J Nutr Health Aging. 2018;

BERBERINE IMPROVES COGNITIVE DEFICIENCY AND MUSCULAR DYSFUNCTION VIA ACTIVATION OF THE AMPK/SIRT1/PGC-1A PATHWAY IN SKELETAL MUSCLE FROM NATURALLY AGING RATS

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Abstract: *Objective:* The manifestations of aging include cognitive deficits and muscular dysfunction, which are closely linked to impairment of mitochondrial biogenesis. Berberine, an isoquinoline alkaloid, presents multiple anti-diabetic pharmacological effects. Evidence has indicated that insulin resistance and cognitive impairment share the same pathogenesis, and berberine could reverse glucose metabolism abnormalities and muscle mitochondrial dysfunction induced by a high-fat diet. This study was used to investigate whether berberine could be used as an anti-aging drug to prevent cognitive deficits and muscular dysfunction in natural aging. *Methods:* Biochemical indicators and an intraperitoneal glucose tolerance test were tested in 5-month-old rats (5 mo group), 24-month-old rats (24 mo group) and 24-month-old rats that had undergone 6 months of berberine treatment (BBR group). A Morris water maze test was conducted to assess the cognitive ability of the rats. Insulin resistance in whole-body was evaluated by intraperitoneal glucose tolerance test (IPGTT). The morphology of the skeletal muscle tissue was observed by hematoxylin-eosin (HE) staining. The levels of total cholesterol, triglyceride, ATP and reactive oxygen species (ROS) were assessed with corresponding reagent kits. The protein expressions of GLUT4, AMPK, SIRT1 and PGC-1α in skeletal muscle were examined by Western blot. *Results:* The results showed that administration of berberine for 6 months significantly improved cognitive deficits and insulin resistance in naturally aging rats ($p<0.01$). Furthermore, berberine treatment helped normalize the disordered alignment and the decreased number of muscle fibers ($p<0.01$) in the skeletal muscle of 24 mo rats. Berberine decreased the levels of ROS in both the serum and the skeletal muscle of 24 mo rats ($p<0.01$). Berberine increased the protein expression of p-AMPK, SIRT1 and PGC-1α and increased the production of ATP in the skeletal muscle of aging rats ($p<0.01$). *Conclusions:* Berberine markedly ameliorates aging-related reductions in cognitive ability and muscular function, and the activation of the AMPK/SIRT1/PGC-1α pathway in skeletal muscle may be the underlying protective mechanism of berberine on muscular function.

Key words: Berberine, aging, cognitive deficits, skeletal muscle, insulin resistance, mitochondrial biogenesis.

Abbreviations: PGC-1α: proliferator-activated receptor γ coactivator 1-α; AMPK: adenosine monophosphate-activated protein kinase; SIRT1: sirtuin type 1; FBG: fasting blood glucose; T-CHO: total cholesterol; TG: triglyceride; BCA: bicinchoninic acid; RIPA: Radiommunoprecipitation Assay; PMSF: phenylmethanesulfonyl fluoride; mo: month-old; IPGTT: intraperitoneal glucose tolerance test; MWM: Morris water maze

Introduction

Aging is associated with a decline in physical function, which often manifests as loss of cognitive ability and muscular function. Cognitive ability declines gradually as a normal process of aging, and one of the most common cognitive changes is the decline of learning and memory ability (1). Structural and functional changes in skeletal muscle are another important manifestations of aging and usually start in the fourth decade of life (2, 3). Decreases in both muscle mass and fiber number are often observed during the aging process (4, 5). Furthermore, these alterations in skeletal muscle can generate and aggravate insulin resistance, type 2 diabetes and the aging process (6, 7). In view of the increased global aging population, taking effective measures for the improvements in cognitive ability and muscular function are meaningful for preventing the aging process.

Cognitive deficiency and muscular dysfunction are two key pathological manifestations in the course of senility. Additionally, in recent years, accumulating evidences have indicated that insulin resistance and cognitive impairment share the same pathogenesis (8, 9). Muscular dysfunction leads to insulin resistance in skeletal muscle and the whole-body. Compared with the insulin resistance caused by decreased insulin sensitivity of target tissue in the diabetic condition, the decline of mitochondrial function plays an important role in insulin resistance in the natural aging process (10). Skeletal muscle is a mitochondrion-rich tissue, accounting for approximately 80% of postprandial blood glucose uptake in normal state (11). Enhancing mitochondrial biogenesis in skeletal muscle facilitates the improvement of muscular strength, reduces insulin resistance, and even indirectly

Received August 16, 2017
Accepted for publication November 30, 2017

mitigates cognitive decline (12, 13). Mitochondrial biogenesis is considerably well regulated by peroxisome proliferator-activated receptor γ coactivator 1-α (PGC-1α), which promotes mitochondrial nuclear gene transcription and increases mitochondrial replication (14). Previous studies have shown that adenosine monophosphate-activated protein kinase (AMPK) can upregulate the activity of PGC-1α by increasing the intracellular concentration of NAD+ to activate sirtuin type 1 (SIRT1), and can ultimately promote mitochondrial biogenesis (15, 16). Therefore, the activation of AMPK/SIRT1/PGC-1α-dependent mitochondrial biogenesis in skeletal muscle might protect against muscular dysfunction, insulin resistance, and even cognitive deficiency in remote during the natural aging processes.

Berberine is an isoquinoline alkaloid originally isolated from extracts of the Chinese herb Coptis chinensis. In recent years, multiple pharmacological effects of berberine have been reported, including mitigation of Alzheimer's disease, diabetes mellitus, and hyperlipidemia (17, 18). Among the effects of berberine, its anti-diabetic activity is most potent. Our and other previous studies have shown that berberine can significantly ameliorate insulin resistance and type 2 diabetes by enhancing the activation of AMPK (19-21). Recently, a group reported that berberine protected against high-fat diet induced metabolism dysfunction in skeletal muscle by inducing SIRT1-dependent mitochondrial biogenesis (22). In line with the abovementioned findings, it might be speculated that berberine could delay the aging process or help normal aging health by mitigating insulin resistance, improving muscular dysfunction and ameliorating cognitive dysfunction via the activation of the AMPK/SIRT1/PGC-1α pathway in the skeletal muscle of aging rats.

Here, a naturally aging rat model was established to investigate the protective effects of berberine on aging-related cognitive ability and muscular function. We demonstrated that berberine mitigated aging-related cognitive deficits, and the effects of berberine against muscular dysfunction and insulin resistance might occur through enhancement of AMPK/SIRT1/PGC-1α-dependent mitochondrial biogenesis in the natural aging phase.

