Restoration of Insulin Responsiveness in Skeletal Muscle of Morbidly Obese Patients after Weight Loss

Effect on Muscle Glucose Transport and Glucose Transporter GLUT4

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Abstract

A major defect contributing to impaired insulin action in human obesity is reduced glucose transport activity in skeletal muscle. This study was designed to determine whether the improvement in whole body glucose disposal associated with weight reduction is related to a change in skeletal muscle glucose transport activity and levels of the glucose transporter protein GLUT4. Seven morbidly obese (body mass index = 45.8±2.5, mean ± SE) patients, including four with non–insulin-dependent diabetes mellitus (NIDDM), underwent gastric bypass surgery for treatment of their obesity. In vivo glucose disposal during a euglycemic clamp at an insulin infusion rate of 40 mU/m² per min was reduced to 27% of nonobese controls (*P* < 0.01) and improved to 78% of normal after weight loss of 43.1±3.1 kg (*P* < 0.01). Maximal insulin-stimulated glucose transport activity in incubated muscle fibers was reduced by ≈ 50% in obese patients at the time of gastric bypass surgery but increased twofold (*P* < 0.01) to 88% of normal in five separate patients after similar weight reduction. Muscle biopsies obtained from vastus lateralis before and after weight loss revealed no significant change in levels of GLUT4 glucose transporter protein. These data demonstrate conclusively that insulin resistance in skeletal muscle of mobidly obese patients with and without NIDDM cannot be causally related to the cellular content of GLUT4 protein. The results further suggest that morbid obesity contributes to whole body insulin resistance through a reversible defect in skeletal muscle glucose transport activity. The mechanism for this improvement may involve enhanced transporter translocation and/or activation. (*J. Clin. Invest.* 1992. 89:701–705.) Key words: insulin resistance • obesity • glucose disposal • noninsulin-dependent diabetes mellitus • glucose metabolism

Introduction

Peripheral insulin resistance is a primary disorder in both obesity and non–insulin-dependent diabetes mellitus (NIDDM)[1] (1–3). This appears to be due at least in part to a defect in skeletal muscle, the major site of whole body insulin-dependent glucose disposal (2–4). Using a novel in vitro human muscle preparation, we recently demonstrated that insulin-stimulated glucose transport was severely decreased by ≈ 50% in muscle of morbidly obese patients with or without NIDDM (5). Although the primary defect(s) responsible for the insulin resistance is still unknown, one possible candidate for this defect is the glucose transporter, since glucose transport is the rate-limiting step for glucose utilization in muscle (6, 7).

The glucose transport response in insulin-sensitive tissues such as muscle and fat is predominantly mediated by the unique glucose transporter isoform GLUT4 (8). Garvey et al. (9) recently demonstrated that the cellular insulin resistance in adipocytes from obese and obese-NIDDM patients is accompanied by a 40–80% depletion of GLUT4 glucose transporter protein. However, the mechanism underlying the severe defect in glucose transport activity in skeletal muscle is much less clear. Pederson et al. (10) and Handberg et al. (11) reported no significant change in levels of muscle GLUT4 protein in vastus lateralis of obese NIDDM patients compared with lean controls. On the other hand, our group recently reported that GLUT4 protein is decreased by ≈ 20% in both rectus abdominis and vastus lateralis muscles in morbidly obese patients, with or without NIDDM (12). This discrepancy may be due in part to the heterogeneity of the obesity syndrome, as well as a large biological variability in muscle GLUT4 protein found in normal subjects (10–12).

Given that decreased glucose transport is a major mechanism underlying insulin resistance, we sought to determine whether muscle GLUT4 transporter protein contributes to the insulin resistance of skeletal muscle by examining levels of glucose transporter protein GLUT4 in the same patients before and after a period of weight loss, which has been shown to be an effective means of improving peripheral glucose disposal (13–16). In addition to muscle glucose transporter GLUT4, we examined whole body glucose disposal and glucose transport activity in muscle from patients after weight loss to permit comparisons of changes in muscle glucose transporter protein with changes in insulin action in vivo and muscle transport activity in vitro.

