

ORIGINAL ARTICLE

Exercise Training Improves Low-Density Lipoprotein Oxidability in Untrained Subjects With Coronary Artery Disease

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ABSTRACT. Ziegler S, Schaller G, Mittermayer F, Pleiner J, Mihaly J, Niessner A, Richter B, Steiner-Boeker S, Penak M, Strasser B, Wolzt M. Exercise training improves low-density lipoprotein oxidability in untrained subjects with coronary artery disease. *Arch Phys Med Rehabil* 2006;87:265-9.

Objective: To test the hypothesis that regular exercise alters low-density lipoprotein (LDL) oxidability in patients with coronary artery disease.

Design: Longitudinal study.

Setting: General hospital and community.

Participants: Thirteen patients.

Interventions: Training program comprising running bouts twice weekly over 2 months.

Main Outcome Measures: Plasma lipid profile, oxidized LDL, and rate (Ox_{rate}) and amount (Ox_{amount}) of LDL reaction products were measured at baseline and after 2 months of training. Brachial artery endothelium-dependent and -independent vasodilation was assessed by use of ultrasound.

Results: Lipid profile and oxidized LDL remained unchanged, but mean Ox_{rate} and $Ox_{amount} \pm$ standard deviation were reduced from $2.5 \pm 1.5 \text{ nmol} \cdot \text{mgLDL}^{-1} \cdot \text{min}^{-1}$ and $120.3 \pm 75.3 \text{ nmol/mgLDL}$ at baseline to $0.4 \pm 0.2 \text{ nmol} \cdot \text{mgLDL}^{-1} \cdot \text{min}^{-1}$ and $21.3 \pm 11.4 \text{ nmol/mgLDL}$ after training ($P < .05$), respectively. Brachial artery vasodilation was suggested to be improved, but statistical significance was not reached in the small cohort under study.

Conclusions: Aerobic training enhances the resistance of LDL to oxidation in patients with coronary artery disease, which may play a role in the favorable effects of exercise.

Key Words: Brachial artery; Coronary artery disease; Exercise; Lipoproteins, LDL; Rehabilitation; Vasodilation.

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PHYSICAL EXERCISE CONTRIBUTES to the long-term reduction of cardiovascular morbidity and mortality. Regular aerobic exercise has been shown to be associated with an

improved cardiac performance by structural and functional changes, such as an increase in parasympathetic tone,¹ cardiac function, and muscle mass.² Furthermore, exercise-induced cardiac improvement is accompanied by reversal of impaired vascular endothelial function, which regulates coronary perfusion.³

This direct protective effect of physical training on the endothelium and vessel function independent of concomitant changes of cardiovascular risk factors has not yet been fully elucidated. Cell-culture and animal experiments have found a reduction of endogenous oxidant species, a modulation of organic antioxidant defenses, and the decrease in low-density lipoprotein (LDL) susceptibility to oxidation associated with physical training.⁴⁻⁶ Increased oxidative stress was found to be linked to atherosclerotic diseases, that is, coronary artery disease (CAD).⁷ Plasma levels of oxidized LDL have been shown to correlate inversely with left ventricular function and, therefore, were suggested as a risk marker of cardiovascular mortality.⁸ Beneficial effects of regular exercise on LDL oxidation have thus been postulated.⁶ A recent study⁹ has suggested that LDL susceptibility to in vitro oxidation is not associated with levels of circulating oxidized LDL.

Endothelial dysfunction is detectable in patients with CAD. This impairment of flow-mediated vasodilation (FMD) is present in different vascular beds.^{10,11} Studies¹²⁻¹⁴ have unanimously shown an improvement of impaired vascular endothelial function of patients with CAD by an aerobic training program over several weeks.

The effect of regular aerobic exercise on LDL susceptibility to oxidation has not been investigated in patients with CAD. The aim of the present study was to assess whether a regular physical training program would increase the resistance of LDL to oxidation and counteract endothelial dysfunction in patients with CAD. We measured oxidation rate (Ox_{rate}) and amount (Ox_{amount}) of conjugated LDL diene products to ex vivo LDL oxidation with copper, in vivo LDL oxidation, and FMD of the brachial artery before and after a structured training program.