Materials and methods

Materials

Glucometers were purchased from Roche (Basel, Switzerland). Radioimmunoprecipitation assay (RIPA) buffer, a total cholesterol (T-CHO) kit, a triglyceride (TG) kit, phenylmethanesulfonyl fluoride (PMSF), hematoxylin, eosin and phosphatase inhibitors were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Enzyme-linked immunosorbent assay (ELISA) for insulin and Adenosine Triphosphate (ATP) were purchased from MyBioSource (San Diego, USA). 2,7-dichlorofluorescein diacetate (H2DCF-DA) was purchased from Sigma (St. Louis, USA). A plasma membrane protein isolation kit was purchased from Invent Biotechnologies, Inc. (Plymouth, USA). Pierce bicinchoninic acid (BCA) protein assay reagents and Pierce ECL Western Blotting Substrate were purchased from Thermo Fisher Scientific (Waltham, USA). Polyvinylidene difluoride (PVDF) membranes were purchased from Bio-Rad Laboratories (Hercules, USA). Anti-AMPK (ab80039), anti-p-AMPK (ab133448), anti-SIRT1 (ab110304), anti-PGC-1α (ab54481), anti-GAPDH (ab8245), anti-sodium potassium ATPase (ab197713) and anti-GLUT4 antibodies were purchased from Abcam (Cambridge, UK). Peroxidase-conjugated AffiniPure goat anti-mouse (SA00001-1) and peroxidase-conjugated AffiniPure goat anti-rabbit (SA00001-2) antibodies were purchased from Proteintech Group (Rosemont, USA). All other chemicals used in this study were purchased from Sigma-Aldrich (St. Louis, USA).

Animal model

Male Wistar rats were purchased from Vital River Laboratory Animals Technology Co, Ltd. The rats were housed under a 12 h/12 h light-dark cycle at 22±3°C and were fed regular rodent chow (Laboratory Animal Center of Jilin University). The animals were divided into 3 groups: 5-month-old rats (5 mo group, n=20), defined as adults; 24-month-old rats (24 mo group, n=20), defined as being aged; and a berberine treatment rats that had received 100 mg/kg Berberine orally once daily for 6 months beginning at the age of 18 months (BBR group, n=20). Before the rats were sacrificed, half the rats in each group were injected with 0.75 IU/kg body weight insulin (i.p.) to activate glucose uptake. Fifteen minutes later, all the rats were anesthetized with chloral hydrate; the soleus muscles were fixed in paraformaldehyde, and the gastrocnemius muscles were frozen immediately in liquid nitrogen and stored at −80°C for protein-related experiments. All animal experimental procedures were approved by The Ethics Committee for the Use of Experimental Animals of Jilin University [SCXK(Jing)2014-0004].

Behavioral testing

The Morris water maze (MWM) test consisted of 4 training trials of searching for the hidden platform on each of 5 consecutive days, followed by a spatial probe test on the 6th day. The study was conducted in a dark circular pool 1.5 m in diameter, and the water temperature was maintained at 25±2°C. The spatial probe was performed on the sixth day, and each probe trial lasted for 120 seconds after the escape platform was removed. In the probe trial, the frequency of platform crossings, swimming speed, swimming path length and time spent in the target quadrant were measured and calculated. The training and probe trials were recorded by a video camera mounted on the ceiling, and the data were analyzed using EthoVision XT 8.0 (Noldus Information Tech., Netherlands).

Intraperitoneal glucose tolerance test (IPGTT)

Rats were injected with 2 g/kg glucose i.p. after fasting overnight for 12 h. Ten microliters of blood was obtained from the tail tip, and the glucose level was measured with a glucometer at 0, 30, 60, 90, and 120 min after the injection.

Hematoxylin-eosin (HE) staining

Soleus muscles were fixed in paraformaldehyde overnight, dehydrated in an ascending graded ethanol series, equilibrated with xylene, embedded in paraffin and sectioned into 5- to 10-μm slices. Then, the samples were dewaxed with xylene and a descending graded ethanol series. Sections were stained with Mayer's hematoxylin and eosin. A BX40 12 J02 system biological microscope (Olympus, Japan) was used for morphological observation.

Total cholesterol and triglyceride testing

The total cholesterol (T-CHO) and triglyceride (TG) levels in the serum were assessed using an enzymatic determination method from a commercial kit, according to the manufacturer's instructions. The reaction mixture was shaken for 10 seconds and then incubated at 37°C for 6 min. The absorption density was determined at 510 nm in an Epoch microplate reader (BioTek, USA).

Reactive oxygen species (ROS) test

Ten microliters of skeletal muscle or lysate or 10 μl of serum was added to 127.5 μl of reaction buffer in 96-well plates [containing 30 mM HEPES (pH 7.2), 200 mM KCl, 1 mM MgCl2, and 7.5 μl of 2,2'-azobis(2-methylpropionamidine) dihydrochloride]. The same volume of ultrapure water was pipetted into the plate as a blank. The microplate was put into a fluorescence microplate reader at 35°C. 10 μl of the fluorescent probe H2DCF-DA was added to each well for a final concentration of 40 μM immediately before reading, and the level of ROS was detected at 488 and 525 nm.

ATP and insulin level determination

The levels of insulin in serum and the ATP concentration in the serum and gastrocnemius muscle were measured using an ELISA according to the manufacturer's instructions. For extraction of serum samples, blood was taken immediately after sacrifice, and the serum was collected after centrifugation at 3,500 g×15 min at 4°C. Before the ELISA was tested, the gastrocnemius muscle lysates were first measured with a BCA protein assay kit, and the total protein concentration of each group was adjusted to the same by RIPA. The concentrations of ATP and insulin were then calculated based on absorption density determined at 450 nm.

Protein sample preparation

For total protein extraction, 100 mg of frozen gastrocnemius muscle was homogenized in RIPA buffer (containing 0.2 μg/mL PMSF and 10 μg/mL phosphatase inhibitor) for 30 min and then centrifuged at 3,500 rpm×15 min and 12,000 rpm×15 min. The supernatants were extracted for Western blotting and enzyme-linked immunosorbent assay measurements.

For plasma membrane protein isolation, 100 mg of frozen gastrocnemius muscle from each sample was used. Plasma membrane proteins were isolated according to the kit manufacturer's instructions, and the end product was dissolved in 100 μl of PBS containing 5% SDS.