Methods

Materials. 2-[1,2-^{3}H(N)]deoxy-D-glucose (30.2 Ci/mmol) and [U-^{14}C]-D-sorbitol (150–250 mCi/mmol) were obtained from Du Pont-NEN (Boston, MA). Reagents for PAGE were purchased from Bio-Rad Laboratories (Richmond, CA). An affinity-purified polyclonal antibody specific for a carboxy-terminal synthetic peptide (30 amino acids) for GLUT4 (8) was generously supplied by Dr. Lawrence Sleiker (Eli Lilly Research Laboratories, Indianapolis, IN). Unless otherwise stated,

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Received for publication 15 August 1991 and in revised form 21 October 1991.

1. *Abbreviations used in this paper:* BMI, body mass index; NIDDM, non–insulin-dependent diabetes mellitus; TBS, Tris-buffered saline.

J. Clin. Invest.
© The American Society for Clinical Investigation, Inc.
0021-9738/92/02/0701/05 $2.00
Volume 89, February 1992, 701–705

all other reagents were purchased from Sigma Chemical Co. (St. Louis, MO).

Subjects. Subject characteristics are listed in Table I. Seven morbidly obese patients (body mass index [BMI] > 40 kg/m^2) scheduled for gastric bypass surgery were studied. Of these patients, four were classified as having NIDDM and three were classified as nondiabetic, according the criteria established by the National Diabetes Data Group (17). None of the subjects had any disease other than diabetes and/or obesity and were weight stable for ≥ 2 mo before entry into the study. Of the four diabetics, two were on insulin and two were being treated with oral agents. The experimental protocol was explained to each subject, and informed consent was obtained. The project was approved by the East Carolina University Policy and Review Committee on Human Research.

Euglycemic clamp and muscle biopsies. In vivo glucose disposal was assessed and muscle biopsies obtained 1 mo before gastric bypass surgery and again after a stable body weight was achieved, ~ 12 mo after gastric bypass surgery. After surgery all patients were followed at 6–8-wk intervals for weighing and dietary counseling. A plateau in weight was declared when three consecutive weights varied by ⩾ 1 kg. 2 wk before the study diabetic patients were withdrawn from therapy with consent and were followed on an outpatient basis. Subjects received a weight-maintaining diet containing 50% of total calories as carbohydrates, 30% as fat, and 20% as protein for 3 d before each clamp. Subjects were admitted to the Outpatient Clinical Research Unit of the East Carolina University School of Medicine at 8:00 a.m. after an overnight fast. A 50–100-mg muscle sample was obtained from the vastus lateralis muscle using the percutaneous muscle biopsy technique as described previously (12). The muscle tissue was rinsed and dissected free of connective tissue, quick frozen on dry ice, and stored at −70°C until analysis for GLUT4 protein. The subjects then underwent a 2-h hyperinsulinemic euglycemic clamp (without radioactivity) to determine in vivo glucose disposal as described in detail previously (18, 19). A continuous insulin infusion rate of 40 mU/m^2 per min was used and plasma glucose was maintained between 80 and 90 mg/dl throughout the test by a variable infusion of 20% dextrose. The glucose disposal rate (M) = mg/m^2 per min was calculated using the exogenous glucose infusion rate during the final 30 min of euglycemia.

Analytical methods. Plasma glucose was analyzed by the glucose oxidase method (glucose analyzer II; Beckman Instruments Inc., Fullerton, CA). Insulin was analyzed by double antibody radioimmunoassay using a kit supplied by Cambridge Diagnostics (Billerica, MA) according to the method of Desbuquois and Aurbach (20). Glycosylated hemoglobin was determined in heparinized blood using the fast hemoglobin test system kit (Isolab, Inc., Akron, OH).

