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Insulin induces the translocation of the fatty acid transporter FAT/CD36 to the plasma membrane

JOOST J. F. P. LUIKEN,[1,3] DAVID J. DYCK,[2] XIAO-XIA HAN,[4] NARENDRA N. TANDON,[4] YOGA ARUMUGAM,[3] JAN F. C. GLATZ,[1] AND AREND BONEN[3]
[1]*Department of Physiology, Maastricht University, 6200 MD Maastricht, The Netherlands;* [2]*Department of Human Biology and Nutritional Sciences, University of Guelph, Guelph, Ontario N1G 2W1;* [3]*Department of Kinesiology, University of Waterloo, Waterloo, Ontario N2L 3G1, Canada; and* [4]*Thrombosis Research Laboratory, Otsuka Maryland Research Institute, Rockville, Maryland 20850*

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Luiken, Joost J. F. P., David J. Dyck, Xiao-Xia Han, Narendra N. Tandon, Yoga Arumugam, Jan F. C. Glatz, and Arend Bonen. Insulin induces the translocation of the fatty acid transporter FAT/CD36 to the plasma membrane. *Am J Physiol Endocrinol Metab* 282: E491–E495, 2002; 10.1152/ajpendo.00419.2001.—It is well known that muscle contraction and insulin can independently translocate GLUT-4 from an intracellular depot to the plasma membrane. Recently, we have shown that the fatty acid transporter FAT/CD36 is translocated from an intracellular depot to the plasma membrane by muscle contraction (<30 min) (Bonen et al. *J Biol Chem* 275: 14501–14508, 2000). In the present study, we examined whether insulin also induced the translocation of FAT/CD36 in rat skeletal muscle. In studies in perfused rat hindlimb muscles, we observed that insulin increased fatty acid uptake by +51%. Insulin increased the rate of palmitate incorporation into triacylglycerols, diacylglycerols, and phospholipids ($P < 0.05$) while reducing muscle palmitate oxidation ($P < 0.05$). Perfusing rat hindlimb muscles with insulin increased plasma membrane FAT/CD36 by +48% ($P < 0.05$), whereas concomitantly the intracellular FAT/CD36 depot was reduced by 68% ($P < 0.05$). These insulin-induced effects on FAT/CD36 translocation were inhibited by the phosphatidylinositol 3-kinase inhibitor LY-294002. Thus these studies have shown for the first time that insulin can induce the translocation of FAT/CD36 from an intracellular depot to the plasma membrane.This reveals a previously unknown level of regulation of fatty acid transport by insulin and may well have important consequences in furthering our understanding of the relation between fatty acid metabolism and insulin resistance.

hindlimb perfusion; glucose transporter 4; palmitate; oxidation; triacylglycerol; diacylglycerol; phospholipids

SKELETAL MUSCLE IS AN IMPORTANT TARGET for insulin action, because this hormone has multiple effects on muscle intermediary metabolism. One of the best known insulin effects is the stimulation of glucose transport through the translocation of GLUT-4 from intracellular vesicular structures to the sarcolemma. Insulin is also able to regulate fatty acid metabolism in the myocyte. For example, in skeletal muscle, insulin can inhibit fatty acid oxidation through the inhibition of carnitine palmitoyltransferase I, an effect that is mediated through increased intracellular levels of malonyl-CoA (4, 14). It has now also been shown that insulin increases fatty acid esterification into triacylglycerols in skeletal muscle while simultaneously reducing fatty acid oxidation (13, 22). From these studies, it can also be inferred that insulin increases net fatty acid uptake into the myocyte, since the insulin-induced increase in fatty acid esterification was markedly greater than the insulin-induced inhibition of fatty acid oxidation.

Fatty acid uptake across the plasma membrane occurs via both diffusion (17) and a protein-mediated mechanism (2), involving a 43-kDa plasma membrane fatty acid binding protein (19) and an 88-kDa heavily glycosylated fatty acid translocase, the rat homolog of human CD36 (FAT/CD36) (1). The 63-kDa fatty acid transport protein (FATP1) (24), originally also thought to be a fatty acid transporter, may not be involved in the transsarcolemmal transport of fatty acid, since it has been shown that FATP1 is a very long chain fatty acyl-CoA synthetase (9, 26). We (6) have recently found evidence that FAT/CD36 is involved in the short-term regulation of fatty acid uptake by muscle; namely, during muscle contraction (<30 min), fatty acid uptake by muscle is increased. This was shown to be due to the

Address for reprint requests and other correspondence: A. Bonen, Dept. of Kinesiology, Univ. of Waterloo, Waterloo, ON, Canada N2L 3G1 (E-mail: abonen@healthy.uwaterloo.ca).

