SREBP-1 Transcription Factors Regulate Skeletal Muscle Cell Size by Controlling Protein Synthesis through Myogenic Regulatory Factors

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Introduction

Loss of skeletal muscle mass occurs during aging (sarcopenia), disease (cachexia), or inactivity (atrophy) and results from an imbalance between the rate of muscle protein synthesis and degradation [1]. In a previous study, we identified SREBP-1 transcription factors as regulators of muscle mass, showing that increasing SREBP-1 nuclear content induces both in vitro and in vivo muscle cell atrophy [2]. The Sterol Regulatory Element Binding Proteins (SREBP) transcription factors belong to the basic helix-loop-helix leucine zipper family of DNA binding proteins [3]. The three isoforms are encoded by two distinct genes, Srebf1 and Srebf2, and vary in structure, regulation, and functions: SREBP-1a and SREBP-1c proteins are key actors of the regulation of genes related to lipid metabolism, whereas SREBP-2 has been more closely associated to cholesterol synthesis and accumulation [4].

Beyond their strong expression in tissues with high lipogenic capacities like liver and adipose tissues, the SREBP-1 proteins are ubiquitous transcription factors with significant expression in muscle cells [5,6]. In muscle, SREBP-1 expression is induced by activation of the PI3K/Akt and the MAP kinase pathways by insulin and growth factors [6–10], suggesting additional functions of these transcription factors in a tissue with a low rate of lipid synthesis. Several studies have been performed to identify SREBP-1 target genes using microarray analysis [11–14] and to characterize the role of SREBP-1a and -1c in skeletal muscle [2,15,16].

In SREBP-1 overexpressing cells, we have previously observed a combined decrease in the expression of the myogenic regulatory factors (MRFs) MYOD1, MYOG and MEF2C [2]. The MRFs are muscle-specific transcription factors that induce the expression of muscle-specific genes during the myogenic differentiation process [17,18]. They are also still expressed in adult fibres and differentially accumulate in the various fibre types [19,20]. MRFs may be required to maintain muscle homeostasis, and may play a role in muscle plasticity in response to both hypertrophic (ie exercise) and atrophic (ie denervation) stimuli [21–23].

We explored here the molecular mechanisms by which SREBP-1 proteins affect the size of differentiated myotubes, and the involvement of MRFs in this process. We investigated in particular the role of MRFs in the regulation of protein synthesis, and in the expression of ubiquitin ligases that control muscle protein degradation.

Materials and Methods

Culture of Skeletal Muscle Cells

For human primary myotubes, muscle biopsies were taken from healthy lean subjects who participated in a global study on insulin action on gene expression. All participants gave their written consent after being informed of the nature, purpose and possible risks of the study. The experimental protocol (‘Clamp-Gene...
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Fusion Index

Cultures were fixed and myotubes were immunostained using anti-myosin antibody [25]. Nuclei were labelled with DAPI using Vectashield mounting medium for fluorescence (Vector, USA). Nuclei were counted at least in eight randomly chosen microscope fields (2 culture dishes for each of the experimental condition, 4 fields in each dish) at a magnification of x100. One microscope field usually contained between 200 and 400 nuclei. The fusion index is defined as the number of nuclei in myotubes divided by the total number of nuclei.

Expression Vectors and Generation of Recombinant Adenoviruses

Construction of expression vectors encoding mature nuclear forms of human SREBP-1a and SREBP-1c was described previously. Recombinant adenoviruses MYOG or MEF2C were generated using the same procedure [7]. Recombinant adenovirus expressing the human MYOD1 protein is a generous gift from Dr Teruhisa Miike (Department of Child Development, Kunamoto University, Japan) [26].

Inhibition of SREBP-1 Expression in Human Muscle Cells

Inhibition of SREBP-1 expression was performed by RNA interference. SiRNA against SREBP-1 (SI02662877) and control (Allstars negControl) were from Qiagen (Courtaboeuf, France). Myotubes were transfected for 48 h with siRNA at 20 nM final using the Hiperfect transfection reagent (Qiagen) according to the manufacturer’s protocol.

