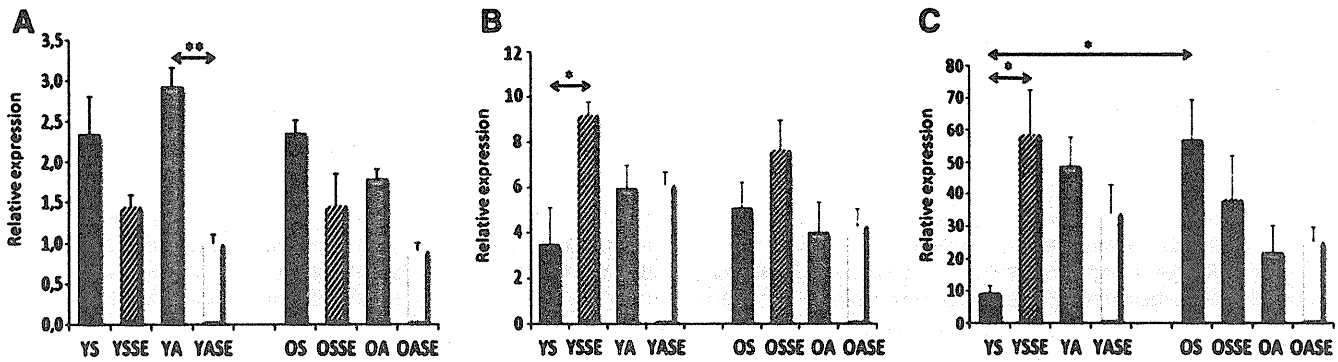

Fig. 3. Effect of SEB on expression of (A) Ku70, (B) Cu,Zn-superoxide dismutase (SOD), and (C) Mn-SOD. RNA was isolated from muscle biopsies excised before and 24 h after SEB. Quantitative RT-PCR was carried out as described under Materials and methods. YS, young sedentary; YSSE, young sedentary after a single bout of exercise; YA, young active; YASE, young active after a single bout of exercise; OS, old sedentary; OSSE, old sedentary after a single bout of exercise; OA, old active; and OASE, old active after a single bout of exercise. Values are means ± SE for six subjects per group. $^*p<0.05$, $^{**}p<0.01$.

preexercise levels in physically active individuals, both OA and YA, its level in DNA remained high in sedentary young and old subjects after a 24-h recovery period (Fig. 1A). For example, 8-oxoG levels were approximately four times higher in untrained older (Fig. 1A) compared to younger individuals without SEB (Fig. 1A). Importantly, there was no change in genomic 8-oxoG levels in muscle biopsies of OA individuals after SEB (Fig. 1A).

The subphysiological level of genomic 8-oxoG in physically active subjects suggested an efficient repair of DNA. We observed that OGG1 levels did not significantly change in younger subjects, but they increased in the older subjects in response to SEB (Fig. 1B). In contrast, Ac-OGG1 levels were significantly increased in younger individuals, whereas in the older subjects no significant change was observed in response to SEB. Ac-OGG1 level was approximately threefold higher in active compared to older, sedentary individuals (Figs. 1E and C). SEB did not change Ac-APE1 (Fig. 2A), which was similar to APE1 levels (data not shown), suggesting that neither Ac-APE1 nor APE1 is limiting in the repair of 8-oxoG.

In response to SEB, the expression of p300/CBP increased approximately fivefold in the younger subjects, but unexpectedly, it significantly decreased in older subjects (Fig. 3A). If indeed p300/CBP is the acetyltransferase in muscle, these results are in line with the levels of Ac-OGG1 (Figs. 1C and E). In physically active subjects SEB did not significantly alter p300/CBP levels (Fig. 2B). Expression of the deacetylase SIRT1 showed a significant increase only in younger sedentary subjects in response to SEB (Fig. 2C). The expression of SIRT3, which has no deacetylase activity, was the highest in muscle biopsies of active, younger subjects (Fig. 2D), and its expression did change upon SEB (Fig. 2D). SIRT6 expression (Fig. 2E), along with Ku70 (Fig. 3A), decreased in both young and old muscles after SEB. Together these data suggest that a physically active lifestyle induces an adaptive response by generating mild oxidative stress and prevents the age-associated increase in genomic 8-oxoG levels possibly due to the age-independent increase in OGG1's acetylation.

Discussion

Age-related and physical exercise-associated changes in DNA damage levels in skeletal muscle of experimental animals have been reported previously [13,14,48]. This study analyzed levels of 8-oxoG in DNA and the abundance of rate-limiting BER enzymes in human muscle biopsies before and after a single exercise bout. We also examined expression of acetyltransferases and deacetylases linked to DNA repair pathways and antioxidant genes that could reflect on cellular redox conditions. We show that the genomic 8-oxoG level is lastingly elevated in sedentary young and old subjects, but it returned rapidly to preexercise levels in physically active individuals independent of age upon a single exercise bout. The 8-oxoG level in DNA inversely correlated with the abundance of Ac-OGG1, but not with total OGG1, APE1, or Ac-APE1. Importantly, our data also demonstrate a physical activity-dependent increase in the acetylated forms of OGG1 in human skeletal muscle. Accordingly, it is possible that an exercise-induced acetylation pathway would enhance OGG1 activity, not only in muscles, but in other tissues, and thereby exercise may decrease the incidence of various pathological conditions, such as inflammation, that have been linked to carcinogenesis, cardiovascular diseases, strokes, or Alzheimer disease.

8-oxoG is arguably one of the important forms of DNA base damage induced by ROS, and it has been proposed to play a role in the aging process and is also linked to age-associated diseases [1–3,5]. This hypothesis is consistent with the severalfold increase in 8-oxoG (and possibly of other oxidized bases) content in nuclear and mtDNA from aged tissues [1–3,5]. A single bout of exercise has been shown to cause mild oxidative stress [32,49,50], and thus we applied a SEB and determined cellular oxidative states, changes in 8-oxoG levels, and abundance of selected repair enzymes. Because of a limited amount of muscle biopsies, we used quantitative fluorescence analysis [36,38,41] to assess 8-oxoG levels, as the quantity of DNA isolated did not allow us to use HPLC with electrochemical detection [7,8], which would provide a better estimates. By using a highly specific, anti-8-oxodG-specific antibody, we observed significantly higher levels of genomic 8-oxoG in human skeletal muscle of sedentary, older individuals compared to the levels in younger subjects, in line with previous observations [13,14,43,44]. In response to SEB-induced ROS, 8-oxoG levels increased further and were not repaired, even after a 24-h period, in sedentary individuals, independent of age. In contrast, 8-oxoG levels returned to preexercise levels in physically active individuals, a finding that may mean regular physical activity could prevent accumulation and/or increase repair efficacy of 8-oxoG and possibly other bases in DNA human skeletal muscle.

The observed increase in 8-oxoG levels in sedentary individuals points to a possible age-dependent decrease in levels of OGG1. In contrast, our data show a significantly increased OGG1 level in elderly subjects and, interestingly, SEB furthered its level. Unexpectedly, the 8-oxoG level was also enhanced. These paradoxical observations suggested to us that OGG1 may have a low DNA glycosylase/AP lyase activity or that BER activities are significantly lower in aged human muscle. Indeed, a recent publication documents decreased overall BER activities in both the nuclei and the mitochondrial extracts from skeletal muscles, compared to those from liver or kidneys of the same mice [51]. Although decreased overall BER activity could be a possibility, our data also imply that a lack of or delayed repair of 8-oxoG could be linked to a deficiency in posttranslationally modified OGG1 in aged muscles. Indeed, OGG1's glycosylase/AP-lyase activity is modulated via acetylation, phosphorylation, and redox [23,25]. For example, OGG1 is acetylated on lysines 338 and 341 and has an approximately 10-fold increase in its 8-oxoG excision activity compared to unacetylated OGG1 [23]. To explore this possibility we show that approximately one-fifth of OGG1 is in an acetylated form in younger individuals and, importantly, Ac-OGG1 was nearly undetectable in the sedentary elderly. This observation is a feasible possibility, as 8-oxoG level in DNA was inversely correlated with levels of Ac-OGG1 in muscles of young and old individuals.

Repair of 8-oxoG is initiated by OGG1 during the BER pathway, followed by APE1-mediated cleavage of the DNA strand at the abasic site. After removal of this 3′-blocking group, the single-nucleotide gap is filled in by a DNA polymerase, and DNA ligase seals the nick to restore DNA integrity [17]. It has also been shown that OGG1 remains tightly bound to its AP product after base excision, and APE1 prevents its reassociation with its product, thus enhancing OGG1 turnover [45]. Accordingly, APE1 is considered to be rate-limiting in the BER of 8-oxoG [17,39]. However, neither APE1 nor Ac-APE1 showed significant changes with aging and/or physical activity. Therefore, it may be proposed that the Ac-OGG1 is limiting in the repair of 8-oxoG lesions in human skeletal muscle during BER processes. As modification by phosphorylation substantially alters the incision activity of only OGG1 [24], our earlier observations of an exercise-induced increase in OGG1 activity in skeletal muscles of human and experimental animals [14,43] may be attributed to Ac-OGG1.

Acetylation levels of OGG1 and APE1 are dependent on the level/activity of the acetyltransferase p300/CBP [23,25] and possibly on a deacetylase(s) such as some of the sirtuins [52]. Results from our studies show that p300/CBP's expression was increased in young individuals by SEB, independent of whether they were sedentary or active. However, we were not able show such consistency in the elderly. SIRT1, a NAD-dependent histone deacetylase [53], has been shown to interact with p300/CBP to regulate its acetyltransferase activity [52]. SIRT1 levels increased in both young and elderly muscles in response to exercise. These observations are in line with the general role of SIRT1 in the DNA damage response and maintenance of genomic integrity, as it promotes proper chromatin structure and DNA damage repair foci formation for repair of DNA base lesions [27,28]; however, the patterns of change in SIRT1 expression in young vs old or sedentary vs physically active suggest an inverse correlation between SIRT1 and the level of Ac-OGG1.

