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Long-term exercise stimulates adenosine monophosphate–activated protein kinase activity and subunit expression in rat visceral adipose tissue and liver

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Abstract

Adenosine monophosphate–activated protein kinase (AMPK) is activated in response to adenosine triphosphate depletion caused by the metabolic and nutritional state. Mammalian AMPK is a heterotrimeric enzyme composed of a catalytic α subunit and 2 regulatory subunits (β and γ). Although much attention has been focused on exercise-induced AMPK activation in skeletal muscle, little information is available on the role of AMPK in adipose tissue and liver. Acetyl-coenzyme A carboxylase (ACC) is a well-known downstream target of AMPK. The ACC contains serine residues that are phosphorylated by AMPK. The present study was undertaken to determine whether long-term exercise of medium intensity (60% of $\dot{V}O_2$max for 12 weeks) may influence AMPK enzyme activity, gene/protein expression, and subsequent ACC phosphorylation in rat adipose tissue (visceral and subcutaneous) and liver. We initially demonstrated that long-term exercise induced a significant increase in phosphorylation of Thr^{172} in the AMPK α_1 subunit and of Ser^{79} in ACC in visceral adipose tissue rather than subcutaneous tissue. We also demonstrated that the AMPK α_1-,α_2-subunit messenger RNA (mRNA) level as well as the corresponding protein levels were increased in response to long-term exercise, whereas the other subunits were not altered significantly. In contrast to that of visceral adipose tissue, long-term exercise did not induce any significant effect on any of the AMPK subunit mRNA levels or α_1-,α_2-subunit protein levels in subcutaneous adipose tissue. In addition to adipose tissue, we demonstrated that long-term exercise induced an increase in both AMPK/ACC phosphorylation and α_1-,α_2-subunit mRNA/protein expression in the liver. Although the precise physiologic relevance of AMPK activation in these tissues remains unknown, it is possible that it might play an important role in long-term exercise-induced adaptation mechanisms and may lead to an improvement in certain metabolic abnormalities in metabolic diseases.

1. Introduction

Adenosine monophosphate–activated protein kinase (AMPK) is activated in response to adenosine triphosphate depletion caused by the metabolic and nutritional state. Mammalian AMPK is a heterotrimeric enzyme composed of a catalytic α subunit and 2 regulatory subunits (β and γ). As for the catalytic α subunit, the α_1 isoform is widely distributed, whereas the α_2 isoform is highly expressed in muscle and liver, as well as in adipose tissue. However, the precise role of the β and γ subunits remains unknown [1-3].

The activity of AMPK is regulated mainly by allosteric activation and phosphorylation. Indeed, AMPK is covalently activated by kinases via phosphorylation on Thr^{172} of the α subunit [4]. In addition, AMPK has longer-term effects, altering both gene expression and protein production. However, the precise mechanisms and physiologic relevance remain unclear [5-8].

A significant amount of attention has been focused on exercise-induced AMPK activation in skeletal muscle. Activation of AMPK in response to short-term exercise results in improved glucose tolerance, which is mainly responsible for increased glucose transporter 4 translocation in these tissues [9]. In addition to short-term exercise, long-term activation of the AMPK system might be due to certain long-term effects of long-term exercise. These studies were

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performed by long-term treatment of rats with 5-amino-imidazole-4-carboxamide riboside (AICAR), an agent that is known to activate the AMPK system [10]. This treatment results in increased expression of glucose transporter 4, increased activity of hexokinase and mitochondrial enzymes, and increased glycogen content [11], which is similar to the response to long-term exercise. Indeed, long-term AICAR treatment improves glucose tolerance in obese mice and rats [12,13]. Recently, long-term exercise was shown to induce an increase in protein expression of the α_1 and γ_3 subunits in human and rat skeletal muscle, respectively, indicating that the protein expression of AMPK is susceptible to long-term exercise in both rodents and humans [5,7,8].

It is well known that acetyl-coenzyme A (CoA) carboxylase (ACC) is a downstream target of AMPK. The ACC contains serine residues (Ser^{79}) that are phosphorylated by AMPK. Acetyl-CoA carboxylase phosphorylation inhibits malonyl-CoA synthesis, enhancing carnitine palmitoyltransferase activity and free fatty acid oxidation [14-17].

In liver, it has been demonstrated that AMPK activation decreases sterol and fatty acid synthesis via inhibition of ACC and 3-hydroxy-methylglutaryl-CoA reductase [18,19]. Adenosine monophosphate–activated protein kinase also inhibits hepatic gluconeogenesis through the suppression of phospho*enol*pyruvate carboxykinase gene expression in these cells [20].

Excessive deposition of visceral adipose tissue is known to be a causative factor for metabolic disease [21]. In this context, the pathophysiologic difference between visceral and subcutaneous adipose tissue has emerged. In particular, abdominal adipose tissue is known to be lipolytically active comparing that of subcutaneous tissue [22]. Consistent with this finding, exercise training increases lipolysis, particularly in abdominal adipose tissue, and may lead to improvement of metabolic disease. Thus, it is reasonable that exercise training has an obvious benefit based on this information. However, the precise pathophysiologic role of visceral adipose rather than lipolysis remains to be determined.

