Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 57 (2008) 170–176
Metabolism
Clinical and Experimental
www.elsevier.com/locate/metabol

Effect of aerobic exercise training on oxidative stress in patients with type 2 diabetes mellitus

Hideki Nojima[a], Hiroshi Watanabe[a], Kiminori Yamane[a,*], Yoshihiro Kitahara[a], Kiyokazu Sekikawa[b], Hideya Yamamoto[a], Akihito Yokoyama[a], Tsutomu Inamizu[b], Toshimasa Asahara[c], Nobuoki Kohno[a], the Hiroshima University Health Promotion Study Group

[a]*Department of Molecular and Internal Medicine, Graduate School of Biomedical Sciences, Hiroshima University, Minami-ku, Hiroshima 734-8551, Japan*
[b]*Division of Physical Therapy and Occupational Therapy Sciences, Department of Sports Medicine, Graduate School of Health Sciences, Hiroshima University, Minami-ku, Hiroshima 734-8551, Japan*
[c]*Division of Frontier Medical Sciences, Programs for Biomedical Research, Department of Surgery, Graduate School of Biomedical Sciences, Hiroshima University, Minami-ku, Hiroshima 734-8551, Japan*

Received 15 May 2007; accepted 23 August 2007

Abstract

The purpose of this study was to determine whether moderate-intensity exercise training reduces oxidative stress in patients with type 2 diabetes mellitus over 12 months. The patients were divided into 3 groups: aerobic training combined with the use of a fitness center (group A, n = 43), aerobic training only (group B, n = 44), or controls (group C, n = 16). The subjects in groups A and B were instructed to exercise at 50% of peak oxygen uptake for more than 30 minutes on at least 3 days per week over a 12 month period. In addition, the subjects in group A were instructed to use a fitness center and were taught how to perform aerobic training in the indicated manner by certified fitness instructors. We measured the levels of urinary 8-hydroxy-2′-deoxyguanosine (8-OHdG) as a parameter of oxidative stress. Serum glycated albumin levels were reduced significantly after 6 and 12 months in groups A and B and after 12 months in group C. Urinary 8-OHdG levels decreased after 12 months in groups A and B, but remained unchanged in group C. There was a significant positive linear association between percentage changes in urinary 8-OHdG and glycated albumin levels over the 12 months. In conclusion, aerobic exercise training improved glycemic control and reduced oxidative stress in patients with type 2 diabetes mellitus. Furthermore, improvement in glycemic control was associated with a reduction in oxidative stress.

1. Introduction

Diabetes mellitus is a risk factor for cardiovascular disease, with the prevalence of cardiovascular mortality being higher in diabetic patients than in the general population [1-4]. Production of reactive oxygen species and lipid peroxidation are increased in diabetic patients [5,6], and it is well established that oxidative stress is an important metabolic abnormality in both cardiovascular disease and diabetic microvascular complications [7].

8-Hydroxy-2′-deoxyguanosine (8-OHdG) is a product of oxidative DNA damage following specific enzymatic cleavage after 8-hydroxylation of the guanosine base. Urinary 8-OHdG is a biomarker of overall systemic oxidative stress in vivo [8]. Urinary 8-OHdG levels have been shown to be higher in diabetic patients than in nondiabetic subjects [6,9].

Aerobic exercise training is one of the most effective interventions for type 2 diabetes mellitus because it does not only improve glycemic control [10], but also can prevent ischemic heart disease [11]. However, the mechanisms underlying the beneficial effects of aerobic exercise that prevent cardiovascular disease are not fully understood.

Depending on its intensity and duration, physical exercise can increase the generation of free radicals and the activity of

* Corresponding author. Tel.: +81 82 257 5196; fax: +81 82 255 7360.
E-mail address: yamanek@hiroshima-u.ac.jp (K. Yamane).

0026-0495/$ – see front matter © 2008 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2007.08.021

antioxidant systems [12-15]. Oxidative stress is determined by the balance between free-radical generation and antioxidant activity. Goto et al [16] showed that high-intensity exercise training in healthy young men increased plasma concentrations of 8-OHdG and serum concentrations of malondialdehyde-modified low-density lipoprotein cholesterol, both of which are indices of oxidative stress. In contrast, the study also showed that moderate-intensity exercise training tended to decrease the levels of these oxidative stress markers. These findings therefore indicated that the effect of exercise on oxidative stress may differ according to the level of exercise intensity. In most patients with type 2 diabetes mellitus, moderate-intensity exercise training is recommended for achieving metabolic improvements [17,18]. However, the impact of moderate-intensity exercise training on oxidative stress in patients with type 2 diabetes mellitus is yet to be fully elucidated.