METHODS

Participants

We studied 13 patients (11 men, 2 women; mean age, 54 ± 9 y) with CAD and stable angina pectoris (Canadian Cardiovascular Society classes I-II). The study protocol was approved by the Ethics Committee of the Medical University of Vienna. Informed consent was obtained from all participants.

Five patients had a history of myocardial infarction and 3 patients had undergone aortocoronary bypass surgery. Concomitant treatment consisted of β -blockers ($n=5$), angiotensin-converting enzyme inhibitors ($n=6$), angiotensin II receptor antagonists ($n=2$), and calcium-channel blockers ($n=2$). All

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patients received platelet antiaggregatory therapy and 7 patients were on statins. Two patients had type 2 diabetes mellitus, one of whom was insulin-dependent. Medication was unchanged during the study period. Before the study, all patients had a sedentary lifestyle with physical exercise of 1 hour or less a week.

Exclusion criteria were: heart failure, New York Heart Association functional class II to IV; angina pectoris, Canadian Cardiovascular Society class III or greater; any acute coronary event; atrial fibrillation or severe arrhythmia; coronary angioplasty or bypass surgery within the last 3 months; any acute disease within 30 days prior to the study; evidence of peripheral artery occlusive disease; or any disease that prevented participation in a regular exercise program.

Training

The outpatient training program comprised running bouts twice weekly for a period of 2 months. The training was performed outdoors on a tarmac surface under more or less constant convenient temperature conditions. In detail, each exercise session lasted for approximately 30 minutes, consisting of a short warm-up period of 3 to 5 minutes and an aerobic exercise session of 20 minutes. The last 5 minutes were used for cooling down and stretching exercise. Training was supervised by a physician, who participated in each training bout. Heart rate during exercise was monitored by wrist watches with coded electrocardiogram transponders. Exercise intensity was set on an individual basis at 50% of each patient's maximal functional capacity. Patients were allowed to carry out additional training. A moderate exercise program was selected for these untrained subjects. This has been proven to result in an equivalent training effect for the cardiovascular system as can be achieved by exercising at higher intensities,¹⁵ with lower risk of adverse side effects.

Parameters

We analyzed the following parameters at baseline and after the 2-month training period.

Peak oxygen uptake. To assess maximal oxygen uptake ($\dot{V}O_{2max}$; in $\text{mL}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$), a symptom-limited, incremental cycle ergometer protocol after the protocol of the Austrian Society for Cardiology was performed in all participants before the training. In brief, starting with a working load of 50W, work rate was increased by 25W every 2 minutes until the subject could not maintain the cycling cadence of 50rpm.

Lipid analysis. Fasting plasma total cholesterol, triglycerides, high-density lipoprotein (HDL) and LDL cholesterol measurements were determined by routine laboratory methods.

In vitro LDL oxidation. The isolation and oxidation of LDL in vitro was performed by a slightly modified procedure from Esterbauer et al.¹⁶ LDL was isolated from heparinized plasma by short-run ultracentrifugation using a density gradient. In brief, 6mL of plasma were adjusted with .327KBr/mL of plasma and layered on the bottom of a centrifuge tube. All solutions contained 1.85mg/mL ethylenediaminetetraacetic acid (EDTA). The tubes were centrifuged^a at 56,000rpm at 4°C for 2 hours. After centrifugation, the distinct LDL band was carefully separated. Thereafter, EDTA was removed from LDL by using small dextran-sulfate affinity columns as previously described.¹⁷

LDL oxidation was initiated by adding 20 $\mu\text{mol/L}$ of copper sulfate (CuSO_4) to LDL at a final concentration of 200 $\mu\text{g/mL}$. We determined the kinetics of LDL oxidation by monitoring the change in absorbance at 234nm in a spectrophotometer. The change in absorbance was recorded every 5 minutes and can

