Cardiac Rehabilitation Increases Exercise Capacity with a Reduction of Oxidative Stress

Taira Fukuda, MD,1,2 Miwa Kurano, BS2 Kazuya Fukumura, BS2 Tomohiro Yasuda, PhD2 Haruko Iida, MD2 Toshihiro Morita, MD2 Yumiko Yamamoto, MD2 Nami Takano, MD2 Issei Komuro, MD3 and Toshiaki Nakajima, MD2

1 Health Center, Hitotsubashi University, Tokyo, 2 Department of Ischemic Circulatory Physiology, University of Tokyo, Tokyo, 3 Department of Cardiovascular Medicine, University of Tokyo, Tokyo, Japan

Background and Objectives: Reactive oxygen species (ROS) mediate various signaling pathways that underlie vascular inflammation in atherogenesis and cardiovascular diseases. Cardiac rehabilitation (CR) has a variety of multiple beneficial effects, including anti-inflammatory effects. The purpose of the present study was to investigate the effects of CR on ROS in patients with cardiovascular diseases.

Subjects and Methods: The serum level of derivatives of reactive oxidative metabolites, an index of oxidative stress, was measured in 100 patients with cardiovascular diseases before, and, subsequently, 3 and 6 months after, CR. A biological antioxidant potential (BAP) test was applied to assess the antioxidant power of the serum.

Results: The resting reactive oxidative metabolite levels decreased 3-6 months after CR {pre: 351±97 Carratelli unit (CARR U), 3 months: 329±77 CARR U, 6 months: 325±63 CARR U, all p<0.01} with the increase of the percentage of the predicted values of VO2peak and the percentage of the predicted values of VO2 at the anaerobic threshold (VO2 AT) and the decrease of the B-type natriuretic peptide (BNP). The BAP test and antioxidative/oxidative stress ratio increased 6 months after CR. The % changes of the antioxidative/oxidative stress ratio was positively correlated with the % changes of VO2 AT, and negatively correlated with the % changes of the BNP.

Conclusion: These results suggest that intensive supervised CR significantly improved exercise capacity, which may be attributable to an adaptive response involving more efficient oxidative metabolites or the increased capacity of endogenous anti-oxidative systems in patients with cardiovascular diseases. (Korean Circ J 2013;43:481-487)

KEY WORDS: Reactive oxygen species; Antioxidants; Exercise therapy; Oxygen consumption; Cardiovascular diseases.

Introduction

Cardiovascular risk factors, such as hypertension, obesity, hypercholesterolemia, diabetes mellitus, and chronic smoking, stimulate the production of reactive oxygen species (ROS) in the vascular wall. Additionally, increases in ROS, such as superoxide and hydrogen peroxide (H2O2), have been reported in patients with cardiovascular diseases and chronic heart failure (CHF).

Decreased nitric oxide (NO) production due to changes in the expression and activity of endothelial NO synthase and increased degradation of NO, through a reaction with superoxide, accounts for the reduction in endothelium-dependent vascular relaxation. Furthermore, although the activation of the renin-angiotensin-aldosterone system occurs in cardiovascular diseases, including hypertension, CHF, and coronary artery diseases, angiotensin II has been shown to induce the activity of nicotinamide-adenine dinucleotide phosphate (NADPH) oxidase, and to increase local ROS production. Thus, the pathophysiologic causes of oxidative stress in cardiovascular diseases are considered likely to involve changes in different oxidative enzyme systems. On the other hand, oxidative stress is a balance between ROS and antioxidant enzymes, such as superoxide dismutase (SOD), glutathione peroxidase (GPX), and catalase. While
a small amount of superoxide is normally produced as a byproduct of the use of molecular oxygen during mitochondrial oxidative phosphorylation, it is inactivated by either NO or SOD, and SOD rapidly converts superoxide to H₂O₂ (which is, itself, broken down by GPX and catalase to water). However, studies in relation to oxidant stress and antioxidant defense mechanisms in patients with cardiovascular diseases remain quite scarce.

