CELL BIOLOGY AND METABOLISM:

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A High Fat Diet Impairs Stimulation of Glucose Transport in Muscle

FUNCTIONAL EVALUATION OF POTENTIAL MECHANISMS*

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A high fat diet causes resistance of skeletal muscle glucose transport to insulin and contractions. We tested the hypothesis that fat feeding causes a change in plasma membrane composition that interferes with functioning of glucose transporters and/or insulin receptors. Epitrochlearis muscles of rats fed a high (50% of calories) fat diet for 8 weeks showed ~50% decreases in insulin- and contraction-stimulated 3-*O*-methylglucose transport. Similar decreases in stimulated glucose transport activity occurred in muscles of wild-type mice with 4 weeks of fat feeding. In contrast, GLUT1 overexpressing muscles of transgenic mice fed a high fat diet showed no decreases in their high rates of glucose transport, providing evidence against impaired glucose transporter function. Insulin-stimulated system A amino acid transport, insulin receptor (IR) tyrosine kinase activity, and insulin-stimulated IR and IRS-1 tyrosine phosphorylation were all normal in muscles of rats fed the high fat diet for 8 weeks. However, after 30 weeks on the high fat diet, there was a significant reduction in insulin-stimulated tyrosine phosphorylation in muscle. The increases in GLUT4 at the cell surface induced by insulin or muscle contractions, measured with the ^{3}H-labeled 2-*N*-4-(1-azi-2,2,2-trifluoroethyl)-benzoyl-1,3-bis-(D-mannose-4-yloxy)-2-propylamine photolabel, were 26–36% smaller in muscles of the 8-week high fat-fed rats as compared with control rats. Our findings provide evidence that (*a*) impairment of muscle glucose transport by 8 weeks of high fat feeding is not due to plasma membrane composition-related reductions in glucose transporter or insulin receptor function, (*b*) a defect in insulin receptor signaling is a late event, not a primary cause, of the muscle insulin resistance induced by fat feeding, and (*c*) impaired GLUT4 translocation to the cell surface plays a major role in the decrease in stimulated glucose transport.

Rodents fed a high fat diet rapidly develop severe whole body and skeletal muscle insulin resistance, hyperinsulinemia, hyperglycemia, and in genetically susceptible strains, diabetes (1–6). The high fat diet-fed rodent is of interest as a research model because it might provide insights regarding the mechanisms underlying insulin resistance in obese individuals with impaired glucose tolerance or type 2 diabetes. For example, there is considerable experimental evidence that insulin signaling is impaired in skeletal muscle of obese, insulin-resistant humans (7–9), although it is still not clear if the insulin receptor defect is a mechanism involved in the development of the insulin resistance or is a consequence of the insulin resistance. One purpose of this study was to determine whether an insulin-signaling defect is involved in the development of muscle insulin resistance in response to a high fat diet.

In addition to insulin, glucose transport in skeletal muscle can be stimulated by muscle contractile activity or hypoxia (for review, see Ref. 10). However, the signaling pathways by which insulin and contractions/hypoxia stimulate glucose transport are distinct, as evidenced by the findings that their maximal effects on glucose transport are additive (11, 12), and the effect of insulin, but not of contractions/hypoxia, is blocked by phosphatidylinositol 3-kinase inhibition (13–15). In this context, the finding that stimulation of glucose transport by muscle contractions is also impaired in high fat diet-fed rodents (6, 16) suggests the alternative possibility that it is a common step beyond the contraction and insulin-signaling pathways that is involved.

Stimulation of skeletal muscle glucose transport by either insulin or contractions is mediated by translocation of the GLUT4 isoform of the glucose transporter to the cell surface (15, 17, 18). It has been postulated, on the basis of findings of Zierath *et al.* (19), that the insulin resistance induced by fat feeding is mediated by decreased movement of GLUT4 transporters to the cell surface, although this has not been a consistent finding (16). The second purpose of this study was to re-evaluate the effect of the high fat diet on insulin-stimulated GLUT4 translocation to the cell surface and to determine whether the diet affects contraction-stimulated GLUT4 translocation.

The catalytic activity of the glucose transporter proteins is sensitive to changes in the compositional and physical properties of the membrane bilayer (20, 21), and it has been postulated that the insulin resistance associated with high fat feeding and obesity is mediated, in part, by membrane composition-related reductions in glucose transporter activity (22, 23). In support of this possibility, Rosholt *et al.* (16) have obtained evidence that a high fat diet results in decreased GLUT4 intrinsic activity in skeletal muscle. The GLUT1 and GLUT4 isoforms of the glucose transporter have a high degree of sequence similarity (24, 25), suggesting that the basic mecha-

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nism by which GLUT1 and GLUT4 transport glucose across the plasma membrane is the same. If the hypothesis that a change in plasma membrane composition interferes with GLUT4-mediated glucose transport in muscle of fat-fed rodents is correct, it seems reasonable that glucose transport by the GLUT1 transporter would be similarly affected. Another purpose of the present study was to test the hypothesis that insulin resistance in high fat diet-fed rodents is mediated by an alteration in plasma membrane structure that interferes with glucose transporter function. To this end, we have evaluated the effect of a high fat diet on glucose transport in muscles of mice overexpressing the GLUT1 glucose transporter. We have also examined the effect of the high fat diet on the activity of the system A amino acid transporter, another protein whose function could be affected by compositional changes in the plasma membrane.