In contrast to skeletal muscle and liver, little attention has been focused on the role of AMPK in adipose tissue. Until now, the role of AMPK on adipocyte metabolism was assumed to be complicated and controversial. Most of previous studies showed that the activation of AMPK induced by AICAR antagonized lipolysis [23,24]. However, recent evidence has shown that expression of a dominant-negative form of AMPK reduces the lipolytic response to the adrenoreceptor agonist isoprenaline in 3T3L1 cells, implying that activation of AMPK may play a positive role in the stimulation of lipolysis [25]. Recently, Park et al [26] reported that short-term exercise had a stimulatory effect on AMPK activation in liver (α_1 and α_2 subunits) and adipose tissue of epididymal fat, supporting the suggestion that AMPK plays an important role in these cells after short-term exercise. In addition, these authors claimed that the net effect of its activation in adipose tissue and liver resulted in increased fatty acid oxidation and diminished glycerolipid synthesis.

Although it is well established that long-term medium-intensity exercise improves glucose tolerance and dyslipidemia, the long-term effect of exercise on AMPK activity in adipose tissue and liver remains unknown.

The present study was undertaken to determine whether long-term medium-intensity exercise (60% of $\dot{V}O_2$max for 12 weeks) influences AMPK enzyme activity as well as gene expression and protein levels in rat adipose tissue (visceral and subcutaneous) and liver. In particular, we focused on regional variations of adipose tissue and compared AMPK activation (visceral and subcutaneous) in response to long-term training.

2. Materials and experimental protocols

2.1. Experimental procedures (experimental animals for exercise study)

Nine-week-old male Wistar rats, weighing 301 ± 9 g, were obtained from Crea (Tokyo, Japan). They were housed 2 or 3 per cage in a temperature-controlled (20°C-22°C) and 12-hour light-dark cycle controlled room. Purina rat chow and water were provided ad libitum. All rats were run on a rodent treadmill (KN-73, Natume, Tokyo, Japan) for 1 week to acclimate them to handling and to running on a treadmill. They were randomly distributed into 2 groups, designated rest and medium-intensity exercise. The medium-intensity group was run for 30 minutes on a rodent treadmill at 15 m/min, 5 d/wk for 12 weeks. This work rate was estimated to elicit about 60% $\dot{V}O_2$max [27]. Before the experiment, all of the animals fasted for 24 hours, with free access to water. Each group remained sedentary for 48 hours to estimate long-term effect. On the day of experimentation, all rats were anesthetized with diethyl ether and sodium pentobarbital (6 mg/100 mg of body weight) at rest. Subsequently, samples of blood were taken from the abdominal aorta. The liver, subcutaneous adipose tissue, and visceral adipose tissue were excised and immediately frozen in liquid nitrogen.

2.2. Assay methods

The plasma concentrations of glucose, cholesterol, and triglycerides were measured by the Hitachi Auto Analyzer (type 7170; Hitachi Electronics, Hitachi, Japan). All parameters in the plasma samples were assayed in duplicate.

2.3. Real-time polymerase chain reaction analysis for AMPK subunit messenger RNA levels

Adenosine monophosphate–activated protein kinase subunit messenger RNA (mRNA) level was determined by using Taq-Man Assay on Demand (Applied Biosystems, Foster City, CA). We cited Assay ID instead of primers and probe sequences, as described below (Rn00569558_m1 [α_1], Rn00576935_m1 [α_2], Rn00583054_m1 [β_1], Rn00575219_m1 [β_2], Rn00564571_m1 [γ_1], Rn00788439_m1 [γ_2]). The polymerase chain reaction (PCR) reaction mixture was prepared using a Taq-Man PCR master reagent kit (Applied

Table 1
Body weight and serum parameters after long-term exercise

	Weight (g)	BG (mg/dL)	TCHO (mg/dL)	HDL-C (mg/dL)	TG (mg/dL)
CONT	484 ± 26.1	224 ± 24.9	67.7 ± 9.8	19.9 ± 4.6	124 ± 25.2
EXC	461 ± 25.5	181.6 ± 24.6	63.1 ± 9.3	24.1 ± 4.5	100 ± 20.7

CONT indicates sedentary control group; EXC, exercised group; BG, fasting blood glucose; TCHO, total cholesterol; TG, triglycerides.

Biosystems) according to the manufacturer's instructions. The thermal cycling protocol was as follows: 50°C for 2 minutes, 95°C for 10 minutes, then 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Thermal cycling, fluorescence detection, and data analysis were performed on an ABI PRISM 7700 Sequence Detector using software provided with the instrument.

Standard curves were plotted with Ct vs the log of the template quantities. The quantities of samples were determined from the standard curves. Adenosine monophosphate–activated protein kinase mRNA levels were normalized to those of 18S ribosomal RNA in each sample.