2-deoxy-D-glucose transport in human muscle fiber strips. Glucose transport activity was determined in rectus abdominis muscle obtained from seven morbidly obese patients during gastric bypass surgery for the treatment of their obesity (21) and from five former gastric bypass patients undergoing elective abdominal surgery, as described previously (5). After an overnight fast, general anesthesia was induced with a short-acting barbiturate and maintained by fentanyl and N_2O–O_2 mixture. Only saline was given intravenously before the biopsy. After exposing the rectus abdominis, a 3 × 2 × 2 cm biopsy was clamped, excised, and quickly transported to the laboratory in oxygenated Krebs-Henseleit buffer. Muscle fiber strips weighing 40–80 mg were teased from the muscle and a smaller clamp (2 cm) was placed on the muscle fiber strip before it was cut free. Muscles were incubated in 4.0 ml of medium consisting of Krebs-Henseleit buffer, 1% bovine serum albumin, 1.0 mM pyruvate, at 0, 1×10^{-9}, and 1×10^{-7} M insulin. Transport was measured in the presence of 5 mM 2-deoxy-D-glucose for 60 min as described previously (5).

Analysis of glucose transporter protein. To minimize any contribution of interassay variability, muscle samples from each subject's pre- and post-weight reduction biopsies were prepared and analyzed for GLUT4 protein in the same assay. The procedures for quantifying GLUT4 are described here and elsewhere in detail (12, 19). Muscle biopsies were homogenized (Polytron; Brinkmann Instruments, Inc., Westbury, NY) in 2 ml of cold (4°C) buffer (pH 7.4) containing 25 mM Hepes, 25 mM benzamidine, 4 mM EDTA, 0.5 mM PMSF, and 1 μM each of leupeptin, pepstatin, and aprotinin. To isolate cellular membranes, samples were centrifuged at 150,000 g for 1 h at 4°C. The resulting pellet was rinsed and resuspended (Polytron) in cold buffer. Triton was added to each resuspension to give a final concentration of 1%. The samples were allowed to sit on ice for 1.5 h to solubilize the membrane-bound protein. After a final spin (150,000 g, 1 h, 4°C), the supernatant was removed and used for Western analysis. Duplicate samples containing 50 μg protein (BCA procedure; Pierce Chemical Co., Rockford, IL) were mixed overnight at 4°C with Laemmli sample buffer (22) containing 2.5% dithiothreitol and 1% SDS. Proteins were separated by SDS-PAGE under reducing conditions on an 8% resolving gel and transferred to Immobilon membrane (Millipore Corp., Bedford, MA) by electrotransfer (500 mA, 2 h). The membrane was blocked for 2 h in 5% Carnation low-fat instant milk in Tris-buffered saline (TBS-blotto), followed by incubation for 16 h at 4°C in polyclonal antibody (1 μg/ml in TBS-blotto) specific for the GLUT4 transporter isoform. The membrane was washed in TBS/TBS-Tween and incubated 1 h at 25°C in 50 ml TBS-blotto containing donkey anti-rabbit antibody conjugated to horseradish peroxidase (1:4,000). GLUT4 was visualized using an enhanced chemiluminescence detection system (Amersham, Corp., Arlington Heights, IL). Membranes were washed in TBS/TBS-Tween and incubated for 1 min in detection reagent (luminol + H_2O_2), followed by exposure to Hyperfilm (Amersham Corp.) for 10–30 s. The resulting films were analyzed by scanning densitometry.

Statistical analysis. All data are given as mean ± SEM. Paired t test was used to determine differences between the same patients before and after weight loss. Glucose transport in five separate morbidly obese

Table I. Clinical and Metabolic Characteristics

Patient	Sex	Age	Height	Weight		BMI		Glucose		Insulin		HbA_{1c}	
				Before	After	Before	After	Before	After	Before	After	Before	After
n	*M/F*	*yr*	*cm*	*kg*		*kg/m²*		*mg/dl*		*μU/ml*		*%*	
1	F	45	168	106.8	72.6	40.3	27.5	103	85	28.0	12.6	5.0	5.4
2	F	53	164	112.0	69.0	40.6	25.8	103	92	23.0	12.6	5.8	5.4
3	M	40	188	186.9	129.5	52.9	35.5	103	88	31.0	11.7	4.6	4.7
4	F	32	160	107.0	68.9	41.6	26.9	128*	71	25.0	5.3	7.1	5.2
5	F	40	158	139.0	91.2	55.6	39.7	138*	88	23.0	12.2	11.1	5.8
6	F	40	162	122.9	81.4	46.9	31.1	122*	81	25.0	6.0	7.1	5.4
7	F	43	175	132.0	92.1	43.2	30.4	149*	85	33.0	7.0	8.4	5.5
Mean	6F/1M	41.8	168	129.5	83.4‡	45.8	30.9‡	121	84‡	26.8	9.6‡	7.0	5.3‡
±SE	—	±2.6	±4	±11.5	±8.7	±2.5	±2.1	±8	±3	±1.6	±1.4	±0.9	±0.1