There is extensive evidence demonstrating the benefits of exercise in the management of type 2 diabetes mellitus [19]. Despite this large volume of evidence, up to 80% of patients with type 2 diabetes mellitus do not perform sufficient physical activity to obtain these beneficial health effects. Furthermore, diabetic patients have a greater tendency to relapse to sedentary behavior compared with the general population [20]. Fitness centers are now readily accessible in major cities in Japan and are used commonly for exercise training and therefore may be useful for treatment of type 2 diabetes mellitus.

The major purpose of this study was to determine whether moderate-intensity exercise training reduces oxidative stress in patients with type 2 diabetes mellitus. We further evaluated whether use of a fitness center in addition to aerobic exercise training could further reduce oxidative stress.

2. Research design and methods

2.1. Subjects

The Hiroshima University Health Promotion Study is an exercise training study, of which the main purpose is to assess the cardiovascular, metabolic, and hormonal responses to aerobic exercise training in patients with type 2 diabetes mellitus. The subjects in the study were 134 patients with type 2 diabetes mellitus, aged between 30 and 74 years, who were recruited from diabetes outpatient clinics. Diabetes was defined according to established criteria [21] and was controlled by either diet or oral hypoglycemic agents. The exclusion criteria were as follows: (1) glycated hemoglobin (HbA_{1C}) ≧9%, (2) clinical findings of diabetic micro- or macrovascular complications, (3) taking insulin, (4) inability to walk for exercise, and (5) medical conditions for which the exercise program might be contraindicated. Thirty-one patients dropped out of the study because of either relocation, loss of motivation, serious disease, or work commitments. The study protocol was approved by the Ethics Committee of Hiroshima University, and written informed consent was obtained from all the participants before commencement of the study.

2.2. Study design and interventions

The subjects were assigned to one of the following 3 groups: aerobic training in combination with the use of a fitness center (group A) (n = 43), aerobic training only (group B) (n = 44), or controls (group C) (n = 16).

The subjects in groups A and B were requested to undertake aerobic exercise at 50% of peak oxygen uptake for more than 30 minutes on at least 3 days per week over a 12-month period. The types of aerobic exercise, which we proposed, were walking, jogging, cycling, and swimming. Furthermore, the subjects in group A were instructed to visit a fitness center at least once a month and were taught how to perform aerobic exercise training in the indicated manner by certified fitness instructors. We did not order the subjects in group A to perform exercise training in the fitness center. No specific advice on dietary habits was given to any of the subjects during the study period. All subjects received regular treatment of their diabetes mellitus at outpatient clinics. The subjects in group C were not advised on exercise training but received regular treatment of their diabetes mellitus at the clinics.

2.3. Determination of peak oxygen uptake

All the participants underwent an exercise test on a bicycle ergometer (Ergometer STB-2400; Nihon Kohden, Tokyo, Japan). The exercise test was performed after a meal to avoid hypoglycemic symptoms because some of the participants took hypoglycemic agents. After a sufficient period of rest on the cycle ergometer, exercise was begun with a 1-minute warm-up at 10 W, followed by a ramp protocol (20 W/min). An electrocardiogram and heart rates were recorded during the test using an electrocardiograph (QP932D; Nihon Kohden). Oxygen uptake (VO_2) was measured using a respiratory gas-exchange analyzer (AE300SRC; Minato Medical Science, Osaka, Japan). The exercise test was terminated when (1) predicted maximum heart rate determined by (210 − age [years]) of the subject was achieved; (2) signs of ischemia in the electrocardiogram were detected; or (3) the patient could no longer sustain a pedaling cadence of at least 50 revolutions per minute because of dyspnea, leg fatigue, or other symptoms. The peak VO_2 was defined as the highest value of VO_2 reached at the end of the exercise.