Among sirtuins, only SIRT3 expression correlates with the life span of humans [54]. Interestingly, SIRT3 expression was increased with physical fitness level only in young subjects in this study. SIRT3 has two isoforms with different molecular masses (44 and 28 kDa), which are localized in mitochondria and nucleus, respectively [55]. The translocation of SIRT3 from the nucleus to the mitochondria has been shown to be induced by oxidative stress [55]. SIRT3 is also a modulator of apoptosis [56]. Recent findings also indicate that SIRT3 is a downstream target of PGC-1α and one of the regulators of mitochondrial ROS production [57].

Exercise has been shown to cause mild oxidative stress [32,49,50,58]. Although the 8-oxoG level is a documented measure of such an oxidative insult [14], MDA levels and expression of superoxide dismutase(s) were used to evaluate further SEB-induced oxidative stress. An increase in MDA levels in plasma correlated with genomic 8-oxoG level in both young and old subjects in response to SEB. Interestingly, only the expression of Cu,Zn-SOD showed age-independent and exercise-associated changes, and Mn-SOD expression was increased only in the younger sedentary group. Based on these observations, it appears that Cu,Zn-SOD expression is a better measure of an adaptive response to ROS than that of mitochondrial Mn-SOD. These data also imply a decline in adaptive response with age at the level of Mn-SOD. These observations are in line with those showing that the adaptive capability of an organism to withstand oxidative stress challenge(s) is markedly decreased as a function of age [59,60]. Based on our data, however, we propose that adaptive responses to ROS are not age dependent, but decided by the physical status of an individual.

In conclusion, this investigation offers insight into interactions between aging processes, exercise, and regulation of the repair of oxidized DNA base lesions in human skeletal muscle. We show for the first time that (1) acetylated forms of OGG1 and APE1 are present in human tissues, but (2) only Ac-OGG1 seems to be rate limiting in the BER processes of 8-oxoG, and (3) repair of 8-oxoG seems to be independent of age, but (4) is dependent on the physical state of muscles. Our data also imply that regular exercise induces an adaptive response that involves an improved, more efficient antioxidant and DNA repair machinery.

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Short-term adenosine monophosphate–activated protein kinase activator 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside treatment increases the sirtuin 1 protein expression in skeletal muscle

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Abstract

Adenosine monophosphate–activated protein kinase (AMPK) has been proposed to stimulate mitochondrial biogenesis and fat and glucose metabolism in skeletal muscle. Nicotinamide adenine dinucleotide–dependent histone deacetylase sirtuin 1 (SIRT1) is also thought to play a pivotal role for such metabolic adaptations. The purpose of the present study was to examine the effect of AMPK activation with the administration of AMPK activator 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) to rats on skeletal muscle SIRT1 protein expression as well as peroxisome proliferator activated receptor γ coactivator–1α (PGC-1α) and glucose transporter 4 (GLUT4) protein expression and hexokinase activity. The AICAR promoted the phosphorylation of AMPK α-subunit (Thr^{172}) and acetyl–coenzyme A carboxylase (Ser^{79}) without any change of total AMPK α-subunit or acetyl–coenzyme A carboxylase protein levels in both the slow-twitch soleus and fast-twitch extensor digitorum longus (EDL) muscles. The SIRT1 protein expression increased at 24 hours after administration of AICAR in the EDL muscle but not in the soleus muscle. The PGC-1α protein expression increased in both the soleus and EDL muscles and GLUT4 did in the EDL muscle at 24 hours after an administration of AICAR. The hexokinase activity increased at 18 and 24 hours in the soleus and at 12, 18, and 24 hours in the EDL after an AICAR treatment. These results suggest that short-term AICAR treatment to rats promotes skeletal muscle AMPK phosphorylation and then coincidently increases the SIRT1 protein expression. In addition, such treatment also enhances the PGC-1α and GLUT4 protein contents and hexokinase activity in skeletal muscle.

1. Introduction

Silence information regulator 2 (Sir2) proteins are the nicotinamide adenine dinucleotide–dependent acetylases that regulate longevity in *Caenorhabditis elegans* [1] and *Saccharomyces cerevisiae* [2] in response to caloric restriction. In mammals, the Sir2 ortholog, sirtuin 1 (SIRT1)/Sir2α plays an important role in various biological processes via functionally interacting and deacetylating several proteins [3]. SIRT1 controls both energy homeostasis and metabolic adaptations [4]. The activation of SIRT1 with its activator resveratrol improved the glucose tolerance and survival in mice fed high-fat diet [5,6]. SIRT1 can promote mitochondrial biogenesis and fatty acid oxidation in skeletal muscle cells via deacetylation and functionally activating the peroxisome proliferator activated receptor γ coactivator–1α (PGC-1α) [7-9]. This metabolic role of SIRT1 is associated with 5′-adenosine monophosphate–activated protein kinase (AMPK), which is also a key regulator of energy metabolism [4].

5′-Adenosine monophosphate–activated protein kinase is a heterotrimer consisting of 3 subunits: α, β, and γ [10]. Two isoforms exist for both the α-subunit (α1 and α2) and β-subunit (β1 and β2) and 3 for the γ-subunit (γ1, γ2, and γ3). The α-subunit contains the catalytic domain. The β-subunit mediates the assembly of the heterotrimeric AMPK complex [11] and glycogen binding [12]. The γ-subunit binds the AMP and following phosphorylation of threonine

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172 in the α-subunit and kinase activation [13]. The AMPK functions as an energy sensor and is activated when the cellular AMP to adenosine triphosphate ratio is increased [10]. The phosphorylation of threonine 172 in α-subunit strongly correlates with the AMPK activity [14]. The AMPK phosphorylation is mainly regulated by an upstream kinase LKB1 in skeletal muscle [15]. Skeletal muscle AMPK is activated by exercise [16], adipocytokines including leptin [17] and adiponectin [18], and antidiabetic drug metformin [19,20]. The activation of AMPK by its activator 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) stimulates both glucose uptake and fatty acid oxidation in skeletal muscle cells [21] and increases insulin-stimulated glucose uptake, insulin signaling such as phosphatidylinositol 3-kinase and protein kinase B activities, glucose transporter 4 (GLUT4) protein expression, hexokinase activity, and mitochondrial oxidative enzyme activities in skeletal muscle [22-24]. The activation of AMPK by AICAR also increases the PGC-1α expression in skeletal muscle [25], which controls mitochondrial biogenesis and glucose metabolism [25,26]. The AMPK is indirectly phosphorylated by SIRT1 through LKB1 deacetylation [27]. In addition, AMPK promotes SIRT1 activation by enhancing the transcription and activity of nicotinamide phosphoribosyltransferase [28].

The skeletal muscle SIRT1 protein expression [29] and activity [30] have been observed to increase with endurance exercise in rat skeletal muscle. Endurance exercise has a great impact on the skeletal muscle metabolic characteristics, including mitochondrial biogenesis and GLUT4 expression [31], while also activating AMPK [16]. The activation of AMPK with AICAR also induces such metabolic adaptations in skeletal muscle [23,24], thus suggesting that the activation of AMPK mediates the effect of endurance exercise training on metabolic characteristics. It is hypothesized that AMPK regulates SIRT1 expression. The purpose of the present study was to investigate whether the activation of AMPK with short-term AICAR treatment to rats induced the expression of SIRT1 protein as well as the expression of PGC-1α and GLUT4 protein and also the hexokinase activity in slow- and fast-twitch skeletal muscles.

2. Materials and methods

2.1. Animals

Male Wistar rats that were 4 weeks of age and with a body weight of 70 to 90 g (Kyudo, Tosu, Saga, Japan) were used for the current study. All rats were handled daily for at least 5 days before beginning their experiment regimen. All rats were housed in a temperature- (22°C ± 2°C) and humidity- (60% ± 5%) controlled room with a 12-hour light (7:00 AM-7:00 PM) and 12-hour dark (7:00 PM-7:00 AM) cycle. Food and water were provided ad libitum. All experimental procedures were strictly conducted in accordance with the Nakamura Gakuen University Guidelines for the Care and Use of Laboratory Animals and were approved by the University Animal Experiment Committee.

2.2. AMPK and acetyl–coenzyme A carboxylase phosphorylation study

The rats were randomly assigned to pre (n = 12) and AICAR treatment (n = 36) groups. The rats of AICAR treatment group were then given a subcutaneous ingestion of AICAR (Toronto Research Chemicals, North York, Ontario, Canada; 1 mg/g body weight). The rats were anesthetized with pentobarbital sodium (60 mg/kg body weight IP), and the slow-twitch soleus and fast-twitch extensor digitorum longus (EDL) muscles were rapidly dissected out at 1 (n = 12), 2 (n = 12), and 4 (n = 12) hours after the AICAR treatment. The rats of the pre group were also anesthetized, and the soleus and EDL muscles were dissected out. The muscles were frozen in liquid nitrogen and stored at −80°C until determinations of phosphorylated and total AMPKα and acetyl–coenzyme A carboxylase (ACC) protein expression were performed.

A lysis buffer was used to inhibit phosphatases and determine the phosphorylated AMPK and ACC protein levels as well as total AMPKα and ACC (50 mmol/L HEPES, 0.1% Triton X-100, 4 mmol/L EGTA, 10 mmol/L EDTA, 15 mmol/L $Na_4P_2O_7$, 100 mmol/L β-glycerophosphate, 25 mmol/L NaF, 5 mmol/L Na_3VO_4, and 1 tablet per 50 mL Complete Protease Inhibitor Cocktail Tablets [Roche Diagnostics, Tokyo, Japan], pH 7.4). The muscle specimens were homogenized in ice-cold lysis buffer (1:10 wt/vol) with a Polytron-type homogenizer operating at maximum speed for 30 seconds. The homogenate was centrifuged at 15 000g (4°C) for 25 minutes. The protein concentration of the supernatant was then determined by use of a protein determination kit (Bio-Rad, Richmond, CA). The muscle protein homogenate was solubilized in sample loading buffer (50 mmol/L Tris-HCl, pH 6.8, 2% sodium dodecyl sulfate (SDS), 10% glycerol, 5% β-mercaptoethanol, and 0.005% bromophenol blue).

