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Metabolism Clinical and Experimental 55 (2006) 946–952

Metabolism
Clinical and Experimental

www.elsevier.com/locate/metabol

Acarbose ameliorates atherogenecity of low-density lipoprotein in patients with impaired glucose tolerance

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Received 7 November 2003; accepted 22 March 2006

Abstract

Acarbose, an α-glucosidase inhibitor, is administered to control blood glucose levels. The drug also reduces the risk of cardiovascular disease, but the underlying mechanism is still to be elucidated. We therefore hypothesized that treatment with acarbose ameliorates the atherogenecity of low-density lipoprotein (LDL), a key molecule in atherogenesis. Patients with impaired glucose tolerance were or were not treated with acarbose (acarbose-treated group [n = 20] and control group [n = 20], respectively) for 3 months under dietary therapy. The oxidative susceptibility of LDL was determined by measuring lag time for the formation of dienes in the presence of $CuSO_4$. The lag time was significantly longer in the acarbose-treated group than in the control group before treatment. Moreover, the density gradient lipoprotein separation and disk polyacrylamide gel electrophoresis analyses showed that acarbose reduced the amount of small dense LDL, a more atherogenic and oxidatively susceptible form of LDL. We also found that the fatty acid composition of LDL changed after the treatment: polyunsaturated (ω-3) fatty acid, a beneficial substance for preventing cardiovascular disease, was significantly increased, whereas saturated fatty acids and triglyceride were decreased in the LDL of the acarbose-treated group. The present findings suggest that acarbose treatment reduces the risk of cardiovascular diseases by ameliorating the atherogenecity of LDL.
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1. Introduction

Cardiovascular disease (CVD) is the leading cause of death in patients with type 2 diabetes mellitus [1]. It is now considered that the macrovascular disease starts before the development of diabetes [2], especially in impaired glucose tolerance (IGT), a prediabetic state characterized by moderate postprandial hyperglycemia [3]. Indeed, recent studies have shown that postprandial hyperglycemia is an independent risk factor for CVD in patients with diabetes and IGT [4,5].

Acarbose, a potent α-glucosidase inhibitor, controls blood glucose levels at postprandial by inhibiting intestinal glucose absorption [6]. The Study To Prevent Non-Insulin Dependent Diabetes Mellitus (STOP-NIDDM) has shown that acarbose treatment reduced the risk of both diabetes and CVD [7,8]. In the multicenter randomized study, the risk factors for CVD, such as body weight, blood pressure, and triglyceride (TG), were reduced by acarbose treatment, which explained in part the beneficial effect of acarbose on the reduced risk for CVD. However, the treatment itself on the outcomes remained statistically significant and independent after adjusting for those variables [7], suggesting that other unknown mechanisms are involved.

Acarbose treatment improves hyperglycemia and dyslipidemia [9], which are associated with the risk of CVD [10,11]. The 2 metabolic abnormalities also change the amount, density, and size of low-density lipoprotein (LDL), and structurally and oxidatively modified LDL particles appear in those conditions. Those changes are the risk of CVD, and, thus, understanding the effect of acarbose treatment on LDL particles is crucial and may explain the beneficial effect of acarbose in macrovascular diseases.

We therefore tried to determine whether acarbose treatment affects the atherogenic properties of LDL by evaluating the oxidative susceptibility, the distribution of size including small dense LDL, and the fatty acid

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doi:10.1016/j.metabol.2006.03.002

Table 1
Clinical characteristics of the control group and the acarbose-treated group before and after the trial

Variables	Control group (n = 20)		Acarbose group (n = 20)	
	Before	After	Before	After
Body height (cm)	161.4 ± 6.6	161.4 ± 6.6	161.6 ± 7.1	161.6 ± 7.1
Body weight (kg)	71.8 ± 11.6	72.1 ± 11.4	71.5 ± 9.0	69.3 ± 8.7*
Body mass index	27.5 ± 4.0	27.7 ± 3.9	27.5 ± 3.8	26.6 ± 3.7*
Systolic blood pressure (mm Hg)	157.3 ± 9.0	167.2 ± 10.1	158.5 ± 8.0	148.4 ± 9.3*
Diastolic blood pressure (mm Hg)	70.5 ± 7.0	70.6 ± 8.2	71.5 ± 7.2	70.5 ± 7.0
Total cholesterol (mg/dL)	196.0 ± 16.5	206.0 ± 18.7	196.0 ± 15.4	184.0 ± 8.9*
TG (mg/dL)	151.0 ± 28.5	192.0 ± 30.5	151.0 ± 30.5	108.0 ± 10.6*
HDL cholesterol (mg/dL)	42.0 ± 3.9	40.0 ± 3.2	41.0 ± 4.0	45.0 ± 2.3*

