Biochimica et Biophysica Acta 1822 (2012) 1716–1726

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbadis

The role of AMPK/mTOR/S6K1 signaling axis in mediating the physiological process of exercise-induced insulin sensitization in skeletal muscle of C57BL/6 mice ☆

Xiaolei Liu [a], Hairui Yuan [a], Yanmei Niu [a], Wenyan Niu [b], Li Fu [a,*]

[a] Department of Rehabilitation and Sports Medicine, Tianjin Medical University, Tianjin 300070, China
[b] Department of Immunology, School of Basic Medical Science, Tianjin Medical University, Tianjin 300070, China

ARTICLE INFO

Article history:
Received 4 February 2012
Received in revised form 9 July 2012
Accepted 21 July 2012
Available online 28 July 2012

Keywords:
Aerobic exercise
Insulin resistance
High-fat diet
Insulin signaling
Lipogenesis

ABSTRACT

The crosstalk between mTORC1/S6K1 signaling and AMPK is emerging as a powerful and highly regulated way to gauge cellular energy and nutrient content. The aim of the current study was to determine the mechanism by which exercise training reverses lipid-induced insulin resistance and the role of AMPK/mTOR/S6K1 signaling axis in mediating this response in skeletal muscle. Our results showed that high-fat feeding resulted in decreased glucose tolerance, which was associated with decreased Akt expression and increased intramuscular triglyceride deposition in the skeletal muscle of C57BL/6 mice. Impairments in lipid metabolism were accompanied by increased total protein and phosphorylation of S6K1, SREBP-1c cleavage, and decreased AMPK phosphorylation. Exercise training reversed these impairments, resulting in improved serum lipid profiles and glucose tolerance. C2C12 myotubes were exposed to palmitate, resulting in an increased insulin-dependent Akt Ser473 phosphorylation, associated with a significant increase in the level of phosphorylation of S6K1 on T389. All these changes were reversed by activation of AMPK. Consistent with this, inhibition of AMPK by compound C induced an enhanced phosphorylation of both S6K1 and Akt, and silencing of S6K1 with siRNA showed no effect on Akt phosphorylation in both the absence and presence of palmitate cultured myotubes. In addition, compound C led to an elevated SREBP-1c cleavage but was blocked by S6K1 siRNA. In summary, exercise training inhibits SREBP-1c cleavage through AMPK/mTOR/S6K1 signaling, resulting in decreased intramyocellular lipid accumulation. Our results provide new insights into the mechanism by which AMPK/mTOR/S6K1 signaling axis mediates the physiological process of exercise-induced insulin sensitization.

1. Introduction

Excessive supply of high-fat diet (HFD) and lack of exercise-induced caloric consumption might be the main factors leading to insulin resistance (IR) associated with type 2 diabetes (T2DM) and obesity. Chronic consumption of a HFD increases the circulation of non-esterified fatty acids (NEFA) resulting in marked skeletal muscle IR characterized by intramuscular triglyceride deposition and hyperlipidemia in rodents and humans [1,2]. Various strategies have been proposed to combat IR, including lifestyle modifications and pharmacologic interventions [3,4]. Regular aerobic exercise has been recognized as one of the most potent means to improve insulin action in muscle. We and others have previously proved that exercise training could reverse the effects of HFD on skeletal muscle IR [1,2]. However, the precise mechanisms by which lipids induce IR and exercise training reverses these defects in skeletal muscle are still elusive.

Mammalian target of rapamycin (mTOR), a serine/threonine kinase, has been implicated in a number of specific human pathologies, including obesity, T2DM and cancer [5,6]. mTOR signals in at least two distinct multiprotein complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2), distinguished by their partner proteins and differing sensitivities to rapamycin [7]. mTORC1 is rapamycin-sensitive and thought to mediate many of its downstream effects through S6K1 and initiation factor 4E-binding protein 1 (4E-BP1) [6]. Some evidence has demonstrated that overactivation of mTOR may be associated with impairments to skeletal muscle insulin action in rodents consuming a HFD [8]. In contrast, S6K1 null mice are protected from HFD-induced IR [9], implicating mTORC1 activation as a possible mechanism for impaired insulin action in response to elevated lipid availability. When nutrients are available, mTOR is activated and drives anabolism as well as energy storage and consumption. mTORC1 activation contributes to obesity by mediating excess fat deposition in white adipose tissue (WAT), liver, and muscle through the transcription factor sterol regulatory element

Abbreviations: 4E-BP1, Initiation factor 4E-binding protein 1; ACC, Acetyl-CoA carboxylase; Akt, Protein kinase B; AMPK, AMP-activated protein kinase; CPT1, Carnitine palmitoyltransferase I; DAG, Diacylglycerol; FAT/CD36, Fatty acid translocase; LCFA, Long-chain fatty acids; mTOR, Mammalian target of rapamycin; NEFA, Non-esterified fatty acids; S6K1, p70 ribosomal protein S6 kinase 1; SREBP-1c, Sterol regulatory element binding protein 1c; WAT, White adipose tissue

☆ Disclosure statement: The authors declare that there is no duality of interest associated with this manuscript.

* Corresponding author at: Tianjin Medical University, 22 Qixiangtai Road, Heping District, Mail Box 162, Tianjin 300070, China. Tel./fax: +86 2223542063.
E-mail address: lifu@tijmu.edu.cn (L. Fu).

0925-4439/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbadis.2012.07.008

binding protein 1c (SREBP1c) [10]. Furthermore, lipid metabolites such as ceramide and diacylglycerol (DAG) in cells have been implicated as a detrimental factor to insulin signaling transduction in a variety of tissues [11]. In cultured cells, chronic mTORC1 hyperactivation has been shown to contribute to IR through a S6K1-dependent, negative-feedback mechanism by promoting inhibitory serine phosphorylation and subsequent degradation of IRS1, leading to an attenuated Akt signaling and insulin desensitization [9].

Apart from insulin and nutrients, cellular energy status is a key regulator of mTORC1 signaling [12]. It has been suggested that mTOR may sense cellular ATP levels because it has a high K_m (around 1 mM). However, this K_m value is still considerably lower than normal cellular ATP levels; therefore, a drastic decrease in ATP would be required to affect the activity of mTOR [12]. The AMP-activated protein kinase (AMPK) has been proposed as a much more sensitive energy sensor in cells. AMPK, a highly conserved heterotrimeric kinase complex composed of a catalytic (α) subunit and two regulatory (β and γ) subunits, is activated under conditions of energy stress when intracellular ATP levels decline and intracellular AMP increases, as occurs during nutrient deprivation, hypoxia, and physical exercise [1,13]. Moreover, AMPK also mediates the suppression of expression of lipogenic genes, including fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC), via reduction of SREBP-1c activity [14].

Recent evidence indicates that mTORC1 and AMPK may represent two antagonistic forces governing muscle adaptation to nutrition, starvation, and growth stimulation [15]. Activation of AMPK leads to the inhibition of mTORC1 in a TSC1/2-dependent or -independent mechanism [10,16,17]. Moreover, in animal models with impaired mTORC1 signaling, AMPK is highly activated [18], suggesting a negative crosstalk between these two pathways. A study by Rivas et al. showed that diet-induced mTORC1 over-stimulation and skeletal muscle IR were reversed by 4 weeks of exercise training, which was associated with enhanced muscle AMPK activation [13]. All of the above results raise the intriguing possibility that AMPK-dependent mTORC1 signaling inhibition in skeletal muscle may be a critical mechanism in the process of endurance exercise ameliorating IR. However, direct evidence of the molecular link between AMPK and mTOR, especially in the insulin-resistant state, and the role and underlying mechanisms of this interaction in the regulating process of aerobic exercise ameliorating IR still need to be determined.

In the present study, we used a model of lipid-induced IR, the HFD-fed C57BL/6 mouse, to examine the health promoting effects of regular exercise training under the condition of an unaltered high-fat dietary pattern. We observed that 6-week aerobic exercise training increased whole-body insulin sensitivity. Furthermore, instead of the mTORC1/S6K1-driven inhibitory feedback mechanism causing attenuation of IRS1/Akt signaling, mTORC1/S6K1-mediated activated lipogenesis through SREBP-1c activation, resulting in excessive lipid accumulation in skeletal muscle may be one of the crucial mechanisms contributing to the decreased insulin sensitivity caused by long-term HFD-feeding. Accordingly, activation of AMPK by aerobic exercise may protect the skeletal muscle from increased triglyceride deposition by exerting an inhibitory effect on mTORC1/S6K1 signaling pathway. Together, our results provide new insights into the molecular connections between AMPK and mTOR/S6K1 signaling pathway and the mechanism by which AMPK/mTOR/S6K1 signaling axis mediates the physiological process of exercise-induced insulin sensitization.

2. Material and methods

2.1. Animals

Thirty 4-week old male wild-type C57BL/6 mice were housed in vented cages in a temperature-controlled room (20–23 °C; 35–55% humidity) with a 12-hour light/dark cycle and free access to food and water. This research was approved by the Tianjin Medical University Animal Care and Use Committee under the guidelines of the Chinese Academy of Sciences.

At the beginning of the experiment, mice were acclimated to a control diet for 7 days and were then divided into control (n = 10) and HFD-fed (n = 20) groups, and fed with normal diet and HFD (45% calories from fat, #D12451, Research Diets), respectively, for 10 weeks. During the subsequent 6-week experimental period, HFD-fed mice continued to eat a high-fat diet and were randomly allocated to HFD control group (n = 10) and HFD exercise group (n = 10). The mice from the exercise group were exercised on a motor-driven rodent treadmill for 5 days a week for a total of 6 weeks. The mice initially ran at the intensity of 50% VO_2 max for 20 min/day during the first week; thereafter, the running intensity and time were increased to 75% VO_2 max (12 m/min) for 60 min/day [19].

2.2. Oral glucose tolerance test (OGTT), fasting serum insulin (FIN), and metabolic profiles

After the desired length of exercise training (6 weeks), animals were food-restricted for at least 12 h overnight. Blood samples were taken for fasting serum insulin and lipid assay and an OGTT was administered. Blood was collected from a cut at the end of the tail immediately before and at 15, 30, 60, 90, and 180 min after glucose administration. Mice were then killed, and a portion of quadriceps was isolated.

