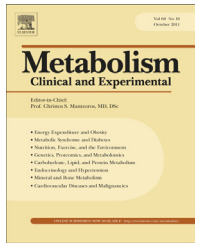
Available online at www.sciencedirect.com

Metabolism

www.metabolismjournal.com



Fatty acid transport proteins chronically relocate to the transverse-tubules in muscle from obese Zucker rats but are resistant to further insulin-induced translocation

*Leslie E. Stefanyk, Arend Bonen, David J. Dyck**

Department of Human Health and Nutritional Science, University of Guelph, Guelph, ON, Canada, N1G 2W1

ARTICLE INFO

Article history:
Received 26 January 2013
Accepted 29 April 2013

Keywords:
FAT/CD36
FABPpm
FATP
Insulin resistance
Plasma membrane

ABSTRACT

Objectives. Recently, we have demonstrated that FA transport proteins are located within the t-tubule fraction of rodent muscle, and that insulin stimulation causes their translocation to this membrane fraction. Chronic relocation of the FA transport protein FAT/CD36 to the sarcolemma is observed in obese rodents and humans, and correlates with intramuscular lipid accumulation and insulin resistance. It is not known whether in an obese, insulin resistant state FA transporters also chronically relocate to the t-tubules. Furthermore, it is not known whether the insulin-stimulated translocation of the various FA transport proteins to the t-tubules is impaired in insulin resistance.

Methods. Sarcolemmal and t-tubule membrane fractions were isolated via differential centrifugation from muscles of lean and obese female Zucker rats during basal or insulin stimulated conditions. FA transport proteins were measured via western blot on both membrane fractions.

Results. Our results demonstrate that in muscle from insulin resistant Zucker rats, FAT/CD36, FABPpm and FATP1 are all increased on the t-tubules in the basal state (+72%, +120%, and +69%, respectively), potentially contributing to the accumulation of intramuscular lipids. Insulin failed to increase the content of the FA transport proteins on either the t-tubule or sarcolemma above the elevated basal levels, analogous to the well characterized impairment of insulin-stimulated GLUT4 translocation to both membrane domains in obesity.

Conclusion. FA transport proteins chronically relocate to the t-tubule domain in insulin resistant muscle, potentially contributing to lipid accumulation. Further translocation of the FA transport proteins to this domain during insulin stimulation, however, is impaired.

© 2013 Elsevier Inc. All rights reserved.

1. Introduction

In recent years it has been shown that fatty acid (FA) uptake into skeletal muscle is a predominantly protein-mediated process [1–4]. A number of FA transport proteins have been identified in skeletal muscle, including FA translocase/CD36 (FAT/CD36), plasma membrane associated FA binding protein (FABPpm), and a family of FA transport proteins (FATP) 1, 4 and 6 [4,5]. All of these FA transporters, except for FATP6, can be induced to translocate to the sarcolemma by muscle

Abbreviations: FA, fatty acid; FAT/CD36, fatty acid translocase; FABPpm, plasma membrane fatty acid binding protein; FATP, fatty acid transport proteins; GLUT4, glucose transporter 4; t-tubules, transverse tubules; NaKATPase, sodium-potassium ATPase; MCT, monocarboxyltransporter; PDH, pyruvate dehydrogenase; HDPR, dihydropyridine receptor; FABPc, cytosolic fatty acid binding protein.

* *Corresponding author*. Department of Human Health and Nutritional Sciences, University of Guelph, ON, Canada, N1G 2W1. Tel.: +1 519 824 4120x56578; fax: +1 519 763 5902.
E-mail address: ddyck@uoguelph.ca (D.J. Dyck).

0026-0495/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.metabol.2013.04.015

contraction or insulin from intracellular depots [4–6] allowing for a tight matching of FA uptake with metabolic demand under normal circumstances [4].

In addition to the sarcolemma, transverse (t)-tubules constitute a large portion of the muscle cell surface area (60%) [7,8]. The discovery of glucose transport (GLUT) proteins on the t-tubules of skeletal muscle revealed that this membrane fraction may be an important site for substrate uptake [7,9,10]. FA transport proteins have recently been identified on the t-tubules [11]. Interestingly, there appears to be a distinct distribution of these FA transport proteins between the sarcolemma and t-tubules, with FAT/CD36, FATP4, and FABPpm being most abundant on the sarcolemma, and FATP1 residing primarily within the t-tubules [5,11].

The regulation of FA uptake is an important factor regulating lipid accumulation in skeletal muscle. A chronic relocation of FAT/CD36 to the sarcolemma correlates with increased lipid storage and insulin resistance [17] in muscle from obese and diabetic humans [12] as well as in obese [13,14] and high-fat fed rats [15,16]. Given the large surface area of t-tubules, it is possible that the FA transporter content in t-tubules is also altered in models of insulin resistance. This however remains to be determined.

