Table 2 Effects of cardiac rehabilitation for 6 months among three groups.

	VALVE		CABG		MI	
	Before	After	Before	After	Before	After
Peak $\dot{V}O_2$ (ml kg^{-1} min^{-1})	15.7 ± 5.5	24.0 ± 7.2$^{\#}$	15.1 ± 3.7†	21.7 ± 5.7$^{\#}$	18.5 ± 5.1	23.8 ± 6.1$^{\#}$
ΔPeak $\dot{V}O_2$ (%)		58.2 ± 45.3†		46.9 ± 33.3		32.9 ± 31.5
AT (ml kg^{-1} min^{-1})	10.0 ± 2.2	14.1 ± 3.7$^{\#}$	9.2 ± 2.0†	12.5 ± 3.2$^{\#}$	11.0 ± 3.0	14.7 ± 3.3$^{\#}$
ΔAT (%)		41.1 ± 34.3		42.8 ± 55.6		38.0 ± 29.0
Peak RER	1.13 ± 0.13	1.16 ± 0.07	1.09 ± 0.11	1.17 ± 0.07$^{\#}$	1.14 ± 0.08	1.16 ± 0.07
Knee Ex (Nm × 100/BW)	170.1 ± 61.4	195.1 ± 58.5$^{\#}$	161.0 ± 48.4	189.1 ± 53.5$^{\#}$	174.3 ± 54.6	189.5 ± 56.1$^{\#}$
ΔKnee Ex (%)		19.5 ± 22.4		20.9 ± 27.5		10.9 ± 17.3
Knee Flex (Nm × 100/BW)	97.3 ± 31.2	116.7 ± 34.6$^{\#}$	92.3 ± 28.7	114.6 ± 30.9$^{\#}$	101.1 ± 30.7	113.0 ± 32.6$^{\#}$
ΔKnee Flex (%)		26.4 ± 35.5		29.9 ± 34.0		14.1 ± 19.3
Thigh circumference (cm)	47.5 ± 4.2†	49.2 ± 4.3$^{\#}$	48.4 ± 3.9	49.8 ± 4.3$^{\#}$	50.7 ± 3.9	51.2 ± 4.9
Δ Thigh circumference (%)		3.5 ± 4.3		2.9 ± 5.3		1.2 ± 9.0
Calf circumference (cm)	33.5 ± 3.2	35.7 ± 3.0$^{\#}$	34.5 ± 2.9	35.5 ± 2.7$^{\#}$	35.3 ± 2.9	35.2 ± 4.4
Δ Calf circumference (%)		6.8 ± 8.0†		2.9 ± 2.7		−0.1 ± 11.1

Values are expressed mean ± SD. Examination of statistical significance was followed by paired *t*-test. VALVE, cardiac valve surgery group; CABG, coronary artery bypass grafting group; MI, acute myocardial infarction group; peak $\dot{V}O_2$, peak oxygen consumption; AT, anaerobic threshold; RER, respiratory exchange ratio; Ex, extensor torque; Flex, flexor torque; BW, body weight.

$^{\#}$ Denotes statistically significant difference from initial value within group ($p < 0.001$).

† Denotes statistically significant difference from the value of MI ($p < 0.01$).

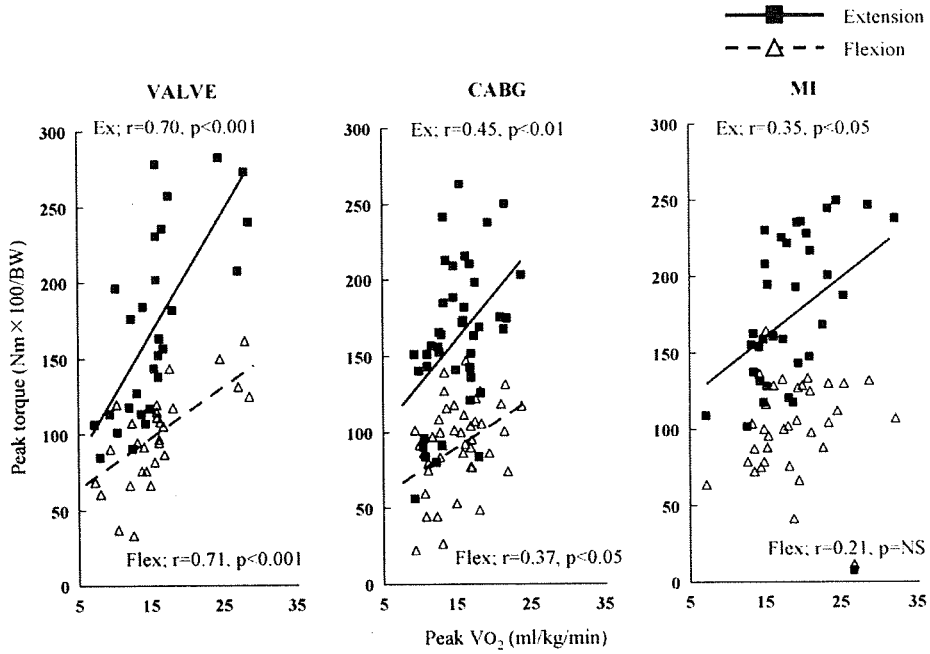

Figure 1 Correlations between peak $\dot{V}O_2$ and peak knee torques before cardiac rehabilitation in each group. VALVE, cardiac valve surgery group; CABG, coronary artery bypass grafting group; MI, acute myocardial infarction group; Ex, knee extensor torque; Flex, knee flexor torque.

Exercise tolerance and muscle strength

At the baseline, the peak $\dot{V}O_2$ (15.1 ± 3.7 ml $kg^{-1} min^{-1}$) vs. 18.5 ± 5.1 ml $kg^{-1} min^{-1}$, $p < 0.01$) and AT (9.2 ± 2.0 ml $kg^{-1} min^{-1}$ vs. 11.0 ± 3.0 ml $kg^{-1} min^{-1}$, $p < 0.01$) in the CABG group were significantly lower than those in the MI group (Table 2). After 6 months, the peak $\dot{V}O_2$, AT, and peak lower limb torques significantly increased in all groups (all $p < 0.01$). There were no differences in the peak $\dot{V}O_2$, AT, or peak torques of the knee extensor/flexor muscles among the three groups after CR. The value of the peak respiratory exchange ratio was similar in the three groups before and after CR. Most patients stopped exercise testing due to leg fatigue and few patients were terminated due to myocardial ischemia (CABG, $n = 1$; MI, $n = 1$) before CR. The reason for stopping exercise was similar after 6 months (leg fatigue, $n = 102$; ischemia, $n = 2$).

Circumferences of thigh and calf

At the baseline, the circumference of thigh was significantly smaller in the VALVE group than in the MI group (47.5 ± 4.2 cm vs. 50.7 ± 3.9 cm, $p < 0.01$) (Table 2). After 6 months, the circumferences of thigh and calf significantly increased in the VALVE and CABG groups (all $p < 0.001$). Although there were no differences in the percent changes of thigh circumferences among the three groups, the percent changes of calf circumferences were significantly larger in the VALVE group than in the MI group ($6.8 \pm 8.0\%$ vs. $-0.1 \pm 11.1\%$, $p < 0.01$).

