$^{13}C/^{31}P$ NMR Studies on the Mechanism of Insulin Resistance in Obesity

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The mechanism of insulin resistance in obesity was examined in ten obese (BMI 33 ± 1 kg/m^2) and nine lean (BMI 22 ± 1 kg/m^2) Caucasian women during a hyperglycemic-hyperinsulinemic clamp using ^{13}C and ^{31}P nuclear magnetic resonance (NMR) spectroscopy to measure rates of muscle glycogen synthesis and intramuscular glucose-6-phosphate (G-6-P) concentrations. Under similar steady-state plasma concentrations of glucose (~11 mmol/l) and insulin (~340 pmol/l), rates of muscle glycogen synthesis were reduced ~70% in the obese subjects (52 ± 8 µmol/[l muscle-min]) as compared with the rates in the lean subjects (176 ± 22 µmol/[l muscle-min]; $P < 0.0001$). Basal concentrations of intramuscular G-6-P were similar in the obese and lean subjects; but during the clamp, G-6-P failed to increase in the obese group (ΔG-6-P obese 0.044 ± 0.011 vs. lean 0.117 ± 0.011 mmol/l muscle; $P < 0.001$), reflecting decreased muscle glucose transport and/or phosphorylation activity. We conclude that insulin resistance in obesity can be mostly attributed to impairment of insulin-stimulated muscle glycogen synthesis due to a defect in glucose transport and/or phosphorylation activity. *Diabetes* 47:381–386, 1998

Carbohydrate intolerance in overweight subjects is associated with decreased sensitivity to insulin and an increased risk of developing type 2 diabetes (1–4). Skeletal muscle accounts for a large percentage of the glucose that is metabolized following a glucose load (5–7). After glucose is transported into muscle, it can be stored as glycogen or fat, undergo oxidation, or be glycolyzed and released as lactate or pyruvate. Direct quantitation of glucose flux through these pathways in muscle is difficult and usually relies on indirect methods such as indirect calorimetry or arteriovenous balance techniques across a limb.

The present study was designed to overcome these limitations of previous studies by addressing several important questions relating to the pathogenesis of insulin resistance in obese humans. First, rates of insulin-stimulated muscle glycogen synthesis were directly assessed in normal and obese subjects using ^{13}C nuclear magnetic resonance (NMR) spectroscopy (7). Second, to examine potential rate-limiting steps in insulin-stimulated muscle glucose uptake, these measurements were combined with ^{31}P NMR measurements of intramuscular glucose-6-phosphate (G-6-P) concentrations (8). Finally, to estimate the contribution of muscle glycogen synthesis to whole-body nonoxidative glucose disposal, whole-body muscle mass was directly measured using whole-body magnetic resonance imaging (MRI).

RESEARCH DESIGN AND METHODS

Subjects. Ten healthy, nonsmoking, obese Caucasian women (35 ± 2 years, BMI 33 ± 1 kg/m^2) with no family history of diabetes, hypertension, or any other systemic disease were studied. The control group consisted of nine lean, nonsmoking, healthy Caucasian women (27 ± 2 years, BMI 22 ± 2 kg/m^2; $P < 0.05$). All had a regular menstrual cycle. Two of the lean control subjects and three of the obese subjects were taking oral contraceptives; no other medications were taken.