Measurement of Protein Synthesis

Myotubes were infected for 48 h with adenovirus overexpressing GFP, SREBP-1a or SREBP-1c with or without MYOD1, MEF2C or MYOG, and protein synthesis rates were assayed as described [25]. Briefly, culture medium was then replaced by experimental medium containing 2 mCi/mL of [3H]-L-Tyrosine and non-radioactive tyrosine up to 2 mM, for 2 hours. Culture monolayer was washed five times with ice-cold PBS. Cells were scraped in lysis buffer, an aliquot was taken for Bradford protein analysis and the rest of protein was precipitated with 10% trichloroacetic acid (TCA). Samples were incubated at least 1 hour at 4°C and then centrifuged at 12,000 g for 10 min to separate pellet containing labelled neosynthetized proteins from supernatant, containing the pool of non-incorporated [3H]-L-Tyrosine. Pellet was then dissolved in basic buffer. Determination of radioactivity was performed in a Packard liquid-scintillation spectrometer.

Measurement of Protein Degradation

Rates of protein degradation were assayed according to [25,27] by monitoring the release of TCA-soluble radioactivity in the culture medium at defined time. Proteins were radiolabelled by incubating the cells with 2 μCi/mL of [3H]-L-Tyrosine in differentiating conditions for 2 days. Human myotubes were then infected with adenovirus overexpressing GFP, SREBP-1a or SREBP-1c for 48 h. Proteolysis was evaluated as the percentage of TCA-soluble radioactivity released in the medium reported to total incorporated radioactivity.

Protein Expression Analysis by Immunocytofluorescence

Measurement of the area of immunofluorescence-labelled myotubes was performed as previously fully described [25].

Protein Expression Analysis by Western Blotting

Cells were harvested, extracted and immunoblotted as previously described [7]. The total cell lysates were immunoblotted with the following antibodies: anti-FoxO1 (#05-1075), anti-phospho(Ser256)FoxO1 (#9461), anti-phospho(Ser253)FoxO3a (#9466) and anti-MEF2C (#5030) were from Cell Signaling Technology (Beverly, MA, USA); anti-FOXO3a (#04-1007) from Millipore (Billerica, MA, USA); anti-MYOD1 (#sc760), anti-MYOG (#sc576) and anti-TroponinI-SS (#sc8119) from Santa Cruz Biotechnology (Santa Cruz, CA, USA); anti-MURF1 (ab77577) from Abcam Biotechnology (Cambridge, UK); and anti-alpha-tubulin (T5168) from Sigma (L’Isle-d’abeau, France); and anti-sarcosomic MHC (MF-20) were from the Developmental Studies Hybridoma Bank (Iowa City, IO, USA).

Luciferase Assay

The MuRF1 reporter plasmid was constructed by ligating PCR fragments of the human genomic region upstream of the transcription start site of the MuRF1 gene into the pGL3-Enhancer reporter (Promega). The MuRF1 genomic fragment was generated by PCR (primers upon request at lefai@univ-lyon1.fr) to obtain the construction −1337/+90 (according to the transcription starting site). Next, deletions were performed on the −1337/+90 construct to obtain the −540/90 and −237/+90 constructs. Cell transfection and luciferase assays were performed as previously described [7].

Quantiﬁcation of mRNAs by Real-time RT-PCR

Total RNA was isolated and ﬁrst-strand cDNAs were synthesized as previously described [7]. A list of the primers and real-time PCR assay conditions are available upon request (lefai@univ-lyon1.fr). The results were normalized using RPLP0 mRNA concentration, measured as reference gene in each sample.

