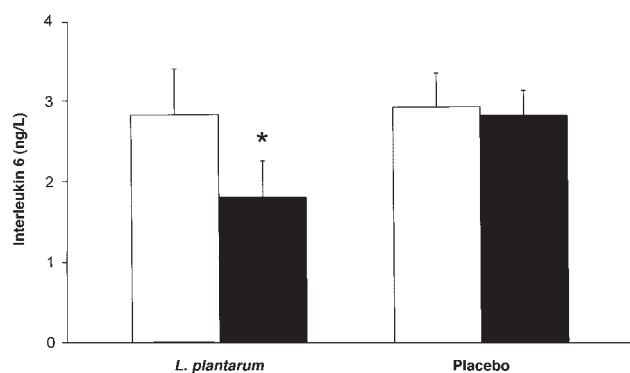


FIGURE 1. Mean (\pm SD) plasma concentrations of interleukin 6 in 18 smokers before (\square) and after (\blacksquare) supplementation with *Lactobacillus plantarum* or placebo. *P* values were calculated by using a two-factor repeated-measures ANOVA followed by Tukey's test. *P* for treatment = 0.005, *P* for time = 0.001, and *P* for the interaction = 0.001. *Significantly different from before treatment, *P* < 0.001.

administration in smokers markedly decreased the adherence of monocytes to resting (40%) and tumor necrosis factor-activated (36%) HUVECs (**Figure 4**). No such effect was seen in the control group.

DISCUSSION

Our results indicate that supplementation of the diet with active cultures of *L. plantarum* 299v leads to significant reductions in fibrinogen and LDL-cholesterol concentrations in smokers, which corroborates our previous findings in patients with mild hypercholesterolemia (14). This time, however, presumably because of the administration of a greater quantity of bacteria, the reduction was more pronounced, ie, fibrinogen concentrations fell by 21% compared with 13% in the earlier study. The marked decrease in concentrations of this acute phase protein with procoagulant activity appears to be the result of the antiinflammatory properties of *L. plantarum*, as evidenced by the lack of reduction in plasma IL-6 concentrations in the control group.

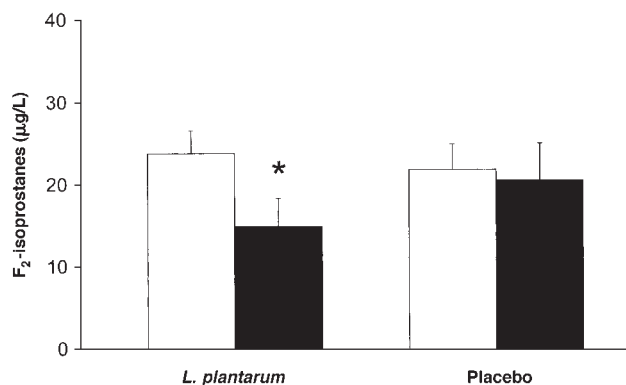


FIGURE 2. Mean (\pm SD) plasma concentrations of F₂-isoprostanes in 18 smokers before (\square) and after (\blacksquare) supplementation with *Lactobacillus plantarum* or placebo. *P* values were calculated by using a two-factor repeated-measures ANOVA followed by Tukey's test. *P* for treatment = 0.003, *P* for time = 0.001, and *P* for the interaction = 0.001. *Significantly different from before treatment, *P* < 0.001.

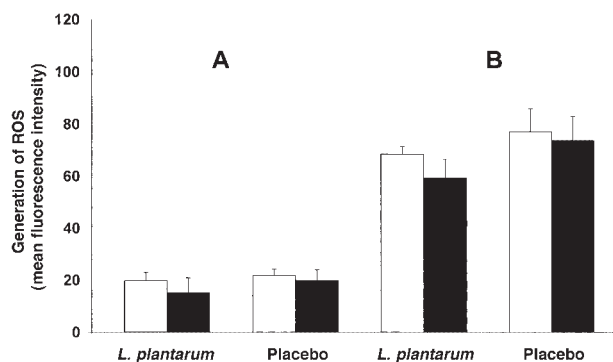


FIGURE 3. Mean (\pm SD) generation of reactive oxygen species (ROS) in resting (A) and phorbol myristate acetate-activated (B) monocytes before (\square) and after (\blacksquare) supplementation with *Lactobacillus plantarum* or placebo in 18 smokers. Freshly isolated peripheral blood mononuclear cells were incubated with 2',7'-dichlorofluorescein with or without phorbol myristate acetate (100 ng/mL) for 30 min in the dark. The fluorescence intensity of fluorophore dichlorofluorescein was detected with a cytofluorimetric assay. During cytometric analysis, the monocytes were gated on the basis of forward and side scatter.

The symptoms of chronic inflammation observed in smokers may be attributed to the toxic compounds inhaled in tobacco smoke and to the oxidative stress associated with a deficit of vitamin C (27). As a result, lipid peroxidation is intensified in tissues and lipoproteins, particularly in the LDL fraction. The presence of oxidized LDL stimulates endothelial cells to release adhesion molecules and monocyte-activating factors. The activation of monocytes leads to the generation of reactive oxygen species and in turn to the activation of kinase C of NADPH oxidase (EC 1.9.3.1) in the cellular membranes of these cells (28).