Experimental protocol. Written consent was obtained from all subjects after explanation of the purpose, nature, and potential risks of the study. The protocol was reviewed and approved by the Human Investigation Committee of Yale University School of Medicine. All studies and screening tests were performed during the follicular phase of the menstrual cycle (between day 0 and 12) to eliminate changes in metabolism due to the hormonal changes during the menstrual cycle (9). The patients were instructed to eat a weight maintenance diet with at least 150 g carbohydrate for 3 days before the tests as well as to abstain from any kind of vigorous physical exercise during this period. During these 3-day periods, the subjects kept a diet journal, which was reviewed to assess dietary compliance. Subjects initially underwent a clinical screening, which included a 2-hour oral glucose tolerance test, before they were included in the study. After an overnight fast, basal blood samples were taken from an antecubital vein for determination of plasma glucose and insulin concentrations, after which the patient was given 150 ml Glucola (Curtin Matheson Scientific, Houston, TX) containing 75 g glucose to drink at $t = 0$ min. Plasma glucose, insulin, and free fatty acid (FFA) concentrations were measured every 30 min for 120 min. Only obese subjects with normal glucose tolerance and insulin resistance—defined as a mean plasma insulin concentration (from 30 to 120 min) 380 pmol/l, which is 2 standard deviations above the value for the normal, lean subjects—were included in the study.

Clamp study. The subjects were admitted to the Yale-New Haven Hospital General Clinical Research Center on the evening before the study, given dinner at 6:00 P.M., and then fasted overnight before the study. At 7:00 A.M., a Teflon catheter was inserted into an antecubital vein in each arm for blood drawing and glucose/hormone infusion. After baseline $^{13}C/^{3}$ ^{1}P NMR spectra were obtained, an infusion of somatostatin (0.1 µg/[kg body weight-min]) was initiated 5 min before start of the glucose and insulin infusions and was continued throughout the study to inhibit endogenous insulin secretion. At $t = 0$ min, insulin (Humulin; Eli Lilly, Indianapolis, IN) was administered as a prime-continuous infusion (240 pmol/[m^2-min]) to raise the plasma insulin concentration rapidly and to maintain it at ~340 pmol/l during the study. Simultaneously, a primed-variable infusion of [1–^{13}C]glucose (1.1 mol/l, 20% ^{13}C atom percent excess [APE]; Cambridge Isotopes, Cambridge, MA) was begun to raise the plasma glucose concentration to ~11 mmol/l and maintain it at this concentration for 135 min. Blood samples were taken for measurement of plasma glucose concentration every 5 min, for plasma glucose ^{13}C enrichment every 15 min, and for plasma lactate, insulin, and FFA every 30 min. At $t \leq 135$ min, the insulin and somatostatin infusions were terminated, and the infusion of

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APE, atom percent excess; FFA, free fatty acid; G-6-P, glucose-6-phosphate; LBM, lean body mass; MRI, magnetic resonance imaging; NMR, nuclear magnetic resonance; P_i, inorganic phosphate.

glucose was tapered off until the plasma glucose concentration stabilized at euglycemia. The mean glucose infusion minus urinary glucose excretion (which was negligible) was used to measure the rates of whole-body glucose metabolism.

Analyses. Plasma glucose concentration was measured by the glucose oxidase method using a Beckman Glucose Analyzer (Glucose Analyzer II; Beckman, Fullerton, CA). Plasma immunoreactive insulin concentrations were measured with a double antibody radioimmunoassay (Diagnostic, Webster, TX). Plasma lactate concentrations were measured by using the lactate dehydrogenase method (10). Plasma FFA concentrations were determined using a microfluorimetric method (11). The ^{13}C APE of plasma glucose was determined by gas chromatography-mass spectrometry after plasma glucose was derivatized as the penta-acetate, followed by $Ba(OH)_2/ZnSO_4$ deproteinization and semipurification by anion/cation exchange chromatography (AG1-X8; AG50W-X8, Bio-Rad, Richmond, CA), as described previously (12). GC-MS analysis was performed with a Hewlett-Packard (Palo Alto, CA) 5890 gas chromatograph (HP-1 capillary column, 12 m < 0.2 mm < 0.33 μm film thickness) interfaced to a Hewlett-Packard 5971A Mass Selective Detector operating in the positive chemical ionization mode with methane as reagent gas. GC conditions were isothermal at 200°C, and ions with m/z 331, 332, and 333 were monitored for glucose C1 through C6 APE. Continuous indirect calorimetry by the ventilated hood technique using a Deltatrack Metabolic Monitor (Sensormedics, Anaheim, CA) was performed to determine rates of total-body glucose and lipid oxidation at baseline and from 45 to 75 min and 100 to 130 min during the clamp, as described previously (13).