2.3. SIRT1, PGC-1α, and GLUT4 proteins and hexokinase activity study

The rats were randomly assigned to pre (n = 12), AICAR treatment (n = 48), and saline treatment (n = 12) groups. The rats of AICAR treatment group were then given a subcutaneous ingestion of AICAR (1 mg/g body weight). The rats were anesthetized with pentobarbital sodium (60 mg/kg body weight IP); and then the soleus and EDL muscles were rapidly dissected out at 6 (n = 12), 12 (n = 12), 18 (n = 12), and 24 (n = 12) hours after the AICAR treatment. The rats of pre group were also anesthetized, and the muscles were dissected out. In the rats of saline treatment group, a comparable volume of saline was administered subcutaneously. The rats were anesthetized, and the muscles were dissected out at 24 hours after the saline injection. The muscles were frozen in liquid nitrogen and stored at −80°C until analyses were performed.

The frozen samples were homogenized with homogenizer in ice-cold homogenizing buffer (1:10 wt/vol) (25 mmol/L HEPES, 250 mmol/L sucrose, 2 mmol/L EDTA, 0.1% Triton X-100, and 1 tablet per 50 mL Complete Protease Inhibitor Cocktail Tablets [Roche Diagnostics], pH 7.4). The homogenate was centrifuged at 15000*g* (4°C) for 25 minutes. The protein concentration of the supernatant was determined by the use of a protein determination kit (Bio-Rad). The muscle homogenate was used for Western blotting to determine the SIRT1, PGC-1α, and GLUT4 protein contents and hexokinase activity. For Western blotting, the muscle protein homogenate was solubilized in sample loading buffer as described above.

2.4. Gel electrophoresis and Western blotting

The proteins (20 μg) of these homogenates were separated by SDS polyacrylamide gel electrophoresis using 5% (phospho- and total ACC), 7.5% (SIRT1 and PGC-1α), and 10% (GLUT4 and phospho- and total AMPKα) resolving gels. The proteins separated by SDS polyacrylamide gel electrophoresis were then electrophoretically transferred onto the polyvinylidene difluoride membrane. The membrane was incubated with a blocking buffer of casein solution (SP-5020; Vector Laboratories, Burlingame, CA) for 1 hour at room temperature. The membrane was reacted with affinity-purified rabbit polyclonal antibody to phospho-AMPKα (Thr^{172}; 1:500 dilution, #2532, Cell Signaling, Beverly, MA), total AMPKα (1:1000 dilution, #2531S, Cell Signaling), phospho-ACC (Ser^{79}; 1:500 dilution, #3661, Cell Signaling), total ACC (1:500 dilution, #3662, Cell Signaling), Sir2 (1:1000 dilution, #07-131, Upstate Biotechnology, Lake Placid, NY), PGC-1α (1:500 dilution, AB3242, Chemicon International, Temecula, CA), or GLUT4 (1:8000 dilution, AB1346, Chemicon International) overnight at 4°C and then was incubated with biotinylated anti-rabbit/mouse immunoglobulin G (1:1000 dilution, BA-1400, Vector

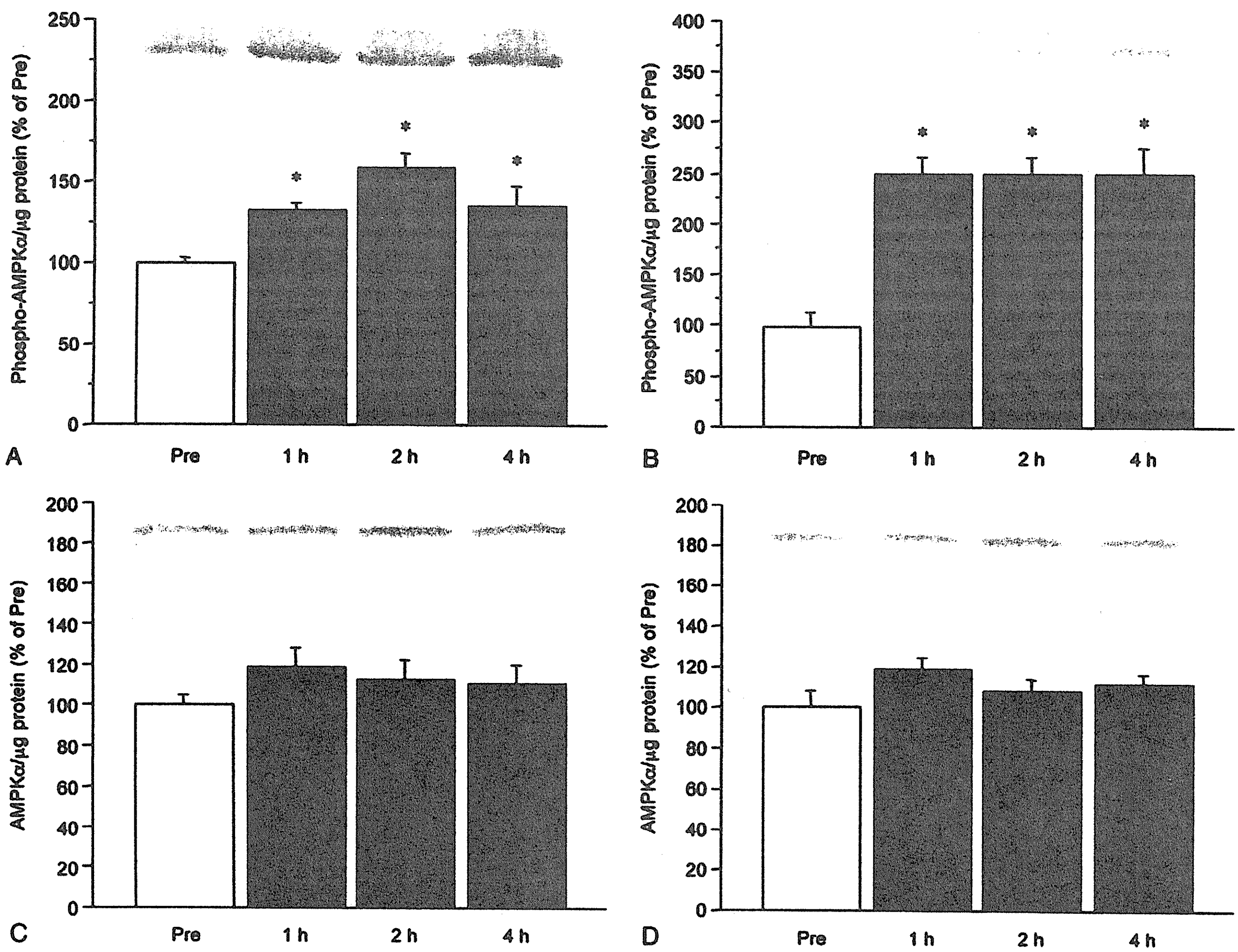

Fig. 1. Phospho- and total AMPKα protein expression in the soleus and EDL muscles before and 1, 2, and 4 hours after AICAR treatment. A and B, Phospho-AMPKα in soleus and EDL muscles, respectively. C and D, Total AMPKα in soleus and EDL muscles, respectively. Values are the means ± SE; n = 12 muscles per group. *$P < .05$ vs pre.

Laboratories) for 30 minutes. The band on the membrane was visualized by avidin and biotinylated horseradish peroxidase macromolecular complex technique (PK-6100, Vector Laboratories). The band densities were determined using the Image 1.62 software package (National Institute of Health, Bethesda, MD).

2.5. Hexokinase activity

The hexokinase activity was measured spectrophotometrically. The enzymatic assay was carried out at 30°C using saturating concentrations of substrates and cofactors as determined in preliminary analyses. The hexokinase activity was measured at 340 nm by following the production of reduced form of beta-nicotinamide adenine dinucleotide phosphate (NADPH) for 3 minutes. The extinction coefficient for NADPH, which is a reference of the hexokinase activity, was 6.22. For the hexokinase assay, 100 mmol/L Tris-HCl, 0.4 mmol/L beta-nicotinamide adenine dinucleotide phosphate (NADP), 5 mmol/L $MgCl_2$, 700 U/mL glucose-6-phosphate dehydrogenase, 1 mmol/L glucose (omitted for the measurement of nonspecific activity), and 5 mmol/L adenosine triphosphate (omitted for the measurement of nonspecific activity), pH 7.0, were used.

2.6. Statistical analysis

All data are expressed as the means ± SE. To estimate the time course of the protein expressions and hexokinase activity with AICAR treatment, we used the 1-way analysis of variance. Dunnett post hoc test was conducted if the analysis of variance indicated a significant difference. The unpaired *t* test was used to compare the saline and AICAR groups. A value of $P < .05$ was considered to be significant.