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translocation of FAT/CD36 from intracellular compartments to the plasma membrane, since the blocking of the plasma membrane FAT/CD36 with sulfo-*N*-succinimidyl oleate prevented the contraction-induced increase of fatty acid uptake (6). Thus fatty acid uptake can be acutely regulated through the subcellular redistribution of FAT/CD36.

Whether FAT/CD36 distribution within the myocyte is also regulated by insulin is not known, but because insulin increases net fatty acid uptake (13, 22) via a phosphatidylinositol (PI) 3-kinase-dependent mechanism (13, 22), and because it it is well known that insulin induces the translocation of the glucose transporter GLUT-4 via PI 3-kinase activation, it is reasonable to hypothesize that insulin increases fatty acid uptake by muscle by translocating FAT/CD36 from its intracellular compartments to the plasma membrane. Therefore, in the present studies, we have examined the effects of insulin on *1*) fatty acid uptake and metabolism in perfused rat skeletal muscle and *2*) on the translocation of FAT/CD36 from its intracellular compartment to the plasma membrane. Our results show *1*) that insulin stimulates fatty acid uptake by 51% in intact muscles, and *2*) that this is closely matched by the insulin-induced increase in plasma membrane FAT/CD36 (+48%) and concomitant reduction in the intracellular FAT/CD36 pool (−68%), effects that were blocked by the PI 3-kinase inhibitor LY-294002.

METHODS

Materials. Fat-free BSA was obtained from Roche Diagnostics (Laval, QC, Canada). [1-^{14}C]palmitate was obtained from Amersham Life Science (Oakville, ON, Canada). LY-294002 was obtained from Calbiochem. Human insulin (Humulin) was purchased from a local pharmacy.

Animals. Male Sprague-Dawley rats were used in all experiments (200–250 g). Animals were housed in a controlled environment on a 12:12-h light-dark cycle and fed Purina rat chow ad libitum. All procedures were approved by the animal care committee at the University of Waterloo. Animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (6 mg/100 g body mass) before all experimental procedures.

Hindlimb perfusion. Rat hindlimb muscles were perfused for 60 min in either the absence or presence of insulin (20 mU/ml) with the use of a cell-free perfusate that contained palmitate (1 mM) and 4% BSA, as we have described previously (18, 20) in detail. In this perfusion model, we have shown that insulin increases glucose transport and GLUT-4 translocation to the plasma membrane (18, 20). Muscles were also perfused with PI 3-kinase inhibitor LY-294002 (60 μM) and with insulin + LY-294002. This concentration of LY-294002 maximally inhibits insulin-stimulated glucose transport in perfused rat muscle (28). Muscles from all of these experiments were used to fractionate rat hindlimb muscles (see *Subcellular localization of transporters in skeletal muscle).*

In separate experiments, we also determined basal and insulin-stimulated palmitate (1 mM) oxidation rates in the rat hindlimb by collecting arterial and venous effluents at regular intervals during the perfusion and measuring the $^{14}CO_2$ in these samples by means of a modification of previous procedures used in our laboratory (10–13). In addition, palmitate incorporation into phospholipids, triacylglycerol, and diacylglycerol was determined by measuring [^{14}C]palmitate accumulation in these depots in selected rat hindlimb muscles (soleus and red and white gastrocnemius) as we have previously reported (10–13). This permitted us to calculate the incorporation rates of palmitate in the whole rat hindlimb musculature from the known muscle fiber composition of these muscles and the known proportions of their muscle fiber mass in the rat hindlimb (3), as we (20) have done previously when determining glucose transport for the whole hindlimb muscle from selected individual muscles.

