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-visceral-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 β-HAD and CS only in the OP group in the current study. The change of β-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 β-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) γ coactivator 1 (PGC-1) (*28*), the co-activator that promotes mitochondrial biogenesis (*29, 30*) and cooperates with PPARα 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 on the enzyme activities of skeletal muscle and the related time course are therefore needed in the future.

It is note-worthy 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 predeterminate 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

1) Commerford SR, Pagliassotti MJ, Melby CL, Wei Y, Gayles EC, Hill JO. 2000. Fat oxidation, lipolysis, and free fatty acid cycling in obesity-prone and obesity-resistant rats. *Am J Physiol Endocrinol Metab* **279**: E875–E885.

2) Lauterio TJ, Barkan A, DeAngelo M, DeMott-Friberg R, Ramirez R. 1998. Plasma growth hormone secretion is impaired in obesity-prone rats before onset of diet-induced obesity. *Am J Physiol Endocrinol Metab* **38**: E6–E11.

3) Pagliassotti MJ, Pan DA, Prach PA, Koppenhafer TA, Storlien LH, Hill JO. 1995. Tissue oxidation capacity, fuel stores and skeletal muscle fatty acid composition in obesity-prone and obesity-resistant rats. *Obes Res* **3**: 459–464.

4) Sant'Ana Pereira JAA, Ennion S, Sargeant AJ, Moorman AFM, Goldspink G. 1997. Comparison of the molecular, antigenic and ATPase determinants of fast myosin heavy chains in rat and human: a single-fibre study. *Pflügers Arch* **435**: 151–163.

5) Essén B, Jansson E, Henriksson A, Taylor AW, Saltin B. 1975. Metabolic characteristics of fibre types in human skeletal muscle. *Acta Physiol Scand* **95**: 153–165.

6) Abou Mrad J, Yakubu F, Lin D, Peters JC, Atkinson JB, Hill JO. 1992. Skeletal muscle composition in dietary obesity-susceptible and dietary obesity-resistant rats. *Am J Physiol Regul Integr Comp Physiol* **262**: R684–R688.

7) Lillioja S, Young AA, Culter CL, Ivy JL, Abbott WGH, Zawadzki JK, Yki-Järvinen H, Christin L, Secomb TW, Bogardus C. 1987. Skeletal muscle capillary density and fiber type are possible determinants of in vivo insulin resistance in man. *J Clin Invest* **80**: 415–424.

8) Wade AJ, Marbut MM, Round JM. 1990. Muscle fibre type and aetiology of obesity. *Lancet* **335**: 805–808.

9) Raben A, Mygind E, Astrup A. 1998. Low activity of oxidative key enzymes and smaller fiber areas in skeletal muscle of postobese women. *Am J Physiol Endocrinol Metab* **38**: E487–E494.

10) Simoneau JA, Veerkamp JH, Turcotte LP, Kelley DE. 1999. Markers of capacity to utilize fatty acids in human skeletal muscle: relation to insulin resistance and obesity and effects of weight loss. *FASEB J* **13**: 2051–2060.

11) Joug-Yeon K, Nolte LA, Hansen PA, Dong-Ho H, Ferguson K, Thompson PA, Holloszy JO. 2000. High-fat diet-induced muscle insulin resistance: relationship to visceral fat mass. *Am J Physiol Regul Integr Comp Physiol* 279: R2057–R2065.

12) Miller WC, Bryce GR, Conlee K. 1984. Adaptations to a high-fat diet that increase exercise endurance in male rats. *J Appl Physiol* **56**: 78–83.

13) Simi B, Sempore B, Mayet MH, Favier RJ. 1991. Additive effects of training and high-fat diet on energy metabolism during exercise. *J Appl Physiol* **71**: 197–203.

14) Gayles EC, Pagliassotti MJ, Prach PA, Koppenhafer TA, Hill JO. 1997. Contribution of energy intake and tissue enzymatic profile to body weight gain in high-fat-fed rats. *Am J Physiol Regul Integr Comp Physiol* **272**: R188–R194.

15) Nemeth PM, Rosser BW, Choksi RM, Norris BJ, Baker KM. 1992. Metabolic response to a high-fat diet in neonatal and adult rat muscle. *Am J Physiol Cell Physiol* **31**: C282–C286.

16) Sam C, Graham B, Yakubu F, Lin D, Peters JC, Hill JO. 1990. Metabolic differences between obesity-prone and obesity-resistant rats. *Am J Physiol Regul Integrative Comp Physiol* **28**: R1103–R1110.

17) Gorza L. 1990. Identification of a novel type 2 fiber population in mammalian skeletal muscle by combined use of histochemical myosin ATPase and anti-myosin monoclonal antibodies. *J Histochem Cytochem* **38**: 257–265.

18) Uyeda K, Racker E. 1965. Regulatory mechanisms in carbohydrate metabolism VII. Hexokinase and phosphofructokinase. *J Biol Chem* **240**: 4682–4688.

19) Kobayashi A, Jiang LL, Hashimoto T. 1996. Two mitochondrial 3-hydroxyacyl-CoA dehydrogenases in bovine liver. *J Biochem* **119**: 775–782.

20) Srere PA. 1969. Citrate synthase. *Methods Enzymol* **13**: 3–6.

21) Holmäng A, Brzezinska Z, Björntorp P. 1993. Effect of hyperinsulinemia on muscle fiber composition and capillarization in rats. *Diabetes* **42**: 1073–1081.

22) Houmard JA, O'Neill DS, Donghai Z, Hickey MS, Dohm GL. 1999. Impact of hyperinsulinemia on myosin heavy chain gene regulation. *J Appl Physiol* **86**: 1828–1832.

23) Suwa M, Kumagai S, Higaki Y, Nakamura T, Katsuta S. 2002. Dietary obesity-resistance and muscle oxidative enzyme activities of the fast-twitch fibre dominant rat. *Int J Obes* **26**: 830–837.

24) Minokoshi Y, Kim YB, Peroni OD, Fryer LG, Muller C, Carling D, Kahn BB. 2002. Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. *Nature* **415**: 339–343.

25) Muoio DM, Dohm GL, Fiedorek FT Jr, Tapscott EB, Coleman RA, Dohn GL. 1997. Leptin directly alters lipid partitioning in skeletal muscle. *Diabetes* **46**: 1360–1363.
26) Steinberg GR, Dyck DJ. 2000. Development of leptin resistance in rat soleus muscle in response to high-fat diets. *Am J Physiol Endocrinol Metab* **279**: E1374–E1382.
27) Steinberg GR, Bonen A, Dyck DJ. 2002. Fatty acid oxidation and triacylglycerol hydrolysis are enhanced after chronic leptin treatment in rats. *Am J Physiol Endocrinol Metab* **282**: E593–E600.
28) Kakuma T, Wang ZW, Pan W, Unger RH, Zhou YT. 2000. Role of leptin in peroxisome proliferator-activated receptor gamma coactivator-1 expression. *Endocrinology* **141**: 4576–4582.
29) Bergeron R, Ren JM, Cadman KS, Moore IK, Perret P, Pypaert M, Young LH, Semenkovich CF, Shulman GI. 2001. Chronic activation of AMP kinase results in NRF-1 activation and mitochondrial biogenesis. *Am J Physiol Endocrinol Metab* **281**: E1340–E1346.
30) Wu H, Kanatous SB, Thurmond FA, Gallardo T, Isotani E, Bassel-Duby R, Williams RS. 2002. Regulation of mitochondrial biogenesis in skeletal muscle by CaMK. *Science* **296**: 349–352.
31) Vega RB, Huss JM, Kelly DP. 2000. The coactivator PGC-1 cooperates with peroxisome proliferator-activated receptor alpha in transcriptional control of nuclear genes encoding mitochondrial fatty acid oxidation enzymes. *Mol Cell Biol* **20**: 1868–1876.
32) Steinberg GR, Parolin ML, Heigenhauser GJF, Dyck DJ. 2002. Leptin increases fatty acid oxidation in lean but not obese human skeletal muscle: evidence of peripheral leptin resistance. *Am J Physiol Endocrinol Metab* **283**: E187–E192.

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Effects of chronic AICAR treatment on fiber composition, enzyme activity, UCP3, and PGC-1 in rat muscles

Masataka Suwa,[1] Hiroshi Nakano,[2] and Shuzo Kumagai[1]
[1]*Institute of Health Science, Kyushu University, Kasuga, Fukuoka 816-8580; and* [2]*Department of Human Development, Nakamura Gakuen University, Jonan-ku, Fukuoka 814-0198, Japan*

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Suwa, Masataka, Hiroshi Nakano, and Shuzo Kumagai. Effects of chronic AICAR treatment on fiber composition, enzyme activity, UCP3, and PGC-1 in rat muscles. *J Appl Physiol* 95: 960–968, 2003. First published May 30, 2003; 10.1152/japplphysiol.00349.2003.—This study was designed to determine the histological and metabolic effects of the administration of 5′-AMP-activated protein kinase (AMPK) activator 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) for 14 successive days. AICAR treatment caused a significant decrease in the percentage of type IIB fibers and the concomitant increase in the percentage of type IIX fibers in extensor digitorum longus (EDL) muscle. The capillary density and the capillary-to-fiber ratio were not altered by AICAR. AICAR treatment increased the glycolytic and oxidative enzyme activities but not the antioxidant enzyme activities. The AICAR treatment increased the uncoupling protein 3 (UCP3) level in EDL and the peroxisome proliferator-activated receptor-γ coactivator-1α protein level in the soleus and EDL muscles, whereas the myogenin level was not altered by AICAR. These results seem to imply that the chronic activation of AMPK alters such muscle histochemical and metabolic characteristics.

5′-AMP-activated protein kinase; antioxidant capacity; mitochondrial enzymes; muscle fiber type composition; peroxisome proliferator-activated receptor-γ coactivator-1α; 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside; uncoupling protein 3

ENDURANCE EXERCISE TRAINING modifies various physiological characteristics of the skeletal muscle, including the muscle fiber composition (16), capillary network (25), metabolic capacity (4), and antioxidant systems (37). Despite the fact that a number of signaling pathways inducing such adaptations have been analyzed for several years (9, 30, 51, 54), the mechanisms of such adaptations remain to be fully elucidated.

Recently, the activation of 5′-AMP-activated protein kinase (AMPK) by the injection of 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) or β-guanidinopropionic acid feeding showed an increase in fatty acid oxidation (31), glucose uptake (31), mitochondrial biogenesis (2, 58), hexokinase (HK) activity (20, 53), mitochondrial enzyme activities (53), glucose transporter 4 (GLUT-4) protein (20), and uncoupling protein 3 (UCP3) (57). AMPK was activated by muscle contraction such as exercise (14, 52) and electrical stimulation (22) by depression of ATP-to-AMP ratio. It is thus hypothesized that such skeletal muscle adaptations to endurance exercise training occur at least in part through the AMPK pathway.

Peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1) is a transcriptional coactivator that interacts with several nuclear transcriptional factors (40). PGC-1 promoted GLUT-4 expression (32), mitochondrial biogenesis (30, 56), and fiber type transformation (30) in skeletal muscle cells. PGC-1 mRNA expression was enhanced by AICAR (46). Acute endurance exercise increased skeletal muscle PGC-1 mRNA (1, 36, 46) and protein (1) levels. Endurance exercise training also increased skeletal muscle PGC-1 mRNA (36). On the basis of these data, it is hypothesized that some skeletal muscle adaptations due to endurance exercise training such as an increase in the oxidative enzyme activities and fiber type transformation are induced by the activation of AMPK and the consequent increase in the PGC-1 expression. However, a study to determine whether such skeletal muscle adaptations are related to the chronic activation of AMPK and increased PGC-1 protein content has yet to be conducted. Therefore, one purpose of the present study was to determine whether the chronic injection of AICAR influences the PGC-1 protein content, muscle histochemical characteristics including the fiber composition and capillary density, and metabolic enzyme activities in rat slow- and fast-twitch skeletal muscles.

Endurance exercise training enhances skeletal muscle antioxidant enzyme activities (37). In addition, acute exercise and AICAR increase skeletal muscle UCP3 (57), which is a possible regulator of free radical production (49). These reports raise the possibility that the activation of AMPK also enhances antioxidant systems. However, very few studies concerning the relationship between AMPK and antioxidant systems have so far been conducted (43, 57). Another purpose of the present study was to test the hypothesis that the chronic activation of AMPK by AICAR increases the skeletal muscle UCP3 content and antioxidant enzyme activities.