Correlations among peak $\dot{V}O_2$, peak torque, and thigh/calf circumferences

At the baseline, significant positive correlations between the peak $\dot{V}O_2$ and knee extensor torque were observed in all groups (VALVE: $r = 0.70$, $p < 0.001$; CABG: $r = 0.45$, $p < 0.01$; and MI: $r = 0.35$ $p < 0.05$) (Fig. 1). There were positive correlations between the peak $\dot{V}O_2$ and knee flexor torque in the CABG and VALVE groups, but not in the MI group (VALVE: $r = 0.71$, $p < 0.001$; CABG: $r = 0.37$, $p < 0.05$; and MI: $r = 0.21$, $p = NS$). There were positive correlations between the knee extensor torques and thigh circumferences in the CABG and VALVE groups, but not in the MI group (VALVE: $r = 0.41$, $p < 0.05$; CABG: $r = 0.32$, $p < 0.05$; and MI: $r = 0.21$, $p = NS$). There were positive correlations between the knee extensor torques and calf circumferences in the CABG and VALVE groups, but not in the MI group (VALVE: $r = 0.46$, $p < 0.05$; CABG: $r = 0.37$, $p < 0.05$; and MI: $r = 0.25$, $p = NS$). There were positive cor-

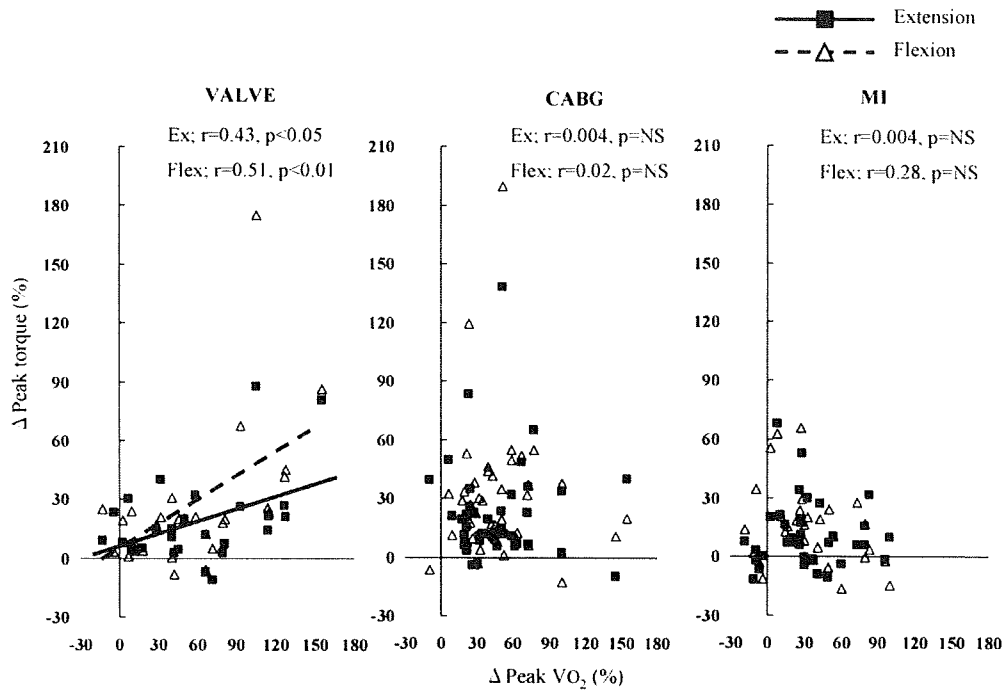

Figure 2 Correlations between changes of peak $\dot{V}O_2$ and peak knee torques before and after cardiac rehabilitation in each group. VALVE, cardiac valve surgery group; CABG, coronary artery bypass grafting group; MI, acute myocardial infarction group; Ex, knee extensor torque; Flex, knee flexor torque.

relations between the knee flexor torques and calf circumferences in the CABG and VALVE groups, but not in the MI group (VALVE: $r = 0.53$, $p < 0.01$; CABG: $r = 0.30$, $p < 0.05$; and MI: $r = 0.27$, $p = NS$).

Correlations between Δpeak $\dot{V}O_2$ and Δpeak torque

The percent changes of peak $\dot{V}O_2$ were significantly correlated with those of knee extensor and knee flexor torques in the VALVE group (Ex: $r = 0.43$, $p < 0.05$; Flex: $r = 0.51$, $p < 0.01$) (Fig. 2). However, no correlation between the percent changes of peak $\dot{V}O_2$ and those of extensor or flexor torques was observed in the CABG or MI groups (CABG: Ex $r = 0.004$, Flex $r = 0.02$; and MI: Ex $r = 0.004$, Flex $r = 0.28$, all $p = NS$). No significant correlation between the percent changes of thigh/calf circumferences and those of exercise capacity was observed in all groups.

Discussion

The present study demonstrated the following major findings: (1) the combined aerobic and resistance training for 6 months significantly increased exercise tolerance and lower limb muscle strength in all groups; (2) the circumferences of the thigh and calf significantly increased in the VALVE and CABG groups; and (3) the improvement in exercise tolerance was significantly correlated with the changes in lower limb muscle strength in the VALVE group, but not in the CABG nor MI group. To the best of our knowledge, this is the first report demonstrating the effect of CR with respect to exercise tolerance, thigh/calf circumferences, and muscle strength in patients after cardiac valve surgery, patients after CABG, and patients with acute MI, simultaneously.

It has been clearly established that exercise tolerance measured by oxygen consumption, is a good predictor of prognosis in patients with cardiovascular diseases [2,11]. In addition, muscular strength is also associated with all-cause mortality [12,13]. Muscular strength is one of the determinant factors of exercise tolerance; however, there are few published data regarding these correlations before and after CR. In the present study, the supervised CR with home-based exercise significantly increased exercise tolerance and lower limb muscle strength without a serious incident during the entire training period. As is widely accepted, these results suggest that hospital-based CR in patients with cardiac disease is safe and can be beneficial [1–3,5–9].

In the present study, the improvement in exercise tolerance was significantly correlated with the changes in lower leg muscle strength only in the VALVE group. It has been demonstrated that patients who received heart valve surgery and patients with congestive heart failure have several disorders, including a disrupted central cardiac function, peripheral maladaptations such as an abnormal skeletal muscle morphology and metabolism, increased oxidative stress, and an inappropriate neurohumoral axis [5,12,14]. Volterrani et al. demonstrated that the muscle strength of the quadriceps and the cross-sectional area of the thigh were correlated with the peak $\dot{V}O_2$ in patients with chronic heart failure [15]. In the present study, the circumference of thigh was smaller in the VALVE group than in the other groups, especially in the MI group (Table 2). Moreover, the change in circumference of the calf was larger than in the MI group. These data might support the reason why the improvement in exercise tolerance was significantly correlated with changes in muscle strength in the VALVE group. The lower limb circumference may be a good marker of physical deconditioning in patients after open-heart surgery and patients with acute MI. The circumference of lower leg includes not only the muscle diameter, but also the thickness of soft tissue, such as subcutaneous fat. In the present study, there were significant positive correlations between the circumference of thigh/calf and lean body mass (LBM) at baseline (data not shown). The LBMs in the VALVE and the CABG groups, but not the MI group, significantly increased after 6 months, as well as the circumferences of thigh and calf. In addition, the percent changes of the circumference of thigh and calf were significantly correlated with the percent change of LBM (data not shown). Therefore, we believe that the circumference in thigh and calf may reflect, at least in part, muscle volume. In general, the duration of deconditioning is much longer in patients with valvular disease than other patients, such as those with acute MI. The mean duration from the diagnosis or onset of clinical symptoms to operation in the VALVE group was 4.7 years. We speculate that the deconditioning of the skeletal muscle structure and metabolism was ameliorated by CR, resulting in improved exercise tolerance, especially in the VALVE group. Further prospective investigations are needed to clarify these points.

Resistance training has been recently recommended in CR for not only patients with atherosclerotic diseases, but also patients with chronic heart failure [12,16]. Indeed, dynamic moderate-load resistance training, such as that found in our exercise program, has been established as a safe and effective mode of exercise in CR [16–19]. Hare et al. reported that resistance training resulted in increased muscle strength and endurance, whereas the peak $\dot{V}O_2$ did not increase [16]. Numerous studies have shown that aerobic training improves exercise tolerance and muscle strength in patients following open-heart surgery and patients with MI [20–24]. However, the peak $\dot{V}O_2$ increased by less than 25% after exercise training, especially in patients who had undergone cardiac valvular surgery [25,26]. In contrast, improvement of the peak $\dot{V}O_2$ was approximately 50% in the VALVE group after CR with combined aerobic and resistance training in the present study. The obvious amelioration might have been derived from the combination of aerobic and resistance training rather than from aerobic or resistance training alone. Marzolini et al. demonstrated that combined aerobic and resistance training elicited more marked changes in physiological adaptations such as muscular strength and endurance, and body composition compared to aerobic training alone in patients with coronary artery disease [27]. We believe that an increased lower limb muscle strength might be an effective strategy for improving exercise tolerance in patients after cardiac valve surgery and patients with chronic heart failure.