* Non–insulin-dependent diabetic; ‡ significantly reduced after weight loss, $P < 0.01$.

patients after weight loss was compared with the obese patients and previously published data on nonobese controls (5) using one-way analysis of variance. Newman-Keuls post-hoc testing was used to determine significant differences between groups.

Results

The subject characteristics are presented in Table I. The mean weight of the group fell from 129.5±11.5 to 83.4±8.7 kg, mean ± SE. ($P < 0.01$), resulting in a mean weight loss of 43.1±2.3 kg (range, 34–57 kg) or 36% of initial mean body weight. Likewise, the BMI, which correlates well with the degree of obesity, fell from 45.8±2.5 to 30.9±2.1 kg/m^2 ($P < 0.01$). Upon entering the study, obese nondiabetics had normal glucose tolerance, whereas the obese NIDDM patients had fasting hyperglycemia. After weight reduction, fasting plasma glucose was normalized in diabetic patients from 134±7 to 81±4 mg/dl ($P < 0.01$). Glycosylated hemoglobin decreased from 7.9±2.2 to 5.5±0.3% ($P < 0.01$) in diabetic patients after weight loss. Significant reductions in fasting insulin were observed in all obese patients as a result of weight loss (from 26.8±1.6 to 9.6±1.4 μU/ml, $P < 0.01$).

To determine the effect of weight loss on in vivo glucose disposal, a euglycemic clamp was performed in all subjects before and after weight loss at an insulin infusion rate of 40 mU/m^2 per min (Fig. 1). Insulin levels during the clamp averaged ≈ 100 μU/ml before and after weight loss. Glucose disposal increased significantly by threefold from 84±5 to 252±32 mg/m^2 per min after weight loss ($P < 0.01$). For comparison purposes, glucose disposal in nonobese nondiabetic patients at similar levels of insulinemia and glycemia in our laboratory average 322±19.6 mg/m^2 per min. Thus, the glucose disposal rate was 27% of normal in obese patients initially and improved to 78% of normal after weight loss.

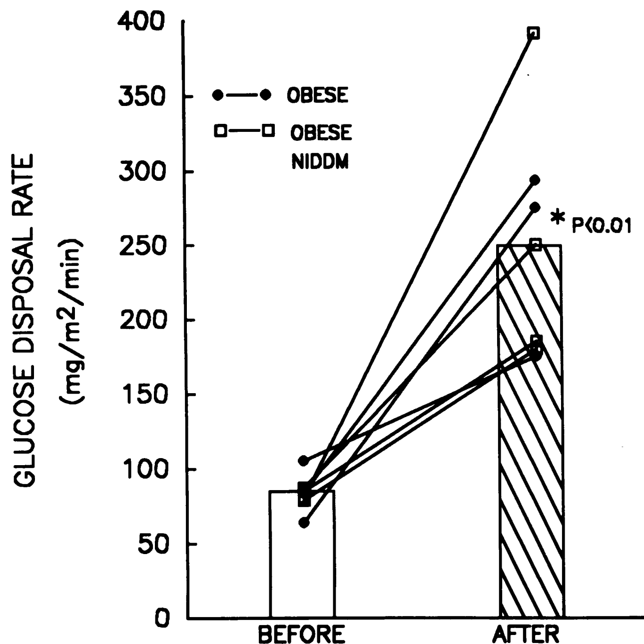

Figure 1. Effect of weight loss on whole body insulin-stimulated glucose disposal rates. Individual and mean glucose disposal rates during 40 mU/m^2 per min insulin infusion rate euglycemic clamp performed before (□) and after weight loss (▨). For comparison, glucose disposal rate in nonobese nondiabetics is 322±19.6 mg/m^2 per min (mean ± SEM) under comparable euglycemic and hyperinsulinemic conditions.