2.4. Clinical examination

Clinical data were obtained at baseline and after 6 and 12 months of the aerobic training program in groups A and B, whereas data were collected only at baseline and after 12 months in group C. Information collected included duration of diabetes, smoking status, habitual exercise status, and kinds of exercise. Anthropometry (height and body

weight), blood pressure measurement, blood sampling, urine collection, and a cycle ergometer exercise test were performed. Body mass index (BMI) was calculated by dividing weight (in kilograms) by height (in meters) squared. Total body fat (percentage) was assessed by bioimpedance measurements (TBF-501; Tanita, Tokyo, Japan). At baseline and after 12 months, total energy intake and intakes of carbohydrate, protein, total fat, and vitamin A were measured by a registered dietitian using food frequency questionnaire software (Excel Eiyoukun FFQg version 1.0; Kenpousha, Tokyo, Japan) [22]. Physical activity was evaluated through an interviewer-administered questionnaire. The status level of aerobic exercise training was categorized into the following 3 groups: (1) active (more than 30 minutes at least 3 days per week), (2) mild (between active and sedentary), and (3) sedentary (no exercise). Venous blood and urine samples were collected before an exercise test. Total cholesterol, high-density lipoprotein (HDL) cholesterol, triglyceride, and HbA_{1C} were measured at the respective outpatient clinics. Glycated albumin was measured by high-performance liquid chromatography. Urine samples were stored in polypropylene tubes at −80°C. Urinary 8-OHdG levels were measured using a competitive enzyme-linked immunosorbent assay kit (New 8-OHdG Check; Japan Institute for the Control of Aging, Nikken SEIL, Fukuroi, Japan). The levels of urinary 8-OHdG were expressed as the ratio to urinary creatinine levels.

2.5. Statistical analysis

To determine the differences between the groups before intervention, categorical variables were tested using the χ^2 test, whereas continuous variables were tested by analysis of covariance (ANCOVA) adjusted for age and sex, followed by Bonferroni multiple comparison test. Serum triglyceride and urinary 8-OHdG were log-transformed because distribution of the data was skewed. Repeated-measures 2-way (time × group) analysis of variance (ANOVA) models were used to analyze the effect of intervention on outcome measures, followed by Dunnett multiple comparisons. Furthermore, we compared the percentage change of urinary 8-OHdG levels between the study groups by ANCOVA adjusted for age and sex, followed by Bonferroni multiple comparisons. Percentage change was calculated by the following formula: percentage change = (value at 12 months − baseline value) × 100/baseline value. The relationship between percentage changes in urinary 8-OHdG and glycated albumin levels after 12 months was determined using simple regression analysis. We performed a multivariate linear regression model that included age, sex, and the percentage changes in BMI and glycated albumin levels as independent variables and the percentage change in urinary 8-OHdG as a dependent variable, respectively. The Wilcoxon matched pairs signed rank test was used to compare physical activity status at baseline and at 12 months. A P value < .05 was considered to be statistically significant. The statistical tests were performed using SPSS 12.0 J (SPSS Japan, Tokyo, Japan).

3. Results

3.1. Baseline characteristics

The baseline characteristics of the study participants are shown in Tables 1 and 2. All the baseline characteristics were similar in the 3 study groups. The level of physical activity at baseline was not significantly different among the 3 study groups (Table 3). Total energy intake and intakes of carbohydrate, protein, total fat, and vitamin E at baseline were similar among the 3 study groups (data not shown).

3.2. Changes in variables according to study group

The physiological and biochemical variables at baseline and after 6 and 12 months for groups A and B and at baseline and after 12 months for group C are shown (Table 2). After 6 months, BMI was decreased significantly in group B, but remained unchanged in group A. A significant decrease in total body fat was found in both groups A and B after 6 months. Lean body mass increased after 6 months in group A, whereas it did not change in group B. There was no significant change in BMI, percentage body fat, and lean body mass over the study period in group C. Systolic blood pressure decreased after 6 and 12 months in groups A and B. On the other hand, no significant changes in systolic blood pressure were observed in group C. Diastolic blood pressure was reduced after 12 months in group A, whereas it remained unchanged in groups B and C. Total cholesterol and triglyceride did not change in any of the groups. High-density lipoprotein cholesterol increased after 12 months in groups A and B, but did not change in group C. Glycated albumin was reduced after 6 and 12 months in groups A and B and was reduced after 12 months in group C. No significant changes in peak VO_2 were observed in any of the groups.