Western blot analysis

The concentration of protein in the gastrocnemius muscle was quantitated by BCA, and 120 μg of total protein was electrophoresed on a 12% SDS polyacrylamide gel and electrotransferred onto PVDF membranes. The membranes were blocked with 5% (w/v) BSA for 2 h at room temperature and then incubated with primary antibodies overnight at 4°C with slight shaking. After being washed with TBST three times, the membranes were incubated with secondary antibody (1:2000) at room temperature for 2 h. The protein bands were visualized by enhanced chemiluminescence with a GENE Imaging system. The images were quantified using Image Analysis Software (Quantity One, USA).

Statistical analysis

All data are expressed as the mean ± standard error (SE). Escape latency data were analyzed using two-way analysis of variance (ANOVA), while the other data were analyzed using one-way ANOVA. These tests were followed by a post hoc Bonferroni test to determine individual differences among groups. Data in Fig. 3 were counted by image pro plus 6.0 (Media Cybernetics, USA). All statistical analyses were performed using SPSS 17.0 version for Windows (IBM, USA).

Results

Berberine mitigates aging-related learning and memory deficits

A decline in learning and memory ability is commonly observed in the elderly population. The Morris water maze test was first conducted to evaluate whether learning and memory deficits existed in our natural old group (24 mo group) and then to determine the effects of berberine on aging-related learning and memory deficits. The rats in 5 mo, 24 mo and BBR groups received 4 training trials per day for 5 consecutive days. The time to find the platform was recorded and analyzed (Fig. 1B). The rats learned the task across sessions in a day-dependent manner ($F=590.291$; $p<0.01$). More importantly, one or more of the groups was different from the others by calculating the group-day interaction ($F=10.417$; $p<0.01$). Then, a Bonferroni's post hoc analysis was conducted to identify changes among the groups. As shown in Fig. 1B, the escape latency in the 24 mo group was longer than that in the 5 mo group from day 2 onward ($p<0.01$), which was remarkably shortened by berberine treatment from day 3 onward ($p<0.01$). The probe test was

performed on the sixth day. The trials were recorded by camera for 120 seconds (Fig. 1A). Based on the tracked paths of the animals, the platform-crossing frequency and path length in the target quadrant were remarkably decreased in the 24 mo group compared with the 5 mo group. However, the frequency of crossing the platform and the path length in the target quadrant in berberine-treated rats exhibited a remarkable increase over the 24 mo group. Furthermore, the frequency of crossing the platform ($F=5.524$, $p<0.01$; Fig. 1D), the percentage of time spent in the target quadrant ($F=5.965$, $p<0.05$; Fig. 1F) and the percentage of total path length traveled in target quadrant ($F=11.26$, $p<0.01$; Fig. 1G) were calculated by one-way ANOVA. The results showed the same trend described previously. In the 24 mo group, the swimming speed, the average of frequency of crossing the platform, the percentage of time in the target quadrant and the percentage of path length in target quadrant were decreased by approximately 10%($p<0.05$; Fig. 1C), 48% ($p<0.01$; Fig. 1D), 37% ($p<0.05$; Fig. 1F) and 58% ($p<0.01$; Fig. 1G), respectively, compared with the values for the 5 mo group. With 6 months of berberine treatment, these indexes elevated approximately 1.1-fold ($p<0.05$; Fig. 1C), 1.8-fold ($p<0.01$; Fig. 1D), 1.3-fold ($p<0.05$; Fig. 1F) and 2.4-fold ($p<0.01$; Fig. 1G) of these in 24 mo group. There was no significant difference among groups in the latency to reach the platform (Fig. 1E). The data from the Morris water maze test show learning and memory deficits in the 24 mo group, which could be improved by berberine treatment.

Table 1

Values of routine physiological and biochemical indicators

Parameter	5 mo	24 mo	BBR
Body weight (g)	294.6±16.3	625.0±15.0**	504.4±9.2#
Body length (cm)	21.6±0.2	24.1±0.6**	23.9±0.3
Lee's index	307.1±5.3	355.0±6.5**	333.1±2.7#
FBG (mmol/L)	4.33±0.24	6.70±0.33**	5.62±0.26#
Insulin (mIU/L)	10.99±1.75	15.61±0.51**	13.84±0.65#
TG (mmol/L)	0.75±0.09	1.41±0.04*	0.62±0.10#
T-CHO (mmol/L)	7.86±0.82	9.93±0.94	7.37±0.42

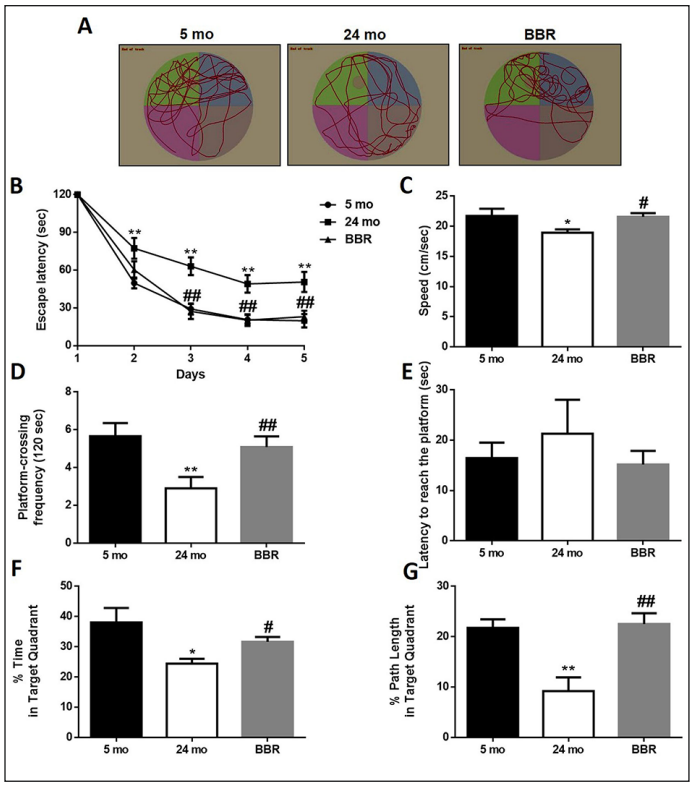
*p<0.05 and **p<0.01 compared to 5 mo group, #p<0.05 compared to the 24 mo group. Lee's index= $\sqrt[3]{}$[body weight (g)×1000]/body length (cm), FBG: fasting blood glucose, T-CHO: total cholesterol, TG: triglyceride.