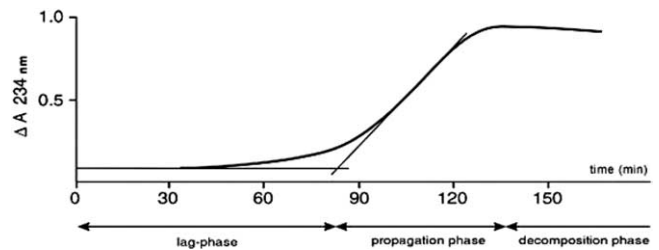


Fig 1. Measurement of the kinetics of LDL oxidation by continuous monitoring the change of absorbance at 234nm ($\Delta A_{234\text{nm}}$). A 1-cm quartz cuvette with the LDL solution supplemented with CuSO_4 as a pro-oxidant was placed in a single-beam ultraviolet spectrophotometer. The initial 234-nm absorbance of the LDL solution was set to 0.1 and the increase recorded over 3 hours. The change in absorbance over time can be divided into 3 phases: a first phase during which the dienes do not or only very slowly increase (lag phase), a second phase during which the dienes very rapidly increase to a maximum value (propagation phase), and a third phase during which the dienes strongly decrease again (decomposition phase). The curve allows one to determine the maximum rate of oxidation and maximum amount of conjugated dienes formed.

be divided into 3 consecutive phases: lag phase, propagation phase, and decomposition phase, which were used as measures of LDL oxidation in vitro (fig 1). The curve allows for determination of the rate (Ox_{rate} ; $\text{nmol dienes}\cdot\text{mgLDL}^{-1}\cdot\text{min}^{-1}$) and the amount of conjugated dienes formed (Ox_{amount} ; $\text{nmol dienes}/\text{mgLDL}$) as a measure of LDL oxidability.

In vivo oxidized LDL. We measured plasma levels of oxidized LDL using a commercially available sandwich enzyme-linked immunosorbent assay.^b In brief, oxidized LDL in the sample reacts with antioxidantized LDL antibodies bound to microtitration well during incubation. Oxidized LDL is recognized by a conjugated antihuman apolipoprotein B antibody, detected by 3,3',5,5'-tetramethylbenzidine and read spectrophotometrically at 450nm. This murine antibody has been commonly used for quantification of oxidized LDL.¹⁸⁻²⁰ Normative reference values from healthy subjects range between 26 and 117U/L in our laboratory.

Forearm conduit artery function. All subjects were studied in the morning after overnight fasting. We conducted the studies in a quiet room with ambient temperature of 22°C. All measurements were made by an experienced observer. We used a high-resolution ultrasound system with a 10-MHz transducer^c and an automated computer-based wall-tracking analysis system^d to measure brachial artery diameter as described previously.²¹ Briefly, each subject was in supine position with the left arm supported on a foam block and a cuff placed on the upper arm. The ultrasound header was fixed to maintain an identical position during the experiments. The brachial artery was scanned in a longitudinal section proximal to its bifurcation, which was used as an anatomic marker and the diameter measured at end-diastole. After a 10-minute resting period, the baseline vessel wall diameter was assessed for 60 seconds. The cuff on the upper arm was then inflated to suprasystolic pressure (250mmHg) for 4.5 minutes and then released. Brachial artery diameter was measured for 180 seconds after cuff release. After a 15-minute rest period to allow restoration, brachial artery diameter was measured for 240 seconds after sublingual administration (0.8mg) of the endothelium-independent vasodilator glyceryl trinitrate (GTN).^e Maximum dilation of the brachial artery was expressed as percentage change of diameter from baseline after reactive hyperemia (FMD) or GTN administration.

Statistical Analysis

Data sets were tested for normal distribution and log transformed if not normally distributed. The effect of training on brachial artery vasodilation, lipids, and parameters of LDL oxidation were compared using Student paired *t* tests. A *P* value of less than .05 was considered significant. Values are expressed as mean \pm standard deviation. We used the SPSS program^f for statistical analysis.

RESULTS

All subjects completed the study and no adverse events were noted. Training compliance was 92%. Total cholesterol, triglycerides, HDL and LDL plasma levels did not change during the observation period. Mean plasma concentrations of oxidized LDL at baseline were 44.9 ± 12.5 U/L before and 44.5 ± 15.1 U/L after the training period (*P* = .93).