Urinary 8-OHdG significantly decreased after 12 months in groups A and B; however, no significant change was

Table 1
Baseline characteristics of the study groups

Characteristic	Group A (n = 43)	Group B (n = 44)	Group C (n = 16)	P
Sex (male/female)	32/11	27/17	7/9	NS
Age (y)	55.4 ± 1.1	55.9 ± 1.1	53.9 ± 2.4	NS
Duration of diabetes (y)	7.4 ± 0.9	5.6 ± 0.8	7.3 ± 2.1	NS
Current smoking (%)	28.0	20.5	37.5	NS
HbA_{1C} (%)	7.0 ± 0.2	6.6 ± 0.2	6.4 ± 0.2	NS
Urinary 8-OHdG [a] (ng/mg creatinine)	10.3 ± 1.1	11.3 ± 1.4	6.7 ± 0.7	NS

Values are expressed as mean ± SE or percentage. The data were analyzed by ANCOVA with age and sex as the covariates. NS indicates not significant.
[a] Analyses were performed using natural logarithms.

Table 2
Characteristics at baseline and during follow-up by the study groups

Characteristic	Baseline	6 mo	12 mo	P
Group A				
BMI (kg/m^2)	23.7 ± 0.6	23.4 ± 0.6	23.5 ± 0.6	NS
Total body fat (%)	24.0 ± 1.2	22.4 ± 1.1 *	23.2 ± 1.1	<.01
Lean body mass (kg)	47.7 ± 1.4	48.4 ± 1.4 *	48.1 ± 1.4	<.01
Systolic BP (mm Hg)	140.5 ± 2.9	135.5 ± 2.4 *	132.1 ± 2.0 *	<.01
Diastolic BP (mm Hg)	84.2 ± 1.8	82.3 ± 1.7	79.0 ± 1.4 *	<.01
Total cholesterol (mmol/L)	5.56 ± 0.13	5.63 ± 0.17	5.46 ± 0.17	NS
Triglyceride [a] (mmol/L)	1.51 ± 0.11	1.70 ± 0.13	1.58 ± 0.18	NS
HDL cholesterol (mmol/L)	1.46 ± 0.05	1.45 ± 0.06	1.55 ± 0.05 *	<.05
Glycated albumin (%)	22.5 ± 0.6	21.5 ± 0.6 *	20.7 ± 0.6 *	<.01
Peak VO_2 (mL/min/kg)	25.4 ± 0.7	25.6 ± 0.8	25.3 ± 0.7	NS
Urinary 8-OHdG [a] (ng/mg creatinine)	10.3 ± 1.1	9.3 ± 1.0	6.7 ± 0.6 *	<.05
Group B				
BMI (kg/m^2)	24.4 ± 0.5	23.9 ± 0.6 *	24.3 ± 0.6	<.01
Total body fat (%)	25.9 ± 1.1	24.1 ± 1.1 *	25.2 ± 1.1	<.01
Lean body mass (kg)	46.3 ± 1.3	46.6 ± 1.3	46.5 ± 1.3	NS
Systolic BP (mm Hg)	139.8 ± 3.3	133.1 ± 2.5 *	134.3 ± 2.7 *	<.05
Diastolic BP (mm Hg)	82.7 ± 1.6	81.6 ± 1.8	80.0 ± 1.3	NS
Total cholesterol (mmol/L)	5.21 ± 0.14	5.13 ± 0.12	5.31 ± 0.12	NS
Triglyceride [a] (mmol/L)	1.41 ± 0.12	1.51 ± 0.13	1.52 ± 0.14	NS
HDL cholesterol (mmol/L)	1.38 ± 0.05	1.44 ± 0.05	1.48 ± 0.05 *	<.01
Glycated albumin (%)	21.5 ± 0.7	20.4 ± 0.5 *	20.0 ± 0.5 *	<.01
Peak VO_2 (ml/min/kg)	23.9 ± 0.8	24.3 ± 0.8	24.6 ± 0.8	NS
Urinary 8-OHdG [a] (ng/mg creatinine)	11.3 ± 1.4	8.1 ± 0.8	6.8 ± 0.7 *	<.01
Group C				
BMI (kg/m^2)	24.3 ± 1.1		24.0 ± 1.0	NS
Total body fat (%)	28.5 ± 2.7		28.4 ± 2.6	NS
Lean body mass (kg)	42.4 ± 2.0		42.2 ± 2.2	NS
Systolic BP (mm Hg)	127.8 ± 4.4		128.2 ± 4.3	NS
Diastolic BP (mm Hg)	76.4 ± 2.8		79.1 ± 3.1	NS
Total cholesterol (mmol/L)	5.67 ± 0.24		6.00 ± 0.33	NS
Triglyceride [a] (mmol/L)	1.33 ± 0.19		1.59 ± 0.24	NS
HDL cholesterol (mmol/L)	1.31 ± 0.08		1.49 ± 0.06	NS
Glycated albumin (%)	20.0 ± 0.9		18.9 ± 0.8	<.05
Peak VO_2 (ml/min/kg)	21.6 ± 1.3		22.2 ± 1.5	NS
Urinary 8-OHdG [a] (ng/mg creatinine)	6.7 ± 0.7		6.2 ± 0.6	NS

Values are expressed as mean ± SE. The data were analyzed by repeated-measures ANOVA. BP indicates blood pressure.

