Skeletal muscle glycolytic and oxidative enzyme capacities are determinants of insulin sensitivity and muscle composition in obese women

JEAN-AIMÉ SIMONEAU, SHERI R. COLBERG, F. LELAND THAETE, AND DAVID E. KELLEY[1]

Department of Veterans Affairs Medical Center and Departments of Medicine and Radiology, University of Pittsburgh School of Medicine, Pittsburgh Pennsylvania, 15261, USA and Physical Activity Sciences Laboratory, Laval University, Sainte-Foy, Quebec, Canada

ABSTRACT **Regional fat distribution is an important determinant of insulin resistance in obesity. In the current study, the relationship between skeletal muscle insulin sensitivity, mid-thigh muscle composition, and the metabolic profile of muscle was investigated. Muscle composition was assessed by computed tomography of the mid-thigh, and by activities of marker enzymes of aerobic-oxidative and glycolytic pathways and muscle fiber typing using biopsies of the vastus lateralis muscle. Muscle with reduced Hounsfield attenuation on computed tomography scans was increased in proportion to obesity, and was strongly related to insulin resistance, reduced muscle oxidative capacity, and increased anaerobic and glycolytic capacities by muscle. These findings suggest that as part of its expression of insulin resistance, skeletal muscle of obese individuals is also poorly equipped for substrate oxidation and manifests increased storage of fat.— Simoneau, J.-A., Colberg, S. R., Thaete, F. L., Kelley, D. E. Skeletal muscle glycolytic and oxidative enzyme capacities are determinants of insulin sensitivity and muscle composition in obese women. *FASEB J.* 9, 273–278 (1995)**

Key Words: computed tomography • limb balance methods • muscle metabolism

OBESITY CAUSES INSULIN RESISTANCE (1). Upper body fat distribution is regarded as a clearer determinant of skeletal muscle insulin sensitivity than lower body predominance of fat distribution (2). Fat deposition within skeletal muscle may be another characteristic related to insulin resistance of obesity (3, 4). Accrual of fat within muscle could result from increased uptake of fatty acids or a decreased capacity for lipid oxidation. Previously we reported that women with visceral obesity have lower fasting rates of skeletal muscle free fatty acid uptake and concomitant decreases in activities of muscle citrate synthase, a marker enzyme of the Krebs cycle, and in muscle carnitine palmitoyl transferase (5). This biochemical profile could favor increased fat deposition in skeletal muscle and thereby contribute to insulin resistance.

Computed tomography (CT)[2] has proven to be a useful method for body composition analysis because it can directly assess the distribution of adipose tissue (6). CT may have some potential as well to assess fat deposition within muscle. Skeletal muscle of obese men has lower computed tomography attenuation than muscle of normal-weight men (7). Since fat has a strongly negative attenuation value on CT imaging, the lower attenuation found in muscle is a useful indicator of fat deposition within muscle (8).

The current study was undertaken to determine the effect of obesity in otherwise healthy women upon skeletal muscle insulin sensitivity, upon the aerobic-oxidative potential of muscle, and on skeletal muscle composition assessed by CT imaging. The findings indicate that insulin resistance of obesity is strongly linked with a reduced oxidative capacity of skeletal muscle and with accretion of muscle displaying reduced CT attenuation.

METHODS

Subjects

Seventeen healthy, premenopausal women participated in the study and their clinical characteristics are summarized in **Table 1.** The group included obese and lean women, all of whom had normal glucose tolerance and were without hyperlipidemia, hypertension, or irregular menstrual cycles. None of these volunteers exercised regularly (less than twice weekly), nor were any on medication, including oral contraceptives. The protocol was approved by the University of Pittsburgh Institutional Review Board, and informed consent was obtained from each subject.