2.4. Western blot analysis

Western blot analyses were performed as previously described [28]. In brief, the sample tissue were solubilized

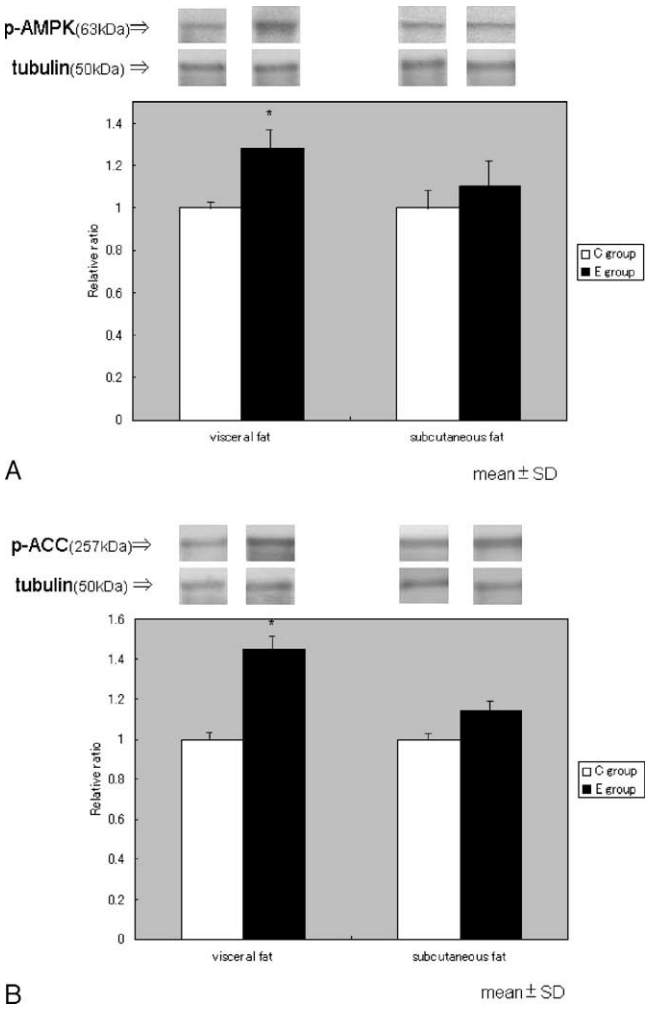

Fig. 1. Effect of long-term exercise either on AMPK α-subunit phosphorylation (Thr^{172}) or on ACC phosphorylation (Ser^{79}) in adipose tissue in rats. A, AMPK. B, ACC. The upper panel shows representative Western blots. Adenosine monophosphate–activated protein kinase α-subunit phosphorylation (Thr^{172}), ACC phosphorylation (Ser^{79}), and tubulin (internal control) were measured as described in Materials and Experimental Protocols. The lower panel shows summarized data of densitometric analyses. Values are represented as the relative ratio of the density of phosphorylated protein against that of tubulin (internal control). Relative ratio in sedentary control was expressed as 1 arbitrary unit. Values are means ± SD (n = 7). *P < .05 vs sedentary controls.

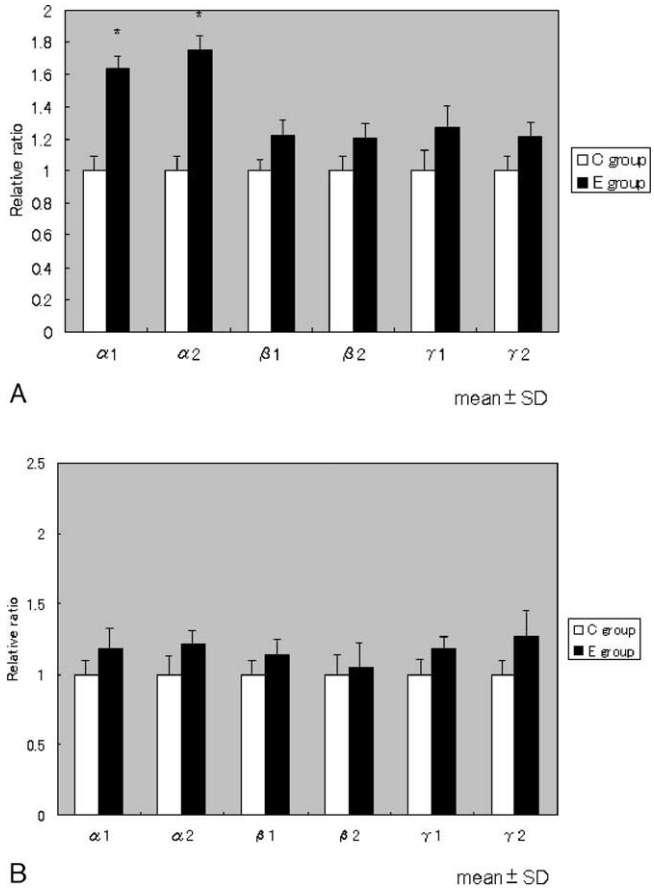

Fig. 2. The mRNA level of AMPK subunit isoforms in adipose tissue of long-term exercised and sedentary control rats. A, Visceral. B, Subcutaneous. Complementary DNA was characterized by real-time PCR as described in Materials and Experimental Protocols. Values are expressed as the relative ratio of the mRNA content against that of 18S ribosomal RNA (internal control). The relative ratio of the sedentary control was expressed as 1 arbitrary unit. Values are means ± SD (n = 7). *P < .05 vs sedentary controls.