EXPERIMENTAL PROCEDURES

Materials—α-[1-^{14}C](Methylamino)isobutyrate, D-[2-^{3}H]-mannitol, 3-*O*-[^{3}H]-methyl-D-glucose, [^{14}C]-mannitol, and [γ-^{32}P]ATP were all purchased from NEN Life Science Products. 2-Deoxy-D-[1,2-^{3}H]glucose was purchased from American Radiolabeled Chemicals. Donkey anti-rabbit ^{125}IgG and reagents for ECL were obtained from Amersham Pharmacia Biotech. Antibodies against the insulin receptor β-subunit, insulin receptor substrate-1, and phosphotyrosine were all purchased from Upstate Biotechnology. Horseradish peroxidase-conjugated donkey anti-rabbit IgG was obtained from Jackson ImmunoResearch Laboratories. All other chemicals were obtained from Sigma. The 2-*N*-4-(1-azi-2,2,2-trifluoroethyl)-benzoyl-1,3-bis-(D-mannose-4-yloxy)-2-propylamine (ATB-[2-^{3}H]BMPA)[1] was kindly provided by Dr. Geoff Holman (University of Bath, United Kingdom). Immunoprecipitation of ATB-[^{3}H]BMPA-labeled GLUT4 was performed using a rabbit polyclonal antibody (G4 829) directed against the 16 carboxyl-terminal amino acids of the GLUT4 glucose transporter.

Treatment of Animals—All experimental procedures were approved by the Washington University Animal Studies Committee. At the time of weaning, colony-bred male Wistar rats were assigned to either high fat or chow diet groups. The semi-purified high fat diet was prepared as described previously (6), with 50% of the total calories derived from fat, 27% from sucrose, and 23% from casein. Control animals were fed constant-formula Purina rodent chow (Purina 5001). All diets were fed *ad libitum*.

Additional high fat feeding experiments were performed using mice that overexpress the human GLUT1 glucose transporter in skeletal muscle and their nontransgenic littermates. The construction of these mice has been described previously (26). The minigene in this construct contains a 2.47-kilobase cDNA fragment encoding the human GLUT1 glucose transporter under the regulation of the 1.2-kilobase rat myosin light chain-2 promoter. Expression of the transgene is restricted to skeletal muscle and does not affect expression of the GLUT4 isoform. Basal glucose transport activity in extensor digitorum longus (EDL) and epitrochlearis muscles from GLUT1 transgenic animals is 4–8-fold higher than in muscles from nontransgenic controls (27).

Muscle Preparation—Rats or mice were anesthetized by an intraperitoneal injection of pentobarbital sodium (5 mg/100 g body weight), and the epitrochlearis and/or EDL muscles were excised. In some experiments, muscles were stimulated to contract *in situ* (see below) prior to dissection. Muscles were incubated with shaking for 1 h at 35 °C in 2 ml of oxygenated Krebs-Henseleit buffer (KHB) supplemented with 8 mM glucose, 32 mM mannitol, and 0.1% bovine serum albumin (BSA), with or without a maximally effective concentration of insulin (2 milliunits/ml). Muscles were then washed in KHB containing 40 mM mannitol and 0.1% BSA, with or without insulin, for 10 min at 30 °C prior to measurement of glucose transport activity. The gas phase throughout the incubations was 95% O_2, 5% CO_2.

In Situ Muscle Contractions—Rat epitrochlearis muscles were stimulated to contract indirectly via the nerve. Square wave pulses (0.1 ms) were delivered with a Grass S48 stimulator at 100 Hz to give 250-ms-long trains at a rate of 60/min for 5 min. After a 1-min rest period, the muscles were stimulated for a second 5-min interval using the same protocol.

Measurement of Glucose Transport Activity—Muscle glucose transport activity was assayed using 1.0 ml of KHB containing 8 mM 3-*O*-[^{3}H]methyl-D-glucose (3-MG; 2.2 μCi/ml), 32 mM [^{14}C]mannitol (0.2 μCi/ml), 0.1% BSA, and insulin if it was present during the previous incubation. Incubations were performed at 30 °C with a gas phase of 95% O_2, 5% CO_2. Extracellular space and intracellular 3-MG concentration (μmol·ml intracellular water^{-1}) were determined as described previously (28). In some experiments, glucose transport activity was measured using 1 mM 2-deoxy-D-[1,2-^{3}H]glucose and 39 mM [^{14}C] mannitol (27).

Measurement of System A Amino Acid Transport Activity—The nonmetabolizable amino acid analog α-(methylamino)isobutyrate (MeAIB) was used to measure system A amino acid transport activity as described previously (29). Muscles were incubated in the presence or absence of 2 milliunits/ml insulin exactly as described above for the measurement of glucose transport activity. After the wash step, muscles were incubated at 30 °C for 20 min in 1.5 ml of KHB containing 0.1 mM [^{14}C]MeAIB (0.075 μCi/ml), 10 mM D-[2-^{3}H]mannitol (0.375 μCi/ml), 0.1% BSA, and insulin if it was present in the previous steps.