There are several limitations in this study. First, we cannot conclude that the improvement in exercise tolerance and muscle strength was only caused by CR because patients in both VALVE and CABG groups underwent open-heart surgery. However, as mentioned above, the improvement in exercise tolerance was clearly greater than in previous reports [25,26]. We therefore believe that CR improves exercise tolerance through the amelioration of peripheral deconditioning in patients after cardiac valvular surgery. Another limitation is that we obtained data regarding neither central function nor peripheral effects, such as muscular structure and metabolism, endothelial function, oxidative markers, nor neurohumoral factors. There are no data regarding pre-surgical state or home-based exercise volume in each group. We need to clarify the precise mechanism of improvement for CR and assess the effects of home-based exercise in the next step. Finally, the present investigation was a single center study with a small sample size. Further randomized investigations involving more subjects and institutions are required to confirm our findings.

In conclusion, exercise intolerance in patients after heart valve surgery may in part depend on decreased muscular strength. Further studies are

needed to assess whether the strategy of increasing muscular strength of lower limb by programmed resistance training could be effective for improving exercise intolerance in patients after heart valve surgery and symptomatic patients with heart failure.

Acknowledgment

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ORIGINAL ARTICLE

Effects of cardiac rehabilitation in patients with metabolic syndrome after coronary artery bypass grafting

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Exercise tolerance;
C-reactive protein

Summary
Background: Cardiac rehabilitation (CR) has numerous beneficial effects, including the modification of coronary risk factors and improvement of the prognosis, in patients with coronary artery disease (CAD). Limited data are available regarding the effects of CR on the physical status and risk factors in patients with metabolic syndrome (MetS) after coronary artery bypass grafting (CABG).
Methods and results: We enrolled 32 patients with MetS after CABG, who participated in a supervised CR program for 6 months. Metabolic parameters, blood chemistry, exercise tolerance, and muscle strength of the thigh were measured before and after CR. After CR: (1) the body mass index, waist circumference, and fat weight significantly decreased; (2) peak $\dot{V}O_2$ and anaerobic threshold were significantly increased; (3) isokinetic peak torques of knee extensor and flexor muscles significantly increased; (4) metabolic scoring defined by the number of the modified Adult Treatment Panel criteria of the US National Cholesterol Education Program

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was significantly improved; (5) serum concentration of high-sensitivity C-reactive protein also significantly decreased.
Conclusions: These results suggest that CR might be useful for patients with MetS after CABG.

Metabolic syndrome (MetS), which is a clustering of cardiac risk factors caused by visceral fat accumulation, is closely linked with the initiation and progression of cardiovascular disease [1,2]. The accumulation of visceral fat is essential for the diagnosis of the MetS, which is considered to locate upstream of multiple risk factors [3]. The main concept of the MetS is to recognize the effective reduction of multiple coronary risk factors and subsequent cardiovascular diseases by the reduction of visceral fat [3]. Recently, much attention has focused on the relationship of inflammation in adipose tissue with the pathogenesis of MetS and atherosclerotic diseases [2–5]. It is clear that cardiac rehabilitation (CR) has numerous benefits involving not only modulating risk factors, but also preventing future cardiac events [6,7]. However, the impact of CR on metabolic parameters in patients with MetS after coronary artery bypass grafting (CABG) is still unclear. The purpose of the present study was to investigate the clinical usefulness of CR, including the improvement of metabolic risk factors and the inflammatory state, in patients with MetS after CABG.

Methods

Subjects

We enrolled 32 patients with MetS, defined by the modified Adult Treatment Panel criteria of the US National Cholesterol Education Program (waist size: male ≥85 cm and female ≥90 cm) after CABG at Juntendo University Hospital. All patients participated in CR after CABG (5–14 days). Patients with ongoing congestive heart failure, liver dysfunction, renal failure (creatinine ≥2.0 mg/dL), or systemic diseases, including malignancy and collagen disease, were excluded. All patients received anti-platelet therapy. The subjects took the following medications: calcium-channel blockers, $n=18$; beta-blockers, $n=13$; angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers, $n=15$; statins, $n=14$; oral hypoglycemic agents, $n=8$ and insulin, $n=5$. No subjects underwent a change of medication throughout the study period except for three patients newly treated with statins. Subjects received full verbal and written explanations of the nature and purposes of the study, and each gave written informed consent. This study was approved by the Ethical Committee of Juntendo University.

Rehabilitation protocol

Supervised CR, which was composed of warm-up stretching, aerobic exercise, resistance training, and cool-down stretching, was performed once or twice a week for 6 months, as we described previously [8,9]. Aerobic exercise consisted of a cycle ergometer, treadmill, and walking on an in-room track. The total aerobic exercise time was approximately 60 min. The exercise intensity was prescribed individually at the anaerobic threshold (AT) level, as measured by an ergometer test using expiratory gas analysis or a rating of 11–13 (from fairly light to somewhat hard) on the standard Borg's perceived exertion scale. Resistance training, which was gradually added to the exercise program at least 6 weeks after the beginning of participation, consisted of four types of training (sit-ups, back kicks and front raises, squats and push-ups) using the patients' own weight. In addition, patients were encouraged to perform home-based aerobic exercise for more than 20 min at a rating of 11–13 of the perceived exertion on Borg's scale twice a week. All subjects were instructed to follow the phase II diet of the American Heart Association after CABG at the beginning. An educational program was also provided for each subject by physicians, nurses, and dietitians regarding ischemic heart disease and risk factors at the baseline.

Measurements

We assessed body composition, exercise tolerance, and muscle strength before and after CR. Anthropometric parameters were assessed using the body mass index (BMI) and waist circumference. The percentages of body fat and lean body weight were measured by a BOD POD® (Life Measurement, Inc., Concord, CA, USA), as we described previously

[8,9]. To measure peak oxygen consumption (peak $\dot{V}O_2$) and the AT, patients underwent ergometer testing (Corival 400, Lode B.V. Groningen, Netherlands) using an expiratory gas analysis machine (Vmax-29S, SensorMedics Co., Yorba Linda, CA, USA). After a period of resting, warm-up was performed for a few minutes at 20 W, followed by ramp loading (15 W/min) until subjective exhaustion, progressive angina, ST-segment depression (≥2 mm), or sustained tachyarrhythmia. The point of AT was determined by the 'V-slope' method. The power of the thigh muscles was measured using the Cybex770 system (Cybex Division of Lumex, Ronkonkoma, NY, USA), as we reported previously [8,9]. The isokinetic peak torques of the knee extensor and flexor muscles were measured at 60°/s, and those were adjusted by body weight according to the following formula: strength (Nm) × 100/body weight (kg). Serum lipid profiles, including total cholesterol (TC), triglycerides (TG), and high-density lipoprotein cholesterol (HDL-C), and high-sensitivity C-reactive protein (hsCRP) were determined by standard methods using an auto-analyzer after at least 12 h of fasting before and 6 months after CABG. Concentrations of low-density lipoprotein cholesterol (LDL-C) were calculated with the Friedewald equation using the concentrations of TC, HDL-C, and TG.

Definition of MetS score

In the present study, the MetS score (from 0 to 5) was defined by the number of criteria of the modified Adult Treatment Panel criteria of the National Cholesterol Education Program. Each criterion, excluding waist size (male ≥85 cm and female ≥90 cm), was utilized following the original definition [10].

Statistical analysis

The results are expressed as the mean value ± standard deviation. Data at the baseline and after 6 months were compared in each patient by the paired *t*-test. A *p*-value of less than 0.05 was considered significant.

Results

Baseline characteristics

The baseline characteristics of the subjects are presented in Table 1. The prevalence of hypertension, diabetes, and dyslipidemia, was relatively high, because this study consisted of patients with MetS. Ninety-four percent of the subjects underwent an off-pump operation, and the mean number of times performing CR was 34.

Table 1 Baseline characteristics.

N	32
Age (year)	66 ± 10
Male (%)	29 (97)
Body mass index (kg m^{-2})	25.0 ± 2.7
Hypertension (%)	30 (94)
Diabetes mellitus (%)	20 (63)
Dyslipidemia (%)	23 (77)
% fat	28.7 ± 5.5
Off pump (%)	30 (94)
Number of bypass grafting (*n*)	3.4 ± 1.3
Exercise in hospital (times)	34 ± 12

Data are presented as the mean value ± S.D.