NMR spectroscopy. During the measurements, the subjects were lying inside the NMR spectrometer (Bruker Biospec Spectrometer, Billerica, MA; 1 m bore 2.1T) with the gastrocnemius muscle of the right leg positioned on top of a concentric surface coil probe for ^{31}P and ^{13}C acquisition, and for ^{1}H acquisition, decoupling, and shimming as earlier described (8). ^{31}P spectra were acquired in 15-min blocks consisting of 288 scans with 4,096 data points. Concentrations of metabolites were calculated from the data as described (8). The area of the β resonance of ATP was used as an internal concentration standard and assumed to represent a concentration of 5.5 mmol/kg of muscle (14). The ^{31}P NMR G-6-P measurement has been validated in an animal model by comparison with chemical measurements of G-6-P in muscle frozen in situ (15). ^{13}C NMR spectra were obtained in 15-min blocks consisting of 4,800 scans using a 90° pulse at coil center and a repetition time of 120 ms. Intramuscular glycogen concentrations were determined by comparison with an external standard glycogen solution (7). To approximate the thickness of the subcutaneous fat, thermal plastic spacers (Orthoplast; Johnson & Johnson, New Brunswick, NJ) that produced fat signals in the same chemical shift regions as human fat were placed between the radiofrequency coil and the leg cast. ^{13}C NMR spectra were processed by methods that have been described in detail in several of our earlier studies (7). The ^{13}C NMR technique for assessing intramuscular glycogen concentrations has been validated in situ in frozen rabbit muscle (16) and by comparison with biopsies from human gastrocnemius muscle tissue (17).

Body composition. On the day of admission for the clamp study, a complete dietary report was obtained by a nutritionist, who also performed anthropometric and bioelectrical impedance measurements. Whole-body MRI and hydrostatic weighing were performed within 1 week of the clamp study.

Waist-to-hip ratio. With a plastic tape measure, the circumferences of the waist and hip were measured at the smallest circumference of the midtorso and the widest circumference between the trochanter major and the iliac crest, respectively.

Skinfold thickness. The skinfold thickness was assessed at four sites: biceps, triceps, subscapular, and suprailiac. Measurements were taken with a Lange caliper on the right side of the body, with the subjects standing in a relaxed position, and rounded to the nearest 0.5–1 mm. The subscapular skinfold measurement was taken at an angle of 45° to the vertical, and the suprailiac skinfold was taken above the iliac crest in the mid-axillary line.

Electrical impedance. Electrical impedance was measured (Model JA-101A; Bioelectrical Impedance Analyzer Systems, La Jolla, CA), and the fat mass was calculated as body weight minus fat-free mass (kilograms) using the algorithm of Heitman (18).

Hydrostatic weighing. Body density was estimated by applying Archimedes's principle. The subjects submerged after maximal expiration in a tank filled with water (Hydrodensitometer, Model II Precision Biomedical Systems), and the underwater weight was recorded. Because any air remaining, especially in the lungs, contributes to buoyancy, the residual lung volume was measured by the closed-circuit nitrogen dilution technique (Survey Spirometer; Warren Times, Brantee, MA) with 100% oxygen as the tracer gas and subtracted from the total body volume.