2.3. Cell culture and treatments

C2C12 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS and maintained at 37 °C in a humidified atmosphere with 5% CO_2 as previously described [20]. Cells were seeded in a six-well plate and differentiated in DMEM with 2% horse serum for 6 days. siRNA transfection was performed with Lipofectamine™ according to the manufacturer's instructions (Invitrogen, Carlsbad, CA). For palmitate treatment, cells were incubated with 0.8 mM palmitate in ethanol vehicle for 15 h in DMEM with 1% fatty acid-free BSA. For insulin-stimulated conditions, 200 nM insulin was added to appropriate wells during the last 30 min of incubations. All experiments were run in triplicate.

2.4. Oil red O (ORO) staining and immunofluorescence assay

Tissues were cut into small cubes and embedded in optimal cutting temperature (OCT) freezing media and cryosectioned at 8 μm. ORO staining was performed in accordance to Koopman et al. [21]. The frozen sections were immunostained with the primary anti-FAT/CD36 antibody (a gift from Dr. Lei Cai, University of Kentucky). First, the section was blocked with blocking buffer (0.2% Triton X-100, 1% BSA, 1% normal goat serum dilution in PBS) for 1 h to avoid unspecific binding and incubated overnight at 4 °C with primary antibody. After being washed three times with PBS, a mixture of Alexa Fluor® 488-conjugated secondary antibody was added for 1 h at room temperature, followed by incubation in Hoechst for 5 min. The images were captured by fluorescence microscope.

2.5. Immunoprecipitation

Anti-CPT1 primary antibody was cross-linked to Dynabeads® Protein A (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. Tissue lysates were precleared with IgG Dynabeads® Protein A for 10 min and then incubated with CPT1-Dynabeads overnight at 4 °C. CPT1 immunoprecipitated complexes were washed five times with washing buffer. Proteins were eluted by boiling in loading buffer and then processed for western blot analysis.

2.6. Real-time reverse transcriptase

PCR Total RNA from quadriceps femoris of each sample was subjected to reverse transcription using SuperScript™ First-Strand Synthesis System for RT-PCR (Invitrogen, Carlsbad, CA) for cDNA synthesis. Synthesized cDNA was used for PCR with specific primers (Table 1) at optimized cycles. Real-time PCR was performed using Bio-Rad iQ5 Real-Time PCR system (Bio-Rad Laboratories, Hercules, CA) and FastStart Universal SYBR Green Master (Rox) (Roche Applied Science, Indianapolis, IN), according to the manufacturer's instructions. We quantified transcripts of the housekeeping gene glyceraldehydes 3-phosphate dehydrogenase (GAPDH) as control. Relative expression level of target gene was determined as 2-ΔΔCT.

2.7. Western blot analysis

Protein extracts from tissues/cells were made in freshly prepared NP-40 lysis buffer. Equal amounts of protein were separated by SDS-PAGE, and transferred to PVDF membranes (Millipore, Bedford, MA). The membranes were blocked and incubated overnight at 4 °C with antibodies specific for Akt, phospho-Akt Ser473/Thr308, ACC, phospho-ACC S79, AMPK, phospho-AMPK T172, IRS1 S636/639 (Cell Signaling Technology, MA), CPT-1 (LifeSpan Biosciences, WA), S6K1, phospho-S6K1 Thr389, or SREBP-1c (Abcam, MA). The immunoreactive proteins were detected with chemiluminescent horseradish peroxidase (HRP) substrate (Millipore, Bedford, MA) and quantified by densitometry. All results are representative of three independent experiments.

2.8. Statistical analysis

All data are expressed as means ± SEM. Significant differences were assessed by two-tailed Student's *t* test or one-way ANOVA followed by the Student–Newman–Keuls test. $P<0.05$ was considered statistically significant.

3. Results

3.1. Effects of HFD and exercise training on body weight, metabolic profiles, and glucose tolerance

As shown previously [2], the body mass of C57BL/6 mice was increased by high-fat feeding and was reversed by aerobic exercise training ($P<0.05$; Fig. 1A). Serum total cholesterol concentration was elevated in the HFD group by 77.02% ($P<0.05$ vs. normal diet) but decreased in the high-fat exercise training group ($P<0.05$ vs. HFD; Fig. 1B). Serum triglyceride concentration was also decreased in mice in the high-fat exercise training group ($P<0.01$ vs. HFD; Fig. 1C). HFD mice had higher fasting insulin levels ($P<0.01$ vs. normal diet), whereas 6-week exercise training caused a significant decrease in the high-fat exercise training group ($P<0.05$ vs. HFD; Fig. 1D). We further examined glucose tolerance and clearance by OGTTs. Consistent with these changes, the curve for glucose levels during OGTT showed that glucose intolerance induced by HFD was also reversed by 6 weeks of aerobic exercise (Fig. 1E).

Table 1
Primer sequences for real-time RT-PCR.

Gene name	Forward primer	Reverse primer
CPT1	CGTGCTGCTTTCTTTGTG	AGTGTTCGGTGTTGAGGC
FAT/CD36	AAAGTTGCCATAATTGAGTC	CGATTTCAGATCCGAACA
S6K1	AGAAGATGCAGGCTCTGA	TTACCAAGTACCCGAAGTA

All primer sequences are shown 5′ to 3′, left to right.

3.2. Effects of HFD and exercise training on lipid metabolism in skeletal muscle of C57BL/6 mice

To further elucidate the effects of HFD and exercise training on lipid metabolism, intracellular lipid accumulation in skeletal muscle was examined. As shown in Fig. 2A, 6 weeks of aerobic exercise training eliminated excess fat accumulation in skeletal muscle that was induced by long-term HFD-feeding, as determined by ORO staining (Fig. 2A).

CPT-1 plays a major role in the regulation of β-oxidation because it functions as a rate-limiting step in long-chain fatty acids (LCFA) transport into the mitochondria [22]. CPT-1 protein expression in skeletal muscle was not changed in HFD-fed mice compared with that of mice maintained on the normal diet, but was increased by ~14% with the combination of HFD and exercise training (Fig. 2C); these results are similar to those in our previous report [23]. These changes in CPT-1 protein expression were accompanied by similar alternations in mRNA levels among groups (Fig. 2B). In contrast, fatty acid translocase (FAT)/CD36, which was also identified on plasma membrane and mitochondria in skeletal muscle and found to be involved in LCFA transport and oxidation [24], was increased in response to high-fat feeding, as reflected by higher levels of mRNA and protein content in skeletal muscle compared with that of mice from control group (Fig. 2B and C). Moreover, exercise training largely abolished the HFD-induced increase in FAT/CD36 expression (Fig. 2B and C). Immunofluorescence assay was carried out to further detect the subcellular localization of FAT/CD36. The results showed that HFD feeding promoted the localization of FAT/CD36 on cell membrane. However, this effect of HFD was retarded by exercise training (Fig. 2D).

Study shows that the amount of FAT/CD36 that coimmunoprecipitated with CPT1 on mitochondrial membrane was strongly correlated with whole body fat oxidation [24]. Therefore, immunoprecipitation techniques were used to determine whether the association between FAT/CD36 and CPT1 is altered after HFD feeding and exercise training intervention. FAT/CD36 was found to coimmunoprecipitate with CPT1, and the amount of FAT/CD36 that coimmunoprecipitated with CPT1 decreased by 58% after 16-week HFD feeding but was increased after exercise training (Fig. 2E).

SREBP-1c is a key lipogenic transcription factor that is regulated by nutrients and insulin [25]. Activated nuclear form of SREBP-1c is translocated into the nucleus and regulates the lipogenic process by activating genes involved in fatty acid and triglyceride synthesis. Herein, we observed elevated protein concentrations of processed SREBP-1c in skeletal muscle of HFD-fed mice, but this trend was totally reversed by exercise training, resulting in a significantly decreased expression of SREBP-1c in exercise training group (Fig. 2C).

3.3. Activity of Akt and AMPK/mTOR/S6K1 signaling axis in skeletal muscle of HFD-fed C57BL/6 mice with or without training intervention

Increased glucose tolerance in mice that underwent exercise training suggests an increase in insulin sensitivity in peripheral tissues. To test the effects of HFD and aerobic training on insulin signaling transduction, we examined the expression of one key component, protein kinase B (Akt), in insulin signaling pathways [26] in skeletal muscle. As reported previously [1], expression of Akt protein was decreased in tissues in HFD-fed mice compared with mice maintained on a control diet, but was increased by 6 weeks of exercise training (Fig. 3A).