Thigh and body composition

A single 1 cm thickness cross-sectional scan of the mid-thigh was obtained by CT (9800 scanner, General Electric, Milwaukee, Wis.) at 120 kV (peak), 100 mA and a scanning time of 3 s, to measure thigh lean and fat tissue composition. Subjects were scanned while supine. The field of view was 32 cm and the matrix was 512 × 512 pixels. Regions of interest for measurement of tissue areas were defined by a range of attenuation values. Thigh adipose tissue was electronically measured by using commercially available CT software (GE Medical Systems, Milwaukee, Wis.), with the region of interest window set to attenuation values of −200 to −1 Hounsfield units (HU). The cross-sectional area for bone was measured within a region of interest greater than 200 HU. The normal range for muscle attenuation in healthy normal-weight adults is 65 HU, with a standard deviation of 12 HU (9). Taking two standard deviations into account, skeletal muscle with normal attenuation was electronically measured within a region of interest from 35 to 100 HU. Muscle below 35 HU was measured electronically within a region of interest of 0 to 34 HU. These methods of thigh composition analyses using CT have been previously described (7). Visceral fat content was also measured by CT, using an accepted method (6), and body mass index was determined from height and weight.

[1]To whom correspondence should be addressed, at: E-1140 Bio-Medical Science Tower, University of Pittsburgh, Pittsburgh, PA, 15261.

[2]Abbreviations: CT, computed tomography; HU, Hounsfield unit; CK, creatine kinase; HK, hexokinase; PFK, phosphofructo kinase; GAPDH, glyceraldehyde phosphate dehydrogenase; CS, citrate synthase; COX, cytochrome *c* oxidase; HADH, 3-hydroxyacyl CoA dehydrogenase; PHOS, glycogen phosphorylase; mATPase; myofibrillar adenosine triphosphatase.

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TABLE 1. *Clinical characteristics and thigh composition of the subjects*

Variables	Mean ± SEM	Min	Max
Age (yrs)	31 ± 1	22	38
BMI (kg/m^2)	28.8 ± 1.5	19.3	38.8
Visceral fat content (cm^2)	80 ± 11	19	184
Mid-thigh muscle with normal attenuation (cm^2)	97 ± 5	60	137
Mid-thigh muscle with low attenuation (cm^2)	24 ± 2	11	44
Mid-thigh adipose area (cm^2)	209 ± 19	54	339

Determination of insulin sensitivity

Skeletal muscle insulin sensitivity was determined using the euglycemic clamp method (10), in conjunction with leg balance methods (11). Briefly, subjects were admitted to the University of Pittsburgh General Clinical Research Center at approximately 6:00 AM, following an overnight fast. Catheters were placed in a radial artery and a femoral vein for leg balance sampling, and a forearm vein was cannulated for insulin and glucose infusions. A percutaneous muscle biopsy of the vastus lateralis was obtained as described previously (11). A portion of the muscle sample was quickly placed in liquid nitrogen, then kept frozen at −70°C, for later analysis of enzyme activity. The remaining portion, used for muscle fiber type distribution and capillary density, was frozen in isopentane cooled to its freezing point (−160°C) by liquid nitrogen. For another project, a continuous infusion of isotopically labeled oleate was given, and these data have been separately reported (5). A two-step (low- and mid-level) insulin infusion was conducted for 5 h, with 3 h at 10 mU/m^2-min and two subsequent hours at 40 mU/m^2-min, while maintaining euglycemia. For the current study, measurement of insulin sensitivity was conducted during steady-state conditions of the final 30 min of the mid-level insulin infusion. These determinations included leg glucose uptake, oxidation, and storage, as described previously (11). Three arterial and femoral vein samples for measurement of glucose, lactate, and alanine were obtained, separated by 15-min intervals. At 5-min intervals, arterial and femoral vein blood samples were obtained for measurement of blood O_2 and plasma CO_2 content. These samples were used to determine gas exchange across the leg for indirect calorimetry estimation of leg glucose oxidation. Leg blood flow was determined in triplicate using mercury strain gauge plethysmography (Hokanson, Bellevue, WA).