3. Results

3.1. AMPK and ACC protein phosphorylation

Fig. 1 shows the change in the phosphorylated and total AMPKα protein expression after an AICAR treatment. In the

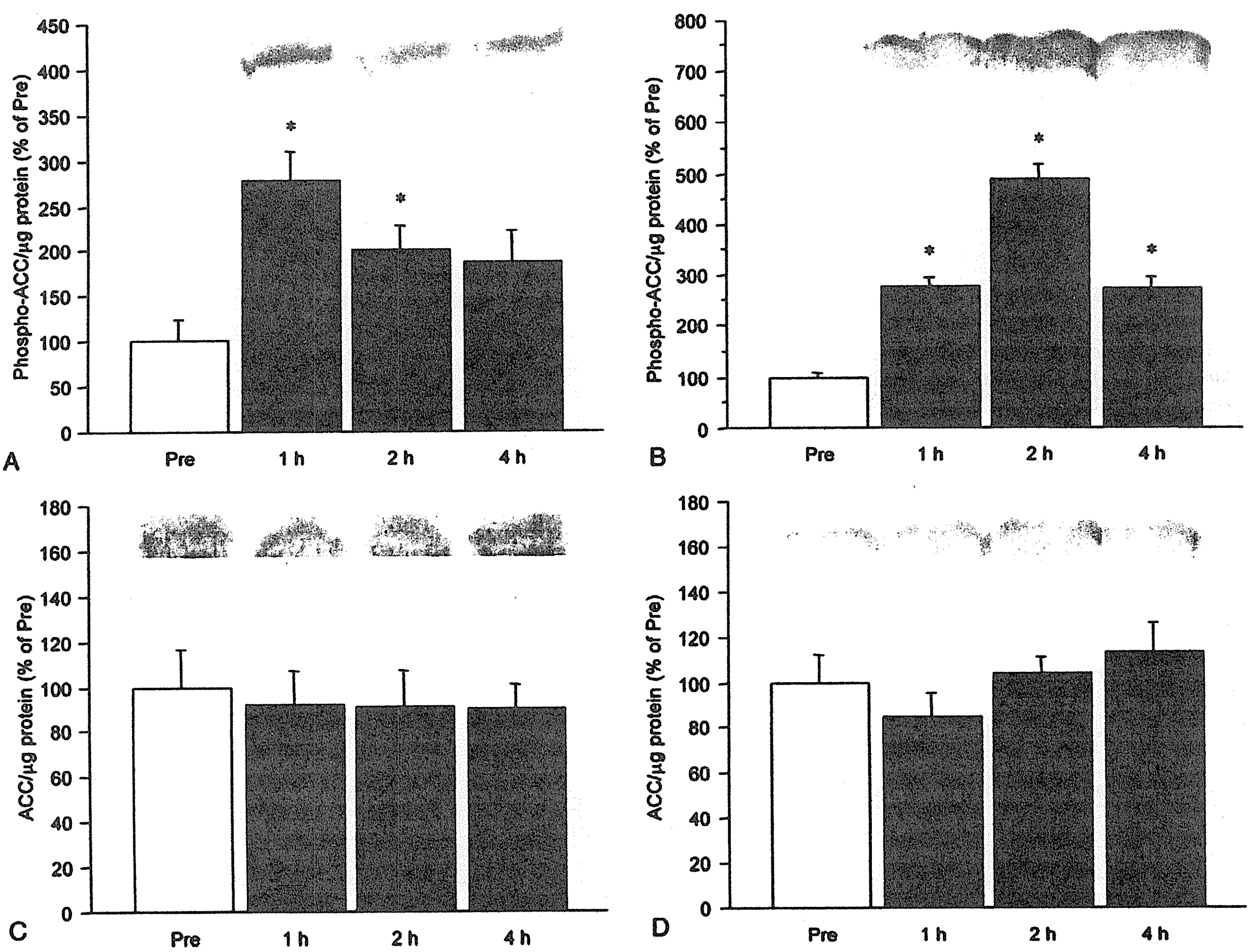

Fig. 2. Phospho- and total ACC protein expression in soleus and EDL muscles before and 1, 2, and 4 hours after AICAR treatment. A and B, Phospho-ACC in soleus and EDL muscles, respectively. C and D, Total ACC in soleus and EDL muscles, respectively. Values are the means ± SE; n = 12 muscles per group. *$P < .05$ vs pre.

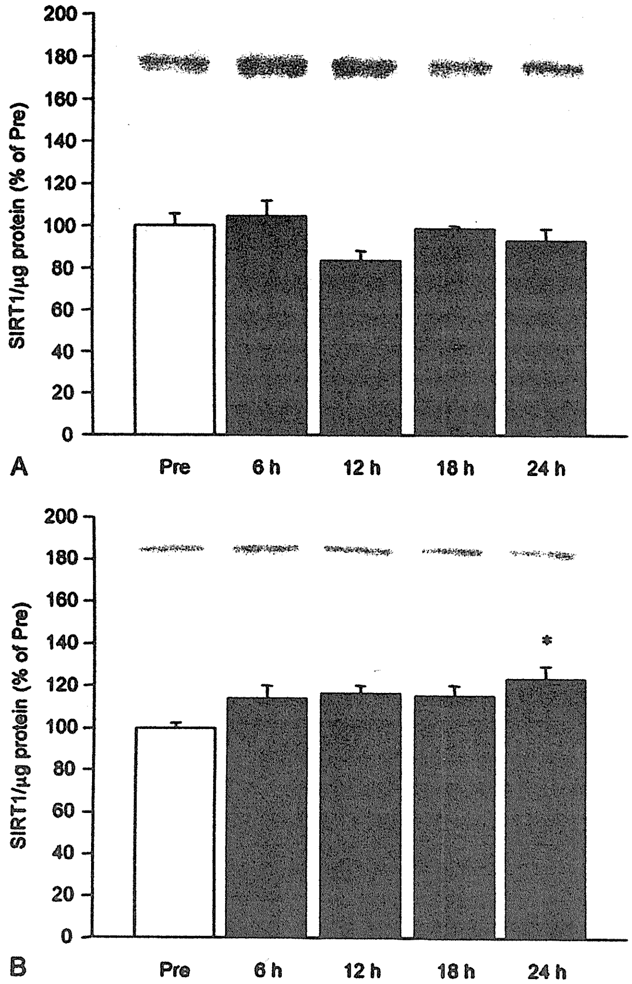

Fig. 3. SIRT1 protein expression in the soleus (A) and EDL (B) muscles before and 6, 12, 18, and 24 hours after AICAR treatment. Values are the means ± SE; n = 12 muscles per group. *$P < .05$ vs pre.

soleus muscle, the phosphorylated AMPKα protein increased at 1, 2, and 4 hours after the AICAR injection from the preinjection period (Fig. 1A; +32%, +59%, and +36%, respectively, from pre; $P < .05$). In the EDL muscle, the phosphorylated AMPKα protein also increased at 1, 2, and 4 hours after the AICAR injection from the preinjection period (Fig. 1B; +150%, +151%, and +150%, respectively, from pre; $P < .05$). Total AMPKα protein expression did not change in the soleus or EDL muscles (Fig. 1C, D).

The effect of AICAR was further examined on the phosphorylation of ACC, a downstream target of AMPK controlling the entry of fatty acids into mitochondrial matrix in skeletal muscle [21]. Fig. 2 shows the change in the phosphorylated and total ACC protein expression after an AICAR treatment. In the soleus muscle, the phosphorylated ACC protein increased at 1 and 2 hours after the AICAR injection from the preinjection period (Fig. 2A; +178% and +101%, respectively, from pre; $P < .05$). In the EDL muscle, the phosphorylated ACC protein also increased at 1, 2, and 4 hours after the AICAR injection from the preinjection period (Fig. 2B; +178%, +392%, and +173%, respectively, from pre; $P < .05$). Total ACC protein expression did not change in the soleus or EDL muscles (Fig. 2C, D).

3.2. SIRT1 protein expression

Fig. 3 shows the change in the SIRT1 protein expression after an AICAR administration. In the soleus muscle, no changes were observed after the treatment (Fig. 3A). In the EDL muscle, the SIRT1 protein increased (+24%) at 24 hours after the treatment from the pretreatment period (Fig. 3B, $P < .05$). In addition, the SIRT1 protein expression in the EDL muscle at 24 hours after the AICAR treatment was significantly higher than that in the saline treatment (Table 1, $P < .05$).

3.3. PGC-1α protein expression

Fig. 4 shows the change of the PGC-1α protein expression after an AICAR administration. The PGC-1α protein increased at 24 hours after an AICAR administration from the pretrial period in both the soleus (Fig. 4A) and EDL (Fig. 4B) muscles (+21% and +26%, respectively, from pre; $P < .05$). In addition, the PGC-1α protein expression in both the soleus and EDL muscles at 24 hours after the AICAR treatment was significantly higher than that in the saline treatment (Table 1, $P < .05$).

3.4. GLUT4 protein expression

Fig. 5 shows the change in the GLUT4 protein expression after an AICAR administration. In the soleus muscle, no changes were observed after the treatment (Fig. 5A). In the

Table 1
Skeletal muscle protein expression and hexokinase activity 24 hours after either saline or AICAR administration

	Soleus muscle		EDL muscle	
	Saline	AICAR	Saline	AICAR
SIRT1 (% of saline)	100.0 ± 1.8	104.1 ± 2.5	100.0 ± 6.2	117.6 ± 2.1*
PGC-1α (% of saline)	100.0 ± 6.0	116.3 ± 3.4*	100.0 ± 6.7	122.0 ± 8.1*
GLUT4 (% of saline)	100.0 ± 4.1	102.5 ± 5.9	100.0 ± 6.9	137.0 ± 5.8*
Hexokinase activity (μmol L^{-1} g^{-1} min^{-1})	2.02 ± 0.07	2.33 ± 0.07*	2.49 ± 0.07	3.45 ± 0.11*

Data are expressed as the mean ± SE; n = 12 muscles per group.
* $P < .05$ vs saline-treated group.

EDL muscle, the GLUT4 protein increased (+38%) at 24 hours after the treatment from the pretreatment period (Fig. 5B, $P < .05$). In addition, the GLUT4 protein expression in the EDL muscle at 24 hours after the AICAR treatment was significantly higher than that in the saline treatment (Table 1, $P < .05$).

3.5. Hexokinase activity

Fig. 6 shows the change in the hexokinase activity after an AICAR administration. In the soleus muscle, the hexokinase activity increased at 18 and 24 hours after an AICAR administration from the pretrial period (Fig. 6A; +12% and +12%, respectively, from pre; $P < .05$). In the EDL muscle, the activity increased at 12, 18, and 24 hours after an AICAR administration from the pretrial period (Fig. 6B; +24%, +36%, and +30%, respectively, from pre; $P < .05$). In addition, the hexokinase activity in both the soleus and EDL muscles at 24 hours after the AICAR treatment was significantly higher than that in the saline treatment (Table 1, $P < .05$).