Exercise training completely abolished the HFD-induced increase in S6K1 mRNA expression such that values were restored to those observed in the normal diet group (Fig. 3B). Similar to the effect of HFD on S6K1 mRNA expression, HFD-feeding significantly increased the S6K1 protein content in skeletal muscle, and this trend was also reversed by aerobic exercise training (Fig. 3C). Furthermore, phosphorylation of

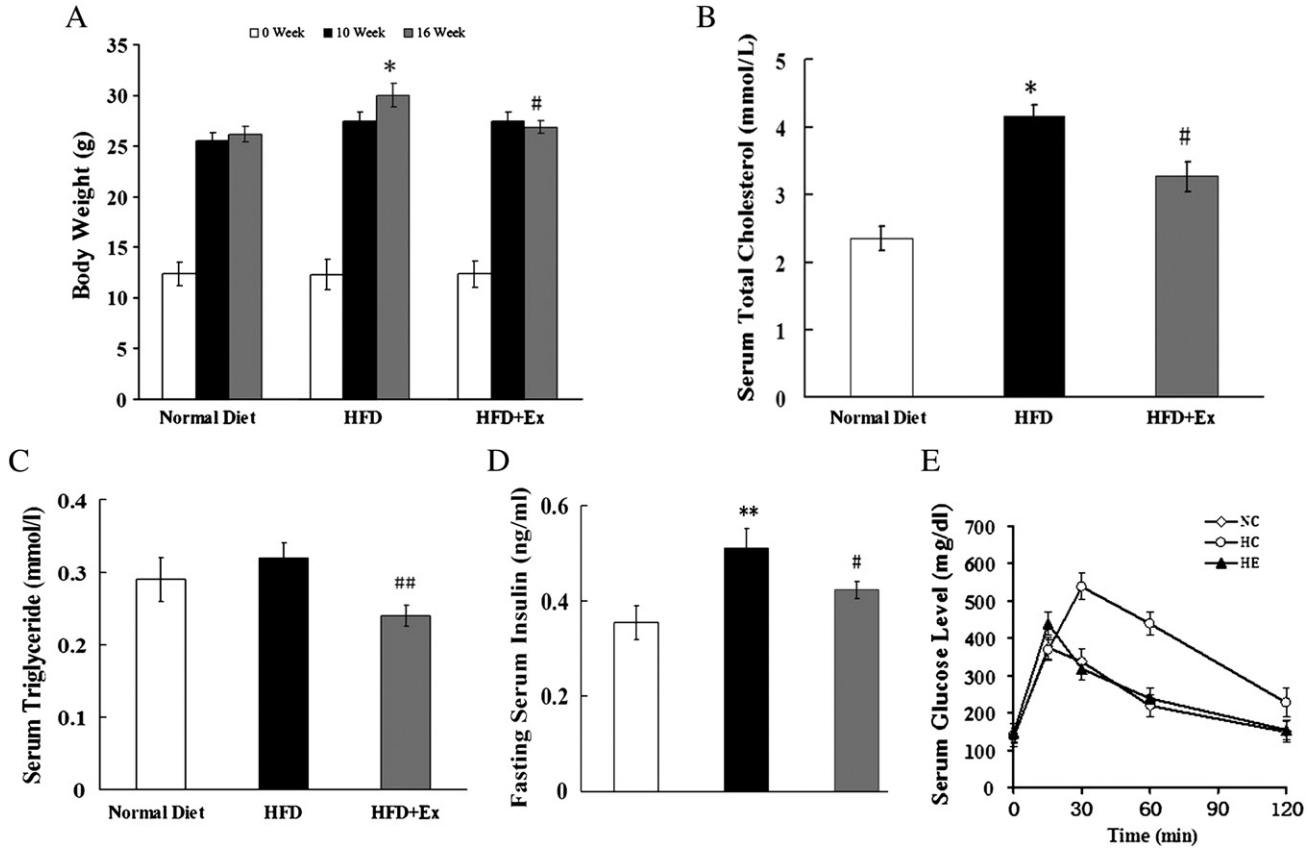

Fig. 1. Exercise training decreased body weight, improved plasma lipid profiles, hyperinsulinemia, and glucose tolerance. (A) High-fat diet (HFD)-fed mice displayed increased body weight but was reversed by exercise training (Ex). After 16 weeks of intervention, serum total cholesterol (B), serum triglyceride (C), and serum insulin levels (D) were measured, followed by fasting overnight. HFD-fed mice displayed hyperinsulinemia but were improved by 6-week exercise training. Results are means ± SEM (n = 10). *P<0.05 vs. normal diet; **P<0.01 vs. normal diet; #P<0.05 vs. HFD; ##P<0.01 vs. HFD. Oral glucose tolerance test (E) was performed in 16-hour-fasted mice (n = 10). Mice were given D-glucose (2 g/kg body wt.) by gavage through a gastric tube (outer diameter 1.2 mm) inserted in the stomach. Blood glucose levels were measured at the time points indicated. Values are means ± SEM.

S6K1 on Thr389, a surrogate measure of mTORC1 kinase activity, was also altered by HFD-feeding, with a ~30% increase in skeletal muscle of mice with high-fat feeding but was normalized by exercise training (Fig. 3C).

Because of its role in suppressing energy consuming processes, AMPK is known as the physiological inhibitor of mTOR/S6K1 signaling [16,17,27]. In our study, no changes were noted in the total protein concentration of the AMPKα. In contrast, phosphorylation of AMPK on Thr172, a marker of AMPK activity, was decreased in skeletal muscle of C57BL/6 mice by high-fat feeding. However, this diet-induced effect was reversed by exercise training (Fig. 3C).

3.4. Role of mTORC1/S6K1 signaling in regulation of IRS1/Akt cascade by palmitate

It has been previously demonstrated that high levels of circulating NEFA cause toxic effects and contribute to the development of metabolic disorders [1,2,28]. Therefore, we determined whether 0.8 mM palmitate incubation could activate mTORC1/S6K1 signaling in C2C12 cell line. Although no changes in the total protein level of S6K1 were observed, there was a significant increase in the level of phosphorylation of S6K1 on T389 with 18 h palmitate treatment (Fig. 4A), demonstrating that in response to the insulin, mTORC1/S6K1 signaling was stimulated by increased lipid availability.

Phosphorylation of total Akt on its Ser473 activation site was also altered by palmitate, with a ~33% increase in palmitate-treated C2C12 cells in response to insulin compared to the control cells (Fig. 4A). There was virtually no signal in control cells in the basal state (Fig. 4A). However, we found that culture of C2C12 cells with palmitate showed an unexpected increase of insulin-stimulated phosphorylation of IRS1 at S636/639 (Fig. 4A), indicating significantly decreased IRS1 activity caused by palmitate treatment.

To determine the role of the mTOR/S6K1 signaling pathway in Akt activation in response to palmitate, C2C12 myotubes were treated with S6K1 RNAi oligos. S6K1 RNAi significantly decreased endogenous S6K1 and pS6K1 protein levels (Fig. 4B). Consistent with previous studies [9], knockdown of S6K1 increased the insulin-dependent Ser473 phosphorylation of Akt and significantly decreased IRS1 phosphorylation at S636/639 (Fig. 4B), revealing the existence of a negative feedback mechanism in regulating insulin signaling activity at the level of IRS1 by mTOR/S6K1. In contrast, increases in phosphorylation of Akt following palmitate treatment resulted in concomitant increase in mTORC1 activity, as demonstrated by phosphorylation of its downstream substrate S6K1. Furthermore, knockdown of S6K1 by RNAi induced no further activation of Akt in C2C12 cells incubated with palmitate, even though phosphorylation of IRS1 at S636/639 showed a drastic reduction under both the absence and presence of palmitate conditions (Fig. 4B). Taken together, these results suggest that lipid-induced Akt phosphorylation is independent of mTORC1/S6K1 signaling pathway and IRS1.

3.5. Role of mTORC1/S6K1 signaling in the regulation of IRS1/Akt cascade by AMPK

It has been demonstrated that AMPK plays an important role in the regulation of fatty acid oxidation [29]. In this study, we examined the effects of prolonged exposure to palmitate on AMPK activity in C2C12 cells. Similar to the results of chronic lipid oversupply in vivo experiments, 18 h palmitate treatment decreased the phosphorylation