Effect of weight loss on 2-deoxyglucose transport. For ethical reasons, we were unable to obtain rectus muscle from the same patients after weight loss. However, we were able to study glucose transport activity in rectus abdominis muscle from a similar group of patients undergoing revision ($n = 2$), hernia repair ($n = 2$), or abdominoplasty ($n = 1$) 15–24 mo after gastric bypass surgery. These patients had stabilized in body weight after a similar weight loss of 27–49 kg (BMI = 31.3±2.8 kg/m^2). As shown in Fig. 2, maximal insulin-stimulated glucose transport activity was increased 1.9±0.3-fold over basal (range, 1.3–2.9) in patients after weight loss ($P < 0.01$). Insulin-stimulated glucose transport activity in morbidly obese patients at the time of gastric bypass was ≈ 50% of nonobese nondiabetic patients, published previously (5), and these data are now presented together with our previous work. The values for glucose transport in patients after weight loss reveal an approximately twofold increase in maximal glucose transport activity from 25.3±3.3 to 48.2±7.3 nmol/g dry wt per min ($P < 0.01$). Thus, weight loss restored maximal insulin-stimulated skeletal muscle glucose transport activity to 88% of normal, nonobese patients. Lack of tissue prevented us from performing a complete insulin dose–response curve in incubated muscle; however, at a single submaximal concentration of insulin (10^{-9} M) glucose transport activity increased significantly from 25.3±3.3 to 37.3±5.1 nmol/g dry wt per min ($P < 0.05$). This value corresponds to 83% of nonobese nondiabetic subjects at the same insulin concentration (data not shown).

To determine whether the improvement in insulin-stimulated glucose transport capacity could be accounted for by increased levels of the insulin-sensitive glucose transporter protein GLUT4, muscle biopsies were obtained from vastus lateralis muscle in the gastric bypass patients before and after weight loss. Identical amounts of total membrane protein were size fractionated by SDS-PAGE, transferred to nitrocellulose membrane, and detected using an affinity purified COOH-terminal anti-GLUT4 antibody. Fig. 3 *A* shows a representative autora-

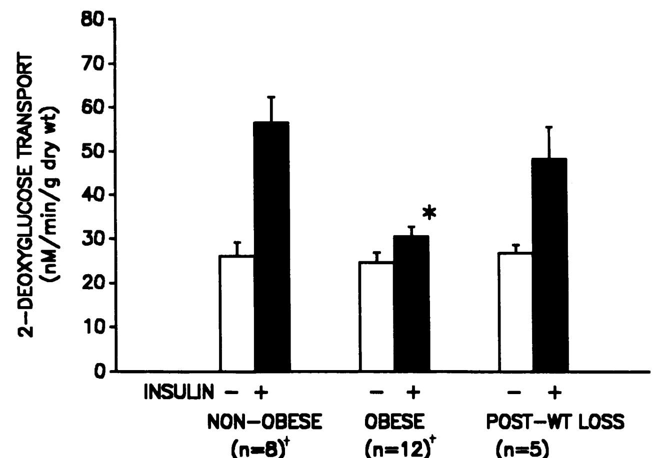

Figure 2. 2-Deoxyglucose transport in muscle fiber strips from nonobese subjects (BMI = 26.3±0.6 kg/m^2), morbidly obese subjects with normal glucose tolerance or NIDDM (BMI = 45.8±2.5 kg/m^2), and morbidly obese patients after weight loss (BMI = 31.3 ± 2.8 kg/m^2). Muscle fiber strips were prepared as outlined previously (5) and rates of 2-deoxyglucose transport measured under basal (0 insulin) conditions and after 60 min of maximal insulin (10^{-7} M) stimulation. *Significantly less than ($P < 0.05$) muscle from nonobese or post-weight loss group (+ insulin). †Values for nonobese and obese ($n = 5$) were taken from reference 5 for comparison.

