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Brief Reports

# Insulin and contraction-induced movement of fatty acid transport proteins to skeletal muscle transverse-tubules is distinctly different than to the sarcolemma

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ABSTRACT

Fatty acid (FA) transport proteins are known to exist on the sarcolemma of skeletal muscle. However, it is unknown whether the t-tubules, which comprise ~60% of the cell surface, also harbor these proteins. We examined FA transport proteins from both membrane fractions in unstimulated, insulin-stimulated and contracted skeletal muscle. Sarcolemmal and t-tubule membrane fractions were isolated from the same muscle homogenate using a discontinuous sucrose gradient. Our results demonstrate that the relative content of FA transport proteins within the two fractions and the magnitude to which they increase when stimulated were distinctly different. In unstimulated muscle FAT/CD36, FATP4, and FABPpm are abundant on the sarcolemma (3-, 8-, and 10-fold greater than t-tubule, respectively), whereas FATP1 resides primarily within the t-tubule fraction (1- to 2-fold greater than the sarcolemma). With both stimuli, in terms of absolute increase, FAT/CD36 predominantly translocated to the sarcolemma and FATP1 to the t-tubules. There are clear differences in the profile of FA transport proteins and the response to stimuli of the sarcolemma and t-tubules. FATP1, a variable and unresponsive protein on the sarcolemma, appears to reside primarily in the t-tubules where it is responsive to stimuli.



## 1. Introduction

Fatty acid (FA) uptake into skeletal muscle is tightly regulated in order to protect the cell from FA accumulation and meet metabolic demands [1–4]. Skeletal muscle expresses several FA transport proteins, including FA translocase (FAT)/CD36, plasma membrane associated FA binding protein (FABPpm) [5], and FA transport proteins (FATP) 1, 4, and 6 [6,7]. In skeletal muscle, FAT/CD36 and FATP4 appear to be more closely associated with increased FA uptake than either FABPpm or FATP1 [7].

Similar to GLUT4, FA transport proteins can be induced to translocate from intracellular stores to the muscle surface when exposed to different stimuli, including insulin and contraction [6,8]. To date, the translocation of FA transport proteins induced by insulin and muscle contraction has only been assessed in the sarcolemma. However, the sarcolemma represents only a portion of the muscle surface (~40%), while the remainder (~60%) is comprised of transverse (t)-tubules [9–12]. T-tubules play a vital role in propagating signals for muscle contraction and potentially glucose transport [9–12]. Subfractionation of muscle [11] allows for the recovery of both the sarcolemmal and t-tubule membranes, and has been used to characterize the translocation of GLUT4 with physiologic stimuli [11]. The presence of FA transport proteins on

*Abbreviations:* FA, fatty acid; GSV, giant sarcolemmal vesicle; AU, arbitrary units.

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t-tubules and their ability to translocate to this domain have not yet been determined. Therefore, we examined the cell surface distribution of FA transporters under unstimulated and metabolically stimulated conditions (insulin, contraction).

## 2. Methods

### 2.1. Animals

Female Sprague–Dawley rats (Charles River, Senneville, QC) ~200 g were provided standard chow and water *ad libitum*. All procedures were approved by the Animal Care Committee at the University of Guelph. Animals were anaesthetized with an intraperitoneal injection of sodium pentobarbital (6 mg/kg body weight) after a 10 h fast.

### 2.2. Experimental treatments

Lower hindlimb muscles (red and white tibialis anterior, gastrocnemius, plantaris and extensor digitorum longus) were used for all analyses. Muscles were either unstimulated (basal), stimulated with an insulin injection into the vena cava (30 min, 1.0 U/kg body weight; Humulin-R, Eli Lilly, Toronto, ON), or stimulated to contract via the sciatic nerve (10 ms impulses, 100 Hz/3 s at 10–20 V; train duration 100 ms) [8]. The limb was stimulated twice for 13 min, separated by a 4 min rest. During contraction, blood glucose remained stable. Insulin administration lowered blood glucose by 56% ± 6% after 30 min.