Address for reprint requests and other correspondence: S. Kumagai, Institute of Health Science, Kyushu Univ., Kasuga, Fukuoka 816-8580, Japan (E-mail: shuzo@ihs.kyushu-u.ac.jp).

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METHODS

Animals. Five-week-old male Wistar rats with a body weight of ~140 g were used for this experiment. All rats were handled daily for at least 6 days before beginning the treatment regimen. All rats were housed two to three per cage (42 × 25 × 20 cm deep) in a temperature- (22 ± 2°C) and humidity-controlled (60 ± 5%) room with a 12-h light (0700 to 1900) and 12-h dark (1900 to 0700) cycle. Food and water were provided ad libitum. All experimental procedures were approved by the University Committee for the Use of Animals in Research and were in strict accordance with the American Physiological Society Guiding Principles in the Care and Use of Animals. The rats were divided into control (n = 9) or AICAR (n = 7) groups. The rats of the control and AICAR groups were given daily subcutaneous injections of saline vehicle and AICAR (Toronto Research Chemicals, North York, ON, Canada) (1 mg/g body wt) in saline, respectively. This dose of AICAR certainly enhanced the skeletal muscle AMPK activity at 60 and 120 min after injection (20, 23, 43, 53). Such procedures were performed between 0800 and 1000 for 14 successive days. The nonfasted rats were anesthetized ~24 h after the last injection, with pentobarbital sodium (60 mg/kg body wt ip). The soleus and extensor digitorum longus (EDL) muscles of both legs were rapidly dissected. The muscles of the right leg were used for an enzyme assay and those of the left leg were used for histochemistry and Western blotting. The abdominal fat pads (perirenal, epididymal, and mesenteric) were also excised and weighed.

Muscle histochemistry. Skeletal muscle fibers were roughly categorized as type I, IIA, and IIB fibers (6). An analysis of single muscle fibers demonstrated that the histochemically defined type I, IIA, and IIB fibers expressed myosin heavy chains 1, 2a, and 2b, respectively (47). Furthermore, an additional myosin heavy chain 2x, which is also called 2d, has been identified (29, 47). In addition, the type IIC fibers that coexpress type 1 and type 2a myosin heavy chain proteins were also observed (42, 47). In this study, the muscle fibers were categorized as type I, IIC, IIA, IIX, and IIB fibers. The rank order of maximum contraction velocity in rat skeletal muscle fibers was I < IIA < IIX < IIB (15). Muscle transverse sections (7 μm) were cut from each muscle by using a cryostat maintained at −20°C, and the sections were then mounted on a cover glass. Myosin adenosine triphosphatase (ATPase) was determined by using the previously described procedures (44). In brief, consecutive serial sections were processed by using three different pretreatments, preincubation at pH 4.3, 4.6, and 10.4. The muscle fibers were identified as type I, IIC, IIA, IIX, and IIB fibers on the basis of the myosin ATPase staining intensity. Each section was photographed by use of an Axioskop 2 plus microscope (Carl Zeiss, Hallbergmoos, Germany) mounted with an Axiocam HRm CCD camera (Carl Zeiss), and then each fiber was identified and counted by use of a hand counter. Next, the muscle fiber composition was determined by evaluating all countable fibers in both muscles. A remaining transverse section was stained to determine the succinate dehydrogenase activity (33).

To visualize the capillaries, another cross-section (7 μm) was also cut. The section was fixed with 100 mM phosphate buffer containing 4% formaldehyde for 4 min at room temperature and then myosin ATPase (preincubation at pH 10.3) was demonstrated as described previously (45). The stained sections were photographed, and then the artifact-free three 0.147-mm^2 areas in each section were analyzed to determine the capillary density (capillaries/mm^2), capillary-to-fiber ratio (capillaries/fibers), and fiber density (fibers/mm^2).

Enzyme assay. The frozen muscle samples were homogenized 1:20 (wt/vol) in 175 mM KCl, 10 mM GSH, 2 mM EDTA, and 0.1% Triton X-100, pH 7.4. Enzyme activities were measured spectrophometrically. All enzymatic assays were carried out at 30°C by using saturating concentrations of substrates and cofactors as determined in preliminary analyses. Citrate synthase (CS; tricarboxylic acid cycle) and carnitine palmitoyltransferase (CPT; transport of fatty acids to mitochondria) activities were measured at 412 nm to detect the transfer of sulfhydryl groups to 5,5′-dithiobis(2-nitrobenzonic acid) (DTNB). HK (mobilization of blood glucose), pyruvate kinase (PK; glycolysis), lactate dehydrogenase (LDH; anaerobic glycolysis), malate dehydrogenase (MDH; tricarboxylic acid cycle), β-hydroxyacyl CoA dehydrogenase (HAD; β-oxidation of fatty acids), glutathione peroxidase (GPX; antioxidant system), and glutathione reductase (GR; antioxidant system) activities were measured at 340 nm by following the production or disappearance of NADH or NADPH.

For the HK (EC 2.7.1.1) assay, 100 mM Tris·HCl, 0.4 mM NADP, 5 mM $MgCl_2$, 700 U/ml glucose-6-phosphate dehydrogenase, 1 mM glucose, and 5 mM ATP, pH 7.0, were used.

For the PK (EC 2.7.1.40) assay, 50 mM Tris·HCl, 0.1 mM KCl, 10 mM $MgCl_2$, 0.28 mM NADH, 1.5 mM ADP, 6 U/ml LDH, and 5 mM phosphoenolpyruvate, pH 7.6, were used.

For the LDH (EC 1.1.1.27) assay, 50 mM Tris·HCl, 0.28 mM NADH, and 2.4 mM pyruvic acid, pH 7.6, were used.

For the MDH (EC 1.1.1.37) assay, 50 mM Tris·HCl, 0.28 mM NADH, and 0.5 mM oxalacetate, pH 7.6, were used.

For the CS (EC 4.1.3.7) assay, 100 mM Tris·HCl, 0.1 mM DTNB, 0.3 mM acetyl-CoA, 3.33 mM K_2HPO_4, and 0.5 mM oxalacetate, pH 8.0, were used.

For the CPT (EC 2.3.1.21) assay, 75 mM Tris·HCl, 0.2 mM DTNB, 1.5 mM EDTA, 2 mM L-carnitine, 0.05 mM palmitoyl-CoA, pH 8.0, were used.

For the HAD (EC 1.1.1.35) assay, 100 mM Tris·HCl, 0.28 mM NADH, 5 mM EDTA, and 0.1 mM acetoacetyl-CoA, pH 6.9, were used.

For the GPX (EC 1.11.1.9) assay, 100 mM Tris·HCl, 0.5 mM EDTA, 2 mM GSH, 0.2 mM NADPH, 1 U/ml GR, and 0.07 mM *t*-butyl hydroperoxide, pH 8.0, were used.

For the GR (EC 1.6.4.2) assay, 50 mM phosphate buffer, 1 mM EDTA, 1 mM GSSG, 0.2 mM NADPH, and 0.1% BSA, pH 7.6, were used.

Primary antibodies. Affinity-purified rabbit polyclonal antibody to UCP3 (AB3046, Chemicon International, Temecula, CA), PGC-1 (H-300, Santa Cruz Biotechnology, Santa Cruz, CA), and myogenin (M-225, Santa Cruz Biotechnology) were used in this study.

Gel electrophoresis and Western blotting. The tissue specimens from each muscle were homogenized (1:10) in 50 mM Tris·HCl pH 7.4, 5 mM EDTA, 10 μg/ml PMSF, 0.5 μg/ml leupeptin, 0.2 μg/ml aprotinin, 0.1% Triton X-100, 0.2% NP-40, 0.05% mercaptoethanol, and 1 mM Na_3VO_4 for 30 s. The homogenate was centrifuged at 15,000 *g* (4°C) for 25 min. The supernatant was removed, and its protein concentration was determined by use of a protein determination kit (Protein Assay II, 500-0006, Bio-Rad, Richmond, CA). Sodium dodecyl sulfate-polyacrylamide gel (12.5% for UCP3 and myogenin and 7.5% for PGC-1) electrophoresis (SDS-PAGE) was performed. The proteins separated by SDS-PAGE were transferred onto the polyvinylidene difluoride membrane electrophoretically. The membrane was incubated with a blocking buffer of casein solution (SP-5020, Vector Laboratories, Burlingame, CA) for 30 min. The membrane was reacted with the primary antibodies for 1 h and then incubated with biotinylated anti-rabbit IgG (1:800 dilution, Vector Laboratories) for

30 min. The membrane was incubated for 30 min with the avidin and biotinylated horseradish peroxidase macromolecular complex technique (PK-6100, Vector Laboratories) and then was visualized with diaminobenzidine and H_2O_2. The band densities were determined by use of the NIH Image 1.62 software (National Institutes of Health, Bethesda, MD).

Statistical analysis. To compare the body weight between the treatment groups, the two-way repeated-measures ANOVA (rat group × day) was used. To compare the other variables, the unpaired *t*-test was used. A value of $P < 0.05$ was considered to be significant.

RESULTS

Body composition. The body mass and abdominal fat content of the rats in each group are shown in Fig. 1. No statistically significant differences in the body mass were observed between the control and AICAR groups ($P = 0.599$) (Fig. 1*A*). The abdominal fat content of AICAR (3.99 ± 0.20 g) was 26% lower than the control (5.39 ± 0.20 g) ($P = 0.0002$) (Fig. 1*B*). Although the rats were fed ad libitum and food consumption was not measured, it is speculated that the observed difference was at least partially due to the chronic activation of AMPK by AICAR rather than any difference in food intake because a previous study using pair-fed rats (53) also indicated a lower fat pad weight in chronic AICAR-injected rats than saline-injected rats.

Histochemical analyses. In the EDL muscle, from the myosin ATPase stained sections (Fig. 2*A*, *a* and *b*), the intermediately stained type IIB fibers appear to decrease in the AICAR group. In addition, in the succinate dehydrogenase-stained sections (Fig. 2*A*, *c* and *d*), the intensely stained oxidative fibers seem to increase in the AICAR group. As shown in Fig. 2*B*, the percentage of type IIB fibers in AICAR was significantly lower than in control (26.9 ± 2.7 and 36.1 ± 2.4%, respectively, $P = 0.024$), and the percentage of IIX fibers in AICAR was significantly higher than in control (49.0 ± 2.6 and 40.9 ± 2.1%, respectively, $P = 0.030$). In the soleus muscle, as shown in Fig. 2*B*, no significant differences were observed in fiber composition of either fiber type. No type IIX or IIB fibers were observed in the soleus muscle.

The capillary density, capillary-to-fiber ratio, and fiber density (an index of fiber size) are indicated in Table 1. No significant differences were observed regarding the capillary network between the groups.

Enzyme activities. Table 2 indicates the muscle enzyme activities. HK, PK, CS, MDH, and HAD activities in the AICAR group was significantly greater than control ($P < 0.05$) in both the soleus and EDL muscles. On the other hand, the LDH, CPT, GPX, and GR activities in AICAR were not significantly different from control in either the soleus or EDL muscles.

Western blotting. Figure 3*A* shows representative Western blot detections of UCP3 in the EDL muscle of rats from both groups. Compared with control, UCP3 was markedly increased in the EDL muscle of the AICAR group. The densitometric data (Fig. 3*B*) indicated that UCP3 protein level in AICAR was significantly higher than in control (1.96 ± 0.28 and 1.00 ± 0.19, respectively, $P = 0.010$). In the soleus muscle, UCP3 was undetected.

Figure 4*A* shows representative Western blot detections of PGC-1 in the soleus and EDL muscles of the rats from both groups. PGC-1 appeared to markedly increase in both the soleus and EDL muscles of the AICAR group. The densitometric data (Fig. 4*B*) indicated that the PGC-1 protein level of the soleus and EDL muscles in AICAR were significantly higher than control (2.14 ± 0.32 and 1.00 ± 0.08, $P = 0.003$ in soleus, and 1.87 ± 0.39 and 1.00 ± 0.07, $P = 0.025$ in EDL, respectively). We also determined the myogenin protein level because it was a possible regulator of oxidative enzyme activities (21). In Fig. 5, the myogenin content was shown to demonstrate no change after chronic AICAR treatment in the soleus muscle (1.00 ± 0.14 in control and 1.26 ± 0.23 in AICAR, $P = 0.33$). In the EDL muscle, no myogenin was detected.

