Adaptation of Skeletal Muscle Characteristics to a High-Fat Diet in Rats with Different Intra-Abdominal-Obesity Susceptibilities

Buhao Zou\(^1\), Masataka SUWA\(^2\), Hiroshi NAKANO\(^3\), Yasuki HIGAKI\(^4\), Tatsumi ITO\(^5\), Shigeru KATSUTA\(^6\) and Shuzo KUMAGAI\(^1,2\)

\(^1\)Graduate School of Human-Environment Studies, and \(^2\)Institute of Health Science, Kyushu University, Kasuga, Fukuoka 816–8580, Japan
\(^3\)Department of Human Development, Nakamura Gakuen University, Jonan-ku, Fukuoka 814–0198, Japan
\(^4\)Department of Preventive Medicine, Saga Medical School, Saga 849–8501, Japan
\(^5\)Department of Bioscience and Biotechnology, Kyushu University Graduate School, Hakozaki, Fukuoka 812–8581, Japan
\(^6\)Graduate School of Integrated Science and Art, University of East Asia, Shimonoseki, Yamaguchi 751–8503, Japan

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Summary The purpose of the present study was to investigate the relationship between intra-abdominal-obesity susceptibility and the adaptation of skeletal muscle metabolic and histochemical characteristics when fed a high-fat diet (HFD) for a short period of time. Twenty-four male Wistar rats were fed a HFD (39.7% calories of fat) for 5 wk. After the 5-wk dietary period, the rats were sacrificed and divided into intra-abdominal-obesity-prone (OP) or obesity-resistant (OR) groups according to the total intra-abdominal fat pads (epididymal, mesenteric, and perirenal) weights. A superficial portion of the Muscle (M.) gastrocnemius tissue obtained from 2 groups before and after feeding the HFD were analyzed to determine their hexokinase (HK), ß-hydroxyacyl CoA dehydrogenase (ß-HAD), and citrate synthase (CS) activities. Muscle fiber composition and capillary density were examined in the deep portion of the M. gastrocnemius, soleus, and extensor digitorum longus (EDL) gained after the HFD. While the OP group had more intra-abdominal fat pads and a heavier final body weight than the OR group, there was no significant difference in the energy intake between the two. Due to the HFD, the OP group showed significant increases in ß-HAD and CS activities, while the OR group did not. Change of ß-HAD activity by HFD in the OP group was significantly greater than that in the OR group. The ratio of fat oxidation, expressed as ß-HAD/CS, significantly increased in the OP group, but not in the OR group. No differences were found in either the muscle fiber composition or capillarization. These results suggest that intra-abdominal-obesity-susceptive rats may have a higher adaptation degree in muscle oxidative enzyme activities as characteristic in the early stage of intra-abdominal adipose accumulation.

Key Words obesity, high-fat diet, muscle fiber composition, enzyme activity

Obesity is characterized by increased fat stores when the energy intake exceeds the energy expenditure. Visceral obesity, among several obese indices, has been more closely associated with chronic diseases and syndromes such as “insulin resistance syndrome” or “Syndrome X” than subcutaneous obesity. High-fat diet (HFD) studies using rats have shown individual differences in the susceptibility to visceral adipose tissue (1–3). However, the metabolic factors that are responsible for this difference remain unclear. Due to its utilization of glucose and fatty acids, the skeletal muscle plays an important role in the energy metabolism. Skeletal muscle fibers are categorized as slow-twitch (type I) and fast-twitch (type II) fibers according to their contraction velocity. Recently, rat muscle type II fibers have been further subclassified into type IIA, type IIX, and type IIB fibers (4). Type I fibers have been demonstrated to have higher oxidative enzyme activity as compared to type II fibers (5). In cross-sectional studies, obese subjects were found to have a lower proportion of type I muscle fibers (6–8) and lower oxidative enzyme activities (9, 10), which were thus suggested to be predeterminate factors for obesity. On the other hand, in longitudinal studies, HFD, which induced visceral obesity (11), increased the oxidative enzyme activities of muscles (12, 13). However, the relationship between visceral-obesity-susceptibility and the adaptation of skeletal muscle characteristics have not been strictly investigated so far. We designed a 5-wk longitudinal protocol and compared the muscle characteristics not only between obesity-prone (OP) and obesity-resistant (OR) groups, but also between the same animals before and after HFD.
which has been done only rarely in previous studies (1, 3, 6, 14–16) in order to rule out any bias from individual diversity. Instead of body weight gain, intra-abdominal adipose accumulation, as a model of visceral fat, which is a more important factor regarding obesity and manifests itself as intra-abdominal fat pads, was chosen to be the main classification criterion. The purpose of this study was to investigate the relationship between intra-abdominal-obesity susceptibility and the adaptation of skeletal muscle characteristics when fed a HFD for a short period of time.

