Exercise Improved Accumulation of Visceral Fat and Simultaneously Impaired Endothelium-Dependent Relaxation in Old Rats

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Exercise decreases plasma total cholesterol and triglycerides, and simultaneously, increases high density lipoprotein (HDL) cholesterol. Furthermore, it has been reported that exercise improves insulin resistance. As a result, exercise is believed to aid in preventing atherosclerosis. However, we do not know whether exercise protects against the development of atherosclerosis in the elderly. The aim of this study was to ascertain whether exercise prevented atherosclerosis in aorta of old rats. Exercise for three months did not affect plasma lipid levels but decreased accumulation of visceral fat and body weight without the reduction of food intake in two year old rats. Exercise also decreased triglycerides in liver and gastrocnemius white muscle. Exercise resulted in an elevation of plasma lipid peroxide (LPO) levels without affecting superoxide dismutase (SOD). Exercise impaired the endothelium-dependent relaxation of the thoracic aorta caused by acetylcholine in old rats. In summary, the results of our study indicate that exercise induces the reduction of visceral fat, body weight and triglyceride contents in tissues in old rats. These results seem to show that exercise improves insulin resistance. However, exercise simultaneously may cause impaired endothelium-dependent relaxation by elevation of LPO in old rats.

Key words exercise; old rat; endothelium-dependent relaxation; visceral fat; lipid peroxide; superoxide dismutase

Hypercholesterolemia, hypertriglyceridemia and hypo high density lipoprotein (HDL) cholesterolemia are independent risk factors for atherosclerosis.1–3 Many studies have reported that exercise decreases plasma total cholesterol and triglyceride levels, and increases HDL cholesterol.4–5 Furthermore, it has been shown that exercise improves insulin resistance in diabetes mellitus.6–8 These results indicate that exercise provides protection against the development of atherosclerosis. However, these studies were performed in adolescence or middle age. In the elderly, we do not know whether exercise protects against the development of atherosclerosis. Accordingly, this study was initiated to ascertain whether exercise prevents atherosclerosis in aorta of two year old rats.

Damage to endothelial cells has been suggested as a potent trigger for the onset of coronary atherosclerosis and coronary vasospasm.7 The impairment of endothelial function exists before the histological findings of atherosclerosis become apparent.8 In this study, we estimated endothelial function instead of histological findings of atherosclerosis.

MATERIALS AND METHODS

Animal Experiments Two year old male SD rats weighing 600–1100 g were obtained from Japan SLC, Inc., Shizuoka, Japan. The animals were maintained under a 12-h light–dark cycle at a constant temperature of 23±2 °C. The rats were stratified by body weight and divided into a control group (no exercise) and an exercise group. The exercise group performed exercise for 3 months. Exercise was performed on a custom-built rodent treadmill (Rotating Motion Recorder, Toyoriko Co., Ltd., Tokyo, Japan) once a day (exercise 10° slope, 15 m/min for 30 min). The animals were given free access to food (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water. Food consumption and body weight were recorded monthly. Following an overnight fasting after the final bout of exercise, the animals were killed by exsanguination under sodium pentobarbital anesthesia. Blood samples were collected from the posterior vena cava for lipid measurement. The thoracic aorta was removed immediately for measurement of vasorelaxation response. The liver and gastrocnemius (red and white) were excised and frozen in liquid nitrogen. These tissues were stored at −80 °C until measurement of lipid contents.

Analytical Methods Plasma total cholesterol, high density lipoprotein cholesterol (HDL-C), triglycerides, nonesterified free fatty acid (NEFA) and glucose were determined by conventional enzymatic methods. The cholesterol C-test Wako (Wako Pure Chemical Industries, Osaka, Japan) was used in the case of total cholesterol, the Nesco test Wako (Wako Pure Chemical Industries, Osaka, Japan) for HDL-C, the triglyceride G-test Wako (Wako Pure Chemical Industries) for triglycerides, the NEFA test Wako for NEFA and the glucose II test Wako for glucose. Insulin was determined by conventional enzyme immunoassay, using the Glazyme insulin-EIA test (Wako Pure Chemical Industries).

Plasma creatinine (Cr), blood urea nitrogen (BUN), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined with an automatic analyzer (Fuji Drichem, Tokyo, Japan).

Superoxide dismutase (SOD) was determined by the nitro blue tetrazolium reduction method.9 Lipid peroxide (LPO) was determined by Yagi’s method.10 The SOD test Wako (Wako Pure Chemical Industries) was used in the case of SOD, the LPO test Wako (Wako Pure Chemical Industries) for LPO.