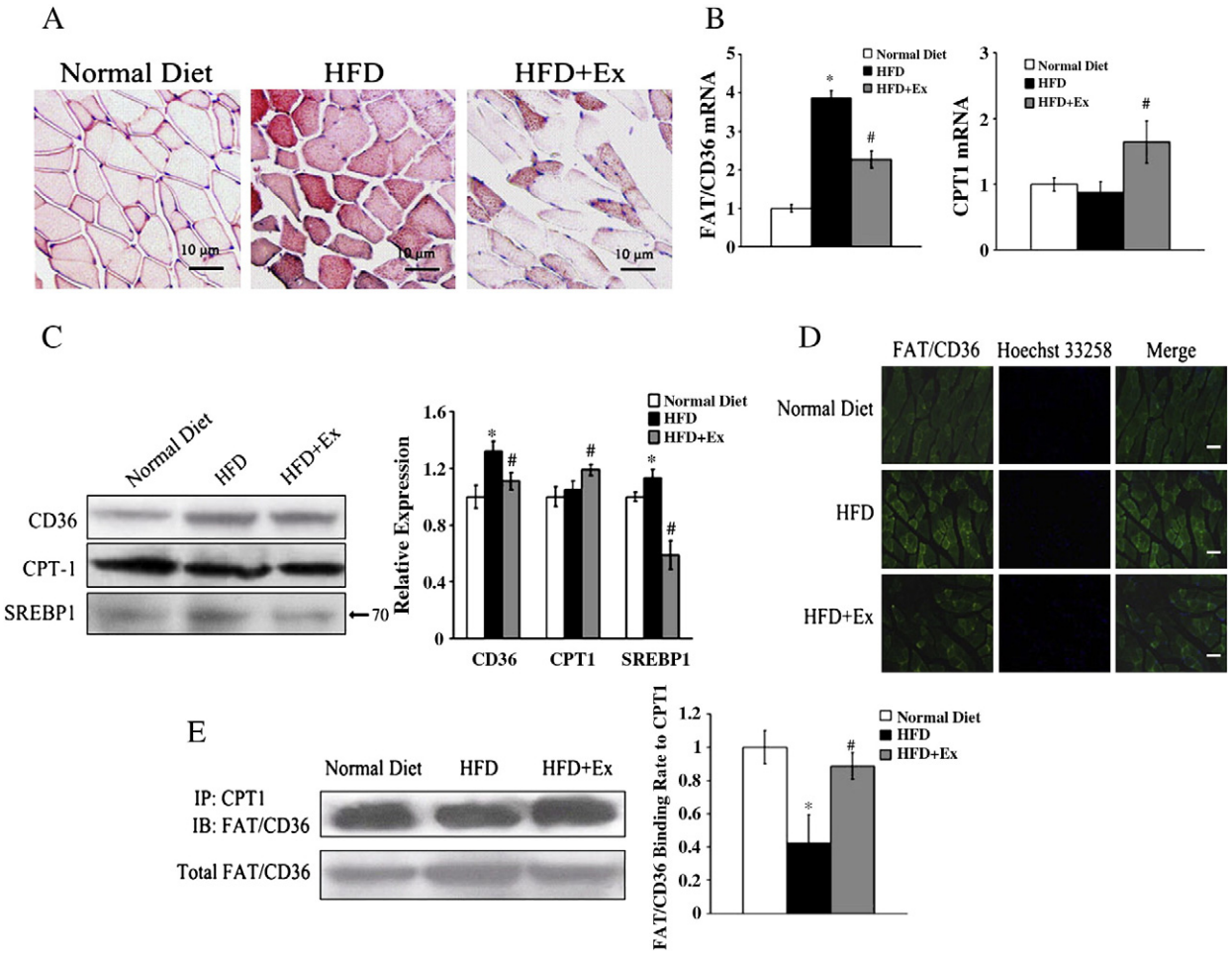

Fig. 2. Effects of high-fat diet (HFD) and exercise training (Ex) on lipid metabolism in skeletal muscle of C57BL/6 mice. (A) Representative oil red O (ORO) staining of muscle sections. Exercise training decreased lipid accumulation in skeletal muscle. (B–C) Relative levels of CPT-1 and FAT/CD36 mRNA and protein, active form of SREBP-1c protein (~70 kDa), were quantified using real-time RT-PCR and western blot analysis. Results are means ± SEM (n = 6). *P<0.05 versus normal diet; #P<0.05 versus HFD. The subcellular localization of FAT/CD36 (D) was measured using immunofluorescence assay. Representative confocal immunofluorescence images show FAT/CD36 staining (green) and nuclear staining with Hoechst (red). Scale bars = 20 μm. (E) Reciprocal immunoprecipitation of endogenous FAT/CD36 and CPT1. Tissue lysates were subject to immunoprecipitation with anti-CPT1 antibody. The immunoprecipitates were then blotted with FAT/CD36 antibody. IP and IB denote immunoprecipitation and immunoblot, respectively.

of AMPK T172 by ~70%. No significant change was noted in total AMPK expression in C2C12 cells in response to palmitate (Fig. 5A).

To test the functional significance of AMPK phosphorylation on mTORC1/S6K1 signaling, C2C12 cells were treated with 5-aminoimidazole-4-carboxamide riboside (AICAR), a specific AMPK activator, and the regulatory phosphorylation of S6K1 was measured using immunoblot analyses. Strikingly, in AMPK-activated cells, palmitate was incapable of activating the mTORC1 pathway as detected by the phosphorylation of S6K1 at T389 (Fig. 5A).

We further examined the function of AMPK in Akt regulation. Activation of AMPK by AICAR resulted in a decrease of insulin-stimulated Akt phosphorylation (Fig. 5A). Our observation is consistent with recent reports that AMPK activation closely correlates with the inhibition on the activating Ser/Thr phosphorylation of Akt [30,31].

Because inappropriate activation of the mTOR/S6K1 pathway imposes a negative feedback program to attenuate IRS/Akt signaling, we applied (6-[4-(2-piperidin-1-yl-ethoxy)-phenyl)]3-pyridin-4-yl-pyrazolo-[1,5-a] pyrimidine, which is also known as compound C [32], a specific AMPK inhibitor, to the cells to determine whether mTOR/S6K functions in Akt regulation by AMPK. Inhibition of AMPK activity by compound C, as reflected by decreased AMPK phosphorylation, induced a significantly enhanced phosphorylation of S6K1 and insulin-stimulated Akt S473 phosphorylation by ~1.6 and 3.3-fold, respectively (Fig. 5B). Furthermore, if S6K1 mediates the inhibitory effects of AMPK on Akt, downregulation of S6K1 activity should cause an elevated activity of Akt in response to compound C. In contrast, in our experiments, knockdown of S6K1 induced Akt phosphorylation in control cells, but not in compound C-treated cells with or without palmitate (Fig. 5B and C). These results suggest that mTORC1/S6K1 signaling is not involved in the process of Akt activity regulation by AMPK in both the absence and presence of palmitate-cultured cells.

3.6. S6K1 is responsible for the inhibition of SREBP-1c by AMPK in C2C12 myotubes

Consistent with our in vivo results, levels of SREBP-1c cleavage were also increased under lipid oversupply conditions in C2C12 cells (Fig. 6A), indicating that expression of the processed SREBP-1c is nutritionally responsive. As AMPK has recently been found to block the processing of SREBP isoforms [33], we next examined the effect of AMPK activation on SREBP-1c expression in C2C12 myotubes. As predicted, SREBP-1c cleavage was significantly decreased by AICAR treatment, indicating a reverse correlation between palmitate-induced SREBP-1c cleavage and AMPK activity (Fig. 6A). A significant body of evidence suggests that, in addition to reducing insulin signaling by promoting IRS-1 phosphorylation or degradation, mTORC1 hyperactivation by overfeeding may promote de novo lipogenesis by inducing SREBP-1c

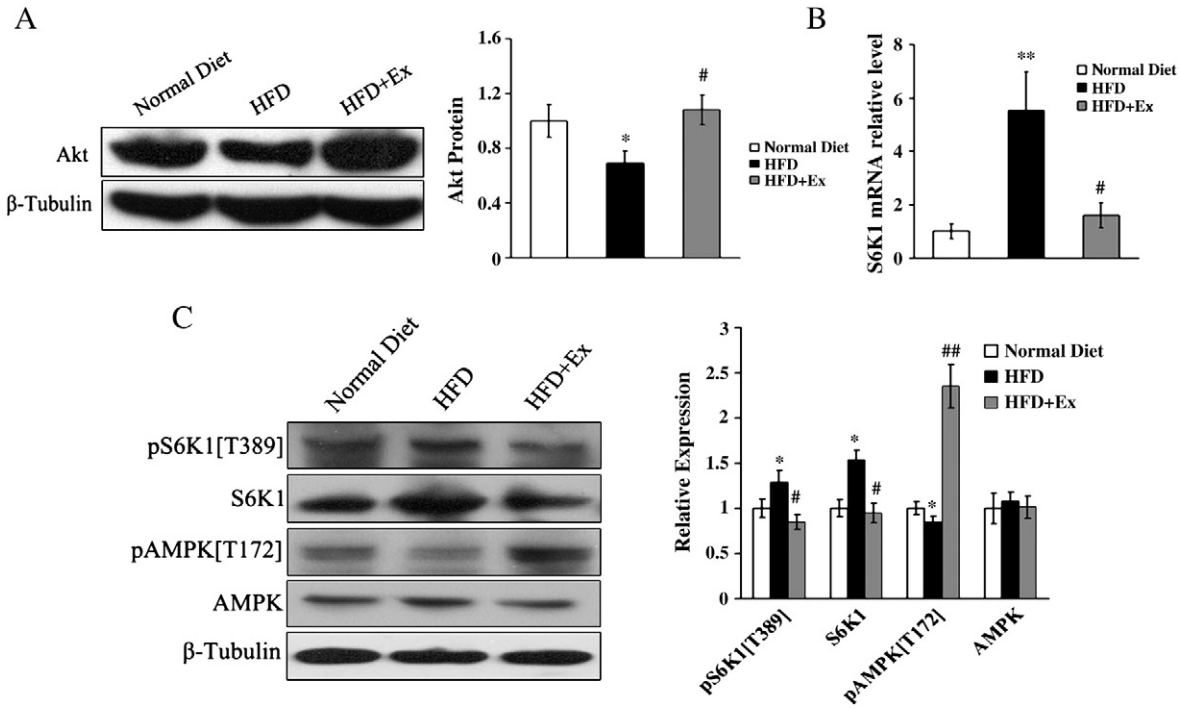

Fig. 3. Activity of Akt and AMPK/mTOR/S6K1 signaling axis in skeletal muscle of high-fat diet (HFD)-fed mice with or without training intervention. Western blot analysis of the expression levels of total Akt (A), S6K1, phospho-S6K1 on T389, AMPK, and phospho-AMPK on T172 (C) in skeletal muscle from HFD-fed or HFD-feeding combined exercise training (Ex) mice. Tubulin was used as the internal housekeeping protein control. Relative level of S6K1 mRNA expression (B) was quantified using real-time RT-PCR analysis. Results are means ± SEM (n = 6) and *P<0.05 vs. normal diet; **P<0.01 vs. normal diet; #P<0.05 vs. HFD; ##P<0.01 vs. HFD.

