

# Interrelationships between muscle morphology, insulin action, and adiposity

A. D. KRIKETOS, D. A. PAN, S. LILLIOJA, G. J. COONEY, L. A. BAUR, M. R. MILNER, J. R. SUTTON†, A. B. JENKINS, C. BOGARDUS, AND L. H. STORLIEN  
*Department of Endocrinology, Royal Prince Alfred Hospital, Sydney, New South Wales 2050; Departments of Medicine and Biomedical Science, University of Sydney, Sydney, New South Wales 2006; Department of Biomedical Science, University of Wollongong, Wollongong, New South Wales 2522, Australia; and Clinical Diabetes and Nutrition Section, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Phoenix, Arizona 85016*

**Kriketos, A. D., D. A. Pan, S. Lillioja, G. J. Cooney, L. A. Baur, M. R. Milner, J. R. Sutton, A. B. Jenkins, C. Bogardus, and L. H. Storlien.** Interrelationships between muscle morphology, insulin action, and adiposity. *Am. J. Physiol.* 270 (*Regulatory Integrative Comp. Physiol.* 39): R1332–R1339, 1996.—There is evidence that insulin resistance and obesity are associated with relative increases in the proportion of glycolytic type IIb muscle fibers and decreases in the proportion of oxidative type I fibers. Furthermore, insulin resistance and obesity are associated with the fatty acid (FA) profile of structural membrane lipids. The present study was undertaken to define interrelationships between muscle fiber type and oxidative capacity, muscle membrane FA composition, and insulin action and obesity. Muscle morphology, insulin action, and body fat content were measured in 48 male nondiabetic Pima Indians. Percent body fat (pFAT, determined by hydrodensitometry) correlated negatively with percentage of type I fibers ( $r = -0.44$ ,  $P = 0.002$ ) and positively with percentage of type IIb fibers ( $r = 0.40$ ,  $P = 0.005$ ). Consistent with this finding, pFAT was also significantly related to oxidative capacity of muscle, as assessed by NADH staining ( $r = -0.47$ ,  $P = 0.0007$ ) and citrate synthase (CS) activity ( $r = -0.43$ ,  $P = 0.008$ ). Insulin action was correlated with oxidative capacity (CS;  $r = 0.41$ ,  $P = 0.01$ ) and weakly correlated with percentage of type IIb fibers ( $r = -0.29$ ,  $P = 0.05$ ). In addition, relationships were shown between muscle fiber type and FA composition (e.g., percentage of type I fibers related to n-3 FA;  $r = 0.37$ ,  $P = 0.01$ ). Thus leanness and insulin sensitivity are associated with increased oxidative capacity and unsaturation of membranes in skeletal muscle. Present studies support the hypothesis that muscle oxidative capacity and fiber type may play a genetically determined or an environmentally modified role in development of obesity and insulin resistance.

skeletal muscle; fiber type; humans; oxidative capacity; fatty acid composition; NIH image; obesity; capillary density

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IMPAIRED INSULIN ACTION (insulin resistance) is central to a cluster of prevalent diseases, including non-insulin-dependent diabetes mellitus, obesity, hypertension, dyslipidemias, and cardiovascular disease (4, 29). The underlying mechanisms of insulin resistance remain poorly understood. Skeletal muscle is the primary site of insulin-stimulated glucose disposal at euglycemia (10), and innate factors have therefore been sought in an attempt to identify the causes of insulin resistance.

As such, morphological characteristics of muscle, including fiber type, capillary density, and fatty acid (FA) composition of membrane, have been linked to alterations in insulin action and adiposity (21, 25). Alterations in skeletal muscle glucose storage and the mechanisms controlling this storage have also been examined. How adiposity affects muscle insulin resistance remains unknown.

A number of studies have shown an increased percentage of type IIb fibers in vastus lateralis muscle biopsies to be associated with in vivo insulin resistance and increased adiposity (16, 21–22, 36). Furthermore, Hickey et al. (15) recently showed increased body mass index (BMI) to be inversely correlated to percentage of type I fibers and to in vitro glucose transport rate in rectus abdominis muscle. Likewise, an increased percentage of type I fibers and increased capillary density in vastus lateralis muscle have been shown to be associated with good insulin action and leanness in a mixed group of Pima Indian and Caucasian subjects (21). This work in humans is in keeping with studies in obesity-prone rats, which have shown a higher percentage of type IIb fibers in gastrocnemius hindlimb muscle than obesity-resistant rats (1). This area is not without controversy, however, with negative results also having been reported (24, 30, 31).

Fiber type is not a direct measure of muscle oxidative capacity because of substantial overlap of oxidative capacity between different fiber types. Oxidative capacity can be specifically measured by histochemical and enzymatic means. For example, an index of mitochondrial density can be obtained by the intensity of NADH staining, and the capacity for metabolic fuel utilization can be assessed by measuring the activity of enzymes such as hexokinase (HK) and citrate synthase (CS). In humans, a few very recent studies have related adiposity and/or insulin action to muscle enzyme activity (7, 16, 32). In general, increased muscle oxidative capacity has been directly related to insulin sensitivity and leanness. Conversely, a relative increase in adiposity was shown to be related to a relative decrease in malate dehydrogenase (MDH) activity (31), an oxidative reducing enzyme.