We recently showed in smokers that ibuprofen, a nonsteroidal antiinflammatory drug, inhibits reactive oxygen species production

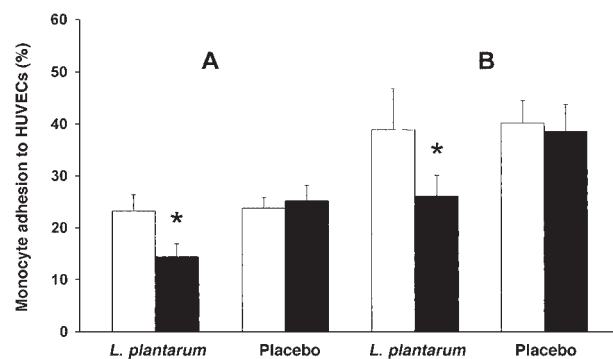


FIGURE 4. Adhesion of resting monocytes to unstimulated (A) and tumor necrosis factor α -stimulated (B) human umbilical vein endothelial cells (HUVECs) before (\square) and after (\blacksquare) supplementation with *Lactobacillus plantarum* or placebo in 18 smokers. Freshly isolated peripheral blood mononuclear cells (PBMCs) were coincubated with HUVECs for 30 min. Nonadherent PBMCs were withdrawn and the cells were stained with monoclonal antibody against CD45 and CD11 and then analyzed by fluorescence-activated cell sorting. The results are expressed as a percentage of monocyte added. *P* values were calculated by using a two-factor repeated-measures ANOVA followed by Tukey's test. For both A and B: *P* for treatment = 0.001, *P* for time = 0.001, and *P* for the interaction = 0.001. *Significantly different from before treatment, *P* < 0.001.


by monocytes, reduces the concentration of fibrinogen, and suppresses the adhesion of monocytes to endothelial cells (19). This property has been traced *inter alia* to the inhibition of cyclooxygenase-2 (EC 1.6.99.6) activity. The results of our *in vitro* study confirmed that ibuprofen inhibits the oxidative modification of LDL by endothelial cells and macrophages (29).

It is surprising that supplementation of the diet with *L. plantarum* is associated with similar effects. This could be due indirectly to the inhibition of nonenzymatic processes of lipid peroxidation, as reflected by the decreased concentrations of F₂-isoprostanes shown in the present study. Because ibuprofen is a derivative of propionic acid and *L. plantarum* can generate large quantities of this acid from dietary fiber, we are inclined to suggest that propionic acid exerts a specific antiinflammatory action through a hitherto unknown mechanism, perhaps related to the activation by ibuprofen of peroxisome proliferator-activated receptor α , which modulates the nuclear transcription factor κ B and reduces the production of inflammatory cytokines by monocytes-macrophages (30). It is also possible that the 10% higher HDL-cholesterol concentrations we observed in smokers taking either 600 mg ibuprofen/d for 2 wk or *L. plantarum* for 6 wk represents the effect of propionic acid on peroxisome proliferator-activated receptor α , a factor stimulating the expression of genes responsible for the synthesis of apolipoproteins A-I and A-II, which form the structure of the HDL particle (31).

The reduced systolic blood pressure we observed in the *L. plantarum* group is of potential clinical significance. Nakajima et al (32) noted that supplementation of the diet with an extract of *Lactobacillus casei* reduces blood pressure in hypertensive patients. As shown in our study, this effect may be related to decreased tissue resistance to insulin associated with lower blood concentrations of leptin. The significance of leptin in the modulation of neuropeptide Y and angiotensinogen or its effects on the pituitary-adrenal axis were recently discussed (33). Kazumi et al (34) showed a close relation between fasting insulin concentrations, leptin, and systolic blood pressure in young males, irrespective of body mass index and body fat percentage.

The sequence of metabolic events we observed during dietary supplementation with *L. plantarum* 299v has hitherto not been described and deserves further clinical investigation. Kawase et al (35) recently showed in healthy volunteers that supplementation of the diet twice daily for 8 wk with 200 mL fermented milk containing *Lactobacillus casei* and *Streptococcus thermophilus* TMC 1543 results in a significant increase in HDL-cholesterol and a decrease in triacylglycerol concentrations and systolic blood pressure. It would be safe to conclude that both studies showed significant improvements in the risk factors for ischemic heart disease, a condition attributed to the metabolic syndrome resulting from tissue resistance to insulin, obesity, low HDL-cholesterol concentrations, increased concentrations of triacylglycerol, and arterial hypertension. Therefore, supplementation of the diet with *L. plantarum* and other acidophilic bacteria may constitute a prospective nonpharmacologic alternative for the management of risk factors and the primary prevention of atherosclerosis. The Dietary Approaches to Stop Hypertension (DASH) study confirmed this line of reasoning by showing reduced blood pressure in subjects consuming more vegetables and fruit, which are a rich source of fiber (36). One could expect the results of the DASH study to have been improved by increasing the quantity of *L. plantarum* in the gastrointestinal tract. However, it is well known that most populations in Western countries suffer from a deficit of acidophilic bacteria, brought about by antibiotics, stress,

and changes in food technologies involving bacteria-free fermentation processes (37).

In summary, the results of our study show that supplementation of the diet with *L. plantarum* may contribute significantly to the prevention and treatment of metabolic disorders in smokers. This positive effect may be directly associated with the production of propionic acid by the bacterial fermentation of fiber. In fact, recent studies in rats by Cheng and Lai (38) showed for the first time that resistant rice starch in the diet increases serum concentrations of propionate, with a concomitant reduction in the concentrations of total and LDL cholesterol. 

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