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Fatty acid transport and transporters in muscle are critically regulated by Akt2

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ABSTRACT

Muscle contains various fatty acid transporters (CD36, FABPpm, FATP1, FATP4). Physiological stimuli (insulin, contraction) induce the translocation of all four transporters to the sarcolemma to enhance fatty acid uptake similarly to glucose uptake stimulation via glucose transporter-4 (GLUT4) translocation. Akt2 mediates insulin-induced, but not contraction-induced, GLUT4 translocation, but its role in muscle fatty acid transporter translocation is unknown. In muscle from Akt2-knockout mice, we observed that Akt2 is critically involved in both insulin-induced and contraction-induced fatty acid transport and translocation of fatty acid translocase/CD36 (CD36) and FATP1, but not of translocation of fatty acid-binding protein (FABPpm) and FATP4. Instead, Akt2 mediates intracellular retention of both latter transporters. Collectively, our observations reveal novel complexities in signaling mechanisms regulating the translocation of fatty acid transporters in muscle.

1. Introduction

Fatty acids and glucose are essential fuels for skeletal muscle. The transport of these substrates into this tissue occurs via highly regulated, protein-mediated processes. Specifically, in skeletal muscle, glucose transport is facilitated by glucose transporter-4 (GLUT4) (cf. [1,2]), while fatty acid transport is facilitated by a number of fatty acid transporters, including fatty acid translocase/CD36 (CD36), plasma membrane associated fatty acid-binding protein (FABPpm), and selected fatty acid transport proteins (FATP1 and -4) (cf. [3]). Acute changes in glucose utilization induced by selected metabolic stimuli (insulin, 5′AMP-activated protein kinase (AMPK) activation, muscle contraction) are largely attributable to the translocation of GLUT4 from intracellular depots to the cell surface, thereby increasing the rate of glucose transport [2,4]. In recent years, similar metabolic flexibility has been shown for FAT/CD36, as this fatty acid transport protein is also induced to translocate to the cell surface by the same metabolic stimuli (cf. [3]).

The signaling mechanisms involved in GLUT4 translocation to the sarcolemma in response to selected metabolic perturbations are well characterized [2,4]. In contrast, the signaling pathways involved in the translocation of fatty acid transporters to the sarcolemma are largely unknown. Nevertheless, CD36 translocation appears to share, in part, a signaling pathway that may be similar to GLUT4. For example, both GLUT4 and CD36 are recruited from intracellular pools to the sarcolemma by insulin-stimulated activation of phosphatidylinositol-3-kinase (PI3K) [5,6]. Similarly, muscle contraction and the activation of muscle AMPK by 5-aminoimidazole-4-carboxamide-1-beta-D-ribofuranoside (AICAR) induce the translocation of both GLUT4 and CD36 to the sarcolemma [3,5,7,8], as well as that of a number of other fatty acid transporters, namely FABPpm, FATP1, FATP4, but not FATP6 [7].

Despite the comparable responses of GLUT4 and fatty acid transporters to metabolic stimulation and the apparent sharing of PI3K signaling, there is evidence that the GLUT4 and the fatty acid transporter signaling pathways diverge at some point. For example, in insulin resistant, obese Zucker rats and in Zucker diabetic fatty acid rats GLUT4 is retained intracellularly [9], while CD36 is permanently relocated to the sarcolemma [10], resulting in an in an inverse relationship between sarcolemmal GLUT4 and

Abbreviations: AMPK, 5′AMP-activated protein kinase; CD36, fatty acid translocase/CD36; FABPpm, fatty acid-binding protein; FATP, fatty acid transport protein; GLUT4, glucose transporter-4

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CD36 ($r = -0.91$) [11] In addition, in muscle from obese Zucker rats insulin-stimulated GLUT4 translocation is impaired [9], while contraction-stimulated GLUT4 translocation is normal [1]. Moreover, both insulin and contraction-induced CD36 translocation are impaired in obese Zucker rat muscle [12]. Since FABPpm translocation is not impaired in these animals, it appears that CD36 and FABPpm may be regulated by different insulin and contraction-mediated signaling pathways [12]. Collectively, these observations indicate that the signaling pathways involved in the translocation of these transporters differ from those involved in GLUT4 translocation. In addition, their signaling pathways remain obscure, and there may be specific signaling pathways for the different fatty acid transporters.

Akt is a family of protein kinases regulating multiple anabolic pathways, and at present there are three isoforms described. In general, Akt1 mediates hypertrophic signaling, Akt2 is involved in insulin signaling, whereas much less is known about Akt3 [13]. In the past few years it has become evident that Akt2 contributes to the regulation of lipid metabolism. For example, Akt2 signaling promotes mammary gland [14] and hepatic triacylglycerol accumulation [15–17]. Yet, it is unknown whether the regulation of lipid metabolism by Akt2 signaling extends to the fatty acid transport process. Moreover, whether a signaling divergence between GLUT4 and selected fatty acid transporters, or among different fatty acid transporters, occurs at the level of Akt2 is also unknown. Therefore, in the present study, we investigated whether Akt2 is involved in the insulin-, and/or contraction-mediated signaling for fatty acid transport and fatty acid transporter translocation (CD36, FABPpm, FATP1 and -4), and compared this to glucose transport and GLUT4 translocation.