Parameters after CR for 6 months

Physiological variables

Physiological variables at the baseline and after CR are presented in Table 2. After CR, waist size (from 87.1 ± 8.5 to 84.0 ± 6.9 cm, $p < 0.01$), body weight (from 65.4 ± 9.5 to 63.5 ± 8.4 kg, $p < 0.01$), fat weight (from 18.5 ± 5.1 to 17.2 ± 4.7 kg, $p < 0.05$), and % fat (from 28.7 ± 5.5 to 26.8 ± 6.0%, $p < 0.01$) were significantly decreased and the lean body weight was increased (from 45.6 ± 6.9 to 46.5 ± 6.7 kg, $p < 0.005$).

Exercise tolerance and strength of thigh muscles

Exercise tolerance and the strength of the thigh muscles at the baseline and after CR are presented in Figs. 1 and 2, respectively. Peak $\dot{V}O_2$ and AT significantly increased after CR (from 14.2 ± 3.7 to 19.2 ± 5.2 ml·kg^{-1}·min^{-1}), $p < 0.001$, and 8.8 ± 1.9 to 11.5 ± 3.1 ml·kg^{-1}·min^{-1}, $p < 0.001$, respectively). The isokinetic peak torques of the knee extensor and flexor muscles were significantly increased after CR (from 149 ± 41 to 169 ± 47 Nm·kg^{-1} × 100, $p < 0.001$, and 85 ± 28 to 98 ± 30 Nm·kg^{-1} × 100, $p < 0.001$, respectively).

Table 2 Physiological variables.

	Pre	Post	*p*
Waist (cm)	87.1 ± 8.5	84.0 ± 6.9	<0.001
Weight (kg)	65.4 ± 9.5	63.5 ± 8.4	0.0005
Fat weight (kg)	18.5 ± 5.1	17.2 ± 4.7	0.019
Lean weight (kg)	45.6 ± 6.9	46.5 ± 6.7	0.003
% fat	28.7 ± 5.5	26.8 ± 6.0	0.008

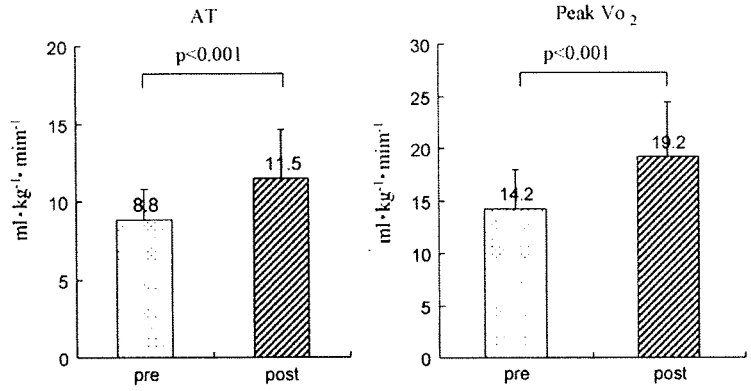

Figure 1 Left: change of peak $\dot{V}O_2$ (oxygen uptake) between pre- and post-training. Right: AT (anaerobic threshold) between pre- and post-training.

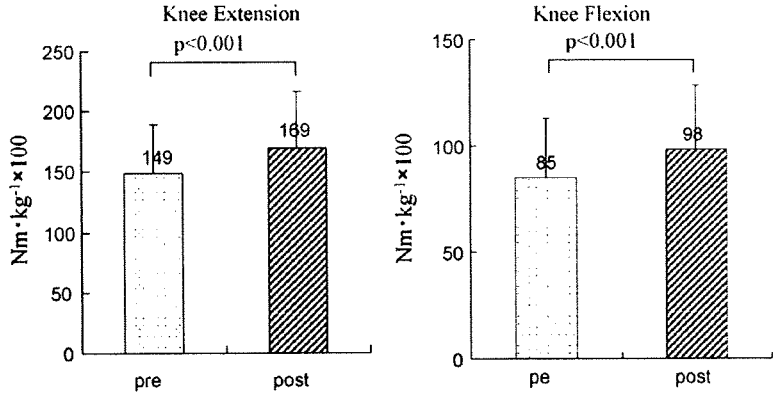

Figure 2 Left: change of knee extension between pre- and post-training. Right: knee flexion between pre- and post-training.

Serum lipid profiles, glucose parameters, and blood pressure

Serum lipid profiles, glucose parameters, and blood pressure at the baseline and after CR are presented in Table 3. Serum concentrations of TC and LDL-C were significantly decreased after CR (from 199 ± 34 to 182 ± 28 mg/dL, $p < 0.01$ and from 122 ± 29 to 111 ± 22 mg/dL, $p < 0.05$, respectively). However, no significant changes were observed in other values.

Table 3 Clinical variables pre- and post-cardiac rehabilitation.

	Pre	Post	p
TC (mg/dL)	199 ± 34	182 ± 28	0.008
LDL-C (mg/dL)	122 ± 29	111 ± 22	0.047
TG (mg/dL)	174 ± 74	139 ± 66	0.060
HDL-C (mg/dL)	43 ± 13	44 ± 11	0.602
FBS (mg/dL)	135 ± 45	131 ± 50	0.420
HbA1c (%)	6.2 ± 1.3	6.2 ± 1.3	0.613
SBP (mmHg)	140 ± 16	134 ± 19	0.143
DBP (mmHg)	76 ± 11	76 ± 8	0.897

Data are presented as the mean value ± S.D. TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; FBS, fasting blood sugar; HbA1c, hemoglobin A1c; SBP, systolic blood pressure; DBP, diastolic blood pressure.

Serum levels of hsCRP

Serum levels of hsCRP were significantly decreased after CR (from 0.30 ± 0.21 to 0.25 ± 0.29 mg/dL, $p < 0.001$) (Fig. 3). A significant decrease was also observed after the exclusion of three patients who were newly prescribed statins after CABG (from 0.27 ± 0.19 to 0.19 ± 0.18 mg/dL, $p < 0.005$).

MetS score

The MetS score was significantly decreased after CR (from 3.6 ± 0.7 to 2.4 ± 1.0, $p < 0.001$) (Fig. 4).

Discussion

The present study showed that CR for 6 months ameliorated not only metabolic parameters, but

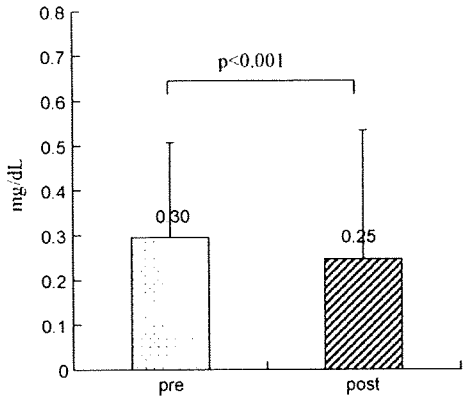

Figure 3 Change of CRP (C-reactive protein) between pre- and post-training.

also the exercise capacity, muscle strength, and inflammatory state in MetS patients after CABG.

The prevalence of MetS, closely linked to the initiation and progression of cardiovascular diseases, has markedly increased in not only developed countries, but also developing nations [11,12]. Indeed, we and other groups reported the impact of MetS on clinical outcomes in patients with coronary artery disease [11–13]. Although it has been elucidated that exercise decreases the prevalence of MetS [14–16], the beneficial effects of CR in patients with MetS, especially after CABG are still unclear. Milani and Lavie [17,18] reported that CR improved multiple metabolic derangements, however, the subjects consisted of patients with various diagnoses after major cardiac events. Shubair et al. [19] showed that CR resulted in a significant improvement in the cardiovascular risk profiles, including body weight, lipid profile, blood glucose, and exercise capacity, however, this study also consisted of patients with various diagnoses of coronary artery disease. Patients who need to undergo CABG show a high risk based on clinical findings, such as multivessel disease and diabetes [20]. It is clear that these clinical manifestations are associated with a poor outcome in the clinical setting [21,22]. Therefore, it is very important to investigate the efficacy of CR for improving metabolic parameters in patients after CABG.

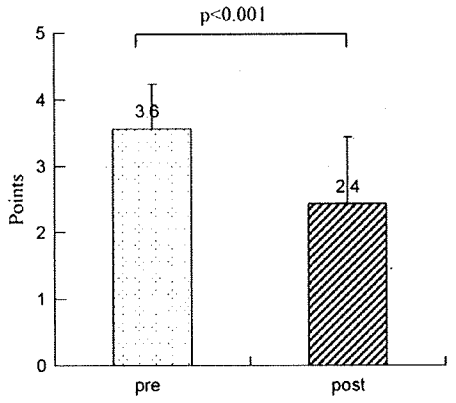

Figure 4 Change of MetS (metabolic syndrome) score between pre- and post-training.