### 2.3. Sarcolemmal and t-tubule fractionation

Sarcolemmal and t-tubule membranes were isolated as previously described [11]. In brief, the muscle was cleaned of fat, minced in buffer A (10 mmol/L $NaHCO_3$, 0.25 mol/L sucrose, 5 mmol/L $NaN_3$, 100 μmol/L phenylmethylsulfonyl-fluoride, pH 7.4) and homogenized by polytron (low for 5 s). The sample was centrifuged for 10 min at 1300 × g. The supernatant was retained, and the previous steps were repeated with the pellet. The two supernatants were combined and centrifuged at 9000 × g for 10 min. The resulting supernatant was spun at 190,000 × g for 1 h. The pellet was layered on top of a discontinuous sucrose gradient of 25%, 32%, and 35% (wt/vol) and centrifuged at 150,000 × g for 16 h, resulting in two interfaces; sarcolemmal membrane (sample to 25%), and t-tubules (32% to 35%). Each interphase was extracted, resuspended in sucrose free buffer A and spun separately at 190,000 × g for 1 h. Pellets were retained and frozen.

### 2.4. Western blotting

Protein concentration of whole muscle and the isolated fractions was determined using a BCA assay. Protein from whole muscle (35 μg), sarcolemmal (10 μg), and t-tubule (10 μg) membrane preparations were solubilized in Laemmli's buffer, boiled, resolved by SDS-PAGE, and wet-transferred to polyvinylidene difluoride membranes. Membranes were blocked and incubated overnight at 4 °C with primary antibodies to measure MO25 FAT/CD36 (gift from Dr. N. Tandon, Otsuka Maryland Medicinal Laboratories); FABPpm (gift from Dr. J. Calles-Escandon, Wake Forest University); FATP1 and FATP4, (Santa Cruz Biotechnology, Santa Cruz, CA), and to test the purity of the membrane fractions, GLUT4 (Millipore, Temecula, CA), monocarboxylate transport protein 1 (MCT1; gift from Dr. Hatta, University of Tokyo); α-tubulin, α1-dihydropyridine Receptor (DHPR; Abcam, Cambridge, MA), α1-NaK ATPase (Upstate Biotechnology, Charlottesville, VA) and pyruvate dehydrogenase (PDH; Life Technologies, Burlington, ON). The immune complexes were detected using enhanced chemiluminescence and quantified with densitometry. Equal loading was confirmed with Ponceau-S stain. Sarcolemma and t-tubule samples were run together on the same gel and all samples were transferred to one membrane so that comparisons between fractions could be made. Changes in protein content with stimulation are reported both as an absolute, and percent change relative to the basal amount due to the large variation in basal concentrations of the various proteins in each fraction.

### 2.5. Statistical analysis

Data are reported as mean ± standard error. Comparisons between the sarcolemma and t-tubules under unstimulated conditions were performed through a two-way ANOVA. The effects of the stimuli within each membrane fraction were analyzed using a one-way ANOVA and a Student Newman Keuls post hoc test. Significance was accepted at $P \leq .05$.

## 3. Results

### 3.1. Characterization of the membrane fractions

The α1-subunit of the sarcolemmal marker NaK ATPase, was enriched in our isolated fraction (Fig. 1). In contrast, a t-tubule marker, DHPR was exclusively located in this fraction. Both membrane fractions were devoid of the microtubular protein α-tubulin and contained only trace amounts of the mitochondrial protein PDH which did not change with metabolic stimulation. As expected, stimulation did not alter the sarcolemmal protein MCT1 which was relatively low in the t-tubules [13,14].

### 3.2. Sarcolemmal and t-tubule content of transport proteins in unstimulated muscle

There was no difference in the GLUT4 protein content between the two fractions (Fig. 2). FAT/CD36, FABPpm, and FATP4 contents were all significantly greater ($P < .001$) in the sarcolemma than the t-tubules (3-, 22-, and 9-fold, respectively). FATP1 content in the t-tubules was 1- to 2-fold greater than in the sarcolemma ($P < .001$).

### 3.3. Sarcolemmal and t-tubule content of transport proteins in stimulated muscle

#### 3.3.1. Sarcolemma

Insulin stimulation increased the content of GLUT4 [+54 arbitrary units (AU) (+54%); $P = .009$], FAT/CD36 (+37 AU and %; $P < .001$), FATP1 (+23 AU and %; $P = .047$) and FATP4 (+29 AU and %; $P = .016$), but not FABPpm (Fig. 2). Contraction