[a] Analyses were performed using natural logarithms.

* $P < .05$ vs baseline (Dunnett test) in group A or B.

Table 3
Physical activity status at baseline and during follow-up by the study groups

Characteristic	Baseline	6 mo	12 mo	P
Group A				<.01
Sedentary	18	10	8	
Mild	10	8	11	
Active	15	25	24	
Group B				.067
Sedentary	19	14	14	
Mild	10	10	8	
Active	15	20	22	
Group C				.180
Sedentary	7		3	
Mild	3		8	
Active	6		5	

Values are expressed as number. The data were analyzed by Wilcoxon matched pairs signed rank test to compare physical activity status at baseline and after 12 months.

found in group C. Analysis by repeated-measures 2-way ANOVA model showed that there was no intergroup difference in the change in urinary 8-OHdG levels before intervention and after 12 months ($P = .368$). The percentage changes in urinary 8-OHdG were not different among the 3 groups after adjustment for age and sex (data not shown). At 12 months, physical activity status level had significantly increased in group A, whereas it showed a nonsignificant increasing trend in group B and no change in group C (Table 3). There was no significant change in total energy intake and intakes of carbohydrate, protein, total fat, and vitamin E during the study in any group (data not shown).

3.3. Correlation between percentage changes in oxidative stress and glycated albumin

The combined data of all the subjects showed a weak but significant positive linear association between the percentage changes in urinary 8-OHdG and glycated albumin over the 12-month period of the study (Fig. 1). In a multivariate linear

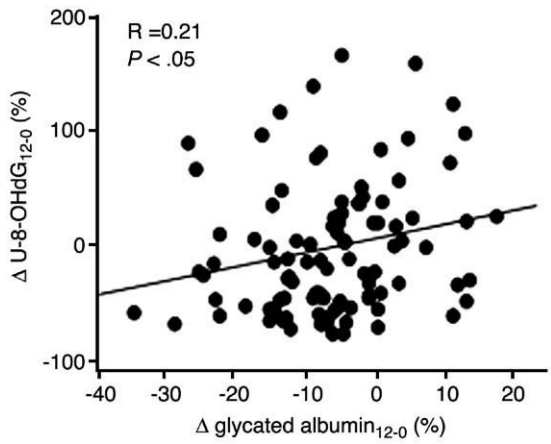

Fig. 1. Correlation between the changes from baseline to 12 months in urinary 8-OHdG and glycated albumin levels for all the subjects.

regression model, the percentage changes in urinary 8-OHdG were correlated with the percentage changes in glycated albumin, even after adjusting for age, sex, and the percentage changes in BMI (data not shown).

4. Discussion

This study demonstrated that moderate-intensity aerobic exercise training over 12 months reduced oxidative stress in patients with type 2 diabetes mellitus. This finding is based on the results that urinary 8-OHdG levels decreased significantly after 12 months in groups A and B, whereas they remained unchanged in group C.

The impact of long-term exercise training on oxidative stress in patients with type 2 diabetes mellitus has not been fully investigated. It has been reported that both intensity and duration of exercise are important for antioxidant adaptation [23]. Although endurance exercise can increase oxidative stress, it also simultaneously induces antioxidant systems [14,15]. Long-term exercise training can reduce oxidative stress by enhancing antioxidant defense mechanisms that include antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase [23]. Based on the results of our study, we propose that endurance exercise training over 12 months is important for reducing parameters of oxidative stress. Mori et al [24] demonstrated that 8 weeks of aerobic exercise training did not significantly reduce urinary isoprostane in 11 dyslipidemic patients with type 2 diabetes mellitus. However, the sample size of the study was smaller and the duration of the intervention period was shorter than those used in our study.