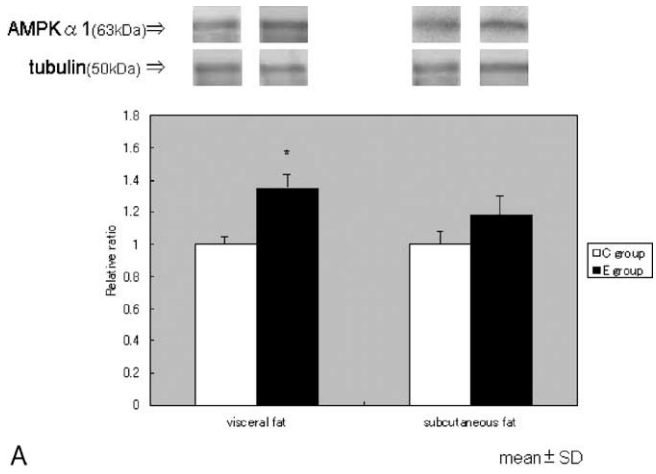

Fig. 3. Protein levels of AMPK α_1-,α_2-subunit isoforms in adipose tissues of exercise-trained and sedentary rats at rest. A, α_1 Subunit. B, α_2 Subunit. The upper panel shows representative Western blots. The lower panel shows densitometric analyses of AMPK protein levels. The AMPK protein levels are represented as the relative ratio of the density of AMPK subunit isoform proteins against that of tubulin (internal control). Relative ratio in sedentary control was expressed as 1 arbitrary unit. Values are means ± SD (n = 7). *P < .05 vs sedentary controls.

with 0.1% sodium dodecyl sulfate containing 1% Triton X-100, 1% sodium deoxycholate, and 20 mmol/L Tris-HCl, pH 7.4. The supernatants, containing 10-μg protein, were separated by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (10% gel) and then transferred to nitrocellulose using a Bio-Rad (Hercules, CA) Transblot apparatus. After transfer, the nitrocellulose sheets were incubated for 1 hour with BLOTTO buffer (5% skimmed milk, 0.05% Triton X-100, 100 mmol/L NaCl, 200 mmol/L Tris-HCl, pH 7.4). The nitrocellulose membranes were washed 3 times for 10 minutes with TBST solution (0.05% Triton X-100, 20 mmol/L Tris-HCl, pH 7.4, 150 mmol/L NaCl) and then for 1 hour with an antibody described below. The nitrocellulose membranes were then washed 3 times for 10 minutes with TBST solution, and then incubated for 1 hour with horseradish peroxidase–labeled protein A (Amersham Biosciences, Amersham, Little Chalfont, UK). Finally, the blots were washed 3 times in TBST solution, incubated with ECL reagent (Amersham) for 1 minute, and then exposed to Polaroid film (ISO 3000). For blotting, antibodies were diluted as follows: anti-α_1-AMPK (Cell Signaling, Beverly, MA) at 1:2000, anti-α_2-AMPK (Cell Signaling) at 1:3000, anti-pThr^{172} AMPK (Cell Signaling) at 1:5000 [17], anti-pSer^{79} ACC (Upstate, Charlottesville, VA) at 1:1000, and anti-tubulin (Chemicon, Temecula, CA) at 1:1000.

2.5. Statistical analysis

The results are expressed as means ± SD. Group means were compared using 1-way analysis of variance. A P value of less than .05 was considered to be statistically significant.

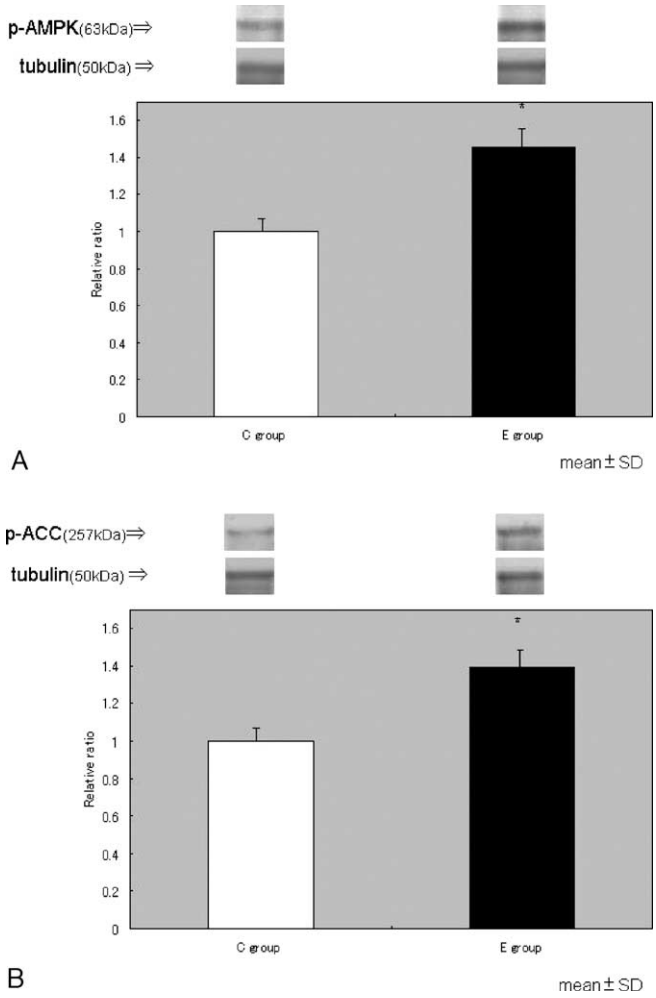

Fig. 4. Effect of long-term exercise either on AMPK α-subunit phosphorylation (Thr^{172}) or on ACC phosphorylation (Ser^{79}) in liver of rats. A, AMPK. B, ACC. The upper panel shows representative Western blots. The AMPK α-subunit phosphorylation (Thr^{172}), ACC phosphorylation (Ser^{79}), and tubulin were measured as described in Materials and Experimental Protocols. The lower panel shows summarized data of densitometric analyses. Values are represented as the relative ratio of the density of phosphorylated protein against that of tubulin (internal control). Relative ratio in sedentary control was expressed as 1 arbitrary unit. Values are means ± SD (n = 7). *P < .05 vs sedentary controls.