Whole-body MRI. Whole-body MRI was performed in a 1.5T imager (Signa III; GE, Milwaukee, WI) with the subjects lying in the supine position with arms extended over the head. The determinations were performed with multi-echo axial scanning with a total echo time of 20 ms/80 ms and 200 ms repetition time. The total scanning time was ~1 h. The data were transferred to an independent work station and processed with a CAMRA S200 program (ISG Technologies, Toronto, Canada), which provides three-dimensional reconstruction and volume calculation as well as distribution of muscle and fat. Determination of fat and muscle tissue was performed by using interactive level detection with thresholds to separate fat and muscle, respectively, from other tissues by pixel signal intensity. Total fat and muscle were measured by outlining the number of pixels in each slice. Determination of fat in the abdominal slice was performed by using interactive level detection with a threshold to separate fat from nonfat by pixel signal intensity. Subcutaneous and intra-abdominal fat were then measured by outlining these areas and measuring the number of bright pixels representing subcutaneous and intra-abdominal fat, respectively. In five of the obese subjects, the MRI determinations of fat and muscle mass in the arms were difficult to perform accurately due to a large body size. In these studies, the percentages of fat and muscle mass in the arms were estimated by the percent fat and muscle in the arms of the other obese subjects.

Calculations

Hyperglycemic-hyperinsulinemic clamp. Under the hyperglycemic, hyperinsulinemic, and hypoglucagonemic conditions of the study, hepatic glucose production can be assumed to be completely suppressed and the rate of the infused glucose can be used as a measure of the total amount of glucose metabolized (glucosuria was negligible under these clamp conditions). This assumption is supported by euglycemic clamp studies performed in obese individuals during similar insulin infusion rates that resulted in a 80% suppression of hepatic glucose production (19–21). The addition of hyperglycemia and hypoglucagonemia in the present study would be expected to further contribute to the suppression of hepatic glucose production in these subjects. The nonprotein respiratory quotient was obtained from the tables of Lusk (13), according to which the respiratory quotient for 100% oxidation of fat is 0.707 and that for oxidation of carbohydrates is 1.00. Nonoxidative glucose metabolism was calculated by subtracting the amount of glucose oxidized from the total amount of glucose infused. Increments in muscle glycogen concentration were calculated from the change in [1-^{13}C]glycogen concentration and the plasma [1-^{13}C]glucose APE, as described (7). The rate of muscle glycogen synthesis was calculated from the slope of the least-squares linear fit to the glycogen concentration curve from t = 60 to 135 min.

Bioelectrical impedance analyses. The fat-free mass, i.e., lean body mass (LBM), was calculated by the formula: LBM (kg) = 4.631 + 0.2050 cm^2/R + 0.2837(kg) – 0.03082 (kg); where R is the electrical impedance ($1/cm^2$) (18). Whole-body fat mass was calculated as the difference between body weight and LBM.

Hydrostatic weighing. Body density and percent body fat were calculated as follows:

$$\text{Body density} = \text{mass/volume} = (\text{weight in air}) / [(\text{weight in air} - \text{weight in water}) / (\text{density of water} - \text{residual volume})]$$

$$\text{Percent body fat} = [(4.95/\text{body density}) - 4.5] \times 100$$

Whole-body MRI. Patients were scanned from jawline to ankles in image sections of 20 cm each in thickness. Muscle and fat tissue volumes within each three-dimensional image were expressed in centimeters cubedx, and net tissue weight was calculated by multiplying tissue volume and density (fat 0.90 g/cm^3; muscle 1.06 g/cm^3), respectively. The volumes of intra-abdominal and subcutaneous fat were quantified using the MRI cross-sectional image at the level of the umbilicus as previously described (22). For comparison with the other techniques for determination of body composition, LBM was calculated as the difference between body weight and fat mass.

Statistical analysis. Measurements are given as the group mean ± SE. Statistical differences between groups were performed using Student's t test.

RESULTS

Hormone and metabolite concentrations. Fasting plasma concentrations of insulin and triglycerides were higher in the obese than in the lean subjects (Table 1). There were no differences in glucose, lactate, or FFA concentrations between the groups in the fasting state or during the clamp (Table 1). Plasma lactate concentrations increased significantly from basal values in the both the lean and the obese subjects during the hyperglycemic-hyperinsulinemic clamp (Table 1) (increment in lactate for lean subjects, 0.52 ± 0.10 mmol/l; increment in lactate for obese subjects, 0.46 ± 0.14 mmol/l).