Results

Overexpression of SREBP-1 in Muscle Cells Reduces Protein Synthesis

To decipher the molecular mechanisms by which SREBP-1 overexpression induces atrophy in muscle cells, we ﬁrst examined the impact of SREBP-1a and -1c overexpression on protein synthesis and degradation rates (Figure 1). In human primary differentiating myotubes, overexpression of both SREBP-1a and SREBP-1c signiﬁcantly decreased protein synthesis rates (−33%, p = 0.002 and −26%, p = 0.003 respectively), compared to GFP overexpression (Figure 1A). Under the same conditions, a slight decrease in protein degradation rates was also observed, (−8% and −10% respectively) (Figure 1B). The amplitude of changes in protein synthesis and degradation we observed thus indicates that SREBP-1 induced atrophy is due to an inhibition of muscle protein synthesis.

Forced Expression of MRFs Reverses the SREBP-1 Effects on Muscle Cells

We previously demonstrated that SREBP-1 overexpression inhibits the expression of several MRFs through an induction of...
both BHLHB2 and BHLHB3 transcriptional repressors [2]. We
examined here if forced expression of these MRFs in SREBP-1
overexpressing myotubes could reverse the induced atrophy. As
shown in Figure 2, immunostaining of myotubes reveals that
MEF2C, MYOD1 and MYOG, when co-expressed with either
SREBP-1a or -1c, restored almost completely the expression of
sarcomeric myosin heavy chain protein (Figure 2A). In all cases,
when lowered MRFs expression was compensated for by viral
expression, the SREBP-1 induced atrophy was limited as shown by
the preservation of myotube area (Figure 2B).

We next quantified the fusion index (ratio of the number of
nuclei in myotubes to the total number of nuclei) in all the
conditions where SREBP-1 and/or MRFs were overexpressed
(Figure 2C). We first observed that the overexpression of either
SREBP-1a or -1c induced a significant decrease in fusion index
(Figure 2C, control). A 15% loss of nuclei in SREBP-1
overexpressing myotubes is observed when myotube areas
showed a reduction of 40% (Figure 2B). Notably, when
overexpressed alone, none of the studied MRFs increased the
fusion index compared to GFP (white bars), suggesting that the
in vitro differentiation was complete and that the remaining
myoblasts were unable to fuse with myotubes. Finally, when
SREBP-1 proteins were co-expressed with MRFs, the atrophy
was prevented and the decrease in fusion index was abolished
(black and grey bars). Thus, the restoration of MRF expression
in SREBP-1 overexpressing myotubes can prevent the atrophic
process and inhibit the loss of nuclei in differentiated myotubes.

### Inhibition of SREBP-1 Expression Increases Protein
Synthesis and Degradation

As overexpression of SREBP-1 proteins mainly affected protein
synthesis, and more mildly decreased protein degradation
(Figure 1), we next studied the effects of siRNA mediated
SREBP-1 depletion on protein metabolism, with or without
MRF overexpression. As shown in Figure 3, compared to control
conditions, overexpression of MRFs increased protein synthesis
and decreased protein degradation (white bars). When the
expression of SREBP-1 was inhibited, protein synthesis was higher
compared to control siRNA conditions (Figure 3A). The same was
observed for protein degradation (Figure 3B), with SREBP-1
extinction inducing a slight but significant increase. As the results
of SREBP-1 down-regulation were opposite of those of SREBP-1
overexpression, we then conclude that changes in SREBP-1
protein content, either positive or negative, can modulate protein
turnover in myotubes.

### Forced Expression of MRFs Prevents the SREBP-1s
Induced Decrease in Protein Synthesis

To explore the mechanisms by which MRFs can counteract the
SREBP-1 induced reduction in cell size, we measured protein
synthesis rates in conditions in which expression of each MRF was
restored. As shown in Figure 4A, the dramatic decrease in protein
synthesis rate induced by SREBP-1 was partly abolished when the
expression of MEF2C, MYOD1 or MYOG was maintained. The
absence of decrease in protein synthesis rate was particularly
evident when MYOG expression was maintained, with no
differences in protein synthesis whether SREBP-1a, SREBP-1c
or GFP were overexpressed.