3. Results

3.1. Body weight and serum parameters after long-term exercise

As shown in the Table 1, long-term medium-intensity exercise (60% of $\dot{V}O_2$max for 12 weeks) tended to decrease body weight, fasting blood glucose, total cholesterol, and triglycerides, and increase high-density lipoprotein cholesterol. However, the changes were not significant.

3.2. Effect of long-term exercise on either AMPK or ACC activity in visceral and subcutaneous adipose tissue

Adenosine monophosphate–activated protein kinase activity was monitored using a phosphospecific antibody to Thr^{172}, a site on the α_1 subunit of AMPK [17]. As shown in Fig. 1A, long-term exercise significantly induced an increase in AMPK activity by about 1.30-fold compared with that of sedentary controls in visceral adipose tissue ($P < .05$). In contrast, long-term exercise did not affect AMPK activity compared with that of sedentary controls in subcutaneous adipose tissue.

To expand these findings, we examined the effect of long-term exercise on ACC phosphorylation in visceral and subcutaneous adipose tissue. As shown in Fig. 1B, long-term exercise significantly induced an increase in ACC phosphorylation by about 1.45-fold compared with that of sedentary controls in visceral adipose tissue ($P < .05$). In contrast, long-term exercise did not affect ACC phosphorylation compared with that of sedentary controls in subcutaneous adipose tissue.

3.3. Effect of long-term exercise on AMPK subunit mRNA levels and resulting AMPK protein levels in visceral and subcutaneous adipose tissue

As shown in Fig. 2A, in response to long-term exercise, the AMPK mRNA levels of the α_1 and α_2 subunits were significantly increased by approximately 1.65- and 1.75-fold, respectively, in visceral adipose tissue ($P < .05$). In contrast to the α_1,α_2 subunits, no significant effect was observed in the mRNA of β_1,β_2 and γ_1,γ_2 in these tissues. Different from visceral adipose tissue, no significant effect was seen in any of the subunit mRNA examined, in subcutaneous adipose tissue (Fig. 2B). To further confirm the mRNA level of the α_1,α_2 subunits in visceral adipose tissue, we carried out Western blots. Similar to the mRNA level, the protein levels of the α_1,α_2 subunits were significantly increased 1.35- and 1.40-fold, respectively, in these tissues (Fig. 3A and B).

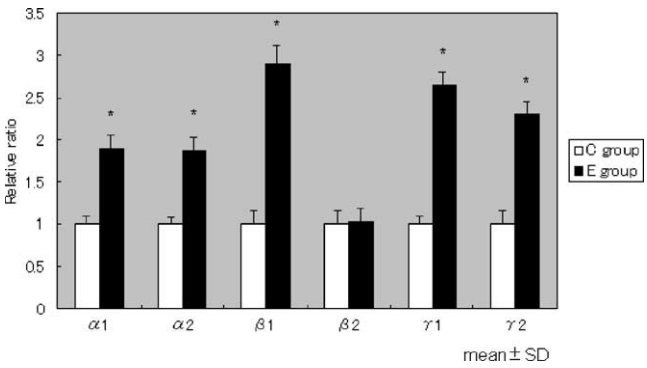

Fig. 5. Messenger RNA levels of AMPK subunit isoforms in the liver of long-term exercised and sedentary control rats. Complementary DNA was characterized by real-time PCR as described in Materials and Experimental Protocols. Values are expressed as the relative ratio of the mRNA content against that of 18S ribosomal RNA (internal control). The relative ratio of the sedentary control was expressed as 1 arbitrary unit. Values are means ± SD (n = 70.) *$P < .05$ vs sedentary controls.

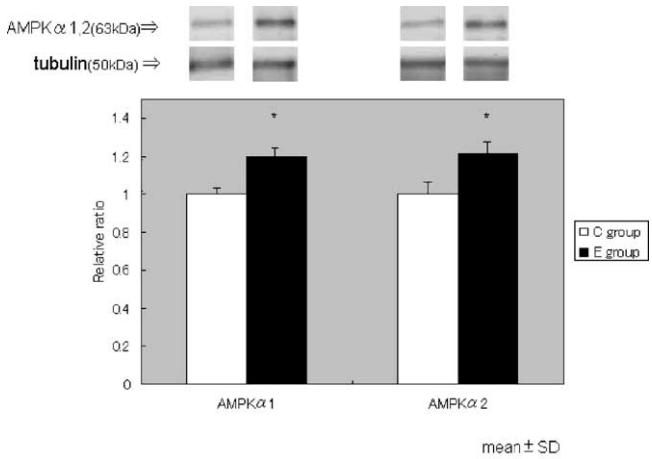

Fig. 6. Protein levels of AMPKα_1-,α_2-subunit isoforms in the liver of exercise-trained and sedentary rats at rest. The upper panel shows representative Western blots. The lower panel shows densitometric analyses of AMPK protein levels. The AMPK protein levels are represented as the relative ratio of the density of AMPK subunit isoform proteins against that of tubulin (internal control). Relative ratio in sedentary control was expressed as 1 arbitrary unit. Values are means ± SD (n = 7). *$P < .05$ vs sedentary controls.