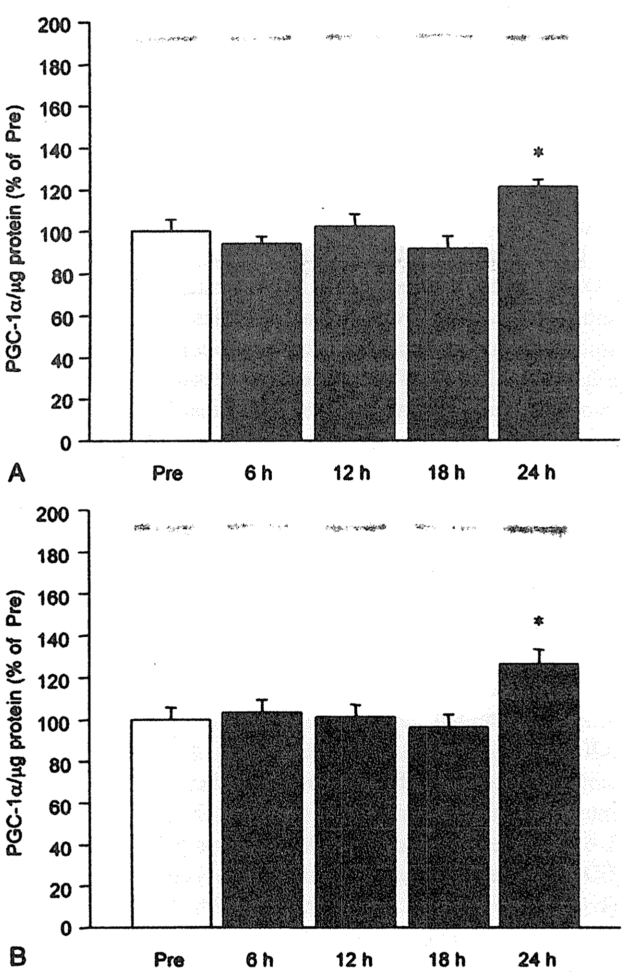

Fig. 4. PGC-1α protein expression in the soleus (A) and EDL (B) muscles before and 6, 12, 18, and 24 hours after AICAR treatment. Values are the means ± SE; n = 12 muscles per group. *$P < .05$ vs pre.

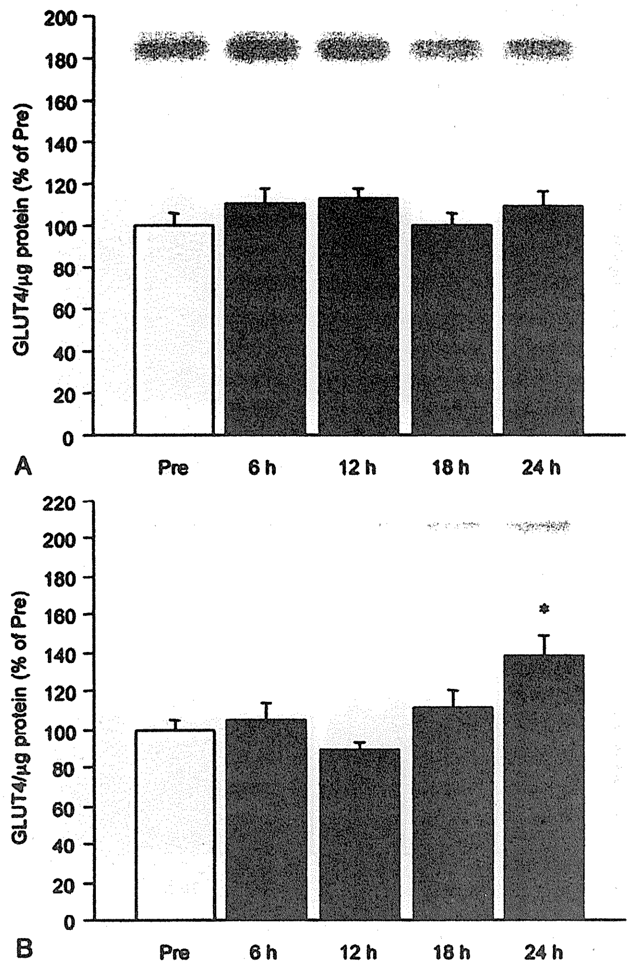

Fig. 5. GLUT4 protein expression in the soleus (A) and EDL (B) muscles before and 6, 12, 18, and 24 hours after AICAR treatment. Values are the means ± SE; n = 12 muscles per group. *$P < .05$ vs pre.

4. Discussion

The current study demonstrated that the activation of AMPK with AMPK activator AICAR treatment in vivo increases the SIRT1 protein expression in the rat EDL muscle. The AMPK phosphorylation level in human hepatoma cell line HepG2 is associated with the SIRT1 protein level [32]. Incubation of HepG2 cells in a high-glucose medium (25 mmol/L) decreases the phosphorylation of AMPK and its downstream target ACC with parallel decline of SIRT1 protein level in comparison to that in low-glucose medium (5 mmol/L). In contrast, incubation of HepG2 cells with pyruvate (0.1 or 1 mmol/L) increases the phosphorylation of AMPK and ACC and SIRT1 protein content. These results suggest that AMPK controls SIRT1 protein content.

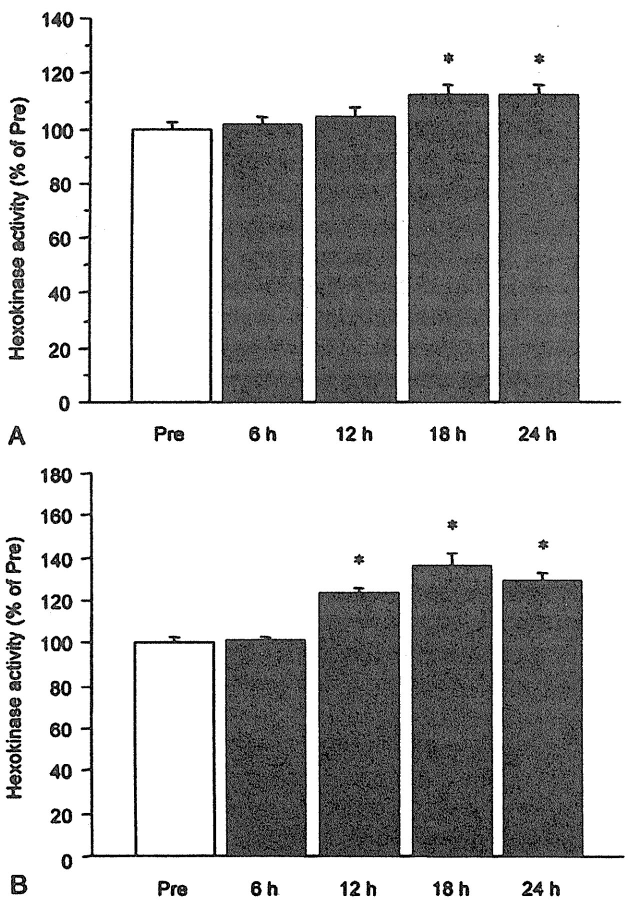

Fig. 6. Hexokinase activity in the soleus (A) and EDL (B) muscles before and 6, 12, 18, and 24 hours after AICAR treatment. Values are the means ± SE; n = 12 muscles per group. *$P < .05$ vs pre.

The effects of AICAR treatment to animals seem similar to those of endurance exercise training with regard to glucose uptake, mitochondrial fatty acid oxidation, and mitochondrial and GLUT4 biogenesis in skeletal muscle [10]. The endurance exercise increased the skeletal muscle SIRT1 protein expression [29]. Consequently, the results regarding SIRT1 in the current study further suggest that the AICAR treatment mimics the benefits of endurance exercise. In skeletal muscle cells, SIRT1 plays an important role in metabolic adaptations including mitochondrial biogenesis, fatty acid oxidation, and glucose homeostasis through deacetylation of PGC-1α [7-9]. Collectively, these observations raise the possibility that the AMPK-SIRT1-PGC-1α pathway may, in part, contribute to the metabolic adaptations with endurance exercise training in skeletal muscle.

However, AMPK may not be the only way to regulate the SIRT1 expression with exercise. The ablation of the AMPK activity experiments using AMPK dominant negative or AMPKα2 knockout mice models demonstrates that AMPK is not always essential for the regulation of downstream targets including ACC, fatty acid oxidation, mitochondrial biogenesis, or the glucose metabolism [33-35], thus suggesting that the redundant signaling pathways cooperate with AMPK in many kinds of adaptations and that signaling other than AMPK may compensate for such metabolic characteristics in the AMPK ablation state. To elucidate the mechanisms, other than AMPK, which regulate the SIRT1 expression with exercise, further experiments using AMPK ablation animal models subjected to various types of exercise are thus called for.

The mechanisms underlying the increase of SIRT1 protein content with AICAR treatment are unclear at present. One potential mechanism for this phenomenon is that nitric oxide synthase (NOS) mediates the SIRT1 expression after an AICAR treatment. The AMPK-induced skeletal and cardiac muscle glucose uptake depends on NOS [36]. In addition, AMPK seems to enhance the NOS activity and phosphorylation of endothelial NOS at Ser^{1177} [36,37]. The level of expression and phosphorylation of endothelial NOS is associated with SIRT1 expression in endothelial cells [38,39]. Furthermore, long-term treatment of NOS inhibitor N^G-nitro-L-arginine-methyl ester decreases the skeletal muscle SIRT1 protein content (M Suwa and S Kumagai, unpublished observation). Overall, it is likely that increasing SIRT1 protein expression with AICAR treatment is mediated by NOS. However, other studies have demonstrated that NOS inhibition does not affect the AICAR- or contraction-induced glucose uptake in rat skeletal muscle [40,41]. Further studies are necessary to clarify the mechanisms in the increase of skeletal muscle SIRT1 dependent on NOS after AMPK activation.