To evaluate the impact of MRFs on sarcomeric protein
content, we next quantified protein levels of Troponin I slow-
twitch isoform (TNNI1), in different conditions. As observed for
MHC expression (Figure 2A), TNNI1 expression quantified by
western blotting was significantly decreased when SREBP-1
were expressed alone, but the decrease was reduced and non
significant when one of the MRFs was co-expressed with
SREBP-1 (Figures 4B and 4C). We thus conclude that the
decrease in protein synthesis rate in atrophic muscle cells
overexpressing SREBP-1 is mainly due to the inhibition of the
expression of MRFs, and that maintenance of MRFs can
protect muscle specific gene expression from the inhibition
induced by SREBP-1 increase.
We previously observed that SREBP-1 overexpression led to opposite regulation of FBXO32 (Atrogin-1) and TRIM63 (MuRF1), the two E3-ubiquitin ligases involved in muscle protein degradation through the Ubiquitin Proteasome System (UPS) [2]. In SREBP-1 overexpressing myotubes, TRIM63 mRNA expression is enhanced in conditions in which MRFs expression is abolished [2], and we verified here that the protein levels were also increased (Figure 5A). To confirm this MRF-independent activation of TRIM63 by SREBP-1, we performed luciferase assays to measure the human TRIM63 proximal promoter activities (Figure 5B). We identified a 303 bp region (−540 to −237) as the main region mediating the effects of SREBP-1s. This region contains a Sterol Regulatory Element.
putative motif [28] and E-boxes. In addition, the proximal promoter contains several MRF motifs, suggesting a possible direct positive regulation by MRFs. However, re-expression of MRFs did not modify TRIM63 expression in SREBP-1 overexpressing myotubes (Figure 5C), showing that the promoter is not controlled by MRFs, and might instead be directly activated by SREBP-1.

By contrast, SREBP-1 overexpression induced a decrease in FBXO32 expression [2]. As MYOG is known to bind atrogenic promoters and activate atrogenic expression [29,30], we measured mRNA levels of FBXO32 when MRFs were re-expressed in the presence of overexpressed SREBP-1. As shown in Figure 5C (right panel), none of them could restore FBXO32 expression in SREBP-1 induced atrophy. We next examined the expression and phosphorylation status of FOXO1 and FOXO3 transcription factors, which are known to be major transcriptional regulators of FBXO32 expression. As shown in Figure 5D, both SREBP-1a and -1c induced a decrease in the total amount of FOXO proteins together with a significant increase in their phosphorylation. Under SREBP-1 overexpression, independently of MRFs expression, FOXO proteins are thus excluded from the nucleus, which likely explains the observed decrease in FBXO32 expression.

Discussion

SREBP-1 transcription factors are known to regulate expression of hundreds of genes in liver [12,13], fibroblasts [11] and muscle cells [15]. In muscle, we previously reported that their overexpression leads to atrophy of both differentiated myotubes in vitro, and tibialis muscle in vivo, via the induction of BHLHB2/B3 transcriptional repressors, and that the expression of MRFs was drastically reduced in these conditions [2]. MRFs are still expressed in differentiated myotubes [21,31], and participate in the expression of sarcomeric proteins [32]. MYOG is involved in the maintenance of the neuromuscular junction [30,33]. It has also been shown that inhibiting MYOD1 degradation can maintain the muscle mass in conditions of FBX032 accumulation [34]. In the present work, we determined the impact of SREBP-1 overexpression on protein metabolism in differentiated myotubes, and we investigated the role of MRF repression in the atrophic response, as evaluated by the reduction in cell size and by the loss of muscle-specific proteins.

In our SREBP-1 induced atrophy model, we observed that the reduction in myotube size could be attributed to a marked decrease in protein synthesis not compensated for by a small decrease in proteolysis. Conversely, the inhibition of SREBP-1 expression affected protein synthesis and degradation rates in an
opposite manner, indicating that both an increase and a decrease in SREBP-1 nuclear content modulate muscle protein content.