diograph of GLUT4 glucose transporter levels in muscle in the same patients before and after weight loss. In all subjects, GLUT4 migrated as an ≈ 45-kD protein. GLUT4 was quantitated by laser densitometry and the values are shown in Fig. 3*B*. The glucose transporter levels decreased slightly in six of seven patients after weight loss, resulting in a nonsignificant decrease of 13% ($P > 0.05$). Correction of glucose transporter protein values based on milligrams of total membrane protein did not substantially alter the results since the amounts of total protein recovered in crude membranes was similar before and after weight loss (7.12±0.72 vs. 8.32±0.86 μg/mg tissue).

Discussion

Previous studies have shown that skeletal muscle is the primary tissue responsible for whole body insulin resistance in human obesity and NIDDM (3). This appears to be due in part to a postbinding defect in insulin action in skeletal muscle, the principal site of whole body glucose disposal. We (5), along with other investigators using a variety of in vivo and in vitro techniques, have suggested that decreased glucose transport activity is a major defect contributing to impaired insulin action in human obesity (3, 6, 9, 23). In morbidly obese subjects peripheral glucose disposal is markedly decreased compared with nonobese controls, and this may be due to the severe decrease in muscle glucose transport activity found here and in our previous study (5).

After weight loss of 36% initial body weight, glucose disposal during a hyperinsulinemic euglycemic clamp increased markedly by threefold. This is consistent with previous studies demonstrating that weight loss in obese and obese-NIDDM subjects improves whole-body insulin sensitivity (13–15), as well as maximal glucose disposal in obese-NIDDM patients after a lesser degree of weight loss (15). The current study reveals the cellular mechanisms underlying the improvement in the effect of insulin to stimulate peripheral glucose disposal. With weight loss, maximal glucose transport activity in vitro in skeletal muscle increased nearly twofold to levels ≈ 88% of nonobese nondiabetic patients. Lack of tissue prevented us from performing a complete insulin dose–response curve in patients after weight loss. However, at 10^{-9} M insulin we observed a significant increase in glucose transport activity, which suggests that insulin sensitivity, as well as responsiveness, improved in human skeletal muscle after weight loss. Increased insulin sensitivity has also been associated with physical training (24, 25). Although daily activity levels may have increased, an effect of exercise is unlikely, however, because none of the subjects were involved in a regular endurance training program. Although only one male subject was studied, his response was in the same direction as in the female subject. An increase in glucose transport activity has also been observed in adipocytes after weight loss (15). Given that skeletal muscle is responsible for > 80% of insulin-mediated glucose uptake in vivo (26, 27), the present findings support the conclusion that obesity contributes to whole body insulin resistance through a reversible defect in skeletal muscle glucose transport activity.

The improvement in muscle glucose transport activity with weight loss could be due to changes in the levels of glucose transporter protein, or part of the signal transduction pathway whereby insulin stimulates glucose transport. Garvey et al. (9) demonstrated a 40–80% depletion in the insulin-sensitive glucose transporter protein GLUT4 in adipocytes from patients with obesity and NIDDM. Similar findings have also been reported by Sinha et al. in obese patients with NIDDM (28). However, the effects of insulin resistance on GLUT4 transporter protein in human skeletal muscle appear to be somewhat controversial. Pederson et al. (10) and others (11, 29) have reported no change in GLUT4 protein in vastus lateralis muscle of obese and obese-NIDDM patients. However, we previously observed a 20% decrease in levels of muscle glucose transporter protein GLUT4 in both rectus abdominis and vastus lateralis muscle in obese patients with and without NIDDM (12). Although this decrease likely contributes to the insulin resistance in these patients, the degree of resistance in vivo or in vitro did not correlate well with the small changes observed in muscle GLUT4 protein, which suggests factors other than the level of glucose transporters regulate insulin responsiveness. Likewise, the slight decrease observed here in muscle GLUT4 protein in the same patients after weight loss was not reflected by in vivo glucose uptake as measured by euglycemic clamp or maximal glucose transport activity in isolated muscle. Taken together, these results clearly show that impaired glucose trans-