Analysis

Plasma glucose was measured using a glucose oxidase system (YSI 23A, Yellow Springs, Ohio). Lactate and alanine were determined by enzymatic assays in whole blood which had been placed in chilled perchloric acid at the time of sample collection as previously described (11). Plasma insulin was measured by radioimmunoassay. Arterial and femoral blood O_2 content was determined with a co-oximeter (IL282 Co-Oximeter System; Allied Instrument Laboratory, Lexington, Mass.). Plasma CO_2 content was measured (System 1304 pH/Blood Gas Analyzer; Allied Instrument Laboratory). Blood gas analysis was performed promptly following sample collection. Blood CO_2 content was calculated from plasma CO_2 using a regression equation (12), employing values for hemoglobin, hemoglobin saturation, and pH from each sample.

Analysis of muscle enzyme activity and fiber type

Muscle samples were obtained at biopsy from 15 of the 17 subjects. Muscle samples were immediately frozen in liquid nitrogen and stored at −70°C until shipment in dry ice to the Physical Activity Sciences Laboratory at Laval University, for analysis by one of the investigators (J. A. S.), who was blinded as to the clinical status of each subject. One of the shipments, containing samples from three volunteers, was destroyed due to thawing during shipment so that muscle from 12 subjects was available for analysis. The activity levels of creatine kinase (CK; EC 2.7.3.2), hexokinase (HK; EC 2.7.1.1), phosphofructokinase (PFK; EC 2.7.1.11), glyceraldehydephosphate dehydrogenase (GAPDH; EC 1.2.1.12) citrate synthase (CS; EC 4.1.3.7), cytochrome *c* oxidase (COX; EC 1.9.3.1), and 3-hydroxyacyl CoA dehydrogenase (HADH; EC 1.1.1.35) were spectrophotometrically determined at 30°C according to previously established techniques from this laboratory (13). Muscle glycogen phosphorylase (PHOS; EC 2.4.1.1) was determined at 25°C as described by Bass et al. (14). Values of enzyme activities were expressed in U/g wet weight muscle. Muscle sections (10 μm thick) were stained for myofibrillar adenosine triphosphatase (mATPase) according to an established technique (15) in order to determine the proportion of the different fiber types (I, IIa, and IIb).

Calculations

Leg balance of glucose, lactate, and alanine was calculated as the product of arterio-venous differences and leg blood flow. Indirect calorimetry equations were adapted for estimation of leg glucose oxidation, as previously described (11). Leg glucose storage was calculated as the difference between leg glucose uptake and the sum of leg glucose oxidation and net balance of lactate and alanine, with negative values representing net glycogenolysis.

Statistics

Data are expressed as mean ± SEM. Linear regression and step-wise multiple regression were used to determine the significance of potential associations between CT measurements, leg glucose metabolism, and muscle enzyme activities (RS1, BBN Software Inc., Cambridge, Mass.).

RESULTS

Thigh composition

Mean values and ranges for the cross-sectional area of mid-thigh adipose tissue, muscle of normal attenuation, and muscle of low attenuation, as determined by CT, are shown in Table 1, together with age, body mass index, and visceral fat content. A mid-thigh cross-sectional CT scan from a normal-weight woman and one from an obese woman are shown in **Fig. 1,** with computer highlighting of muscle for areas of normal and low attenuation. The area of mid-thigh adipose tissue was strongly correlated with body mass index ($r = 0.85$, $P < 0.01$). Thigh muscle area (sum of normal and low attenuation) was also positively correlated with obesity ($r = 0.52$, $P = 0.03$). The area of mid-thigh muscle with normal attenuation was not significantly correlated with either body mass index ($r = 0.28$, $P = 0.25$), or mid-thigh adipose area ($r = 0.04$). The area of mid-thigh muscle with reduced attenuation was, however, significantly correlated with body mass index ($r = 0.47$, $P = 0.05$) and mid-thigh adipose area ($r = 0.55$, $P = 0.02$), but not with mid-thigh area of muscle with normal attenuation ($r = -0.32$, $P = 0.21$). The relationship between mid-thigh area of adipose tissue and mid-thigh areas of muscle with normal and reduced attenuation is shown in **Fig. 2.**

