Biochemical and Biophysical Research Communications 458 (2015) 449–455

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Muscle-selective knockout of AMPKα2 does not exacerbate diet-induced obesity probably related to altered myokines expression

Ting Chen [a], Zhongwen Li [a], Yanyan Zhang [a], Fu Feng [a], Xiaobin Wang [a], Xinxia Wang [b, **], Qingwu W. Shen [a, *]

[a] Department of Animal Science, Northwest A&F University, Yangling, Shaanxi 712100, China
[b] College of Animal Sciences, Zhejiang University, No. 866 Yuhangtang Road, Hangzhou, Zhejiang, 310058, China

ARTICLE INFO

Article history:
Received 8 January 2015
Available online 28 January 2015

Keywords:
AMPKα2
Obesity
Insulin resistance
Lipid metabolism
Myokine

ABSTRACT

Dysregulation of lipid metabolism has been believed to be central in the development of skeletal muscle insulin resistance. Since first being described in 1989, the role of AMPK in energy metabolism, especially its role in lipid metabolism in skeletal muscle has been well studied. However, some recent literature report that fatty acid oxidation in skeletal muscle is not directly associated with AMPK activation and ACC phosphorylation. To further understand the role of AMPK in lipid metabolism and the development of induced obesity and insulin resistance, muscle specific AMPKα2 knockout mice (mAMPKα2-KO) was employed in this study. The results showed that AMPKα2 ablation in muscle did not exacerbate high fat diet induce obesity in mice. On the contrary, it improved animal glucose tolerance and insulin sensitivity, with reduced triglyceride content in skeletal muscle and fat mass in various adipose tissues, when mice were fed high fat diet for 14 weeks. Gene expression analysis revealed that AMPKα2 knockout up-regulated the expression of genes related to lipid catabolism and down-regulated that of genes related to triglyceride synthesis. More importantly, ablation of AMPKα2 altered the expression of several myokines related to adipogenesis and muscle regeneration. Our data suggest that defect in AMPKα2 signaling does not necessarily lead to the development of muscle insulin resistance and obesity. AMPKα2 may regulate whole body lipid metabolism by regulating myokine secretion.

1. Introduction

Insulin resistance, associated with high fat feeding or obesity, is believed to be the combined result of chronic low-grade inflammation [1] and accumulation of bio-active lipid species such as fatty acyl-CoAs, diacylglycerol and ceramide in skeletal muscle [2]. In turn, this leads to impairment of insulin signaling to GLUT4 translocation and reduced insulin sensitivity [3]. As the pathogenic role of intramuscular lipid accumulation, transgenic mouse models have been used for reaching the lipid oxidation in muscle and insulin signaling against adverse effects of high fat feeding [4,5].

AMP-activated protein kinase (AMPK), a phylogenetically conserved serine/threonine protein kinase, has been proposed to function as a 'fuel gauge' to regulate energy balance that, once activated by low energy status, switches on ATP-producing catabolic pathways (such as fatty acid oxidation and glycolysis), and switches off ATP-consuming anabolic pathways (such as lipogenesis), both by short-term effect on phosphorylation of regulatory proteins and by long-term effect on gene expression [6]. The effect of AMPK activation on the regulation of free fatty acid oxidation in skeletal muscle is well described [7], in which AMPK phosphorylates ACC2, reducing its activity and malonyl-CoA production, in turn, increasing lipid oxidation [8–10]. However, some literature reported that fatty acid oxidation in skeletal muscle was not directly associated with AMPK and ACC phosphorylation [8,9,11,12]. Thus, more study is needed to further our understanding of AMPK in skeletal muscle fatty acid oxidation and energy metabolism.

In skeletal and cardiac muscles, AMPK complexes containing the α2 catalytic subunit are predominant [13,14]. To investigate the role of AMPK in skeletal muscle, several genetically engineered mice have been developed. In AMPKα2 Knockout mice, ablation of AMPKα2 leads to the development of obesity when animals are fed a high-fat diet, as a result of enhanced lipid accumulation in

* Corresponding author. Fax: +86 29 87092164.
** Corresponding author. Fax: +86 571 88982650.
E-mail addresses: xinxiawang@zju.edu.cn (X. Wang), yaoyao3153@aliyun.com (Q.W. Shen).

http://dx.doi.org/10.1016/j.bbrc.2015.01.075
0006-291X/

adipocytes but not in other tissues. However, the AMPKα2 KO mice exhibit similar glucose tolerance and insulin sensitivity to wild-type mice despite an increase in adipose tissue mass [15]. As AMPK reduces ACC activity and malonyl-CoA content, ACC2 KI (Acc2 S212A knock-in) mice was used for assessing whole-body and skeletal muscle fatty acid oxidation and insulin sensitivity, These mice had normal adiposity and liver lipids but elevated content of triacylglycerol and ceramide in skeletal muscle, which were associated with glucose intolerance and skeletal muscle insulin resistance [8]. However, In the muscle-specific transgenic mice carrying cDNAs of inactive AMPKα2 (aα2i TG), the glucose tolerance was slightly impaired in male mice compared to wild type littermates [12], while the transgenic mice overexpressing a kinase-dead AMPKα2 mutant (K45R mutation) in cardiac and skeletal muscles (AMPKα2-KD) were slightly smaller than old WT mice independent of diet as indicated by both reduced body weight and fat mass. In addition, the development of insulin resistance in response to high fat feeding was not exacerbated in old AMPKα2-KD mice [16,17]. Some of these observations are seemingly conflict with the well described function of AMPK to increase insulin sensitivity, up-regulate fatty acidy oxidation and inhibit adipogenesis and fat accumulation [8,18–20]. For this reason, further study with the use of new animal models should be recommended.