In the current study, the SIRT1 protein expression in the EDL muscle increased with AICAR treatment but not in the soleus. In addition, other characteristics examined in this study indicate inconsistent results between EDL and soleus muscles. The GLUT4 protein expression significantly increased with AICAR in the EDL muscle but not in the soleus muscle. In the hexokinase activity, AICAR treatment also seems more effective to the EDL than soleus muscle. The increase of AMPK phosphorylation level with AICAR in the EDL (~+150% from pre) seems greater than that in soleus (+32%-59% from pre) as well as ACC phosphorylation level (EDL, +173%-391%; soleus, +89%-179%; from pre), raising the possibility that such difference in the effect of AICAR against the AMPK phosphorylation partially causes the different results between soleus and EDL muscles. Another potential cause for such differences in regard to AICAR treatment is the difference in the AMPK subunit isoform distribution between muscle fiber types. The soleus muscle possesses dominantly slow-twitch type I fibers (type I, 84%; type IIA, 7%; type IIX, 9%; type IIB, 0%), whereas EDL muscle possesses dominantly fast-twitch type II fibers (type I, 4%; type IIA, 20%; type IIX, 38%; type IIB, 38%) in rats [42]. In rodents, the γ3-subunit of AMPK is dominantly expressed in the fast-twitch muscle in comparison to the slow-twitch muscle [43]. The γ3-containing AMPK complexes contain only α2- and β2-subunits [43], thus suggesting that α2/β2/γ3 heterotrimer preferentially expressed in the fast-twitch muscle. Because α2- and β3-subunits play an important role for metabolic and contractile properties in skeletal muscle [44-46], it is likely that the different effects between soleus and EDL muscles on AMPK activation observed in this study are, at least in part, attributable to such differences in the subunit expression pattern between muscle fiber types.

The current study demonstrated that short-term AICAR treatment to rats promotes the skeletal muscle SIRT1 protein expression. On the other hand, a previous study has shown that long-term AICAR treatment to rats for 5 successive days decreases (white gastrocnemius and red and white tibialis anterior muscles) or fails to change (heart and red gastrocnemius muscles) the SIRT1 protein expression [47]. In addition, AICAR treatment for 14 successive days does not alter the SIRT1 protein expression in the rat red and white gastrocnemius muscles (M Suwa and S Kumagai, unpublished observation). These observations suggest that the effect of AICAR treatment on SIRT1 protein expression may thus differ depending on the treatment period. The SIRT1 transcription is regulated by the transcriptional factors E2F transcriptional factor 1 and hypermethylated in cancer 1 [48]. SIRT1 binds to these transcriptional factors, and the complexes repress its transcription [49,50]. This negative feedback loop in SIRT1 regulation might be at least partially associated with the inconsistent results observed among the different treatment period.

Although several previous studies have demonstrated that long-term AICAR treatment enhances the PGC-1α and GLUT4 protein expression and hexokinase activity in the skeletal muscles of rodents in vivo [23,24], the present study is the first to demonstrate that short-term administration of AICAR to rats also promotes them. These results suggest that only a single AICAR treatment is sufficient to promote such phenotypes. Previous studies have demonstrated that short-term endurance exercise augments the PGC-1α and GLUT4 expression and the hexokinase activity and expression [51-53]. These short-term exercise–induced changes may be at least partially associated with AMPK.

Several observations may explain the mechanisms in such changes with AICAR treatment. The PGC-1α and hexokinase II genes have a cyclic AMP–response element, and their transcription is thought to be controlled by the transcriptional factor cyclic AMP–response element binding protein [54-56]. The GLUT4 transcription is regulated by the transcriptional factors myocyte enhancer factor 2 and GLUT4 enhancer factor [57,58]. All these transcriptional factors are phosphorylated and/or transcriptionally activated by AMPK [55,59]. Presumably, such mechanisms are the possible causes for the increase in PGC-1α and GLUT4 expression and hexokinase activity with short-term AICAR treatment.

SIRT1 is associated with insulin sensitivity [7], insulin [60] and adiponectin [61] secretion, mitochondrial biogenesis, fatty acid oxidation [9], protection of neurodegenerative disorders, [62], and longevity [7]. The current study contributes to the understanding of the role of AMPK in the regulation of SIRT1 protein expression and further supports the strategies aimed to activate AMPK as a means of improving the outcome of chronic diseases.

In summary, these results show that short-term AMPK activator AICAR treatment to rats enhances the skeletal muscle AMPK and ACC phosphorylation and then coincidently increases the SIRT1 protein expression. The PGC-1α and GLUT4 protein expression and hexokinase activity also increases with AICAR treatment. Some of these changes preferentially occur in fast-twitch EDL muscles. Therefore, the observations in this study may provide new insights into the mechanisms of SIRT1 regulation and thereby help in both the prevention of and therapy for some chronic diseases including insulin resistance, type 2 diabetes mellitus, metabolic syndrome, and neurodegenerative disorders.

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高齢者における膝痛の強度と罹患側の違いがメンタルヘルスに及ぼす影響

Effects of differences in level of knee pain and affected side on mental health in elderly

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要旨：【目的】本研究の目的は，膝痛有訴者の諸特性について調査し，さらに膝痛の強度と罹患側の違いがメンタルヘルスに及ぼす影響について検討することである。【方法】本研究は，65歳以上の自立高齢者750名を対象とし，膝痛，メンタルヘルス（うつ，quality of life（QOL）および認知機能），運動機能，喫煙習慣および社会経済的要因を調査した。そして，膝痛の強度と罹患側の違いからみた諸特性を男女で比較検討した。【結果】女性のみ，右膝痛有訴者で弱群と比較して中等度～強群の方が有意に身体的QOLおよび認知機能の得点が低かった。男女ともに，膝痛の強度と罹患側の違いでうつの評価尺度であるCES-D得点に有意差は認められなかった。【結論】女性は膝痛の強度と罹患側の違いでメンタルヘルスへの影響が異なる可能性が示唆された。

キーワード：膝痛，罹患側，メンタルヘルス

Abstract: [Purpose] This study explored the characteristics of elderly patients with knee pain and examined the effects of differences in the level of knee pain and affected side on patients' mental health. [Methods] The study examined knee pain, mental health (depression, quality of life (QOL), cognitive functioning), physical functioning, smoking habits, and socioeconomic factors involving 750 elderly subjects aged over 65. The obtained characteristics were compared between male and female subjects. [Results] Female patients with moderate to severe knee pain showed significantly lower physical QOL and cognitive function scores. No significant correlation was observed between the CES-D score and differences in the level of knee pain and affected side in both genders. [Con-

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clusion] The results suggest that the level of knee pain and affected side have different effects on the mental health of female patients.

Key words: Knee pain, affected side, mental health

Ⅰ．緒　言

運動器疾患の中でも膝痛は高齢者において高い有訴率であることが諸外国で報告されている[1]。我が国においても，大規模コホート調査(ROAD: Research on osteoarthritis against disability）により，膝痛の有訴者が800万人を超えることが報告されている[2]。高齢者では膝痛は一般的に変形性関節症（OA: Osteoarthritis）に起因しており，臨床での診断はしばしばＸ線上でのOA所見に基づいて行われるが，Ｘ線所見と臨床症状との間の不一致性が指摘されている[1]。特に，女性ではＸ線所見に関係なく膝痛の有訴率が高いことから[2]，メンタルヘルスとの関連性が考えられる。

膝痛に関する疫学研究では，心理的健康状態[3]，主観的健康観[4]，うつ[5]などのメンタルヘルスや喫煙[6]，肥満[6]，低い教育歴[7]，低い社会経済状態[5]などの様々な生活習慣および社会経済的要因との関連性が報告されている。一方，膝痛と認知機能との関連性を報告した研究は数少ない。

疼痛は，感覚・識別的側面と情動・認知的側面とを含む多面的要素が混在していると考えられるが，特に慢性疼痛における情動・認知的側面への影響が問題視されており，慢性疼痛とメンタルヘルスとの関連性が数多く報告されている[8]。

左右大脳半球の機能的差異に関する知見によれば，古典的には精神分析や神経心理学の分野が知られているが，右半球は空間認知などの全体的／同時的な情報処理に，左半球は書字，計算などの分析的／順次的情報処理と関連するとされている[9]。一般的に，侵害刺激は身体刺激側とは反対側の脳領域で情報処理されることから[10]，罹患側と脳機能への影響には左右差が存在することが推察されるが，我々の知る限り，膝痛有訴者において疼痛の強度と罹患側の違いがメンタルヘルスに及ぼす影響を検討した研究はない。

そこで我々は，まず膝痛有訴者の諸特性について調査し，次に膝痛の強度と罹患側の違いがメンタルヘルスに及ぼす影響について検討することとした。

Ⅱ．研究方法

1. データ収集と対象者

本研究は，福岡県太宰府市（人口約69,000人，高齢化率20.8％：2009年，男女比率１：1.10，全国高齢化率22.1％：2008年，男女比率１：1.10）において2009年と2010年の８～12月に行った測定会のデータを用いた横断的研究である。対象者は，全44地区を年齢と性別で層別化し，それぞれの層から太宰府市全体の高齢化率，男女比率とほぼ一致した５地区に住む，2009年４月時点での65歳以上の全住民2,166名とした。そのうち要介護認定者，死亡，施設入所，転居，入院および調査を拒否した者（358名）などを除外し（戸別訪問，電話確認），アンケート調査ならびに認知機能・体力測定会に参加し，欠損データが認められなかった自立高齢者750名（参加率：41.4％）とした（男性；360名，48％，女性；390名，52％）。測定は，地区公民館において，保健師，理学療法士および健康運動実践指導士などの管理下で行った。本研究は，九州大学健康科学センター倫理委員会での審査，承認を得て実施され，参加者に研究の主旨を説明し，書面による同意を得た後に実施した。

2. 調査内容

1）形態測定

調査項目として，身長，体重（体重体組成計；オムロン社製，HBF-361），BMI（Body mass index），握力（スメドレー握力計）を測定した。また，利き手も問診にて確認した。

2）喫煙習慣と社会経済的状況

喫煙習慣は，喫煙の有無を確認した。教育歴は，これまでに受けた教育すべての就学年数を尋ね，その合計値を算出した。世帯所得は，同居内家族全体の１ヶ月当たりの合計収入（税込み）を最低３万円未満から最高80万以上までの５万円刻みで17分割した項目から対象者に選択してもらった。次に得られた結果を第１三分位数で２群に分類した（25万/月未満群，25万/月以上群）。国民生活基礎調査（平成19年）によると高齢者世帯の総所得平均は298.9万円（月24.9万円）とある。本研究では，第１三分位数が国民生活基礎調査