Data are shown as the mean ± SD.
* $P < .05$ vs control group.

composition of LDL to speculate a possible mechanism for the reduced risk of CVD.

2. Research design and methods

2.1. Subjects and dietary control

We recruited 40 outpatients with IGT but who do not have clinically overt diabetes or are not taking any medications. Impaired glucose tolerance was diagnosed by measuring plasma glucose level 2 hours after a 75-g oral glucose challenge according to the World Health Organization criteria [3]. All the patients were urged to maintain usual physical activity and were enrolled in an intensive dietary education program. The program included diet history, the formulation of a personalized diet, and a 2-hour session with a dietitian who provided recipes and written suggestions for eating out and instructions on food choices. The dietitian met each patient once a month during the study and inspected the dietary record. We prescribed a standardized controlled energy diet to the patients whose dietary record indicated low compliance to our instruction. All subjects gave written informed consent, and the study was approved by the Ethics Committee of Saitama Medical School.

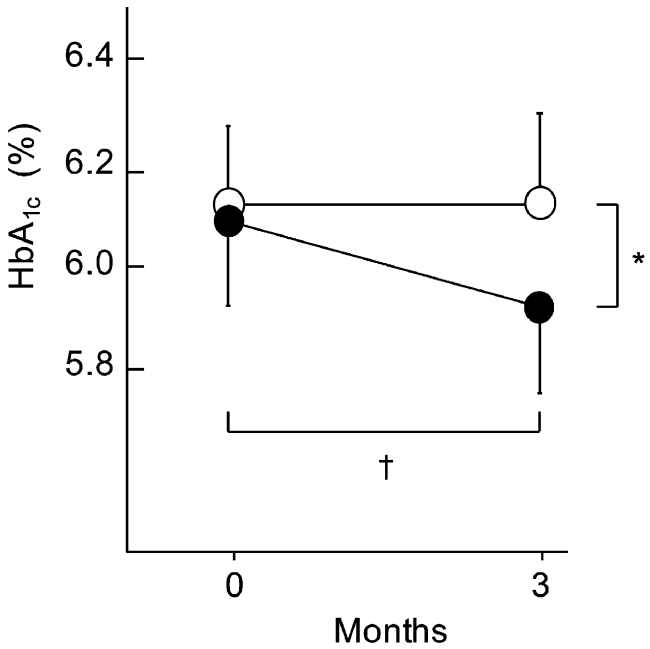

Fig. 1. Change in HbA_{1c} values after acarbose treatment. Plasma HbA_{1c} levels of the acarbose-treated group (closed circles) and the control group (open circles) were determined before and after the 3-month period. Data are shown as the mean ± SD. *$P < .05$ vs control group; †$P < .05$ vs before acarbose treatment.

2.2. Acarbose treatment

To examine the effect of acarbose treatment, we randomly divided the participants into 2 groups: one group received treatment with acarbose for 3 months (acarbose-treated group, n = 20) and the other did not (the control group, n = 20). The acarbose-treated group was administered 100 mg of acarbose once a day during the first week of the trial. The intake was increased 3 times a day (300 mg/d) if no side effects were observed. The patients were instructed to take acarbose 30 minutes before each meal. Some of the patients showed intestinal symptoms, such as flatulence, during the first few weeks, but those were tolerable in all cases. No patients had hypoglycemia or liver dysfunction during the study.