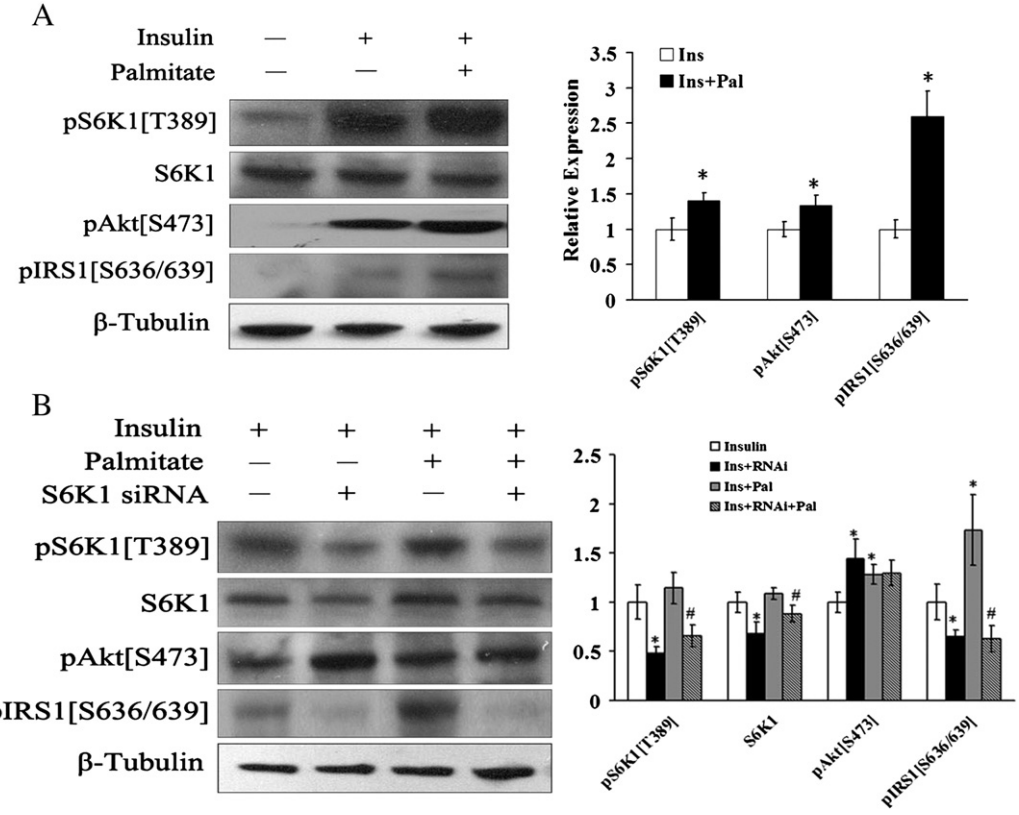

Fig. 4. Lipid-induced Akt phosphorylation is independent of mTORC1/S6K1 signaling pathway and IRS1. (A) Expression levels of S6K1, phosphor-S6K1 on T389, phosphor-Akt on S473, and phosphor-IRS1 on S636/639 in lysates of C2C12 cells. C2C12 myotubes were incubated in medium with or without 0.8 mM palmitate for 18 h, followed with or without 200 nM insulin stimulation for 30 min. *P<0.05 vs. Ins (Insulin). (B) Knockdown of S6K1 by RNA interference induced no further activation of Akt in C2C12 cells incubated with palmitate. C2C12 myotubes were transfected with S6K1 siRNA or negative control as indicated. Cells were incubated with or without 0.8 mM palmitate for 18 h followed by 200 nM insulin stimulation for 30 min. Tubulin was used as the internal housekeeping protein control. Data are means ± SEM of at least three independent experiments. *P<0.05 vs. Ins; #P<0.05 vs. Ins + Pal.

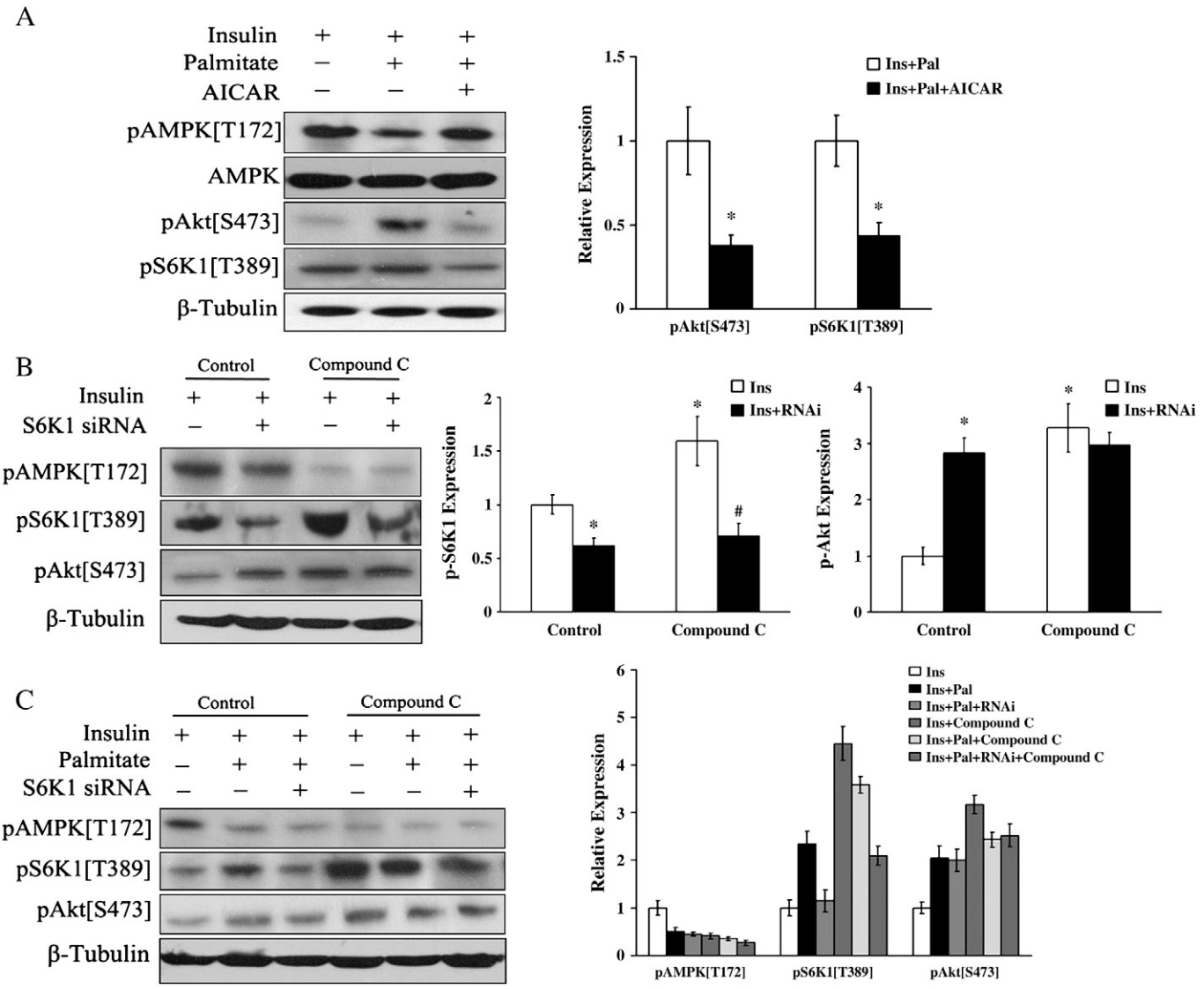

Fig. 5. mTORC1/S6K1 signaling was not involved in the process of Akt activity regulation by AMPK. (A) Activation of AMPK by AICAR decreased insulin-stimulated Akt phosphorylation. C2C12 myotubes were incubated in medium with or without 0.8 mM palmitate and 2 mM AICAR for 18 h, followed with 200 nM insulin (Ins) stimulation for 30 min. *$P<0.05$ vs. Ins + Pal. (B and C) mTORC1/S6K1 signaling was not involved in the process of Akt activity regulation by AMPK in both the absence (B) and presence (C) of palmitate-cultured cells. C2C12 myotubes were transfected with S6K1 siRNA or negative control as indicated. Then cells were cultured in media with indicated concentration of palmitate for 18 h in the presence or absence of compound C (60 μM), followed with 200 nM insulin stimulation for 30 min. Phosphorylation of proteins was determined by western blot using specific phosphospecific antibodies as indicated on the left of each strip. Results are means ± SEM and *$P<0.05$ vs. Ins; #$P<0.05$ vs. Ins + RNAi.

cleavage and activation [33,34]. Consistent with this possibility, in our experiments, knockdown of S6K1 as demonstrated by decreased protein levels of S6K1 and pS6K1 at T389 induced significantly decreased levels of processed active form of SREBP-1c protein in C2C12 cells (Fig. 6B).

Then the effect of AMPK on SREBP-1c cleavage and the role of mTOR/S6K1 signaling in these processes were determined in C2C12 myotubes under high palmitate plus insulin conditions. Inhibition of AMPK by compound C led to an elevated SREBP-1c expression by over 3-fold (Fig. 6C). This effect was blocked by siRNA targeting S6K1 (Fig. 6C). These findings indicate that AMPK is sufficient to suppress an SREBP-1c-dependent increase in de novo lipogenesis, at least in part, through the downregulation of mTOR/S6K1 signaling.

We also found that, as illustrated in Fig. 6D, activation of AMPK by AICAR led to an increased expression of CPT-1 in palmitate plus insulin treated C2C12 myotubes, while silencing of S6K1 with siRNA showed no effect on CPT-1 expression (Fig. 6D). To test the effect of mTOR/S6K1 signaling on lipogenic genes, we investigated the effects of silencing of S6K1 and activation of AMPK on ACC expression. As shown in Fig. 6D, expression of ACC protein was down-regulated in response to S6K1 RNAi treatment, indicating a positive correlation between mTOR/S6K1 signaling and ACC expression. Both S6K1 inhibition and AMPK activation increased ACC phosphorylation on serine 79, which inhibits its activity (Fig. 6D).

4. Discussion

Exercise training is known to activate a number of transcriptional regulators and kinases in skeletal muscles that contribute to metabolic reprogramming, among which AMPK has profound effects on skeletal muscle gene expression and oxidative metabolism [35,36]. Narkar et al. have validated the belief that orally active AMPK agonist AICAR is sufficient as a single agent to improve running endurance in nonexercised mice [37]. Indeed, since AMPK is traditionally described as a central integrator of signal that controls energy balance, this should come as no surprise. In vivo studies have also proved that activation of AMPK was associated with increased skeletal muscle insulin sensitivity and prevention of obesity-related complications [38]. AMPK plays key roles in glucose and lipid metabolism by promoting

