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Regular exercise prevents high-sucrose diet-induced fatty liver via improvement of hepatic lipid metabolism

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ABSTRACT

Fatty liver is known as the initial stage in nonalcoholic fatty liver disease. Epidemiological studies have shown that regular exercise prevents accumulation of hepatic lipids, although the underlying mechanism is unclear. The purpose of this study was to investigate the effect of exercise on fatty liver associated with hepatic lipid metabolism. KK/Ta mice (6 weeks old) were divided into sedentary and exercise groups and compared with sedentary Balb/c mice. All the mice were fed a high-sucrose diet for 12 weeks. The KK/Ta mice in the exercise group performed a treadmill running exercise at 20 m/min for 30 min (3 times per week). Twelve weeks of regular exercise suppressed the accumulation of lipid in the liver, along with reduction in the level of lipid in the plasma. The levels of carnitine palmitoyl transferase II, acyl-coenzyme A dehydrogenase, and trifunctional enzyme, which are rate-limiting enzymes in fatty acid oxidation in the liver, were elevated by exercise. In addition, the expression of fatty acid synthase, a key lipogenetic enzyme, was reduced by exercise. Furthermore, regular exercise decreased the expression of heat shock protein 47, a marker of hepatic fibrosis, in the liver. Our results suggest that regular exercise prevents fatty liver via improvement of hepatic lipid metabolism.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD), which has pathologic features that include histological abnormalities such as steatosis, steatohepatitis, and cirrhosis, is the most common chronic liver disease [1]. The prevalence estimates in the general populations in developed countries are 20–30% [2,3]. Because fatty liver is characterized by excess accumulation of triglyceride in the liver and is the initial step of progression to NAFLD, treatment and prevention of hepatic lipid accumulation can prevent the development of NAFLD.

It is recognized that the accumulation of hepatic lipids is accelerated by disturbance in dietary habits such as overfeeding. Excess entry of free fatty acid (FFA) from the blood into hepatocytes leads to lipid accumulation. Long-term intake of high-fat or high-carbohydrate meals elevates the lipid levels in the blood, leading to accelerated FFA entry into the liver. In addition, overfeeding often results in insulin resistance with accumulation of visceral adipose tissue, referred to as the metabolic syndrome, which results in abnormal release of FFA from the adipose tissue into the blood and an elevation of circulating FFA levels [4,5]. Therefore, a healthy lifestyle with negative energy balance is recommended for the prevention of NAFLD.

Along with reduction of energy intake, exercise is a novel tool for the treatment and prevention of fatty liver. Several epidemiological studies [6–9] have demonstrated that hepatic lipid contents are lower in individuals with high physical activity than in sedentary individuals. It is well-known that a single bout of aerobic exercise promotes the use of lipids as an energy substrate in the skeletal muscle and other tissues during exercise. In addition, regular exercise adaptively improves the lipid profile of blood including a reduction in FFA levels [10,11]. Thus, we think that the immediate and long-term effects of exercise contribute to the reduction of hepatic lipid content. However, little is known about the molecular mechanism that explains hepatic metabolic changes induced by exercise, although numerous studies [12–15] have shown the mechanism of exercise-induced metabolic improvement in skeletal muscle and adipose tissue. On the other hand, we previously reported that regular exercise could change the expression of a number of genes, including metabolic modulators, by a microarray analysis [16], which suggests that exercise may improve hepatic lipid metabolism via regulation of metabolic modulators in the liver.

In the liver, FFA is aerobically metabolized in the mitochondria, resulting in supply of adenosine triphosphate (ATP), and used as a component in the synthesis of circulating lipoproteins and triglyc-

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eride. The reduction of FFA oxidation and the elevation of lipogenesis accelerate the accumulation of triglyceride in the liver. Thus, it is important for the prevention of fatty liver to regulate FFA oxidation and lipogenesis. Exercise may improve the hepatic lipid metabolism by regulating enzymes that relate to FFA oxidation and lipogenesis. Therefore, the present study investigated that the inhibitory effect of regular exercise on fatty liver is associated with FFA oxidation and lipogenesis in the liver of high-sucrose diet-fed mice.

2. Materials and methods

2.1. Animals and experimental design

The present study complied with the principles and guidelines of the Japanese Council on Animal Care, and it was also approved by the Committee for Animal Research of Kyoto Prefectural University of Medicine. KK/Ta and Balb/c mice (5 weeks old) were obtained from Clea Japan Inc. (Osaka, Japan) and were acclimatized to an air-conditioned (22 ± 2 °C) room with a 12-h light–dark cycle (lights on from 07:30 to 19:30 h) for 1 week. The mice were divided into three groups of eight animals each, namely, the Balb/c sedentary group, the KK/Ta sedentary group, and the KK/Ta exercise group. All the mice were freely fed a high-sucrose diet, which contained 68.7% carbohydrates (49.1% sucrose, 14.6% corn starch, and 5% cellulose) and 6.3% fats (beef tallow), throughout the experimental period. During the first 2 weeks, the level of exercise was gradually increased from running for 15 min with the treadmill set at a speed of 15 m/min to running for 30 min at 20 m/min; then, the running speed was kept at 20 m/min for the following 10 weeks. The animals were euthanized after overnight fasting, and the liver, the gastrocnemius, the epididymal fat, and the blood were removed. The tissues were weighed; 1 portion was fixed flat in 10% buffered formalin; and the remaining portion was rapidly frozen in −80 °C.