The SREBP-1 induced atrophy was also characterized by a loss of myonuclei that accompanied the decrease in protein content. The loss of nuclei (mainly by apoptosis) during atrophic process has been extensively documented, even if the opposite (i.e. conservation of nuclei number) is also described in vivo (recently discussed in [35]). In our model, whether the number of nuclei in myotubes is directly controlled by MRFs, or whether the loss is a consequence of the dramatic decrease in cytoplasm volume...
remains to be determined. Nevertheless, when MRF expression was maintained in SREBP-1 overexpressing myotubes, the loss of myonuclei was prevented, excluding a direct effect of SREBP-1 on transcription factors in pathological situations inducing muscle wasting, and also to evaluate their impact on muscle tissue in pathological situations.

SREBP-1 proteins also tended to decrease protein degradation rate, but examination of the atrogenes TRIM63 and FBXO32 showed that SREBP-1 promoted a dramatic increase in TRIM63 expression together with a decrease in FBXO32. These changes in expression remained when MRFs expression was restored and the atrophy was prevented. We can then exclude the participation of MRfs in the SREBP-1 regulation of atrogenes. We conclude that the main factor inducing myotube atrophy under SREBP-1 overexpression is the inhibition of MRF expression by the SREBP-1/BHLH2/B3 pathway, and that MRF expression in differentiated myotubes is necessary for the maintenance of mature fibre size and protein content.

The control of the amount of SREBP-1 proteins in the nucleus involves regulation at several levels, including SREBP-1 gene expression, proteolytic cleavage in the endoplasmic reticulum, nuclear import and activation/degradation within the nucleus (for review see [4]). It has been recently demonstrated in liver that SREBP-1 expression is enhanced through the PKB/mTOR pathway [36,37], and repressed through the AMPK pathway [38]. SREBP-1 is also a target of the deacetylase SIRT1, leading to an inhibition of its transcriptional activity [39,40]. Whether these regulations have the same relevance in muscle tissue remains to be elucidated, but SREBP-1 could thus integrate signalling from nutrient sensing, energetic status and metabolic requirements towards muscle mass regulation, in pathways involving MRfs.

It will therefore be of particular interest to further study these transcription factors in pathological situations inducing muscle wasting, and also to evaluate their impact on muscle tissue in metabolic diseases where abnormalities in SREBP-1 have already been reported, such as insulin-resistance and type 2 diabetes [5,41,42].

Author Contributions

Conceived and designed the experiments: CS EL. Performed the experiments: KD JD SC. Analyzed the data: KD VE SR GN CS. Contributed reagents/materials/analysis tools: IF. Wrote the paper: KD HV GN EL.