3.4. Effect of long-term exercise on either AMPK or ACC activity in liver

As shown in Fig. 4A and B, long-term exercise significantly induced an increase in phosphorylation of AMPK and ACC by 1.45- and 1.40-fold, respectively, in liver ($P < .05$).

3.5. Effect of long-term exercise on AMPK mRNA levels and protein levels in liver

As shown in Fig. 5, in response to long-term exercise, the AMPK mRNA levels of α_1,α_2 subunits were significantly increased (both 1.9-fold) in the liver ($P < .05$). Similarly, long-term exercise induced significant increases in the mRNA levels of β_1 and γ_1-,γ_2, (2.9-fold, and 2.6- and 2.3-fold, respectively). To further confirm the mRNA level of the α_1,α_2 subunits in liver, we carried out Western blots. Similar to the mRNA level, the AMPK protein levels of the α_1,α_2 subunits were significantly increased (both 1.2-fold) in liver (Fig. 6).

4. Discussion

In the present study, we initially demonstrated that long-term exercise induced significantly increased phosphorylation of Thr^{172} in the α_1 subunit of AMPK in visceral adipose tissue ($P < .05$) but not subcutaneous adipose tissue (Fig. 1). Recently, Park et al [26] reported that short-term exercise has a stimulatory role on AMPK activation in visceral adipose tissue (epididymal fat). Thus, it would appear that short-term as well as long-term training could stimulate basal AMPK enzyme activity in visceral adipose tissue. We also demonstrated that the AMPK α_1-,α_2-subunit mRNA level, as well as the corresponding protein levels, was increased in response to long-term exercise, whereas the other subunits were not altered significantly (Figs. 2A and 3).

Repeated bouts of short-term exercise over a prolonged period (long-term exercise) induce "adaptations." Adenosine monophosphate–activated protein kinase is assumed to be a candidate for many of the adaptations in skeletal muscle [5,7,8]. Indeed, Frøsig et al [8] demonstrated that the protein content of α_1, β_2 and γ_1 increased and the γ_3 isoform decreased in response to long-term training and claimed that basal AMPK activity as well as protein levels are highly susceptible for adaptations in these tissues.

Based on the present study, it appears that basal AMPK activity as well as α_1-,α_2-subunit mRNA levels and the corresponding protein levels in visceral adipose tissue could be involved in long-term exercise adaptations. Recently, Villena et al [29] reported that AMPK-α_2 knockout mice exhibited higher body weight, with a specific increase in adipose tissue mass, which could be due to enhanced lipogenesis and changes in oxidative metabolism as a consequence of AMPK-α_2 ablation and the subsequent reduction in AMPK activity. This study supports the notion that α_2- protein levels are positively correlated with long-term AMPK activity, which is in agreement with our results.

Another interesting point made in this study was that exercise-induced AMPK activation was dependent on the location of the adipose tissue. We demonstrated that long-term exercise significantly induced an increase in AMPK activity/expression of α_1 and α_2 subunits compared with that of sedentary rats in visceral adipose tissue. In contrast, long-term exercise did not affect AMPK activity/expression in subcutaneous fat. These findings indicate that long-term exercise stimulates AMPK activity/expression in visceral adipose tissue rather than subcutaneous tissue (Figs. 1-3). It is of note that sustained AMPK activation in obese Zucker rats by long-term administration of AICAR diminished the mass of epididymal and retroperitoneal fat pads up to 30% to 40%, although no difference in total body weight was observed in this study, in agreement with our present study [30]. The authors claimed that the adipose mass reduction observed in this study was due to an increase in lipolysis in visceral adipose tissue by AICAR activation of AMPK, which was previously reported by Winder et al [12]. However, the precise mechanisms responsible for this adipose mass reduction remain unknown.

In addition to adipose tissue, we demonstrated that long-term exercise induced an increase in both AMPK activity and α_1-,α_2-subunit mRNA/protein expression in the liver (Figs. 4-6). Previous studies showed that constitutively active α_1 subunit in hepatocytes were significantly increased in AMPK activity and resulted in the inhibition of glucose activated genes, such as fatty acid synthesis, L-type pyruvate kinase, and ACC, which is in agreement with AICAR antagonism of these genes [31]. Considering that the effect of long-term exercise shown in this study is assumed to be similar to those earlier findings, it is reasonable to speculate that the activation of α_1-protein levels in the liver may be involved, at least part, in long-term exercise-induced adaptation mechanisms.