In our study, improvement in glycemic control was associated with a reduction in oxidative stress, supported by the result that the percentage change in urinary 8-OHdG was weakly but significantly related with the percentage change in glycated albumin after age, sex, and the percentage changes in BMI were adjusted. There are 2 mechanisms that underlie this relationship. The first mechanism is that improvement in glycemic control associated with aerobic exercise training may result in a decrease in oxidative stress. Aerobic exercise training improves insulin sensitivity [25] and glycemic control [10]. Hyperglycemia can induce oxidative stress via several mechanisms including glucose autoxidation, formation of advanced glycation end products, and activation of the polyol pathway [26]. Chugh et al [27] reported previously that 6 weeks of glycemic control with sulfonylurea resulted in an improvement of glycemic control and a reduction in serum malondialdehyde, a reliable measure of lipid peroxidation [28]. The other mechanism is that a decrease in oxidative stress caused by aerobic exercise training may lead to an improvement in glycemic control. Aerobic exercise may increase antioxidant activity and reduce oxidative stress. Elosua et al [29] reported that aerobic exercise training increased the activity of the endogenous antioxidants, glutathione peroxidase, and glutathione reductase and decreased oxidized low-density lipoprotein concentration. There is evidence that oxidative stress is associated with insulin resistance, as Urakawa et al [30] demonstrated that plasma isoprostane levels were negatively correlated with glucose infusion rates in men. These results therefore indicate that a reduction in oxidative stress caused by aerobic exercise training may improve insulin sensitivity and glycemic control.

During the study period, the status level of physical activity significantly increased in group A, whereas it increased in group B, but not significantly. This finding suggested that the recommendation to use a fitness center had resulted in increased physical activity. Despite this difference in physical activity, there were no differences in either the improvement in glycemic control or decrease in oxidative stress between groups A and B. The average frequency of visit to the fitness center was 0.5 ± 0.2 times per month in group A. We assume that use of a fitness center did not have an additional effect of improving glycemic control or decreasing oxidative stress because the subjects in group A did not go to the fitness center once a month.

We requested the subjects in groups A and B to undertake aerobic exercise for more than 30 minutes on at least 3 days per week. The US Surgeon General's report recommended that most people accumulate $\geq$30 minutes of moderate-intensity activity on most days of the week [31]. However, most clinical trials evaluating exercise interventions in people with type 2 diabetes mellitus have used a frequency of 3 times per week [19]. The effect on insulin sensitivity of a single bout of aerobic exercise lasts 24 to 72 hours [32]. Therefore, we also used a frequency of at least 3 times per week.

In this study, we measured serum glycated albumin to monitor glycemic control because glycated serum protein is a more sensitive index than HbA_{1C}. Serum glycated albumin levels have been shown to reflect overall glycemic control during the previous 2 weeks [33,34], whereas HbA_{1C} provides an integrated measurement of blood glucose during the previous 2 to 3 months [35,36]. Schleicher et al [37] proposed that glycated serum protein is a more sensitive index than HbA_{1C}, possibly as a consequence of the higher albumin content of serum. Furthermore, Monnier et al [38] reported that urinary isoprostane level, which is one of the oxidative stress markers [39], was positively correlated with short-term glucose fluctuations, but not HbA_{1C}, in patients with type 2 diabetes mellitus. We therefore used serum glycated albumin as a sensitive marker of glycemic control, as the study subjects did not have severe abnormal glycemic control.

Our study has several limitations. First, it was limited by its relatively small sample size, especially in the control group. Thus, larger-scale studies are warranted to further establish the beneficial effects of aerobic exercise training on oxidative stress. Second, no significant changes in peak VO_2 were observed in any of the groups. Baseline physical activity status was "active" in nearly one third of the

subjects in our study (Table 3); and therefore, initial peak VO_2 in group A or B might be relatively higher than that of the common subjects with type 2 diabetes mellitus reported in the previous studies [40]. Third, we could not perform intention-to-treatment analysis because of the relatively large number of patients who did not finish the trial after randomization.

In conclusion, moderate-intensity aerobic exercise training over 12 months reduced oxidative stress and improved glycemic control in patients with type 2 diabetes mellitus. Furthermore, improvement of glycemic control was associated with a reduction in oxidative stress. Our results suggest that long-term aerobic exercise training can be regarded as an effective antioxidant therapy, as well as being crucial for improving glycemic control in patients with type 2 diabetes mellitus.

