Muscle glucose transport and phosphorylation in type 2 diabetic, obese nondiabetic, and genetically predisposed individuals

Merri Pendergrass,1 Alessandra Bertoldo,2 Riccardo Bonadonna,3 Gianluca Nucci,2 Lawrence Mandarino, Claudio Cobelli,2 and Ralph A. DeFronzo1

1University of Texas Health Science Center, San Antonio, Texas; 2Department of Information Engineering, University of Padova, Padua; and 3Division of Endocrinology and Metabolic Diseases, Verona School of Medicine, Verona, Italy

Submitted 6 December 2005; accepted in final form 21 July 2006

Pendergrass M, Bertoldo A, Bonadonna R, Nucci G, Mandarino L, Cobelli C, DeFronzo RA. Muscle glucose transport and phosphorylation in type 2 diabetic, obese nondiabetic, and genetically predisposed individuals. Am J Physiol Endocrinol Metab 292: E92–E100, 2007. First published August 8, 2006; doi:10.1152/ajpendo.00617.2005.—Our objectives were to quantify insulin-stimulated inward glucose transport and glucose phosphorylation in forearm muscle in lean and obese nondiabetic subjects, in lean and obese type 2 diabetic (T2DM) subjects, and in normal glucose-tolerant, insulin-resistant offspring of two T2DM parents. Subjects received a euglycemic insulin (40 mU·m−2·min−1) clamp with brachial artery/deep forearm vein catheterization. After 120 min of hyperinsulinemia, a bolus of 3-O-methyl-3-[13C]glucose/3-[3-18]H]glucose (triple-tracer technique) was given into brachial artery and deep vein samples obtained every 12–30 s for 15 min. Insulin-stimulated forearm glucose uptake (FGU) and whole body glucose metabolism (M) were reduced by 40–50% in obese nondiabetic, lean T2DM, and obese T2DM subjects (all P < 0.01); in offspring, the reduction in FGU and M was ~30% (P < 0.05). Inward glucose transport and glucose phosphorylation were decreased by ~40–50% (P < 0.01) in obese nondiabetic and T2DM groups and closely paralleled the decrease in FGU. The intracellular glucose concentration in the space accessible to glucose was significantly greater in obese nondiabetic, lean T2DM, obese T2DM, and offspring compared with lean controls. We conclude that 1) obese nondiabetic, lean T2DM, and offspring manifest moderate-to-severe muscle insulin resistance (FGU and M) and decreased insulin-stimulated glucose transport and glucose phosphorylation in forearm muscle; these defects in insulin action are not further reduced by the combination of obesity plus T2DM; and 2) the increase in intracellular glucose concentration under hyperinsulinemic euglycemic conditions in obese and T2DM groups suggests that the defect in glucose phosphorylation exceeds the defect in glucose transport.

insulin resistance; muscle; glucose phosphorylation; type 2 diabetes; obesity

INSULIN RESISTANCE IN MUSCLE, the primary tissue responsible for glucose disposal (18), is a characteristic feature of type 2 diabetes mellitus (T2DM) (19, 20, 31) and obesity (4, 5, 19, 20, 31, 33). In T2DM, impaired insulin action is an inherited trait (41, 65), whereas in obesity insulin resistance is acquired (58) secondarily to disturbances in free fatty acid (FFA) metabolism (27, 53), altered fat topography (13), and deranged secretion of adipocytokines (3). Skeletal muscle insulin resistance in T2DM and obesity affects both the glycogen synthetic and glucose oxidative pathways (22), suggesting a proximal defect in insulin action. Recent studies have demonstrated multiple disturbances in insulin signaling in T2DM subjects (15, 35, 36). However, there has been considerable debate about the contributions of impaired glucose transport vs. reduced glucose phosphorylation to the defect in insulin action in T2DM (6, 7, 12, 36, 56, 66–68). Only one previous study (7) has examined the contributions of impaired muscle glucose transport vs. reduced glucose phosphorylation to the defect in insulin action in lean T2DM subjects. Inward transmembrane glucose transport was markedly impaired in lean T2DM subjects under conditions of euglycemic hyperinsulinemia. However, glucose phosphorylation was impaired to an even greater extent, leading to an increase in intracellular free glucose concentration. When the rate of inward glucose transport was normalized by performing a hyperglycemic hyperinsulinemic clamp in the lean T2DM subjects, the defect in glucose phosphorylation could not be overcome, and the intracellular free glucose concentration in the space to which glucose is accessible increased markedly (7). Conflicting results have been reported by others (12, 34, 66–68). Using a nuclear magnetic resonance approach with carbon-13 and phosphorus-31, Cline et al. (12) concluded that impaired glucose transport was the primary defect responsible for muscle insulin resistance in T2DM. Interpretive problems in the 13C-31P NMR approach have been reviewed in detail by Murphy and Hellerstein (45). Because of the different methodologies and different underlying assumptions, it is difficult to compare the contrasting results of the triple-tracer (7) and NMR (12) techniques. Moreover, Cline et al. compared obese T2DM subjects (BMI 30.7 kg/m2) with very lean controls (BMI 22.7 kg/m2), making it impossible to distinguish whether obesity or diabetes was responsible for the reported defect in glucose transport.