Photolabeling of Epitrochlearis Muscles—Cell surface GLUT4 was assessed using the ATB-[2-^{3}H]BMPA exofacial photolabeling technique as described previously (30), except that the labeled GLUT4 was immunoprecipitated using a rabbit polyclonal antibody followed by protein A-Sepharose.

Preparation of Solubilized Insulin Receptors and Receptor Tyrosine Kinase Assay—Insulin receptors were prepared from the pooled gastrocnemius and plantaris muscles of one hind limb using previously described methods (31, 32). Insulin receptors were partially purified from solubilized muscle extracts using a wheat germ agglutinin (WGA)-Sepharose column. The ability of the solubilized insulin receptors to phosphorylate exogenous substrates was tested using the synthetic peptide poly(Glu-Tyr (4:1)). Aliquots of the WGA eluate (100 μl containing 4 μg of protein) were incubated in the presence or absence of insulin (16.7 milliunits/ml) for 60 min at 23 °C. Poly(Glu-Tyr (4:1)) (28 μg in 20 μl) was then added, and the reaction was initiated by the addition (20 μl) of a mixture containing 8 mM $MgCl_2$, 600 μM Na_3VO_4, 45 μM ATP, and 2 μCi of [γ-^{32}P]ATP (32). The reaction was allowed to proceed for 30 min at 23 °C, and the reaction was terminated by spotting aliquots of the reaction mixture on Whatman 31 ET filter paper. The papers were washed extensively in 1% H_3PO_4, rinsed in acetone, and then allowed to air dry prior to scintillation counting.

Insulin Signaling in Skeletal Muscle—Following an overnight fast, rats were anesthetized, and a bolus of saline (0.5 cc) was injected via a catheter placed in a jugular vein; after 120 s, the soleus muscle from the right hind limb was excised and clamp frozen. A bolus of insulin (10 units/kg body weight in 0.5 cc saline) was then injected into the catheter, and after 120 s, the left soleus muscle was excised and clamp frozen. Muscles were stored at −80 °C until analysis of insulin-stimulated tyrosine phosphorylation.

Soleus muscle extracts were prepared by the method of Saad *et al.* (33). For analysis of insulin receptor β-subunit autophosphorylation, aliquots of the solubilized muscle extract containing 100 μg were subjected to SDS-polyacrylamide gel electrophoresis (6.25% gel) and then transferred to polyvinylidene difluoride membranes. The membranes were blocked overnight at 4 °C in 1% BSA in 10 mM Tris, pH 7.5, 150 mM NaCl, 0.1% Tween 20 and then incubated with a polyclonal anti-phosphotyrosine antibody (1.5 μg/ml) followed by donkey anti-rabbit ^{125}IgG and exposure to x-ray film for 12–48 h. For determination of IRS-1 tyrosine phosphorylation, an aliquot of the muscle extract containing 1 mg of protein was incubated overnight at 4 °C with 4 μg of anti-rat IRS-1 antibody, followed by adsorption with protein A-Sepharose for 60 min at 4 °C. The tyrosine phosphorylation of immunoprecipitated IRS-1 was assessed by immunoblotting with anti-phosphotyrosine antibody exactly as described above for the insulin receptor β-subunit.

IRS-1 protein content in the soluble muscle extracts (10 μg of protein) was assessed by immunoblotting using an antibody directed against the 14 carboxyl-terminal amino acid residues of IRS-1, followed by horseradish peroxidase-conjugated IgG. Antibody-bound protein was visualized using enhanced chemiluminescence according to the manufacturer's specifications. Protein bands were quantitated by densitometry.

For analysis of muscle insulin receptor protein content, a crude total membrane fraction was prepared (9). Membrane proteins (70 μg) were resolved by SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose, and then immunoblotted with a polyclonal antibody directed against the carboxyl terminus of the β-subunit of the insulin

[1] The abbreviations used are: ATB-[2-^{3}H]BMPA, 2-*N*-4-(1-azi-2,2,2-trifluoroethyl)-benzoyl-1,3-bis-(D-mannose-4-yloxy)-2-propylamine; EDL, extensor digitorum longus; BSA, bovine serum albumin; 3-MG, 3-*O*-methyl-D-glucose; MeAIB, α-(methylamino)isobutyrate; WGA, wheat germ agglutinin; IR, insulin receptor; IRS-1, insulin receptor substrate-1.

TABLE I
Effect of the high fat diet on body weight and plasma glucose and insulin concentrations

	Diet group	
	Chow	High fat
Body weight (g)		
8 weeks	385 ± 5	460 ± 9[a]
30 weeks	632 ± 21	758 ± 29[b]
Plasma glucose (mg/dl)		
8 weeks	115 ± 4	110 ± 2
30 weeks	102 ± 4	136 ± 15[b]
Plasma insulin (μU/ml)		
8 weeks	22 ± 2	44 ± 9[c]
30 weeks	26 ± 4	81 ± 9[a]

[a] $p < 0.001$ *vs.* age-matched chow control.
[b] $p < 0.05$ *vs.* age-matched chow control.
[c] $p < 0.01$ *vs.* age-matched chow control.

receptor, followed by horseradish peroxidase-conjugated IgG. Detection was performed using enhanced chemiluminescence. Protein bands were quantitated using densitometry.