In this study, the mice carrying an AMPKα2 allele in which loxP sites up- and down-stream of exon2 were inserted were crossbred with MCK-Cre mice to study the effect of muscle specific knockout of AMPKα2 on animal growth, insulin tolerance and lipid accumulation. The results showed that, unexpectedly, muscle specific knockout of AMPKα2 did not exacerbate high fat diet induced obesity and insulin resistance in mice. Further analysis revealed that AMPKα2 knockout altered the expression of several cytokine in skeletal muscle, which are related to adipogenesis, inflammation and satellite cell function. This study provided new insight into the role of AMPK in energy metabolism and lipid accumulation.

2. Materials and methods

2.1. Generation of muscle-specific knockout AMPKα2 mice

Mice carrying a floxed AMPKα2 gene in the C57BL/6J background (Jackson laboratory, USA) were crossbred with MCK-Cre transgenic mice (Jackson laboratory, USA) expressing Cre recombinase under the muscle creatine (Cr) kinase promoter. The floxed AMPKα2 and MCK-Cre genotypes were confirmed by PCR analysis of genomic DNA. After weaning, wild-type and mAMPKα2-KO mice were fed high-fat diet (60% kcal fat) (Research Diets, HFK Bioscience, China) ad libitum for 14 weeks. At 18 weeks of age, animals were sacrificed by cervical dislocation. All animal procedures were performed in compliance with the Institutional Animal Care.

2.2. Food-intake measurement

Mice were maintained on a 14-h light, 10-h dark cycle, and allowed ad libitum access to food and water. Food consumption was calculated every Sunday following 14 weeks after weaning.

2.3. Glucose and insulin tolerance tests

Glucose tolerance test (GTT) was performed on mice fasted for 12 h. Blood samples were collected from the tail vein at 0, 15, 30, 60, 90, and 120 min after an intraperitoneal injection of glucose (2 g/kg body wt). For insulin tolerance test (ITT), animals were fasted for 6 h and then intraperitoneally injected with 0.5 units insulin/kg body wt. Blood samples were collected as described above at 0, 15, 30, 45, and 60 min post injection. Blood glucose was determined by a glucometer (Roche, Germany).

2.4. RNA extraction and RT-PCR

Total RNA was extracted from *gastrocnemius* muscle using TRIzol Reagent (TaKaRa, Japan) according to the manufacturer's instruction. The concentration of RNA was determined using a NanoDrop® ND-1000 (Thermo, Pittsburgh, PA). 500 ng of RNA was transcribed into single-stranded cDNA using the PrimerScript TM RT reagent (TaKaRa, Japan). qPCR was performed using the SYBR premix ExTaq II (TaKaRa, Japan) and the IQ5 real-time PCR detection system (Bio-Rad, Hercules, CA).

2.5. Western blotting analysis

Western blot analysis was performed as previously described [21]. Briefly, frozen tissue was homogenized in Mueller buffer and protein concentration was determined by the BCA method (Cwbiotech, China). 50 μg of protein was fractionated on 10% SDS-polyacrylamide gels. Gels were transferred to polyvinylidene difluoride (PVDF) membranes. Membranes were blocked overnight in 5% nonfat dry milk in Tris-buffered saline with 0.1% Tween-20 (TBS-T). Levels or phosphorylation of proteins was immunoblotting using the following primary antibodies: AMPKα1 (Santa Cruz, USA), AMPKα2 ((Santa Cruz, USA)) and β-actin (Santa Cruz, USA), phospho-ACC (Ser 79) (Beyotime, China); Secondary antibodies were HRP-conjugated antimouse, antigoat and antirabbit IgG (Santa Cruz, USA). Proteins on membranes were visualized by application of Enhanced chemiluminescence ((Millipore) and quantified by using Quantity One software (Bio-Rad).

2.6. Tissue triglyceride and FFA analysis

One gram of *Gastrocnemius* muscle was grinded in 9 ml of saline. After centrifuged at 12,000g, 4 °C for 10 min, the supernatant was collected for the measurement of triglyceride and free fatty acids (FFAs) in muscle by using triglyceride determination kit (Sigma, USA) and NEFA kit (Nanjing Jiancheng, China), respectively.

2.7. Metabolite measurements

Blood metabolites from wild-type and mAMPKα2-KO mice fed or fasted for 10 h were analyzed. Blood glucose, serum triglycerides, and serum FFAs were determined as described above. Serum total cholesterol (CHOL) was analyzed by using cholesterol assay kit (Nanjing jiancheng, China), low density lipoprotein cholesterol assay kit (Nanjing jiancheng, China) and high density lipoprotein cholesterol assay kit (Nanjing jiancheng, China). Serum insulin concentrations were determined using a enzyme-linked immunosorbent assay kit (Hufeng, China).

2.8. Statistical analysis

Data are expressed as means ± SEM. The statistical significance of differences in mean values between groups was assessed by Student's t test.