Body composition. Despite similar heights (obese, 168 ± 1 cm; lean, 166 ± 2 cm), whole-body muscle mass as estimated by MRI was 24% higher in the obese than in the lean subjects (P < 0.001; Table 2); whereas muscle mass expressed as percent of body weight was significantly higher in the lean sub-

TABLE 1
Plasma metabolite concentrations in the basal state and during a hyperglycemic-hyperinsulinemic clamp in lean and obese subjects

	Basal		Hyperglycemic-hyperinsulinemic clamp	
	Lean	Obese	Lean	Obese
n	9	10	9	10
Glucose (mmol/l)	5.1 ± 0.1	5.5 ± 0.1	10.5 ± 0.2	10.1 ± 0.2
Insulin (pmol/l)	30 ± 6	60 ± 12*	318 ± 48	360 ± 18
Lactate (mmol/l)	0.68 ± 0.05	0.88 ± 0.13	1.19 ± 0.12‡	1.34 ± 0.09‡
FFA (μmol/l)	444 ± 40	541 ± 46	160 ± 7	139 ± 9
Triglyceride (mg/dl)	2.2 ± 0.2	3.9 ± 0.2†	ND	ND
HDL (mmol/l)	1.4 ± 0.1	1.3 ± 0.1	ND	ND
LDL (mmol/l)	2.5 ± 0.3	2.6 ± 0.2	ND	ND

Data are means ± SE. *P < 0.05, †P < 0.0001 vs. lean subjects. ‡P < 0.05 compared with basal. ND, not determined.

jects (27.2 ± 0.8%) than in the obese women (22.2 ± 0.5%; P < 0.001; Table 2). LBM (as calculated by the difference between body weight and body fat mass) as determined by MRI (obese, 62.9 ± 1.1% of body weight; lean, 80.6 ± 3.5% of body weight; P = 0.015) was in excellent agreement with the anthropometric, bioelectrical impedance, and hydrodensitometric determinations of LBM (Table 2). There was a considerable difference between muscle mass and LBM in both lean and obese subjects as determined by the MRI technique (Table 2). Whole-body muscle mass in the obese subjects was 20.6 ± 1.1 kg (thus accounting for 38.4 ± 1.4% of LBM as determined by hydrodensitometry). In the lean subjects, whole-body muscle mass was 16.5 ± 0.6 kg (P < 0.001 vs. obese subjects; accounting for 36.7 ± 0.9% of LBM). These findings show that LBM is not necessarily an accurate reflection of muscle mass. All methods found a similar increase in fat mass in the obese subjects expressed either as weight of adipose tissue or percent of body weight.

Glucose and lipid oxidation. The metabolic rates were similar in lean and obese subjects during both the basal, fasting state (lean, 6,339 ± 330 kJ; obese, 7,351 ± 238 kJ) and the hyperglycemic/hyperinsulinemic clamp (lean, 7,293 ± 431 kJ; obese, 7,983 ± 469 kJ). However, the percent increase in metabolic rate from the basal, fasting state to the clamp conditions was significantly higher in the lean subjects (15.9 ± 4.4%) than in the obese subjects (8.6 ± 3.2%; P < 0.05). The respiratory quotients were similar in the obese and lean subjects both in the basal, fasting state (lean, 0.78 ± 0.01 vs. obese, 0.81 ± 0.01) and during the clamp (lean, 0.92 ± 0.02 vs. obese, 0.96 ± 0.03). Basal rates of whole-body glucose oxidation were similar in the obese and in the lean subjects (obese, 446 ± 44; lean, 437 ± 45 μmol/min), whereas basal rates of lipid oxidation were higher in the obese subjects than in the lean subjects (obese, 853 ± 95; lean, 390 ± 116 μmol/min; P = 0.006). During the clamp, similar increments in rates of glucose oxidation (obese, 1,317 ± 162; lean, 815 ± 105 μmol/min; both P < 0.05 vs. basal) and percent suppression of rates of lipid oxidation were observed in the lean and obese subjects (lean, 53.5 ± 9.4 vs. obese, 63.8 ± 8.9%; both P = 0.05 vs. basal).