Statistical Analysis—Data are presented as means ± S.E. Analysis of differences between the high fat-fed and chow-fed control groups was performed using a Student's t test. $p < 0.05$ was considered to be significant.

RESULTS

Effect of High Fat Diet on Weight Gain, Plasma Insulin, and Glucose—Rats that had been on the high fat diet for 8 weeks were significantly heavier and had higher plasma insulin levels than chow-fed controls of the same age (Table I). After 30 weeks on the high fat diet, the rats had become grossly obese and showed a further increase in plasma insulin levels. In addition, most of the animals on the high fat diet for 30 weeks had diabetic blood glucose levels (Table I).

Insulin- and Contraction-stimulated Glucose Transport Activity—The effect of 8 weeks of the high fat diet on glucose transport activity in the isolated epitrochlearis muscle is shown in Fig. 1. 3-MG transport stimulated by a maximally effective concentration of insulin was ~50% lower in muscles from fat-fed animals. Glucose transport activity stimulated by muscle contractions was similarly reduced (~50%) in muscles from animals fed the high fat diet. These results are similar to those reported previously by our group (6). We have also previously shown that insulin-stimulated glucose transport is decreased in the soleus and extensor digitorum longus muscles (6), so these measurements were not repeated. In addition, it was previously found that the effect of fat feeding is on insulin responsiveness, rather than insulin sensitivity (6), and the effect of a submaximal insulin stimulus was therefore not examined in this study.

Fat Feeding in GLUT1 Overexpressing Mice—To investigate the possibility that the high fat diet might be influencing glucose transporter protein function by altering plasma membrane composition, we studied the effect of high fat feeding on glucose transport in skeletal muscle of mice overexpressing the GLUT1 glucose transporter. Nontransgenic mice fed a high fat diet for 4 weeks were heavier than chow-fed control mice (27.8 ± 1.3 g *versus* 23.4 ± 1.7 g for high fat and chow, respectively) and exhibited a marked reduction in insulin-stimulated and hypoxia-stimulated glucose transport activities in epitrochlearis and EDL muscles (Fig. 2). Mice overexpressing GLUT1 were also fed a high fat diet or standard rodent chow *ad libitum* for 4 weeks. Like the wild-type mice, GLUT1 transgenic mice fed the high fat diet were heavier than their chow-fed littermates (27.4 ± 1.4 g *versus* 23.8 ± 0.5 g for high fat-fed and chow, respectively). The high fat diet had no effect on GLUT1 protein expression (2.04 ± 0.09 arbitrary units *versus* 2.02 ± 0.05 arbitrary units in chow and high fat-fed, respectively) or on basal glucose transport activity in the isolated EDL or epitrochlearis muscles of the GLUT1 transgenic mice (Fig. 3), suggesting that GLUT1-mediated transport was not affected by the diet.

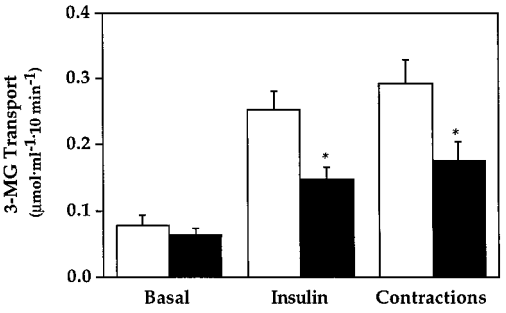

FIG. 1. **Effect of 8 weeks of high fat feeding on 3-MG transport in rat epitrochlearis muscles.** Epitrochlearis muscles were incubated for 60 min in the absence (basal) or presence (insulin) of 2 milliunits/ml insulin or were stimulated to contract indirectly via the nerve (contractions). Muscles were then washed for 10 min in glucose-free medium, followed by measurement of glucose transport activity using 3-MG as described under "Experimental Procedures." Values are means ± S.E. for 5–10 muscles per group. *Open bars*, chow-fed; *solid bars*, high fat diet-fed. Significantly different from chow-fed, $*p < 0.05$.