It has been clearly established that exercise tolerance is a good predictor of the prognosis in patients with cardiovascular diseases [23,24]. In addition, muscular strength is also associated with all-cause mortality [25]. A report from the WHO suggested that increased muscle strength results in an improved long-term prognosis [26]. The present study did not include non-participating patients. It is possible that exercise tolerance might be improved by not only the natural course after CABG, but also the effect of CR. Indeed, Perk and Hedback reported that patients participating in CR following CABG showed a greater increase in work capacity and a more favorable effect on myocardial oxygen consumption compared with non-participating patients [27,28]. Our recent study also demonstrated that muscle strength of the lower limbs did not change in non-participating patients with coronary artery disease, including post CABG [9]. Therefore, we believe that CR, at least partly, brought about the amelioration of exercise tolerance and muscular strength, and may be an important strategy in patients with MetS after CABG.

Chronic inflammation plays an important role in the initiation and progression of atherosclerosis [2,5,29,30]. Indeed, circulating levels of hsCRP, which is one of the acute phase-reactant proteins in inflammatory reactions, are elevated in patients with coronary heart diseases [26,29]. Moreover, high levels of hsCRP can predict mortality and future cardiac events in various patients with cardiovascular diseases [31]. Some previous studies have reported the improvement of hsCRP elevation in participants with CR [32,33]. The result of hsCRP reduction in patients with CR after CABG is consistent with previous results. No reports have investigated the changes of hsCRP comparing CR and non-CR groups in patients after CABG. Previous studies showed that the levels of hsCRP remained unchanged in the control group after percutaneous coronary intervention [34,35]. Eyileten et al. reported that hsCRP levels significantly decreased 1 month after CABG. However, the subjects in their study must be different from those of the present study. Because the mean levels of hsCRP in Eyileten's study were relatively high (from 1.315 ± 0.240 to 0.725 ± 0.189 mg/dL), suggesting that subjects who had ongoing acute inflammation

were included [36]. Moreover, the levels of hsCRP at the baseline were significantly higher in patients with than in those without MetS after CABG (data not shown), suggesting that CR is a useful strategy to improve the inflammatory state even in MetS patients after CABG.

Limitations

There are several limitations to the present study. First, this is a small sample-sized study. Second, the supervised exercise in the outpatient clinic was performed only once or twice a week. Therefore, the protocol of the present study might not be sufficient to improve the metabolic status, such as TG and HDL-C levels. Third, the present study consisted of only subjects who participated in CR after CABG. We assessed the levels of serum lipids and hsCRP in 14 patients (12 males, 66 ± 8 years) with MetS, who did not participate in supervised CR after elective CABG in the outpatient clinic because of difficulty undergoing CR (a long distance from home to hospital) during the same period. Before and 6 months after CABG, no significant changes in TC (from 189 ± 26 to 208 ± 37 mg/dL, $p = 0.11$), LDL-C (from 112 ± 23 to 125 ± 34 mg/dL, $p = 0.25$), TG (from 214 ± 72 to 212 ± 97 mg/dL, $p = 0.93$), or hsCRP (from 0.22 ± 0.13 to 0.26 ± 0.22 mg/dL, $p = 0.51$) were observed. The results of this study need to be confirmed using a prospective and randomized study design involving patients participating and not participating in CR. However, we believe that the present study is a pioneering and valuable report promoting further investigations.

Conclusion

CR might have effects on not only the modification of metabolic risk factors, but also improving exercise tolerance and muscular strength in patients with MetS after CABG. Further studies with a control group are required to confirm these findings and to assess the efficacy of CR for improving the clinical prognosis in MetS patients after CABG.

Acknowledgments

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ORIGINAL ARTICLE

Serum levels of remnant lipoprotein cholesterol and oxidized low-density lipoprotein in patients with coronary artery disease

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Coronary artery disease;
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Summary
Background: Oxidized low-density lipoprotein (OxLDL) and remnant lipoprotein play a crucial role in the development of atherosclerosis. Recently, a novel method for measuring remnant cholesterol levels (remnant lipoproteins cholesterol homogenous assay: RemL-C) has been established. However, the correlation between OxLDL and remnant lipoprotein, including RemL-C, has not been fully investigated.
Methods: We enrolled 25 consecutive patients with documented coronary artery disease (CAD) and 20 controls. Remnant-like particle cholesterol (RLP-C) and RemL-C were used to determine the levels of remnant lipoprotein cholesterol. Serum levels of malondialdehyde-modified LDL (MDA-LDL) and OxLDL using a monoclonal antibody DLH3 (OxPC) were used to measure the concentration of circulating OxLDL.
Results: The CAD group had high levels of fasting glucose and glycosylated hemoglobin (HbA1c), and low levels of high-density lipoprotein cholesterol compared with the control group. Serum levels of total cholesterol or LDL cholesterol were not significantly different between the two groups. The levels of RemL-C ($p=0.035$), MDA-LDL ($p=0.018$), and MDA-LDL/LDL-C ($p=0.036$) in the CAD group were significantly higher than those in the control group. The levels of RLP-C tended to be higher in the CAD group than those in the control group ($p=0.096$). Positive correlations were demonstrated between remnant lipoprotein cholesterol and OxLDL (RLP-C and MDA-LDL/LDL-C, $r=0.45$, $p=0.0024$, RLP-C and OxPC, $r=0.51$, $p=0.0005$, RemL-C and MDA-LDL/LDL-C, $r=0.42$, $p=0.0044$, RemL-C and OxPC, $r=0.43$, $p=0.0043$).

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Similar trends were observed in non-diabetic subjects and in subjects without metabolic syndrome. Positive correlations were also observed between RLP-C and RemL-C ($r = 0.94$, $p < 0.0001$) and between MDA-LDL/LDL-C and OxPC ($r = 0.40$, $p = 0.0074$).
Conclusions: These results suggest that the association between high levels of remnant lipoprotein cholesterol and high OxLDL levels might be linked to atherogenesis in patients with CAD.

Introduction

Remnant lipoproteins, which are produced by hydrolysis of chylomicrons and very low-density lipoproteins, are thought to be atherogenic [1,2]. Remnant lipoproteins, not only activate surface molecules of monocytes and endothelial cells, but also induce foam cell formation and proliferation of smooth muscle cells [2]. Indeed, high levels of remnant-like particles cholesterol (RLP-C) determined by a widely used method, are considered to be a coronary risk factor and a predictor of cardiovascular events independent of high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol in healthy subjects and patients with coronary artery disease (CAD) [3–6].

Oxidized low-density lipoprotein (OxLDL) plays a crucial role in atherogenesis via a number of initiative and accelerative functions, including adherence induction between endothelial cells and monocytes, recruitment of monocyte-derived macrophages into the vessel wall, and foam cell formation of macrophages [7–9]. Previous reports have shown that the level of circulating OxLDL is a marker for identifying patients with CAD or coronary spastic angina [10–12], and has a positive relationship with acute coronary syndromes [13,14]. Moreover, we and other groups demonstrated that high levels of OxLDL are independent predictors of future cardiovascular events in apparently healthy subjects and patients with CAD [15–18].

Until now, several methodologies have been available for determining the levels of remnant lipoprotein cholesterols as well as circulating OxLDL [8,9,19]. In the present study, we measured the levels of remnant lipoprotein cholesterols determined by RLP-C and a recently established method (remnant lipoproteins cholesterol homogenous assay: RemL-C), malondialdehyde-modified (MDA)-LDL, and oxidized phosphatidylcholine (OxPC) in patients with CAD and control subjects. Moreover, we assessed each correlation between remnant lipoprotein cholesterol and OxLDL in those subjects.

Methods

Subjects

We enrolled 25 consecutive patients who underwent diagnostic angiography at Juntendo University between August 2006 and October 2006, and 20 controls who had no clinical history of CAD and hospitalization at the same period. All patients had documented CAD defined as more than 50% stenosis in at least one major coronary artery. Patients with acute coronary syndrome and/or ongoing congestive heart failure were excluded. Control subjects had no abnormal electrocardiographic finding and no evidence of coronary ischemia examined by stress cardiac testing at our outpatient clinic. Subjects who had liver and/or renal dysfunction, or were taking medications, including insulin, lipid-lowering drugs, and vitamin E were also excluded. All subjects gave written informed consent and the Ethical Committee of the Institution approved this study.