Insulin sensitivity

The mean value of glucose uptake across the leg during the final 30 min of euglycemic hyperinsulinemia (504 ± 28 pMol) was 2.29 ± 0.34 μmol/min-100 ml leg tissue, with a mean rate for leg glucose oxidation of 0.64 ± 0.10 μmol/min-100 ml leg tissue, and of −0.12 ± 10 μmol/min-100 ml leg tissue for the sum of lactate and alanine net balance across the leg, expressed in glucose equivalents. Accordingly, the mean calculated rate of insulin stimulated leg glucose storage was 1.54 ± 0.32 μmol/min-100 ml leg tissue. Neither leg glucose oxidation (or leg respiratory quo-

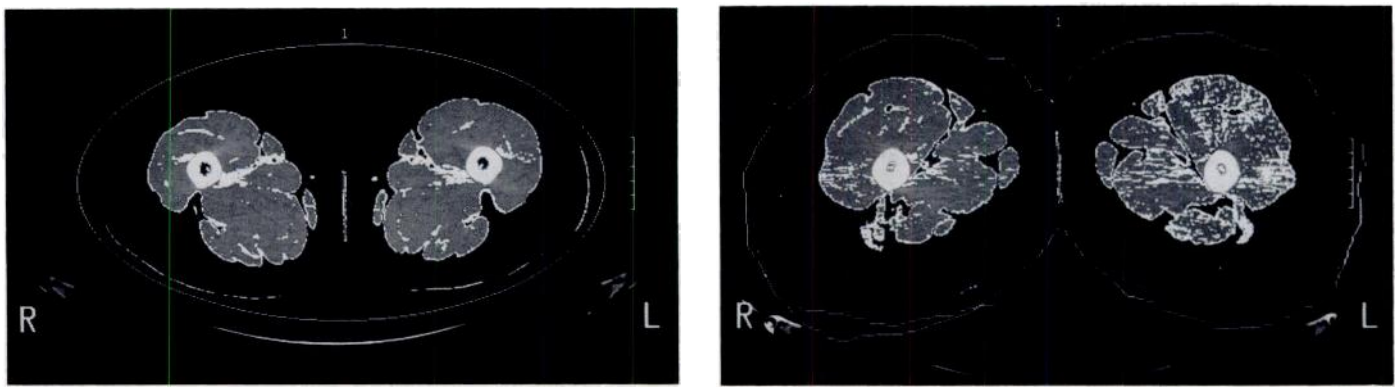

Figure 1. Cross-sectional computed tomography scans of mid-thigh from two women are shown with low attenuation muscle highlighted using a region of interest set to attenuation values of 0 to 35 Hounsfield units. The scan on the left is from a lean woman (BMI = 19.2 kg/m^2) and the scan on the right is from an obese woman (BMI = 33.4 kg/m^2).

tient, 0.90 ± 0.03), nor net balance of lactate and alanine were correlated with any of the components of mid-thigh composition. There was, however, a strong negative correlation between rates of leg glucose storage and the area of muscle with low attenuation ($r = -0.63$, $P < 0.01$), as shown in **Fig. 3.** Leg glucose storage was also negatively correlated with area of mid-thigh adipose tissue ($r = -0.57$, $P = 0.02$), and with visceral adiposity ($r = -0.62$, $P < 0.01$), although not with the area of muscle with normal attenuation ($r = 0.19$, ns). In a step-wise multiple regression model using each component of mid-thigh composition and visceral fat content, with leg glucose storage as the dependent variable, muscle with low attenuation had the strongest predictive value for insulin resistance, with visceral fat content adding independent significance. Low attenuation muscle and visceral fat content accounted for 57 percent of the variance in leg glucose storage ($F = 9.19$, $P < 0.01$); thigh adiposity did not add additional significance.