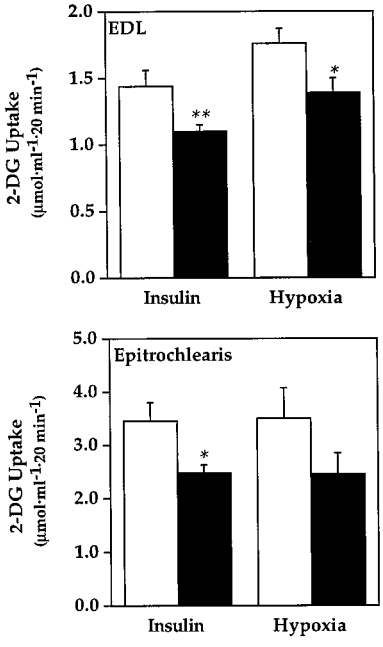

FIG. 2. **Effect of 4 weeks of high fat feeding on stimulated glucose transport activity in epitrochlearis and EDL muscles of wild-type mice.** Muscles were incubated for 60 min at 35 °C in oxygenated KHB containing 8 mM glucose, 32 mM mannitol, 0.1% BSA, and 2 milliunits/ml insulin (Insulin) or in KHB gassed with 95% N_2, 5% CO_2 containing 8 mM glucose, 32 mM mannitol, and 0.1% BSA (Hypoxia). Muscles were then washed in oxygenated, glucose-free buffer prior to measurement of glucose transport activity using 2-deoxy-D-[^{3}H]glucose (2-DG) as described under "Experimental Procedures." When insulin was present during the initial incubation, it was also present throughout the wash and transport assay. Values are means ± S.E. for four (epitrochlearis) or seven to nine (EDL) muscles per group. *Open bars*, chow-fed; *solid bars*, high fat diet-fed. Significantly different from chow-fed, $*p < 0.05$; $**p < 0.01$.

Insulin-stimulated Amino Acid Transport Activity—The system A amino acid transporter is the primary insulin-regulatable neutral amino acid transport system in skeletal muscle (34). In addition, insulin stimulation of system A transport in skeletal muscle cells is completely abolished by the phosphatidylinositol 3-kinase inhibitor wortmannin (35), providing evidence that insulin stimulation of system A amino acid trans-

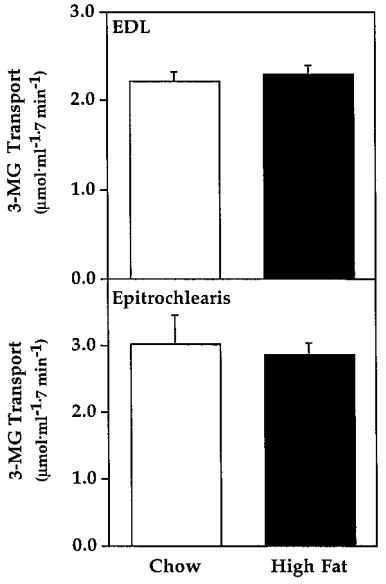

FIG. 3. **Effect of 4 weeks of high fat feeding on basal glucose transport activity in epitrochlearis and EDL muscles of GLUT1 overexpressing mice.** Muscles were incubated for 60 min at 35 °C in KHB containing 8 mM glucose and 32 mM mannitol. Muscles were then transferred to glucose-free KHB for 10 min at 30 °C prior to measurement of 3-MG transport as described under "Experimental Procedures." Values are means ± S.E. for six to eight muscles per group. *Open bars*, chow-fed; *solid bars*, high fat diet-fed.

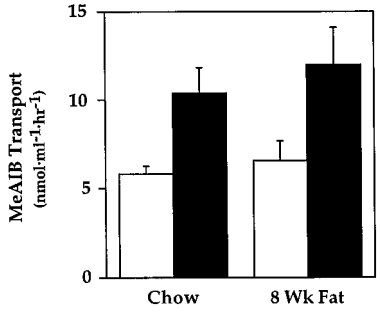

FIG. 4. **Effect of 8 weeks of high fat feeding on insulin-stimulated system A amino acid transport activity in rat epitrochlearis muscles.** Muscles were incubated at 35 °C for 60 min in the presence or absence of 2 milliunits/ml insulin. They were then washed for 10 min at 29 °C prior to measurement of system A amino acid transport activity using 0.1 mM MeAIB as described under "Experimental Procedures." If insulin was present during the 35 °C incubation, it was also added to the wash and transport media. Values are means ± S.E. for five to six muscles per group. *Open bars*, basal; *solid bars*, 2 milliunits/ml insulin.

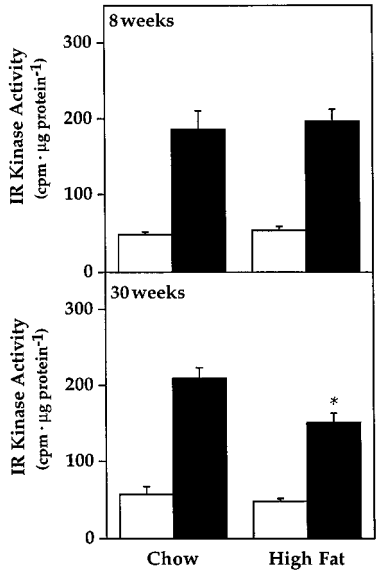

FIG. 5. **Effect of high fat feeding on tyrosine kinase activity of insulin receptors isolated from hind limb skeletal muscle.** Phosphorylation of poly(Glu-Tyr (4:1)) by WGA-purified insulin receptors was measured in the absence (*open bars*) or in the presence (*solid bars*) of 16.7 milliunits/ml insulin at 23 °C. Data are means ± S.E. for four to eight samples per group. Significantly different from chow-fed, $*p < 0.01$.

port and of glucose transport occurs via the same proximal steps of the insulin signaling pathway. To obtain preliminary information regarding the effect of the high fat diet on the insulin signaling pathway and to further investigate the possibility that a diet-induced alteration in plasma membrane composition might be interfering with the function of membrane transporter proteins, we examined the effect of 8 weeks of high fat feeding on system A amino acid transport activity in the epitrochlearis muscle (Fig. 4). Basal MeAIB transport was unchanged in muscles from high fat-fed animals compared with chow-fed controls. In contrast to the defect in insulin-stimulated glucose transport activity that occurs in muscles of the fat-fed animals, MeAIB transport was stimulated to the same extent by insulin (~2-fold above basal) in muscles from control and high fat-fed animals. This finding provides further evidence that the high fat diet does not cause alterations in plasma membrane composition that nonspecifically interfere with function of membrane transporter proteins. In addition, these results suggest that, after 8 weeks of high fat feeding, the early insulin signaling events are intact in skeletal muscle and that the high fat diet is acting on distal steps involved in stimulation of glucose transport.