2.3. Blood collection and lipoprotein preparation

At the beginning and end of the trial, blood samples were obtained under fasting conditions from the median cubital vein, placed in EDTA (0.1%)-containing vials, and centrifuged at 4°C within 20 minutes after the collection. To prevent the oxidation of LDL after the collection, we

Table 2
Lipid profiles in plasma and lipoproteins of the control group

Variables	Total cholesterol (mg/dL)		TG (mg/dL)	
	Before	After	Before	After
Plasma	196.0 ± 16.1	206.8 ± 18.7	151.0 ± 28.5	192.0 ± 30.5*
VLDL	24.9 ± 3.5	29.1 ± 2.2	63.1 ± 10.1	86.9 ± 10.3*
IDL	19.1 ± 1.9	22.6 ± 3.0	25.1 ± 6.5	28.3 ± 1.9
LDL	105.1 ± 6.5	112.1 ± 3.6	50.2 ± 8.9	62.9 ± 8.4*
HDL	42.3 ± 4.0	40.4 ± 3.1	8.2 ± 5.6	8.6 ± 2.9

Data are shown as the mean ± SD.
* $P < .05$ vs before the trial.

Table 3
Lipid profiles in plasma and lipoproteins of the acarbose-treated group

Variables	Total cholesterol (mg/dL)		TG (mg/dL)	
	Before	After	Before	After
Plasma	196.0 ± 15.4	184.8 ± 8.9	151.0 ± 30.5	108.4 ± 10.6*
VLDL	25.0 ± 3.9	21.4 ± 2.9*	50.1 ± 9.8	40.9 ± 1.9*
IDL	20.1 ± 2.1	10.6 ± 1.0*	19.9 ± 6.1	9.5 ± 1.0*
LDL	110.1 ± 5.8	108.1 ± 3.1	39.8 ± 8.8	22.8 ± 5.9*
HDL	40.0 ± 1.9	45.1 ± 1.9*	8.9 ± 5.9	10.0 ± 2.8

Data are shown as the mean ± SD.
* $P < .05$ vs before the trial.

separated the lipoproteins immediately from the EDTA plasma by means of stepwise density sequential ultracentrifugation [12,13]. Briefly, lipoproteins were divided into 12 fractions according to density, dialyzed against phosphate-buffered saline containing 0.02% sodium azide in the dark at 4°C, and used for oxidative susceptibility test and lipid and fatty acid analyses within 48 hours. We also obtained serum and citrate plasma from the patients at the same time. All the specimens were stored at −80°C before use.

2.4. Disk polyacrylamide gel electrophoresis

We used a disk polyacrylamide gel electrophoresis (Lipophor Gel, Joko, Tokyo, Japan) to identify small dense LDL in the LDL fractions by stepwise density sequential ultracentrifugation according to manufacturer's instruction. Briefly, test samples were placed in glass gel tubes, and then a loading gel containing Sudan Black B was added to each tube. Electrophoresis was done at a constant current of 3 mA per tube for 1 hour. The gel in each glass was scanned at 610 nm, and small dense LDL was identified by the migration distance [14].

2.5. Assay methods for lipids and hemoglobin A1c

Total cholesterol, high-density lipoprotein (HDL) cholesterol, and TGs were measured with commercial kits (Wako Pure Chemical, Osaka, Japan). Hemoglobin A_{1c} (HbA_{1c}) was measured with a high-performance liquid chromatography (HPLC) method. Vitamin E contents in plasma and LDL particles were determined using an HPLC method described previously [15].

2.6. Oxidative susceptibility test of LDL

Oxidative susceptibility of the LDL isolated from patients with IGT was determined as described previously [13]. Low-density lipoprotein was diluted to give a concentration of 0.14 mg LDL protein per milliliter, and 1.66 μmol/L $CuSO_4$ was added to initiate the oxidation of LDL. The formation of conjugated dienes was monitored continuously at 234 nm using a spectrophotometer.

2.7. Determination of fatty acid composition of LDL

Fatty acids in LDL were analyzed as described in the literature [16]. Briefly, fatty acids in the specimens were extracted and methylated. The resulting fatty acid methyl esters were identified using a GC17A gas chromatography (Shimazu, Kyoto, Japan) with an Omegawax 30-m/0.25-nm capillary column (Supelco, PA).

