Cardiac Rehabilitation Reduces Serum Levels of Oxidized Low-Density Lipoprotein

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Background: Oxidized low-density lipoprotein (oxLDL) levels have been found to play an important role in the progression of atherosclerosis. However, methods for effectively reducing oxLDL levels have not been established. Comprehensive cardiac rehabilitation (CR) with exercise training prevents the progression of atherosclerosis, and might reduce oxLDL levels.

Methods and Results: We measured the serum levels of malondialdehyde-modified LDL (MDA-LDL), a marker of oxLDL, in 136 patients who were enrolled in a 6-month CR program. Peak oxygen consumption (V\(\text{O}_2\)) and MDA-LDL levels were analyzed, before and 6 months after enrolment. In total, 67 patients completed the CR program (CR group) and 69 patients failed to complete the program (non-CR group). Peak V\(\text{O}_2\) increased significantly in the CR group (P<0.01). The levels of MDA-LDL decreased significantly in the CR group (P<0.01) but not in the non-CR group. ∆V\(\text{O}_2\) (peak V\(\text{O}_2\) after CR–peak V\(\text{O}_2\) before CR) was negatively associated with ∆MDA-LDL (MDA-LDL after CR–MDA-LDL before CR) (R²=0.11, P=0.01). Multiple regression analysis showed that continuing CR was an independent determining factor for lowering MDA-LDL levels.

Conclusions: CR decreases oxLDL levels in patients with cardiovascular diseases. Moreover, CR may prevent cardiovascular events through an antioxidative effect. (Circ J 2014; 78: 2682–2687)

Key Words: Cardiac rehabilitation; Lipoproteins; Oxidative stress

Oxidized low-density lipoprotein (oxLDL) plays a key role in the initiation of atherosclerosis and enhances the formation and vulnerability of atherosclerotic plaque.\(^1\) Malondialdehyde-modified LDL (MDA-LDL) is a marker of oxLDL in which the lysine groups of apolipoprotein B are fused with malondialdehyde, a lipid peroxide. MDA-LDL is reported to significantly influence the progression of arteriosclerosis,\(^5,6\) and is associated with cardiovascular events, including unstable angina and acute myocardial infarction (AMI).\(^4,6\) In addition, the degree of oxidation of LDL cholesterol (LDL-C) is represented as the MDA-LDL/LDL-C ratio; this ratio increases in patients with arteriosclerotic disease.\(^7\) However, established treatment methods for suppressing the oxidation of LDL-C and reducing the serum levels of oxLDL are lacking, and even pharmacotherapy, including statin therapy, is controversial.\(^8\)

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Cardiac rehabilitation (CR) improves the prognosis of patients with ischemic heart disease and chronic heart failure,\(^9,10\) and has been reported to improve atherosclerosis (eg, coronary arteriosclerosis and carotid intima-media thickness).\(^12,13\) However, the influence of CR on oxLDLs remains unclear, so the present was aimed at clarifying the effect of CR on the levels of oxLDL and on the degree of LDL oxidation.

Methods

Patients
The study population included 136 patients with cardiovascular disease who were already enrolled in a CR program between...
April 2009 and November 2012. Patients who had ischemic heart disease, or chronic heart failure, as well as those who had undergone cardiac surgery, were included in the program based on the guidelines of the American Heart Association and the American College of Cardiology Foundation. Exclusion criteria were untreated unstable angina pectoris, serious arrhythmia, uncontrolled heart failure, or an aortic aneurysm. Eligible patients were given individually tailored exercise prescriptions on their entry to the study. All patients received exercise instructions and were interviewed about their diet, smoking habits and exercise. The implementation status of the CR program was determined using outpatient rehabilitation records, history taking, and telephone interviews. Based on this information, the patients were divided into 2 groups: those who successfully completed the CR program, performed at least twice a week (CR group, 67 patients), and those who failed to complete the program (non-CR group, 69 patients). In the non-CR group, the reason why most patients could not continue with the CR program was poor adherence. The results of blood tests, including MDA-LDL levels, were compared between the 2 groups on entry into the CR program and 6 months after the CR program. Additionally, we used maximal symptom-limited cardiopulmonary exercise testing, an indicator of the effect of CR, to evaluate whether exercise capacity was associated with changes in the levels of MDA-LDL. We also used multivariate analysis to identify independent factors that might have affected MDA-LDL levels.