Berberine attenuates aging-related metabolic disorder

Aging is accompanied by changes in metabolic biochemical indicators such as body weight, glucose, insulin and lipids. To evaluate aging-related changes, we first tested body weight and body length, and then calculated Lee's index (Table 1). Significant increases in body weight, body length and Lee's index were observed in the 24 mo group compared with the 5 mo group, which were all reversed by administration of berberine. To evaluate changes in aging-related glucose and lipids metabolic abnormalities, we measured insulin, fasting blood glucose (FBG), triglycerides and total cholesterol in serum (Table 1). In the 24 mo group, FBG, serum insulin and triglycerides were remarkably elevated by 1.54-fold ($p<0.01$), 1.43-fold ($p<0.01$) and 1.88-fold ($p<0.05$), respectively, compared with those of the 5 mo group, while berberine treatment decreased the values by approximately 17% ($p<0.05$), 13% ($p<0.05$) and 57% ($p<0.05$) of these in 24 mo group, respectively. No significant difference was detected in total cholesterol among the three groups. These results suggest obvious glucose and lipid metabolic abnormalities and hyperinsulinism appearing in the 24 mo rats, an age at which rats already have spatial learning deficits and weight gain. In contrast, 100 mg/kg berberine administration for 6 months ameliorates glucose and lipids metabolism and insulin level.

Figure 1

Morris water maze test for each group. (A) The visual traces of the 5 mo, 24 mo and BBR group rats in the spatial probe test. (B) The learning ability curve for escape latency across 5 days in each group (n=6). (C-E) The speed, platform-crossing frequency and latency to reach the platform for each group (n=6). (F, G) The percentages of time and path length spent in the target quadrant for each group (n=6). Escape latency data were analyzed using two-way analysis of variance (ANOVA), while the other data were analyzed using one-way ANOVA. These tests were followed by a post hoc Bonferroni test to determine individual differences among groups



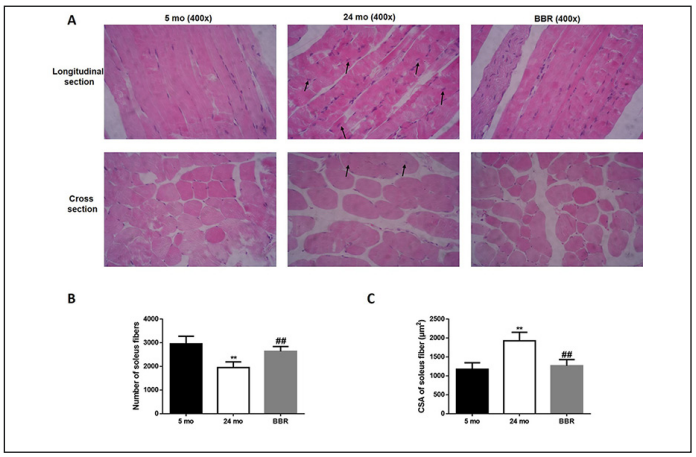
*p<0.05 and **p<0.01 compared with the 5 mo group, #p<0.05 and ##p<0.01 compared with the 24 mo group.

Berberine improves aging-related insulin resistance in the whole body and skeletal muscle

IPGTT and insulin stimulating membrane translocation of glucose transporter type 4 (GLUT4) were used to assess insulin resistance in the whole body and skeletal muscle among the groups, respectively. The results of the area under the curve (AUC) of IPGTT showed a significant difference among the three groups ($F=18.37$, $p<0.01$; Fig. 2B). Bonferroni's post hoc analysis showed that AUC of IPGTT in the 24 mo group was dramatically increased compared to that in the 5 mo group ($p<0.01$), and this increase was attenuated by administration of berberine for 6 months ($p<0.01$). To evaluate insulin resistance in skeletal muscle, we determined the membrane protein expression of GLUT4 in skeletal muscle tissue samples (Fig. 2C-D). Consistent with the IPGTT results, the membrane translocation of GLUT4 was increased faintly in the 24 mo group after 15 min of insulin stimulation; however, the membrane translocation of GLUT4 was remarkably increased after insulin stimulation in both the 5 mo and BBR groups ($p<0.01$). Meanwhile, the membrane translocation of GLUT4 in the BBR group after insulin stimulation was notably greater than that in the 24 mo group ($p<0.05$). Overall, these data confirm that insulin resistance both in whole-body and skeletal muscle existed in the 24 mo rat. In contrast, the administration of berberine ameliorated aging-related insulin resistance in whole-body and skeletal muscle.

Figure 2

Examination of systemic and skeletal muscle insulin resistance. (A, B) The curve for the intraperitoneal glucose tolerance test and its related calculation of AUC (n=6). **p<0.01 compared with the 5 mo group, ##p<0.01 compared with the 24 mo group. (C, D) Membrane protein Western blot and relative expression of GLUT4 in skeletal muscle among insulin-treated and untreated groups (n=6). Fifteen minutes before the rats were sacrificed, half the rats in each group were injected with 0.75 IU/kg body weight insulin (i.p.) to activate glucose uptake



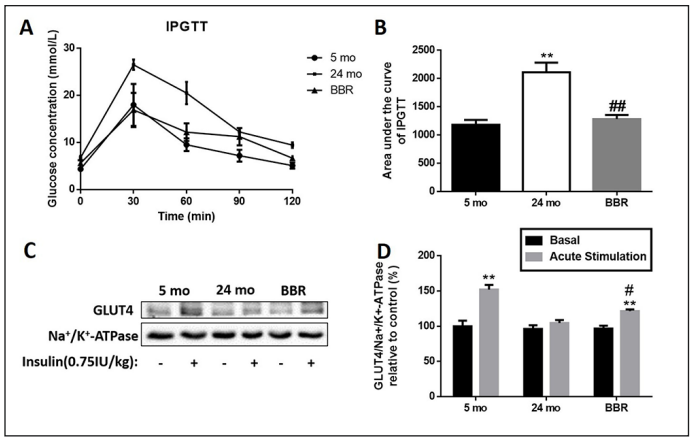
**p<0.01 compared with the corresponding basal group, #p<0.05 compared with the insulin-stimulated 24 mo group.

Berberine ameliorates aging-induced morphological changes in muscle tissue

To evaluate the morphological changes of skeletal muscle, we conducted HE staining of soleus in the groups (Fig. 3A). In longitudinal muscle sections from the 5 mo group, the muscle fibers were spindle shaped, neatly aligned, and closely packed with adjacent muscle fibers; however, in the 24 mo group, the number of skeletal muscle fibers decreased significantly, the muscle fiber became thicker and less orderly, and there were obvious gaps between adjacent fibers. With berberine treatment, the morphological alteration of muscle fibers in aging was improved: the adjacent muscle fibers were closely arranged, and most of the muscle fibers returned to normal. In cross sections from the 5 mo group, the muscle fiber number was large (Fig. 3B), and the relative cross-sectional area per fiber was small (Fig. 3C). In the 24 mo group, the number of muscle fibers decreased significantly ($p<0.01$; Fig. 3B), and the relative cross-sectional area of the muscle fibers increased significantly compared with the 5 mo group ($p<0.01$; Fig. 3C); in addition, the nuclei migrated inward. In the berberine treatment group, the number of skeletal muscle fibers returned to normal ($p<0.01$; Fig. 3B), and the relative cross-sectional area per muscle fiber dropped below that of the 24 mo group ($p<0.01$; Fig. 3C).