2.2. Oral glucose tolerance test

The oral glucose tolerance test was performed at 12 weeks of the experiment after overnight fasting. The mice received a 20% glucose solution at 100 μl per 10 g body weight. Blood was collected from the tail just before and 30, 60, and 120 min after the loading, and the glucose level was measured with a Glutest Ace R (Sanwa Kagaku Kenkyusho Co. Ltd., Nagoya, Japan).

2.3. Blood analysis

Each blood sample obtained from the mice was centrifuged at 3500 rpm for 15 min at 4 °C immediately after collection. Then, the levels of nonesterified fatty acids (NEFA), aspartate 2-oxoglutarate aminotransferase (AST), triglyceride, low-density lipoprotein (LDL)–cholesterol, and high-density lipoprotein (HDL)–cholesterol of plasma were measured with a Shimadzu CL8000 Clinical Chemical Analyzer (Shimadzu Co. Ltd., Tokyo, Japan). The plasma insulin level was measured using the mouse insulin enzyme-linked immunosorbent assay (ELISA) kit (Morinaga Institute of Biological Science Inc., Yokohama, Japan) according to the manufacturer's instructions. The absorbance was measured with a microplate reader, and the concentration of insulin was calculated by comparison with a calibration curve.

2.4. Liver triglyceride measurement

The liver of each mouse was mechanically homogenized in liquid nitrogen. The triglyceride contents were measured with a Serum Triglyceride Determination kit (Sigma–Aldrich Corporation, St. Louis, MO, USA) according to the manufacturer's instructions.

2.5. Histological analysis

Liver tissue sections were cut at a thickness of 7 μm and mounted on silanized slides (Dako Japan, Tokyo, Japan). The sections were stained with hematoxylin–eosin (HE) to observe the histological features of the livers.

2.6. Real-time polymerase chain reaction

Reverse-transcription polymerase chain reaction (PCR) was performed using the total RNA samples obtained from the liver tissues with an ABI 7300 system (Applied Biosystems, Foster City, CA). Real-time PCR using the DNA-binding dye SYBR Green was used for the detection of PCR products for the RNA samples obtained from cells after synthesis of complementary DNA. The following PCR primers (Sigma–Aldrich, Japan, Hokkaido, Japan) were used: acyl-coenzyme A dehydrogenase (ACD): 5′-GGCACAAAAGAACAGATCGAGAA (forward), 5′-TGGCTATGGCACCGATACAC (reverse); carnitine palmitoyl transferase II (CPT-II): 5′-GGGCGAGCTTCAGCATATG (forward), 5′-GGCCCATCGCTGCTTCTT (reverse); trifunctional enzyme: 5′-CCCCAAAGCCCGTTGTG (forward), 5′-GCATGCTATGGCAAGCTCAA (reverse); acetyl-CoA carboxylase (ACC): 5′-CTGGCTGCATCCATTATGTCA (forward), 5′-TGGTAGACTGCCCGTGTGAA (reverse); ATP citrate lyase (ACL): 5′-TCACACTGCCAACTTCCTCCTTA (forward), 5′-ACTCAGAAAAAGATGCTGTCCTACTG (reverse); fatty acid synthase (FAS): 5′-CCTGGATAGCATTCCGAACCT (forward), 5′-AGCACATCTCGAAGGCTACACA (reverse); heat shock protein 47 (HSP47): 5′-GCAGCAGCAAGCAACACTACA (forward), 5′-GGGCGCTGCGCTTGT (reverse); and β-actin: 5′-TATCCACCTTCCAGCAGATGT (forward), 5′-AGCTCAGTAACAGTCCGCCTA (reverse). The ratio of the other signals to that of β-actin was calculated for every sample.

2.7. Statistical analysis

The results are reported as mean ± SE. Analysis of variance (ANOVA) or Student's *t*-test was used to determine statistically significant differences between groups. If the ANOVA indicated a significant difference, the Tukey–Kramer test was used to determine the significance of the differences between the mean values. In all the analyses, $p < 0.05$ was considered to indicate statistical significance.

3. Results

3.1. Body weight and tissue mass

The body weight, the liver mass, and the epididymal fat mass were significantly higher in the KK/Ta mice than in the Balb/c mice (Table 1). Regular exercise significantly reduced increases in body weight and liver mass in the KK/Ta mice (Table 1). On the other hand, the weights of the epididymal fat and gastrocnemius muscle did not significantly change as a result of exercise.

3.2. Oral glucose tolerance test

The fasting blood glucose level was identical between the sedentary and exercise groups of KK/Ta mice (Fig. 1). However, the blood glucose levels at 30, 60, and 120 min after glucose loading had the tendency to be lower in the exercise group than in the sedentary group.