References

1. Evans WJ (2010) Skeletal muscle loss: cachexia, sarcopenia, and inactivity. Am J Clin Nutr 91: 112S–1127S.
2. Lecomte V, Meugnier E, Euthine V, Durand C, Freysenet D, et al. (2010) A new role for sterol regulatory element binding protein 1 transcription factors in the regulation of muscle mass and muscle cell differentiation. Mol Cell Biol 30: 1192–1198.
3. Horton JD (2002) Sterol regulatory element-binding proteins: transcriptional activators of lipid synthesis. Biochem Soc Trans 30: 1091–1095.
4. Raghu R, Yellaturu C, Deng X, Park EA, Elam MB (2008) SREBPs, the crossroads of physiological and pathological lipid homeostasis. Trends Endocrinol Metab 19: 65–75.
5. Duchezau PH, Perretti N, Laville M, Andreffl E, Vegas N, et al. (2001) Regulation by insulin of gene expression in human skeletal muscle and adipose tissue. Evidence for specific defects in type 1 diabetes. Diabetes 50: 1134–1142.
6. Guillet-Deniau I, Mieulet V, Le Lay S, Achouri Y, Carre D, et al. (2002) Sterol regulatory element binding protein-1c expression and action in rat muscles: insulin-like effects on the control of glycolytic and lipogenic enzymes and UCP3 gene expression. Diabetes 51: 1722–1728.
7. Di N, Euthine V, Gonnert E, Laville M, Vital H, et al. (2006) Insulin activates human sterol regulatory-element-binding protein-1c promoter through SRE motifs. Biochem J 400: 179–180.
8. Boomsong T, Norton L, Chokkalingam K, Jewell K, Macdonald I, et al. (2007) 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: 1310–1311.
9. Kozuka J, Muller-Wieland D, Roth G, Kremer I, Mueck M, et al. (2000) Sterol regulatory element binding proteins (SREBP)-1a and SREBP-2 are linked to the MAP kinase cascade. J Lipid Res 41: 99–106.
10. Nadeau KJ, Leitner JW, Gurerich I, Draznin B (2004) Insulin regulation of sterol regulatory element-binding protein-1 expression in L-6 muscle cells and 3T3-L1 adipocytes. J Biol Chem 279: 34330–34331.
11. Kallin A, Johanssen LE, Cani PD, Muller-Wieland D, Rodth L, et al. (2010) SREBP-1 regulates the expression of heme oxygenase 1 and the phosphotyrosine phosphatase 1c gene in skeletal muscle cells. Acta Physiol Scand 180: 281–289.
12. Im SS, Hammond LE, Yousef L, Nugas-Selby C, Shin DJ, et al. (2009) Sterol regulatory element-binding protein-1a and SREBP-1c target genes identify new regulatory pathways in muscle. Physiol Genomics 34: 327–337.
13. Abiola M, Favier M, Christodoulou-Vafeiadou E, Pichard AL, Martelly I, et al. (2009) Activation of Wnt/beta-catenin signaling increases insulin sensitivity through a reciprocal regulation of Wnt10b and SREBP-1c in skeletal muscle cells. PLoS One 4: e8509.
14. Edmondson DG, Olson EN (1993) Helix-loop-helix proteins as regulators of muscle-specific transcription. J Biol Chem 268: 735–758.
15. Kitzmann M, Carnac G, Vandromme M, Primig M, Lamb NJ, et al. (1998) The muscle regulatory factors SREBP-1 and SREBP-2 downstream of distinct cell cycle-specific expression in muscle cells. J Cell Biol 142: 1447–1459.
16. Hughes SM, Taylor JM, Tapsf SJ, Gurley GM, Carter WJ, et al. (1993) Selective accumulation of MyoD and myogenin mRNAs in fast and slow adult skeletal muscle is controlled by innervation and hormones. Development 118: 1137–1147.
17. Hughes SM, Koishi K, Rudnicki M, Maggs AM (1997) MyoD protein is differentially accumulated in fast and slow skeletal muscle fibres and required for normal fibre type balance in rodents. Mech Dev 61: 151–163.
18. Walters EH, Stickland NC, Loughna PT (2000) The expression of the myogenic regulatory factors in denervated and normal muscles of different phenotypes. J Muscle Res Cell Motil 21: 647–653.
19. Ishido M, Kami K, Masahara M (2004) In vivo expression patterns of MyoD, p21 and Myf5 in muscles and satellite cells of denervated rat skeletal muscle. Am J Physiol Cell Physiol 284: C484–C493.