This study was approved by the ethics committee of Tokushima University and the patients gave informed consent.

### CR Program

The CR program began soon after each physician gave permission, and was continued for 6 months after hospitalization for cardiovascular disease. In our CR program, exercise included supervised exercise sessions (bicycle ergometer) and home exercise (walking). The duration of the exercise was 30 min per session, and the session was performed at least twice-weekly for 6 months. The intensity of the exercise was individually tailored to achieve a heart rate equivalent to the anaerobic threshold, which was determined by cardiopulmonary exercise testing. For patients who had not performed cardiopulmonary exercise testing, either the Borg scale was used at level 12–13 (“a little hard”) of the 20-point scale of perceived rating of exercise or Karvonen’s equation at 50–60% of the heart rate.
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The consumption (VO₂), and carbon dioxide consumption, and then calculated anaerobic threshold according to the V-slope method.

Peak VO₂ was defined as the highest VO₂ value when patients reached their peak exercise.

Statistical Analysis
All data are expressed as mean ± SD. Statistical analyses were performed using the JMP10 software (SAS Institute, Cary, NC, USA). The baseline patient characteristics in the 2 groups were analyzed and compared using the unpaired t-test and the chi-square test, assuming equal variances. Laboratory data at baseline and after 6 months were compared by using Student’s paired t-test. The relationship between MDA-LDL level and exercise capacity was evaluated using simple regression analysis. Multiple regression analysis was performed to evaluate the independent factors contributing to changes in MDA-LDL levels. A P-value <0.05 was considered statistically significant.

Results

Subject Characteristics
The baseline patient characteristics are shown in Table 1. The CR and non-CR groups had 67 and 69 patients, respectively. The number of males in the CR group was greater than that in the non-CR group. Ischemic heart disease and heart failure were the major reasons for rehabilitation in both the groups. The risk factors, including current smoking, and medications used, including statins, did not differ between the 2 groups. The number of smokers at 6 months after the enrolment was 2 in the CR group and 3 in non-CR group, and this number did not significantly differ between the 2 groups (P=0.67). Moreover, although 29 patients of the CR group and 20 patients of the non-CR group had received statins for the first time, no significant difference in this regard was noted between the 2 groups.

Effect of CR on LDL-C, HDL-C, and MDA-LDL Levels
LDL-C levels were significantly decreased after enrolment in the CR group. The supervised exercise program was continued until discharge, and then home exercise was combined with once- or twice-weekly supervised exercise for the remaining 6 months. The home exercise involved walking at a speed that resulted in the prescribed heart rate for at least 30 min, 1–7 times a week. The patients received individual education or counseling on exercise prescriptions, secondary prevention of cardiovascular diseases, diet, smoking cessation, medications, and physical activities from physicians, nurses, and exercise instructors whenever they desired them.

Laboratory Tests
Venous fasting blood samples were collected to determine the level of serum lipids and other laboratory parameters (eg, MDA-LDL, LDL-C, high-density lipoprotein cholesterol (HDL-C)) before and after the 6-month CR program. Levels of serum LDL-C were determined by enzymatic methods using an autoanalyzer (Cholestest LDL; Sekisui Medical, Tokyo, Japan) in the hospital laboratory, and HDL-C levels were also determined using an autoanalyzer (Metabolead HDL-C; Kyowa Medex, Tokyo, Japan). MDA-LDL levels were assayed by ELISA (anti-MDA-LDL antibody, SRL Co) at a commercially available laboratory (SRL Co, Tokyo, Japan).