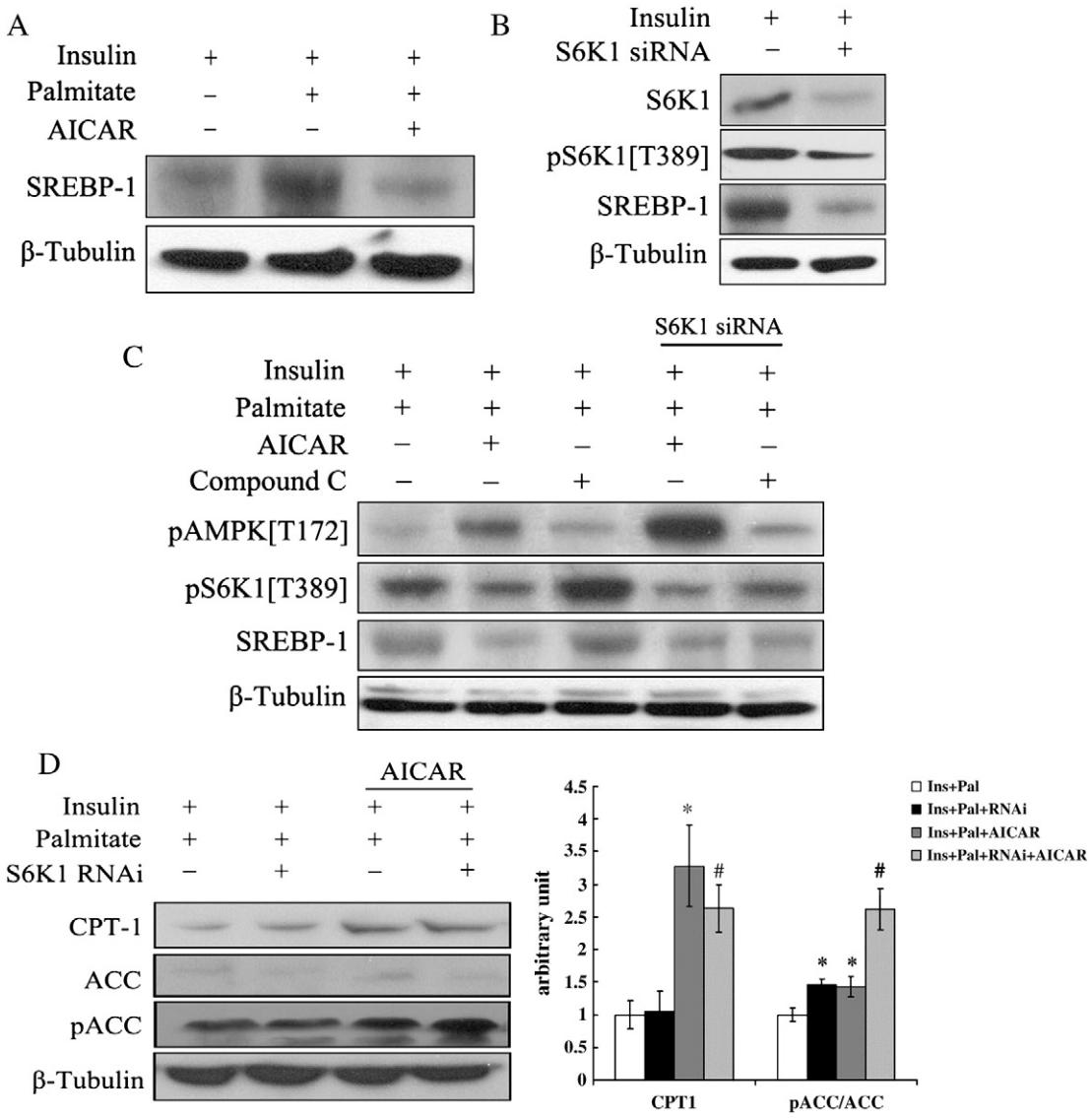

Fig. 6. S6K1 is responsible for the inhibition of SREBP-1c by AMPK. (A) AICAR decreased SREBP-1 cleavage in C2C12 myotubes. Experiments were similar to Fig. 5A. Levels of processed active form of SREBP-1c protein were determined. (B) Knockdown of S6K1 by siRNA inhibits SREBP-1c cleavage. C2C12 myotubes were transfected with S6K1 siRNA or negative control, followed with 200 nM insulin stimulation for 30 min. (C) Activation of SREBP-1c cleavage by AMPK inhibition is blocked by S6K1 RNAi. C2C12 myotubes were transfected with S6K1 siRNA or negative control. Then cells were incubated in 0.8 mM palmitate with or without 2 mM AICAR and 60 μM compound C for 18 h, followed with 200 nM insulin stimulation for 30 min. (D) Expression levels of CPT-1, ACC, and phosphor-ACC on S79 in lysates of C2C12 cells. C2C12 myotubes were transfected with S6K1 siRNA or negative control. Then cells were incubated in 0.8 mM palmitate (Pal) with or without 2 mM AICAR for 18 h, followed with 200 nM insulin (Ins) stimulation for 30 min. Results are means ± SEM and $^{*}P<0.05$ vs. Ins + Pal; #$P<0.05$ vs. Ins + Pal + RNAi.

mitochondrial biogenesis and fatty acid oxidation [37,39]. This link is likely to hold true in vivo, because regular aerobic exercise indeed results in elevated AMPK activity (Fig. 3C), as demonstrated by increased AMPK T172 phosphorylation, associated with increased glucose tolerance as determined by OGTT (Fig. 1E) and eliminated excess fat accumulation in skeletal muscle as determined by ORO staining (Fig. 2A).

Recent findings suggest that AMPK's metabolic effects might be mediated, at least in part, through modulation of mTORC1 activity. Over the past decade, mTOR kinase has emerged as an essential factor for cell metabolism, growth, and proliferation [5,6]. Feedback inhibition of the IRS1/Akt pathway by mTORC1/S6K1 has emerged as an important signaling event in insulin resistance [9]. However, it is becoming increasingly clear that mTOR also controls cell metabolism by promoting the activation of other anabolic processes, leading to the synthesis of many classes of lipids [34]. Given the potential for mTORC1 to both negatively regulate insulin action and positively regulate lipid biosynthesis, it is plausible to hypothesize that mTORC1/S6K1 signaling may be involved in the differential regulation of high-fat feeding and exercise training on insulin sensitivity in skeletal muscle. We demonstrated in vivo that mTORC1/S6K1 activity was up-regulated by chronic lipid oversupply and that these changes were completely reversed by exercise training (Fig. 3B and C). Results of the present investigation are also consistent with those of Rivas et al., who demonstrated that HFD-stimulated S6K1 activation was decreased following endurance training [13].

The hyperactivation of mTORC1 by nutrient excess has been implicated in decreased insulin signal transduction [9]. Recent in vitro works in skeletal muscle cell culture have directly implicated palmitate with the inhibition of insulin-stimulated glucose uptake through the activation of mTORC1 and its inhibition of insulin signaling [40]. Contrary to these reports, in the present study we observed an unexpected increase in palmitate-induced phosphorylation of S473 on Akt despite elevated S6K1 activity in C2C12 cells (Fig. 4A), which led to a concomitant increase in the serine phosphorylation of IRS1 (Fig. 4A). Discrepancies between Akt activation and upstream insulin signaling

have been reported in several different models of IR [41,42], suggesting that mTORC1/S6K1/IRS1-independent pathways also might contribute to insulin-stimulated Akt activation in response to palmitate. More interestingly, our data that increased Akt activity with palmitate treatment seems a paradoxical finding given the ability of the fatty acids to inhibit insulin action. We are not the first, however, to report an increased Akt activity in response to elevated fatty acid availability [13]. It is important to consider the fact that deleterious effects in skeletal muscle are frequently reported after prolonged exposure to elevated levels of fatty acids [43]. This increase in Akt activity induced by LCFA could serve as an acute protective mechanism against the potential toxic effects of elevated circulating concentrations of fatty acids. However, it still remains to be determined whether a sustained increase could be observed under conditions where prolonged exposure to lipid occurs. In our in vivo experiment, expression of Akt protein was decreased in skeletal muscle of HFD-feeding mice compared with mice maintained on a control diet, but was normalized by 6 weeks of exercise training (Fig. 3A). It is possible that the longer duration of high-fat feeding (10 weeks) used in the present study provided sufficient time to allow for the appearance of insulin resistance.

AMPK is a well-known physiological inhibitor of the energy-consuming mTOR signaling pathway [15–17]. Accordingly, AMPK activation in this study may be one possible mechanism to explain the inhibition of mTOR/S6K1 activity in response to exercise training. Indeed, when treating palmitate-incubated C2C12 myotubes with AMPK activator AICAR, we observed a significantly decreased mTORC1 activity (Fig. 5A). Furthermore, our results proved that activation of AMPK exerts an inhibitory effect on Akt and that this occurs independent of mTOR/S6K1 signaling (Fig. 5B and C). A previous report also found Akt to be dephosphorylated following AICAR treatment in C6 glioma cells [30], further verifying our conclusion. AMPK and Akt generally have opposing roles in cellular metabolism. AMPK is activated when AMP levels increase in conjunction with decreased ATP levels, and activated AMPK switches off ATP-consuming processes, such as glycogen, fatty acid, and protein synthesis pathways, and activates alternative pathways for ATP regeneration. Akt, on the other hand, generally promotes anabolic cellular functions that utilize ATP. Therefore, suppressing the activity of energy-consuming signaling pathways by AMPK to avoid the insurgence of conflicting metabolic signals act as a protective mechanism for survival of the energy-deprived cells.

In previous studies, exercise training resulted in increased [44], unchanged [45,46], or decreased [1] insulin-stimulated Akt Ser473 phosphorylation, indicating that Akt activation may not be necessary for exercise-induced insulin sensitization. As mentioned above, in addition to the S6K1-dependent negative-feedback mechanism in regulating insulin signaling activity, many lines of evidence suggest that mTORC1 also mediates the effect of AMPK on de novo lipogenesis [34]. As expected, we find that SREBP-1c, the gene involved in the regulation of fatty acid storage, is upregulated in palmitate-treated C2C12 myotubes, indicating an elevated lipogenesis in response to increased lipid availability. However, treating palmitate-incubated cells with AICAR reversed this effect (Fig. 6A). We prove that AMPK suppresses SREBP-1c, as well as its target enzyme ACC-dependent increase in de novo lipogenesis through the downregulation of mTOR/S6K1 signaling in C2C12 myotubes (Fig. 6C and D). Our results are in agreement with a recent study that has demonstrated that inhibition of mTORC1 with rapamycin blocks Akt-induced SREBP-1c nuclear localization and the expression of lipogenic genes [47]. Despite advances, the exact mechanism by which mTORC1 promotes SREBP-1c cleavage and nuclear localization in skeletal muscle remains to be determined.