DISCUSSION

In this study, we demonstrated that chronic AICAR treatment for 2 wk decreased the percentage of type IIB fibers and concomitantly increased the percentage of type IIX fibers, which had a slower shortening ve-

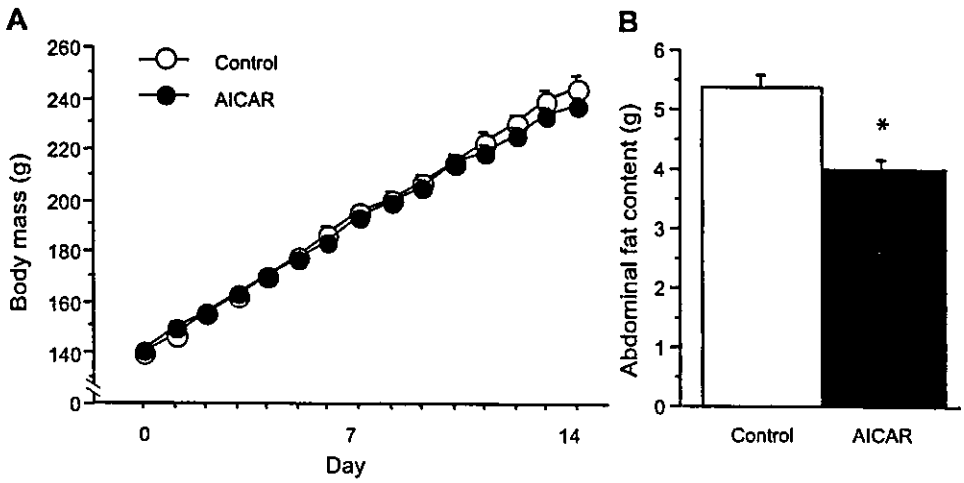
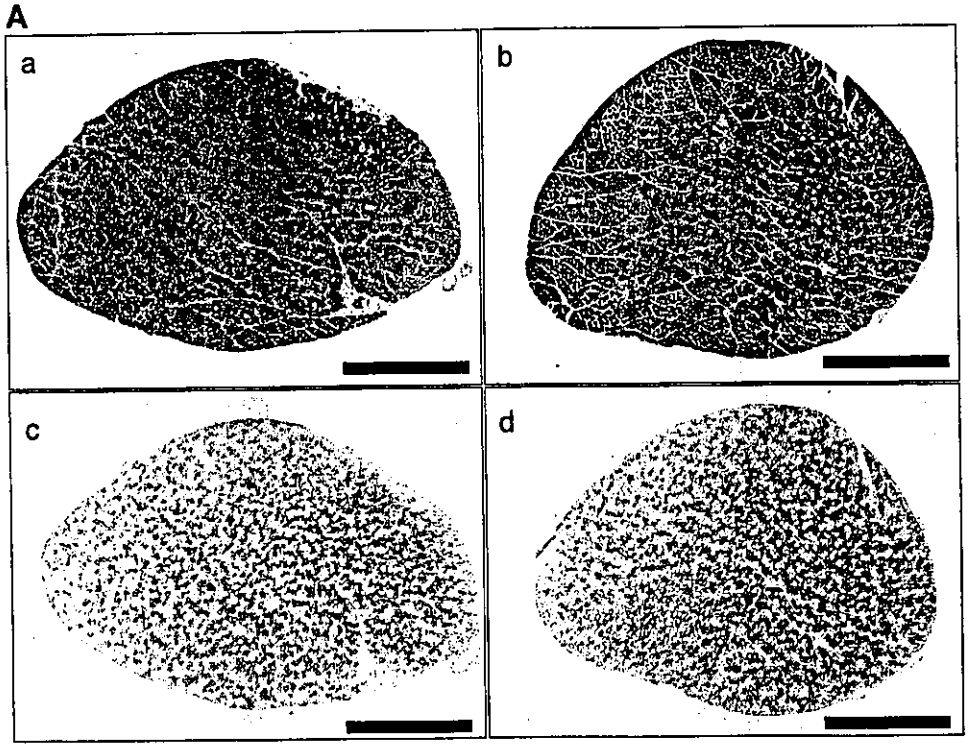


Fig. 1. Body mass (*A*) and abdominal fat content (*B*) of rats. AICAR, 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside. Values are means ± SE; $n = 7$–9 rats per group. *$P < 0.001$ vs. control.



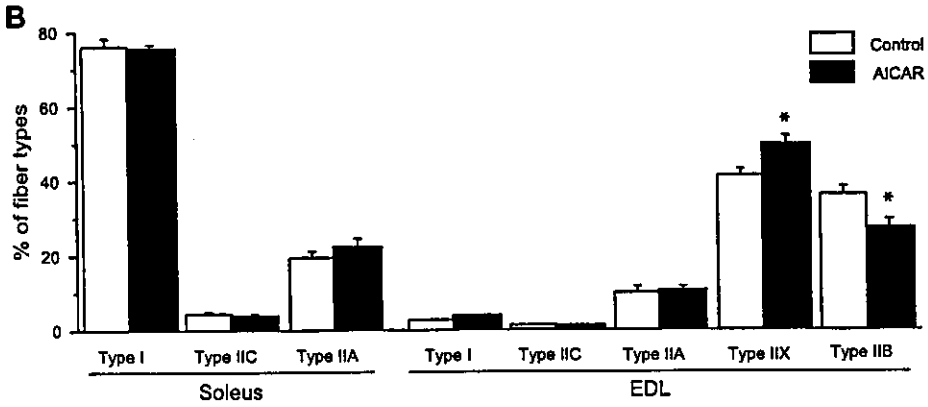


Fig. 2. *A*: representative transverse sections of the extensor digitorum longus (EDL) muscle in control (*a* and *c*) and AICAR groups (*b* and *d*). *a* and *b*: Myosin ATPase preincubation at pH 10.4. Light, intermediate, and dark fibers are types I, IIB, and IIA/IIX, respectively. *c* and *d*: Succinate dehydrogenase activity. Bars = 1 mm. *B*: average percentage of fiber composition values for the soleus and EDL muscles. Values are means ± SE; n = 6–9 muscles per group. *$P < 0.05$ vs. control.

locity (5) and a more oxidative capacity (38) than IIB fibers, in fast-twitch EDL muscle. It is well recognized that exercise training and chronic electrical stimulation induced skeletal muscle fiber type transformation (35). The chronic low-frequency stimulation for 28 days in rat EDL muscle showed a marked decrease in the percentage of type IIB fibers and an increase in the percentage of type IIX fibers (11). Voluntary running exercise over 45 days in rats resulted in a reduced percentage of type IIB fibers and an increased percentage of type IIA/IIX fibers in the EDL muscle (27). These reports suggested that IIB → IIX conversion occurred in the early phase of chronic muscle contraction. On the basis of these data, AICAR treatment appears to mimic the effect of chronic electrical stimulation and endurance exercise training on muscle fiber type trans-

Table 1. *Muscle capillary density, capillary-to-fiber ratio, and fiber density*

	Soleus		EDL	
	Control	AICAR	Control	AICAR
Capillary density, capillaries/mm^2	1,047 ± 40	1,185 ± 54	1,107 ± 37	1,161 ± 37
Capillary-to-fiber ratio, capillaries/fibers	2.32 ± 0.07	2.41 ± 0.10	1.59 ± 0.04	1.61 ± 0.06
Fiber density, fibers/mm^2	453 ± 21	498 ± 35	697 ± 26	726 ± 35

Values are means ± SE; n = 6–9 muscles. EDL, extensor digitorum longus; AICAR, 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside.

Table 2. *Effect of chronic AICAR treatment on enzyme activities*

	Soleus		EDL	
	Control	AICAR	Control	AICAR
HK	2.29 ± 0.15	3.01 ± 0.17*	2.32 ± 0.09	4.82 ± 0.20*
PK	25.9 ± 1.1	31.0 ± 1.3*	166.3 ± 5.7	216.6 ± 8.9*
LDH	166 ± 8	187 ± 9	623 ± 20	568 ± 26
CS	13.9 ± 0.7	16.7 ± 0.5*	10.8 ± 0.4	12.5 ± 0.2*
MDH	735 ± 32	874 ± 37*	614 ± 26	746 ± 46*
CPT	0.115 ± 0.007	0.118 ± 0.009	0.073 ± 0.003	0.076 ± 0.004
HAD	36.2 ± 1.4	46.2 ± 1.5*	14.2 ± 0.5	17.1 ± 0.6*
GPX	184 ± 2	189 ± 5	134 ± 2	135 ± 2
GR	1.91 ± 0.05	1.91 ± 0.06	1.02 ± 0.02	0.99 ± 0.03

Values are means ± SE (in $\mu mol \cdot g^{-1} \cdot min^{-1}$); n = 7–9 muscles. HK, hexokinase; PK, pyruvate kinase; LDH, lactate dehydrogenase; CS, citrate synthase; MDH, malate dehydrogenase; CPT, carnitine palmitoyl transferase; HAD, β-hydroxyacyl CoA dehydrogenase; GPX, glutathione peroxidase; GR, glutathione reductase. *$P < 0.05$ vs. control.

formation. The reason why AICAR treatment alters the muscle fiber composition is unclear at present. One possible mechanism for this is that increased PGC-1 protein by AICAR alters the fiber type-related gene expression. PGC-1 coactivated transcriptional factor myocyte enhancer factor-2 (30, 32) and skeletal muscle fiber type transformation (30). Myocyte enhancer factor-2 activated the expression of slow or oxidative muscle genes (55). The induction of PGC-1 has been proposed to play an important role in coordinating the activation of various genes linking to the skeletal muscle fiber phenotype.

It should be noted that the fiber-type transformation from type II to type I fibers was not observed in the present study. Although any evidences of the transformation from type II to type I fibers with AICAR treatment have not yet been observed, our results did not completely negate the potential role of AMPK for such transformation. In the early phase of high-intensity endurance exercise training (26) and chronic electrical

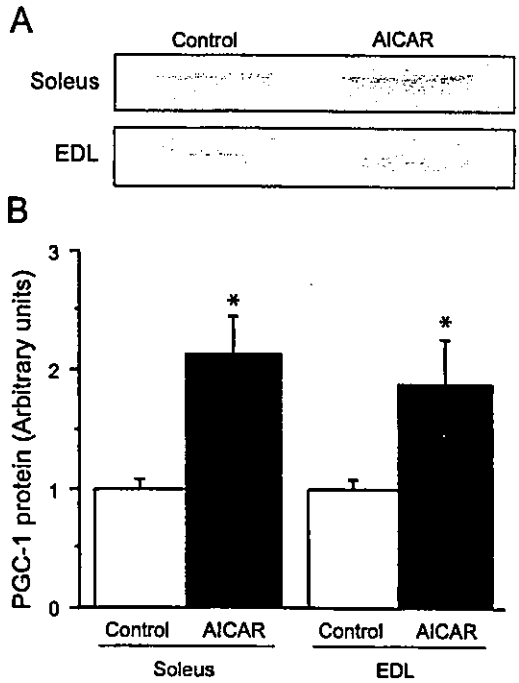


Fig. 4. Western blot analysis of peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1) in the soleus and EDL muscles. *A*: representative blots made by using an antibody against PGC-1 showing increases in PGC-1 in both the soleus and EDL muscles by AICAR treatment. *B*: average values for the PGC-1 in soleus and EDL muscles. Values are means ± SE; n = 7–9 muscles per group. *$P < 0.05$ vs. control.