の結果と近似していたため，第1三分位数で2群に分類することとした。

3）膝痛

（1）膝痛有訴者

「過去1ヶ月で膝に疼痛がありましたか」[11]という問いに対して，“ある”と解答した者には「普段生活している中で，右膝もしくは左膝の痛みはだいたいどれくらいですか」と尋ね，視覚的アナログスケール（VAS: Visual analog scale）を用いて疼痛の程度を評価した。左右膝痛の分類は，両膝に疼痛を訴えた者はVASにて高値の方を罹患側とした。VASの値が同値であった21名は，本対象から除外した。膝痛強度での分類は，男女別にVAS値の平均値から中等度～強群と弱群の2群に分類した（男性：右膝43.9㎜，左膝40.4㎜，女性：右膝40.4㎜，左膝45.2㎜）。Collinsら[12]はVAS値の強度別分類について，4区分（なし群，弱群，中等度群および強群）の中で，中等度群はVAS平均値49㎜（標準偏差±17），境界値30㎜で，強群はVAS平均値75㎜（標準偏差±18），境界値54㎜であることを報告している。本研究では，男女の両膝ともにVASの平均値がCollinsらの報告の中等度群に近似していたため，膝痛の分類に平均値を用いた。

（2）治療歴と手術歴

治療（服薬，注射およびリハビリテーションなどを含む）の有無と手術（人工関節，関節鏡などを含む）の有無を自記式質問紙にて確認した。

4）メンタルヘルス

メンタルヘルスにはNegative Mental Health（うつなど）とPositive Mental Healthがあり，後者はまだグローバルスタンダードな評価尺度ではないが，QOL評価を用いることが多い[13]。さらに近年，うつ，QOLおよび認知機能との関連性を示した研究が散見される[14,15]。そこで，本研究ではメンタルヘルスとして，うつ，QOLおよび認知機能を測定することとした。

（1）うつ

うつは，CES-D（Center for epidemiological studies depression Scale）[16]を用いて評価した。CES-Dは一般人におけるうつ病をスクリーニングするための質問紙であり，米国国立精神保健研究所により開発されたものである。我が国においても，CES-D日本語版の妥当性・信頼性が確認されている[17]。

（2）QOL

QOLは，日本語版WHO-QOL26[18]を用いて評価した。日本語版WHO-QOL26は身体的領域・心理的領域・社会的関係・環境領域の4領域の24項目と，全体を問う2項目を加えた26項目から構成されている。本研究では，膝痛有訴者と非有訴者との比較以外は，膝痛の罹患側の違いに伴う身体的な認知の影響を検討するために身体的QOLを用いた。結果は，各項目と全項目でそれぞれ平均値を算出した。

（3）認知機能

認知機能は，ファイブコグテスト[19]を用いて測定した。ファイブコグテストは認知症に関連した5つの認知機能を調べるテストであり，「記憶機能」，「注意機能」，「言語機能」，「視空間機能」および「思考機能」が含まれている。上記課題を年齢，教育年数および性別を調整した後に得点化し，その偏差値をそれぞれランク1から3までに区分し判定した。

5）日常生活動作

日常生活動作は，手段的日常生活動作（IADL: Instrumental activity of daily living）を自記式質問紙[19]にて測定した。

6）運動機能

椅子からの立ち上がり測定は，30秒間で何回できるか確認した。5m歩行速度は，5m歩行速度をストップウォッチにて測定した。

7）統計解析

解析対象者は，膝痛有訴者と非有訴者との比較以外は，脳機能の特異性を考慮して，全て右利きとした。統計解析は，各調査項目を従属変数とし，t検定ならびにWilcoxonの順位和検定を用い，カテゴリー変数についてはχ^2検定を行った。有意水準は危険率5％未満とした。統計ソフトにはSAS（Var9.2）を用いた。

Ⅲ．結　果

1. 膝痛有訴者と膝痛非有訴者での諸特性の比較

膝痛有訴率は，33.6％（252名）であった。膝痛非有訴者と比較して，膝痛有訴者は有意に女性が多く（$p<0.0001$），BMI高値（$p<0.01$），少ない喫煙者（$p<0.05$），低いQOL（$p<0.0001$），高いCES-D得点（$p<0.01$），低いIADL（$p<0.05$），少ない立ち上がり回数（$p<0.05$），遅い歩行速度（$p<0.01$）であった（表1）。

2. 膝痛の罹患側の違いからみた諸特性の男女比較

膝痛有訴率は，男性が右膝痛46％（36名），左膝痛54％（43名），女性が右膝痛60％（91名），左膝痛40％

表1 膝痛有訴者と膝痛非有訴者での諸特性の比較

	膝痛非有訴者	膝痛有訴者	p
n	498 (66.4%)	252 (33.6%)	
年齢（歳）a)	72.5 (5.8)	73.3 (6.1)	
性別，女性（%）	223 (44.8%)	167 (66.3%)	***c)
BMIa)	22.8 (2.9)	23.5 (3.2)	**d)
喫煙者（%）	159 (32%)	61 (24.4%)	*c)
教育歴（年）a)	12.1 (2.5)	11.9 (2.8)	
世帯所得，25万／月未満群（%）（vs25万／月以上群）	85 (19%)	52 (23.7%)	
QOL（点）a)	3.6 (0.5)	3.4 (0.5)	***d)
身体的	3.8 (0.5)	3.5 (0.6)	***
心理的	3.6 (0.6)	3.5 (0.6)	*
社会的	3.5 (0.5)	3.5 (0.5)	
環境的	3.6 (0.5)	3.4 (0.5)	***
全体的	3.4 (0.6)	3.2 (0.6)	***
CES-D（点）b)	4（1～10）	6（2～12）	**e)
認知機能（点）b)	15 (13～15)	15 (14～15)	
IADL（点）b)	14 (13～15)	13 (12～15)	*e)
椅子からの立ち上がり（回）a)	18.8 (5.5)	17.8 (6.1)	*d)
5m歩行速度（秒）a)	2.9 (0.8)	3.3 (1.2)	**d)

BMI; Body mass index, QOL; Quality of life, IADL: Instrumental activity of daily living
CES-D; the Center of epidemiologic studies depression Scale
a)平均値±標準偏差，b)中央値（四分位範囲），c)χ^2検定，d)対応のないt検定
e)Wilcoxonの順位和検定，*p<0.05 **p<0.01 ***p<0.0001

表2 膝痛の罹患側の違いからみた諸特性の男女比較

	男性		p	女性		p
	右膝痛	左膝痛		右膝痛	左膝痛	
n	36 (46%)	43 (54%)		91 (60%)	61 (40%)	
年齢（歳）a)	74.7 (6.8)	72.2 (5.0)		73.8 (6.3)	73.1 (6.5)	
教育歴（年）a)	12.1 (2.0)	12.2 (2.1)		11.6 (2.0)	11.7 (2.1)	
BMIa)	23.9 (3.7)	23.4 (2.75)		22.9 (3.3)	24.6 (3.2)	
VAS（mm）a)	43.9 (25.3)	40.4 (24.0)		40.4 (23.3)	45.2 (29.0)	*c)
治療歴（%）	25 (42%)	20 (46.5%)		45 (49.4%)	28 (46%)	
手術歴（%）	2 (5.5%)	1 (2.3%)		5 (5.5%)	4 (6.5%)	
身体的QOL（点）a)	3.5 (0.5)	3.4 (0.4)		3.6 (0.5)	3.4 (0.4)	
CES-D（点）b)	3.3（1～10）	6.3（2～14）		7（3～12）	9.5（2～15）	
認知機能（点）b)	15 (13～15)	15 (13.5～15)		14.5 (13～15)	15 (13～15)	
IADL（点）b)	14 (12～15)	14 (12～15)		13 (12～15)	13 (12～15)	
椅子からの立ち上がり（回）a)	19.3 (8.1)	19.1 (5.8)		17.8 (5.0)	16.5 (5.8)	
5m歩行速度（秒）a)	2.8 (0.7)	2.9 (0.9)		3.5 (1.2)	3.5 (1.1)	

BMI; Body mass index, VAS; Visual analog scale, QOL; Quality of life
CES-D; the Center of epidemiologic studies depression Scale, IADL: Instrumental activity of daily living
a)平均値±標準偏差，b)中央値（四分位範囲），c)対応のあるt検定，*p<0.05

（61名）であった。女性のみ右膝痛有訴者と比較して，左膝痛有訴者は有意にVASが高値（p<0.05）であった。その他に有意差は観察されなかった（表2）。

3. 膝痛の強度と罹患側の違いからみた諸特性の男女比較

男女ともに，両膝で弱群と比較して中等度～強群の方がVASは高値であった（p<0.01）。女性のみであるが，右膝痛有訴者で弱群と比較して中等度～強群の方が有意に身体的QOL（p<0.05）および認知機能（p<0.01）の得点が低かった。男女ともに，膝痛の強度と罹患側の違いでCES-D得点に有意差は認められなかった（表3，4）。

表3　男性における膝痛の強度と罹患側の違いからみた諸特性の比較

	右膝痛		p	左膝痛		p
	中等度～強群	弱群		中等度～強群	弱群	
n	19	17		18	25	
年齢（歳）a)	75.5 (6.7)	73.8 (6.9)		70.5 (4.6)	73.9 (5.3)	*c)
教育歴（年）a)	12.2 (2.1)	12.1 (2.0)		12.2 (2.2)	12.1 (2.1)	
BMIa)	23.2 (2.4)	24.6 (2.6)		23.0 (1.9)	23.8 (3.6)	
VAS（mm）a)	63.6 (18.2)	30.7 (17.3)	**c)	60.4 (17.2)	29.4 (17.0)	**c)
身体的QOL（点）a)	3.6 (0.3)	3.5 (0.5)		3.3 (0.4)	3.5 (0.4)	
CES-D（点）b)	3.5 (1.0～10.0)	3 (1～7)		8.5 (2.0～14)	4 (2～10)	
認知機能（点）b)	15 (14～15)	15 (13～15)		15 (13.5～15)	15 (14～15)	
IADL（点）b)	15 (14～15)	15 (14～15)		15 (13～15)	15 (14～15)	
椅子からの立ち上がり（回）a)	19.3 (8.1)	19.2 (8.1)		19 (6.2)	19.1 (5.3)	
5m歩行速度（秒）a)	2.9 (0.7)	2.7 (0.6)		2.8 (0.6)	3.0 (1.2)	