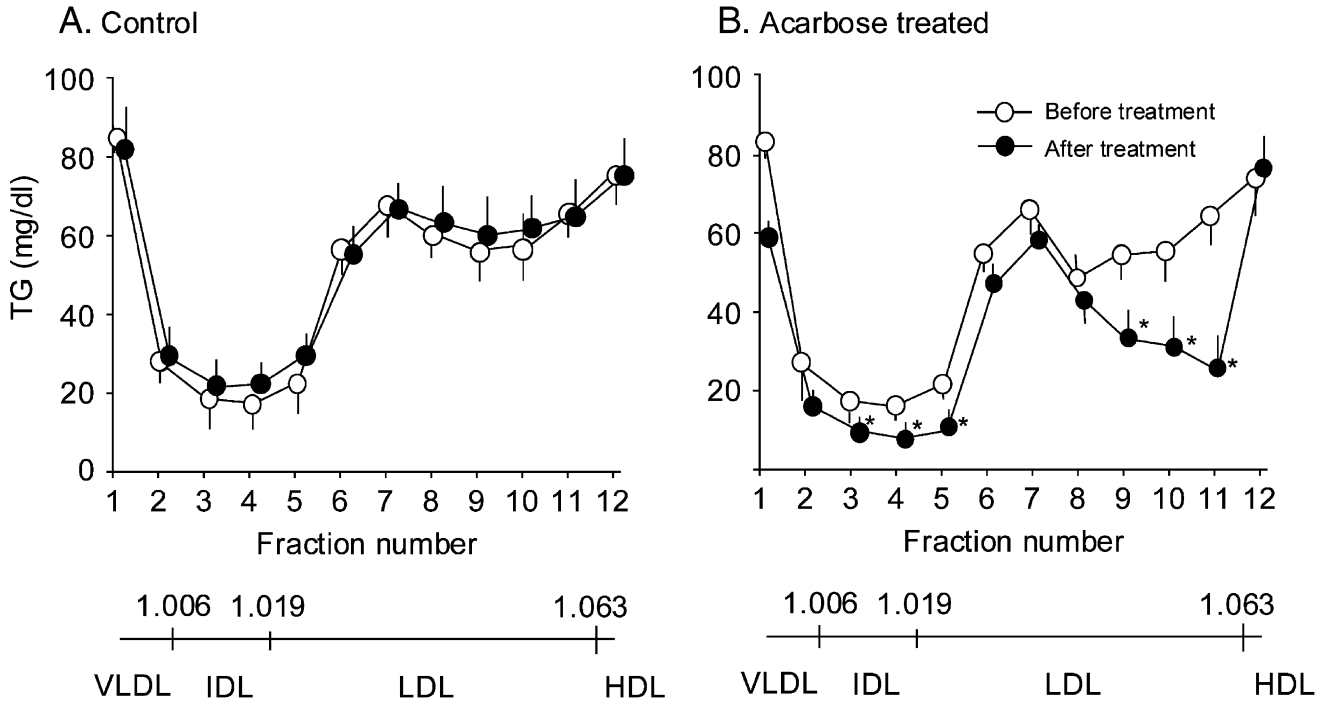

Fig. 2. Triglyceride contents in the size-fractioned lipoproteins. Lipoproteins were separated by means of stepwise density sequential ultracentrifugation according to size, and TG content in each fraction was determined using an enzymatic method. A, Control group; B, acarbose-treated group. Open circles and closed circles indicate the values before and after the trial, respectively. *$P < .05$ vs before the start of the trial. Bars indicate the SD of each point.