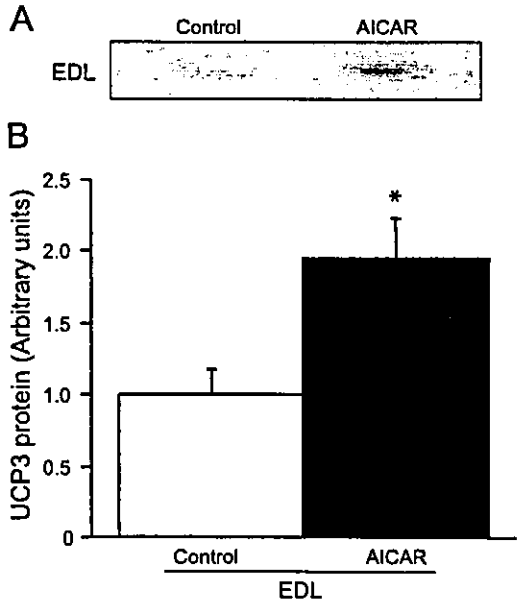


Fig. 3. Western blot analysis of uncoupling protein 3 (UCP3) in the EDL muscle. *A*: representative blots made by use of an antibody against UCP3 showing increases in UCP3 by AICAR treatment. *B*: average values for the UCP3 in EDL muscle. Values are means ± SE; n = 7–9 muscles per group. *$P < 0.05$ vs. control.

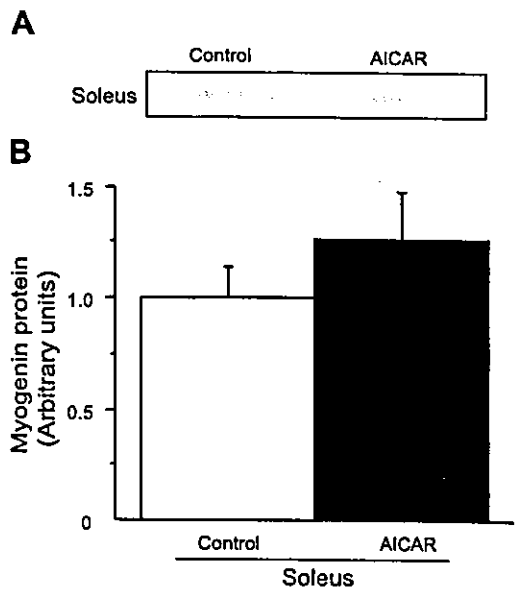


Fig. 5. Western blot analysis of myogenin in the soleus muscle. *A*: representative blots made by using an antibody against myogenin. *B*: average values for the myogenin in soleus muscle. Values are means ± SE; n = 7–9 muscles per group.

stimulation (11), the fiber-type transformation within subtypes of type II fibers was only seen. However, in the later phase, alternation from type II to type I fibers may possibly occur (11, 16, 26). Therefore, it might be possible that AICAR treatment for over 14 days induces the transformation from type II to type I fibers. Further research is needed to examine whether AICAR treatment affects all fiber types.

Acute and chronic AICAR treatment increased the skeletal muscle glucose uptake (8, 18, 28, 31) and GLUT-4 protein content (20, 23), respectively. Like previous studies (20, 53), we demonstrated that the AICAR treatment increased the skeletal muscle HK activity, which is a possible determinant of the glucose uptake in skeletal muscle during exercise and hyperinsulinemic conditions (17). Interestingly, the present study also demonstrated that the glycolytic PK activities of the soleus and EDL muscles were also increased by AICAR. Collectively, these findings provide evidence that the activation of AMPK by AICAR totally enhances the glucose metabolism. We demonstrated that chronic AICAR treatment increased the tricarboxylic acid cycle and β-oxidation enzyme activities in skeletal muscle. Furthermore, a previous study demonstrated that acute AICAR treatment enhanced fat oxidation through the inhibition of the acetyl-CoA carboxylase activity and the consequent reduction in the malomyl-CoA content, which inhibits the transport of fatty acids to the mitochondria, in skeletal muscle (31). Collectively, such evidence suggests that the activation of AMPK may totally enhance the capacity of energy utilization.

It is noteworthy that the HK activity of EDL was increased 108% by 14 successive days of AICAR treatment, whereas all other enzyme activities affected by AICAR increased ~15–31%. Winder et al. (53) demonstrated that the 4 wk of AICAR treatment increased GLUT-4 protein content in fast-twitch quadriceps muscle but not in slow-twitch soleus muscle. In addition, Buhl and coworkers (7, 8) indicated that chronic AICAR treatment enhanced GLUT-4 protein expression, insulin-stimulated glucose uptake, and GLUT-4 translocation in primarily fast-twitch glycolytic muscles. Collectively, these findings provide evidence that chronic AICAR treatment improves the capacity of skeletal muscle glucose uptake especially in fast-twitch glycolytic muscles.

Because PGC-1 and myogenin controlled the mitochondrial biogenesis and oxidative enzyme activities (21, 30, 56), we herein examined their protein content in skeletal muscles. As shown in the results, the PGC-1 protein contents in the rat soleus and EDL muscles increased after chronic AICAR treatment. In addition, a previous study indicated that acute AICAR treatment increased the PGC-1 mRNA expression in rat epitrochlearis muscle (46). Therefore, the activation of AMPK with AICAR should enhance the PGC-1 expression. PGC-1 increased the mRNA expression and transcriptional activity of nuclear respiratory factors 1 and 2 (1, 56), which were transcriptional factors related to mitochondrial function (40). Although the transcriptional activities of nuclear respiratory factors were not determined in this study, on the basis of these data we speculate that the increase in the muscle mitochondrial oxidative enzyme activities with chronic AICAR treatment is at least partially induced by the interaction of PGC-1 and nuclear respiratory factors. On the other hand, myogenin protein was not altered in the soleus muscle by AICAR or undetected in the EDL muscle. Therefore, an upregulation of myogenin by exercise (19, 50) is probably not related to the AMPK pathway.

Both the present study (treatment for 2 wk) and a previous study (treatment for 4 wk) (53) demonstrated that chronic AICAR treatment increased the mitochondrial tricarboxylic acid cycle enzyme activities. In addition, the HAD activity, which catalyzes mitochondrial β-oxidation of fatty acids, was also increased by AICAR in the present study. On the other hand, Winder et al. (53) failed to demonstrate such an increase. They also showed that treatment with AICAR for 2 wk increased the CS activity in the rat red quadriceps muscle, whereas 4 wk of such treatment did not. These results raise the possibility that the increase in the mitochondrial oxidative enzyme activities by AICAR occurs in the early phase but downregulation occurs if the treatment period is extended. To resolve the effect of the time course on the AICAR-treated muscle oxidative enzyme activities, further studies are thus called for.

Although the HAD activity was enhanced by AICAR treatment, this study and the previous study (53) demonstrated that CPT activity, which may be the rate-limiting step in fatty acid uptake and oxidation by mitochondria, was not changed by chronic AICAR treatment. Because both HAD and CPT are the enzymes of mitochondrial fatty acid metabolism, our results suggested that CPT was not regulated by the AMPK pathway. The upregulation of CPT in response to endurance exercise training (3, 48) has thus been proposed to depend on the other signaling pathways. It should be noted that such results did not exactly negate the effect of AICAR treatment on fatty acid uptake by mitochondria. Merrill et al. (31) demonstrated that acute AICAR perfusion to the hindlimb immediately inactivated acetyl-CoA carboxylase and decreased malonyl-CoA, an inhibitor of CPT, and then the fatty acid oxidation was increased. In the present study, the muscles were dissected ~24 h after the last AICAR injection. It is possible that such an acute effect is not maintained at least 24 h after injection.

In this study, we demonstrated that chronic AICAR treatment increased the UCP3 protein content in EDL muscle. UCPs inhibited the production of reactive oxygen species in the mitochondria (12, 34, 49). Exercise and hypoxia, which are considered to produce reactive oxygen species, immediately enhanced the skeletal muscle UCP3 protein level as well as AICAR (57). In addition, exercise (14, 52), hypoxia (13), and hydrogen peroxide (10) all activated AMPK. As a result, the increased muscle UCP3 protein by the AMPK pathway

thus appears to inhibit the reactive oxygen species production in fast-twitch muscle. On the other hand, this study indicated that the antioxidant enzyme activities, including GPX and GR, were not altered after chronic AICAR treatment in either the soleus or EDL muscles. The increase in the antioxidant enzyme activities by exercise training (37) is thus suggested to be independent of the AMPK pathway. As a result, our original hypothesis was proven to be incorrect. To resolve the underlying process for the increased antioxidant enzyme activities in response to exercise, further experimental studies are called for.

In the EDL muscle, both the UCP3 levels and mitochondrial enzyme activities except for CPT increased after chronic AICAR treatment. These results were consistent with previous reports describing that the UCP3 appeared to increase as a component of the exercise-induced increase in skeletal muscle mitochondria (24). These results seem plausible because the PGC-1 protein, which regulated both mitochondrial biogenesis (30, 56) and expression of UCPs (56), increased with chronic AICAR treatment. In other words, it is speculated that the activation of AMPK increased the PGC-1 protein level, and it consequently increased both mitochondrial enzyme activities and UCP3.

Both this study and a previous one (41) demonstrated that UCP3 protein was not detectable in the control rat slow-twitch soleus muscle. In addition, the rank order of the UCP3 protein content in human skeletal muscle fibers is IIX > IIA > I (39). These results suggest that UCP3 protein preferentially accumulates in fast-twitch muscle fibers. The factors causing a difference in the UCP3 protein content between slow- and fast-twitch muscles remain to be elucidated. One possibility might be the difference in the recruitment of muscles. Because the slow-twitch soleus is an antigravity and postural muscle, it would be recruited to a much greater extent than EDL muscle at least in the sedentary condition. The muscle UCP3 protein content in endurance-trained subjects was lower than in untrained subjects (39), thus suggesting that an increase in the muscle activity results in a decrease in the UCP3 expression. As a result, the UCP3 protein of the soleus muscle dramatically decreased until reaching an undetectable level.

In summary, we herein demonstrated that chronic AICAR treatment for 2 wk decreased the percentage of type IIB fibers and increased the percentage of type IIX fibers in EDL muscle. In addition, such treatment also increased the glycolytic and oxidative enzyme activities in the soleus and EDL muscles and the UCP3 protein content in the EDL muscle of rats. It is speculated that at least several such adaptations with AICAR treatment may be due to an increased PGC-1 protein content. On the other hand, the capillary network, antioxidant enzyme activities, and myogenin protein content may be independent of the AMPK pathway.