Using leg catheterization with 2-deoxy-2[18F]fluoro-d-glucose and positron emission tomography, Kelly et al. (34) and Williams and colleagues (66–68) examined skeletal muscle glucose transport and phosphorylation in T2DM. In an initial study (34), they concluded that stimulation of the rate constant for inward glucose transport by insulin was severely and equally impaired in T2DM and obese nondiabetic subjects, whereas the rate constant for glucose phosphorylation was reduced in T2DM but not in obese individuals. However, T2DM subjects were significantly more obese (BMI 34.1 kg/m2) compared with the nondiabetic obese group (BMI 31.5

Address for reprint requests and other correspondence: R. A. DeFronzo, Univ. of Texas Health Science Center, Diabetes Division, 703 Floyd Curl Dr., San Antonio, TX 78229.

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
kg/m²). Thus it was not possible to define whether the defects in glucose transport and phosphorylation were due to T2DM or to obesity. Last, those authors reported normal muscle hexoki-

nase II activity in both T2DM and obese groups, a finding at variance with many published studies (40, 48, 63) and with their own results that the rate constant for glucose phosphor-

ylation was diminished in the T2DM group. In a follow-up publication (66), using similar methodology, the same group reported a normal rate constant for glucose transport in obese individuals but a decreased rate constant for glucose phosphory-

lation. In subsequent publications (67, 68), the investigators used a muscle-specific compartmental model and demonstrated a reduction in the insulin-stimulated glucose transport param-

eter in obese nondiabetic subjects vs. obese T2DM subjects. Since a lean T2DM group was not studied (67, 68), it is difficult to discern whether T2DM or obesity was responsible for the reported defects in glucose transport and glucose phosphorylation. Moreover, there was a marked difference in the severity of insulin resistance in the obese nondiabetic groups in these two studies (67, 68). This is of importance because most investigators have reported a similar magnitude of insulin resistance in obese nondiabetic and lean T2DM subjects (19, 31). Finally, without knowledge of the intracel-

lular free glucose concentration, it is not possible to calculate absolute flux rates, and extrapolation from rate constants to absolute fluxes of glucose transport and phosphorylation is hazardous (34, 66–68).

Although an overwhelming body of evidence indicates an underlying genetic basis for the insulin resistance in T2DM (20, 41, 65), acquired factors, including glucotoxicity (52, 55), lipotoxicity (43, 60), and obesity (18, 19, 53, 58), have also been shown to play an important role in the development of impaired insulin action. The aims of the present study were to 1) quantitate transmembrane inward glucose transport and intracellular glucose phosphorylation in insulin-resistant obese nondiabetic subjects; 2) examine whether obesity and T2DM exert additive effects to impair transmembrane glucose transport and intracellular glucose phosphorylation in insulin-resistant obese nondiabetic subjects; 3) examine whether normal-glucose-tolerant, insulin-resistant, lean offspring of two T2DM parents, at high risk to develop T2DM, manifest defects in muscle glucose transport and glucose phosphorylation similar to those in overtly diabetic individuals; if so, this would argue in favor of an inherited basis for the observed defects; and 4) examine the relationship between Hb A₁c and impaired glucose transport and glucose phosphoryla-

tion to gain insight into the potential contribution of glucose toxicity to the muscle insulin resistance.

METHODS

Subjects. Seven lean control subjects, seven obese subjects, six lean T2DM subjects, five obese T2DM subjects, and eight offspring of two T2DM parents participated in the study. Results from five of the six lean T2DM subjects were reported previously (7). None of the results from any of the other groups (including the lean controls, who were different from those presented in Ref. 7) have been reported previ-

ously. The clinical and laboratory characteristics are shown in Table 1. Lean control subjects, obese subjects, and offspring had a normal 75-g oral glucose tolerance test according to American Diabetes Association criteria (24). None of the lean controls or obese subjects had any family history of diabetes. Other than diabetes, no subject had any evidence of systemic disease as determined by physical exami-

nation, medical history, screening blood chemistry and hematologic tests, and urinalysis. In all subjects, body weight was stable for ≥3 mo prior to study. No subject participated in any strenuous exercise program. Five of the lean and four of the obese T2DM subjects were treated with sulfonylureas, which have no known direct effects on insulin sensitivity (21). T2DM subjects who were taking or had previously received treatment with metformin, a thiazolidinedione, or insulin were excluded from study. Other than sulfonylureas, no T2DM or control subject was taking any medication. All of the offspring of two T2DM parents were of Mexican American ethnicity, reflecting the high incidence of diabetes in the predominantly Mexican American population of San Antonio. There was a disproportionate number of females in the offspring group. All groups were well matched for age. Subjects were instructed to eat a weight-maintaining diet contain-

ing ≥200 g of carbohydrate for 3 days prior to study. T2DM subjects taking sulfonylureas omitted their medication for 3 days prior to study. The purpose, nature, and potential risks of the study were explained to all subjects, and informed written consent was obtained before their participation. The protocol was reviewed and approved by the Human Investigation Committee of the University of Texas Health Science Center in San Antonio, Texas.