MATERIALS AND METHODS

Study design. Male Wistar rats (n=24) at 10 wk of age and weighing 317–386 g were used for this study. Rats were housed individually under controlled conditions (12:12-h light-dark cycle and 20°C room temperature) and given food and water ad libitum. All experimental procedures were approved by the Guide for the Care and Use of Laboratory Animals.

At the beginning of the study, all rats were weighed and anaesthetized with pentobarbital sodium (50 mg/kg ip). The lateral side of the right leg was shaved and then sterilized with 70% ethanol. The skin was opened (1 cm) with a blade, and muscle samples (about 100 mg) were obtained from the superficial portion of the Muscle (M.) gastrocnemius. Samples were immediately frozen and stored in liquid nitrogen until assayed. The skin was thereafter closed with stainless steel autoclips and the rats were injected with penicillin (2.5 mg/kg im). During the 2-wk recovery period after biopsy, all rats were fed a normal rat chow diet. After the recovery period, they were fed a HFD for 5 wk. The HFD was prepared by mixing lard with normal rat chow materials to make it contain the following percentages of calories, 39.7% fat, 18.5% protein, and 41.9% carbohydrate (KBT Oriental, JP). The energy content of the diet was 39,7% fat, 18.5% protein, and 41.9% carbohydrate.

Muscle histochemical analysis. In the deep portion of the M. gastrocnemius, soleus, and EDL, complete cross-sectional segments were cut at the muscle belly. Each muscle piece was mounted on a specimen holder in OCT embedding medium (Miles Tissue-Tec L), and frozen in isopentane previously cooled in a viscous fluid with liquid nitrogen. Transverse sections (10 μm) were cut from each muscle using a cryostat maintained at −20°C and the sections were mounted on a cover glass. Myosin adenosine triphosphatase (ATPase) was demonstrated using previously described procedures. In brief, consecutive serial sections were processed using three different pretreatments, preincubation pH 4.3, 4.6, and 10.4. The muscle fibers were identified as type I, IIA, IIX, IIB, and IIC fibers based on the myosin ATPase staining intensity (17). A composite photomontage of each ATPase preparation was made using micrographs, and then each fiber was identified and counted using a handcounter. The fibers counted for the deep portion of the M. gastrocnemius were >500, and all countable M. soleus and EDL fibers on the cross section were counted. Muscle capillaries were stained with ATPase staining. In brief, each muscle cross section was fixed in 4% formaldehyde with 0.1 m phosphate buffer, pH 7.4 in room temperature, followed by pre-incubation at pH 10.3. After that, incubation was performed as above.

Enzyme Assay. Enzyme assays were performed on the samples extracted from a superficial portion of the M. gastrocnemius. The 10% homogenates made from the respective muscles in 175 mm KCl, 10 mm GSH, and 2 mm EDTA, pH 7.4, were frozen and thawed three times to disrupt the mitochondrial membrane and then mixed thoroughly before performing the enzymatic measurements. Assays were performed using a UV/Vis spectrophotometer (JAS V-530) equipped with a temperature-controlled cell holder by previously established techniques: HK (18), β-HAD (19), and CS (20) at 30°C. The coefficients of variation for the enzyme assay were 1.8% for HK, 1.2% for β-HAD, and 1.7% for CS by the same sample repeated measurements.

Statistical analysis. Based on the weights of the total intra-abdominal fat pads, the data of the rats were divided into two groups: 12 for obesity-prone rats (OP) and 12 for obesity-resistant rats (OR). All data were presented as means±SE. Two-way ANOVA was used to compare the body weights, enzyme activities, and changes in ratio of enzyme activities. Fisher’s PLSD was conducted if ANOVA indicated a significant difference. The unpaired t-test was used in comparisons for weight gain, total energy intake, muscle weight, intra-abdominal fat pads, intra-abdominal fat pads/final body weight, change in enzyme activities, muscle fiber composition, and indexes of capillarization. Variables were considered significantly different when the p value was less than 0.05.