REFERENCES

1. **Baar K, Wende AR, Jones TE, Marison M, Nolte LA, Chen M, Kelly DP, and Holloszy JO.** Adaptations of skeletal muscle to exercise: rapid increase in the transcriptional coactivator PGC-1. *FASEB J* 16: 1879–1886, 2002.
2. **Bergeron R, Ren JM, Cadman KS, Moore IK, Perret P, Pypaert M, Young LH, Semenkovich CF, and Shulman GI.** Chronic activation of AMP kinase results in NRF-1 activation and mitochondrial biogenesis. *Am J Physiol Endocrinol Metab* 281: E1340–E1346, 2001.
3. **Berthon PM, Howlett RA, Heigenhauser GJ, and Spriet LL.** Human skeletal muscle carnitine palmitoyltransferase I activity determined in isolated intact mitochondria. *J Appl Physiol* 85: 148–153, 1998.
4. **Booth FW and Baldwin KM.** Muscle plasticity: energy demand and supply processes. In: *Handbook of Physiology. Exercise: Regulation and Integration of Multiple Systems.* Bethesda, MD: Am. Physiol. Soc., 1996, sect. 12, chapt. 24, p. 1075–1123.
5. **Bottinelli R, Betto R, Schiaffino S, and Reggiani C.** Unloaded shortening velocity and myosin heavy chain and alkali light chain isoform composition in rat skeletal muscle fibres. *J Physiol* 478: 341–349, 1994.
6. **Brooke MH and Kaiser KK.** Three "myosin adenosine triphosphatase" systems: the nature of their pH lability and sulfhydryl dependence. *J Histochem Cytochem* 18: 670–672, 1970.
7. **Buhl ES, Jessen N, Pold R, Ledet T, Flyvbjerg A, Pedersen SB, Pedersen O, Schmitz O, and Lund S.** Long-term AICAR administration reduces metabolic disturbances and lowers blood pressure in rats displaying features of the insulin resistance syndrome. *Diabetes* 51: 2199–2206, 2002.
8. **Buhl ES, Jessen N, Schmitz O, Pedersen SB, Pedersen O, Holman GD, and Lund S.** Chronic treatment with 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside increases insulin-stimulated glucose uptake and GLUT4 translocation in rat skeletal muscles in a fiber type-specific manner. *Diabetes* 50: 12–17, 2001.
9. **Chin ER, Olson EN, Richardson JA, Yang Q, Humphries C, Shelton JM, Wu H, Zhu W, Bassel-Duby R, and Williams RS.** A calcineurin-dependent transcriptional pathway controls skeletal muscle fiber type. *Genes Dev* 12: 2499–2509, 1998.
10. **Choi SL, Kim SJ, Lee KT, Kim J, Mu J, Birnbaum MJ, Soo Kim S, and Ha J.** The regulation of AMP-activated protein kinase by H_2O_2. *Biochem Biophys Res Commun* 287: 92–97, 2001.
11. **Delp MD and Pette D.** Morphological changes during fiber type transitions in low-frequency-stimulated rat fast-twitch muscle. *Cell Tissue Res* 277: 363–371, 1994.
12. **Duval C, Negre-Salvayre A, Dogilo A, Salvayre R, Penicaud L, and Casteilla L.** Increased reactive oxygen species production with antisense oligonucleotides directed against uncoupling protein 2 in murine endothelial cells. *Biochem Cell Biol* 80: 757–764, 2002.
13. **Fisher JS, Gao J, Han DH, Holloszy JO, and Nolte LA.** Activation of AMP kinase enhances sensitivity of muscle glucose transport to insulin. *Am J Physiol Endocrinol Metab* 282: E18–E23, 2002.
14. **Fujii N, Hayashi T, Hirshman MF, Smith JT, Habinowski SA, Kaijser L, Mu J, Ljungqvist O, Birnbaum MJ, Witters LA, Thorell A, and Goodyear LJ.** Exercise induces isoform-specific increase in 5'AMP-activated protein kinase activity in human skeletal muscle. *Biochem Biophys Res Commun* 273: 1150–1155, 2000.
15. **Galler S, Schmitt TL, and Pette D.** Stretch activation, unloaded shortening velocity, and myosin heavy chain isoforms of rat skeletal muscle fibres. *J Physiol* 478: 513–521, 1994.
16. **Green HJ, Klug GA, Reichmann H, Seedorf U, Wiehrer W, and Pette D.** Exercise-induced fibre type transitions with regard to myosin, parvalbumin, and sarcoplasmic reticulum in muscles of the rat. *Pflügers Arch* 400: 432–438, 1984.
17. **Halseth AE, Bracy DP, and Wasserman DH.** Overexpression of hexokinase II increases insulin and exercise-stimulated muscle glucose uptake in vivo. *Am J Physiol Endocrinol Metab* 276: E70–E77, 1999.

18. **Hayashi T, Hirshman MF, Kurth EJ, Winder WW, and Goodyear LJ.** Evidence for 5′ AMP-activated protein kinase mediation of the effect of muscle contraction on glucose transport. *Diabetes* 47: 1369–1373, 1998.
19. **Hespel P, Op't Eijnde B, Van Leemputte M, Urso B, Greenhaff PL, Labarque V, Dymarkowski S, Van Hecke P, and Richter EA.** Oral creatine supplementation facilitates the rehabilitation of disuse atrophy and alters the expression of muscle myogenic factors in humans. *J Physiol* 536: 625–633, 2001.
20. **Holmes BF, Kurth-Kraczek EJ, and Winder WW.** Chronic activation of 5′-AMP-activated protein kinase increases GLUT-4, hexokinase, and glycogen in muscle. *J Appl Physiol* 87: 1990–1995, 1999.
21. **Hughes SM, Chi MM, Lowry OH, and Gundersen K.** Myogenin induces a shift of enzyme activity from glycolytic to oxidative metabolism in muscles of transgenic mice. *J Cell Biol* 145: 633–642, 1999.
22. **Ihlemann J, Ploug T, Hellsten Y, and Galbo H.** Effect of stimulation frequency on contraction-induced glucose transport in rat skeletal muscle. *Am J Physiol Endocrinol Metab* 279: E862–E867, 2000.
23. **Jessen N, Pold R, Buhl ES, Jensen LS, Schmitz O, and Lund S.** Effects of AICAR and exercise on insulin-stimulated glucose uptake, signaling, and GLUT-4 content in rat muscles. *J Appl Physiol* 94: 1373–1379, 2003.
24. **Jones TE, Baar K, Ojuka E, Chen M, and Holloszy JO.** Exercise induces an increase in muscle UCP3 as a component of the increase in mitochondrial biogenesis. *Am J Physiol Endocrinol Metab* 284: E96–E101, 2003.
25. **Klausen K, Andersen LB, and Pelle I.** Adaptive changes in work capacity, skeletal muscle capillarization and enzyme levels during training and detraining. *Acta Physiol Scand* 113: 9–16, 1981.
26. **Kovanen V and Suominen H.** Effects of life-time physical training on fibre composition of slow and fast skeletal muscle in rats. *Pflügers Arch* 408: 543–551, 1987.
27. **Kriketos AD, Pan DA, Sutton JR, Hoh JF, Baur LA, Cooney GJ, Jenkins AB, and Storlien LH.** Relationships between muscle membrane lipids, fiber type, and enzyme activities in sedentary and exercised rats. *Am J Physiol Regul Integr Comp Physiol* 269: R1154–R1162, 1995.
28. **Kurth-Kraczek EJ, Hirshman MF, Goodyear LJ, and Winder WW.** 5′ AMP-activated protein kinase activation causes GLUT4 translocation in skeletal muscle. *Diabetes* 48: 1667–1671, 1999.
29. **LaFramboise WA, Daood MJ, Guthrie RD, Moretti P, Schiaffino S, and Ontell M.** Electrophoretic separation and immunological identification of type 2X myosin heavy chain in rat skeletal muscle. *Biochim Biophys Acta* 1035: 109–112, 1990.
30. **Lin J, Wu H, Tarr PT, Zhang CY, Wu ZD, Boss O, Michael LF, Puigserver P, Isotani E, Olson EN, Lowell BB, Bassel-Duby R, and Spiegelman BM.** Transcriptional co-activator PGC-1 alpha drives the formation of slow-twitch muscle fibres. *Nature* 418: 797–801, 2002.
31. **Merrill GF, Kurth EJ, Hardie DG, and Winder WW.** AICA riboside increases AMP-activated protein kinase, fatty acid oxidation, and glucose uptake in rat muscle. *Am J Physiol Endocrinol Metab* 273: E1107–E1112, 1997.
32. **Michael LF, Wu Z, Cheatham RB, Puigserver P, Adelmant G, Lehman JJ, Kelly DP, and Spiegelman BM.** Restoration of insulin-sensitive glucose transporter (GLUT4) gene expression in muscle cells by the transcriptional coactivator PGC-1. *Proc Natl Acad Sci USA* 98: 3820–3825, 2001.
33. **Nachlas M, Tsou K, Desouza E, Cheng C, and Seligman A.** Cytochemical demonstration of succinic dehydrogenase by the use of a new P-nitrophenyl substituted ditetrazone. *J Histochem Cytochem* 5: 420–436, 1957.
34. **Nègre-Salvayre A, Hirtz C, Carrera G, Cazenave R, Troly M, Salvayre R, Penicaud L, and Casteilla L.** A role for uncoupling protein-2 as a regulator of mitochondrial hydrogen peroxide generation. *FASEB J* 11: 809–815, 1997.
35. **Pette D and Staron RS.** Transitions of muscle fiber phenotypic profiles. *Histochem Cell Biol* 115: 359–372, 2001.
36. **Pilegaard H, Saltin B, and Neufer PD.** Exercise induces transient transcriptional activation of the PGC-1α gene in human skeletal muscle. *J Physiol* 546: 851–858, 2003.
37. **Powers SK, Ji LL, and Leeuwenburgh C.** Exercise training-induced alterations in skeletal muscle antioxidant capacity: a brief review. *Med Sci Sports Exerc* 31: 987–997, 1999.
38. **Rivero JLL, Talmadge RJ, and Edgerton VR.** Interrelationships of myofibrillar ATPase activity and metabolic properties of myosin heavy chain-based fibre types in rat skeletal muscle. *Histochem Cell Biol* 111: 277–287, 1999.
39. **Russell P, Wadley G, Hesselink C, Schaart G, Lo S, Leger B, Garnham A, Kornips E, Cameron-Smith D, Giacobino JP, Muzzin P, Snow R, and Schrauwen P.** UCP3 protein expression is lower in type I, IIa and IIx muscle fiber types of endurance-trained compared to untrained subjects. *Pflügers Arch* 445: 563–569, 2003.
40. **Scarpulla RC.** Nuclear activators and coactivators in mammalian mitochondrial biogenesis. *Biochim Biophys Acta* 1576: 1–14, 2002.
41. **Short KR, Nygren J, Barazzoni R, Levine J, and Nair KS.** T_3 increases mitochondrial ATP production in oxidative muscle despite increased expression of UCP2 and -3. *Am J Physiol Endocrinol Metab* 280: E761–E769, 2001.
42. **Staron RS and Hikida RS.** Histochemical, biochemical, and ultrastructural analyses of single human muscle fibers, with special reference to the C-fiber population. *J Histochem Cytochem* 40: 563–568, 1992.
43. **Stoppani J, Hildebrandt AL, Sakamoto K, Cameron-Smith D, Goodyear LJ, and Neufer PD.** AMP-activated protein kinase activates transcription of the UCP3 and HKII genes in rat skeletal muscle. *Am J Physiol Endocrinol Metab* 283: E1239–E1248, 2002.
44. **Suwa M, Kumagai S, Higaki Y, Nakamura T, and Katsuta S.** Dietary obesity-resistance and muscle oxidative enzyme activities of the fast-twitch fibre dominant rat. *Int J Obes* 26: 830–837, 2002.
45. **Suwa M, Nakano H, Higaki Y, Nakamura T, Katsuta S, and Kumagai S.** Increased wheel-running activity in the genetically skeletal muscle fast-twitch fiber-dominant rats. *J Appl Physiol* 94: 185–192, 2003.
46. **Terada S, Goto M, Kato M, Kawanaka K, Shimokawa T, and Tabata I.** Effects of low-intensity prolonged exercise on PGC-1 mRNA expression in rat epitrochlearis muscle. *Biochem Biophys Res Commun* 296: 350–354, 2002.
47. **Termin A, Staron RS, and Pette D.** Myosin heavy chain isoforms in histochemically defined fiber types of rat muscle. *Histochemistry* 92: 453–457, 1989.
48. **Tunstall RJ, Mehan KA, Wadley GD, Collier GR, Bonen A, Hargreaves M, and Cameron-Smith D.** Exercise training increases lipid metabolism gene expression in human skeletal muscle. *Am J Physiol Endocrinol Metab* 283: E66–E72, 2002.
49. **Vidal-Puig AJ, Grujic D, Zhang CY, Hagen T, Boss O, Ido Y, Szczepanik A, Wade J, Mootha V, Cortright R, Muoio DM, and Lowell BB.** Energy metabolism in uncoupling protein 3 gene knockout mice. *J Biol Chem* 275: 16258–16266, 2000.
50. **Willoughby DS and Nelson MJ.** Myosin heavy-chain mRNA expression after a single session of heavy-resistance exercise. *Med Sci Sports Exerc* 34: 1262–1269, 2002.
51. **Winder WW.** Energy-sensing and signaling by AMP-activated protein kinase in skeletal muscle. *J Appl Physiol* 91: 1017–1028, 2001.
52. **Winder WW and Hardie DG.** Inactivation of acetyl-CoA carboxylase and activation of AMP-activated protein kinase in muscle during exercise. *Am J Physiol Endocrinol Metab* 270: E299–E304, 1996.
53. **Winder WW, Holmes BF, Rubink DS, Jensen EB, Chen M, and Holloszy JO.** Activation of AMP-activated protein kinase increases mitochondrial enzymes in skeletal muscle. *J Appl Physiol* 88: 2219–2226, 2000.
54. **Wu H, Kanatous SB, Thurmond FA, Gallardo T, Isotani E, Bassel-Duby R, and Williams RS.** Regulation of mitochon-

drial biogenesis in skeletal muscle by CaMK. *Science* 296: 349–352, 2002.