Skeletal muscle metabolic profile

The mean values and range of the six marker enzymes of skeletal muscle aerobic-oxidative and glycolytic potential are shown in **Table 2.** Also shown are the simple correlations of

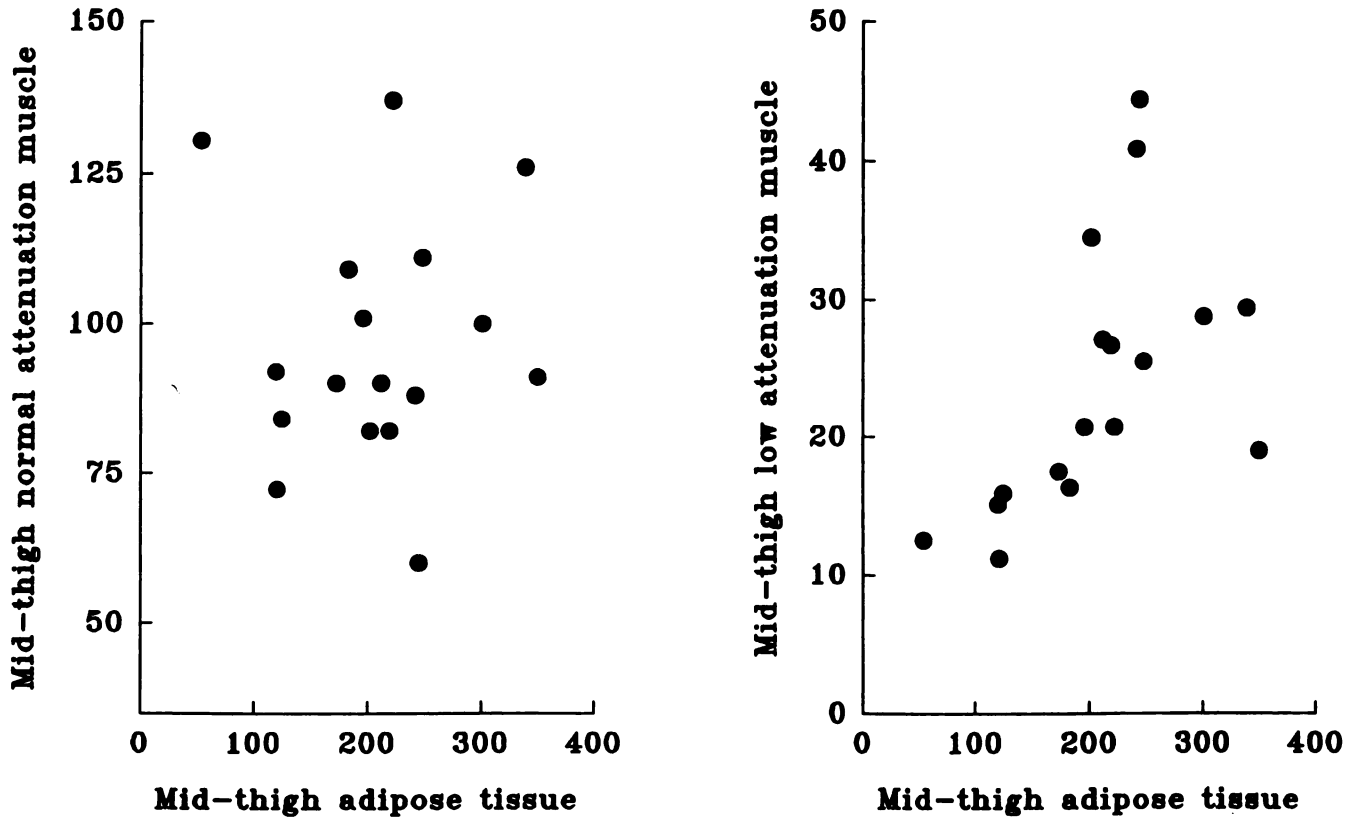

Figure 2. The left panel shows mid-thigh cross-sectional area (cm^2) of adipose tissue plotted against mid-thigh cross-sectional area of skeletal muscle within the normal range (mean ± 2 SD) for attenuation values on computed tomography ($r = 0.04$, ns). The right panel shows mid-thigh adipose area plotted against mid-thigh cross-sectional area of skeletal muscle with attenuation values below the normal range ($r = 0.54$, $P < 0.01$).

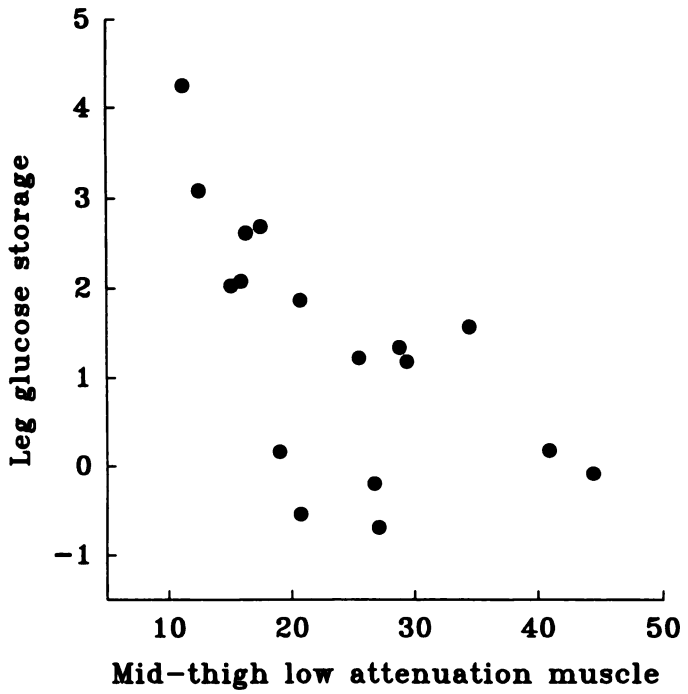

Figure 3. The values for insulin-stimulated glucose storage (μmol/min-100 ml leg tissue) across the leg are plotted against the cross-sectional area (cm^2) of mid-thigh skeletal muscle with computed tomography attenuation below the normal range ($r = -0.63$, $P < 0.01$).

each enzyme activity with skeletal muscle of low attenuation and with rates of insulin-stimulated leg glucose storage, as the marker pathway of insulin sensitivity. Insulin sensitivity was positively associated with a high oxidative potential of muscle as reflected by its significant correlations with CS activity. Conversely, insulin sensitivity was negatively associated with a heightened capacity to anerobically generate ATP from creatine phosphate, as reflected by its relationship with muscle CK activity. Although simple correlations between insulin sensitivity and marker enzymes of muscle glycolytic capacity did not reach significance, the ratio of PFK to CS activity did have a strong negative correlation with leg glucose storage ($r = -0.79$, $P < 0.01$). This suggests that an increased ratio of glycolytic relative to oxidative capacity of muscle is associated with insulin resistance. In a multiple regression model involving muscle enzymes, the activity of CS and CK had independent significance (each $P < 0.01$), together accounting for 82 percent of the variance in insulin-stimulated leg glucose storage ($F = 20.8$, $P < 0.01$).