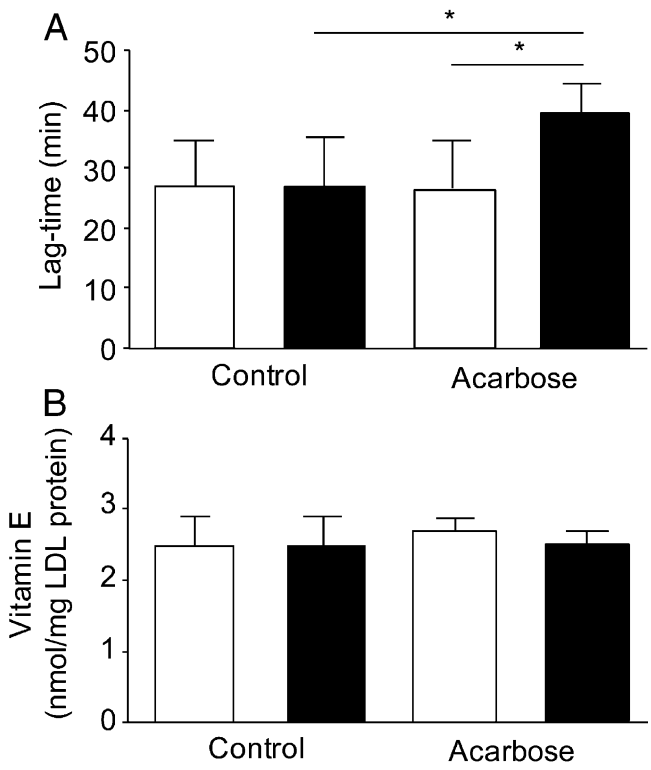

Fig. 3. Lag time before initiation of LDL oxidation (A) and vitamin E contents (B). Low-density lipoprotein fractions from the acarbose-treated group and the control group before (open column) and after (closed column) the 3-month trial were analyzed for the lag time before the initiation of LDL oxidation. The LDL fractions were also assayed for vitamin E. Data are shown as the mean ± SD. *P < .05 vs before the treatment and vs the control group after the trial.

2.8. Statistical analysis

Data are expressed as the mean ± SD. Differences between the mean measured values before and after the trial were assessed with the paired t test. We considered P values less than .05 to be significant. All the analyses were performed using StatFlex, a statistical software package (Artech, Osaka, Japan).

3. Results

3.1. Changes in clinical data after acarbose treatment

All patients designated in the acarbose-treated group continued taking 100 mg acarbose 3 times a day during the trial after an initial low dose of acarbose (100 mg daily) for a week. The treatment with acarbose slightly but significantly reduced body weight by 3.1%, body mass index by 3.3%, systolic blood pressure by 6.4%, and total cholesterol by 6.1% (Table 1). On the other hand, the mean serum HDL cholesterol level was increased by 9.8%. Marked decreases were observed in the levels of TG (28.5%), but TG levels in the control group increased after the study period. The HbA_{1c} levels in the acarbose-treated group also showed considerable decrease over the 3 months (Fig. 1). In contrast, we observed no difference in the clinical data of the control group before and after the trial.

3.2. Acarbose treatment reduces lipid contents in lipoproteins

Acarbose treatment resulted in decreases in total cholesterol and TG. Profiling the lipid contents in the lipoprotein fractions showed that acarbose treatment reduced plasma TG content in very low-density lipoprotein (VLDL) by 18%, in LDL by 43%, and in intermediate-density lipoprotein (IDL) by 52% (Table 2). In addition, the total cholesterol contents were decreased in VLDL by 14% and in IDL by 47%. The TG contents in the lipoproteins of the control group were increased in VLDL by 38% and in LDL by 25% (Table 3), but there were no significant changes in the cholesterol contents.

3.3. Changes in TG contents in size-fractioned lipoproteins with acarbose treatment

Determination of TG in the size-fractioned lipoproteins showed that TG contents in the high-density subfractions (nos. 9-11) of LDL decreased approximately by half after acarbose treatment (Fig. 2B). We further analyzed electrophoretically those fractions and found substantial decrease in the amount of small dense LDL (data not shown).

Moreover, slight decreases in TG content were found in the subfractions of IDL (fraction nos. 3-5). In contrast, low-density subfractions of LDL (fraction nos. 6-8) and those of HDL and VLDL showed no significant changes in the TG contents before and after the trial. On the other hand, there was no significant change in the control group (Fig. 2A).

3.4. Acarbose treatment reduces oxidative susceptibility of LDL

We evaluated the oxidative susceptibility of LDL obtained from the participants by measuring the lag time for LDL oxidation and found that the lag time was longer after the treatment in the acarbose-treated group (Fig. 3A), whereas no significant change was observed in the control group before and after the trial. To estimate the cause of the prolonged effect, we determined vitamin E content in LDL, but there was no significant change in the content before and after the treatment (Fig. 3B). Similarly, there was no significant change in vitamin E content in the plasma specimens (data not shown).