3. Result

3.1. High-efficiency ablation of AMPKα2 in skeletal muscle

To validate muscle-specific knockout of AMPKα2, AMPKα2 protein in four different skeletal muscles and heart was analyzed by immunoblotting. Compared with wild type mice, AMPKα2 protein

was not detectable in skeletal and cardiac muscles of mAMPKα2-KO mice (Fig. 1A), while AMPKα1 expression was unaffected (Fig. 1B), indicating that muscle-specific knockout of AMPKα2 was successful. The knockout of AMPKα2 in skeletal muscle was also confirmed by the reduced phosphorylation of ACC (Fig. 1C), a substrate of AMPK.

3.2. Physiological effects of muscle-specific knockout of AMPKα2

mAMPKα2-KO mice tend to be retarded in growth. Body weights were reduced for mAMPKα2KO mice, no matter feeding high-fat diet (Fig. 2A) or chow diet (Fig. S1A). Knockout of AMPKα2 not only decreased the deposition of fat (Fig. 2C), but also inhibited the development of skeletal muscle (Fig. 2D) and kidney (Fig. 2E). Other organs, including heart, liver, spleen and lung are not affected by ablation of AMPKα2 (Fig. 2E).

GTT and ITT analysis showed that glucose and insulin tolerance was similar between wild type and mAMPKα2-KO mice when fed a normal diet (Fig. S1C and D). As expected, both mAMPKα2-KO and wild type mice showed the impairment of glucose tolerance (Fig. 2F) and insulin sensitivity (Fig. 2G) after being fed a high-fat diet for 14 weeks. However surprisingly, AMPKα2 depletion in muscle did not aggravate the impairment, but resulted in some improvement in insulin sensitivity and glucose tolerance in mAMPKα2-KO mice when compared to wild type mice.

Along with the reduced fat deposition in adipose tissues, triglyceride content in skeletal muscle was decreased in AMPKα2 knockout mice when fed a high fat diet (Fig. 2H), which was not affected in heart and liver by the absence of AMPKα2 in muscle (Fig. 2I and J), while higher triglyceride was determined in heart of mAMPKα2-KO mice when fed a normal diet (Fig. 2I). We also measured the intramuscular FFA content. More FFAs in *gastrocnemius* muscle of mAMPKα2-KO mice was detected when mice were fed high fat diet (Fig. 2K). Storage of glycogen in both skeletal muscle and liver was not altered by AMPKα2 knockout (Fig. 2L and M).

Compared to wild-type mice, we observed mAMPK2-KO mice had decreased basal blood glucose levels and similar insulin levels when fed a high-fat diet. Serum triglyceride, high-density lipoprotein and FFA were decreased in both fasting and fed conditions. Serum low density lipoprotein concentrations was not altered in mAMPKα2KO mice (Table 1).

3.3. AMPKα2 ablation in muscle alters the expression of genes related to lipid metabolism

As triglyceride decreased while FFA increased in the skeletal muscle of AMPKα2 ablation, we analyzed the expression of genes related to lipid metabolism. Generally, the expressions of genes related to triglyceride synthesis, like aP2, was decreased and that of genes related to triglyceride catabiosis, including peroxisome proliferator-activated receptor alpha (PPARα), adipose triglyceride lipase (ATGL) and carnitine palmitoyl transterase-1 (CPT-1), was increased in mouse skeletal muscle after AMPKα2 knockout (Fig. 3). Exceptionally, the expression of glycerol-3-phosphate acyltransferase 2 (GPAT2), an enzyme catalyzing the production of diacyle glycerol 3-phosphate in the synthesis of triglyceride, was detected to be up-regulated in the skeletal muscle of mAMPKα2-KO mice, which could be compensatory as previously reported [22].

3.4. AMPKα2 knockout changes the expression of myokines in skeletal muscle

As muscle specific knockout of AMPKα2 reduced not only triglyceride in skeletal muscle under high fat diet, but also fat mass in different adipose tissues that is far away from skeletal muscle, we