Exercise training is associated with a decreased risk of many of the diseases linked to excessive oxidative stress. Studies of experimental animals also suggested that long-term voluntary exercise can reduce mitochondrial ROS production in the heart of old rats. Linke et al. showed that anti-oxidative enzymes in skeletal muscles were lower among patients with CHF than in normal subjects, and were improved 6 months after aerobic training compared with control groups. However, the effects of cardiac rehabilitation (CR) on oxidative stress in patients with cardiovascular diseases remain unclear. The purpose of the present study was to investigate the effects of CR on ROS in patients with cardiovascular diseases.

Subjects and Methods

Subjects

One hundred patients with cardiovascular diseases who had been referred to CR, male/female 88/12; age: 63±10 years; height: 166±8 cm; weight: 67.5±13.9 kg; body mass index (weight/height²): 24.4±3.5 kg/m² participated in the present study. Patients were enrolled in the present study if they had visited the hospital for CR as a new patient between July 2009 and March 2012. The underlying cardiovascular diseases included ischemic heart diseases in 90 patients, dilated cardiomyopathy in seven patients, a dilated phase of hypertrophic cardiomyopathy in one patient, idiopathic ventricular tachycardia in one patient, and a complete atrioventricular block in one patient. According to the New York Heart Association classification of functional capacity, nine patients were in class I, 72 patients were in class II, and 19 patients were in class III (Table 1). Left anterior descending artery, left circumflex artery, and right coronary artery lesions were observed in 79 (88%), 39 (43%), and 47 (52%) of coronary patients, suggesting that many patients with ischemic heart diseases have multiple coronary lesions. Myocardial infarction before 2 months was identified in 12 (24%) patients. Twelve patients had had coronary artery bypass grafting surgery, especially among those with left anterior descending artery involvement. Percutaneous coronary intervention, using drug-eluting stents in 68 (76%) patients, was performed for not less than 75% of stenosis in 80 (89%) patients. On the other hand, seven patients with dilated cardiomyopathy were not ischemic. Hypertension, diabetes mellitus, and past smoking were identified in 83 (83%), 46 (46%), and 64 (64%) patients, respectively. More than half of the patients used statins, and almost half of the patients used angiotensin converting enzyme inhibitors and angiotensin receptor blockers. The treatment regimen had not been changed during the 6-month follow-up period, indicating no treatment had interfered with the patients’ inflammatory status. Although the proportion of smokers was relatively high (64%), a large majority had quit smoking before they were enrolled in this study. The B-type natriuretic peptide (BNP) level before CR was 142.2±213.8 pg/mL. The present study was approved by the institutional review board of this hospital.

| Age (years old) | Male/Female | LVEF (%) | Height (cm) | Weight (kg) | BMI (kg/m²) | CABG (%) | LAD (%) | LCX (%) | RCA (%) | NYHA I/II/III | Smoking (%) | Diabetes mellitus (%) | Hypertension (%) | Statins (%) | ACEIs (%) | ARBs (%) | LDL-C (mg/dL) | HDL-C (mg/dL) | HbA1c (%) | BNP (pg/mL) | VO₂peak (mL/kg/min) | %VO₂peak (%) | VO₂at (mL/kg/min) | %VO₂at (%) | d-ROMs (CARR U) | BAP (μmol/L) |
|----------------|-------------|----------|-------------|-------------|-------------|----------|----------|--------|---------|-------------|------------|----------------------|----------------|------------|----------|--------|------------|-------------|----------|----------|-----------------|-------------|----------------|-------------|----------------|-------------|
| 63±10          | 88/12       | 98±14    | 166±8       | 67.5±13.9   | 24.4±3.5    | 12 (12)  | 79 (88)  | 39 (43) | 47 (82) | 9/72/19     | 64 (64)    | 46 (46)               | 83 (83)       | 80 (80)    | 45 (45)  | 46 (46) | 90.5±24.7   | 51.9±13.7   | 6.0±0.9   | 142.2±213.8 | 15.6±3.2     | 64±14       | 11.2±2.0    | 68±12      | 351±97     | 2135±371   |