In the present study, we showed that long-term exercise induced an increase of AMPK α_1- and α_2-subunit mRNA followed by an increase in protein levels both in adipose and liver tissue, suggesting that they could be transcriptionally regulated. Recently, Mahlapuu et al [32] demonstrated that the AMPK subunit mRNA level has a good correlation with the protein expression pattern in rat skeletal muscle, which is in agreement with our idea. However, Neilsen et al [7] showed that long-term exercise stimulated the AMPK protein level without a significant increase in the mRNA in human skeletal muscle and claimed that the increase of AMPK protein expression may be due to posttranscriptional regulation. Furthermore, we showed that long-term exercise induced an increase in AMPK subunit mRNA level of α_1,α_2 in visceral adipose tissue (Fig. 2A), whereas the AMPK mRNA levels of α_1,α_2,β_1,γ_1, and γ_2 in liver also increased (Fig. 5). Although the precise role of the 2 regulatory subunits (β and γ) in liver remains unclear, it could reflect a difference in the adaptation mechanisms in these organs. Further studies are needed to clarify this point.

Although we did not directly measure the activity of the catalytic unit of AMPK, it was demonstrated that Thr^{172} phosphorylation of AMPK is required for and correlates with actual enzyme activity [14-17]. Acetyl-CoA carboxylase is a well-established downstream target of AMPK. In addition, Park et al [17] demonstrated that negative linear correlations were observed between phosphor-ACC and ACC activity. Corresponding to AMPK activity, there was also significant increase in ACC phosphorylation after long-term exercise in visceral adipose tissue and liver. Taken as a whole, our data support the notion that long-term exercise stimulates AMPK activity and a subsequent down-regulation of ACC activity in these tissues.

From the point of intensity of exercise, it should be noted that long-term medium-intensity exercise (60% of $\dot{V}O_2max$ for 12 weeks), although it did not affect body weight and serum parameters such as fasting blood glucose, total cholesterol, triglyceride, and high-density lipoprotein cholesterol, could be sufficient to activate AMPK activity in

visceral adipose tissue and liver, which is assumed to be the major organ for regulating the energy state. Thus, it appears that long-term medium-intensity exercise may have a benefit by activating AMPK in these tissues.

In conclusion, we observed an increase in AMPK activity as well as protein expression in visceral adipose tissue and liver in response to medium-intensity long-term exercise. Although the precise physiologic relevance of AMPK activation in these tissues remains unknown, it is possible that they might play an important role in adaptation mechanisms and may lead to an improvement of some metabolic abnormalities in metabolic diseases.

References

[1] Cheung PC, Salt IP, Davies SP, Hardie DG, Carling D. Characterization of AMP-activated protein kinase gamma-subunit isoforms and their role in AMP binding. Biochem J 2000;346:659-69.

[2] Stapleton D, Mitchelhill KI, Gao G, Widmer J, Michell BJ, Teh T, et al. Mammalian AMP-activated protein kinase subfamily. J Biol Chem 1996;271:611-4.

[3] Thornton C, Snowden MA, Carling D. Identification of a novel AMP-activated protein kinase beta subunit isoform that is highly expressed in skeletal muscle. J Biol Chem 1998;273:12443-50.

[4] Davies SP, Helps NR, Cohen PT, Hardie DG. 5′-AMP inhibits dephosphorylation, as well as promoting phosphorylation, of the AMP-activated protein kinase. Studies using bacterially expressed human protein phosphatase-2C alpha and native bovine protein phosphatase-2AC. FEBS Lett 1995;377:421-5.

[5] Durante PE, Mustard KJ, Park SH, Winder WW, Hardie DG. Effects of endurance training on activity and expression of AMP-activated protein kinase isoforms in rat muscles. Am J Physiol Endocrinol Metab 2002;283:E178-86.

[6] Langfort J, Viese M, Ploug T, Dela F. Time course of GLUT4 and AMPK protein expression in human skeletal muscle during one month of physical training. Scand J Med Sci Sports 2003;13:169-74.

[7] Nielsen JN, Mustard KJ, Graham DA, Yu H, MacDonald CS, Pilegaard H, et al. 5′-AMP–activated protein kinase activity and subunit expression in exercise-trained human skeletal muscle. J Appl Physiol 2003;94:631-41.

[8] Frøsig C, Jørgensen SB, Hardie DG, Richter EA, Wojtaszewski JFP. 5′-AMP–activated protein kinase activity and protein expression are regulated by endurance training in human skeletal muscle. Am J Physiol Endocrinol Metab 2004;286:E411-7.

[9] Holmes BF, Kurth-Kraczek EJ, Winder WW. Chronic activation of 5′-AMP–activated protein kinase increases GLUT-4, hexokinase, and glycogen in muscle. J Appl Physiol 1999;87:1990-5.

[10] Buhl ES, Jessen N, Schmitz O, Pedersen SB, Pedersen O, Holman GD, et al. Chronic treatment with 5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside increases insulin-stimulated glucose uptake and GLUT4 translocation in rat skeletal muscles in a fiber type–specific manner. Diabetes 2001;50:12-7.

[11] Jessen N, Pold R, Buhl ES, Jensen LS, Schmitz O, Lund S. Effects of AICAR and exercise on insulin-stimulated glucose uptake, signaling, and GLUT-4 content in rat muscles. J Appl Physiol 2003;94:1373-9.

[12] Winder WW, Holmes BF, Rubink DS, Jensen EB, Chen M, Holloszy JO. Activation of AMP-activated protein kinase increases mitochondrial enzymes in skeletal muscle. J Appl Physiol 2000;88:2219-26.