Subcellular localization of transporters in skeletal muscle. Muscles were fractionated as we (6) have recently described to determine the subcellular location of FAT/CD36. Briefly, muscles (1.5 g) were minced for 5 min in 10 mM $NaHCO_3$ (pH 7.0), 0.25 M sucrose, 5 mM NaN_3, and 100 mM phenylmethylsulfonyl fluoride (*buffer A*). The minced muscles were homogenized (1 g/15 ml dilution) using a Polytron (Brinkman Instruments) at a low setting of 3 for 5 × 1 s. The resulting homogenate was centrifuged at 1,300 *g* for 10 min. The supernatant was saved, and the low-speed pellets were resuspended in *buffer A* (2 g/15 ml), homogenized, and centrifuged again. Both supernatant fractions were pooled. The low-speed pellet was resuspended in 0.5 M LiBr, 50 mM Tris (pH 8.5), and 100 mM phenylmethylsulfonyl fluoride (*buffer B*) at a ratio of 1 g tissue/25 ml and stirred for 4 h. The pooled 1,300-*g* supernatant fractions were centrifuged at 9,000 *g* for 10 min. The 9,000-*g* pellet was saved, and the supernatant was spun at 190,000 *g* for 1 h. The 190,000-*g* pellet was resuspended in 800 ml of *buffer A* by five strokes with the use of a tightly fitting Potter-Elvehjem glass homogenizer and subjected to a continuous Percoll gradient as described below. The LiBr-treated membranes were centrifuged first at 1,200 *g* for 5 min and then at 10,000 *g* for 30 min. The LiBr-10,000-*g* pellet was saved. The supernatant fraction was centrifuged at 190,000 *g* for 1 h. The LiBr-190,000-*g* pellet was resuspended in 1.2 ml of *buffer A* by 40 strokes with the use of a tightly fitting Potter-Elvehjem glass homogenizer. Five hundred milliliters of both the 190,000-*g* pellet and the LiBr-190,000-*g* pellet were layered on top of a 7-ml Percoll suspension (60%) in 120 mM KCl, 25 mM HEPES (pH 7.0), 5 mM NaN_3, and 0.5 mM EGTA (initial density 1.12 g/ml) resting on a cushion of 1 ml of 1.25 M sucrose. The resulting gradients were centrifuged at 62,000 *g* for 1 h. After centrifugation, 0.5-ml fractions were collected starting from the top. All procedures were performed at 0°C. The fractions were stored at −80°C and, upon thawing, were used for SDS-polyacrylamide gel electrophoresis followed by Western blotting. Before electrophoresis, Percoll particles were removed by alkalinizing aliquots of the fractions to pH 12.0 by the addition of NaOH, followed by centrifugation in a microcentrifuge for 2 min. The supernatant was then neutralized to pH 7–8 by the addition of HCl. In the resulting Percoll-free fraction samples, the protein content was determined by the bicinchoninic acid method, and 5 mg of each fraction were subjected to SDS-polyacrylamide gel electrophoresis and Western blotting. For detection of FAT/CD36, the antibody MO25 was used (21). The GLUT-4 was detected with a commercially available antibody (East Acres Biologicals). The monocarboxylate transporter MCT4 was detected by using an antibody prepared in our laboratory (7).

Statistics. Statistical analyses were performed using analyses of variance and *t*-tests where appropriate. All data are presented as means ± SE.

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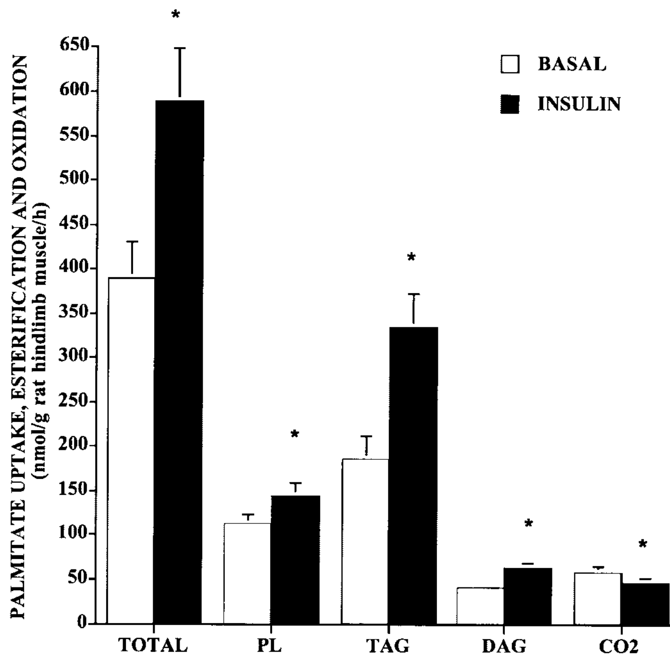

Fig. 1. Effect of insulin on palmitate oxidation (CO_2) and incorporation into phospholipid (PL) and triacylglycerol (TAG), and diacylglycerol (DAG) palmitate esterification. Total palmitate uptake is the sum of CO_2 + TAG + DAG +PL by perfused rat hindlimb muscles. Muscles were perfused in the absence (basal) or presence of insulin with the use of a cell-free perfusate containing palmitate (1 mM) complexed to BSA (4%). Results are based on 5 independent preparations for each experimental treatment (means ± SE). *$P < 0.05$, insulin vs. basal.