Insulin Receptor Tyrosine Kinase Activity—To further evaluate the effect of the high fat diet on insulin signaling, we measured the tyrosine kinase activity of WGA-purified, solubilized skeletal muscle insulin receptors toward an exogenous substrate. Insulin stimulated a ~4-fold increase in ^{32}P incorporation into poly(Glu-Tyr (4:1)) when receptors obtained from control animals were used. Phosphorylation of the substrate was increased to a similar extent (~4-fold above basal) in preparations from animals fed the high fat diet for 8 weeks (Fig. 5*A*), indicating that this duration of high fat feeding does not induce a defect in the tyrosine kinase activity of the insulin receptor. In contrast, assay of the receptors isolated from animals fed the high fat diet for 30 weeks shows that longer term high fat feeding resulted in a decrease (~30%) in tyrosine kinase activity (Fig. 5*B*).

Insulin-stimulated Tyrosine Phosphorylation—Impaired insulin receptor function that was related to a change in the composition of its membrane lipid environment would not necessarily be observed when studying a solubilized receptor preparation. Therefore, to further evaluate the effect of the high fat diet on insulin receptor function in intact skeletal muscle, the phosphorylation of the insulin receptor and its endogenous substrate IRS-1 were studied following *in situ* administration of insulin (Fig. 6). There was no difference in insulin-stimulated tyrosine phosphorylation of the β-subunit of the insulin receptor between the chow control and 8-week fat-fed groups. In addition, 8 weeks of the high fat diet had no effect on tyrosine phosphorylation of IRS-1. However, after 30 weeks on the high fat diet, insulin-stimulated tyrosine phosphorylation of both the insulin receptor and IRS-1 were reduced by ~30% (Fig. 6). The high fat feeding had no significant effect on the content of muscle insulin receptor (as assessed by β-subunit content) or IRS-1 protein at either 8 or 30 weeks.

Insulin- and Contraction-stimulated Cell Surface GLUT4 Labeling—Although the early signaling events through which insulin and contractions increase muscle glucose transport ac-

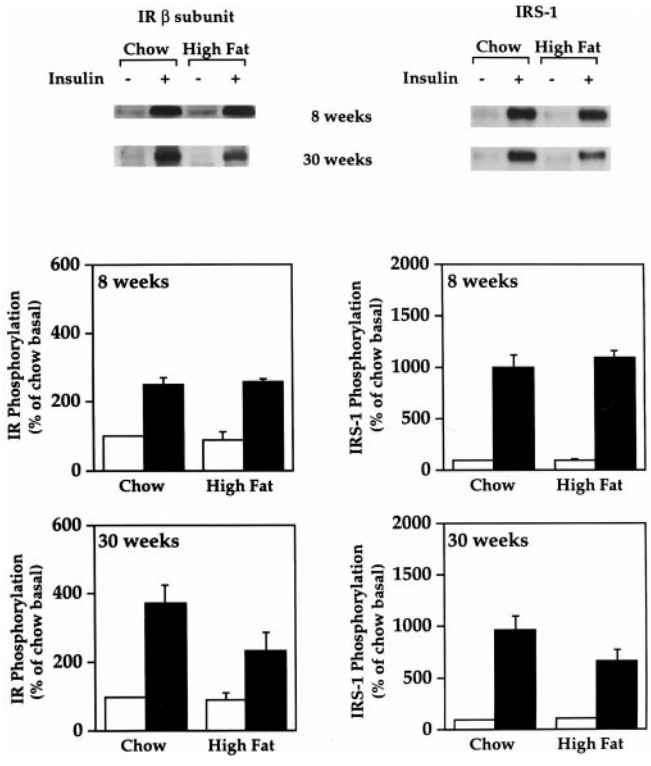

FIG. 6. **Effect of high fat feeding on insulin-stimulated tyrosine phosphorylation of IR and IRS-1.** Animals were studied after 8 or 30 weeks on the high fat or chow control diets. Animals were anesthetized, catheterized, and then given an intravenous bolus of saline (basal) or insulin (10 units/kg body wt). Soleus muscles were quickly excised and frozen 90–120 s after administration of the saline or insulin. Frozen muscles were prepared for quantitation of IR β-subunit and IRS-1 tyrosine phosphorylation by Western blotting as described under "Experimental Procedures." Representative blots are shown at *top* of the figure. For each experiment, the optical density of each chow-fed, basal sample was set at 1.0. Values for all other samples from that blot were then calculated relative to that sample. Values are means ± S.E. from four to six rats. *Open bars*, basal; *solid bars*, insulin. Significantly different from chow-fed, $^*p < 0.05$.