55. **Wu H, Naya FJ, McKinsey TA, Mercer B, Shelton JM, Chin ER, Simard AR, Michel RN, Bassel-Duby R, Olson EN, and Williams RS.** MEF2 responds to multiple calcium-regulated signals in the control of skeletal muscle fiber type. *EMBO J* 19: 1963–1973, 2000.
56. **Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, Troy A, Cinti S, Lowell B, Scarpulla RC, and Spiegelman BM.** Mechanisms controlling mitochondrial biogenesis and respiration through the thermogenic coactivator PGC-1. *Cell* 98: 115–124, 1999.
57. **Zhou M, Lin BZ, Coughlin S, Vallega G, and Pilch PF.** UCP-3 expression in skeletal muscle: effects of exercise, hypoxia, and AMP-activated protein kinase. *Am J Physiol Endocrinol Metab* 279: E622–E629, 2000.
58. **Zong H, Ren JM, Young LH, Pypaert M, Mu J, Birnbaum MJ, and Shulman GI.** AMP kinase is required for mitochondrial biogenesis in skeletal muscle in response to chronic energy deprivation. *Proc Natl Acad Sci USA* 99: 15983–15987, 2002.

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Decreased serum leptin and muscle oxidative enzyme activity with a dietary loss of intra-abdominal fat in rats

Buhao Zou[a], Masataka Suwa[b], Hiroshi Nakano[c], Yasuki Higaki[d], Tatsumi Ito[e], Shigeru Katsuta[f], Shuzo Kumagai[b,*]

[a]*Graduate School of Human-Environment Studies, Kyushu University, Kasuga, Fukuoka 816-8580, Japan*
[b]*Institute of Health Science, Kyushu University, Kasuga, Fukuoka 816-8580, Japan*
[c]*Department of Human Development, Nakamura Gakuen University, Fukuoka 814-0198, Japan*
[d]*Department of Preventive Medicine, Saga Medical School, Saga 849-8501, Japan*
[e]*Department of Bioscience and Biotechnology, Kyushu University Graduate School, 6-10-1 Hakozaki, Fukuoka 812-8581, Japan*
[f]*Graduate School of Integrated Science and Art, University of East Asia, Shimonoseki, Yamaguchi 751-8503, Japan*

Abstract

The purpose of the present study was to investigate the relationship among intra-abdominal adipose storage, adaptation in the serum leptin concentration and skeletal muscle enzyme activity after a 4-week energy restriction (ER). Thirty-one male Wistar rats were divided into 40% energy restricted (n=24) or ad libitum-fed control (CL) rats (n = 7). The energy-restricted rats were grouped into the most fat (MF, n=7), medium (n 10) and the least fat (LF, n=7) by their intra-abdominal fat pads mass (epididymal, mesenteric, and perirenal) after ER. A superficial portion of M. gastrocnemius tissue obtained before and after the diet period were analyzed to determine the activities of hexokinase (HK), β-hydroxyacyl CoA dehydrogenase (β-HAD) and citrate synthase (CS). Blood samples were also collected for a serum leptin assay. At the baseline, no difference was found in either the leptin concentration or the enzyme activities among LF, MF and CL. The serum leptin concentration was positively correlated with the muscle activities of β-HAD and CS, while it negatively correlated with HK/β-HAD. After ER, the activities of HK, β-HAD and CS were all significantly lower in LF than in CL. Among the energy-restricted rats, the intra-abdominal fat pad weight, leptin concentration and the activities of β-HAD, CS, β-HAD/CS all significantly correlated with one another. The changes in leptin and the activity of β-HAD were also positively correlated. These findings indicate that parallel decreases in the serum leptin and skeletal muscle enzyme activities with the energy restriction-induced intra-abdominal adipose reduction, thus may suggest the leptin to have a regulative effect on the muscle enzyme activity during ER.

Keywords: Energy restriction; Intra-abdominal adipose; Leptin; Muscle oxidative enzyme activity

1. Introduction

The association between the risk of chronic diseases, including type II diabetes and coronary heart disease, and visceral adipose accumulation [1, 2], rather than subcutaneous adipose accumulation [3–6], has well been recognized. The prevalence of these chronic metabolic diseases has made it urgent to clarify the mechanism of visceral adipose storage and utilization.

Skeletal muscle represents more than 30% of body mass and plays important role in the consumption of carbohydrates and lipids. As a result, the metabolic characteristics of such muscle has been investigated in both cross-sectional and longitudinal studies regarding its relationship with body fat accumulation [7–13].

Leptin, the adipose-derived hormone, has been shown to regulate food intake and energy metabolism [14–18]. High correlations have been shown between plasma leptin and either the body mass index (BMI) [16, 19, 20] or the percentage of body fat [20, 21]. In the rodent skeletal muscle, fatty acid oxidation can be enhanced by leptin [22–25]. Leptin thus appears to play a protective role which helps to inhibit further energy accumulation under energy-sufficient conditions.

We recently reported a greater upregulation in the oxidative enzyme activity in skeletal muscle of rats with

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* Corresponding author. Tel. and fax: +81-92-583-7853.
E-mail address: shuzo@ihs.kyushu-u.ac.jp (S. Kumagai).

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greater high-fat diet-induced intra-abdominal adipose accumulation [26]. From the results of that study, we considered that the adipose storage, as manifested by intra-abdominal fat pads, might therefore induce the increase in serum leptin, thus also increasing the oxidative potential. In other words, the oxidative capacity of skeletal muscle might change in parallel with the changes in both the intra-abdominal adipose accumulation and serum leptin levels. However, little is still known about the relationship between leptin and the skeletal muscle metabolism under energy-insufficient conditions. In the current study, we focused on the relationship among the remnants of intra-abdominal fat deposition, changes in the serum leptin concentration, and the skeletal muscle enzyme activity after a 4-week ER. If the regulation of the enzyme activity in skeletal muscle is regulated by the serum leptin, then the decrease in leptin concentration owing to the consumption of adipose storage by ER should be able to induce a comparable decrease in the skeletal muscle enzyme activity.

2. Methods and materials

2.1. Animals

Male Wistar rats (n=31) at 15 weeks of age weighing 440.5 ± 2.7g were used in this study. All rats were housed individually under controlled conditions (12:12-hr light-dark cycle and a 20°C room temperature) and given a regular rat chow diet (Oriental Yeast Co. Tokyo Japan, consisting of 12.9% fat, 26.6% protein, 60.5% carbohydrate, and 3.6 kcal/g) and water ad libitum. All experimental procedures were conducted strictly in accordance with the Guiding Principles for Research Involving Animals and Human Beings.

2.2. Experimental protocol

At the beginning of the study, all rats were weighed and anesthetized with pentobarbital sodium (50 mg/kg ip). Blood samples were taken from the tail. 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 (~100 mg) were obtained from the superficial portion of M. gastrocnemius. All samples were immediately frozen in liquid nitrogen and stored −80°C until assayed. The skin was closed with stainless steel autoclips and then the rats were injected with penicillin (2.5 mg/kg im). After a 2-week recovery period, 24 randomly selected rats underwent a 40% reduction of their baseline average calorie intake (18g/day) for 4 weeks. The remaining 7 rats continued to feed ad libitum as the control group. The body weights were recorded once a week. After the 4-week ER period, and an over-night fast (12 hr), all rats were weighed and anesthetized with pentobarbital sodium (50 mg/kg ip). The superficial potion of the M. gastrocnemius in the left leg was dissected. The intra-abdominal fat pads (epididymal, mesenteric, and perirenal) were excised and weighed after blood samples were taken.

2.3. Enzyme Assay

An enzyme assay was carried out in samples extracted from the superficial portion of the M. gastrocnemius. The activity of hexokinase (HK, key enzyme of glucose utilization), β-hydroxyacyl CoA dehydrogenase (β-HAD, key enzyme of β-oxidation of fatty acids) and citrate synthase (CS, key enzyme of tricarboxylic acid cycle) were spectrophotometrically determined at 30°C according to previously established techniques [27–29]. The coefficients of variation for the enzyme assay were 1.8% for HK, 1.2% for β-HAD and 1.7% for CS by same sample repeated measurements.

2.4. Serum measurements

Serum leptin, glucose, free triiodothyronine (FT3) and free fatty acid (FFA) concentration were measured before and after ER. The leptin concentrations were assayed using a sensitive commercially available radioimmunoassay kit (Rat Leptin RIA, Linco Research Inc., St. Charles, MO, USA) according to the manufacturer's instructions [30]. The assay lower limit of detection is 0.5ng/mL. The serum glucose concentration was assayed using a glucose analyzer (YSI 2300STAT, OH). The FT3 concentrations were assayed using an automated chemiluminescent immunoassay system (Advia Centaur, Bayer Medical Ltd., Tokyo, Japan). FFA concentrations were assayed using an enzymatic determination device (Bio Majesty JCA-BM1650, Japan Electron Optics Laboratory Co., Ltd., Tokyo, Japan).

2.5. Statistical analysis

Among the energy-restricted rats, based on the weight of the intra-abdominal fat pads, the data were divided into 3 groups: the 2 tertiles on both sides with the same number as in the control group for the least fat (LF, n=7) and the most fat (MF, n=7), the 10 between LF and MF for medium. All data were presented as the means ± SE. To investigate the relationship among intra-abdominal adipose, leptin, and muscle enzyme activities, one-way ANOVA with post-hoc comparisons made using the Tukey-Kramer test was assessed only among the LF, MF and control (CL) groups, the 3 groups with a significant difference in intra-abdominal fat pads (the medium group was not significantly different from MF or LF when it was added to ANOVA). Differences in the body weight, weight loss, intra-abdominal fat pads, intra-abdominal fat pads/final body weight, enzyme activity, serum leptin concentration, change in the enzyme activity and leptin concentration among these 3 groups were assessed in this way. Significant differences in the variables were determined with a 5% significant criterion. Data from the medium group were only used for analyses in all energy-restricted rats, such as pre-post comparisons (using the

Table 1
Effects of ER on the food intake and body composition

	Food intake (g)	Body weight (g)					Weight change (g)	Intra-abdominal fat pads (g)
		Week 0	Week 1	Week 2	Week 3	Week 4		
CL(n=7)	770.0 ± 17.2	481.3 ± 10.1	498.6 ± 11.3	509.4 ± 12.0	526.9 ± 11.6	539.4 ± 13.4	36.9 ± 4.4	18.6 ± 1.9
MF(n=7)	504.0 ± 0.0*	480.3 ± 8.2	443.9 ± 6.3*	437.7 ± 5.2*	435.7 ± 6.0*	437.4 ± 6.0*	−42.9 ± 8.3*	10.9 ± 0.2*#
LF(n=7)	504.0 ± 0.0*	486.1 ± 9.2	451.9 ± 6.7*	445.7 ± 5.9*	440.7 ± 5.0*	438.0 ± 5.9*	−48.1 ± 8.0*	6.3 ± 0.3*

Values are the means ± SE.
*Significantly different from CL, $p < 0.05$.
#Significantly different from LF, $p<0.05$.

paired *t*-test) and correlation analyses. The correlations at baseline were analyzed using the data from all rats before ER. The correlations after ER or concerning changes in variables were analyzed in either energy-restricted or CL rats, respectively. Correlations were considered significant only when the *P*-value was less than 0.05. All statistical analyses were performed using StatView 5.0 software (SAS Institute, Cary, NC).

3. Results

3.1. Food intake and body composition

The total food intake, body weight, weight change and intra-abdominal fat pad weight are presented in Table 1. During the 4-week ER period, the food intake in CL was totally 770 ± 17g. Although energy-restricted groups were restricted to 60% (18g/day) of their baseline intake at the beginning of the ER period, their total intake (504g) consequently reached about 65% that of the CL. Significantly much food was consumed by CL than by the energy-restricted groups of rats.

CL had a higher body weight than LF or MF after the first week of ER ($P < 0.05$). This significant difference persisted for the remainder of the dietary restriction period. No significant difference was observed between LF and MF at either time point. The changes in body weight were not different between LF and MF whereas those of CL were higher than those of LF and MF ($P < 0.05$).