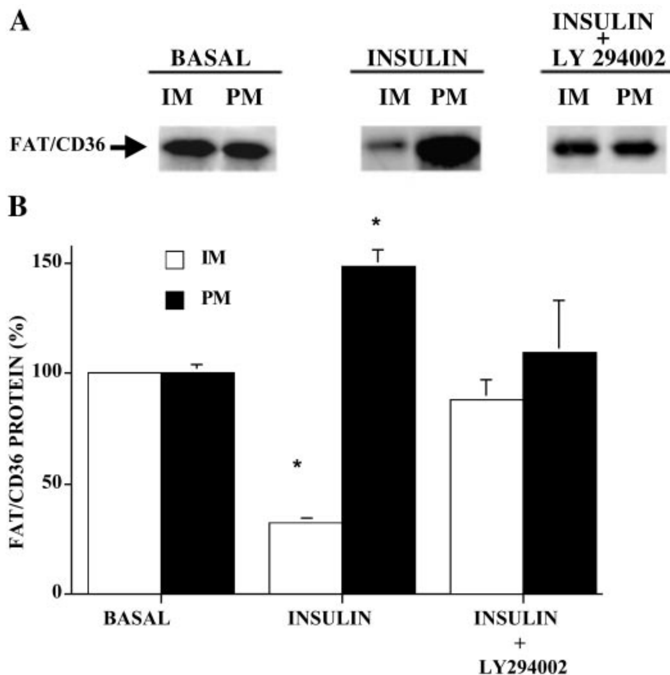

Fig. 2. Representative Western blots of fatty acid transporter FAT/CD36 (*A*) and their quantification (*B*) in rat hindlimb muscle perfused in the absence of insulin (basal), with insulin, and with insulin + LY-294002 [a phosphatidylinositol (PI) 3-kinase inhibitor]. PM, plasma membrane; IM, intracellular membrane. *$P < 0.05$, insulin vs. basal.

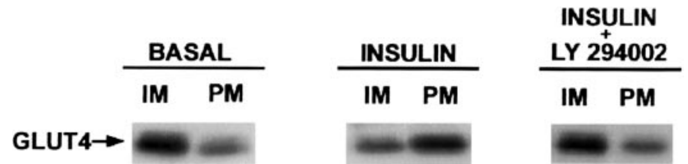

Fig. 3. Representative Western blots of the glucose transporter GLUT-4 at the PM and IM of rat hindlimb muscles that had been perfused in the absence of insulin (basal), with insulin, and with insulin + the PI 3-kinase inhibitor LY-294002.

RESULTS AND DISCUSSION

In the perfused rat hindlimb muscles, insulin increased the incorporation of palmitate into skeletal muscle phospholipids (+29%, $P < 0.05$), triacylglycerols (+80%, $P < 0.05$), and diacylglycerols (+49%, $P < 0.05$). Concomitantly, insulin reduced palmitate oxidation (−20%, $P < 0.05$; Fig. 1). We can ignore the palmitate incorporated into monoacylglycerols, because that typically represents <1% of the palmitate taken up by muscle (10, 12). Similarly, free palmitate in muscle was not determined, as the unesterified fatty acid content in muscle is negligible (25). Thus, in rat hindlimb muscles, the net uptake of palmitate was increased 51% ($P < 0.05$; Fig. 1) by insulin.

These observations in the perfused rat hindlimb muscles are consistent with a recent report from our laboratory, which also demonstrated that insulin increased palmitate incorporation into phospholipids, triacylglycerols, and diacylglycerols, and reduced fatty acid oxidation in isolated soleus muscle (13). In these studies (Fig. 1 and Ref. 13), insulin increased the total fatty acid taken up by the muscles to a similar extent [+51% in perfused muscles and +43% in isolated soleus muscles (13)], since the insulin-stimulated increase in fatty acid esterification was much greater than the concomitant reduction in fatty acid oxidation (Fig. 1).

The insulin-induced increments in fatty acid uptake are not attributable to changes in the delivery of fatty acids, because *1*) in perfused muscles (present study) the perfusate flow is controlled, *2*) in isolated muscle preparations (13) the muscle is maintained in a constant volume of buffer, and *3*) in both preparations the fatty acid concentration remains constant. However, we (6) have recently reported that the fatty acid transporter FAT/CD36 can be translocated from an intracellular pool to the plasma membrane by muscle contraction to meet the increased metabolic demand of the contracting muscle (6). Therefore, we speculated that

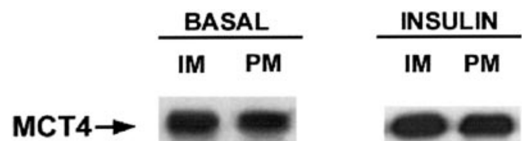

Fig. 4. Representative Western blots of the monocarboxylate transporter MCT4 at the PM and IM of rat hindlimb muscles that had been perfused in the absence of insulin (basal) and with insulin. Perfusions with insulin + LY-294002 were not performed, because insulin did not translocate MCT4.