Total and nonoxidative glucose disposal. Rates of glucose metabolism were not significantly different between the obese and lean subjects when expressed per whole body (3,096 ± 318 in the obese vs. 3,850 ± 281 μmol/min in the lean control subjects; P = 0.096), but they were significantly reduced in the obese subjects when expressed per kilogram LBM (25.0 ± 3.4 in the obese vs. 69.4 ± 6.2 μmol/kg LBM in the lean subjects; P < 0.001) or when expressed per kilogram muscle mass (88.8 ± 12.1 in the obese vs. 188.1 ± 13.1 μmol/[l muscle-min] in the control subjects; P < 0.001). Rates of whole-body nonoxidative glucose metabolism accounted for 57 ± 4% of rates of whole-body muscle glucose metabolism in the obese group and for 79 ± 2% in the lean group (P < 0.001 vs. obese).

Muscle glycogen synthesis. The fasting concentrations of intramuscular glycogen were significantly lower in the obese subjects (56 ± 4 mmol/l) than in the lean subjects (76 ± 6 mmol/l; P < 0.05). Rates of insulin-stimulated muscle glycogen synthesis (Table 3) were 70% lower in the obese subjects (52 ± 8 μmol/[l muscle min]) compared with the rates of muscle glycogen synthesis in the lean subjects [176 ± 22 μmol/(kg min)] (P < 0.0001). When the individual rates of muscle glycogen synthesis were extrapolated to the whole body by multiplying by the individual MRI-determined muscle mass, the mean rate of whole-body muscle glycogen synthesis accounted for 99 ± 14 and 67 ± 10% of whole-body nonoxidative glucose metabolism in lean and obese subjects, respectively.

Intracellular G-6-P concentrations. The basal concentrations of intramuscular G-6-P were similar in the lean (0.106 ± 0.014 mmol/l) and obese (0.103 ± 0.009 mmol/l) subjects, as were the concentrations of intracellular phosphate (P_i; lean, 2.91 ± 0.13; obese, 2.33 ± 0.12 mmol/l). During the clamp, the concentrations of G-6-P increased to 0.227 ± 0.019 mmol/l muscle in the lean subjects and to 0.148 ± 0.016 mmol/l muscle in the obese subjects (P < 0.005). The insulin-stimulated increase in intramuscular concentrations of G-6-P was significantly lower in the obese women (ΔG-6-P 0.044 ± 0.011 mmol/l muscle) than the lean subjects (0.117 ± 0.011 mmol/l muscle; P < 0.001), reflecting reduced insulin-stimulated glucose transport/phosphorylation activity in the obese subjects (Table 3). There was no difference in the insulin-stimulated net increase in intracellular phosphate concentrations between the groups (ΔP_i lean, 0.35 ± 0.11 mmol/l; ΔP_i obese, 0.41 ± 0.13 mmol/l).

DISCUSSION

It is well established that obese individuals are insulin resistant at the whole-body level (23–31). Using the arteriovenous catheterization approach across a limb, Rabinowitz and Zierler (32), followed by others (33–35), have demonstrated