20. Ishido M, Kami K, Masahara M (2004) Localization of MyoD, myogenin and cell cycle regulatory factors in hypothyroid rat skeletal muscles. Acta Physiol Scand 180: 281–289.
21. Cozzone D, Debard C, Di N, Ricard N, Disse E, et al. (2006) Activation of liver X receptors promotes lipid accumulation but does not alter insulin action in human skeletal muscle cells. Diabetologia 49: 990–999.
22. De Larichaudy J, Zulferi A, Serra F, Josidi AM, Naro F, et al. (2012) TNFalpha- and tumor-induced skeletal muscle atrophy involves sphingolipid metabolism. Skelet Muscle 2: 2.
23. Fuji I, Matsuoka I, Ikekawa M, Suzuki S, Shimada T, et al. (2006) Adenoviral mediated MyoD gene transfer into fibroblasts: myogenic disease diagnosis. Brain Dev 28: 420–425.
24. Galve EA, Dice JF (1989) Regulation of protein synthesis and degradation in L3 myotubes. Effects of serum, insulin and insulin-like growth factors. Biochem J 260: 377–387.
25. Aminssina-Kawo M, Shimano H, Hasty AH, Yagaeh N, Yoshikawa T, et al. (2002) Selective accumulation of nuclear SREBP-1a, -1c, and -2 to different target promoters of lipogenic and cholesterologenic genes. J Lipid Res 43: 1220–1235.
26. Macpherson PC, Wang X, Goldman D (2011) Myogenin regulates differentiation-dependent muscle atrophy in mouse soleus muscle. J Cell Biochem 112: 2149–2159.
27. Moreisi V, Williams AH, Meadowes E, Flynn JM, Pothoff MJ, et al. (2010) Myogenin and class II HDACs control neurogenic muscle atrophy by inducing E3 ubiquitin ligases. Cell 143: 33–45.
28. Charge SB, Brack AS, Bayd SA, Hughes SM (2008) MyoD- and nerve-dependent maintenance of MyoD expression in mature muscle fibres acts through the DRR/PRR element. BMC Dev Biol 8: 5.
32. Li H, Capetanaki Y (1993) Regulation of the mouse desmin gene: transactivated by MyoD, myogenin, MRF4 and Myf5. Nucleic Acids Res 21: 335–343.
33. Merlie JP, Mudd J, Cheng TC, Olson EN (1994) Myogenin and acetylcholine receptor alpha gene promoters mediate transcriptional regulation in response to motor innervation. J Biol Chem 269: 2461–2467.
34. Lagirand-Cantaloube J, Cornille K, Cabi A, Batonnier-Pichon S, Leibovitch MP, et al. (2009) Inhibition of atrogin-1/MAFbx mediated MyoD proteolysis prevents skeletal muscle atrophy in vivo. PLoS One 4: e973.
35. Bruusgaard JC, Egner IM, Larsen TK, Dupre-Aucouturier S, Desplanches D, et al. (2012) No change in myonuclear number during muscle unloading and reloading. J Appl Physiol 113: 290–296.
36. Peterson TR, Sengupta SS, Harris TE, Carmack AE, Kang SA, et al. (2011) mTOR complex 1 regulates lipin 1 localization to control the SREBP pathway. Cell 146: 408–420.
37. Porstmann T, Santos CR, Griffiths B, Cully M, Wu M, et al. (2008) SREBP activity is regulated by mTORCl and contributes to Akt-dependent cell growth. Cell Metab 8: 224–236.
38. Li Y, Xu S, Mihaylova MM, Zheng B, Hou X, et al. (2011) AMPK phosphorylates and inhibits SREBP activity to attenuate hepatic steatosis and atherosclerosis in diet-induced insulin-resistant mice. Cell Metab 13: 376–388.
39. Ponugoti B, Kim DH, Xiao Z, Smith Z, Miao J, et al. (2010) SIRT1 deacetylates and inhibits SREBP-1C activity in regulation of hepatic lipid metabolism. J Biol Chem 285: 33959–33970.
40. Walker AK, Yang F, Jiang K, Ji JY, Watts JL, et al. (2010) Conserved role of SIRT1 orthologs in fasting-dependent inhibition of the lipid/cholesterol regulator SREBP. Genes Dev 24: 1403–1417.
41. Sewter C, Berger D, Considine RV, Medina G, Rochford J, et al. (2002) Human obesity and type 2 diabetes are associated with alterations in SREBP1 isoform expression that are reproduced ex vivo by tumor necrosis factor-alpha. Diabetes 51: 1035–1041.
42. Commerford SR, Peng L, Dube JJ, O’Doherty RM (2004) In vivo regulation of SREBP-1c in skeletal muscle: effects of nutritional status, glucose, insulin, and leptin. Am J Physiol Regul Integr Comp Physiol 287: R218–227.