Experimental design. All studies took place at the General Clinical Research Center (GCRC) of the Audie L. Murphy Memorial Veterans Administration Hospital. On the evening before the study, the lean T2DM subjects were admitted to the GCRC. They received an overnight intravenous infusion of insulin (0.6–1.5 pmol·min⁻¹·kg⁻¹), which was adjusted every hour to slowly achieve and maintain normoglycemia. Therefore, diabetic and nondiabetic subjects were studied with comparable basal plasma glucose concentra-

tions. The obese T2DM and all of the nondiabetic subjects were admitted to the GCRC on the morning of the study. All studies began at 0800 after a 10- to 12-h overnight fast. On the morning of the study,

Table 1. Clinical and laboratory characteristics

<table>
<thead>
<tr>
<th></th>
<th>Lean Control</th>
<th>Obese</th>
<th>Lean T2DM</th>
<th>Obese T2DM</th>
<th>Offspring</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>10</td>
<td>7</td>
<td>6</td>
<td>5</td>
<td>8</td>
</tr>
<tr>
<td>Age, yr</td>
<td>39±3</td>
<td>40±4</td>
<td>49±5</td>
<td>41±4</td>
<td>32±2</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>6/4</td>
<td>3/4</td>
<td>5/1</td>
<td>2/3</td>
<td>0/8</td>
</tr>
<tr>
<td>Ethnicity (MA/Cauc)</td>
<td>7/3</td>
<td>3/4</td>
<td>3/3</td>
<td>4/1</td>
<td>7/1</td>
</tr>
<tr>
<td>T2DM duration, yr</td>
<td>0.4±0.4</td>
<td>3.0±2.5</td>
<td>1/5</td>
<td>2/3</td>
<td></td>
</tr>
<tr>
<td>T2DM Rx (diet/SU)</td>
<td>BMI, kg/m²</td>
<td>25.0±1</td>
<td>36.6±2.0*</td>
<td>24.8±1.0</td>
<td>33.3±2.0*</td>
</tr>
<tr>
<td></td>
<td>Fasting glucose, mM</td>
<td>5.3±0.1</td>
<td>5.3±0.1</td>
<td>10.9±1.21*</td>
<td>5.6±0.3</td>
</tr>
<tr>
<td></td>
<td>Fasting insulin, pM</td>
<td>33±6</td>
<td>56±6*</td>
<td>72.2±11*</td>
<td>124±24*</td>
</tr>
<tr>
<td></td>
<td>Systolic BP, mmHg</td>
<td>118±5</td>
<td>131±7</td>
<td>131±5</td>
<td>137±5*</td>
</tr>
<tr>
<td></td>
<td>Diastolic BP, mmHg</td>
<td>81±4</td>
<td>81±6</td>
<td>81±3</td>
<td>81±5</td>
</tr>
</tbody>
</table>

Values are means ± SE. T2DM, type 2 diabetic; MA/Cauc, Mexican American/Caucasian; BP, blood pressure; SU, sulfonylurea; *P < 0.05–0.1 vs. lean controls.
catheters were introduced percutaneously into the brachial artery and retrogradely into an ipsilateral deep forearm vein draining muscle. All blood samples were obtained through these two catheters. The tip of the deep forearm vein catheter was advanced for a distance of 2 in. from the puncture site and could not be palpated in any of the subjects. Previous studies have documented that such catheter placement allows sampling of the muscle bed perfused by either the radial or ulnar artery. Catheter patency was maintained by a slow infusion of normal saline. To exclude blood flow from the hand, a pediatric sphygmomanometric cuff was inflated around the wrist to 100 mmHg above the systolic pressure for 2 min before and 15 min after tracer injection. A third catheter was inserted into a contralateral arm vein for infusion of insulin and glucose. Following a 70-min basal period, a euglycemic hyperinsulinemic clamp was performed for 140 min (17). Human regular insulin (Humulin; Lilly, Indianapolis, IN) was administered as a primed continuous (240 pmol·min⁻¹·m⁻²) infusion to achieve plasma insulin concentrations in the physiological range. Arterial plasma glucose concentration was measured every 5–10 min, and a variable infusion of 20% glucose was adjusted on the basis of the negative feedback principle to maintain the fasting plasma glucose concentration at basal levels. At −70, −30, −15, 40, 80, 100, and 140 min, simultaneous arterial and venous samples were obtained for determination of plasma glucose concentrations, and forearm blood flow was determined by indocyanine green dye dilution (57). Arterial blood samples were obtained at these same time points for measurement of plasma insulin and FFA concentrations. Forearm volume was measured in all subjects by water displacement. Forearm specific gravity was assumed to be 1. At 120 min, a triple-tracer technique was as previously described (7, 57) to determine transmembrane glucose transport and intracellular glucose phosphorylation. A bolus of unlabelled d-mannitol (580 µmol) plus 3-O-methyl-d-[14C]glucose (4–8 µCi) plus d-[3-3H]glucose (8–16 µCi) was injected rapidly (over 2 s) into the artery via the same syringe. Frequent (every 12–30 s) blood samples were drawn from the deep vein for 15 min thereafter for determination of the three tracer concentrations. The radioactive tracers were purchased from New England Nuclear (Boston, MA) and were tested to be sterile and pyrogen free before use. Analytic determinations. Plasma glucose concentration was determined in duplicate by the glucose oxidase method on a Glucose Analyzer II (Beckman, Fullerton, CA). Plasma insulin concentration was measured by radioimmunoassay [Coat-a-Count Insulin Kits; Diagnostic Products, Los Angeles, CA] intra-assay coefficient of variation (CV) = 5%; inter assay CV = 5%. Free fatty acids were measured by an enzymatic method (NEFA C Kit; Wako Chemicals, Dallas, TX). Indocyanine green dye concentration was determined spectrophotometrically at 810 nm. To determine plasma radioactive tracer concentrations in deep venous effluent, plasma proteins were precipitated according to the Somogyi procedure. Aliquots of the supernatant were evaporated to dryness, reconstituted with water, mixed with scintillation fluid, and counted for 3H and 14C radioactivity in a dual-channel scintillation counter with external standard correction (Beckman). Known volumes of all tracer infusates were added to plasma samples obtained from the same subject before the injection of tracers, and plasma radioactivity was determined after Somogyi precipitation as described above. Plasma d-mannitol concentration in deep venous effluent blood was determined as described previously (57).