Figure 3

Morphological changes in the soleus muscles. (A) The morphological changes in soleus muscles were visualized by HE staining for each group. The three pictures in the top row are longitudinal sections, and the three in the bottom row are cross sections. The magnification of each image is 400x. Aged soleus muscle showed the presence of centrally located nuclei (arrowhead) in the myofibers. (B, C) The number of soleus fibers and the cross section area of soleus fiber (n=6). Data were counted by image pro plus 6.0, then statistical analyses were used by SPSS 17.0



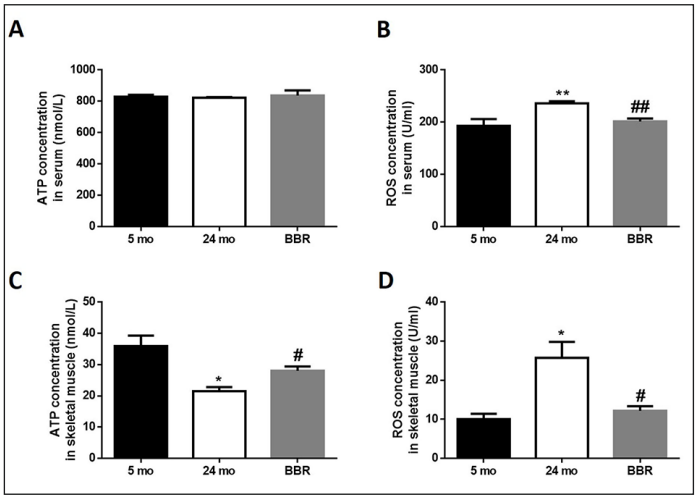
**p<0.01 compared with the 5 mo group, ##p<0.01 compared with the 24 mo group.

Berberine ameliorates aging-induced mitochondrial dysfunction and activates the AMPK/SIRT1/PGC-1α mitochondrial biogenesis signaling pathway in muscle tissue

Based on the results of impaired morphological structure of skeletal muscle, decreased fiber number and insulin resistance in our aging model, we speculated that mitochondrial function in skeletal muscle was also impaired. ATP and ROS levels in serum and skeletal muscle were further determined for the representation of mitochondrial function. The ROS levels in serum and skeletal muscle was dramatically increased in the 24 mo group compared with that of the 5 mo group ($p<0.01$; Fig. 4B and 4D), while 6-month administration of berberine effectively inhibited increased ROS in serum ($p<0.01$) and skeletal muscle ($p<0.05$). Although serum ATP levels were unchanged across the three groups (Fig. 4A), the concentration of ATP in the skeletal muscle of the 24 mo group was only 55% of that of the 5 mo group. With berberine treatment, the production of ATP was increased significantly ($p<0.05$; Fig. 4C). The data show that berberine administration decreased the ROS level both in serum and skeletal muscle and increased the production of ATP in skeletal muscle. This indicates that berberine might improve aging related mitochondrial dysfunction of skeletal muscle.

Figure 4

The levels of ATP and ROS in serum and skeletal muscle. (A, B) The levels of ATP and ROS in serum (n=6). (C, D) The concentrations of ATP and ROS in skeletal muscle in each group (n=6)

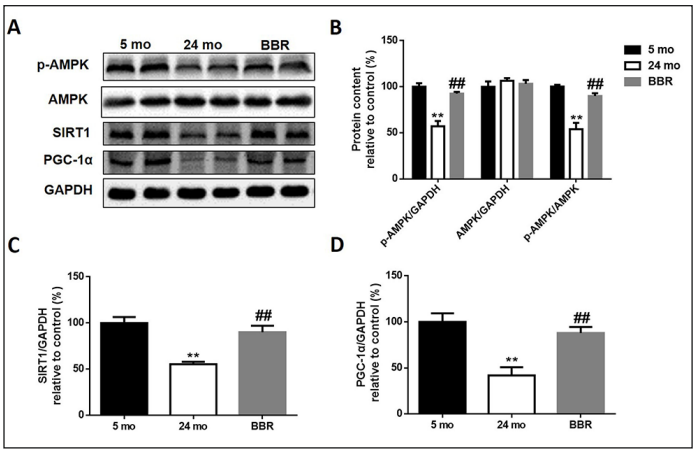


*p<0.05 and **p<0.01 compared with the 5 mo group, #p<0.05 and ##p<0.01 compared to 24 mo group.

AMPK/SIRT1/PGC-1α are the key regulators of mitochondrial biogenesis, and berberine has been proven to be the activator of AMPK in various diseases. Therefore, we speculated the protective mechanism of berberine on mitochondrial function of skeletal muscle may be via the AMPK/SIRT1/PGC-1α-dependent mitochondrial biogenesis pathway. In the present study, there was no significant difference in total AMPK protein expression among the three groups. However, the protein expression of p-AMPK was significantly reduced in the 24 mo group compared to that in the 5 mo group ($p<0.01$; Fig. 5B). With berberine treatment, the protein expression of p-AMPK was significantly upregulated ($p<0.01$; Fig. 5B). SIRT1 and PGC-1α presented the same protein expression trend as p-AMPK. In the 24 mo group, the protein expressions of SIRT1 and PGC-1α decreased to 56% and 42% of the levels in the 5 mo group ($p<0.01$; Fig. 5C-D). With berberine treatment, the protein expressions of SIRT1 and PGC-1α were significantly upregulated by approximately 1.6- and 2.1- fold compared to these of the 24 mo group ($p<0.01$; Fig. 5C-D). These results suggest that the protective mechanism of berberine on mitochondrial function of skeletal muscle may be via the AMPK/SIRT1/PGC-1α-dependent mitochondrial biogenesis pathway.

Figure 5

The protein expression of AMPK/SIRT1/PGC-1α in skeletal muscle. (A) Western blot of p-AMPK/AMPK/SIRT1/PGC-1α protein in skeletal muscle. (B-D) Relative expression levels of p-AMPK/AMPK/SIRT1/PGC-1α protein in skeletal muscle (n=6)



*p<0.05 and **p<0.01 compared with the 5 mo group, #p<0.05 and ##p<0.01 compared to 24 mo group.