Cardiopulmonary Exercise Testing
Symptom-limited cardiopulmonary exercise testing was performed at the start and end of the 6-month CR program. Each patient was examined using 12-lead ECG, automated sphygmomanometer, and exhalation gasmask. After a 2-min rest on the bicycle ergometer, the patients started pedaling at a workload of 10 or 20 W (depending on the patient) for 3 min as warm up, and then pedaled the ergometer with a gradually increasing ramp workload at 10 or 20 W/min to the limit of their endurance. The patients’ condition was monitored by a continuous 12-lead ECG and by minute-by-minute blood pressure measurements. Exhaled gas was analyzed continuously using a gas analyzer (Expired Gas Analysis System, Cpx–1; Inter Reha, Tokyo, Japan). We recorded ventilation, oxygen consumption (VO₂), and carbon dioxide consumption, and then calculated anaerobic threshold according to the V-slope method. Peak VO₂ was defined as the highest VO₂ value when patients reached their peak exercise.
both the groups (CR group: 103±33 mg/dl to 86±25 mg/dl, P<0.01; non-CR group: 99±34 mg/dl to 86±21 mg/dl, P<0.01) (Figure 1). The HDL-C levels did not significantly change in either group (CR group: 52±15 mg/dl to 54±15 mg/dl, P=0.06; non-CR group: 53±16 mg/dl to 55±15 mg/dl, P=0.10) (Figure 1). The MDA-LDL levels were significantly decreased in the CR group (113±46 U/L to 90±25 U/L, P<0.01), but not in the non-CR group (105±40 U/L to 106±35 U/L, P=0.75) (Figure 2). In addition, the MDA-LDL/LDL-C ratio was significantly increased in the non-CR group (1.10±0.39 to 1.25±0.35, P<0.01), but not in the CR group (1.14±0.38 to 1.09±0.29, P=0.36) (Figure 2).
Relationship Between Exercise Tolerance and MDA-LDL Levels

Changes in exercise tolerance were evaluated by cardiopulmonary exercise testing in 56 patients before and 6 months after the enrolment. Of these 56 patients, 39 were in the CR group and 17 were in the non-CR group. The peak V˙O₂ increased in the enrolment. Of these 56 patients, 39 were in the CR group and 17 were in the non-CR group. The peak V˙O₂ increased in the CR group (17.9±5.8 to 21.8±6.2 ml min⁻¹ kg⁻¹, P<0.01), but not in the non-CR group. ∆V˙O₂ (peak V˙O₂ after CR−peak V˙O₂ before CR) was negatively associated with ∆MDA-LDL (MDA-LDL after CR−MDA-LDL before CR) (Figure 3A). To eliminate the effects of statins on MDA-LDL, 35 patients who were newly administered statin therapy were excluded, and we re-analyzed the relationship between ∆V˙O₂ and ∆MDA-LDL in 21 patients (Figure 3B). Of these 21 patients, 15 were in the CR group and 6 were in the non-CR group. ∆V˙O₂ was negatively associated with ∆MDA-LDL in these 21 patients.

Multiple Regression Analysis

Multiple regression analyses were performed to elucidate the independent determinants of ∆MDA-LDL, which were CR and history of AMI (Table 2).

Discussion

We demonstrated that MDA-LDL levels were decreased by CR, and that this decrease was associated with a reduction in peak VO₂. Multiple regression analyses indicated that CR was an independent determinant for a reduction in the MDA-LDL level, indicating that CR lowers MDA-LDL levels and improves exercise capacity.

Although the effects of exercise therapy on lipid profiles have been reported, including reductions in the levels of neutral fat and an increase in HDL-C levels,23,24 there have been not many previous reports of the effect of CR on oxLDLs. Appropriate exercise has been reported to suppress oxidative stress,25 so, we hypothesized that exercise-induced suppression of oxidative stress reduced the oxidation of LDLs, leading to a decrease in MDA-LDL level. This hypothesis was supported by the result form the present study that although the LDL-C levels decreased in both groups, the MDA-LDL/LDL-C ratio was increased in only the non-CR group. Appropriate intensity and duration of exercise following our CR program may have contributed to the decrease in oxidative stress via induction of antioxidant enzymes and a decrease in the production of reactive oxygen species.26-27

Exercise increases circulating blood flow, which in turn increases the shear stress on the endothelium.28 Long-term laminar shear stress increases nitric oxide generation by endothelial cells, which causes downregulation of nicotinamide adenine dinucleotide phosphate oxidase subunits. Consequently, the formation of oxidants such as superoxide anion are decreased, thus ameliorating oxidative stress.29 CR suppresses cardiovascular events and improves the prognosis of patients with various cardiovascular diseases.30-33 Although the mechanisms by which MDA-LDL is decreased by CR remain unclear, the antioxidative properties of CR may contribute to lower levels of oxLDL.