Blood sampling and biochemical analysis

Whole blood samples were drawn after overnight fasting. Serum levels of total cholesterol, triglyceride (TG), HDL cholesterol, and high sensitivity C-reactive protein (hs-CRP), were measured by standard methods. LDL cholesterol values were measured by the direct assay (Sekisui Medical Co., Ltd., Tokyo, Japan). Serum levels of RLP-C were measured by widely using an immunoaffinity mixed gel containing anti-apolipoprotein A-1 and anti-apolipoprotein B-100 monoclonal antibodies method (JIMRO II, Otsuka Pharmaceutical Co., Ltd., Tokyo, Japan), as previously described [3,20,21]. RemL-C, which has recently been established, was

also employed in this study (Kyowa Medex Co., Ltd., Tokyo, Japan). In brief, RemL-C utilizes a selective solubilizing and degradative method by using surfactant and phospholipase-D. After this reaction, released cholesterol was measured enzymatically [22]. The levels of malondialdehyde-modified LDL (MDA-LDL) recognized by the monoclonal antibody, ML25, and OxPC defined by the monoclonal antibody, DLH3, were measured by enzyme-linked immunosorbent assay, as we and other groups previously reported [8,16,17,23,24].

Statistical analysis

Statistical intergroup differences were analyzed by the Chi-square test and the Student's *t*-test. Correlation between the two parameters was determined by simple linear regression analysis. A value of $p < 0.05$ was considered to be significant.

Results

Characteristics of the study subjects

The characteristics of the subjects are shown in Table 1. There was no significant difference in body mass index, prevalence of hypertension, smoking history, or family history, between the two groups. The CAD group had a higher prevalence of diabetes mellitus ($p = 0.012$) and metabolic syndrome ($p = 0.005$) defined by the Evaluation Committee of Diagnostic Criteria for Metabolic syndrome [25], and significantly lower levels of HDL cholesterol ($p = 0.003$) than the control group. The levels of fasting glucose and glycosylated hemoglobin (HbA1c) in the CAD group were significantly higher than in the control group ($p = 0.019$, $p = 0.001$, respectively). Total cholesterol and LDL cholesterol levels were not significantly different between the two groups.

Comparison of RLP-C, RemL-C, MDA-LDL, and Ox-PC between the two groups

As shown in Fig. 1, the CAD group had significantly higher levels of RemL-C than the control group (12.3 ± 9.0 mg/dl vs. 7.4 ± 5.1 mg/dl, $p = 0.035$). The level of RLP-C tended to be higher in the CAD group than that in the control group (9.1 ± 8.6 mg/dl vs. 5.7 ± 3.3 mg/dl, $p = 0.096$). As demonstrated in Fig. 2, the level of MDA-LDL and the ratio of MDA-LDL to LDL cholesterol (MDA-LDL/LDL-C) were significantly higher than those in the control group (181 ± 53 IU/l vs. 147 ± 36 IU/l, $p = 0.018$; 1.36 ± 0.36 vs. 1.16 ± 0.21, $p = 0.036$, respectively). There was no significant difference of Ox-PC level between the two groups (9.5 ± 6.9 IU/l vs. 8.0 ± 2.9 IU/l, $p = 0.38$).

The levels of RLP-C (6.6 ± 3.5 mg/dl vs. 4.5 ± 1.8 mg/dl, $p = 0.050$) and RemL-C (9.0 ± 5.2 mg/dl vs. 5.9 ± 3.7 mg/dl, $p = 0.066$) tended to be higher in the CAD group than in the control group even in subjects without metabolic syndrome. The levels of RLP-C (6.9 ± 3.1 mg/dl vs. 5.4 ± 3.2 mg/dl, $p = 0.1$) and RemL-C (9.9 ± 4.7 mg/dl vs. 7.0 ± 4.9 mg/dl, $p = 0.085$) tended to be higher in the CAD group than in the control group in subjects without diabetes. The levels of MDA-LDL were significantly higher in the CAD group than in the control group in the subjects without diabetes (193 ± 61 IU/l vs. 144 ± 36 IU/l, $p = 0.0065$).

Associations between remnant lipoprotein cholesterol and OxLDL

As shown in Fig. 3, the levels of RLP-C were positively correlated with MDA-LDL/LDL-C ($r = 0.45$, $p = 0.0024$) and OxPC ($r = 0.51$, $p = 0.0005$) in all subjects. The serum levels of RemL-C were also positively correlated with MDA-LDL/LDL-C ($r = 0.42$, $p = 0.0044$) and OxPC ($r = 0.43$, $p = 0.0043$) in all subjects. The positive correlations between RLP-C and MDA-LDL/LDL-C ($r = 0.43$, $p = 0.030$), RLP-C and OxPC ($r = 0.53$, $p = 0.0062$), RemL-C and MDA-LDL/LDL-C ($r = 0.46$, $p = 0.019$), and RemL-C and OxPC ($r = 0.53$, $p = 0.0059$) were also observed in the CAD group. The trends of positive correlations between RLP-C and MDA-LDL/LDL-C ($r = 0.40$, $p = 0.08$), and RemL-C and MDA-LDL/LDL-C ($r = 0.31$, $p = 0.1$) were observed in the control group.

The positive correlations between levels of RLP-C and OxPC ($r = 0.34$, $p = 0.057$), between levels of RLP-C and MDA-LDL/LDL-C ($r = 0.47$, $p = 0.0048$), between levels of RemL-C and OxPC ($r = 0.32$, $p = 0.071$), and between levels of RemL-C and MDA-LDL/LDL-C ($r = 0.42$, $p = 0.013$), were observed in non-diabetic patients as well as in all subjects (Fig. 4).

Correlations between each measurement of remnant lipoprotein cholesterol and OxLDL

As demonstrated in Fig. 5, a strong correlation was observed between RLP-C and RemL-C levels ($r = 0.94$, $p < 0.0001$). The MDA-LDL/LDL-C levels were positively correlated with OxPC ($r = 0.40$, $p = 0.0074$).

Table 1 Patient characteristics.

	Control	CAD	*p*-Value
Number	20	25	
Age (years)	58 ± 12	64 ± 12	0.126
Male (%)	16 (80)	23 (92)	0.239
BMI (kg/m^2)	24.5 ± 4.8	24.6 ± 2.4	0.935
Hypertension (%)	11 (55)	19 (76)	0.138
Diabetes mellitus (%)	1 (5)	10 (40)	0.012
Metabolic syndrome (%)	3 (15)	14 (56)	0.005
Smokers (%)	10 (50)	16 (64)	0.448
Family history (%)	3 (15)	5 (20)	0.922
Number of diseased vessels			
One (%)	–	9 (36)	
Two (%)	–	10 (40)	
Three (%)	–	6 (24)	
Gensini score	–	52.9 ± 48.6	
TC (mg/dl)	215 ± 28	220 ± 32	0.587
TG (mg/dl)	125 ± 89	189 ± 130	0.066
HDL-C (mg/dl)	60 ± 14	46 ± 16	0.003
LDL-C (mg/dl)	127 ± 27	135 ± 27	0.343
FBS (mg/dl)	99 ± 12	120 ± 36	0.019
HbA1c (%)	5.2 ± 0.5	6.3 ± 1.2	0.001
hs-CRP (mg/dl)	0.097 ± 0.152	0.133 ± 0.118	0.409

Data are mean ± S.D. CAD, coronary artery disease; BMI, body mass index; TC, total cholesterol; TG, triglyceride; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FBS, fasting blood sugar; hs-CRP, high-sensitivity C-reactive protein.

Discussion

This study demonstrated that: (1) patients with CAD had high levels of remnant lipoprotein cholesterol, especially when measured by RemL-C method; (2) CAD patients had high levels of MDA-LDL; (3) the serum levels of remnant lipoprotein cholesterol were positively correlated with OxLDL, suggesting that the association between high levels of remnant lipoprotein cholesterol and high OxLDL levels might be linked to atherogenesis in patients with CAD.