Discussion

In the current study, we examined whether berberine could function as an anti-aging drug via increasing cognitive and muscular function. The principal findings of our study are as follows: 1. berberine improves cognitive deficiency and insulin resistance in naturally aging rats; 2. berberine preserves the morphology of skeletal muscle; 3. berberine ameliorates mitochondrial function and activates AMPK, SIRT1, and PGC-1α, key proteins for mitochondrial biogenesis, in skeletal muscle.

Cognitive decline is a common pathological feature of aging and many neurodegenerative diseases, and manifests with the death of neurons in hippocampus (23-25). Recent studies

have shown that cognitive decline and insulin resistance share the same pathological pathway, and the disruption of insulin level can lead to neuron death (8, 26). In addition, the ability of the elderly to control glycolipid metabolism decreases, and hyperglycemia can further lead to neuron death (27). Berberine is a clinical anti-diarrhea drug in common use. Recent studies have shown that berberine is a potential multi-target drug for the therapy of diabetes and diabetic complications (28). Therefore, it is speculated that berberine may have a potential role in the treatment of cognitive decline. Durairajan et al. (20) has reported that berberine could diminish toxic protein accumulation in neurons and thereby improve symptoms of Alzheimer's disease. The study of Zhang et al. (19) has confirmed that berberine alleviates cognitive dysfunction by reducing the generation of TNF-α and IL-1β. A recent study has reported that berberine ameliorates cognitive impairment in Alzheimer's disease induced by a high-fat diet (29). Our previous work also suggested that berberine protected hippocampal neuron damage in type 2 diabetic rats by the activation of the AMPK-dependent autophagy pathway (30). In the present study, based on cognitive behavioral test, we found that berberine could significantly improve the learning and memory deficits of naturally aging rats, which is consistent with the effects of berberine on other neurodegenerative diseases mentioned above. The failure to control metabolic function is closely related to cognitive decline in the elderly (31-33). This study further examined the levels of the serum metabolic indicators and insulin resistance in aging rats with berberine treatment. Numerous studies have demonstrated that berberine can improve insulin resistance via protecting islet β cell damage, promoting glucose uptake of skeletal muscle, improving hepatic gluconeogenesis and reducing blood lipid levels in various diabetic models (34-37). Our results showed the same trend that berberine can significantly reduce the level of triglyceride and insulin in serum and improve insulin resistance. It indicates that berberine could slow aging process by improving cognitive function and insulin resistance.

Another character of aging is muscular dysfunction. Skeletal muscle is a prior research object in insulin resistance. Studies have confirmed that structural and functional alterations in skeletal muscle are the critical factors related to insulin resistance in the aging state (38-41). The effect of aging on skeletal muscle is site specific, and the morphological changes of weight-bearing skeletal muscle of hind limbs are more prominent than that of other parts (42). Therefore, we investigated the main morphological characteristics of the soleus muscle, which is the weight-bearing muscle in hind limbs of rats. Notably, we report for the first time that berberine can help normalize the number and alignment of soleus muscle fibers.

We further explored the skeletal muscle function. Compared to the insensitive response of skeletal muscle to insulin in diabetic condition, the decline of muscular function play a more critical role for insulin resistance in skeletal muscles caused by aging (10). Moreover, as a mitochondrion-rich tissue, mitochondrial function is vital in maintaining muscular function in skeletal muscle (43, 44). ATP and ROS levels in tissues are two key indicators used to describe mitochondrial function (44-46). In the present study, increased ATP production and decreased ROS production were observed in the skeletal muscle after berberine treatment, indicating that berberine participated in the recovery of mitochondrial function in skeletal muscle.

A recent study reported that berberine protects against high-fat diet induced muscle dysfunction via SIRT1-related mitochondrial biogenesis (22). Additionally, mitochondrial biogenesis is a vital factor affecting mitochondrial function under aging, which is regulated by the AMPK/SIRT1/PGC-1α pathway (10, 14, 47). PGC-1α initiates mitochondrial biogenesis by upregulating the expressions of nuclear DNA-encoded mitochondrial proteins, and the process is enhanced by the NAD+-dependent protein deacetylase SIRT1 (48). Interestingly, SIRT1 is also a popular anti-aging target at present. Resveratrol, a SIRT1 activator, has been confirmed to protect against obesity and development of insulin resistance in rodents receiving a high-calorie diet for a long period of time[49]. Controversial reports suggest that AMPK activates PGC-1α directly or indirectly by activating SIRT1. Our previous studies demonstrated that berberine exerts anti-diabetic effects primarily by activating AMPK (50, 51). Therefore, we speculated that by the protective effects of berberine against aging-related structural and functional impairments of skeletal muscle maybe via the activation of the AMPK/SIRT1/PGC-1α-dependent mitochondrial biogenesis pathway. Our results showed significant decreases in p-AMPK, SIRT1 and PGC-1α in the aged group, while berberine treatment reversed the protein expressions of p-AMPK, SIRT1 and PGC-1α, which were consistent with the results of Gomes et al. concerning skeletal muscle in a high-fat diet model (22). In the current study, berberine treatment of aging rats activated the AMPK/SIRT1/PGC-1α-dependent mitochondrial biogenesis pathway, and attenuated the mitochondrial dysfunction and structural changes in skeletal muscle. These data provide further evidences for the protective effects of berberine in natural aging.

In conclusion, the present study sheds new light on the crosstalk involved in aging-related cognitive deficits, insulin resistance and muscle dysfunction. Our studies may have important implications for the use of berberine as an anti-aging therapeutic agent to mitigate cognitive decline and improve muscle function via activation of the AMPK/SIRT1/PGC-1a pathway in skeletal muscle.

Ethical standards: This study was not a clinical trial. All animal experimental procedures were approved by The Ethics Committee for the Use of Experimental Animals of Jilin University [SCXK(Jing)2014-0004].

Conflict of interest: The authors declare that they have no conflicts of interest in this study.

Acknowledgements: This work was conducted at the Preclinical Pharmacology R&D Center of Jilin Province and the Key Lab of Traditional Medicine for Diabetes

of Jilin Province. We thank Ming Chang from the 1st Hospital of Jinlin University for providing help with the Morris water maze test. This study was supported by funds from the Science and Technology Development Projects of Jilin Province (20140203011YY, 20150311013YY, 20170520020JH), the National Natural Science Foundation of China (81503122), and the Norman Bethune Program of Jilin University (2015224, 2015401).