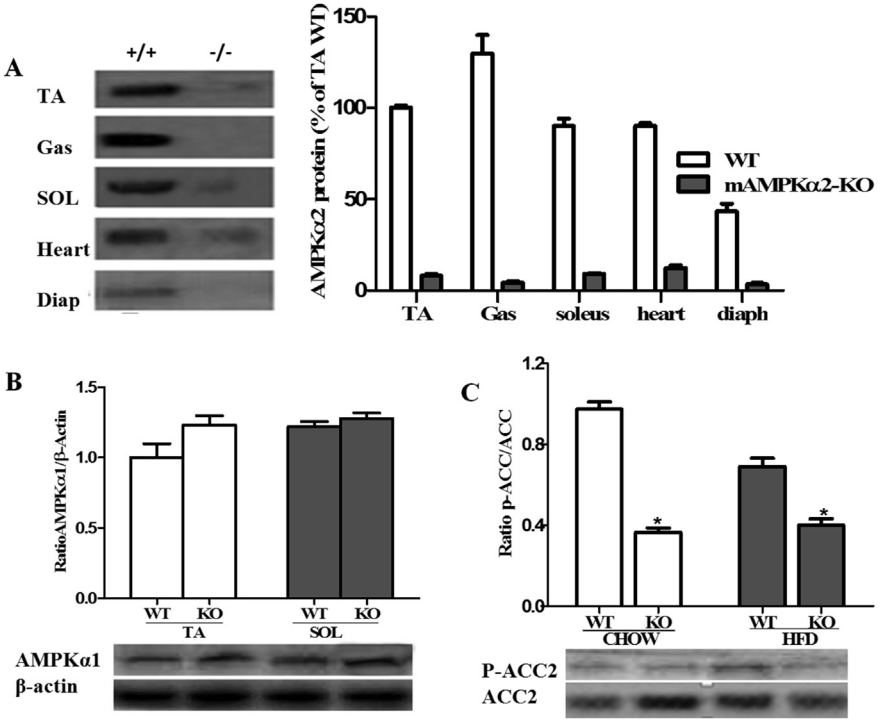

Fig. 1. AMPK expression and ACC2 phosphorylation. (A) High-efficiency ablation of the AMPKα2 protein selectively in skeletal and cardiac muscles. Protein was assessed by immunoblotting. Muscles from mAMPK-KO mice show nearly complete depletion of the AMPK protein. (B) The AMPKα1 protein in mouse tibialis anterior and soleus muscles was unaffected by the ablation of AMPKα2. (C) ACC2 S79 phosphorylation was reduced in mouse skeletal muscle after AMPKα2 knockout no matter mice were fed a chow or a high-fat diet. TA, tibialis anterior; Gas, gastrocnemius; Sol, soleus. Data are means ± SEM; n = 4–6. *, $P < 0.05$; **, $P < 0.001$ versus control (+/+).