Calculations. Whole body insulin-stimulated glucose metabolism (M) was calculated during the final 40 min of the clamp according to the following formula: M = glucose infusion rate ± pool correction, where the pool correction takes into account the change in the whole body glucose pool, as estimated from the change in plasma glucose concentration (17). This correction was <2% of the glucose infusion rate during all insulin clamp studies.

Forearm glucose uptake (FGU) was quantitated at the previously indicated time points according to the Fick principle: FGU = (artery – vein glucose concentration) × blood flow. Blood glucose concentration was estimated from plasma glucose concentration and the hematocrit (Hct) (23). Forearm blood flow was measured by indocyanine green dye dilution in the deep forearm vein according to the following formula: blood flow rate = (dye infusion rate/deep vein dye concentration) / (1 – Hct) (70). Forearm blood flow is expressed per kilogram of forearm volume.

Measurement of glucose transport and phosphorylation. In vivo rates of transmembrane glucose transport and intracellular glucose phosphorylation were determined by analyzing the dilution curves of d-mannitol, 3-O-methyl-d-[14C]glucose, and d-[3-3H]glucose, using a multicompartmental model of glucose kinetics in forearm tissues (57). Details of the model and criteria to assess its validity have been discussed thoroughly in previous publications (7, 57) and will be briefly outlined here. d-Mannitol follows the distribution of blood, crosses the capillary membrane, and diffuses into the interstitial fluid but cannot permeate the cell membrane. It therefore reflects extracellular kinetic events. 3-O-[14C]methyl-d-glucose follows blood flow and diffusion into the interstitial tissues but is, in addition, transported into the muscle cell. It therefore reflects both extracellular kinetic events and transmembrane glucose transport. The difference between the washout curves for d-mannitol and 3-O-methyl-d-[14C]glucose provides a measure of glucose transport. d-[3-3H]glucose is transported into the cell and is phosphorylated to glucose 6-phosphate by the enzyme hexokinase II. It therefore traces intracellular glucose phosphorylation and is equivalent to irreversible metabolism of glucose by muscle. The difference between the washout curves for 3-O-methyl-d-[14C]glucose and d-[3-3H]glucose provides a measure of intracellular glucose phosphorylation. Simultaneous mathematical modeling of the three tracer dilution curves allows the quantitation of transmembrane inward (Fm) and outward (Fo) glucose fluxes, the intracellular glucose phosphorylation (Fmax) flux (µmol·min⁻¹·kg⁻¹), and the extracellular (CBe) and intracellular (CIC) glucose concentrations (µmol/l). To improve model numerical identifiability, a priori information on heterogeneity of transit times was exploited based on previous studies (7, 57).

Statistical analysis. All data are presented as means ± SE. All baseline values are reported as the mean of the samples taken at time points −70, −30, and −15 min. Values during the insulin clamp are reported as the mean of samples taken at time points 80, 100, and 140 min. Overall differences among the three groups were compared using a one-way analysis of variance. When significant overall differences were found, Fisher’s protected least significant difference test for multiple comparisons was performed. Basal and test period values for each group were compared using the paired Student’s t-test. Correlations between variables of interest were assessed using Pearson’s correlation coefficient. Because values for all parameters of whole body and forearm glucose metabolism were very similar in lean control, obese nondiabetic, lean and obese T2DM Mexican American and Caucasian subjects, no adjustment for ethnicity was made.

RESULTS

Substrate levels. Basal arterial plasma glucose concentrations were similar in all five groups [5.3 ± 0.1, 5.3 ± 0.1, 5.4 ± 0.4, 5.6 ± 0.3, and 4.9 ± 0.2 mmol/l in lean controls, obese nondiabetic, lean T2DM, obese T2DM, and offspring, respectively, P = not significant (NS)] and were unchanged during hyperinsulinemia (5.4 ± 0.1, 5.3 ± 0.1, 5.4 ± 0.4, 5.4 ± 0.2, and 4.9 ± 0.2 mmol/l). Basal arterial insulin levels were lower in lean controls (33 ± 6 pmol/l, P < 0.01) vs. obese nondiabetics (56 ± 6 pmol/l), which in turn were lower than in the lean (174 ± 24) and obese (124 ± 24 pmol/l)
T2DM (both \( P < 0.01 \)). Basal arterial insulin levels in the offspring (54 ± 9 pmol/l) were higher than in controls (\( P < 0.01 \)) but less than in the two T2DM groups (\( P < 0.01 \)). During the insulin clamp, the steady-state arterial insulin concentration in the control subjects (378 ± 30 pmol/l) was less than in the obese (540 ± 30 pmol/l), lean T2DM (531 ± 102), obese T2DM (522 ± 54), and offspring (545 ± 32 pmol/l) subjects (\( P < 0.05 \)–0.01). Compared with controls (531 ± 70 pmol/l), basal plasma FFA levels were slightly but not significantly higher in the obese nondiabetic (658 ± 55 μmol/l), obese T2DM (587 ± 14), and offspring (560 ± 64 μmol/l) groups (\( P = NS \)). Basal FFA concentration was lower in the lean T2DM subjects (473 ± 42 μmol/l), presumably because of the higher insulin concentrations brought about by the overnight low-dose insulin infusion. The basal plasma FFA concentration in offspring (555 ± 58 μmol/l) was similar to that in controls. During hyperinsulinemia, FFA levels were suppressed to a greater degree in the control (162 ± 23 μmol/l) and obese (147 ± 12 μmol/l) subjects compared with lean (203 ± 41) and obese (236 ± 32 μmol/l) T2DM subjects and compared with the offspring (220 ± 30 μmol/l; all \( P < 0.05 \)).