References

1. Harada CN, Natelson Love MC, Triebel KL: Normal cognitive aging. Clin Geriatr Med 2013, 29(4):737-752.
2. Fischer L, Baker J, Rienhoff R, Strauss B, Tirp J, Busch D, Schorer J: Perceptual-cognitive expertise of handball coaches in their young and middle adult years. J Sports Sci 2016, 34(17):1637-1642.
3. Nair KS: Aging muscle. Am J Clin Nutr 2005, 81(5):953-963.
4. Purves-Smith FM, Sgarioto N, Hepple RT: Fiber typing in aging muscle. Exerc Sport Sci Rev 2014, 42(2):45-52.
5. Scott D, Park MS, Kim TN, Ryu JY, Hong HC, Yoo HJ, Baik SH, Jones G, Choi KM: Associations of Low Muscle Mass and the Metabolic Syndrome in Caucasian and Asian Middle-aged and Older Adults. J Nutr Health Aging 2016, 20(3):248-255.
6. Groen BB, Hamer HM, Snijders T, van Kranenburg J, Frijns D, Vink H, van Loon LJ: Skeletal muscle capillary density and microvascular function are compromised with aging and type 2 diabetes. J Appl Physiol (1985) 2014, 116(8):998-1005.
7. Maddison LA, Joest KE, Kammeyer RM, Chen W: Skeletal muscle insulin resistance in zebrafish induces alterations in beta-cell number and glucose tolerance in an age- and diet-dependent manner. Am J Physiol Endocrinol Metab 2015, 308(8):E662-669.
8. Morris JK, Vidoni ED, Honea RA, Burns JM, Alzheimer's Disease Neuroimaging I: Impaired glycemia increases disease progression in mild cognitive impairment. Neurobiol Aging 2014, 35(3):585-589.
9. Kim TE, Lee DH, Kim YJ, Mok JO, Kim CH, Park JH, Lee TK, Yoo K, Jeong Y, Lee Y et al: The relationship between cognitive performance and insulin resistance in non-diabetic patients with mild cognitive impairment. Int J Geriatr Psychiatry 2015, 30(6):551-557.
10. Chistiakov DA, Sobenin IA, Revin VV, Orekhov AN, Bobryshev YV: Mitochondrial aging and age-related dysfunction of mitochondria. Biomed Res Int 2014, 2014:238463.
11. DeFronzo RA, Gunnarsson R, Bjorkman O, Olsson M, Wahren J: Effects of insulin on peripheral and splanchnic glucose metabolism in noninsulin-dependent (type II) diabetes mellitus. J Clin Invest 1985, 76(1):149-155.
12. Sandri M, Lin J, Handschin C, Yang W, Arany ZP, Lecker SH, Goldberg AL, Spiegelman BM: PGC-1alpha protects skeletal muscle from atrophy by suppressing FoxO3 action and atrophy-specific gene transcription. Proc Natl Acad Sci U S A 2006, 103(44):16260-16265.
13. Loprinzi PD: Epidemiological investigation of muscle-strengthening activities and cognitive function among older adults. Chronic Illn 2016, 12(2):157-162.
14. Anderson R, Prolla T: PGC-1alpha in aging and anti-aging interventions. Biochim Biophys Acta 2009, 1790(10):1059-1066.
15. Lan F, Cacicedo JM, Ruderman N, Ido Y: SIRT1 modulation of the acetylation status, cytosolic localization, and activity of LKB1. Possible role in AMP-activated protein kinase activation. J Biol Chem 2008, 283(41):27628-27635.
16. Gerhart-Hines Z, Rodgers JT, Bare O, Lerin C, Kim SH, Mostoslavsky R, Alt FW, Wu Z, Puigserver P: Metabolic control of muscle mitochondrial function and fatty acid oxidation through SIRT1/PGC-1alpha. EMBO J 2007, 26(7):1913-1923.
17. Lan J, Zhao Y, Dong F, Yan Z, Zheng W, Fan J, Sun G: Meta-analysis of the effect and safety of berberine in the treatment of type 2 diabetes mellitus, hyperlipemia and hypertension. J Ethnopharmacol 2015, 161:69-81.
18. Zhang Q, Li Y, Chen L: [Effect of berberine in treating type 2 diabetes mellitus and complications and its relevant mechanisms]. Zhongguo Zhong Yao Za Zhi 2015, 40(9):1660-1665.
19. Zhang Z, Li X, Li F, An L: Berberine alleviates postoperative cognitive dysfunction by suppressing neuroinflammation in aged mice. Int Immunopharmacol 2016, 38:426-433.
20. Durairajan SS, Liu LF, Lu JH, Chen LL, Yuan Q, Chung SK, Huang L, Li XS, Huang JD, Li M: Berberine ameliorates beta-amyloid pathology, gliosis, and cognitive impairment in an Alzheimer's disease transgenic mouse model. Neurobiol Aging 2012, 33(12):2903-2919.
21. Zhang M, Lv X, Li J, Meng Z, Wang Q, Chang W, Li W, Chen L, Liu Y: Sodium caprate augments the hypoglycemic effect of berberine via AMPK in inhibiting hepatic gluconeogenesis. Mol Cell Endocrinol 2012, 363(1-2):122-130.
22. Gomes AP, Duarte FV, Nunes P, Hubbard BP, Teodoro JS, Varela AT, Jones JG, Sinclair DA, Palmeira CM, Rolo AP: Berberine protects against high fat diet-induced dysfunction in muscle mitochondria by inducing SIRT1-dependent mitochondrial biogenesis. Biochim Biophys Acta 2012, 1822(2):185-195.
23. Deibel SH, Zelinski EL, Keeley RJ, Kovalchuk O, McDonald RJ: Epigenetic alterations in the suprachiasmatic nucleus and hippocampus contribute to age-related cognitive decline. Oncotarget 2015, 6(27):23181-23203.
24. Lin CH, Wu RM: Biomarkers of cognitive decline in Parkinson's disease. Parkinsonism Relat Disord 2015, 21(5):431-443.
25. Lo RY, Jagust WJ, Alzheimer's Disease Neuroimaging I: Effect of cognitive reserve markers on Alzheimer pathologic progression. Alzheimer Dis Assoc Disord 2013, 27(4):343-350.
26. Kullmann S, Heni M, Hallschmid M, Fritsche A, Preissl H, Haring HU: Brain Insulin Resistance at the Crossroads of Metabolic and Cognitive Disorders in Humans. Physiol Rev 2016, 96(4):1169-1209.
27. Capiotti KM, De Moraes DA, Menezes FP, Kist LW, Bogo MR, Da Silva RS: Hyperglycemia induces memory impairment linked to increased acetylcholinesterase activity in zebrafish (Danio rerio). Behav Brain Res 2014, 274:319-325.