Mean±SD. LVEF: left ventricular ejection fraction, BMI: body mass index, CABG: coronary artery bypass grafting, LAD: left anterior descending artery, LCX: left circumflex artery, RCA: right coronary artery, NYHA: New York Heart Association, ACEI: angiotensin converting enzyme inhibitor, ARB: angiotensin receptor blocker, LDL-C: low density lipoprotein-cholesterol, HDL-C: high density lipoprotein-cholesterol, HbA1c: hemoglobin A1c, BNP: B-type natriuretic peptide, %VO₂peak: percentage of the predicted values of VO₂peak, %VO₂at: percentage of the predicted values of VO₂at, d-ROMs: derivatives of reactive oxidative metabolites, BAP: biological antioxidant potential, CARR U: carotene unit
by the Ethics Committee of the University of Tokyo. All subjects were informed of the methods, procedures, and risks of the study, and signed an informed consent document prior to their participation.

**Study protocol**

Before CR, a symptom-limited exercise test on an electromagnetically braked upright cycle ergometer (Corival, Load, Holland) at least 2 hours after a meal was performed to determine peak VO\(_2\) (V\(\dot{O}_2\)peak) and anaerobic threshold (AT). After a 4 minutes rest on the cycle ergometer, exercising was commenced at 20 W for a 4 minutes warm up, and the work rate was then increased in 1-W increments every 6 seconds. Blood pressure was measured by an automatic indirect cuff manometer (FB-300, Fukuda Denshi, Tokyo, Japan) every minute. Subjects stopped exercising because of leg fatigue or dyspnea. Expired gases were measured continuously in all subjects on a breath-by-breath basis, using an expired gas analyzer (AE-300S, Minato Medical Science, Osaka, Japan). Ventilatory parameters, including VO\(_2\), were calculated. AT was determined by gas exchange criteria as the point of nonlinear increase in the ventilatory equivalent for oxygen. Electrocardiogram (ECG) was monitored throughout the test to detect any possible ECG signal abnormalities (ML-9000, Fukuda Denshi, Tokyo, Japan). Subsequently, each subject visited the laboratory to receive CR for 40 minutes using aerobic bicycle exercise training at the d-ROMs level of 70-75% AT levels or 1 minute before AT levels, 2 or 3 times per week, for 3-6 months. The number of scheduled exercise sessions attended was very high. On average, each subject attended 87% of the scheduled exercise sessions. Only two subjects attended less than 50% of the scheduled sessions and 16 attended all of the scheduled sessions. As the results indicate, adherence to the exercise protocol was high in this study, with 87% of all sessions attended. Drug compliance was good and diet change during CR was attended. A mean value of V\(\dot{O}_2\)peak and AT was not evident. At 3 and 6 months, a symptom-limited exercise test was again performed to determine V\(\dot{O}_2\)peak and AT. A mean value of V\(\dot{O}_2\)peak was found, as shown in Table 1. The percentage of the predicted values of V\(\dot{O}_2\)peak for healthy Japanese of the same age and sex was 64±14%, suggesting that the subjects exhibited mild exercise intolerance. An echocardiography was performed inside of two months of enrollment. Systolic dysfunction, left ventricular ejection fraction less than 55% (yes, no), New York Heart Association class III (yes, no), and ischemic heart disease (yes, no). Differences were considered significant if the p were less than 0.05. All values were expressed as a mean±SD. Statistical analyses were performed using JMP 9.0.1 software (SAS Institute, Cary, NC, USA).

**Results**

Firstly, we investigated the relationship between rest d-ROMs and BAP in patients with cardiovascular diseases receiving CR. Fig. 1 shows that there was no significant relationship between d-ROMs and BAP at rest.