Whole body and forearm glucose uptake. Rates of insulin-stimulated whole body glucose metabolism (M) during the insulin clamp were higher in the control (32.8 ± 4.1 μmol·min⁻¹·kg⁻¹) compared with nondiabetic obese, lean T2DM, and obese T2DM groups, in whom they were similarly reduced by ~50% (15.0 ± 2.8, 9.3 ± 1.8, and 14.4 ± 3.3 μmol·min⁻¹·kg⁻¹, respectively; all \( P < 0.01 \) vs. controls; Fig. 1). In the offspring, M (19.9 ± 3.5 μmol·min⁻¹·kg⁻¹) was reduced compared with controls (\( P < 0.01 \)) and was slightly, although not significantly, greater than in obese nondiabetic and both lean and obese T2DM groups. Basal FGU was similar in all five (lean controls, obese nondiabetic, lean T2DM, obese T2DM, offspring) groups (9.5 ± 1.3, 6.9 ± 1.0, 7.4 ± 1.2, 7.3 ± 0.9, and 4.9 ± 0.9 μmol·min⁻¹·kg forear tissue⁻¹, respectively, \( P = NS \)). During the insulin clamp, the rates of FGU mirrored the rates of whole body glucose disposal and were significantly higher in control (35.7 ± 5.7 μmol·min⁻¹·kg forear tissue⁻¹) compared with nondiabetic obese, lean T2DM, and obese T2DM (16.4 ± 3.1, 9.2 ± 1.8, and 8.4 ± 2.4 μmol·min⁻¹·kg forear tissue⁻¹, respectively; all \( P < 0.01 \) vs. lean controls). FGU (20.3 ± 3.6 μmol·min⁻¹·kg forear tissue⁻¹) in the offspring was slightly greater (\( P < 0.01 \)) than in lean and obese T2DM groups and slightly, although not significantly, less than in controls. The basal rate of forearm blood flow was not changed by hyperinsulinemia in any group: lean controls (28.2 ± 2.9 vs. 30.9 ± 4.4 ml·min⁻¹·kg forearm tissue⁻¹), obese nondiabetic subjects (35.1 ± 3.1 vs. 34.7 ± 4.1), lean T2DM (41.7 ± 5.6 vs. 40.2 ± 3.2), obese T2DM (22.2 ± 2.1 vs. 25.4 ± 4.4), and offspring (36.1 ± 3.9 vs. 37.2 ± 3.8 ml·min⁻¹·kg forearm tissue⁻¹).

Muscle glucose fluxes. During the insulin clamp, transmembrane glucose influx increased to 60.6 ± 5.7 μmol·min⁻¹·kg forear tissue⁻¹ in the lean control group (Fig. 2). In the offspring, insulin-stimulated transmembrane glucose influx (43.5 ± 4.6 μmol·min⁻¹·kg forear tissue⁻¹) was significantly reduced compared with the lean controls (\( P < 0.05 \)). In the nondiabetic obese (33.8 ± 4.9), lean T2DM (21.6 ± 4.7), and obese T2DM (29.0 ± 6.0 μmol·min⁻¹·kg forear tissue⁻¹) groups, insulin-stimulated transmembrane glucose influx was significantly reduced compared with lean controls (\( P < 0.01 \)) and offspring (\( P < 0.05 \); Fig. 2). In response to insulin, the transmembane outflux of glucose was similarly reduced in all four groups compared with controls (\( P < 0.05 \)).

In response to hyperinsulinemia, the rate of intracellular glucose phosphorylation in control subjects increased to 34.4 ± 7.5 μmol·min⁻¹·kg forear tissue⁻¹. In obese nondiabetic subjects (16.6 ± 3.7) and in offspring (18.8 ± 3.1 μmol·min⁻¹·kg forear tissue⁻¹), insulin-stimulated intracellular glucose phosphorylation was significantly less than in controls (\( P < 0.01 \)). In lean T2DM (8.1 ± 1.6) and obese T2DM (11.7 ± 4.1 μmol·min⁻¹·kg forear tissue⁻¹), the rates of insulin-stimulated glucose phosphorylation were markedly reduced compared with controls (\( P < 0.01 \)) and compared with offspring (\( P < 0.05 \); Fig. 3).

FGU equals the irreversible metabolism of glucose by cells and is equivalent to the rate of glucose phosphorylation by the forearm tissues. There was a highly significant correlation between insulin-stimulated intracellular glucose phosphorylation and FGU (\( r = 0.62, P < 0.001 \)) as well as with whole body glucose metabolism (\( r = 0.59, P = 0.001 \)) when all subjects were examined collectively.

During the 120- to 135-min time period of the euglycemic insulin clamp, intracellular glucose concentration in the space

![Fig. 1. Insulin-stimulated whole body glucose metabolism and forearm glucose uptake in lean controls (open bars), obese nondiabetic subjects (stippled bars), lean type 2 diabetic (T2DM) subjects (filled bars), obese T2DM subjects (striped bars), and normal glucose-tolerant offspring of 2 T2DM parents (checkered bars). *P < 0.01 vs. lean controls; †P < 0.05 vs obese nondiabetic, lean T2DM, and obese T2DM subjects.](http://ajpendo.physiology.org/ by 10.22033.3 on October 29, 2017)
to which glucose is accessible was 0.72 ± 0.06 mmol/l in the lean control group. Compared with the lean controls, the intracellular glucose concentration after 120–135 min of euglycemic hyperinsulinemia was increased in the obese non-diabetic (1.23 ± 0.28), lean T2DM (2.00 ± 0.34), obese T2DM (1.50 ± 0.29), and offspring (1.31 ± 0.29 mmol/l; all P < 0.05 vs. controls) groups (Fig. 4).