TABLE 2
Body weight, BMI, and MRI-determined muscle mass

	Lean subjects	Obese subjects	*P* value
n	9	10	
Body weight (kg)	61.1 ± 2.1	92.5 ± 4.0	<0.001
BMI (kg/m^2)	22.2 ± 0.7	32.5 ± 1.3	<0.001
Muscle mass (kg)	16.5 ± 0.6	20.6 ± 1.1	<0.001
(% BW)	(27.2 ± 0.8)	(22.2 ± 0.5)	
LBM			
Hydrodensitometry (kg)	45.4 ± 1.1	53.8 ± 3.0	0.022
(% BW)	(73.1 ± 0.9)	(58.3 ± 2.0)	
Anthropometry (kg)	44.9 ± 1.4	52.4 ± 1.7	0.004
(% BW)	(73.0 ± 1.1)	(58.0 ± 1.4)	
Electrical bioimpedance (kg)	42.6 ± 0.8	58.2 ± 5.6	<0.0001
(% BW)	(70.3 ± 1.5)	(60.9 ± 1.0)	
MRI (kg)	49.0 ± 2.3	58.0 ± 2.4	0.015
(% BW)	(80.6 ± 3.5)	(62.9 ± 1.1)	
Fat mass			
Hydrodensitometry (kg)	16.8 ± 1.0	38.6 ± 2.9	<0.0001
(% BW)	(26.9 ± 0.9)	(41.7 ± 2.0)	
Anthropometry (kg)	16.8 ± 1.3	38.6 ± 2.8	<0.0001
(% BW)	(27.0 ± 1.1)	(42.0 ± 1.4)	
Electrical bioimpedance (kg)	18.6 ± 1.5	36.2 ± 1.8	0.01
(% BW)	(29.7 ± 1.5)	(39.1 ± 1.0)	
MRI (kg)	15.5 ± 1.0	34.4 ± 2.1	<0.0001
(% BW)	(24.9 ± 1.1)	(37.1 ± 1.1)	

Data are means ± SE. LBM and fat mass as determined by MRI, skin-fold thickness, bioelectrical impedance, and hydrodensitometric weighing expressed in kilograms and percent of body weight (% BW) in lean and obese subjects.

that this insulin resistance can be primarily attributed to a defect located in skeletal muscle. Although this technique is tissue specific, it does not directly assess the intracellular fate of glucose once it is taken up by the muscle bed. The purpose of this study was to directly measure the rate of insulin-stimulated muscle glycogen synthesis in obese subjects for the first time using ^{13}C NMR spectroscopy and to assess potential rate-limiting steps in this process under hyperglycemic-hyperinsulinemic conditions. These conditions were chosen to simulate postprandial conditions, when most of the muscle glycogen synthesis typically occurs. We found that obese subjects had a profound impairment in insulin-stimulated muscle glycogen synthesis, which was 70% lower than in the lean control subjects. Felber et al. (36) have previously used indirect calorimetry to demonstrate that obese individuals have a defect in nonoxidative glucose metabolism under euglycemic-hyperinsulinemic conditions, and they concluded that because glycogen synthesis represents a major component of nonoxidative glucose metabolism, this abnormality may reflect a defect in glycogen synthase activation. However, in contrast to the findings of the present study, Felber et al. found that hyperglycemia following oral glucose ingestion was able to overcome this defect. Although it is possible that differences in the route of the glucose administration could have accounted for these observations, it is also possible that limitations in the indirect calorimetry technique due to the relatively high basal rates of glucose oxidation by brain and heart might obscure any small differences in rates of insulin-stimulated muscle glucose oxidation between the two groups.

To assess the contribution of muscle glycogen synthesis to whole-body insulin-stimulated nonoxidative glucose disposal, we also measured muscle mass directly using a whole-body MRI technique. Previous estimates of whole-body muscle mass in humans have ranged from 26% of body weight by a prompt γ-neutron-activation technique measuring ^{14}N/^{15}N

TABLE 3
Metabolic parameters in lean and obese women during the hyperglycemic-hyperinsulinemic clamp study

	n	Whole-body glucose metabolism (μmol/min)	Whole-body oxidative glucose metabolism (μmol/min)	Whole-body nonoxidative glucose metabolism (μmol/min)	Glycogen synthetic rates (μmol/[l muscle-min])	ΔG-6-P (mmol/l)
Lean	9	3,850 ± 281	815 ± 105	3,035 ± 216	176 ± 22	0.117 ± 0.011
Obese	10	3,096 ± 318	1,317 ± 162	1,778 ± 249	52 ± 8	0.044 ± 0.011
P value	—	0.10	0.02	<0.05	<0.0001	<0.001