There were significant simple correlations between muscle enzyme activity and the area of mid-thigh muscle with low attenuation, as also shown in Table 2. In women with increased muscle of low attenuation, CS activity was lower and activities of CK, PFK, and GAPDH were higher. This suggests that accumulation of this type of tissue is linked to decreased oxidative capacity and increased glycolytic and anerobic capacities of skeletal muscle. This assessment is supported by multiple regression analysis. By placing muscle with low attenuation as the dependent variable, CS and PFK activities were found to have independent significance (each $P < 0.01$), together accounting for 71 percent of the variability in the area of muscle with low attenuation ($F = 10.9$, $P < 0.01$).

The relative proportions of Type I, IIa, and IIb muscle fiber types was 43 ± 7, 38 ± 4 and 19 ± 5 percent, respectively. There were no significant correlations between muscle fiber type and either area of low attenuation muscle or insulin sensitivity.

DISCUSSION

Insulin resistance of obesity is characterized by impaired skeletal muscle glucose uptake and, more specifically, by reduced glycogen storage (2). These metabolic defects in obesity are strongly associated with increased visceral fat content. Another aspect of regional fat deposition of potential relevance to insulin resistance is fat content within muscle (3, 4). Utilizing computed tomography, we have previously found that obese men have a specific increase of thigh muscle possessing lower than normal values for computed tomography attenuation (7). Because adipose tissue has a negative attenuation value on imaging with CT, this suggests increased fat deposition within muscle as reported in patients with muscular dystrophy (8). The current study was undertaken to determine whether skeletal muscle of obese women is characterized by lower attenuation values, and if so, whether such a characteristic is linked to both the presence of skeletal muscle insulin resistance and a metabolic profile of muscle predisposing toward fat storage within this tissue.

TABLE 2. *Enzyme activities (U/g wet weight) of skeletal muscle and their relationships with insulin sensitivity and low attenuation skeletal muscle tissue*

				Simple correlation	
Enzyme activities	Mean ± SEM	Min	Max	Insulin sensitivity	Low attenuation muscle area
CK	415 ± 30	195	532	−0.64*	0.56*
HK	2.1 ± 0.1	1.4	2.7	0.74*	−0.42
PHOS	18.7 ± 1.3	11.6	25.3	0.10	0.16
PFK	58 ± 4	36	75	−0.24	0.41
GAPDH	437 ± 32	331	589	−0.29	0.52*
CS	9.9 ± 0.7	7.1	14.2	0.70*	−0.54*
COX	6.3 ± 0.4	3.6	8.0	0.41	−0.37
HADH	15.7 ± 0.7	12.6	19.2	0.07	−0.08
PFK/CS	6.0 ± 0.5	3.7	9.6	−0.79*	0.83*

*$P < 0.05$.

Among healthy, pre-menopausal women obesity was strongly associated with increased areas of muscle with low attenuation. This is consistent with the previous finding in obese men. A strong relationship between muscle with low attenuation and insulin resistance was also observed. Whether reduced attenuation of skeletal muscle is indeed a manifestation of fat deposition remains to be firmly established. It is also possible that reduced glycogen content could contribute to reduced attenuation. CT-guided needle biopsy of precise areas of muscle for determination of lipid and glycogen content would resolve this issue. However, the current findings suggest that the pattern of attenuation values of skeletal muscle determined by CT is a new and potentially useful body composition determination relevant to skeletal muscle insulin resistance. This CT measurement provides information about lean tissue composition that is not feasible to obtain with compartmental models of fat and fat-free body composition, because these methods commonly assume uniform density of lean tissue.

In the current study, a strong relationship between visceral fat content and skeletal muscle insulin resistance was found, consistent with previous studies (2, 16). Step-wise regression analysis was used to compare the effects of muscle with low attenuation and visceral fat content upon insulin sensitivity. This analysis revealed that muscle with low attenuation was as strong a predictor of insulin resistance as was visceral obesity. The current findings extend the concept that regional fat deposition has major effects upon insulin sensitivity by indicating that muscle with low attenuation values on CT imaging add to the adverse impact of visceral fat on insulin sensitivity.