The epididymal, mesenteric, and perirenal fat pads were measured and the total intra-abdominal fat pad weight was determined in this study. The energy-restricted rats were divided into LF, medium or MF groups based on this criterion. The ranges of the intra-abdominal fat pad weight were 13.75 to 27.70g for CL, 9.91 to 11.59g for MF, 8.15 to 9.77g for medium, and 4.87 to 7.26g for LF. The rank order (CL > MF > LF) of the intra-abdominal fat pads or intra-abdominal fat pads/final body weight showed significant differences between all 3 groups ($P < 0.05$).

3.2. Muscular enzyme activity

The enzyme activities in the superficial portion of the M. gastrocnemius tissue obtained before and after the diet period are shown in Fig. 1. Before ER, no significant differences were seen between LF, MF and CL. After ER, the activities of HK, β-HAD, and CS were all significantly lower in LF than in CL ($P < 0.05$). When the medium group, which was not analyzed by ANOVA, was evaluated together with MF and LF, enzyme activities of HK, β-HAD, and CS in energy-restricted rats were found to significantly decrease, and the enzyme activities showed the following rank order: CL > MF > medium > LF after ER.

3.3. Fasting serum values

The fasting serum leptin, glucose, FT3, FFA concentrations are shown in Table 2. Before ER, there was no significant difference among LF, MF and CL. The serum leptin concentration decreased in all of the energy-restricted groups while it increased in CL during the experimental period. After ER, the serum leptin concentration was significantly lower in LF than in CL. The rank order of the leptin level among all groups after ER was CL > MF > medium > LF. No significant differences were found in the glucose, FT3 or FFA concentrations before or after ER between LF, MF and CL. The changes in the glucose, FT3 or FFA concentrations were not significant between LF, MF or CL.

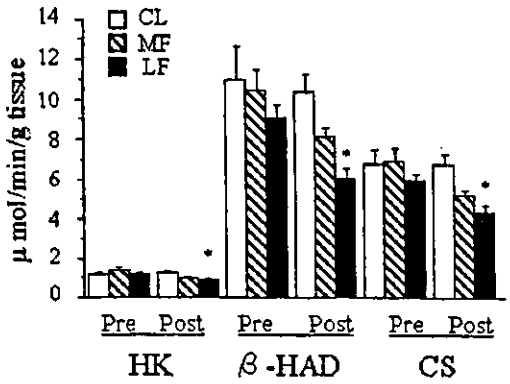

Fig. 1. Enzyme activities of HK, β-HAD and CS. *Significantly different from the activity in CL after ER.

Table 2
Fasting serum values in the CL, MF and LF rats

	Glucose (mg/dl)		Leptin (ng/ml)		FT3 (pg/ml)		FFA (mEq/l)	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
CL(n=7)	112.3 ± 2.6	113.0 ± 3.6	3.0 ± 0.5	4.4 ± 0.6	3.3 ± 0.1	3.0 ± 0.2	0.7 ± 0.1	0.6 ± 0.1
MF(n=6)	116.9 ± 5.9	118.5 ± 4.3	3.7 ± 0.7	3.3 ± 0.5	3.4 ± 0.2	3.0 ± 0.1	0.5 ± 0.1	0.4 ± 0.0
LF(n=5, 7)	109.1 ± 2.8	116.0 ± 2.4	1.7 ± 0.1	1.3 ± 0.1*	3.0 ± 0.1	2.7 ± 0.1	0.6 ± 0.0	0.5 ± 0.0

Values are the means ± SE.
*Significantly different from CL.

3.4. Correlation analysis

Before ER, the serum leptin concentration correlated positively with the activities of β-HAD ($r = 0.570$, $P < 0.01$, Fig. 2A) and CS ($r = 0.630$, $P < 0.01$), but negatively with HK/β-HAD ($r = -0.396$, $P < 0.05$), which is an indicator of the fat oxidation capacity. No significant correlation was found regarding the HK activity ($r = 0.189$, $P = 0.34$) or β-HAD/CS ($r = 0.234$, $P = 0.23$) ($n=28$).

After ER, correlations were made in energy-restricted or CL rats, respectively. Significant positive correlations with intra-abdominal fat mass were observed regarding the activities of β-HAD ($r = 0.550$, $P < 0.01$) and CS ($r = 0.409$, $P < 0.05$), and β-HAD/CS ($r = 0.465$, $P < 0.05$) in energy-restricted rats ($n=24$) but not in CL ($n=7$). In energy-restricted rats ($n=21$), the serum leptin concentration correlated positively with the intra-abdominal fat pads ($r = 0.718$, $P < 0.01$), the activities of HK ($r = 0.546$, $P < 0.01$), β-HAD ($r = 0.700$, $P < 0.01$, Fig. 2B) and CS ($r = 0.513$, $P < 0.05$), as well as β-HAD/CS ($r = 0.586$, $P < 0.01$). The changes in the serum leptin concentration and the activity of β-HAD also correlated significantly ($r = 0.432$, $P < 0.05$, Fig. 2C). In the CL rats ($n=7$), the leptin concentration correlated positively with the intra-abdominal fat ($r = 0.836$, $P < 0.05$).

4. Discussion

In the current study, we compared MF, LF and CL, the three groups of rats with a significant difference in the intra-abdominal adipose pads. Other differences among these rats in skeletal muscle or blood variables were expected to clarify their roles in the adaptation to energy restriction, especially regarding the change in intra-abdominal adipose fat.

Energy-restricted rats took in a total amount of 1814.4 kcal from chow. This was 957.6 kcal less than what the CL rats took and corresponded to approximately 106.4 g adipose tissue. In fact, the LF rats lost 48.1 g, while the MF rats lost 42.9 g of body weight. Regarding the caloric intake of the CL rats as the standard, the smaller loss of body weight (nearly 40%) than would normally be expected is due to the fact that the energy-restricted rats exhibited a resistance to weight loss. Significant correlations were found between intra-abdominal fat, leptin and muscle enzyme activities in energy-restricted rats.

Before ER, there was no difference in the leptin concentration or muscular enzyme activity among LF, MF and CL. However, after ER, LF showed a lower serum leptin concentration and lower muscle enzyme activities than CL. Regarding the CL rats, LF had a relatively greater adaptation in both serum leptin concentration and enzyme activity in skeletal muscle than MF under an energy-insufficient

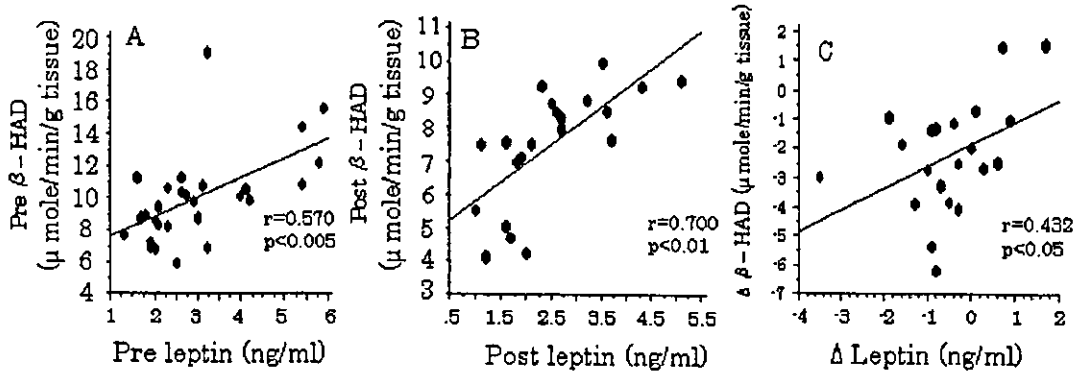

Fig. 2. Correlation between the serum leptin concentration and the activity of β-HAD in all rats ($n = 28$) before ER (A). Correlation between the serum leptin concentration and the activity of β-HAD in energy-restricted rats ($n = 24$) after ER (B). Correlation between the changes in serum leptin concentration and the activity of β-HAD (C) in energy-restricted rats ($n = 21$) after ER.

condition. The decreased leptin concentration observed owing to the consumption of adipose storage, manifested by intra-abdominal adipose in this study, was accompanied with a parallel decrease in the skeletal muscle enzyme activities. To our knowledge, this is the first report concerning the relationship between the serum leptin concentration and the skeletal muscle enzyme activity.

Before ER, these rats had a relatively higher serum leptin concentration and also a higher activity of skeletal muscle enzyme activity due to the free access to food. During the ER, the consumption of adipose storage, such as in the intra-abdominal adipose pads, caused a comparable descent of leptin. When the leptin level dropped, the muscle enzyme activity began to decline from the relatively higher level of the baseline. This is supported by the fact that the leptin concentration correlated with the muscle enzyme activities (except pre HK) both at the baseline and in the energy restricted rats after the ER. Taken together, these findings demonstrate an adaptive effect to cope with the metabolic balance with a restricted energy intake to prevent any further energy consumption or weight loss.

In rodent skeletal muscle, leptin has shown regulative effect on fatty acid oxidation [22–25] or expression of peroxisome proliferator-activated receptor γ-coactivator 1 (PGC-1) [31], the co-activator that promotes mitochondrial biogenesis and cooperates in transcriptional control of nuclear genes encoding mitochondorial fatty acid oxidation enzymes [32–34]. Together with the significant correlation found in the current study between the ER-induced changes in serum leptin concentration and in β-HAD in energy-restricted rats, although there is no direct evidence for causality, leptin thus play a putative role in regulating the activity of skeletal muscle enzymes, especially oxidative enzymes.

In summary, the present study demonstrated a lower leptin concentration and enzyme activity of the skeletal muscle in rats who had less remaining of intra-abdominal fat after a 4-week ER in comparison to CL rats. These findings indicate that skeletal muscle enzyme activity, especially oxidative enzymes, adapted to ER in a parallel way to that of intra-abdominal adipose consumption. Together with similar findings from another study based on a high-fat diet [26], leptin is therefore considered to play a regulative role in this process. It is possible that the change of adipose storage induces an adaptation in the serum leptin concentration and then the change in leptin causes the mitochondrial enzyme activity in skeletal muscle to adapt in a parallel way. This adaptation occurs in the early stage of diet change and has a significantly positive protective effect on the body.

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References

[1] Despres JP, Lemieux I, Tchernof A, Couillard C, Pascot A, Lemieux S. Fat distribution and metabolism. Diabetes Metab 2001;27:209–14.
[2] Frayn KN. Visceral fat and insulin resistance—causative or correlative? Br J Nutr 2000;83:S71–S77.
[3] Enzi G, Busetto L, Jimenez G, d'Alessio M, Biasion P. Metabolic abnormalities in visceral obesity. Front Diabetes 1992;11:119–23.
[4] Fujioka S, Matsuzawa Y, Tokunaga K, Kawamoto T, Kobatake T, Keno Y, Kotani K, Yoshida S, Tarui S. Improvement of glucose and lipid metabolism associated with selective reduction of intra-abdominal visceral fat in premenopausal women with visceral fat obesity. Int J Obes Relat Metab Disord 1991;15:853–9.
[5] Lefebvre AM, Laville M, Vega N, Riou JP, van Gaal L, Auwerx J, Vidal H. Depot-specific differences in adipose tissue gene expression in lean and obese subjects. Diabetes 1998;47:98–103.
[6] Weinsier RL, Hunter GR, Grower BA, Schutz Y, Darnell BE, Zuckerman PA. Body fat distribution in white and black women: different patterns of intraabdominal and subcutaneous abdominal adipose tissue utilization with weight loss. Am J Clin Nutr 2001;74:631–6.
[7] Aspnes LE, Lee CM, Weindruch R, Chung SS, Roecker EB, Aiken JM. Caloric restriction reduces fiber loss and mitochondrial abnormalities in aged rat muscle. FASEB J 1997;11:573–81.
[8] Hansen PA, Han DH, Marshall BA, Nolte LA, Chen MM, Mueckler M, Holloszy JO. A high fat diet impairs stimulation of glucose transport in muscle. J Biol Chem 1998;273:26157–63.
[9] Helge JW, Fraser AM, Kriketos AD, Jenkins AB, Calvert GD, Ayre KJ, Storlien LH. Interrelationships between muscle fibre type, substrate oxidation and body fat. Int J Obes Relat Metab Disord 1999; 23:986–91.
[10] Imbeault P, Tremblay A, Simoneau JA, Joanisse DR. Rise in plasma pollutant in response to weight loss is associated with a reduction in human skeletal muscle oxidative capacity. Am J Physiol Endocrinol Metab 2002;282:E574–E579.
[11] Simoneau JA, Bouchard C. Skeletal muscle metabolism and body fat content in men and women. Obes Res 1995;3:23–9.
[12] Simoneau JA, Veerkamp JH, Turcotte LP, Kelley DE. Markers of capacity to utilize fatty acids in human skeletal muscle: relation to insulin resistance and obesity and effects of weight loss. FASEB J 1997;13:2051–60.
[13] Wade A, Marbut MM, Round JM. Muscle fibre type and aetiology of obesity. Lancet 1990;335:806–8.
[14] Campfield L, Smith F, Guisez Y, Devos R, Burn P. Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. Science 1995;269:546–9.
[15] Halaas J, Gajiwala K, Maffei M, Cohen S, Chait B, Rabinowitz D, Lallone R, Burley S. Weight-reducing effects of the plasma protein encoded by the obese gene. Science 1995;269:543–6.
[16] Maffei M, Halaas J, Ravussin E, Pratley RE, Lee GH, Zhang Y, Fei H, Kim S, Lallone R, Ranganathan S, Kerm PS, Friedman JM. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nature Med 1995;1: 1155–61.
[17] Pellymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F. Effects of the obese gene product on body weight regulation in ob/ob mice. Science 1995;269:540–3.
[18] Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman J. Positional cloning of the mouse obese gene and its human homologue. Nature 1994;372:425–32.
[19] Lonnqvist F, Wennlund A, Arner P. Relationship between circulating leptin and peripheral fat distribution in obese subjects. Int J Obes Relat Metab Disord 1997;21:255–60.
[20] Ostlund RE, Yang JW, Klein S, Gingerich R. Relation between plasma leptin concentration and body fat, gender, diet, age, and metabolic covariates. J Clin Endocrinol Metab 1996;81:3909–13.