Tissue Lipid Content: Tissue samples of approximately 0.2 g, together with 2 ml of chloroform–methanol (2:1) solution,11 were added into Pyrex centrifuge tubes and homogenized by Polytron (PCU-2-110, KINEMATICA GmbH, Switzerland). The samples were extracted with chloroform–methanol (2:1) solution, and the lipid extraction was done by the method of Folch et al.12 After extraction, the samples were dried in a vacuum oven at 60 °C. After the samples were desiccated in vacuo, the sample weight was determined.
Tokyo, Japan). The tubes were then centrifuged at 3000 rpm. An aliquot of chloroform–methanol extract was transferred to another Pyrex tube and dried under a stream of nitrogen gas. These samples were redissolved in 100 μl isopropyl alcohol, after which cholesterol and triglyceride levels in the isopropyl alcohol were measured by conventional enzymatic methods.

Relaxation Responses: Sections of the thoracic aorta between the aortic arch and the diaphragm were dissected from the same animal following exsanguination. These were mounted as ring preparations and normalized for the measurement of isometric tension in a 5 ml organ bath (VFER micro easy magnus, Medical Kishimoto Co., Ltd., Kyoto, Japan) containing a Krebs solution with the following composition: NaCl 118.4 mM, KCl 4.9 mM, CaCl2 2.5 mM, MgCl2 1.2 mM, NaHCO3 25.0 mM, KH2PO4 1.2 mM, glucose 11.1 mM and ascorbic acid 1.2 mM. The medium was maintained at 34°C and bubbled with 95% O2 and 5% CO2. The arteries were contracted with phenylephrine at a concentration corresponding to EC80 values (10^-6 M). Acetylcholine and sodium nitroprusside were added cumulatively. Relaxation responses were measured with an isometric transducer (AP-5, Medical Kishimoto Co., Ltd., Kyoto, Japan). The tubes were then centrifuged at 3000 rpm. An aliquot of chloroform–methanol extract was transferred to another Pyrex tube and dried under a stream of nitrogen gas. These samples were redissolved in 100 μl isopropyl alcohol, after which cholesterol and triglyceride levels in the isopropyl alcohol were measured by conventional enzymatic methods.

Statistical Analysis: The results are expressed as means±S.D. Significant differences between the experimental groups were calculated by Student’s t-test or Aspin–Welch’s t-test.

RESULTS

Effects of Exercise on Body Weight, Visceral Fat Weight and Food Consumption  Body weights decreased 13.3%, 18.1% and 20.4% at one month, 2 months and 3 months in the exercise group, respectively, compared to before exercise. Body weights in the control group did not change after 3 months (Fig. 1). Visceral fat weight of the exercise group was 36.4% lower than the control group after 3 months in the exercise group, respectively, compared to before exercise (Table 1). However, the plasma glucose level of the exercise group was 25.7% lower than the control group after 3 months of exercise, although not significantly (p<0.20). Insulin level in the exercise group was also 39% lower than the control group, although not significantly (p<0.20).

Effects of Exercise on Plasma Lipids, Glucose and Insulin  Plasma total cholesterol, HDL cholesterol, triglycerides and NEFA and glucose levels were not changed by exercise, compared to before exercise (Table 1). However, the plasma glucose level of the exercise group was 25.7% lower than the control group after 3 months of exercise, although not significantly (p<0.20). Insulin level in the exercise group was also 39% lower than the control group, although not significantly (p<0.20).

Effects of Exercise on Plasma Cr, BUN, AST and ALT  Plasma Cr, BUN, AST and ALT levels were not changed by exercise.
exercise (Table 2).

Effects of Exercise on SOD Activity and LPO Plasma SOD activity was not affected by exercise. Plasma LPO levels were increased after 3 months of exercise (Table 3).

Effects of Exercise on Tissue Lipid Contents Liver cholesterol content was not affected by exercise, but triglyceride content decreased after 3 months of exercise. Triglyceride contents in gastrocnemius white muscle decreased significantly after exercise but cholesterol showed only a slight decrease (p<0.10). In gastrocnemius red muscle, both cholesterol and triglyceride contents were unaffected by exercise (Table 4).

Effects of Exercise on Relaxation Responses Endothelium-dependent relaxation by acetylcholine was significantly impaired by exercise in old rats at low concentrations of acetylcholine (Fig. 3). However, endothelium-independent relaxation by nitroprusside was not affected by exercise after 3 months (Fig. 4).

DISCUSSION

Age is a risk factor for atherosclerosis. Aging is associated with progressive development of dislipidemia, insulin resistance, hypertension and obesity. These symptoms are also risk factors for cardiovascular disease and atherosclerosis. Therefore, a reduction of plasma cholesterol and triglycerides, an elevation of HDL-C, and an improvement of insulin resistance and obesity is important to protect against the development of atherosclerosis in the elderly.