RESULTS

Body composition and food intake

The body weights are presented in Fig. 1. On the biopsy day, the body weights were 338.4±5.3 g for the OP group and 329.8±2.5 g for the OR group. On the first day of the HFD, body weights reached 407.6±4.1 g for the OP group and 393.9±5.6 g for the OR group. There was no significant difference between the OP and OR groups on either day. The final body weights on the day of sacrifice were statistically different (OP: 551.7±7.4 g; OR: 520.2±10.9 g, p<0.05). The difference between the two groups in body weight increase after feeding the HFD was also almost significant (p<0.006).

Total energy intake was calculated according to a food intake record. Despite the apparent difference in the final body weights, there was no significant difference in the total energy intake between the groups (OP:
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Fig. 1. Left: the average body weights of OP and OR rats. Biopsies were conducted in week 0, and the HFD started in week 2 and finished in week 7. *Significant difference (p<0.05) between OP and OR groups. Right: total weight gain of the two groups during feeding of HFD.

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Fig. 2. Enzyme activities of hexokinase (HK), β-hydroxyacyl CoA dehydrogenase (β-HAD), and citrate synthase (CS). *Significantly different (p<0.05) compared to the activity of the same rat group before feeding the HFD.

Fig. 3. Changes in HK, β-HAD, and CS caused by the HFD. The change in enzyme activity was determined as follows: change of enzyme activity = post−pre of same group. *Significant difference (p<0.05) between OP and OR groups.

Fig. 4. Ratio of enzyme activities. *Significantly different (p<0.05) compared to the activity of the same rat group before feeding the HFD.

Enzyme activity

Muscle enzyme activities are shown in Fig. 2. Over the 5-wk HFD period, the maximal activity of β-HAD (key enzyme of β-oxidation of fatty acids) and CS (key enzyme of tricarboxylic acid cycle) significantly increased in the OP group but did not change in the OR group. The activity of HK (key enzyme of glucose utilization) did not change in either group. Comparisons between the OP and OR groups were also made, but the activities of these three enzymes were not significantly different before and after HFD. As for the change of enzyme activity, the change in β-HAD of the OP rats was nearly fourfold that of the OR rats (p<0.05, Fig. 3).

The ratios of glycolysis and oxidation were determined based on the enzyme ratios (Fig. 4). In the OP group, after HFD, HK/β-HAD (an indication of the relative capacity for glycolysis vs. β-oxidation) and HK/CS (an indication of the relative capacity for glycolysis vs. total oxidative capacity) decreased while an increase was found in β-HAD/CS (an indication of the capacity for β-oxidation relative to total oxidative capacity). All of these differences were significant (p<0.05). On the other hand, in the OR group, significant differences were only observed in HK/β-HAD and HK/CS.

DISCUSSION

This study examined a number of important properties of skeletal muscle in OP and OR rats before and/or after feeding a HFD. In most of the former studies (3, 14, 15), muscle samples were compared only between OP and OR groups after feeding the HFD. We addition-
ally compared samples from the same animals before and after diet intervention in this study. The method of extracting muscle tissue specimens from the same rats from the contraposition in the leg before and after feeding the HFD was considered to be an effective method for obtaining closely similar samples.

The second meaningful characteristic of this study was the similar energy intake of the two groups. The average cumulative energy intake by the OP group was not significantly different from that by the OR group. Without this prerequisite (1, 2, 6, 14), the difference in food intake may weaken such a comparability. In most of the former studies on HFD, the subjects were divided into OP and OR groups based on their body weight gain, and the total energy intake was usually greater in OP rats than in OR rats. The classification method in this study was not the same. We defined OP and OR rats according to their visceral fat pads weight. It is possible that the intra-abdominal fat pad weight is less closely correlated with total energy intake than body weight gain.

Lillioja et al. showed a significant correlation between the degree of obesity and muscle fiber composition (r=-0.32 for percent type I and r=0.32 for percent type IIX) (7). This observation was consistent with the report of Wade et al. (8) that the proportion of type I fibers was negatively correlated with the percentage of body fat. Therefore, the muscle fiber composition was considered to be a predeterminate factor for obesity. When rats were classified by their intra-abdominal fat pad weight in this study, no differences were observed between OP and OR rats regarding either fiber type of either muscle. Based on our data, skeletal muscle fiber composition may not be a predeterminate factor for visceral obesity. The OP group was in the early stages of the normal-to-viscerally obese process. This differs from former studies, which mostly observed only obese subjects. Obesity, especially visceral obesity, induces marked endocrinal changes such as hyperinsulinemia in its later stage, and hyperinsulinemia has been reported to be able to induce an alteration in the muscle fiber composition (21, 22). Such alterations might not have been induced in this study due to the short length of the study. Unfortunately, we did not collect blood samples in this study. The presence of hyperinsulinemia cannot be negated directly. However, in another HFD (45.2% energy from fat) study we conducted, even 8 wk of loading a diet much higher in fat% failed to induce hyperinsulinemia in the male Wistar rats (unpublished data). The data of the current study negated the muscle fiber composition as a predeterminate factor for intra-abdominal obesity. Interestingly, another study we conducted, which showed the fast-twitch fiber dominant rat was more obesity-resistant than the control rat after feeding a HFD, threw doubt on the muscle fiber composition as a predeterminate factor even for body weight-based obesity (23).