3.5. Acarbose treatment changes the fatty acid composition of LDL

Vitamin E is a major antioxidant in lipoproteins, but we found no changes in the content before and after acarbose treatment. Then, we speculated that acarbose treatment affects the fatty acid composition of LDL and decreases the oxidative susceptibility of LDL because acarbose treatment improves dyslipidemia in patients with diabetes [7,8]. The analysis of the fatty acid composition of LDL showed that the level of saturated fatty acids in LDL was

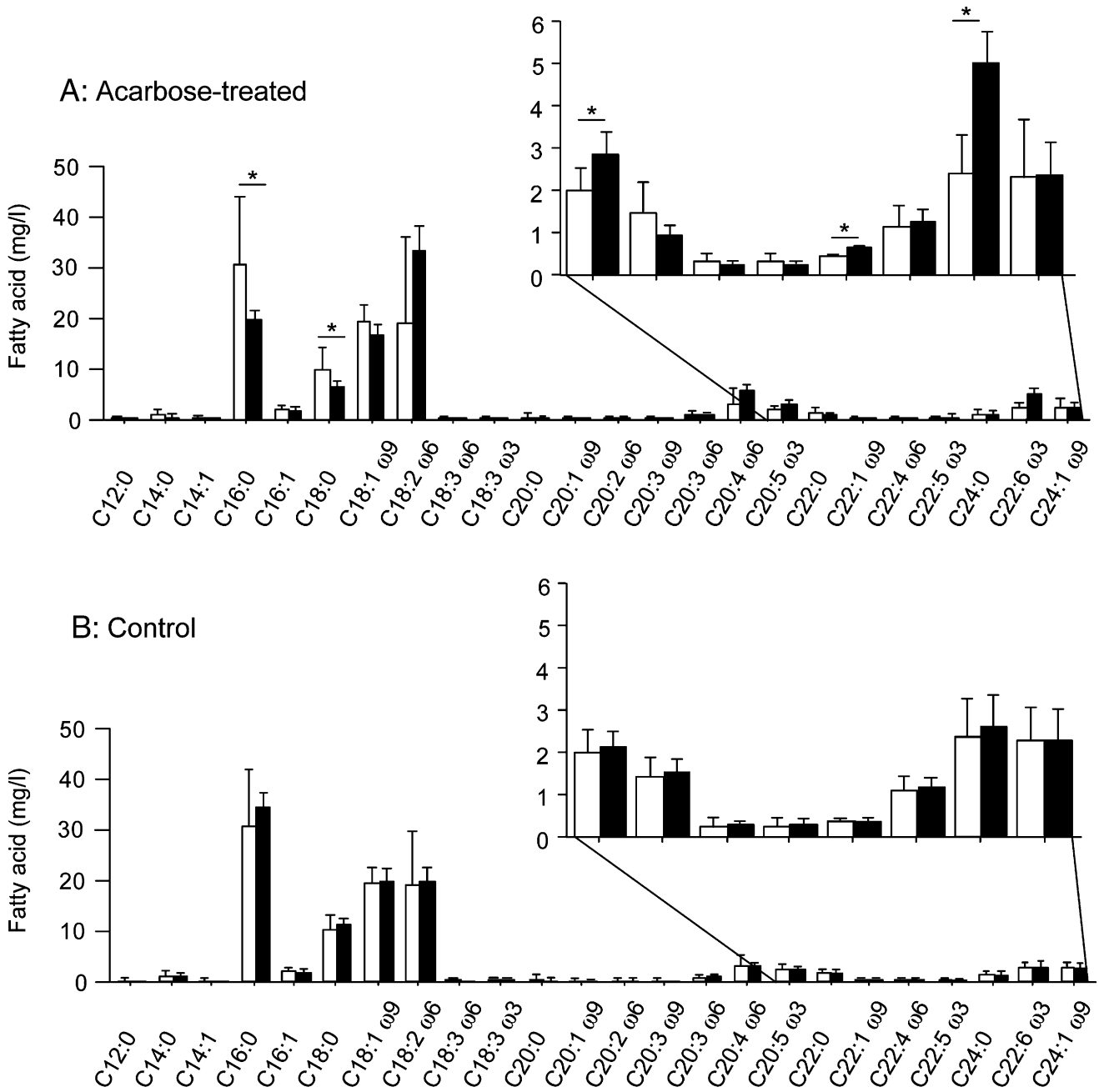

Fig. 4. Changes in fatty acid composition of LDL in acarbose-treated group (A) and control group (B). Isolated LDL was analyzed for fatty acid composition and compared before (open column) and after (closed column) the trial. Data are shown as the mean˘ SD. *$P < .05$ vs before the trial.

decreased, whereas the level of polyunsaturated (ω-3) fatty acids in LDLs was increased after acarbose treatment (Fig. 4A). We also observed increased levels of such ω-3 fatty acids in the plasma of the acarbose-treated group (data not shown). Such changes were not found in the control group (Fig. 4B).