**DISCUSSION**

Considerable evidence, both in vitro and in vivo, indicates that muscle glucose transport and/or glucose phosphorylation are impaired in insulin-resistant conditions, including T2DM and obesity (7, 12, 30, 34, 36, 37, 40, 48, 50, 56, 57, 63, 66–68, 70). However, conflicting reports have appeared concerning 1) the relative magnitude of importance of these defects to the observed muscle insulin resistance in vivo; 2) whether the defects in muscle glucose transport and/or phosphorylation are a characteristic feature of obesity, T2DM, or both; 3) whether the observed defects in muscle glucose transport and/or phosphorylation in obesity and T2DM are additive; and 4) whether the defects in muscle glucose transport and/or phosphorylation in vivo are acquired, i.e., secondarily to glucotoxicity or lipotoxicity or some other metabolic disturbance that accompanies the diabetic state, or are present in normal glucose-tolerant, insulin-resistant individuals at high risk to develop T2DM later in life, i.e., the offspring of two diabetic parents of Mexican American descent (28). In large part, these conflicting reports are the result of different methodologies (triple tracer, MRI with $^{13}$C-$^{31}$P, positron emission topography with $^{18}$F-fluoro-2-deoxyglucose) employed to evaluate the separate contributions of impaired muscle glucose transport and/or phosphorylation and the failure to distinguish between the insulin resistance of obesity vs. that of T2DM. Thus, Cline et al. (12), Williams et al. (66), and Kelley et al. (34) compared obese T2DM subjects (BMI > 30.5 kg/m²) with lean controls (BMI < 25) or lean nondiabetic with obese nondiabetic (68) subjects, making it difficult to distinguish between the separate effects of obesity vs. those of diabetes. In their initial study (34), Kelley et al. reported a decreased inward rate constant for muscle glucose transport in both nondiabetic obese and T2DM subjects, but in a subsequent paper (68) they demonstrated a normal inward rate constant for glucose transport in obesity but a decreased rate constant in
T2DM. In contrast to glucose transport, Kelley et al. (34) described a normal rate constant for muscle glucose phosphorylation in obese individuals (decreased in diabetic subjects) and normal muscle hexokinase II activity in both obese and T2DM subjects, whereas in a subsequent publication [Williams et al. (66)] they demonstrated a decreased rate constant for muscle glucose phosphorylation and reduced muscle hexokinase II activity in both obese and diabetic subjects. Of note, the obese nondiabetic subjects who served as the control group for the obese diabetics (67) were very insulin sensitive compared with the obese nondiabetic subjects previously reported by the same group (67) and by others (19, 31). Last, because the rate constants for inward glucose transport and phosphorylation cannot be equated with flux rates without knowledge of the intracellular glucose concentration, and because of concern over changes in the lumped constant from the basal to the insulin-stimulated state and between insulin-sensitive and insulin-resistant groups (61), the results of Kelley et al. (34) and Williams and colleagues (66–68) are difficult to interpret.

Despite the interpretive problems described above, it is clear that obese and T2DM subjects are characterized by defects in both glucose transport and phosphorylation, although the relative contributions of these abnormalities to muscle insulin resistance are difficult to sort out from the studies of Kelley et al. (34) and Williams and colleagues (66–68). The interpretive problems inherent in the $^{13}$C-$^{31}$P NMR approach (12) have been reviewed by Murphy and Hellerstein (45) and include the following: 1) assumption of a literature value for total creatinine to calculate the ratio of Hellerstein (45) and include the following: 2) overlap of the NMR signal of C-1 of free glucose with the C-1 signal of glucose 6-phosphate; 3) use of a literature-derived value for ATP with which to compare the glucose 6-phosphate; 4) the effect of even small changes in intracellular pH to shift the location of the glucose 6-phosphate peak; 5) use of a correction factor (0.7× the plasma glucose enrichment) from normal rats to estimate the glucose 6-phosphate enrichment; and 6) inability to account for differences in any of the preceding values between normal glucose-tolerant/insulin-sensitive vs. glucose-intolerant/insulin-resistant subjects. Of greater concern is the failure of Cline et al. (12) to employ arterial or arterialized venous blood in the studies. The MRI approach requires knowledge of the extracellular volume of distribution and the glucose concentration in the extracellular space. Since the “euglycemic” insulin clamp was performed using blood drawn from the antecubital vein, one would expect the venous glucose concentration to be ~20–30 mg/dl lower than that in the artery (6, 18). The use of venous rather than arterial or some intermediate glucose concentration would have greatly influenced the results reported by these investigators (12).