[13] Pold R, Jensen LS, Jessen N, Buhl ES, Schmitz O, Flyvbjerg A, et al. Long-term AICAR administration and exercise prevents diabetes in ZDF rats. Diabetes 2005;54:928-34.

[14] Dean D, Daugaard JR, Young ME, Saha A, Vavvas D, Asp S, et al. Exercise diminishes the activity of acetyl-CoA carboxylase in human muscle. Diabetes 2000;49:1295-300.

[15] Winder WW, Hardie DG. Inactivation of acetyl-CoA carboxylase and activation of AMP-activated protein kinase in muscle during exercise. Am J Physiol 1996;270(2 Pt 1):E299-E304.

[16] Kudo N, Gillespie JG, Kung L, Witters LA, Schulz R, Clanachan AS, et al. Characterization of 5′AMP–activated protein kinase activity in the heart and its role in inhibiting acetyl-CoA carboxylase during reperfusion following ischemia. Biochim Biophys Acta 1996;1301:67-75.

[17] Park SH, Gammon SR, Knippers JD, Paulsen SR, Rubink DS, Winder WW. Phosphorylation-activity relationships of AMPK and acetyl-CoA carboxylase in muscle. J Appl Physiol 2002;92:2475-82.

[18] Lochhead PA, Salt IP, Walker KS, Hardie DG, Sutherland C. 5-Aminoimidazole-4-carboxamide riboside mimics the effects of insulin on the expression of the 2 key gluconeogenic genes PEPCK and glucose-6-phosphatase. Diabetes 2000;49:896-903.

[19] Hardie DG, Carling D, Carlson M. The AMP-activated/SNF1 protein kinase subfamily: metabolic sensors of the eukaryotic cell? Ann Rev Biochem 1998;67:821-55.

[20] Hawley SA, Davison M, Woods A, Davies SP, Beri RK, Carling D, et al. Characterization of the AMP-activated protein kinase kinase from rat liver, and identification of threonine-172 as the major site at which it phosphorylates and activates AMP-activated protein kinase. J Biol Chem 1996;271:27879-87.

[21] Kissebah AH. Central obesity: measurement and metabolic effects. Diabetes Rev 1997;5:8-20.

[22] Arner P. Differences in lipolysis between human subcutaneous and omental adipose tissues. Ann Med 1995;27:435-8.

[23] Sullivan JE, Brocklehurst KJ, Marley AE, Carey F, Carling D, Beri RK. Inhibition of lipolysis and lipogenesis in isolated rat adipocytes with AICAR: a cell-permeable activator of AMP-activated protein kinase. FEBS Lett 1994;353:33-6.

[24] Corton JM, Gillespie JG, Hawley SA, Hardie DG. 5-Aminoimidazole-4-carboxamide ribonucleoside: a specific method for activating AMP-activated protein kinase in intact cells? Eur J Biochem 1995;229:558-65.

[25] Yin W, Mu J, Birnbaum MJ. Role of AMP-activated protein kinase in cyclic AMP-dependent lipolysis in 3T3-L1 adipocytes. J Biol Chem 2000;3278:43074-80.

[26] Park H, Kaushik VK, Constant S, Prentki M, Przybytkowski E, Ruderman NB, et al. Coordinate regulation of malonyl-CoA decarboxylase, *sn*-glycerol-3-phosphate acyltransferase, and acetyl-CoA carboxylase by AMP-activated protein kinase in rat tissues in response to exercise. J Biol Chem 2002;277:32571-7.

[27] Ohmori H, Kawai K, Yamashita K. Characterization of exercise-induced hyperketonemia in streptozotocin-diabetic rats and the effects on it of prolonged training. Endocrinol Jpn 1989;36:417-27.

[28] Fukuda T, Takekoshi K, Nanmoku T, Ishii K, Isobe K, Kawakami Y. Inhibition of the RhoA/Rho kinase system attenuates catecholamine biosynthesis in PC 12 rat pheochromocytoma cells. Biochim Biophys Acta 2005;1726:28-33.

[29] Villena JA, Viollet B, Andreelli F, Kahn A, Vaulont S, Sul HS. Induced adiposity and adipocyte hypertrophy in mice lacking the AMP-activated protein kinase-alpha2 subunit. Diabetes 2004;53:2242-9.

[30] Buhl ES, Jessen N, Pold R, Ledet T, Flyvbjerg A, Pedersen SB, et al. Long-term AICAR administration reduces metabolic disturbances and lowers blood pressure in rats displaying features of the insulin resistance syndrome. Diabetes 2002;51:2199-206.

[31] Woods A, Azzout-Marniche D, Foretz M, Stein SC, Lemarchand P, Ferre P, et al. Characterization of the role of AMP-activated protein kinase in the regulation of glucose-activated gene expression using constitutively active and dominant negative forms of the kinase. Mol Cell Biol 2000;20:6704-11.

[32] Mahlapuu M, Johansson C, Lindgren K, Hjalm G, Barnes BR, Krook A, et al. Expression profiling of the gamma-subunit isoforms of AMP-activated protein kinase suggests a major role for gamma3 in white skeletal muscle. Am J Physiol Endocrinol Metab 2004;286:E194-E200.