Acknowledgments

The Hiroshima University Health Promotion Study was conducted by Hiroshima University in cooperation with the Corporations of Hiroshima Prefecture, Hiroshima City, and Higashi-Hiroshima City. This work was also a special enterprise for social contribution and was supported by the Ministry of Education, Culture, Sports, Science, and Technology. We thank Rie Shimooka, Department of Nutrition and Health Promotion, Hiroshima Jogakuin University, for dietary assessment.

Appendix A. Committee Members of The Hiroshima University Health Promotion Study group

Akira Eboshida, Yasuo Hashimoto, Kawasaki Hiromi, Norihisa Kato, Hidemi Kurihara, Mitsuo Ochi, Megu Ohtaki, Kiyoshi Onari (Hiroshima University); Ryozo Harada (Hiroshima University High School).

References

[1] The Diabetes Control and Complications Trial Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 1993;329:977-86.
[2] Haffner SM, Lehto S, Ronnemaa T, Pyorala K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. N Engl J Med 1998;339:229-34.
[3] Kannel WB, McGee DL. Diabetes and cardiovascular disease. The Framingham study. JAMA 1979;241:2035-8.
[4] Stratton IM, Adler AI, Neil HA, Matthews DR, Manley SE, Cull CA, et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. BMJ 2000;321:405-12.
[5] Giugliano D, Ceriello A, Paolisso G. Oxidative stress and diabetic vascular complications. Diabetes Care 1996;19:257-67.
[6] Nakanishi S, Suzuki G, Kusunoki Y, Yamane K, Egusa G, Kohno N. Increasing of oxidative stress from mitochondria in type 2 diabetic patients. Diabetes Metab Res Rev 2004;20:399-404.
[7] Baynes JW. Role of oxidative stress in development of complications in diabetes. Diabetes 1991;40:405-12.
[8] Wu LL, Chiou CC, Chang PY, Wu JT. Urinary 8-OHdG: a marker of oxidative stress to DNA and a risk factor for cancer, atherosclerosis and diabetics. Clin Chim Acta 2004;339:1-9.
[9] Nishikawa T, Sasahara T, Kiritoshi S, Sonoda K, Senokuchi T, Matsuo T, et al. Evaluation of urinary 8-hydroxydeoxy-guanosine as a novel biomarker of macrovascular complications in type 2 diabetes. Diabetes Care 2003;26:1507-12.
[10] Vanninen E, Uusitupa M, Siitonen O, Laitinen J, Lansimies E. Habitual physical activity, aerobic capacity and metabolic control in patients with newly-diagnosed type 2 (non–insulin-dependent) diabetes mellitus: effect of 1-year diet and exercise intervention. Diabetologia 1992;35:340-6.
[11] Bassuk SS, Manson JE. Epidemiological evidence for the role of physical activity in reducing risk of type 2 diabetes and cardiovascular disease. J Appl Physiol 2005;99:1193-204.
[12] Davies KJ, Quintanilha AT, Brooks GA, Packer L. Free radicals and tissue damage produced by exercise. Biochem Biophys Res Commun 1982;107:1198-205.
[13] Inoue T, Mu Z, Sumikawa K, Adachi K, Okochi T. Effect of physical exercise on the content of 8-hydroxydeoxyguanosine in nuclear DNA prepared from human lymphocytes. Jpn J Cancer Res 1993;84:720-5.
[14] Sen CK. Oxidants and antioxidants in exercise. J Appl Physiol 1995;79:675-86.
[15] Viguie CA, Frei B, Shigenaga MK, Ames BN, Packer L, Brooks GA. Antioxidant status and indexes of oxidative stress during consecutive days of exercise. J Appl Physiol 1993;75:566-72.
[16] Goto C, Higashi Y, Kimura M, Noma K, Hara K, Nakagawa K, et al. Effect of different intensities of exercise on endothelium-dependent vasodilation in humans: role of endothelium-dependent nitric oxide and oxidative stress. Circulation 2003;108:530-5.
[17] Albright A, Franz M, Hornsby G, Kriska A, Marrero D, Ullrich I, et al. American College of Sports Medicine position stand. Exercise and type 2 diabetes. Med Sci Sports Exerc 2000;32:1345-60.
[18] Sigal RJ, Kenny GP, Wasserman DH, Castaneda-Sceppa C. Physical activity/exercise and type 2 diabetes. Diabetes Care 2004;27:2518-39.
[19] Boule NG, Haddad E, Kenny GP, Wells GA, Sigal RJ. Effects of exercise on glycemic control and body mass in type 2 diabetes mellitus: a meta-analysis of controlled clinical trials. JAMA 2001;286:1218-27.
[20] Krug LM, Haire-Joshu D, Heady SA. Exercise habits and exercise relapse in persons with non–insulin-dependent diabetes mellitus. Diabetes Educ 1991;17:185-8.
[21] American Diabetes Association. Clinical practice recommendations 2002. Diabetes Care 2002;25(Suppl 1):S1-147.
[22] Takahashi K, Yoshimura Y, Kaimoto T, Kunii D, Komatsu T, Yamamoto S. Validation of a food frequency questionnaire based on food groups for estimating individual nutrient intake. Japanese Journal of Nutrition and Dietetics 2001;59:221-31.
[23] Ji LL. Antioxidants and oxidative stress in exercise. Proc Soc Exp Biol Med 1999;222:283-92.
[24] Mori TA, Dunstan DW, Burke V, Croft KD, Rivera JH, Beilin LJ, et al. Effect of dietary fish and exercise training on urinary F2-isoprostane excretion in non–insulin-dependent diabetic patients. Metabolism 1999;48:1402-8.
[25] Devlin JT, Hirshman M, Horton ED, Horton ES. Enhanced peripheral and splanchnic insulin sensitivity in NIDDM men after single bout of exercise. Diabetes 1987;36:434-9.
[26] Jay D, Hitomi H, Griendling KK. Oxidative stress and diabetic cardiovascular complications. Free Radic Biol Med 2006;40:183-92.
[27] Chugh SN, Dhawan R, Kishore K, Sharma A, Chugh K. Glibenclamide vs gliclazide in reducing oxidative stress in patients of noninsulin dependent diabetes mellitus—a double blind randomized study. J Assoc Physicians India 2001;49:803-7.