In the present study, we have employed a triple-tracer technique that has received extensive validation (57) to quantitate transmembrane inward glucose transport (Fig. 2) and glucose phosphorylation (Fig. 3). Compared with lean controls, both obese nondiabetic and lean T2DM subjects were severely and equally insulin resistant with respect to insulin-stimulated whole body glucose disposal and FGU (both primarily reflect muscle glucose uptake; Fig. 1). As expected, insulin-stimulated whole body glucose disposal and FGU were strongly correlated ($r = 0.77$, $P < 0.0001$). Of note, both insulin-stimulated whole body glucose disposal and FGU in obese T2DM subjects were not significantly different from values in obese nondiabetic and lean T2DM individuals (both $P > 0.50$). Thus both obesity and T2DM are moderately to severely insulin-resistant states, and the combination of the two, i.e., the obese T2DM group, results in little further worsening of the muscle insulin resistance. This observation is consistent with previous publications from our laboratory (19) and others’ (31). The effect of insulin on inward transmembrane glucose transport (Fig. 2) and intracellular glucose phosphorylation (Fig. 3) closely mirrored the results of whole body and forearm glucose uptake. Both obese nondiabetic and lean T2DM subjects manifested severe and quantitatively similar defects in inward glucose transport and glucose phosphorylation. In obese T2DM subjects, the defects in inward glucose transport and glucose phosphorylation were of similar magnitude to those in obese nondiabetic and lean T2DM subjects, indicating that both obesity (an acquired disorder) and T2DM (an inherited disease) are associated with maximal or near-maximal defects in inward muscle glucose transport and phosphorylation. Despite an increase in the intracellular glucose concentration, outward glucose transport flux was similarly reduced in all insulin-resistant groups compared with controls (Fig. 4).

The triple-tracer technique was not performed during the postabsorptive state prior to insulin infusion in the present study. However, in a previous publication (7), we documented that the muscle intracellular glucose concentration in the space accessible to glucose declined from 1.27 to 0.75 mmol/l in lean controls during the euglycemic insulin clamp, whereas it increased from 0.75 to 2.00 mmol/l in lean T2DM subjects. In the present study, the muscle intracellular glucose concentrations in lean controls (0.72 ± 0.05 mmol/l) and obese T2DM subjects (1.50 ± 0.29 mmol/l) in response to insulin were similar to those previously reported by us in lean controls and lean T2DM subjects (7) and are consistent with the concept that the defect in muscle glucose phosphorylation exceeds the defect in glucose transport. In obese nondiabetic and obese T2DM subjects, the intracellular glucose concentration in the space accessible to glucose in response to physiological hyperinsulinemia was similar to that observed in lean T2DM subjects, again suggesting that the defect in intracellular glucose phosphorylation was greater than the defect in glucose transport (Fig. 4). In the offspring, the intracellular glucose concentration in the space to which glucose is accessible during the insulin clamp was slightly, but not significantly, greater than in lean controls, also suggesting that the defect in intracellular glucose phosphorylation exceeded the defect in inward transmembrane glucose transport. The finding that the defect in glucose phosphorylation exceeds that of inward glucose transport is consistent with previous studies from our laboratory that have demonstrated a severe defect in insulin-stimulated muscle hexokinase II activity, mRNA levels, and protein content (37, 40, 48, 63, 64). This observation is also consistent with prior studies demonstrating that, under insulin-stimulated conditions, post-glucose transport defects can be rate limiting for intracellular glucose metabolism (10, 29, 38, 42, 44, 47, 49, 69). However, it should be emphasized that, even if the defect in hexokinase II were corrected, a severe defect in glucose transport most likely would remain (11) and become rate limiting. It also should be pointed out that the observed defect in vivo muscle glucose phosphorylation observed in the present study could result from a defect distal to hexokinase,
i.e., glycogen synthase (15, 26, 32, 48, 59, 62) or glycolysis/glucose oxidation (1, 22, 59), with an increase in glucose 6-phosphate levels (37, 40, 42, 63, 64) and product inhibition of hexokinase. Consistent with this scenario, previous studies have indicated a rate-limiting step for glucose metabolism beyond glucose phosphorylation/glucose 6-phosphate (46, 51), and elevated muscle glucose 6-phosphate concentrations have been demonstrated in insulin-resistant rhesus monkeys during insulin infusion (46). Although this could explain, in part, the decrease in insulin-stimulated hexokinase II activity in T2DM and obesity, it cannot explain the markedly reduced ability of insulin to increase muscle hexokinase mRNA levels and protein content reported by us (48, 64) and others (37, 40, 63).

It might, at first glance, appear disturbing that the muscle intracellular glucose concentration in both insulin-sensitive and insulin-resistant subjects ranged from 0.75 to 2.00 mmol/l. However, we have previously shown that only a fraction (25–40%) of the intracellular water space is accessible to glucose under our experimental conditions (7, 57). This finding is not surprising, since the intracellular space is filled with structures, such as mitochondria, endoplasmic reticulum, Golgi system, and nucleus, which are separated by their own lipophilic membranes from the cytoplasm and, therefore, would not be expected to be receptive to a hydrophilic molecule such as glucose. Both d-mannitol and 3-O-methyl-d-glucose are much more appropriate markers for glucose distribution volumes than water, because their molecular mass is very similar to that of glucose (2) and because 3-O-methyl-d-glucose, being a substrate for all glucose transporters (9), can enter all and only the cellular spaces that are accessible to glucose. Moreover, since d-mannitol and 3-O-methyl-d-glucose are nonmetabolizable molecules, their mean transit times and distribution volumes are model independent and therefore provide quite robust numbers (39).