2. Materials and methods

2.1. Animals

Akt2-knockout (KO) mice were a gift from Pfizer Inc. (New York, NY [18]) and corresponding DBA/1 wildtype (WT) mice were purchased from Jackson Laboratories (Bar Harbor, Maine). WT and KO female mice were carefully matched for age (8 weeks). At this age, the body weights of WT mice (20.8 ± 0.7 g) were greater than in KO mice (16.3 ± 0.5 g) $P < 0.05$, as has been reported by others [18,19]. The animals were housed in controlled temperature and humidity conditions on a 12:12-h light–dark cycle and were given standard laboratory chow and water *ad libitum*. Mice were anesthetized with sodium pentobarbital (6 mg/100 g body wt ip; MTC Pharmaceuticals, Cambridge, ON), and all procedures were approved by the University of Guelph Animal Care Committee.

2.2. Genotyping

Genotypes of KO mice were confirmed using standard DNA isolation and PCR methods (Extract-N-Amp, Sigma–Aldrich, St. Louis, MO) using forward 5′-GCA-GGA-TCT-CCT-GTC-ATC-TCA-CC-3′ and reverse 3′-GAT-GCT-CTT-CGT-CCA-GAT-CAT-CC-5′ primer sets targeted towards the neo cassette.

2.3. Experimental treatment

Basal tail vein glucose concentrations were determined using a glucose meter (Ascensia Elite XL, Bayer Inc., Toronto, ON). Intraperitoneal glucose and insulin tolerance tests were conducted in separate groups of overnight fasted WT and KO animals. Mice were injected intraperitoneally with either glucose (0.75 g/kg body wt) or insulin (1.0 U/kg body wt) and blood glucose was recorded at 15, 30, 45, 90 and 120 min. To examine the effects of insulin and muscle contraction on glucose and fatty acid transport and transporters, fasted WT and KO mice were assigned to the following groups: (i) control (no treatment), (ii) insulin treatment for 15 min (Humulin, 1.0 U/kg body wt, ip; Eli Lilly, Toronto, ON), or (iii) muscle contraction via the sciatic nerve (train delivery 100 Hz/3 s at 5 V, train duration 200 ms, pulse duration 10 ms) applied for 3 repetitions of 5 min with 2 min of rest between stimulations [7]. Following treatment periods, hindlimb muscles were immediately harvested for isolation of giant sarcolemmal vesicles or were freeze-clamped in liquid N_2 for muscle homogenate preparation.

2.4. Isolation of giant sarcolemmal vesicles and substrate transport

Fatty acid and glucose transport as well as sarcolemmal presence of transport proteins were determined in giant sarcolemmal vesicles isolated from hindlimb muscles [7,20]. Vesicle protein yield was determined using the bicinchoninic acid assay. Vesicles were used immediately for substrate transport assays [7,20].

2.5. Western blotting

Protein expression was determined in giant sarcolemmal vesicles and muscle homogenates via Western blotting [7,20]. Blotting protocols with antibodies against total Akt2, AS160, AMPK, phosphorylated Akt-Thr^{308}, AS160-Thr^{642}, AMPK-Thr^{172} (Cell Signaling, Danvers, MA), CD36, FATP1, and -4 (Santa Cruz Biotechnology, Santa Cruz, CA, [21]) and GLUT4 (Millipore, Temecula, CA) were performed according to manufacturer's instructions. Antibodies against FABPpm were kindly donated by Dr. J. Calles-Escandon, Wake Forest University. Following incubation with secondary antibodies, membranes were detected and quantified using chemiluminescence (Perkin Elmer Life Science, Boston, MA) and ChemiGenius2 Bioimaging (SynGene, Cambridge, UK). Equal loading of protein was confirmed via Ponceau-S staining.

2.6. Statistics

All data are reported as mean ± S.E.M. and were analyzed using analysis of variance and Fisher's LSD post hoc test when appropriate. Significance was accepted at $P < 0.05$.

3. Results

3.1. Akt2-KO phenotype

Pre-diabetic state: Basal blood glucose concentrations were lower in WT (4.2 ± 0.2 mM) than in Akt2-KO animals (5.4 ± 1.0 mM, $P < 0.05$), and Akt2-KO mice exhibited whole body glucose intolerance (Fig. 1A). These observations confirm previous results reported elsewhere for these animals [18,19,22].

3.2. Expression of insulin signaling proteins, AMPK and substrate transporters

As expected in Akt2-KO mice, Akt2 protein was not detected (Fig. 1B). The presence of insulin signaling proteins insulin receptor substrate-1, phosphatidylinositol-3 kinase and AS160 (the downstream target of Akt2) in skeletal muscle did not differ between WT and Akt2-KO mice (Fig. 1B). Neither the metabolic regulator AMPK (Fig. 1B), nor GLUT4 or fatty acid transporter (CD36, FABPpm, FATP1 and -4) protein contents differed between WT and Akt2-KO mice (Fig. 1C).