[28] Gutteridge JM. Lipid peroxidation and antioxidants as biomarkers of tissue damage. Clin Chem 1995;41:1819-28.
[29] Elosua R, Molina L, Fito M, Arquer A, Sanchez-Quesada JL, Covas MI, et al. Response of oxidative stress biomarkers to a 16-week aerobic physical activity program, and to acute physical activity, in healthy young men and women. Atherosclerosis 2003;167:327-34.
[30] Urakawa H, Katsuki A, Sumida Y, Gabazza EC, Murashima S, Morioka K, et al. Oxidative stress is associated with adiposity and insulin resistance in men. J Clin Endocrinol Metab 2003;88:4673-6.
[31] US Department of Health and Human Services. A report of the Surgeon General. Physical activity and health. Atlanta (GA): US Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion; 1996.
[32] Wallberg-Henriksson H, Rincon J, Zierath JR. Exercise in the management of non–insulin-dependent diabetes mellitus. Sports Med 1998;25:25-35.
[33] Guthrow CE, Morris MA, Day JF, Thorpe SR, Baynes JW. Enhanced nonenzymatic glucosylation of human serum albumin in diabetes mellitus. Proc Natl Acad Sci U S A 1979;76:4258-61.
[34] Olufemi S, Talwar D, Robb DA. The relative extent of glycation of haemoglobin and albumin. Clin Chim Acta 1987;163:125-36.
[35] Bunn HF, Gabbay KH, Gallop PM. The glycosylation of hemoglobin: relevance to diabetes mellitus. Science 1978;200:21-7.
[36] Koenig RJ, Peterson CM, Jones RL, Saudek C, Lehrman M, Cerami A. Correlation of glucose regulation and hemoglobin A1c in diabetes mellitus. N Engl J Med 1976;295:417-20.
[37] Schleicher ED, Gerbitz KD, Dolhofer R, Reindl E, Wieland OH, Edelmann E, et al. Clinical utility of nonenzymatically glycosylated blood proteins as an index of glucose control. Diabetes Care 1984;7:548-56.
[38] Monnier L, Mas E, Ginet C, Michel F, Villon L, Cristol JP, et al. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. JAMA 2006;295:1681-7.
[39] Montuschi P, Barnes PJ, Roberts II LJ. Isoprostanes: markers and mediators of oxidative stress. FASEB J 2004;18:1791-800.
[40] Boule NG, Kenny GP, Haddad E, Wells GA, Sigal RJ. Meta-analysis of the effect of structured exercise training on cardiorespiratory fitness in type 2 diabetes mellitus. Diabetologia 2003;46:1071-81.