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the insulin-induced increase in fatty acid uptake by intact muscles, when the fatty acid delivery remained constant, may also be attributable to the insulin-stimulated translocation of the fatty acid transporter FAT/CD36.

When the muscles were perfused with insulin, the plasma membrane FAT/CD36 was increased by 48% ($P < 0.05$, Fig. 2, *A* and *B*) whereas the intracellular FAT/CD36 was reduced by 68% ($P < 0.05$, Fig. 2, *A* and *B*). Insulin also translocated GLUT-4 in the perfused muscles (Fig. 3), as we have shown previously in perfused muscles (18, 20). The monocarboxylate transporter MCT4, which is also present at the plasma membrane and in an intracellular pool (7), was not translocated by insulin (Fig. 4). When the muscles were perfused with insulin along with the PI 3-kinase inhibitor LY-294002, no changes in FAT/CD36 redistribution were observed (Fig. 2, *A* and *B*). GLUT-4 appearance at the plasma membrane was also blocked when LY-294002 was included with insulin in the perfusate (Fig. 3). This inhibition of insulin-stimulated GLUT-4 translocation by LY-294002 confirms previous observations in skeletal muscle (27) and adipocytes (8). In addition, we (13) and others (22) have demonstrated recently that the insulin-induced alterations in fatty acid metabolism are prevented when the activity of PI 3-kinase is inhibited by either wortmannin (22) or LY-294002 (13).

The novel observation in the present study is that insulin can induce the translocation of FAT/CD36 from an intracellular depot to the plasma membrane, involving a PI 3-kinase signaling step. Thus this hormone will simultaneously translocate GLUT-4 and FAT/CD36. Also, it appears that insulin signaling of FAT/CD36 (present study) and GLUT-4 translocation in muscle (15, 20) occur via the same early postreceptor signaling steps. Moreover, both FAT/CD36 translocation (6) and GLUT-4 translocation (15, 20) can also be provoked by muscle contraction. Whether there are separate signaling pathways for inducing the translocation of FAT/CD36, as has been shown for the translocation of GLUT-4 by insulin and muscle contraction (16, 20), is not known. But in recent studies in our laboratories, we have shown that insulin and muscle contraction additively stimulate fatty acid uptake into intact isolated muscles (13) and cardiac myocytes (J. J. F. P. Luiken, J. F. C. Glatz, and A. Bonen, unpublished data). Thus these observations suggest that there may also be insulin-sensitive and contraction-sensitive intracellular FAT/CD36 compartments in muscle. This is currently under investigation.

By increasing FAT/CD36 at the plasma membrane, the rate of fatty acid uptake by muscle is increased (5, 6). Thus the insulin-induced increase in the rate of fatty acid esterification (Fig. 1) is attributable, in part, to the insulin-induced translocation of FAT/CD36. This is also suggested by the similar insulin-induced increases in plasma membrane FAT/CD36 (+48%) (Fig. 3) and the total fatty acid uptake (+51%) by the perfused muscles (Fig. 1).

The regulation of fatty acid uptake and metabolism by insulin may have considerable implications for furthering our understanding of insulin resistance in muscle. It has been shown that insulin resistance in this tissue is positively related to the the intramuscular content of triacylglycerol (23). Because insulin stimulates fatty acid uptake and esterification to triacylglycerols (Fig. 1), the hyperinsulinemia observed in animal models of insulin resistance (e.g., obese Zucker rats) may promote fatty acid uptake due to an increase in plasma membrane FAT/CD36. Indeed, we now have evidence for this (J. J. F. P. Luiken and A. Bonen, unpublished data). This permanent relocation of FAT/CD36 to the plasma membrane is then directly linked to an increased rate of fatty acid uptake and a progressive accumulation of intracellular triacylglycerol depots.

In summary, we have shown that insulin stimulates FAT/CD36 translocation from an intracellular compartment to the plasma membrane in skeletal muscle. This reveals a previously unknown level of regulation of fatty acid transport by insulin and may have important consequences for furthering our understanding of the relation between fatty acid metabolism and insulin resistance.

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