Fig. 2 depicts the relationships between d-ROMs and exercise capacity or plasma BNP before CR. Resting d-ROM levels were negatively correlated with the percentage of the predicted values of V\(\dot{O}_2\)peak (%V\(\dot{O}_2\)peak) (Fig. 2A) and the percentage of the predicted values of VO\(_2\) at AT (%\(\dot{O}_2\) AT) (Fig. 2B) (all p<0.05), and were positively correlated with BNP (p<0.01) (Fig. 2C), whereas resting BAP test levels were not correlated with either %\(\dot{O}_2\)peak, %\(\dot{O}_2\) AT or BNP (data not shown).

Fig. 3 shows that d-ROMs significantly decreased time-dependently (3 months vs. pre-CR, 329±77 vs. 351±97 CARR U; p<0.01, 6
months vs. pre-CR, 325±63 vs. 351±97 CARR U, p<0.005) (Fig. 3A). The BAP level increased after 6 months compared with pre-CR (6 months vs. pre-CR, 2424±404 vs. 2135±371 μmol/L, p=0.001) (Fig. 3B). The antioxidative/oxidative stress ratio also increased after 6 months compared with pre-CR levels (6 months vs. pre-CR, 7.6±1.7 vs. 6.8±3.1 μmol/L/CARR U, p<0.05) (Fig. 3C).

The two way ANOVA with repeated measures on d-ROMs during the CR program showed significant main effects (for age more than 63 years old, F=3.79 and p<0.05; for New York Heart Association class III, F=8.34 and p<0.005), and no significant interaction effects (for gender; for ischemic heart disease; for left ventricular ejection fraction less than 55%). The two way ANOVA with repeated measures on BAP during the CR program also indicated significant main effects (for age more than 63 years old, F=5.03 and p<0.01; for New York Heart Association class III, F=7.08 and p<0.01), and no significant interaction effects (for gender; for ischemic heart disease; for left ventricular ejection fraction less than 55%). These multivariate analysis results suggested that both age and functional class may be potential covariates of CR on oxidative stress and antioxidant potential persistence.

The effects of CR on %VO₂peak (Fig. 4A), %VO₂_AT (Fig. 4B), and plasma BNP (Fig. 4C) are as shown in Fig. 4. VO₂peak and VO₂_AT increased after 3-6 months (VO₂peak: 3 months vs. pre-CR, 17.5±4.3 vs. 15.6±3.2 mL/kg/min, p<0.001; 6 months vs. pre-CR, 17.8±4.5 vs. 15.6±3.2 mL/kg/min, p<0.001). %VO₂peak and %VO₂_AT also increased after 3-6 months (%VO₂peak: 3 months vs. pre-CR, 71±17 vs. 64±14%, p<0.001; 6 months vs. pre-CR, 73±17 vs. 64±14%, p<0.001), and plasma BNP decreased 3-6 months after CR.

Fig. 5 illustrates that the % changes of VO₂_AT from pre-CR to 6 months after CR were positively correlated with the % changes of the antioxidative/oxidative stress ratio from pre-CR to 6 months after CR (p<0.05) (Fig. 5A). In addition, the % changes of BNP from pre-CR to 6 months after CR were negatively correlated with the % changes of the antioxidative/oxidative stress ratio from pre-CR to 6 months after CR (p<0.05) (Fig. 5B).

Discussion

The major findings of the present study are as follows. 1) There was no relationship between resting d-ROMs and BAP in patients with cardiovascular diseases. 2) The resting levels of d-ROMs were negatively correlated with %VO₂peak and %VO₂_AT, and positively correlated with plasma BNP. However, the BAP test did not suggest a correlation with either %VO₂peak, %VO₂_AT, or BNP. 3) D-ROMs levels significantly decreased 3-6 months after CR with the increase of %VO₂peak and %VO₂_AT and the decrease of BNP. Furthermore, the BAP test significantly increased 6 months after CR. Furthermore, the % changes of the antioxidative/oxidative stress ratio were positively correlated with the % changes of VO₂_AT, and negatively corre-
lated with the % changes of BNP. These results suggest that CR increased exercise capacity with the reduction of oxidative stress and the increase of antioxidant capacity.