(37) to 38% determined by dissection of cadavers (38). In the current study, total-body muscle mass for the lean subjects as determined by whole-body MRI was 27.2 ± 0.5% of total body weight, which is in excellent agreement with the neutron activation technique. Despite the subjects being approximately matched for height, muscle mass was 24% greater in the obese (20.6 ± 1.1 kg) than in the lean (16.5 ± 0.6 kg; $P < 0.001$) subjects but accounted for a smaller percentage of body weight in the obese subjects. When extrapolated to the whole body, rates of muscle glycogen synthesis accounted for 67 ± 10% of whole-body nonoxidative glucose metabolism in the obese subjects and for 99 ± 14% in the lean subjects. Although these differences were not statistically different ($P = 0.083$), it is of interest to speculate that the lower percent contribution of muscle glycogen synthesis to whole-body nonoxidative glucose disposal might reflect a greater contribution of adipose tissue glycolysis to nonoxidative glucose disposal in the obese subjects. This might be attributed to their twofold increase in adipose tissue mass and would be consistent with the tendency toward higher plasma lactate concentrations observed in these subjects in the basal state and during the hyperglycemic-hyperinsulinemic clamp.

To determine the biochemical factors underlying the reduced rates of insulin-stimulated muscle glycogen synthesis in the obese subjects, ^{31}P NMR was used to measure intramuscular concentrations of G-6-P before and during the hyperglycemic-hyperinsulinemic clamp. If glycogen synthase were rate-controlling, as suggested by some (26,39), a buildup of intramuscular G-6-P would be expected concomitant with the reduced rates of glycogen synthesis. Given that the increment in intramuscular G-6-P concentration was lower in the obese subjects than in the lean control subjects during the hyperglycemic-hyperinsulinemic clamp, we can deduce that glucose transport and/or phosphorylation is impaired in the obese subjects. This defect in glucose transport/phosphorylation in obese subjects is similar to that observed in subjects with type 2 diabetes (8) and normoglycemic, insulin-resistant offspring of type 2 diabetic parents (40) and may reflect a common etiology. The lower insulin-stimulated intramuscular G-6-P concentrations, along with the lower glycogen concentrations, observed in the obese subjects argues against the "retrograde regulation" hypothesis as a putative mechanism for the insulin resistance of obesity as proposed by Golay et al. (39) and Felber and Golay (41) . These data are consistent with previous in vitro results where reduced 2-deoxyglucose uptake was observed in muscle strips obtained from obese individuals and incubated under hyperinsulinemic conditions, which implies a defect in glucose transport/phosphorylation (42,43). The data are also consistent with a recent positron emission tomography imaging study, using 2-deoxy-2[^{18}F]fluoro-D-glucose, where it was found that the rate constant for inward transport of glucose did not increase in obese subjects under hyperinsulinemic conditions, leading these authors to suggest an impairment in glucose transport activity (44). However, it should be pointed out that 2-deoxyglucose can also be incorporated into glycogen, which suggests that 2-deoxyglucose accumulation in tissue does not solely reflect rates of glucose transport and phosphorylation (45–47).

In summary, we examined the mechanism of insulin resistance in moderately obese women using ^{13}C/^{31}P NMR spectroscopy and found a profound impairment in insulin-stimulated muscle glycogen synthesis, which could be attributed to a defect in muscle glucose transport/phosphorylation activity. These abnormalities in insulin-stimulated muscle glucose metabolism are identical to what is observed in type 2 diabetic subjects (8) and normoglycemic, insulin-resistant offspring of type 2 diabetic parents (40) and may reflect a common etiology.

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