Recent prospective studies and meta-analysis clearly demonstrated that a high level of TG is an independent predictor for CAD independent of other cardiovascular risk factors [6,26–28]. Indeed, the deterioration of TG-rich lipoproteins, such as remnant lipoproteins, is frequently observed in high risk patients for CAD, such as patients with metabolic syndrome and/or diabetes [29–31]. Therefore, it is important to establish assays for the measurement of remnant lipoprotein. Four methods, including ultracentrifugation, polyacrylamide

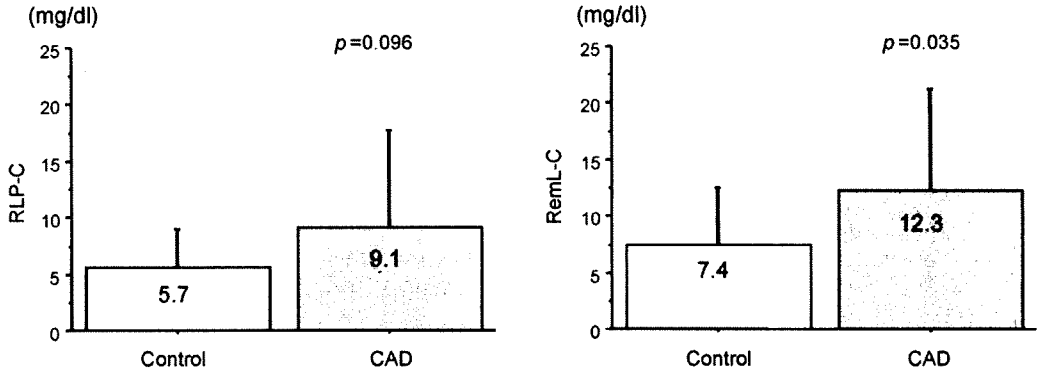

Figure 1 Comparison of serum levels of remnant lipoprotein cholesterol between the control and the CAD groups: CAD, coronary artery disease; RLP-C, remnant-like particle-cholesterol; RemL-C, remnant lipoproteins cholesterol homogeneous assay-cholesterol.

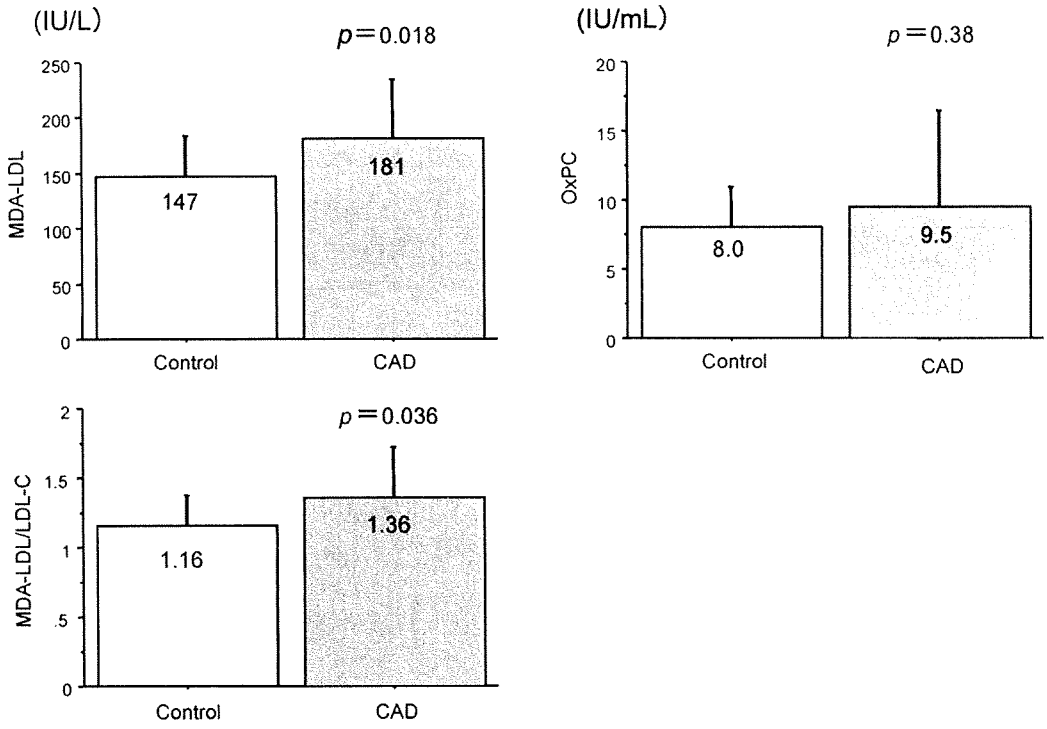

Figure 2 Comparison of serum levels of OxLDL between the control and the CAD groups: CAD, coronary artery disease; OxLDL, oxidized low-density lipoprotein; MDA, malondialdehyde; OxPC, oxidized phosphatidylcholine.

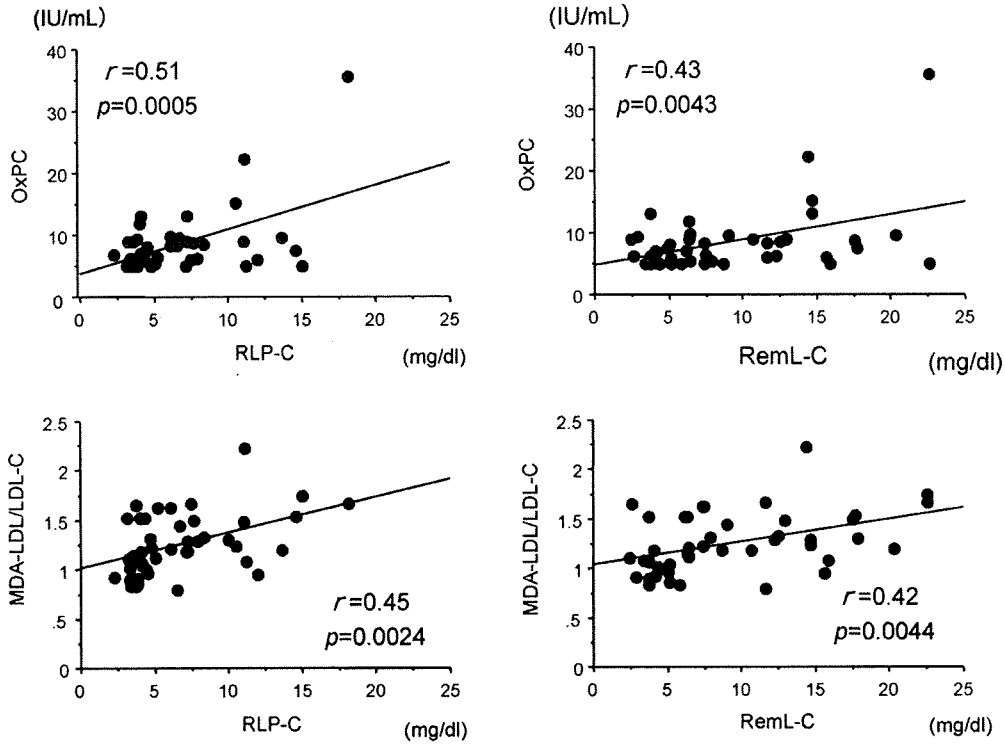

Figure 3 Correlations between remnant lipoprotein cholesterol and OxLDL in all subjects: RLP-C, remnant-like particle-cholesterol; RemL-C, remnant lipoproteins cholesterol homogeneous assay-cholesterol; OxLDL, oxidized low-density lipoprotein; MDA, malondialdehyde; OxPC, oxidized phosphatidylcholine.