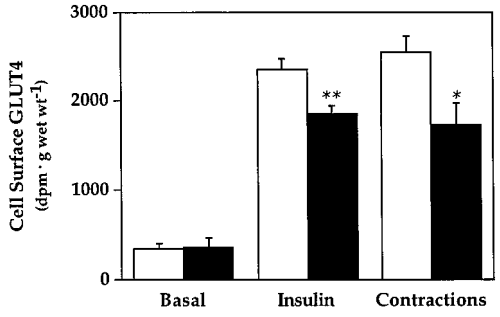

FIG. 7. **Effect of 8 weeks of fat feeding on insulin- and contraction-stimulated GLUT4 cell surface labeling in rat epitrochlearis muscles.** Muscles were incubated for 60 min in the absence or presence of 2 milliunits/ml insulin or were stimulated to contract, as described in the legend to Fig. 1. Muscles were washed for 10 min in glucose-free KHB and then further incubated in the dark at 18 °C for 8 min in buffer containing 0.75 mCi/ml ATB-[^{3}H]BMPA and insulin, if it was present in the previous incubations. Muscles were then irradiated for 2 × 1 min. Muscle membranes were prepared, and labeled GLUT4 glucose transporters were immunoprecipitated and analyzed as described previously. Values are means ± S.E. for six to nine muscles/group. *Open bars*, chow-fed; *closed bars*, fat-fed. Significantly different from chow-fed, $^*p < 0.05$, $^{**}p < 0.01$.

tivity are independent, both stimuli increase glucose transport by increasing the number of GLUT4 transporters at the cell surface (15, 17, 18). Since the insulin- and the contraction-stimulated activations of muscle glucose transport are similarly impaired in the fat-fed animals, it seemed possible that the diet-induced defect might involve a step in the translocation process. To test the effect of fat feeding on GLUT4 translocation, we used the bis-mannose derivative ATB-[2-^{3}H]BMPA to label transporters at the cell surface in the epitrochlearis muscle following treatment with insulin or muscle contractions. The increase in GLUT4 labeling following incubation with a maximally effective concentration of insulin was 26% lower in muscles of fat-fed animals compared with controls (Fig. 7). Similarly, high fat feeding resulted in a 36% smaller increase in cell surface GLUT4 labeling following *in situ* muscle contractions. Thus, it seems that a smaller increase in cell surface GLUT4 plays a major role in the impaired stimulation of glucose transport in the fat-fed animals. The disparity between the magnitudes of the defects in transport and GLUT4 translocation, however, suggests that another mechanism also contributes to the resistance of glucose transport to stimulation in muscles of fat-fed animals.

DISCUSSION

Feeding rodents a high fat diet results in visceral obesity, muscle insulin resistance, and, if continued sufficiently long enough, impaired glucose tolerance or diabetes (1–6). One mechanism that has been suggested to explain the insulin resistance of muscle glucose transport that develops with fat feeding and obesity is a change in the lipid composition of the plasma membrane (4, 22). This seemed a reasonable possibility, as membrane lipid composition can affect the functioning of membrane-associated proteins (reviewed in Ref. 36), and two of the key proteins involved in the regulation of glucose transport, the glucose transporter and the insulin receptor, are constituents of the plasma membrane. There is considerable evidence that the catalytic activities of the glucose transporters (20, 21) and the binding properties of the insulin receptor (37–39) are markedly sensitive to changes in the properties of the membrane lipid bilayer.

Two isoforms of the glucose transporter, GLUT4 (24, 25, 40–42) and GLUT1 (43, 44), are expressed in skeletal muscle. The GLUT4 isoform mediates the increase in glucose transport in response to insulin or muscle contractions by a process that involves the movement of GLUT4 to plasma membrane domains (15, 17, 18). The less abundant isoform, GLUT1, seems to reside constitutively in the plasma membrane (45, 46) and is thought to mediate basal glucose transport (26, 27). These two transporters are structurally similar (24, 25) and are thought to transport glucose across the plasma membrane by the same process. In this context, we used the muscles of transgenic mice that overexpress GLUT1 to evaluate the possibility that a high fat diet causes a change in membrane composition that impairs glucose transporter function. Our results show that a high fat diet that results in severe muscle insulin resistance in wild-type mice has no effect on the high rate of glucose transport in the muscles of GLUT1 overexpressing mice. This finding suggests that an impairment of glucose transporter function mediated by a change in membrane composition is not responsible for the decrease in muscle glucose transport activity caused by a high fat diet. We cannot rule out the possibility that GLUT1 and GLUT4 might respond differently to changes in the composition of the plasma membrane lipid bilayer, but the similarities in the structure of the two transporter isoforms, especially in the putative membrane-spanning regions (24, 25), make this unlikely. Further evidence against a diet-induced change in the composition or physical properties of the plasma membrane that causes a nonspecific decrease in membrane-associated protein activity is provided by our finding that skeletal muscle system A amino acid transport activity is not impaired after 8 weeks on the high fat diet.