Cardiac rehabilitation has a variety of multiple beneficial effects, including improved exercise capacity and anti-inflammatory effects. Cardiovascular risk factors, such as hypertension, obesity, hypercholesterolemia, diabetes mellitus, and chronic smoking, stimulate the production of ROS in the vascular wall. ROS mediate various signaling pathways that underlie vascular inflammation in atherogenesis and cardiovascular diseases. While statins and angiotensin receptor blockers are useful drugs to help prevent cardiovascular diseases, both drugs reduce superoxide by NADPH oxidase.

The results of the present study have several important clinical implications. First, we demonstrated that, among patients with es-
tablished cardiovascular diseases, there was no relationship observed between d-ROMs and BAP. There had been only a small number of previous studies investigating the relationship between d-ROMs and BAP. D-ROMs levels were negatively correlated with BAP levels in a relatively small number of unselected outpatients and subjects who had undergone a health checkup examination, which differed from the present study. These previous data on this issue were obtained from studies of subjects without cardiovascular diseases, suggesting that the relationship between d-ROMs and BAP is different for those with and without cardiovascular diseases, probably due to the higher levels of oxidative stress in patients with cardiovascular diseases.

Second, the present study showed that d-ROMs levels at rest were negatively correlated with %VO2peak and %VO2max, and positively correlated with plasma BNP, yet the BAP test failed to correlate with either %VO2peak, %VO2max or BNP. To the best of our knowledge, there have been no previous studies investigating the relationship between d-ROMs and BAP and exercise capacity in patients with cardiovascular diseases. Thus, the present study for the first time showed that systemic oxidative stress, as measured by d-ROMs, was negatively correlated with exercise capacity. This result suggests that exercise intolerance is related to oxidative stress in patients with cardiovascular diseases.

Third, in the present study, d-ROMs levels significantly decreased 3-6 months after CR with the increase of %VO2peak and %VO2max, and the decrease of BNP. Mercken et al. found that patients with chronic obstructive pulmonary disease had increased pulmonary and systemic oxidative stress levels, as measured by urinary malondialdehyde and H2O2, both at rest and during exercise. They also found that pulmonary rehabilitation increased exercise capacity and reduced exercise-induced oxidative stress. However, as far as we are aware, only one previous study has investigated the effect of CR on oxidative stress. In that study, using left internal mammary artery rings sampled during bypass surgery, Adams et al. showed that exercise training reduced the vascular expression of the NADPH oxidase and angiotensin II receptor type 1 receptor, resulting in the decreased local ROS generation in patients with coronary artery disease, which is consistent with the present findings.

The present study also showed that the BAP test, an index of the antioxidant power of the serum, was improved 6 months after aerobic training compared with control groups. A previous study found that anti-oxidative enzymes assessed in skeletal muscle biopsies were lower in patients with CHF than in normal subjects, and were also improved 6 months after aerobic training compared with controls. These results are also in accordance with our results. Furthermore, the present study showed that the % changes of the antioxidant/oxidative stress ratio were positively correlated with the % changes of VO2peak and negatively correlated with the % changes of BNP. These results show that CR increased exercise capacity, and the observed improvement may be attributable to an adaptive response involving more efficient oxidative metabolites or an increased capacity of endogenous anti-oxidative systems. The possible mechanisms underlying improved exercise capacity and decreased oxidative stress require further investigations.

Notwithstanding these findings, the present study is subject to several limitations. First, our research did not have a control group. Thus, the results were unable to differentiate the effects of CR itself and possible nonspecific changes over time. In addition, about one quarter of the subjects had had recent myocardial infarctions, and many patients had undergone a percutaneous coronary intervention, which may also contribute to the improvement of a patient's condition over time in addition to the CR. Second, the present study was performed among patients with cardiovascular diseases primarily involving ischemic heart diseases. Therefore, further research is required to examine the effect of CR on the ROS and antioxidant potential among patients with other cardiovascular disease types, such as dilated cardiomyopathy and valvular heart disease.