An association among insulin resistance, mid-thigh muscle with low attenuation, and enzyme activities that demarcate regulatory steps of aerobic-oxidative and glycolytic metabolism was found. The amount of muscle with low attenuation was increased in women with lower oxidative capacity, higher anaerobic capacity, and an increased ratio of glycolytic to oxidative capacity in skeletal muscle. This same enzymatic profile of muscle was also associated with insulin resistance in these women. The known function of these enzymes offers some insight into potential mechanisms for these associations. Citrate synthase is involved in the oxidative generation of cellular energy and is found in proportion to muscle mitochondrial content (17), whereas cytosolic CK catalyzes a key step in anaerobic regeneration of ATP. PFK can subserve either oxidative or non-oxidative utilization of glucose for energy production, and is a rate-limiting enzyme for glycolysis. The reduced oxidative capacity (CS activity) found in association with accretion of muscle possessing low attenuation values would suggest that a relative impairment in capacity for substrate oxidation by muscle could favor fat storage within muscle in conjunction with insulin resistance. These data also suggest that muscle manifesting insulin resistance displays increased capacity for anaerobic resynthesis of ATP, as exemplified by increased cytosolic CK activity, and this is also suggested by increased glycolytic capacity (PFK activity). These are novel findings with respect to the pathogenesis of skeletal muscle insulin resistance in obesity.

Fiber type distribution per se was not predictive of muscle composition as measured by CT attenuation, in contrast to the strong correlations found with the biochemical profile established from enzyme activities of glycolytic, anaerobic, and aerobic-oxidative pathways. From our perspective, this is not an unexpected finding (18). When type I, IIA, and IIB fibers are established on the basis of the histochemical myosin ATPase reaction, it provides only a qualitative assessment of the myosin isoform content of the muscle fibers (19). In human muscle, fiber type information is largely independent of aerobic-oxidative and glycolytic enzyme indicators (20–22). Accordingly, in vitro measurements of maximal enzyme activities provide a more useful approach for studying metabolic characteristics of human skeletal muscle than fiber type distribution. The activities of enzymes catalyzing non-equilibrium reactions provide a semi-quantitative index of both maximal metabolic flux and fuel utilization, while the activities of enzymes catalyzing reactions close to equilibrium provide only qualitative information about the importance of particular metabolic pathways and the principal fuels supporting activity (23). Except for CK, GAPDH, and HADH, which are enzymes catalyzing reactions close to equilibrium, the other marker enzymes used in our study are enzymes catalyzing non-equilibrium reactions and are considered as regulators of their respective metabolic pathways.

It is significant in this context of altered enzyme activities and muscle composition that the women with insulin resistance in the current study have also been found to have reduced skeletal muscle fat oxidation during fasting conditions (5). These results are also consistent with additional studies that have suggested that a relative impairment in lipid oxidation is a risk factor for weight gain (24), and that this impairment of lipid utilization may reside in skeletal muscle (25). Whereas visceral obesity is often considered to cause skeletal muscle insulin resistance, an alternative perspective can be suggested from the current findings. An impaired capacity of skeletal muscle for fat oxidation may predispose to intra-muscular fat deposition, and likely elsewhere, including visceral adipocytes.

In summary, using computed tomography to partition mid-thigh skeletal muscle into components of normal and low attenuation, it was found that obese women have increased areas of muscle with low attenuation. There was a strong negative correlation between muscle with low attenuation and insulin stimulation of muscle glucose storage. This indicates that muscle with low attenuation is an additional body composition marker of insulin resistance. It was also found that the amount of muscle with low attenuation was greater in women whose muscle had low aerobic-oxidative capacities and increased anaerobic and glycolytic capacities. This constellation of findings from measurements of tissue composition, muscle enzyme activity, and in vivo insulin sensitivity provides a new direction for understanding the pathogenesis of skeletal muscle insulin resistance in obese individuals. FJ

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