[21] Considine RV, Sinha MK, Heiman ML, Kriauciunas A, Stephens TW, Nyce MR, Ohannesian JP, Marco CC, McKee LJ, Bauer TL, Caro JF. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med 1996;334:292–5.

[22] Muoio DM, Dohm GL, Fiedorek FT, Tapscott EB, Coleman RA, Dohn GL. Leptin directly alters lipid partitioning in skeletal muscle. Diabetes 1997;46:1360–3.

[23] Steinberg GR, Bonen A, Dyck DJ. Fatty acid oxidation and triacylglycerol hydrolysis are enhanced after chronic leptin treatment in rats. Am J Physiol Endocrinol Metab 2002;282:E593–E600.

[24] Steinberg GR, Dyck DJ. Development of leptin resistance in rat soleus muscle in response to high-fat diets. Am J Physiol Endocrinol Metab 2000;279:E1374–E1382.

[25] Minokoshi Y, Kim YB, Peroni OD, Fryer LG, Muller C, Carling D, Kahn BB. Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. Nature 2002;415:339–43.

[26] Zou, B, Suwa, M, Nakano, H, Higaki, Y, Ito, T, Katsuta, S, Kumagai, S. Adaptation of skeletal muscle characteristics to a high-fat diet in rats with different intra-abdominal-obesity susceptibilities. J Nutr Sci Vitaminol. In press.

[27] Kobayashi A, Jiang LL, Hashimoto T. Two mitochondrial 3-hydroxyacyl-CoA dehydrogenases in bovine liver. J Biochem 1996;119: 775–82.

[28] Srere PA. Citrate synthase. Methods Enzymol 1969;13:3–6.

[29] Uyeda K, Racker E. Regulatory mechanisms in carbohydrate metabolism VII. Hexokinase and phosphofructokinase. J Biol Chem 1965; 240:4682–8.

[30] Landt M, Gingerich RL, Havel PJ, Mueller WM, Schoner B, Hale JE, Heiman ML. Radioimmunoassay of rat leptin: sexual dimorphism reversed from humans. Clin Chem 1998;44:565–70.

[31] Kakuma T, Wang ZW, Pan W, Unger RH, Zhou YT. Role of leptin in peroxisome proliferator-activated receptor gamma coactivator-1 expression. Endocrinology 2000;141:4576–82.

[32] Wu Z, Puigserver P, Andersson U, Zhang C, Adelmant G, Mootha V, Troy A, Cinti S, Lowell B, Scarpulla RC, Spiegelman BM. Mechanisms Controlling Mitochondrial Biogenesis and Respiration through the Thermogenic Coactivator PGC-1. Cell 1999;98:115–24.

[33] Lin J, Wu H, Tarr PT, Zhang CY, Wu Z, Boss O, Michael LF, Puigserver P, Isotani E, Olson EN, Lowell BB, Bassel-Duby R, Spiegelman BM. Transcriptional co-activator PGC-1 alpha drives the formation of slow-twitch muscle fibres. Nature 2002;418:797–801.

[34] Vega RB, Huss JM, Kelly DP. The coactivator PGC-1 cooperates with peroxisome proliferator-activated receptor alpha in transcriptional control of nuclear genes encoding mitochondrial fatty acid oxidation enzymes. Mol Cell Biol 2000;20:1868–76.

地域在宅高齢者における高次生活機能を規定する認知機能について：要介護予防のための包括的健診（「お達者健診」）についての研究(2)

岩佐　一[*,2*]　鈴木　隆雄[2*]　吉田　英世[2*]　金　憲経[2*]
新名　正弥[2*]　吉田　祐子[2*]　古名　丈人[2*]　杉浦　美穂[2*]
西澤　哲[2*]　胡　秀英[2*]　新開　省二[3*]　熊谷　修[3*]
藤原　佳典[3*]　渡辺修一郎[4*]　湯川　晴美[5*]

目的　本研究は，包括的健診（「お達者健診」）において試行された認知機能検査および老研式活動能力指標を用いて，認知機能の年齢差，および認知機能と高次生活機能の関連について明らかにすることを目的とした。

方法　東京都板橋区内在宅の70歳以上高齢者438人が本研究に参加した。認知機能は，Wechsler Adult Intelligence Scale-Revised（WAIS-R）符号問題，語想起検査，WAIS-R 数唱問題によって測定した。高次生活機能は，老研式活動能力指標で測定し，「手段的自立」，「知的能動性」，「社会的役割」の3つの下位尺度得点を分析に使用した。

成績　認知機能における年齢差を検討するため，3つの認知機能検査を従属変数，教育年数を共変量とした共分散分析により検討したところ，WAIS-R 符号問題，語想起検査およびWAIS-R 数唱問題において顕著な年齢差が認められ，80歳以上高齢者の成績が80歳未満高齢者のそれよりも低いことが明らかになった。

認知機能と高次生活機能の関連について，年齢と教育年数を統制変数とした偏相関分析により検討したところ，手段的自立では符号検査および語想起検査と，知的能動性では符号検査，語想起検査および数唱検査と，社会的役割では語想起検査とそれぞれ正の相関関係が認められた。老研式活動能力指標下位尺度をそれぞれ従属変数，認知機能検査，年齢，教育年数を独立変数とする重回帰分析を行ったところ，手段的自立，知的能動性，社会的役割における分散のそれぞれ4%，9%，4%が独立変数によって説明されることが明らかになった。

結論　80歳以上高齢者と80歳未満高齢者間においてWAIS-R 符号問題，語想起検査およびWAIS-R 数唱問題の成績に年齢差が認められたことから，後期高齢期以降における認知機能（情報処理速度，遂行機能および一次記憶）の低下が推測された。

高次生活機能は認知機能と正の関連性を有することが確認され，なかでも知的能動性は認知機能によって規定される傾向が最も強い高次生活機能であることが示唆された。

Key words：認知機能，高次生活機能，老研式活動能力指標

* 財長寿科学振興財団
2* 東京都老人総合研究所　疫学部門
3* 同　地域保健部門
4* 桜美林大学大学院国際学研究科
5* 國学院大学栃木短期大学
連絡先：〒173-0015 板橋区栄町35-2
東京都老人総合研究所　岩佐　一

I　はじめに

わが国の平均寿命は，1950年には男性59.6歳，女性63.0歳であったが，2000年には男性77.6歳，女性84.6歳と大きく伸長した。さらには，2050年には90歳を超えると推測されている[1]。このよう

な高齢社会において，痴呆や老年性うつ病，失禁，転倒（骨折），低栄養といった高齢者に特徴的な疾患・病態（老年症候群）を早期発見し，生活機能の維持を促進することは，高齢者保健医療領域における重要な課題として位置付けられる[2]。

生活機能の維持には，身体機能だけでなく認知機能も大きく影響を及ぼすとされている[3]。Cahn-Weiner ら[4]や Carlson ら[5]は，年齢，教育年数，人種といった人口学的変数や疾病状況などの交絡要因を統制してもなお認知機能が生活機能と正の関連性を有することを見出している。また，徘徊を伴う痴呆性高齢者のように，身体機能は比較的保持されているが，認知機能の低下によって生活機能が大きく障害されるケースも報告されている[6,7]。これらのことから，地域在宅高齢者における生活機能の規定要因として認知機能を捉え，その実態について明らかにすることは，痴呆を含む老年症候群の早期発見および要介護状態の予防的取り組みに対して，有益な資料を提供すると考えられる。

Lawton[8]は，生活機能を7つの水準に体系化し，最も低次の「生命維持」から，「機能的健康度」,「知覚-認知」,「身体的自立」,「手段的自立」,「状況対応」を経て，最高次の「社会的役割」へと至る階層的モデルを提唱した。地域在宅高齢者における生活機能と認知機能間の関連性について調べた先行研究の多くは，Lawton の階層的モデルにおける身体的自立および手段的自立の水準に相当する生活機能に限定し検討を行っている[9~11]。しかしながら，地域在宅高齢者が独立した生活，張りのある生活を維持してゆくには，ある種の余暇活動，家族や友人との交流などの活動において具現化される状況対応や社会的役割といったより高次の生活機能の維持が重要な用件となると考えられる[12,13]。そこで本研究では，古谷野ら[12]が開発した老研式活動能力指標（表1参照）を用いて生活機能を評価し，認知機能との関連について検討する。

本研究は，地域在宅高齢者を対象として認知機能の年齢差，および認知機能と高次生活機能間の関連性について検討し，地域高齢者を対象とした要介護予防活動に対して有用な基礎資料を提供することを目的とした。

Ⅱ 研究方法

1. 対象者

東京都板橋区在宅の70歳から84歳の高齢者438人（男性168人，女性270人）が健診に参加した。この対象者は，東京都老人総合研究所が1991年度（平成3年度）に開始した特別研究プロジェクト「中年からの老化予防総合的長期追跡研究心理班」[14]における最終年度調査（平成12年度）の参加者である。1991年度の初回調査において，東京都板橋区在宅の50歳から74歳の中高年者を住民基本台帳から等間隔抽出法によって4,510人をサンプリングした。初回調査の応答率は70.1%，その後毎年同一時期に追跡調査を行い，約80%から90%の応答率を維持しながら2000年の最終年調査までを行い，1,997人について追跡を完了した。このうち平成13年10月1日時点で70歳以上の者863人に対して健診の勧誘を行い，最終的に438人が健診に参加した[2]。

健診は板橋区内3か所における公共施設にて行ったが，全ての対象者は徒歩，家族による送迎，もしくは公共交通機関を利用して参加することが可能であった。

健診参加者438人のうち，教育年数が不明であった者1人を全ての分析から除外し，437人（男性167人，女性270人）のデータを分析に使用した。

健診参加時点での年齢により5歳階級で対象者を3群に分割した。70-74歳を年齢群1，75-79歳を年齢群2，80-84歳を年齢群3とした。表2は，対象者基本属性（対象者数，女性率，年齢，教育年数，健康度自己評価，Mini-Mental State Examination（MMSE）[15]を年齢群間で比較したものである。

2. 測度

1) 認知機能検査

本研究では，認知機能の測定に Wechsler Adult Intelligence Scale-Revised（WAIS-R）符号問題[16]（以下，符号検査と表記する），語想起検査[17]，WAIS-R 数唱問題[16]（以下，数唱検査と表記する）の3つの認知機能検査を用いた。これらの検査は，高齢者の認知機能を説明する上で，中核的な役割を担う概念である「情報処理速度（information processing speed）」[18]，「遂行機能（executive function）」[19,20]，「一次記憶（primary memory）」[21]