In muscle, as well as in all cells within the body, insulin-stimulated glucose phosphorylation represents the irreversible step for intracellular glucose metabolism and is equivalent to the rate of muscle glucose uptake measured with the forearm catheterization technique [(A_{glu} - V_{glu}) \times \text{forearm blood flow}]. A strong correlation \((r = 0.77, P < 0.0001)\) between these two variables was observed. Similarly, a strong correlation \((r = 0.72, P < 0.0001)\) between muscle glucose phosphorylation and insulin-stimulated whole body glucose disposal (which primarily reflects muscle) also was observed. These observations serve to further validate the triple-tracer approach to quantitate inward muscle glucose transport and intracellular glucose phosphorylation and to demonstrate that, under insulin-stimulated conditions, the defect in muscle glucose phosphorylation becomes predominant over the defect in inward glucose transport.

The present results are in contrast to those obtained using $^{13}$C and $^{31}$P MRI in T2DM patients (7). The assumptions and interpretive problems inherent in the MRI approach to measuring muscle rates of glucose transport and phosphorylation have been discussed in great detail in previous publications (7, 45, 57) and will not be discussed further in the present paper. Until studies are performed simultaneously employing the triple-tracer and MRI approaches, the assumptions underlying each technique and the interpretation of the MRI and triple-tracer techniques will remain unresolved. Nonetheless, the two techniques unequivocally demonstrate that severe defects in both inward muscle glucose transport and intracellular glucose phosphorylation are present in obese T2DM subjects. The present results extend these observations by demonstrating that 1) severe defects in in vivo muscle glucose transport and phosphorylation are characteristic findings in lean T2DM and obese nondiabetic individuals; 2) the defects in insulin-stimulated muscle glucose transport are maximally/near-maximally expressed in nondiabetic obese and lean T2DM individuals and that the combination of obesity and T2DM (i.e., the obese T2DM group) does not further exacerbate these abnormalities in glucose transport and phosphorylation; and 3) both glucose transport and phosphorylation are impaired in the normal glucose-tolerant, insulin-resistant offspring of two T2DM parents.

The present results also provide some insight into the underlying mechanisms responsible for the defects in inward muscle glucose transport and phosphorylation in states of insulin resistance. The normal glucose-tolerant, insulin-resistant offspring of two T2DM parents are at very high risk to develop T2DM later in life (65). In these individuals, fasting and postprandial plasma glucose and HbA1c levels were virtually identical to those of the lean controls. These observations exclude glucose toxicity (52, 55) as a cause of the defects in insulin-stimulated whole body/forearm glucose uptake, inward muscle glucose transport, and muscle glucose phosphorylation in the offspring. Although the fasting plasma FFA concentration was similar in control and offspring groups, the suppression of plasma FFA by insulin was impaired in the offspring. We did not observe any significant correlations between either the fasting or insulin-inhibited plasma FFA concentrations or any measure of insulin-stimulated glucose metabolism in the offspring. These observations argue against a role for lipotoxicity as a cause of the defects in insulin-stimulated glucose disposal in the offspring. However, because intramyocellular levels of fatty acid and triglyceride metabolites (i.e., fatty acyl-CoA, diacylglycerol, and ceramides) were not measured in the present study, a role for lipotoxicity cannot be excluded completely. Last, it should be noted that all of the offspring were females and seven of eight offspring were of Mexican American ethnicity. Although we have not observed significant differences between males and females or between Mexican Americans and Caucasians with respect to insulin sensitivity or the metabolic pathways involved in the insulin resistance (8, 16, 25), a cautionary note is warranted.

In summary, defects in insulin-stimulated inward muscle glucose transport and glucose phosphorylation are characteristic features of the muscle insulin resistance observed in the normal glucose-tolerant offspring of two T2DM parents, obese nondiabetic individuals, and lean T2DM subjects. The impairment in inward muscle glucose transport and glucose phosphorylation are maximally/near-maximally expressed in obese nondiabetic and lean T2DM subjects, and the combination of obesity and T2DM does not further worsen the severity of the defects in glucose muscle transport and phosphorylation. In obese nondiabetic, lean T2DM, obese T2DM, and offspring subjects the magnitude of the defect in insulin-stimulated intracellular glucose phosphorylation exceeds that of the defect in glucose transport, and the intracellular glucose concentration in the space that is accessible to glucose rises significantly compared with controls during insulin stimulation. The magnitude of impairment in muscle glucose transport and phos-
phorylation in normal glucose-tolerant offspring is intermediate between that of lean healthy controls and lean T2DM subjects and suggests an inherited component to the muscle insulin resistance. Worsening of the defects in inward muscle glucose transport and phosphorylation in the diabetic compared with offspring groups suggests that acquired components, i.e., metabolic abnormalities that accompany the diabetic state, contribute to the further decline in insulin-stimulated glucose disposal. The defects in both inward muscle glucose transport and intracellular glucose phosphorylation are strongly correlated with the defects in insulin-stimulated whole body and forearm glucose uptake.

ACKNOWLEDGMENTS

We thank our nurses at the Clinical Research Center for the highly competent care of the volunteers and Lorrie Albarado and Elva Chapa for their expert assistance in preparation of the manuscript.

C. de F. is supported by the Howard Medical School, Division of Endocrinology, Diabetes and Hypertension, Brigham and Women’s Hospital, 221 Longwood Ave., RFB-381, Boston, MA 02115.

Current address of M. Pendergrass: Department of Information Engineering, University of Padova, Via Gradenigo 6/b, 35131 Padua, Italy 37126.

Current address of G. Nucci: GlaxoSmithKline, Verona, Italy.

Current address of L. Mandarino: Dept. of Kinesiology, Arizona State University PO Box 87071, Tempe, AZ 85287.

GRANTS

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-24092, a Veterans Administration Merit Grant, General Clinical Research Center Grant MO1-RR-01346, and funds from the Veterans Administration Medical Research Service.

REFERENCES