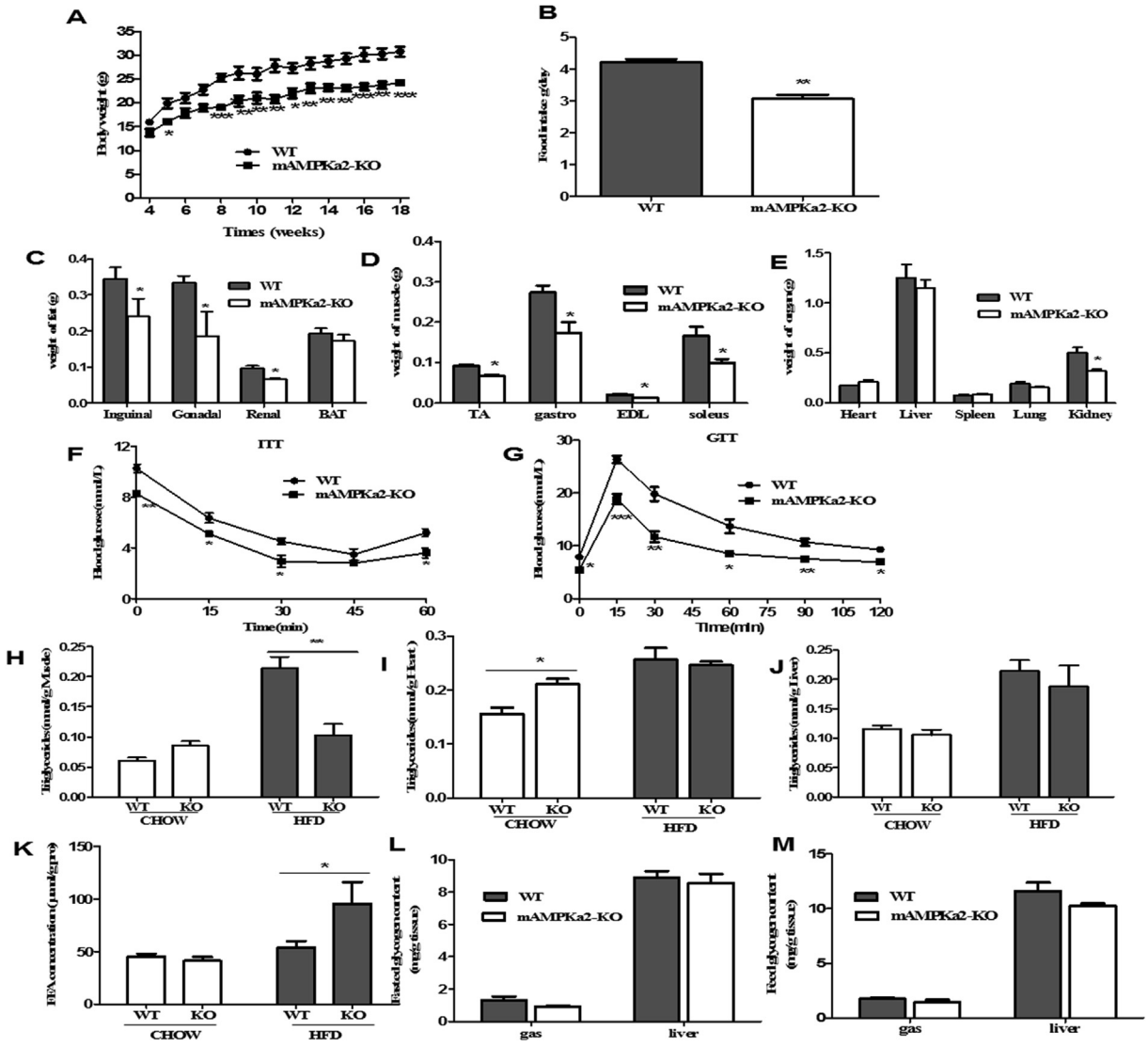

Fig. 2. mAMPKa2-KO mice showed reduced growth and improved whole-body glucose homeostasis after being fed a high-fat diet for 14 weeks. Body weight (A) and food intake (B) were tracked for 14 weeks in male mice fed a high-fat diet (HFD), beginning at 4 weeks of age. The weight of fat (C), muscle (D) and organs (E) were recorded after mice were sacrificed. Glucose tolerance (F) and insulin tolerance (G) tests showed that AMPKa2 knockout mice had improved glucose tolerance and insulin sensitivity after being fed a high fat diet for 14 weeks. Triglyceride content in gastrocnemius muscle (H), heart (I), and liver (J) was estimated for mice fed a chow or high-fat diet (HFD) for 14 weeks. FFA content (K) in gastrocnemius muscle was measured for mice fed a high fat diet for 14 weeks. Glycogen content in skeletal muscle (L) and liver (M) was unaffected by AMPKa2 ablation. Values are presented as mean ± SEM. *P 0.05; **P < 0.01; ***P < 0.001; n = 5–10.

proposed that there was a long range way by which AMPKα2 ablation in muscle altered whole body fat accumulation, which is probably myokines secreted by skeletal muscle. We then analyzed the expression of myokines in skeletal muscle. The result showed that the mRNA expression of *interleukin-6 (IL-6)*, *myostatin (MSTN)*, *FGF21 (fibroblast growth factor)*, *Insl6*, and *CXCL1* was up-regulated, while the expression of *FNDC5* and *myonectin* was down-regulated in the muscle of AMPKα2 knockout mice (Fig. 4A). IL-6 protein in serum (Fig. 4B) and muscle (Fig. 4C) was also detected to be elevated in mAMPKα2-KO mice. The results suggest that myokines might be involved in the altered whole body lipid metabolism of muscle specific AMPKα2 knockout mice.

Table 1
Metabolic variables from high-fat diet-fed wild-type and mAMPKα2-KO mice. Values are mean ± SEM, n = 4–6. Levels measured in serum *p < 0.05 for difference in fasted condition; &p < 0.05 for difference in fed condition n = 4–6; &&p < 0.01.

Serum parameters	Fasted		Fed	
	Wild type	mAMPKα2-KO	Wild type	mAMPKα2-KO
Glucose (mmol/l)	7.925 ± 0.81	5.475 ± 0.55*	11.750 ± 1.02	8.667 ± 1.17&
Insulin (ng/ml)	0.850 ± 0.33	0.901 ± 0.059	1.200 ± 0.25	1.347 ± 0.11
CHOL (mmol/l)	2.290 ± 0.67	2.195 ± 0.20	3.745 ± 0.33	3.400 ± 0.21
Triglycerides (mmol/l)	1.435 ± 0.42	0.595 ± 0.09*	1.095 ± 0.24	0.800 ± 0.22&
HDL (mmol/l)	1.330 ± 0.042	0.766 ± 0.08*	2.060 ± 0.21	0.795 ± 0.41&&
LDL (mmol/l)	0.380 ± 0.08	0.410 ± 0.03	0.615 ± 0.007	0.520 ± 0.01
FFA (umol/l)	1515 ± 20.2	961.66 ± 11.7*	712.43 ± 10.2	661.67 ± 9.5

4. Discussion

The dysregulation of lipid metabolism in resting muscle and the accumulation of detrimental intramuscular lipids and lipid derivates have been believed to be central in the development of skeletal muscle insulin resistance [23]. Since first being described in 1989 [24], the role of AMPK in energy metabolism, especially lipid metabolism in skeletal muscle has been extensively studied [25]. Due to its vital role in the regulation of fatty acid oxidation,

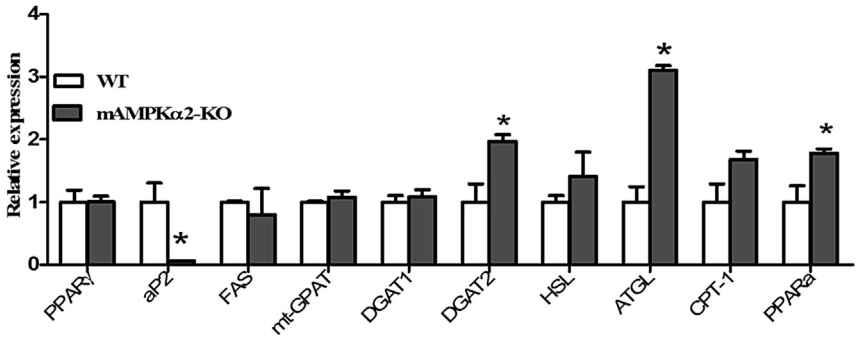

Fig. 3. The mRNA expression of genes related to lipid metabolism in skeletal muscle. WT: wild type. mAMPKα2-KO: AMPKα2 knockout mice. n = 5. *P < 0.05.