右膝痛：中等度～強群；平均値43.9（標準偏差±25.3）mm以上，弱群；平均値43.9（標準偏差±25.3）mm未満
左膝痛：中等度～強群；平均値40.4（標準偏差±24.0）mm以上，弱群；平均値40.4（標準偏差±24.0）mm未満
BMI; Body mass index, QOL; Quality of life, CES-D; the Center of epidemiologic studies depression Scale
IADL: Instrumental activity of daily living，a)平均値±標準偏差，b)中央値（四分位範囲），c)対応のあるt検定，*$p<0.05$　**$p<0.01$

表4　女性における膝痛の強度と罹患側の違いからみた諸特性の比較

	右膝痛		p	左膝痛		p
	中等度～強群	弱群		中等度～強群	弱群	
n	40	51		29	32	
年齢（歳）a)	74.3 (6.4)	73.3 (6.2)		73.7 (6.3)	72.5 (6.6)	
教育歴（年）a)	11.1 (2.0)	12.1 (2.0)		11.2 (2.1)	12.1 (2.1)	
BMIa)	23.5 (3.8)	22.3 (2.9)		24.5 (2.6)	24.7 (3.7)	
VAS（mm）a)	66.7 (17.6)	30.2 (16.8)	**c)	71.6 (19.2)	35.7 (18.3)	**c)
身体的QOL（点）a)	3.4 (0.6)	3.7 (0.5)	*c)	3.4 (0.4)	3.4 (0.5)	
CES-D（点）b)	8 (3～12)	6 (3～11)		12 (3～15)	7 (2～12)	
認知機能（点）b)	14 (13～15)	15 (14～15)	**d)	14.5 (13～15)	15 (14～15)	
IADL（点）b)	13 (13～14)	13 (12～15)		13 (12～15)	12 (12～14)	
椅子からの立ち上がり（回）a)	15.8 (4.2)	18.2 (5.7)		16.1 (6.1)	16.9 (5.5)	
5m歩行速度（秒）a)	3.6 (1.3)	3.3 (1.0)		3.7 (1.5)	3.2 (0.7)	

右膝痛：中等度～強群；平均値40.4（標準偏差±23.3）mm以上，弱群；平均値40.4（標準偏差±23.3）mm未満
左膝痛：中等度～強群；平均値45.2（標準偏差±29.0）mm以上，弱群；平均値45.2（標準偏差±29.0）mm未満
BMI; Body mass index, QOL; Quality of life, CES-D; the Center of epidemiologic studies depression Scale, IADL: Instrumental activity of daily living
a)平均値±標準偏差，b)中央値（四分位範囲），c)対応のあるt検定，d)Wilcoxonの順位和検定，*$p<0.05$　**$p<0.01$

Ⅳ．考　察

1．膝痛有訴者の諸特性について

本研究における膝痛有訴率は，33.6%（252名）であった。研究により膝痛の定義や測定方法に違いはあるが，諸外国における過去1ヶ月での膝痛有訴率が18～19.3%の範囲[20~22)]であることから比較すると，本研究では高い有訴率であることが判明した。同様な対象集団（60歳以上の地域在住高齢者）での我が国の報告では，過去1年で1ヶ月以上持続する膝痛の有訴率が32.8%であったとしている[2)]。また，多くの研究で，男性よりも女性の方が，有訴率が高いことが報告されている[2,20~22)]。以上より，本研究における膝痛有訴率は，邦人を対象とした有訴率と比較的一致しており，特に女性の有訴率は高い傾向にあった。

本研究では，膝痛非有訴者と比較して膝痛有訴者のBMIが高値であった。膝痛とBMIとの関連性に関する先行研究は，ほとんどの研究が膝痛の発生の危険因子の一つとして認めている[6)]。過体重は，荷重関節に力学的ストレスを生じることに加え，脂肪組織（adipose tissue）から分泌される炎症性サイトカインが関節軟骨代謝に影響を与える可能性が提起されている[23)]。

喫煙習慣について検討した結果，膝痛非有訴者と比較して膝痛有訴者の喫煙者が有意に少なかった。膝OAと喫煙との関連性に関する先行研究は，膝OAの発生に対してわずかに予防因子としての影響を示している[6)]。

社会経済的状況においては，膝痛有訴者と教育歴および世帯所得との関連性は観察されなかった。2,113

名の集団を対象とした20年間の縦断研究では，膝関節炎の発生に教育歴および収入との関連性はなかったとしており[5]，本研究と一致する結果となった。しなしながら，本研究との研究デザインの違いや収入の測定法が個人所得ではなく世帯所得であることの違いなどからも見解の一致をみておらず，更なる検討が必要である。

本研究では，膝痛非有訴者と比較して膝痛有訴者のCES-D得点が有意に高値であった。うつは，セロトニン，ノルエピネフリンおよびドーパミンなどの神経伝達物質の発現量低下もしくは神経化学的不均衡の結果であるとされる。また，疼痛は下行性疼痛抑制系により調整され，うつと同様の神経伝達物質により，侵害刺激に対して疼痛を抑制するとされている。そのため，疼痛とうつは合併率が高いものと考えられる[24]。本研究においても，膝痛有訴者は有意にCES-D得点が高値で，膝痛とうつとの関連性を示唆する結果であった。

認知機能での比較においては，膝痛有訴者と認知機能との関連性を認めなかった。先行研究では，線維筋痛症[25]，頸椎軟部組織損傷[26]および関節リウマチ[27]などの運動器疾患において，慢性疼痛者の注意機能，情報処理／精神運動速度および記憶の低下が観察されている。

2. 膝痛の強度と罹患側の違いがメンタルヘルスに及ぼす影響について

膝痛の罹患側の違いからみた諸特性の比較においては，女性のみ右膝痛有訴者と比較して，左膝痛有訴者が有意にVASは高値であった。QOL，うつおよび認知機能に関しては，膝痛の左右差は認められなかった。近年，PETやfMRIなどの画像診断の発展により左右大脳半球の機能的差異が明らかにされている。

疼痛の訴えが身体の右側よりも左側に多いことは先行研究において報告されている[28]。近年では，右半球の情動的側面の機能的優位性から，特に右扁桃体が重要な機能を有することが示唆されている[29]。また，うつ病患者の感情処理が右背外側前頭前野周囲の高い活動と関連することが報告され，うつが背外側前頭前野の異常な機能的非対称性と関連することが示唆されている[30]。慢性的局所疼痛症候群患者を対象に灰白質量を比較検討したところ，右島部，右腹内側前頭野および側坐核の灰白質が萎縮していたことが観察されている[31]。

左半球の認知的側面の機能的優位性に関する研究では，内言語を必要とするような課題では左半球の脳活動が大きく，注意と覚醒に関する課題では右半球の脳活動が大きいことが報告されている[32]。また，左扁桃体は感情の認知的制御と，右扁桃体は感情の自動的処理と関連することも観察されている[33]。さらに，左半球は自我や自己意識に関する機能の優位性が高いことから，左半球に脳腫瘍のある患者では，右半球と比較してQOLが低下している可能性が指摘されている[34]。

これらの知見から，罹患側と脳機能への影響には左右差の存在が推察されるものの，本研究では，QOL，うつおよび認知機能に膝痛の左右差は認められなかった。これは，情動的側面[29]や認知的側面[27]への脳機能の影響は疼痛強度に依存するとの報告から，疼痛強度とは独立して，罹患側の違いがメンタルヘルスに影響することは少ないことが示唆された。

膝痛の強度と罹患側の違いからみた諸特性の比較においては，女性のみに右膝痛有訴者で弱群と比較して中等度～強群が，有意に身体的QOLと認知機能の得点が低かった。しかし，CES-Dで評価されたうつ得点においては左右差が観察されなかった。

この背景として，本研究では膝痛を過去1ヶ月で評価しているが，うつは持続的な疼痛と関連する[35]ことからも，急性疼痛と慢性疼痛が混在していることが影響したのではないかと考える。

次に，性差が認められたことに関しては，疼痛の認知処理に関連する部位の活動レベルの差異が疼痛知覚の性差に影響することが観察されている[36]。特に，女性は不安，うつなどの情動・認知的側面に関連する脳活動レベルが高いのに対して，男性は識別的側面に関連する脳活動レベルが高いとされる[37]。事実，女性において心理的治療により，慢性疼痛や歩行障害，健康関連QOLが改善し，さらに鎮痛薬使用量が低下したことが報告されている[38]。このように，女性と男性では，侵害刺激に対する情報処理において活動部位の違いや特異的差異があり，女性の方が疼痛に対してより影響されやすいようである。これらの知見より，特に女性では右膝痛の強度に依存してメンタルヘルスに影響を及ぼすのかもしれない。

本研究の限界としては，以下の4点が考えられる。まず第1に，参加率が41.4%と低いことである。低い参加率は，他の集団に一般化できない可能性がある。第2に，本研究では膝痛の分類を疼痛強度の強い側で2群した点である。疼痛強度が脳機能に影響する点は

あるが，実際は単関節と多関節に疼痛がある場合での影響を比較していない。さらに，疼痛強度の分類に平均値を用いたことも結果に影響した可能性がある。第3に，本研究では，膝痛を過去1ヶ月で調査しているが，疼痛の持続期間を定めていないために，急性疼痛と慢性疼痛が混在している可能性を否定できない。第4に，本研究デザインは横断研究であるため，その因果関係が不明なことである。すなわち，メンタルヘルスへの影響は，膝痛が原因なのか結果なのか，もしくはその両方の影響を有する可能性が考えられる。今後は，標本数を増やすと共に，方法論の妥当性から同一対象とした縦断的調査および介入研究の必要性まで課題として残された。

Ⅴ. 結 論

本研究では，女性においてのみ，右膝痛有訴者で弱群と比較して中等度～強群の身体的 QOL と認知機能の得点が低いことが観察された。一方，膝痛強度と CES-D で評価されたうつとの関連性においては左右差が観察されなかった。これにより，女性は膝痛の強度と罹患側の違いでメンタルヘルスへの影響が異なる可能性が示唆された。

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