The most important observation of this HFD study is that the OP group showed a greater increase in oxidative enzyme activities than the OR group. The HFD induced visceral obesity (11) and increased the oxidative enzyme activities of muscles (12, 13). On the other hand, feeding of the HFD increased the activities of \( \beta \)-HAD and CS only in the OP group in the current study. The change of \( \beta \)-HAD in OP rats was nearly fourfold that in the OR rats. Together with a significant difference of intra-abdominal adipose between them, it is proper to regard these rats as obesity-prone/resistant rats, respectively. In addition, the increased oxidative adaptation in the skeletal muscle of the OP group was considered to correlate with the HFD-induced intra-abdominal adipose accumulation.

The change in the ratio of glycolysis to oxidation showed that metabolic dominance in the skeletal muscle adapted to the HFD. In both the OP and OR groups, the proportion of glycolysis decreased and that of oxidation increased. Except for the \( \beta \)-HAD/CS in the OR group, all changes were significant. This suggests that, due to the HFD, the metabolic balance in skeletal muscle tends to rely on fat oxidation in order to consume such excessive adipose. The OP group showed a greater change in this regard as compared to the OR group. This was compatible with the difference in intra-abdominal adipose accumulation between the two groups.

Although the difference was not significant, the OP rat consumed a total of 210.4 kcal more than the OR rat did on average, and this corresponded to approximately 23.4 g of adipose tissue. The average increase in body weight gain was 144.0 g for OP rats and 126.3 g for OR rats. In fact, the OP rat showed a body weight gain of only 17.7 g more on average than the OR rat. Namely, OP rat body weight increased an average of about 6 g less than they should have in theory. Regarding the intake calories of the OR rats as the standard, the OP rats tended to exhibit weight gain-resistance.

To explain these phenomena, adipocytokines are considered to be a possible contributor. In rodent skeletal muscle, leptin has shown a regulatory effect on fatty acid oxidation (24-27) or peroxisome proliferator-activated receptor (PPAR) \( \gamma \) coactivator 1 (PGC-1) (28), the co-activator that promotes mitochondrial biogenesis (29, 30) and cooperates with PPAR\( \alpha \) in the transcriptional control of nuclear genes encoding mitochondrial fatty acid oxidation enzymes (31). It is possible that the accumulation of adipose tissue increased the serum leptin concentration in the early stage of obesity, and as a result, fatty acid oxidation in the skeletal muscle occurred. The OP rats showed significant adaptation to the muscle oxidative enzyme activities due to the significant accumulation of adipose tissue, as shown by the greater intra-abdominal fat pad weight and final weight.

In the early stage of obesity, the greater oxidative adaptation in the skeletal muscle of the OP rat might imply a protective effect that inhibits further fat accumulation. This phenomenon correlates with the known function of leptin. However, obese subjects in the latter stage of obesity were found to have lower oxidative enzyme activities (9, 10). The concept of leptin resis-
tance that has been verified in obese subjects (32) may explain this contradiction. Further studies focusing on the regulatory effects of adipocytokines in the enzyme activities of skeletal muscle and the related time course are therefore needed in the future.

It is noteworthy that the differences in weight, intra-abdominal fat, and increase in the oxidative enzyme activities between the OP and OR groups in this study were small. Even though they were statistically significant, there is need to use a larger number of animals or use a control group to confirm the findings in the future. In addition, due to the different patterns of obesity in male and female animals, further studies extended to female rats with the same study protocol are needed to examine whether or not both sexes respond similarly.

In summary, the present study found no differences in the muscle fiber composition or capillarization between OP and OR rats, but a greater increase in the oxidative enzyme activity in OP rats after feeding a HFD. This suggests that the skeletal muscle fiber composition does not seem to be a predeterminant factor for visceral obesity. Instead, intra-abdominal-obesity-susceptible rats may characteristically be more adaptive in terms of muscle oxidative enzyme activities in the early stage of intra-abdominal adipose accumulation.

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REFERENCES