adipogenesis and insulin sensitivity [8,11,26,27], AMPK has been alleged to be a feasible target in the treatment of type 2 diabetes [7]. However, this statement has been challenged recently by some studies using genetically engineered animal models. Over-expression of inactive AMPKα2 in skeletal muscle did not worse animal glucose tolerance and obesity-induced insulin resistance when compared to wild type mice, indicating that dysfunction in AMPKα2 does not lead to the development of diabetes [12,16,17]. To further clarify the role of AMPK in the regulation of insulin sensitivity, lipid accumulation and the development of obesity, muscle specific AMPKα2 knockout mice were employed in this study. Unexpectedly, the loss of AMPKα2 in muscle did not increase the content of triglyceride in skeletal muscle (Fig. 2H). Further, it decreased lipid accumulation in various adipose tissues, including inguinal, gonadal, renal and brown fat tissues after being induced for obesity for 14 weeks by high fat diet (Fig. 2C). Thus, the knockout of AMPKα2 in muscle reduced animal body weight and prevented the development of high fat induced obesity (Fig. 2A). Both glucose and insulin tolerance tests showed that, though high fat diet impaired glucose tolerance and insulin sensitivity in both mAMPKα2-KO and wild type mice, knockout of AMPKα2 in muscle did not exacerbate the impairment. On the contrary, mAMPKα2-KO showed some improvement in insulin sensitivity and glucose tolerance after fed high fat diet for 14 weeks (Fig. 2F and G). Both fasted and fed blood glucose, triglyceride and HDL decreased in mAMPKα2-KO (Table 1). All these data showed that muscle specific knockout of AMPKα2 did not exacerbated high fat diet induced obesity and insulin resistance.

AMPK regulates fatty acid oxidation in muscle by AMPK-dependent phosphorylation of ACC2 to reduce ACC2 activity and malonyl-CoA production [7,28]. Although AMPKα2 knockout reduced the basal phosphorylation of ACC (Fig. 1C), the mAMPKα2-KO mice did not show greater accumulation of whole body lipid. Gene expression analysis showed that genes related to fatty acid oxidation was up-regulated and that related to triglyceride synthesis was down-regulated in skeletal muscle (Fig. 3), indicating that knockout of AMPKα2 increased lipid catabolism, but inhibited lipid deposition, which is probably the reason why triglyceride was decreased in skeletal muscle of mAMPKα2-KO mice (Fig. 2H). Interestingly, after feeding high fat diet, the mAMPKα2-KO mice

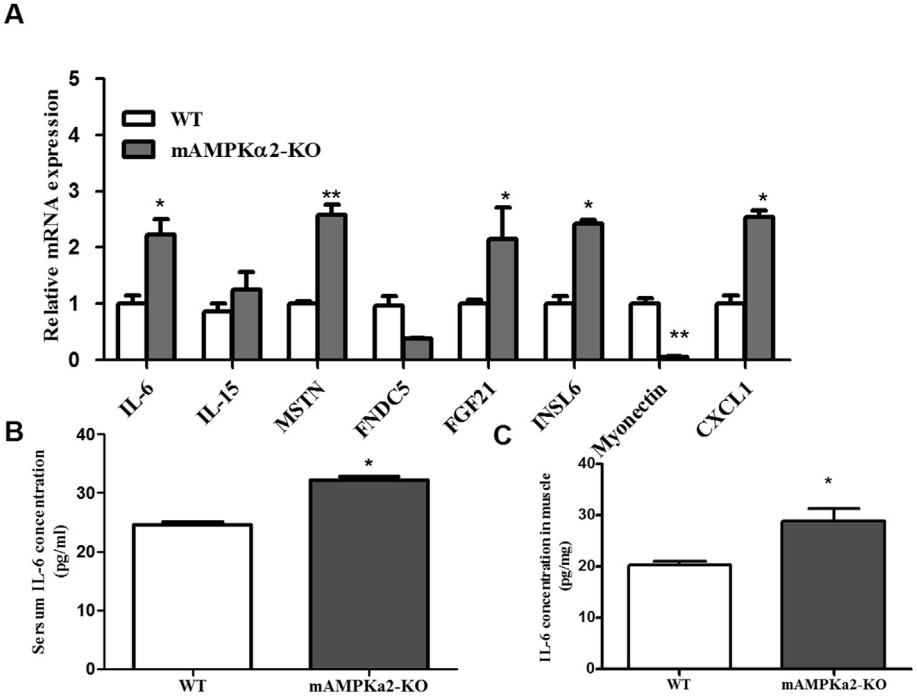

Fig. 4. The expression of myokines was altered in mAMPKα2-KO mice after 14 weeks of high-fat diet. The relative mRNA expression of myokines (A) in muscle was detected by RT-PCR. The IL-6 protein in serum (B) and muscle (C) was measured by ELISA. WT: wild type. mAMPKα2-KO: AMPKα2 knockout mice:. n = 4. *P < 0.05, **P < 0.01.

secreted amounts of sweat containing much triglycerides, which maybe another reason for lower weight and blood lipid level in mAMPKα2-KO mice (Fig. S1 E). Taken together, the substantial reduction in ACC2 phosphorylation did not translate into increasing lipid accumulation with obesity, this may suggest either that ACC2 is not critical for or other factors play main role in regulating basal fatty acid oxidation in resting muscle, which are in line with recent reports [9,16,29].