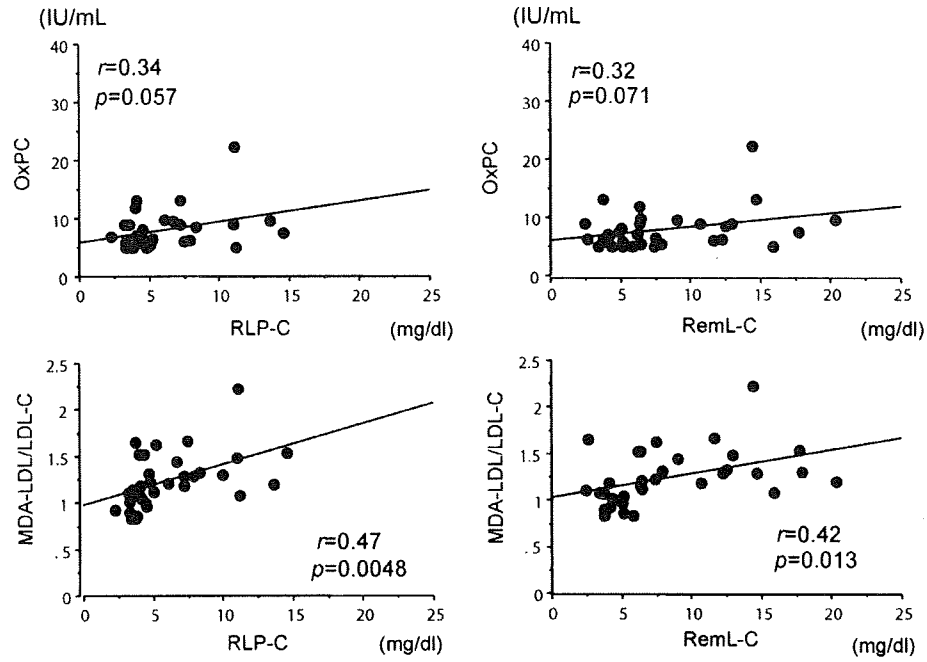

Figure 4 Correlations between remnant lipoprotein cholesterol and OxLDL in non-diabetic patients: RLP-C, remnant-like particle-cholesterol; RemL-C, remnant lipoproteins cholesterol homogeneous assay-cholesterol; OxLDL, oxidized low-density lipoprotein; MDA, malondialdehyde; OxPC, oxidized phosphatidylcholine.

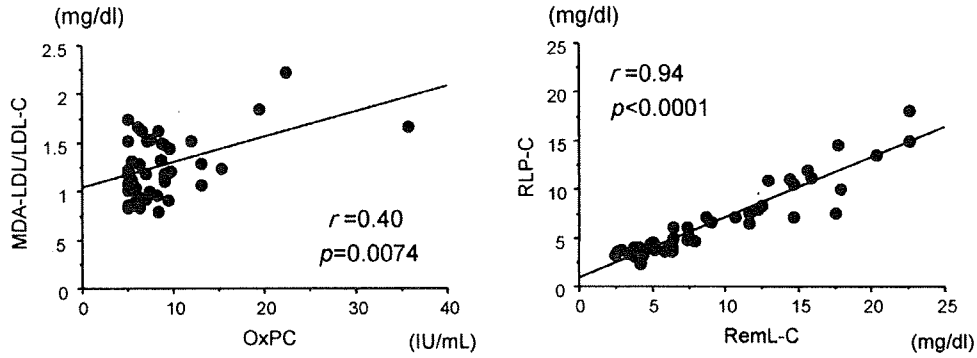

Figure 5 Correlations between each measurement of remnant lipoprotein cholesterol and OxLDL: RLP-C, remnant-like particle-cholesterol; RemL-C, remnant lipoproteins cholesterol homogeneous assay-cholesterol; OxLDL, oxidized low-density lipoprotein; MDA, malondialdehyde; OxPC, oxidized phosphatidylcholine.

gel electrophoresis, RLP-C, and automated analysis of RemL-C, are available for determining remnant lipoproteins levels. The former two methods have some limitations for widespread use in the clinical setting, as they are time- and labor-intensive, complicated to perform, and are complex to quantify. In contrast, the latter two methods, especially RLP-C, are commonly used to measure remnant lipoprotein cholesterol. The correlation between RLP-C and RemL-C levels was consistent with the previous study ($r = 0.95$) [22], however, serum levels of RemL-C, but not RLP-C, were significantly higher in patients with CAD than in the control subjects. One reason for this difference may be due to the small sample number of the present study. Another explanation might be derived from the difference in the method of measurement. The widely used RLP-C assay measures cholesterol levels in the heterogeneous unbound fraction of anti-apoA1 and anti-apoB-100 antibodies utilizing immuno-affinity methodology [3,20,21]. In contrast, the RemL-C assay, which can be carried out by an automated analyzer, utilizes selective agents, such as surfactant and phospholipase-D, for the determination of

remnant lipoproteins [22]. The RemL-C assay might be sensitive enough to identify high-risk subjects, such as patients with CAD.

Oxidative modification of LDL plays a crucial role in the pathogenesis of initiation and progression of atherosclerosis [7–9]. Until recently, several bioassay systems, such as MDA-LDL recognized by a monoclonal antibody, ML25, OxPC determined by the monoclonal antibody, DLH3, and OxLDL utilizing monoclonal antibodies, 4E6 and E06, for the measurement of circulating OxLDL have been developed [8,19,23,32,33]. In the present study, the serum levels of MDA-LDL, but not OxPC, were significantly higher in patients with CAD than in the control subjects. This finding may be caused by the difference in OxLDL determination measured from the use of different antibodies. ML25 for detecting MDA recognizes part of a lipid peroxide product [23], and DLH3 for determining OxPC levels specifically recognizes oxidized phosphatidylcholine [34]. Indeed, a modest correlation between MDA-LDL and OxPC levels was observed in this study (Fig. 3). Further analyses, including a large number of subjects, are needed to investigate the differences and clinical significance of each method.

The result of positive correlations between remnant lipoprotein cholesterols and OxLDL levels was a novel finding in the present study. Although the precise mechanism of these correlations is uncertain, the following possibilities are raised. Holvoet et al. reported that metabolic syndrome was associated with higher levels of OxLDL [35]. These authors also demonstrated the OxLDL levels positively correlated with waist circumstances, serum TG, insulin, and glucose levels, and were negatively correlated with levels of HDL cholesterol [35]. This study found positive correlations not only between body mass index and remnant cholesterol levels, but also between body mass index and OxLDL levels in the present subjects (data not shown). The levels of total cholesterol and LDL cholesterol in the present study were identical between the two groups. The features of the current patients with CAD might be represented by metabolic syndrome. It was reported that metabolic syndrome shows reduced concentrations of antioxidant vitamins [36]. In addition, the high levels of small dense LDL, which is more susceptible to oxidative stress, appear to be proportional to the degree of deterioration of TG rich lipoproteins, such as remnant particles, in metabolic syndrome [37]. However, the results of the present study have consistency regardless of diabetes or metabolic syndrome. Further studies are needed to clarify the reason of these correlations. Another possibility is that enhanced oxidative stress could be induced by low-grade inflammation in subjects with abdominal obesity [38,39]. These indirect mechanisms may be linked to positive correlations between remnant lipoprotein cholesterols and OxLDL levels.

The mechanics and kinetics of OxLDL in the blood stream remain unclear. It is possible that OxLDL might be partly released from atherosclerotic plaques in not only the coronary arteries, but also in the systemic arteries. The levels of OxLDL correlated with plaque morphology, especially macrophage-rich plaque [40], and the amount of OxLDL in the coronary plaque [41]. These results suggest that circulating OxLDL may be released from the atherosclerotic lesions. In the present study, there were no significant associations between remnant lipoprotein cholesterol, OxLDL, and number of diseased vessels. Then, we assessed correlations between remnant lipoprotein cholesterol, OxLDL, and extent of CAD defined by Gensini score [42]. The levels of OxPC were positively correlated with Gensini score. In addition, the trend of positive associations between RLP-C and Gensini score and between RemL-C and Gensini score were observed in the CAD group (data not shown). These results could explain the possibility that OxLDL might be partly released from atherosclerotic lesions.

There are several limitations to the current study. First, this investigation was a small sample-size study. However, this is, to the best of our knowledge, the first report to demonstrate the correlations of remnant lipoprotein cholesterols and OxLDL determined by different measurement methods. Studies with larger sample sizes are required to confirm these results. Secondly, the serum levels of other oxidative and inflammatory markers except hsCRP were not measured. It has been proposed that LDL oxidation may be part of local and systemic inflammatory reaction [43]. Thirdly, there were no significant differences in total or LDL cholesterol between the CAD patients and the control subjects. Therefore the results of the present study may not be representative of all patients with CAD.

In conclusion, this study showed that the association between high levels of remnant lipoprotein cholesterol and high OxLDL levels might be linked to atherogenesis in patients with CAD.

References

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