Oxidation of LDL

The mean maximal Ox_{rate} was 2.5 ± 1.5 nmol·mgLDL⁻¹·min⁻¹ at baseline and decreased to 0.4 ± 0.2 nmol·mgLDL⁻¹·min⁻¹ after training (*P* < .05). Mean Ox_{amount} was 120.3 ± 75.3 nmol/mgLDL before and 21.3 ± 11.4 nmol/mgLDL after the training period (*P* < .05) (fig 2).

Vascular Function

Brachial artery FMD tended to increase from $0.9\% \pm 4.5\%$ at baseline to $6.3\% \pm 6.7\%$ after the 2-month training period, which was not statistically significant (*P* = .055). Further, no significant differences were observed for the increase in GTN-induced vasodilation between baseline ($4.0\% \pm 5.5\%$) and after training ($7.4\% \pm 5.7\%$) (*P* = .09). There was no correlation between changes over time of endothelium-dependent and endothelium-independent vasodilation (*r* = .08, *P* = 0.8).

DISCUSSION

This study showed that regular exercise results in a significant decrease in the susceptibility of LDL to ex vivo copper oxidation in patients with stable CAD. This improvement in circulating LDL characteristics in vitro was independent from modifications of circulating in vivo oxidized LDL or the lipid profile. Further, responsiveness to reactive hyperemia (FMD), which was reduced compared with healthy subjects at baseline,²² was suggested to be improved after the training period, but these changes were not statistically significant. Even if the present results might suggest that physical exercise ameliorates endothelial dysfunction in CAD, the clinical implication of these findings in a small homogenous sample is yet unclear.

Exercise-induced improvement of endothelial function is apparently not limited to the lower extremities. It has been shown that bicycle exercise training improves also coronary endothelium-dependent vasodilation in patients with CAD.^{23,24} This systemic beneficial effect of physical activity on the endothelium is compatible with the data from the present study, in which a structured running program was employed. The present findings are further corroborated by increased plasma concentrations of soluble vascular cellular adhesion molecules, representing markers of endothelial integrity, that is, vascular cellular adhesion molecule-1 and intracellular adhesion molecule-1 by exercise.²⁵

The pathophysiologic aspects underlying early functional changes that cause impaired endothelium-dependent vasodilation are not fully characterized. Reduced nitric oxide bioactivity has been discussed as a possible mechanism, resulting from impaired formation of vasoactive nitric oxide, increased inactivation of the mediator,^{3,26} or altered sensitivity of the under-

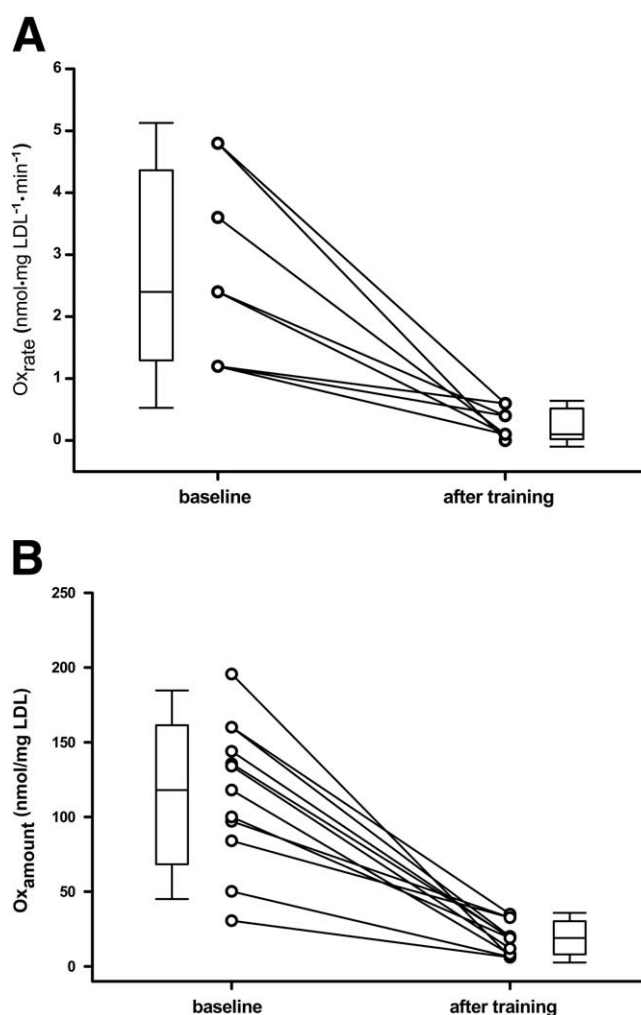


Fig 2. Individual data of maximal (A) Ox_{rate} and (B) Ox_{amount} at baseline and after the training period. Bars represent median values; whiskers show the 5% to 95% confidence intervals.