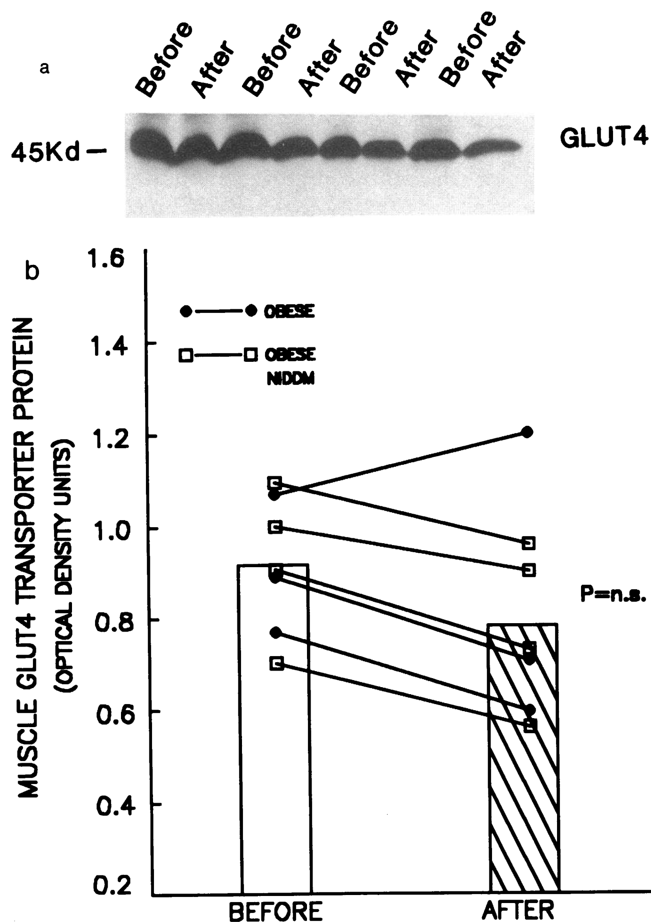

Figure 3. (*A*) Effect of weight loss on immunodetection of GLUT4 glucose transporters in human skeletal muscle. Muscle biopsies were obtained from vastus lateralis before and after weight loss and crude membranes prepared as described in Methods. Identical quantities of protein (50 μg) were applied to each lane, subjected to SDS-PAGE, transferred to nitrocellulose, and immunoblotted using affinity-purified polyclonal antibody to COOH-terminal portion (30 amino acids) of GLUT4 transporter protein. The figure represents an autoradiogram from a typical experiment. (*B*) Quantification by scanning densitometry of individual and group mean GLUT4 transporters obtained before (□) and after (▨) weight loss.

port activity in obesity cannot be causally related to the cellular content of GLUT4 protein in human skeletal muscle. Results similar to these have been reported in muscle of obese and obese-NIDDM rats (30–32).

The contribution of an insulin receptor signalling defect to the insulin resistance of obesity and NIDDM was investigated by Freidenberg et al. (13), who found that weight loss reversed the insulin receptor tyrosine kinase defect present in adipocytes from obese-NIDDM patients. A similar defect exists in skeletal muscle of the obese and obese-NIDDM patients studied here (33) and elsewhere (34, 35). Although the regulation of glucose transport activity probably involves factors other than just activation of tyrosine kinase, it is likely that this or some other defect of insulin signaling more distal to the insulin receptor is responsible for decreased glucose transport activity observed in human obesity. Such a defect may impair the function of the GLUT4 transporter either through decreased activation and/or translocation to the plasma membrane.

Acknowledgments

The authors thank Linda Roy for assistance in these studies and Gloria Castellow for preparation of this manuscript.

This research was supported in part by grant 5 PO1 DK-36296 from the National Institute of Diabetes and Digestive and Kidney Diseases and National Research Service Award 1 F32 DK-08477 (to J. E. Friedman).

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