With insulin stimulation, FA transport proteins translocate to both the t-tubules and the sarcolemma [5,11]. The response of the various FA transporters to insulin differs. FATP1 and 4 increase primarily on the t-tubules, FAT/CD36 translocates predominantly to the sarcolemma, and the response of FABPpm is variable [5,11,14]. The trafficking of FA transport proteins to the cell surface in the insulin resistant state, particularly the t-tubules, is relatively unexamined. In muscle from insulin resistant obese Zucker rats, sarcolemmal FAT/CD36 is increased under basal conditions and is not further increased with insulin stimulation, while insulin-stimulated FABPpm translocation is unimpaired [14]. Functionally, this correlates to increased FA uptake under basal conditions, with no further increase under insulin-stimulated conditions [14]. It is not known whether insulin-stimulated FATP translocation to the sarcolemma is affected. To date, the trafficking of the FA transport proteins to the t-tubules in insulin resistant muscle is unknown. The t-tubules are likely to be of considerable interest, as it is the impairment of GLUT4 translocation to the t-tubules, rather than to the sarcolemma, that is more closely linked to hyperglycemia [7,10,18,19]. These observations stress the potential importance of t-tubules in the regulation of substrate uptake, including FAs.

The present study is the first to examine whether the localization of the FA transporters to the t-tubules in both basal and insulin-stimulated conditions is altered in insulin resistant skeletal muscle. For this purpose, we utilized obese Zucker rats, a well-characterized model of skeletal muscle insulin resistance.

2. Methods

2.1. Animals

Female lean (250 g, n = 6) and obese (350 g, n = 6) Zucker rats were purchased from Charles River (Charles River, Wilmington, MA) and maintained on a 12:12 h light–dark cycle. Rats were allowed ad libitum access to standard chow (Research Diets D06052601M, New Brunswick, NJ) and water. All experimental procedures were approved by the Animal Care Committee of the University of Guelph. Animals were anaesthetized with an intraperitoneal injection of sodium pentobarbital (6 mg/kg body weight) before all experimental procedures, which took place in the morning after a 10 h fast.

2.2. Surgical preparation and insulin stimulation

Six lean and obese Zucker rats were used for both basal and insulin stimulated conditions. All analyses were conducted using pooled red and white tibialis anterior, red and white gastrocnemius, plantaris and extensor digitorum longus muscles. Basal (unstimulated) muscles were removed and frozen from one limb of each rat after all major arteries were ligated at the hip to prevent blood loss. Insulin (1.0 U/kg body weight; Humulin-R, Eli Lilly, Toronto, ON) was then injected directly into the vena cava with the needle kept in place to prevent blood loss during the experiment [5]. After 30 min the same lower hindlimb muscles were extracted from the contralateral limb and immediately frozen in liquid nitrogen. This dosage and duration have previously been used to demonstrate translocation of glucose and FA transporters to the sarcolemma [5]. Blood glucose remained stable during the removal of the muscles in the basal condition. Fasting blood glucose concentration was significantly greater in the obese compared to the lean rats (9.5 ± 0.8 vs. 5.6 ± 0.2 mmol/L, $p < 0.05$). The decline in blood glucose 30 min following insulin injection was less in the obese than the lean rats (−18% vs. −31%, $p < 0.05$).

2.3. Sarcolemmal and t-tubule fractionation

T-tubule and sarcolemmal membranes were isolated as described by Dombrowski et al. [9]. This isolation procedure was carried out in parallel with muscles from one control, and one insulin-treated set of muscles from a lean and an obese animal to ensure that differences were not due to day-to-day variations. In brief, the muscle sample was cleaned of fat, minced in 15 vol of buffer A (10 mmol/L $NaHCO_3$, 0.25 mol/L sucrose, 5 mmol/L NaN_3, 100 μmol/L phenylmethylsulfonylfluoride, pH 7.4), and homogenized by Polytron at low speed for 5 s. The sample was then centrifuged for 10 min at 1300×*g*. The supernatant was retained, and the pellet was resuspended in buffer A, homogenized with the Polytron and centrifuged at 1300×*g* for 10 min. The resulting supernatant was combined with that from the first spin and centrifuged at 9000×*g* for 10 min. The resulting supernatant was spun at 190,000×*g* for 1 h. The pellet was then layered on top of a discontinuous sucrose gradient of 25%, 32%, and 35% (wt/vol in 50 mmol/L Tris HCL, pH 7.5, 1 mmol/L EDTA, 0.05% lauryl maltoside) and centrifuged at 150,000×*g* for 16 h, resulting in two interfaces with their respective membranes; sarcolemmal (sample to 25%) and t-tubule (32–35%) membranes. Each interface was extracted, resuspended in sucrose free buffer A, and spun separately at 190,000×*g* for 1 h. The resulting pellet was frozen at −80 °C.

Download English Version:

https://daneshyari.com/en/article/5903574

Download Persian Version:

https://daneshyari.com/article/5903574

Daneshyari.com