Morris et al. reported in 1953 that men in physically active jobs (bus conductors) had a lower incidence of coronary heart-disease than men in physically inactive jobs (bus drivers) in middle age. Following this, many papers reported about the relationship between exercise and atherosclerosis. We now know that long-term physical exercise improves dislipidemia, insulin resistance and obesity, and helps prevent atherosclerosis. However, these reports only showed studies that were performed in adolescent and middle age subjects. We do not know whether exercise protects against the development of atherosclerosis in the elderly.

In this study, 3 months of exercise did not affect plasma total cholesterol, HDL-C, triglyceride and NEFA levels in old rats. However, exercise resulted in suppressed elevation of plasma glucose by aging and decreased plasma insulin levels (p<0.20).

Simonelli and Eaton reported similar results to ours, whereby plasma total cholesterol and HDL-C levels in 5 months old Zucker-obese rats were not affected by 3 weeks of free access to exercise wheels. However, their data showed that plasma TG levels were decreased by exercise. Since plasma the TG levels of Zucker-obese rats are significantly high (407 mg/dl), high levels of TG may be decreased by exercise. Furthermore, they also reported similar results to our study regarding the effects of exercise on insulin and glucose levels.

Insulin resistance causes higher lipid contents in tissues,
especially in liver and skeletal muscle.\textsuperscript{16,17} In this study, exercise decreased triglyceride in liver and gastrocnemius white muscle in old rats. Also, cholesterol content in gastrocnemius white muscle showed a tendency to decrease by exercise ($p<0.10$). Similar to our work, Narayan\textsuperscript{18} reported that cholesterol and triglyceride contents in the livers of high-fat fed Holtzman rats (initial body weight: 340 g) were decreased by 15 min/d of running for 4 d on a treadmill. Furthermore, Oscail\textsuperscript{19} reported that TG contents in skeletal muscle (red fibers) were decreased by 120 min at 31 m/min/d of running for 12 weeks on a treadmill, using Wistar strain rats (initial body weight: 90 g). However, our data showed that the triglyceride contents in white muscle decreased with exercise but that the triglyceride contents in red muscle were not affected by exercise in old rats.

In this study, exercise decreased visceral fat weight causing a reduction in body weight without affecting food consumption, nor resulting in liver and kidney injury in old rats. These results indicate that exercise may also improve insulin resistance in old rats, which would be consistent with the findings in young and middle age rats.\textsuperscript{20}

On the other hand, exercise elevated plasma LPO levels, but did not affect plasma SOD levels in old rats. LPO is produced from unsaturated fatty acid by oxidation and is a risk factor for atherosclerosis.\textsuperscript{21} SOD is an enzyme that catalyzes the dismutation of superoxide radicals related to fatty acid oxidation. There are many reports showing that aging is associated with progressive reduction of SOD activity, which is a catabolizing enzyme of LPO.\textsuperscript{22,23} In old rats, SOD activity did not increase although there was an elevation of LPO levels. In old rats, the endothelium-dependent relaxation of the thoracic aorta by physiological concentration of acetylcholine reflecting \textit{in vivo} conditions was significantly impaired by exercise.

These results may suggest that exercise improves insulin resistance and the reduction of plasma insulin level, resulting in activation of hormone sensitive lipase and improvement of visceral fat accumulation in old rats. Released fatty acids from visceral fat were oxidized to CO$_2$, and a portion of the unsaturated fatty acids were oxidized to LPO. LPO is produced by oxidized LDL.\textsuperscript{24} LPO and oxidized LDL impair endothelium\textsuperscript{25} and results in a decrease in nitric oxide (NO) production and impairs the endothelium-dependent relaxation of the aorta by acetylcholine.\textsuperscript{26} The degree of impaired endothelium-dependent relaxation in aorta of old rats may be stronger than young rats because SOD activity is reduced in old rats.

Nakamura \textit{et al.}\textsuperscript{27} reported that oxidation was accelerated by exercise. An increased oxidation of low density lipoprotein (LDL) is a risk factor for atherosclerosis. However, loading of vitamin E, which is a known anti-oxidant, prevented the increase in oxidized LDL levels. Prevention of lipid oxidation by exercise is very important for the prevention of progression of atherosclerosis in the elderly.

In summary, long-term physical exercise improved accumulation of visceral fat and insulin resistance, but simultaneously may have caused impaired endothelium-dependent relaxation by elevation of plasma LPO in old rats. These results indicate that the elderly must exercise with caution.

\textbf{REFERENCES}