4. Discussion

Acarbose is an effective drug to prevent diabetes [8]. Recent studies have revealed that this drug also reduces the risk of CVD [7]; however, the underlying mechanism for the reduced risk by acarbose treatment is still to be elucidated. The purpose of this study was to determine whether acarbose treatment alters the characteristics of LDL. After acarbose treatment, 3 major alterations were observed in LDL particles: reduced oxidative susceptibility, decreased TG content and small dense LDL particles, and change in fatty acid composition. These alterations are associated with the reduction of the atherogenecity and oxidative susceptibility of LDL, suggesting that acarbose treatment reduces the risk of CVD by ameliorating atherogenecity of LDL.

Low-density lipoprotein is heterogeneous in size, density, lipid composition, and oxidative susceptibility [14]. In patients with IGT and diabetes, more atherogenic forms of LDL particles, such as oxidized, advanced glycation end products–modified or small dense LDL, are increased [17-19]. In the present study, we found that acarbose treatment decreased the oxidative susceptibility of LDL, indicating that acarbose exerts the beneficial effect on CVD by reducing oxidized LDL. We speculate that 3 factors contributed to this prolonged effect: (1) the decreased small dense LDL particles, which is more susceptible to oxidation than buoyant LDL; (2) the alteration of fatty acid composition with acarbose treatment; polyunsaturated ω-3 fatty acids, beneficial substances for preventing CVD [20], were significantly increased, whereas saturated fatty acids were decreased in the LDL of the acarbose-treated group; (3) a decrease in the TG content of the LDL [13].

The fermentation in the gastrointestinal tract induced by acarbose is probably associated with the changes in the fatty acid composition [21]. The inhibition of glucose uptake in the small intestine produces short-chain fatty acids, such as caproic acid [22,23]. Those short-chain fatty acids induce an increase in the level of ω-3 fatty acids, such as eicosapentaenoic acid and docosahexaenoic acid, that may be incorporated into LDL particles. The production of ω-3 fatty acids may also contribute to the prevention of macrovascular complications by acarbose [24] because studies have shown that dietary intake of polyunsaturated fatty acid, especially linoleic fatty acid, is beneficial for primary prevention [24].

We also suggest that the alterations on LDL particles by acarbose treatment are associated with the increased activity of peroxisome proliferator–activated receptor-alpha (PPARα), which regulates many genes involved in the β oxidation of fatty acids. In fact, PPARα is activated by free fatty acids including linoleic, arachidonic, and oleic acids [23]. Moreover, Chang et al [25] have reported that PPARα agonists act on both the fatty acid elongation and desaturation pathways. This may explain the increases in polyunsaturated fatty acids in the LDL particles. Assuming that PPARα is a key molecule in the improvement of atherogenecity of LDL, then the evaluation of the activity of PPARα is important. Indeed, Andrulionyte et al [26] reported that the common polymorphisms in the gene of PPARα affected the progression from IGT to type 2 diabetes mellitus in participants in the STOP-NIDDM trial. Further studies are needed to clarify whether the activity of PPARα is involved in the disease progression and how acarbose changes the PPARα-related metabolism including fatty acids.

In conclusion, we have found that acarbose treatment reduced the oxidative susceptibility of LDL. Further analyses of the LDL particles indicated that the effect of acarbose was probably due to the alteration of fatty acid composition, decreased TG contents, and small dense LDL. These findings suggest that acarbose exerts the reduced risk of CVD by ameliorating the atherogenecity of LDL in patients with IGT and type 2 diabetes mellitus.

Acknowledgment

This study was supported by research grant no. 17590947 from the Ministry of Education, Culture, Sports, Science and Technology, Japan.

The authors thank Sawako Satoh, Rina Shinozaki, and Akiko Kawano for the secretarial assistance.

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