It has been hypothesized on the basis of studies on human skeletal muscle that a defect at the level of the insulin receptor contributes to the insulin resistance associated with obesity (7–9). There is much evidence that it is abdominal, particularly visceral, obesity that is associated with insulin resistance (the so called abdominal or central obesity syndrome) (47, 48). Rodents fed a high fat diet rapidly develop an increase in visceral fat. In rats fed the high fat diet used in the present study, total visceral fat weight (retroperitoneal, mesenteric, and epididymal fat depots) is already ~50% greater than in chow-fed controls after 8 weeks (6). In this context, it seemed possible that the insulin resistance induced by a high fat diet could be mediated either by a membrane composition-related change in receptor function (38, 39, 49) or as a consequence of decreased receptor number and/or activity associated with the obesity (7–9, 50). The initial steps in the stimulation of system A amino acid transport by insulin are the same as those involved in the stimulation of glucose transport (35). Our finding that stimulation of MeAIB transport by insulin is not decreased in muscles of rats fed the high fat diet for 8 weeks, therefore, argues against an impairment of insulin receptor function early in the development of the insulin resistance induced by a high fat diet.

The interpretation that muscle insulin receptor function is normal in rats after 8 weeks on the high fat diet is supported by our finding that stimulated tyrosine kinase activity of solubilized receptors from 8-week fat-fed and chow-fed control rats is similar. Our results are consistent with previous studies in muscle in which relatively short periods of high fat feeding (4–5 weeks) were shown to have little or no effect on insulin binding (51, 52) and insulin-stimulated autophosphorylation (52) or tyrosine kinase activity (51) of partially purified receptors. In addition, the lack of effect of 8 weeks of fat feeding on insulin-stimulated tyrosine phosphorylation of the receptor β-subunit and IRS-1 is in agreement with the findings of Okamoto *et al.* (52), in which phosphorylation of the insulin receptor and pp190 in rat skeletal muscle following *in vivo* insulin administration was not changed by 4 weeks of high fat feeding. However, rats fed the high fat diet for 30 weeks in our studies did have decreases in insulin receptor tyrosine kinase activity and in insulin-stimulated insulin receptor and IRS-1 tyrosine phosphorylation; by this time, plasma insulin levels in the high fat-fed animals had been elevated for ~4 months. Thus, our results are consistent with the finding of an insulin receptor defect in muscles of insulin-resistant patients with obesity and glucose intolerance or diabetes (7–9), as such individuals have generally been hyperinsulinemic for years. We interpret these findings to indicate that a defect in insulin signaling is a late event that is probably secondary to prolonged hyperinsulinemia and is not a primary cause of the insulin resistance.

In keeping with the interpretation that the insulin resistance of muscle glucose transport induced by 4–8 weeks on a high fat diet is not due to impairment of insulin signaling is the finding that stimulation of glucose transport by contractile activity, or hypoxia, is also reduced. Contractions and hypoxia stimulate glucose transport by a pathway that is separate from, and independent of, the insulin signaling pathway (13–15). Since both insulin-stimulated and contraction-stimulated glucose transport are impaired by fat feeding, it seems likely that it is a late, common step that is affected. Our finding that the increases in GLUT4 at the cell surface in response to both insulin and contractions were smaller in muscles of fat-fed rats as compared with controls provides evidence that a step(s) involved in translocation of GLUT4-containing vesicles to, or fusion with, the sarcolemma is impaired. Reduced insulin-stimulated GLUT4 translocation in soleus muscles of mice fed a high fat diet has also been reported by Zierath *et al.* (19). This smaller increase in GLUT4 at the cell surface is not due to a decrease in total muscle GLUT4, as muscle GLUT4 protein content is unaffected in rats fed a high fat diet such as was used in this study (6, 16).

Our finding that the impairment of stimulated transport was greater than the decrease in GLUT4 movement to the cell surface raises the possibility that GLUT4 intrinsic activity may also be reduced in the fat-fed animals. Evidence that a high fat diet may alter GLUT4 intrinsic activity has been provided by Rosholt *et al.* (16), who found that the GLUT4 in plasma membrane vesicles prepared from muscles of high fat diet-fed rats had a lower transport capacity than GLUT4 in vesicles prepared from chow-fed controls. There is currently no information regarding how high fat feeding might bring about a change in intrinsic activity. One possibility is that the GLUT4 protein is modified prior to translocation. Another possibility is that the composition of the GLUT4 vesicle-derived lipid annulus of the transporter protein is changed. The third possibility, that reduced intrinsic activity of GLUT4 could be mediated by changes in the membrane lipid composition that result in impaired function of the transporter after its insertion into the plasma membrane, now seems unlikely in light of our finding that activity of the GLUT1 transporter, which is constitutively targeted to the plasma membrane, is unaffected.

In conclusion, our findings on muscles of transgenic mice overexpressing the GLUT1 glucose transporter provide evidence that the impairment of muscle glucose transport induced by a high fat diet is not due to a change in sarcolemmal composition that interferes with glucose transporter function. Our results further show that insulin receptor down-regulation does not play a primary role in causing the muscle insulin resistance induced by feeding a high fat diet. Both the contraction-stimulated and insulin-stimulated increases in GLUT4 at the cell surface are reduced in muscles of fat-fed rats, suggesting an impairment of one or more of the steps involved in the GLUT4 translocation process.

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