Myokines are secreted into circulation by muscle to mediate metabolism in muscles or regulate inter-tissue crosstalk to control integrated physiology [30–32]. We proposed that myokines might participate in the whole body lipid metabolism through circulation. Gene expression analysis revealed that AMPKα2 knockout in muscle altered the expression of myokines at both mRNA and protein levels (Fig. 4). *MSTN* was up-regulated after AMPKα2 knockout. MSTN not only regulates muscle growth and development, but inhibit fat mass and improve insulin sensitivity [33]. FNDC5 and FGF21 are reported to activate adipose thermogenesis, providing a robust defense against hypothermia [34,35]. In this study, the *FNDC5* was down-regulated by AMPKα2 knockout. However, higher expression of *FGF21* mRNA was detected in the muscle of mAMPKα2-KO mice, which might contributed to the resistance to high fat induced obesity. Overexpression of *CXCL*1 within a physiological range attenuates diet-induced obesity, likely mediated through improved fatty acid oxidation [36]. Higher level of *CXCL1* mRNA in the muscle of mAMPKα2-KO mice suggests that the lack of AMPKα2 activity alters the expression of *CXCL1*, thus improving fatty acid oxidation and antagonizing obesity. Administration of recombinant myonectin to mice reduces circulating free fatty acid [37], The expression of this myokine was detected to be down-regulated after AMPKα2 knockout. Seemingly, the reduced expression of *myonectin* in mAMPKα2-KO mice should lead to higher FFAs in serum, but lower blood FFAs were determined in mutant mice (Table 1). We do not know the exact mechanism, but we proposed that the altered expression of *myonectin* might be associated with the increased FFAs in the skeletal muscle (Fig. 2K). Muscle IL-6 and Insl6 are important factors for muscle regeneration [38,39]. In this study, the expression of these two chemokines was detected to be up-regulated (Fig. 4), but the muscle growth and development in mAMPKα2-KO was inhibited. More studies about their role in adipogenesis and lipid accumulation are recommended.

In conclusion, a muscle-specific ablation in AMPKα2 reduces ACC phosphorylation, but does not exacerbate high fat diet induced obesity and insulin resistance in mice, suggesting that defect in AMPKα2 signaling does not necessarily lead to dysfunction in lipid metabolism, obesity and insulin resistance. Knockout of AMPKα2 alters the expression of several genes related to lipid metabolism. More importantly, AMPKα2 may regulate whole body lipid metabolism and obesity by regulating myokine expression.

Conflict of interest

None.

Acknowledgments

This work was supported by the initial funding from Northwest Agriculture and Forestry University (Z111021006) and National Basic Research Program of China (2012CB124705).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.01.075.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.01.075.