In the present study, multiple regression analysis revealed that a history of acute myocardial infraction was another independent determinant of ∆MDA-LDL. Holvoet et al reported that the levels of MDA-LDL increase in patients with AMI because of the marked tissue damage or inflammation caused by myocardial ischemia.5 Therefore, the level of MDA-LDL decreases to a greater extent after therapy, including revascularization and medication, in patients with AMI vs. those with other cardiovascular diseases.

Statins are the representative agents for lowering LDL-C levels.34 We speculated that the LDL-C levels decreased in both the groups in the present study because approximately 80% of the patients in both groups had a history of statin administration during the study period. However, statins have also been reported as unable to decrease oxLDL levels.35 When excluding patients in whom statin therapy was initiated after enrolment, the CR still resulted in a decline in MDA-LDL levels; this decline was independent of statin administration.

In this study, we showed a correlation between the increase in peak VO₂ and the decline in oxLDL levels; in addition, continuing CR was an independent determining factor that lowered the MDA-LDL levels. Thus, we believe that continuing CR leads to an increase in exercise capacity, which may improve atherosclerosis by reducing MDA-LDL levels. Therefore, a large clinical study should be performed to clarify the importance of lowering oxLDL levels through CR in order to prevent cardiovascular events.

| Table 2. Multiple Regression Analysis for Changes in Levels of MDA-LDL* |
|-------------------------------|-----------------|-----------------|-----------------|-----------------|
| Variable                      | Coefficient     | 95% CI          | Standardized   | P value         |
| Age (years)                   | 0.19            | −0.55 to 0.93   | 0.05           | 0.61            |
| Male sex                      | −15.8           | −34.5 to 2.87   | −0.16          | 0.10            |
| Body mass index               | −0.55           | −2.31 to 1.21   | −0.05          | 0.54            |
| AMI                           | −23.6           | −40.2 to −7.02  | −0.26          | <0.01           |
| Angina pectoris               | 10.1            | −4.72 to 25.0   | 0.13           | 0.18            |
| Cardiac surgery               | 13.5            | −9.23 to 36.2   | 0.10           | 0.24            |
| CHF                           | −3.04           | −20.6 to 15.5   | −0.03          | 0.73            |
| Hypertension                  | 7.82            | −11.4 to 26.7   | 0.07           | 0.43            |
| Diabetes mellitus             | −1.86           | −16.2 to 12.5   | −0.02          | 0.80            |
| Dyslipidemia                  | −12.2           | −27.7 to 3.36   | −0.14          | 0.12            |
| Current smoking               | 5.38            | −11.9 to 22.7   | 0.06           | 0.54            |
| Statin                        | 3.42            | −19.1 to 26.0   | 0.03           | 0.76            |
| CR                            | −9.94           | −17.4 to −2.43  | −0.23          | 0.01            |

*R²=0.23, P=0.001. CI, confidence interval. Other abbreviations as in Table 1.
Study Limitations
Our study had certain limitations. First, it was a single-facility, retrospective, and observational study. Second, the patients’ baseline characteristics differed between the 2 groups. Therefore, we used multivariate analysis to identify independent determining factors that lower MDA-LDL levels. Third, cardiopulmonary exercise testing after the CR program could not be performed in approximately half the patients in this study population. Therefore, we could not measure peak VO2, particularly in the non-CR group.

Conclusions
CR decreased MDA-LDL levels, and the decrease in MDA-LDL correlated with an increase in exercise capacity. Consequently, CR decreased the oxLDL levels, and may prevent cardiovascular events through an antioxidative effect in patients with cardiovascular diseases.

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Disclosures
None.

References