Analogous to the effects of palmitate treatment on SREBP-1c expression in vitro, we demonstrated that SREBP-1c cleavage was increased in response to HFD-feeding in skeletal muscle but reversed by exercise training (Fig. 2C). Nutritional regulation of SREBP-1c expression is well described in liver [32] and adipose tissue [48]. The current study extends these observations to demonstrate that there is a significant stimulation of skeletal muscle SREBP-1c gene expression with HFD-feeding. The result that exercise-induced reduction in skeletal muscle SREBP-1c expression is in accordance with the study of Boonsong et al., who demonstrated that although SREBP-1c mRNA content was not affected by exercise, its protein level was lower in the exercised group than the control leg [49]. Several studies have also shown that muscle SREBP-1c exhibits responsiveness to acute exercise and training [50,51]. These observations of decreased muscle SREBP-1c expression and lipid storage in skeletal muscle (Fig. 2A) provide a potential mechanism for AMPK-dependent exercise training-induced insulin sensitization.

Prolonged exposure to elevated levels of lipid leads to impairment of the cellular capacity to oxidize LCFA and causes an increase in intracellular lipid accumulation, which could ultimately lead to lipotoxic effects in this tissue. Therefore, the effects of HFD and exercise training on components of the oxidative metabolism of fatty acids were further analyzed by measurement of the gene expression levels of selective biomarkers for fatty acid metabolism. As expected, we found that CPT1, the gene involved in fatty acid uptake, was upregulated by exercise training (Fig. 2C), indicating an elevated fatty acid transport into mitochondria. FAT/CD36 has been isolated in adipocytes [52] and on plasma membranes [53] and has been shown to influence the transport of LCFA across plasma membrane in skeletal tissue. In the present study, expression and translocation of FAT/CD36 to plasma membrane were both elevated in response to HFD feeding but were reversed by exercise training (Fig. 2C and D). In contrast, we found that the amount of FAT/CD36 that co-immunoprecipitated with CPT1 increased after endurance exercise training under the condition of high-fat feeding (Fig. 2E). It has been demonstrated that FAT/CD36 is present on mitochondrial membranes of rat and human skeletal muscle, and that the amount of FAT/CD36 that coimmunoprecipitated with CPT1 on mitochondrial membrane was strongly correlated with whole body fat oxidation [24]. Taken together, the increase in fatty acid oxidation after endurance exercise training might also be the mechanism responsible for improvements in insulin sensitivity and overall metabolic health.

In vitro study, we found that activation of AMPK induced an increased expression of CPT-1 protein and decreased ACC activity in palmitate pre-treated C2C12 myotubes (Fig. 6D), suggesting the beneficial lipid metabolic effects of AMPK in increasing fatty acid oxidation and decreasing lipogenesis. The mTOR/S6K1 signaling has been proved to be involved in regulating the process of lipogenesis through SREBP-1c. The AMPK-mTORC1 gauge adjusts muscle plasticity to environmental signals by altering the glycolytic-oxidative properties of the muscle [54]. The serine phosphorylation of IRS1 protein has been proposed to explain S6K1 effects on insulin sensitivity, although direct evidence is lacking. In the present study, we demonstrate that changes in the mTORC1/S6K1-mediated lipid metabolism may be one mechanism to explain the altered insulin sensitivity in response to HFD and aerobic exercise training. Furthermore, the impairments in the regulation of fuel metabolism caused by HFD could be referred to as “metabolic inflexibility”, which is defined as the impaired capacity to switch between fat and glucose as the primary fuel source [55]. Further verifying our hypothesis, chronic exercise training improves the capacity of skeletal muscle to utilize fatty acids for fuel during exercise, indicating an improved metabolic flexibility, thereby reducing lipid intermediates and improving insulin sensitivity [55]. In conclusion, our results suggest that the control of mTORC1/S6K1 activity by AMPK in response to regular aerobic exercise contributes to decreased intramyocellular lipid accumulation and muscle plasticity, providing a molecular mechanism that may represent an attractive target in metabolic syndromes.

Acknowledgements

This work was supported by grants (30871213 to L.F., 31100856 to YM.N) from the National Natural Science Foundation of China and

grants (09JCZDJC17400 to L.F., and 09ZCZDSF04500 to WY.N) from the Tianjin Municipal Science and Technology Commission. We thank Dr. Lei Cai (University of Kentucky, Lexington, KY) for kindly providing the FAT/CD36 primary antibody.