lying vascular smooth muscle cells. FMD and GTN reactivity tended to improve in this study. This was also found in healthy men, in whom training improved the dilating capacity of coronary arteries.²⁷ However, the positive impact of training on vascular function seems to be saturable and may even follow a bell-shaped curve.²⁸

Susceptibility of LDL to oxidation was substantially increased in the subjects under study before training. The peroxidation of LDL is initiated by oxygen free radicals.²⁹ Under physiologic conditions, sufficient antioxidants are available to inactivate free oxygen radicals.³⁰ In patients with CAD, an enhanced production of reactive oxygen species may exceed the endogenous antioxidant capacity, as shown by the salutary effects of vitamin C.^{31,32}

Widespread beneficial effects of regular exercise on the lipoprotein profile have been reported. Interestingly, exercise-induced changes in LDL subfractions are related to the amount of activity rather than its intensity, fitness improvement, or changes of body weight.³³ In our study, plasma concentrations of oxidized LDL were in the normative range and, therefore, were not expected to be lowered further. This finding is in agreement with a previous report on subjects with CAD.³⁴ A

quantitative comparison of oxidized LDL reference ranges among laboratories is difficult because of slightly different methods and different standard plasma pools used as reference. The lipid profile, which was under pharmacologic control in most subjects under study, was not improved by training in patients with stable CAD.

Several studies in healthy subjects have found modifications of LDL susceptibility to oxidation in response to physical exercise.⁶ Shern-Brewer⁴ and Liu³⁵ and colleagues described an increased resistance of LDL to oxidation in healthy subjects in response to aerobic training over several months, whereas an overall shorter exercise duration elevated only plasma LDL-oxidation products. The reduction in LDL oxidability was a consistent finding in this group of untrained patients. However, the mechanisms involved in exercise-related resistance of LDL to oxidation have not been fully clarified. LDL oxidability is influenced by several physiologic factors, including LDL proportion, composition and density, HDL concentration, and smoking habits,³⁶ and qualitative modifications such as glycation.³⁷ The oxidant status is also influenced indirectly by enzymes such as superoxide dismutase^{38,39} and other antioxidant cofactors, such as α -tocopherol, carotenoids, and phenols.^{40,41} Among these potential mechanisms, none has been identified as the principal determinant of the increased LDL resistance to oxidation observed in trained subjects.

Obviously, LDL susceptibility to in vitro oxidation is not associated with levels of circulating in vivo oxidized LDL, which is consistent with previous observations.⁹ Therefore, in vivo LDL oxidant products are not a suitable measure for in vitro susceptibility of LDL oxidability. The rate of LDL oxidation in the intima depends on endothelial barrier function rather than on the in vivo concentration of oxidized LDL.⁹ Further, evidence suggests that compositional differences of LDL determine the susceptibility to peroxidative modification. It was shown that subjects with more dense LDL subfractions have a smaller resistance to LDL oxidation, which is linked to greater foam cell formation and thus to a higher atherogenic risk.⁴² Further prospective studies are needed to determine the prognostic value of susceptibility of LDL to oxidation.

Study Limitations

Results of the present study are limited by the small sample size and the fairly short time period during which the training program was conducted. Further, from the present data, no conclusions on a permanent protective effect of a 2-month training period can be drawn. However, the fact that even this limited training program resulted in such a notable increase in resistance of LDL to oxidation highlights the powerful role of exercise in the protection of the cardiovascular system.

CONCLUSIONS

Our findings indicate that physical exercise increases LDL resistance to oxidation. This might play a crucial role in the restoration of endothelial function or reduction of atherosclerotic risk in patients with stable CAD.

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Suppliers

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