References

[1] M. Navab, N. Gharavi, A.D. Watson, Inflammation and metabolic disorders, Curr. Opin. Clin. Nutr. Metab. Care 11 (4) (2008) 459–464.
[2] D.B. Savage, K.F. Petersen, G.I. Shulman, Disordered lipid metabolism and the pathogenesis of insulin resistance, Physiol. Rev. 87 (2) (2007) 507–520.
[3] J. Hirosumi, et al., A central role for JNK in obesity and insulin resistance, Nature 420 (6913) (2002) 333–336.
[4] C.S. Choi, et al., Continuous fat oxidation in acetyl-CoA carboxylase 2 knockout mice increases total energy expenditure, reduces fat mass, and improves insulin sensitivity, Proc. Natl. Acad. Sci. U. S. A. 104 (42) (2007) 16480–16485.
[5] B. Viollet, et al., AMPK: lessons from transgenic and knockout animals, Front. Biosci. (Landmark Ed.) 14 (2009) 19–44.
[6] B.B. Kahn, et al., AMP-activated protein kinase: ancient energy gauge provides clues to modern understanding of metabolism, Cell. Metab. 1 (1) (2005) 15–25.
[7] G.R. Steinberg, S.B. Jorgensen, The AMP-activated protein kinase: role in regulation of skeletal muscle metabolism and insulin sensitivity, Mini Rev. Med. Chem. 7 (5) (2007) 519–526.
[8] H.M. O'Neill, et al., AMPK phosphorylation of ACC2 is required for skeletal muscle fatty acid oxidation and insulin sensitivity in mice, Diabetologia 57 (8) (2014) 1693–1702.
[9] H. Alkhateeb, G.P. Holloway, A. Bonen, Skeletal muscle fatty acid oxidation is not directly associated with AMPK or ACC2 phosphorylation, Appl. Physiol. Nutr. Metab. 36 (3) (2011) 361–367.
[10] G.F. Merrill, et al., AICA riboside increases AMP-activated protein kinase, fatty acid oxidation, and glucose uptake in rat muscle, Am. J. Physiol. 273 (6 Pt 1) (1997) E1107–E1112.
[11] N. Dzamko, et al., AMPK-independent pathways regulate skeletal muscle fatty acid oxidation, J. Physiol. 586 (Pt 23) (2008) 5819–5831.
[12] N. Fujii, et al., Role of AMP-activated protein kinase in exercise capacity, whole body glucose homeostasis, and glucose transport in skeletal muscle -insight from analysis of a transgenic mouse model, Diabetes Res. Clin. Pract. 77 (Suppl. 1) (2007) S92–S98.
[13] J.B. Birk, J.F. Wojtaszewski, Predominant alpha2/beta2/gamma3 AMPK activation during exercise in human skeletal muscle, J. Physiol. 577 (Pt 3) (2006) 1021–1032.
[14] K. Sakamoto, et al., Deficiency of LKB1 in heart prevents ischemia-mediated activation of AMPKalpha2 but not AMPKalpha1, Am. J. Physiol. Endocrinol. Metab. 290 (5) (2006) E780–E788.
[15] J.A. Villena, et al., Induced adiposity and adipocyte hypertrophy in mice lacking the AMP-activated protein kinase-alpha2 subunit, Diabetes 53 (9) (2004) 2242–2249.
[16] S. Beck Jorgensen, et al., Reduced AMP-activated protein kinase activity in mouse skeletal muscle does not exacerbate the development of insulin resistance with obesity, Diabetologia 52 (11) (2009) 2395–2404.
[17] C. Frosig, et al., AMPK and insulin action – responses to ageing and high fat diet, PLoS One 8 (5) (2013) e62338.
[18] H.C. Dreyer, et al., Fat oxidation and glucose uptake are increased following an acute bout of resistance exercise: role of AMPK, ACC, Akt/PKB, and AS160, Faseb J. 21 (5) (2007). A580–A580.
[19] J. Gamble, et al., The role of acetyl CoA carboxylase (ACC) and 5' AMP-activated protein kinase (AMPK) in the altered metabolism of insulin resistant rat hearts, Circulation 94 (8) (1996), 730–730.
[20] A. Janovska, et al., AMPK and ACC phosphorylation: effect of leptin, muscle fibre type and obesity, Mol. Cell. Endocrinol. 284 (1–2) (2008) 1–10.
[21] J. Wang, et al., KLF15 regulates slow myosin heavy chain expression through NFATc1 in C2C12 myotubes, Biochem. Biophys. Res. Commun. 446 (4) (2014) 1231–1236.
[22] J.W. Wu, et al., Deficiency of liver adipose triglyceride lipase in mice causes progressive hepatic steatosis, Hepatology 54 (1) (2011) 122–132.
[23] G.I. Shulman, Cellular mechanisms of insulin resistance, J. Clin. Invest. 106 (2) (2000) 171–176.
[24] D. Carling, et al., Purification and characterization of the AMP-activated protein kinase. Copurification of acetyl-CoA carboxylase kinase and 3-hydroxy-3-methylglutaryl-CoA reductase kinase activities, Eur. J. Biochem. 186 (1–2) (1989) 129–136.
[25] G.R. Steinberg, et al., AMP-activated protein kinase – the fat controller of the energy railroad, Can. J. Physiol. Pharmacol. 84 (7) (2006) 655–665.
[26] M. Friedrichsen, et al., Exercise-induced AMPK activity in skeletal muscle: role in glucose uptake and insulin sensitivity, Mol. Cell. Endocrinol. 366 (2) (2013) 204–214.
[27] W.J. Lee, et al., AMPK activation increases fatty acid oxidation in skeletal muscle by activating PPARalpha and PGC-1, Biochem. Biophys. Res. Commun. 340 (1) (2006) 291–295.
[28] A. Dziewulska, P. Dobrzyn, A. Dobrzyn, The role of AMP-activated protein kinase in regulation of skeletal muscle metabolism, Postepy Hig. Med. Dosw 64 (2010) 513–521.

[29] G.P. Holloway, et al., Increasing skeletal muscle fatty acid transport protein 1 (FATP1) targets fatty acids to oxidation and does not predispose mice to diet-induced insulin resistance, Diabetologia 54 (6) (2011) 1457–1467.
[30] T. Henriksen, C. Green, B.K. Pedersen, Myokines in myogenesis and health, Recent Pat. Biotechnol. 6 (3) (2012) 167–171.
[31] B.K. Pedersen, Muscles and their myokines, J. Exp. Biol. 214 (Pt 2) (2011) 337–346.
[32] B.K. Pedersen, et al., Role of myokines in exercise and metabolism, J. Appl. Physiol. (1985) 103 (3) (2007) 1093–1098.
[33] T. Guo, et al., Myostatin inhibition in muscle, but not adipose tissue, decreases fat mass and improves insulin sensitivity, PLoS One 4 (3) (2009) e4937.
[34] S. Wu, et al., Fibroblast growth factor 21 (FGF21) inhibits chondrocyte function and growth hormone action directly at the growth plate, J. Biol. Chem. 287 (31) (2012) 26060–26067.
[35] P. Bostrom, et al., A PGC1-alpha-dependent myokine that drives brown-fat-like development of white fat and thermogenesis, Nature 481 (7382) (2012) 463–468.
[36] L. Pedersen, et al., Muscle-derived expression of the chemokine CXCL1 attenuates diet-induced obesity and improves fatty acid oxidation in the muscle, Am. J. Physiol. Endocrinol. Metab. 302 (7) (2012) E831–E840.
[37] M.M. Seldin, G.W. Wong, Regulation of tissue crosstalk by skeletal muscle-derived myonectin and other myokines, Adipocyte 1 (4) (2012) 200–202.
[38] T.A. Washington, et al., Skeletal muscle mass recovery from atrophy in IL-6 knockout mice, Acta Physiol. (Oxf) 202 (4) (2011) 657–669.
[39] L. Zeng, et al., Insulin-like 6 is induced by muscle injury and functions as a regenerative factor, J. Biol. Chem. 285 (46) (2010) 36060–36069.