28. Perez-Rubio KG, Gonzalez-Ortiz M, Martinez-Abundis E, Robles-Cervantes JA, Espinel-Bermudez MC: Effect of berberine administration on metabolic syndrome, insulin sensitivity, and insulin secretion. Metab Syndr Relat Disord 2013, 11(5):366-369.
29. Chen Q, Mo R, Wu N, Zou X, Shi C, Gong J, Li J, Fang K, Wang D, Yang D et al: Berberine Ameliorates Diabetes-Associated Cognitive Decline through Modulation of Aberrant Inflammation Response and Insulin Signaling Pathway in DM Rats. Front Pharmacol 2017, 8:334.
30. Xue H, Ji Y, Wei S, Yu Y, Yan X, Liu S, Zhang M, Yao F, Lan X, Chen L: HGSD attenuates neuronal apoptosis through enhancing neuronal autophagy in the brain of diabetic mice: The role of AMP-activated protein kinase. Life Sci 2016, 153:23-34.
31. Calvo-Ochoa E, Arias C: Cellular and metabolic alterations in the hippocampus caused by insulin signalling dysfunction and its association with cognitive impairment during aging and Alzheimer's disease: studies in animal models. Diabetes Metab Res Rev 2015, 31(1):1-13.
32. Kim B, Feldman EL: Insulin resistance as a key link for the increased risk of cognitive impairment in the metabolic syndrome. Exp Mol Med 2015, 47:e149.
33. Yin Q, Ma Y, Hong Y, Hou X, Chen J, Shen C, Sun M, Shang Y, Dong S, Zeng Z et al: Lycopene attenuates insulin signaling deficits, oxidative stress, neuroinflammation, and cognitive impairment in fructose-drinking insulin resistant rats. Neuropharmacology 2014, 86:389-396.
34. Gao N, Zhao TY, Li XJ: The protective effect of berberine on beta-cell lipoapoptosis. J Endocrinol Invest 2011, 34(2):124-130.
35. Yang TC, Chao HF, Shi LS, Chang TC, Lin HC, Chang WL: Alkaloids from Coptis chinensis root promote glucose uptake in C2C12 myotubes. Fitoterapia 2014, 93:239-244.
36. Xia X, Yan J, Shen Y, Tang K, Yin J, Zhang Y, Yang D, Liang H, Ye J, Weng J: Berberine improves glucose metabolism in diabetic rats by inhibition of hepatic gluconeogenesis. PLoS One 2011, 6(2):e16556.
37. Pirillo A, Catapano AL: Berberine, a plant alkaloid with lipid- and glucose-lowering properties: From in vitro evidence to clinical studies. Atherosclerosis 2015, 243(2):449-461.
38. Potes Y, de Luxan-Delgado B, Rodriguez-Gonzalez S, Guimaraes MRM, Solano JJ, Fernandez-Fernandez M, Bermudez M, Boga JA, Vega-Naredo I, Coto-Montes A: Overweight in elderly people induces impaired autophagy in skeletal muscle. Free Radic Biol Med 2017, 110:31-41.
39. Kenny HC, Rudwill F, Breen L, Salanova M, Blottner D, Heise T, Heer M, Blanc S, O'Gorman DJ: Bed rest and resistive vibration exercise unveil novel links between skeletal muscle mitochondrial function and insulin resistance. Diabetologia 2017.
40. Zhang N, Valentine JM, Zhou Y, Li ME, Zhang Y, Bhattacharya A, Walsh ME, Fischer KE, Austad SN, Osmulski P et al: Sustained NFkappaB inhibition improves insulin sensitivity but is detrimental to muscle health. Aging Cell 2017.
41. Ou YC, Chuang HH, Li WC, Tzeng IS, Chen JY: Gender difference in the association between lower muscle mass and metabolic syndrome independent of insulin resistance in a middle-aged and elderly Taiwanese population. Arch Gerontol Geriatr 2017, 72:12-18.
42. Brown M, Ross TP, Holloszy JO: Effects of ageing and exercise on soleus and extensor digitorum longus muscles of female rats. Mech Ageing Dev 1992, 63(1):69-77.
43. Crescenzo R, Bianco F, Mazzoli A, Giacco A, Liverini G, Iossa S: Skeletal muscle mitochondrial energetic efficiency and aging. Int J Mol Sci 2015, 16(5):10674-10685.
44. Javadov S, Jang S, Rodriguez-Reyes N, Rodriguez-Zayas AE, Soto Hernandez J, Krainz T, Wipf P, Frontera W: Mitochondria-targeted antioxidant preserves contractile properties and mitochondrial function of skeletal muscle in aged rats. Oncotarget 2015, 6(37):39469-39481.
45. Yamamori T, Yasui H, Yamazumi M, Wada Y, Nakamura Y, Nakamura H, Inanami O: Ionizing radiation induces mitochondrial reactive oxygen species production accompanied by upregulation of mitochondrial electron transport chain function and mitochondrial content under control of the cell cycle checkpoint. Free Radic Biol Med 2012, 53(2):260-270.
46. Bagkos G, Koufopoulos K, Piperi C: ATP synthesis revisited: new avenues for the management of mitochondrial diseases. Curr Pharm Des 2014, 20(28):4570-4579.
47. Russ DW, Acksel C, McCorkle KW, Edens NK, Garvey SM: Effects of Running Wheel Activity and Dietary HMB and beta-alanine Co-Supplementation on Muscle Quality in Aged Male Rats. J Nutr Health Aging 2017, 21(5):554-561.
48. Gurd BJ: Deacetylation of PGC-1alpha by SIRT1: importance for skeletal muscle function and exercise-induced mitochondrial biogenesis. Appl Physiol Nutr Metab 2011, 36(5):589-597.
49. Park SJ, Ahmad F, Philp A, Baar K, Williams T, Luo H, Ke H, Rehmann H, Taussig R, Brown AL et al: Resveratrol ameliorates aging-related metabolic phenotypes by inhibiting cAMP phosphodiesterases. Cell 2012, 148(3):421-433.
50. Jager S, Handschin C, St-Pierre J, Spiegelman BM: AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha. Proc Natl Acad Sci U S A 2007, 104(29):12017-12022.
51. Iwabu M, Yamauchi T, Okada-Iwabu M, Sato K, Nakagawa T, Funata M, Yamaguchi M, Namiki S, Nakayama R, Tabata M et al: Adiponectin and AdipoR1 regulate PGC-1alpha and mitochondria by Ca(2+) and AMPK/SIRT1. Nature 2010, 464(7293):1313-1319.