In conclusion, the present study for the first time revealed that oxidative stress was related to exercise intolerance, and CR reduced oxidative stress and increased antioxidant capacity in patients with cardiovascular diseases. Furthermore, the % changes of exercise capacity were positively correlated with the % changes of the antioxidant/oxidative stress ratio. These results suggest that CR may increase exercise capacity with the reduction of oxidative stress and the increase of antioxidant capacity.

References

1. Toussulis D, Briasoulis A, Papageorgiou N, et al. Oxidative stress and endothelial function: therapeutic interventions. Recent Pat Cardiovasc Drug Discov 2011;6:103-14.
2. Harrison D, Griendling KK, Landmesser U, Hornig B, Drexler H. Role of oxidative stress in atherosclerosis. Am J Cardiol 2003;91:7A-11A.
3. Seddon M, Looi YH, Shah AM. Oxidative stress and redox signalling in cardiac hypertrophy and heart failure. Heart 2007;93:903-7.
4. Giordano FJ. Oxygen, oxidative stress, hypoxia, and heart failure. J Clin Invest 2005;115:500-8.
5. Förstermann U, Münzel T. Endothelial nitric oxide synthase in vascular disease: from marvel to menace. Circulation 2006;113:1708-14.
6. Adams V, Linke A, Kränkel N, et al. Impact of regular physical activity on the NADPH oxidase and angiotensin receptor system in patients with coronary artery disease. Circulation 2005;111:555-62.
7. Finkel T, Holbrook NJ. Oxidants, oxidative stress and the biology of ageing. Nature 2000;408:239-47.
8. Tsutsui H, Kinugawa S, Matsushima S. Oxidative stress and heart failure. Am J Physiol Heart Circ Physiol 2011;301:H2181-90.

http://dx.doi.org/10.4070/kcj.2013.43.7.481 www.e-kcj.org
9. Paffenbarger RS Jr, Hyde RT, Wing AL, Lee IM, Jung DL, Kampert JB. The association of changes in physical-activity level and other lifestyle characteristics with mortality among men. *N Engl J Med* 1993;328:538-45.

10. Church TS, Barlow CE, Earnest CP, Kampert JB, Priest EL, Blair SN. Associations between cardiorespiratory fitness and C-reactive protein in men. *Arterioscler Thromb Vasc Biol* 2002;22:1869-76.

11. LaMonte MJ, Blair SN, Church TS. Physical activity and diabetes prevention. *J Appl Physiol* 2005;99:1205-13.

12. Judge S, Jang YM, Smith A, et al. Exercise by lifelong voluntary wheel running reduces subsarcolemmal and interfibrillar mitochondrial hydrogen peroxide production in the heart. *Am J Physiol Regul Integr Comp Physiol* 2005;289:R1564-72.

13. Linke A, Adams V, Schulze PC, et al. Antioxidative effects of exercise training in patients with chronic heart failure: increase in radical scavenger enzyme activity in skeletal muscle. *Circulation* 2005;111:1763-70.

14. Cesarone MR, Belcaro G, Carratelli M, et al. A simple test to monitor oxidative stress. *Int Angiol* 1999;18:127-30.

15. Gerardi G, Usberti M, Martini G, et al. Plasma total antioxidant capacity in hemodialyzed patients and its relationships to other biomarkers of oxidative stress and lipid peroxidation. *Clin Chem Lab Med* 2002;40:104-10.

16. Lubrano V, Vassalle C, L’Abbate A, Zucchelli GC. A new method to evaluate oxidative stress in humans. *Immunol Biol Spec* 2002;17:172-5.

17. Fukui T, Yamauchi K, Maruyama M, Yasuda T, Kohno M, Abe Y. Significance of measuring oxidative stress in lifestyle-related diseases from the viewpoint of correlation between d-ROMs and BAP in Japanese subjects. *Hypertens Res* 2011;34:1041-5.

18. Mercken EM, Hageman GJ, Schols AM, Akkermans MA, Bast A, Wouters EF. Rehabilitation decreases exercise-induced oxidative stress in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2005;172:994-1001.