References

[1] S.J. Lessard, D.A. Rivas, Z.P. Chen, A. Bonen, M.A. Febbraio, D.W. Reeder, B.E. Kemp, B.B. Yaspelkis III, J.A. Hawley, Tissue-specific effects of rosiglitazone and exercise in the treatment of lipid-induced insulin resistance, Diabetes 56 (2007) 1856–1864.
[2] L. Fu, X.L. Liu, Y.M. Niu, H. Yuan, N. Zhang, E. Lavi, Effects of high-fat diet and regular aerobic exercise on global gene expression in skeletal muscle of C57BL/6 mice, Metabolism 61 (2011) 146–152.
[3] L.U. Monzillo, O. Hamdy, E.S. Horton, S. Ledbury, C. Mullooly, C. Jarema, S. Porter, K. Ovalle, A. Moussa, C.S. Mantzoros, Effect of lifestyle modification on adipokine levels in obese subjects with insulin resistance, Obes. Res. 11 (2003) 1048–1054.
[4] T.A. Buchanan, A.H. Xiang, R.K. Peters, S.L. Kjos, A. Marroquin, J. Goico, C. Ochoa, S. Tan, K. Berkowitz, H.N. Hodis, S.P. Azen, Preservation of pancreatic beta-cell function and prevention of type 2 diabetes by pharmacological treatment of insulin resistance in high-risk Hispanic women, Diabetes 51 (2002) 2796–2803.
[5] S. Marshall, Role of insulin, adipocyte hormones, and nutrient-sensing pathways in regulating fuel metabolism and energy homeostasis: a nutritional perspective of diabetes, obesity, and cancer, Sci. STKE 346 (2006) re7.
[6] S.H. Um, D. D'Alessio, G. Thomas, Nutrient overload, insulin resistance, and ribosomal protein S6 kinase 1, S6K1, Cell Metab. 3 (2006) 393–402.
[7] D.R. Alessi, L.R. Pearce, J.M. García-Martínez, New insights into mTOR signaling: mTORC2 and beyond, Sci. Signal. 67 (2009) pe27.
[8] L. Khamzina, A. Veilleux, S. Bergeron, A. Marette, Increased activation of the mammalian target of rapamycin pathway in liver and skeletal muscle of obese rats: possible involvement in obesity-linked insulin resistance, Endocrinology 146 (2005) 1473–1481.
[9] S.H. Um, F. Frigerio, M. Watanabe, F. Picard, M. Joaquin, M. Sticker, S. Fumagalli, P.R. Allegrini, S.C. Kozma, J. Auwerx, G. Thomas, Absence of S6K1 protects against age- and diet-induced obesity while enhancing insulin sensitivity, Nature 431 (2004) 200–205.
[10] R. Zoncu, A. Efeyan, D.M. Sabatini, mTOR: from growth signal integration to cancer, diabetes and ageing, Nat. Rev. Mol. Cell Biol. 12 (2011) 21–35.
[11] M. Kolak, J. Westerbacka, V.R. Velagapudi, D. Wågsäter, L. Yetukuri, J. Makkonen, A. Rissanen, A.M. Häkkinen, M. Lindell, R. Bergholm, A. Hamsten, P. Eriksson, R.M. Fisher, M. Oresic, H. Yki-Jarvinen, Adipose tissue inflammation and increased ceramide content characterize subjects with high liver fat content independent of obesity, Diabetes 56 (2007) 1960–1968.
[12] P.B. Dennis, A. Jaeschke, M. Saitoh, B. Fowler, S.C. Kozma, G. Thomas, Mammalian TOR: a homeostatic ATP sensor, Science 294 (2001) 1102–1105.
[13] D.A. Rivas, B.B. Yaspelkis III, J.A. Hawley, S.J. Lessard, Lipid-induced mTOR activation in rat skeletal muscle reversed by exercise and 50-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside, J. Endocrinol. 202 (2009) 441–451.
[14] G. Zhou, R. Myers, Y. Li, Y. Chen, X. Shen, J. Fenyk-Melody, M. Wu, J. Ventre, T. Doebber, N. Fujii, N. Musi, M.F. Hirshman, L.J. Goodyear, D.E. Moller, Role of AMP-activated protein kinase in mechanism of metformin action, J. Clin. Invest. 108 (2011) 1167–1174.
[15] R. Mounier, L. Lantier, J. Leclerc, A. Sotiropoulos, M. Foretz, B. Viollet, Antagonistic control of muscle cell size by AMPK and mTORC1, Cell Cycle 10 (2011) 2640–2646.
[16] K. Inoki, H. Ouyang, T. Zhu, C. Lindvall, Y. Wang, X. Zhang, Q. Yang, C. Bennett, Y. Harada, K. Stankunas, C.Y. Wang, X. He, O.A. MacDougald, M. You, B.O. Williams, K.L. Guan, TSC2 integrates Wnt and energy signals via a coordinated phosphorylation by AMPK and GSK3 to regulate cell growth, Cell 126 (2006) 955–968.
[17] D.M. Gwinn, D.B. Shackelford, D.F. Egan, M.M. Mihaylova, A. Mery, D.S. Vasquez, B.E. Turk, R.J. Shaw, AMPK phosphorylation of raptor mediates a metabolic checkpoint, Mol. Cell 30 (2008) 214–226.
[18] Y.C. Long, Z. Cheng, K.D. Copps, M.F. White, Insulin receptor substrates Irs1 and Irs2 coordinate skeletal muscle growth and metabolism via the Akt and AMPK pathways, Mol. Cell. Biol. 31 (2011) 430–441.
[19] P. Fernando, A. Bonen, L. Hoffman-Goetz, Predicting submaximal oxygen consumption during treadmill running in mice, Can. J. Physiol. Pharmacol. 71 (1993) 854–857.
[20] F. Xiao, Z. Huang, H. Li, J. Yu, C. Wang, S. Chen, Q. Meng, Y. Cheng, X. Gao, J. Li, Y. Liu, F. Guo, Leucine deprivation increases hepatic insulin sensitivity via GCN2/mTOR/S6K1 and AMPK pathways, Diabetes 60 (2011) 746–756.
[21] R. Koopman, G. Schaart, M.K. Hesselink, Optimisation of oil red O staining permits combination with immunofluorescence and automated quantification of lipids, Histochem. Cell Biol. 116 (2001) 63–68.
[22] J.D. McGarry, Banting lecture 2001: dysregulation of fatty acid metabolism in the etiology of type 2 diabetes, Diabetes 51 (2002) 7–18.
[23] Y.M. Niu, H. Yuan, L. Fu, Aerobic exercise's reversal of insulin resistance by activating AMPKα–ACC–CPT1 signaling in the skeletal muscle of C57BL/6 mice, Int. J. Sport Nutr. Exerc. Metab. 20 (2010) 370–380.
[24] S. Campbell, N.N. Tandon, G. Woldegiorgis, J.J. Luiken, J.F. Glatz, A. Bonen, A novel function for fatty acid translocase (FAT)/CD36: involvement in long chain fatty acid transfer into the mitochondria, J. Biol. Chem. 279 (2004) 36235–36241.
[25] J.D. Horton, J.L. Goldstein, M.S. Brown, SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver, J. Clin. Invest. 109 (2002) 1125–1131.
[26] A.R. Saltiel, C.R. Kahn, Insulin signalling and the regulation of glucose and lipid metabolism, Nature 414 (2001) 799–806.
[27] A.S. Deshmukh, J.T. Treebak, Y.C. Long, B. Viollet, J.F. Wojtaszewski, J.R. Zierath, Role of adenosine 5′-monophosphate-activated protein kinase subunits in skeletal muscle mammalian target of rapamycin signaling, Mol. Endocrinol. 22 (2008) 1105–1112.
[28] C.J. Homko, P. Cheung, G. Boden, Effects of free fatty acids on glucose uptake and utilization in healthy women, Diabetes 52 (2003) 487–491.
[29] D.M. Thomson, W.W. Winder, AMP-activated protein kinase control of fat metabolism in skeletal muscle, Acta Physiol. (Oxf.) 196 (2009) 147–154.
[30] R. Rattan, S. Giri, A.K. Singh, I. Singh, 5-Aminoimidazole-4-carboxamide-1-β-D-ribofuranoside inhibits cancer cell proliferation in vitro and in vivo via AMP-activated protein kinase, J. Biol. Chem. 280 (2005) 39582–39593.
[31] T.D. King, L. Song, R.S. Jope, AMP-activated protein kinase (AMPK) activating agents cause dephosphorylation of Akt and glycogen synthase kinase-3, Biochem. Pharmacol. 71 (2006) 1637–1647.
[32] G. Zhou, R. Myers, Y. Li, Y. Chen, X. Shen, J. Fenyk-Melody, M. Wu, J. Ventre, T. Doebber, N. Fujii, N. Musi, M.F. Hirshman, L.J. Goodyear, D.E. Moller, Role of AMP-activated protein kinase in mechanism of metformin action, J. Clin. Invest. 108 (2001) 1167–1174.
[33] Y. Li, S. Xu, M.M. Mihaylova, B. Zheng, X. Hou, B. Jiang, O. Park, Z. Luo, E. Lefai, J.Y. Shyy, B. Gao, M. Wierzbicki, T.J. Verbeuren, R.J. Shaw, R.A. Cohen, M. Zang, AMPK phosphorylates and inhibits SREBP activity to attenuate hepatic steatosis and atherosclerosis in diet-induced insulin-resistant mice, Cell Metab. 13 (2011) 376–388.
[34] M. Laplante, D.M. Sabatini, An emerging role of mTOR in lipid biosynthesis, Curr. Biol. 19 (2009) R1046–R1052.
[35] Z.P. Chen, T.J. Stephens, S. Murthy, B.J. Canny, M. Hargreaves, L.A. Witters, B.E. Kemp, G.K. McConell, Effect of exercise intensity on skeletal muscle AMPK signaling in humans, Diabetes 52 (2003) 2205–2212.
[36] R.M. Reznick, G.I. Shulman, The role of AMP-activated protein kinase in mitochondrial biogenesis, J. Physiol. 574 (2006) 33–39.
[37] V.A. Narkar, M. Downes, R.T. Yu, E. Embler, Y.X. Wang, E. Banayo, M.M. Mihaylova, M.C. Nelson, Y. Zou, H. Juguilon, H. Kang, R.J. Shaw, R.M. Evans, AMPK and PPARdelta agonists are exercise mimetics, Cell 134 (2008) 405–415.
[38] T.R. Koves, P. Li, J. An, T. Akimoto, D. Slentz, O. Ilkayeva, G.L. Dohm, Z. Yan, C.B. Newgard, D.M. Muoio, Peroxisome proliferator-activated receptor-gamma co-activator 1alpha-mediated metabolic remodeling of skeletal myocytes mimics exercise training and reverses lipid-induced mitochondrial inefficiency, J. Biol. Chem. 280 (2005) 33588–33598.
[39] N.B. Ruderman, A.K. Saha, E.W. Kraegen, Minireview: malonyl CoA, AMP-activated protein kinase, and adiposity, Endocrinology 144 (2003) 5166–5171.
[40] S. Mordier, P.B. Iynedjian, Activation of mammalian target of rapamycin complex 1 and insulin resistance induced by palmitate in hepatocytes, Biochem. Biophys. Res. Commun. 362 (2007) 206–211.
[41] T.G. Kurowski, Y. Lin, Z. Luo, P.N. Tsichlis, M.G. Buse, S.J. Heydrick, N.B. Ruderman, Hyperglycemia inhibits insulin activation of Akt/protein kinase B but not phosphatidylinositol 3-kinase in rat skeletal muscle, Diabetes 48 (1999) 658–663.
[42] Y.B. Kim, O.D. Peroni, T.F. Franke, B.B. Kahn, Divergent regulation of Akt1 and Akt2 isoforms in insulin target tissues of obese Zucker rats, Diabetes 49 (2000) 847–856.
[43] G. Boden, Interaction between free fatty acids and glucose metabolism, Curr. Opin. Clin. Nutr. Metab. Care 5 (2002) 545–549.
[44] A.V. Chibalin, M. Yu, J.W. Ryder, X.M. Song, D. Galuska, A. Krook, H. Wallberg-Henriksson, J.R. Zierath, Exercise-induced changes in expression and activity of proteins involved in insulin signal transduction in skeletal muscle: differential effects on insulin-receptor substrates 1 and 2, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 38–43.
[45] J.F. Wojtaszewski, B.F. Hansen, Gade, B. Kiens, J.F. Markuns, L.J. Goodyear, E.A. Richter, Insulin signaling and insulin sensitivity after exercise in human skeletal muscle, Diabetes 49 (2000) 325–331.
[46] C.Y. Christ, D. Hunt, J. Hancock, R. Garcia-Macedo, L.J. Mandarino, J.L. Ivy, Exercise training improves muscle insulin resistance but not insulin receptor signaling in obese Zucker rats, J. Appl. Physiol. 92 (2002) 736–744.
[47] T. Porstmann, C.R. Santos, B. Griffiths, M. Cully, M. Wu, S. Leevers, J.R. Griffiths, Y.L. Chung, A. Schulze, SREBP activity is regulated by mTORC1 and contributes to Akt-dependent cell growth, Cell Metab. 8 (2008) 224–236.
[48] J.B. Kim, P. Sarraf, M. Wright, K.M. Yao, E. Mueller, G. Solanes, B.B. Lowell, B.M. Spiegelman, Nutritional and insulin regulation of fatty acid synthetase and leptin gene expression through ADD1/SREBP1, J. Clin. Invest. 101 (1998) 1–9.
[49] T. Boonsong, L. Norton, K. Chokkalingam, K. Jewell, I. Macdonald, A. Bennett, K. Tsintzas, Effect of exercise and insulin on SREBP-1c expression in human skeletal muscle: potential roles for the ERK1/2 and Akt signalling pathways, Biochem. Soc. Trans. 35 (2007) 1310–1311.
[50] S. Ikeda, H. Miyazaki, T. Nakatani, Y. Kai, Y. Kamei, S. Miura, N. Tsuboyama-Kasaoka, O. Ezaki, Up-regulation of SREBP-1c and lipogenic genes in skeletal muscles after exercise training, Biochem. Biophys. Res. Commun. 296 (2002) 395–400.
[51] R.J. Tunstall, K.A. Mehan, G.D. Wadley, G.R. Collier, A. Bonen, M. Hargreaves, D. Cameron-Smith, Exercise training increases lipid metabolism gene expression in human skeletal muscle, Am. J. Physiol. Endocrinol. Metab. 283 (2002) E66–E72.

[52] N.A. Abumrad, M.R. el-Maghrabi, E.Z. Amri, E. Lopez, P.A. Grimaldi, Cloning of a rat adipocyte membrane protein implicated in binding or transport of long-chain fatty acids that is induced during preadipocyte differentiation. Homology with human CD36, J. Biol. Chem. 268 (1993) 17665–17668.
[53] A. Bonen, M. Parolin, G. Steinberg, J. Calles-escandon, G. Heigenhauser, D. Dyck, Triacylglycerol accumulation in human obesity and type 2 diabetes is associated with increased rates of skeletal muscle fatty acid transport and increased sarcolemmal FAT/CD36, FASEB J. 18 (2004) 1144–1146.
[54] V. Aguilar, S. Alliouachene, A. Sotiropoulos, A. Sobering, Y. Athea, F. Djouadi, S. Miraux, E. Thiaudière, M. Foretz, B. Viollet, P. Diolez, J. Bastin, P. Benit, P. Rustin, D. Carling, M. Sandri, R. Ventura-Clapier, M. Pende, S6 kinase deletion suppresses muscle growth adaptations to nutrient availability by activating AMP kinase, Cell Metab. 5 (2007) 476–487.
[55] E. Corpeleijn, W.H. Saris, E.E. Blaak, Metabolic flexibility in the development of insulin resistance and type 2 diabetes: effects of lifestyle, Obes. Rev. 10 (2009) 178–193.