Another characteristic of muscle morphology associated with insulin action and adiposity is the FA profile of structural membrane lipids (5, 25, 34, 35). Overall, these studies have shown that the more unsaturated

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† Deceased 7 February 1996.

the FA profile of muscle membrane lipids, the better the insulin action and the leaner the individual. Also, close relationships were found between leanness/insulin sensitivity and activities of enzymes controlling desaturation and elongation (5, 25). Furthermore, work in rats has shown muscle fiber type and FA composition of membrane to be interlinked (18). In this study, increased amounts of polyunsaturated FAs (PUFAs) were found in the more oxidative insulin-sensitive red quadriceps and soleus muscles, whereas reduced levels of PUFAs were found in the primarily glycolytic white quadriceps muscles. Investigations in humans of relationships between the membrane structural FA composition and fiber type have not been carried out.

The present study was undertaken to investigate the interrelationships between skeletal muscle fiber type, oxidative capacity, muscle membrane FA composition, insulin action, and obesity in humans. In a group of male nondiabetic Pima Indians, data were collected on muscle fiber type, oxidative capacity, capillary density, and muscle membrane FA composition. Each of these morphological characteristics was examined in relation to measures of insulin action and body fatness.

## METHODS

### Subjects

Individuals in this study were 48 male volunteer Pima Indians of the Gila River Indian Community who were participating in a longitudinal study of the development of non-insulin-dependent diabetes mellitus. The metabolic studies were performed in the clinical research unit of the National Institutes of Health (NIH) in Phoenix, AZ. Subjects were  $\leq 44$  yr of age and in good health, as assessed by medical history and physical examination. The current analysis considered only those with a mean fasting plasma glucose concentration of  $< 140$  mg/dl ( $< 7.8$  mmol/l). Subject characteristics are listed in Table 1. All subjects gave informed consent, and the studies were approved by the ethics committees of the NIH, the Indian Health Service, and the Gila River Indian Community.

Upon admission to the clinical research unit, all subjects received a weight maintenance diet consisting of 50% carbohydrate, 30% fat, and 20% protein. A 75-g oral glucose tolerance test was performed after  $\geq 2$  days on the diet. At this baseline test, none of the subjects were diabetic. Percent body fat (pFAT) was determined by hydrodensitometry, with simultaneous determination of lung residual volume (20).

### Euglycemic Clamp

In vivo insulin-mediated glucose disposal rate was measured by a two-step euglycemic-hyperinsulinemic clamp according to a modification of the method of DeFronzo et al. (10), which has been described previously (20). The clamp was performed by primed continuous low- and high-dose insulin infusions (290 and 2,900 pmol $\cdot$ min $^{-1}\cdot$ m $^{-2}$ , respectively), which were continued for 100 min while the plasma glucose was maintained at  $\sim 98$  mg/dl. The plasma insulin concentrations were determined at 0, 75, 95, 175, and 195 min. The plasma glucose concentrations were determined before the start of insulin infusion and every 5 min to the end of the clamp. The in vivo insulin action was determined from 60 to 100 min and from 160 to 200 min. During these time periods, plasma glucose uptake approached steady state, but it is unlikely that a precise steady state is achieved. Nevertheless,

Table 1. *Subject characteristics*

Variable	Mean $\pm$ SE	Minimum	Maximum
Age, yr	27.3 $\pm$ 0.8	18.1	43.8
Height, cm	171.4 $\pm$ 0.8	150.5	182.5
Weight, kg	94.0 $\pm$ 2.6	56.1	132.3
BMI, wt/ht $^2$	32 $\pm$ 1	19	52
%Body fat	28 $\pm$ 1	14	44
Waist, cm	40.9 $\pm$ 1.0	27.0	55.0
Thigh, cm	25 $\pm$ 0.5	19.0	30.8
Waist-to-thigh ratio	1.64 $\pm$ 0.02	1.32	2.18
Fasting plasma glucose, mg/dl	88 $\pm$ 2	70	107
Fasting plasma insulin, log $_{10}$ mU/l	1.50 $\pm$ 0.03	1.14	2.05
Log $_{10}$ total M, mg $\cdot$ min $^{-1}\cdot$ kg FFM $^{-1}$ + 17.7	0.49 $\pm$ 0.03	0.20	1.06
Log $_{10}$ total MZ, mg $\cdot$ min $^{-1}\cdot$ kg FFM $^{-1}$ + 17.7	0.95 $\pm$ 0.02	0.59	1.21
Mean fiber area, $\mu$ m $^2$	6,234 $\pm$ 195	3,820	10,110
Capillaries/fiber	2.57 $\pm$ 0.1	1.5	3.8
Capillary density, capillaries/mm $^2$	417 $\pm$ 9	267	570
%Type I fibers	39.4 $\pm$ 1.4	18.2	60.2
%Type IIa fibers	29.6 $\pm$ 1.7	8.8	55.6
%Intense NADH fibers	40.7 $\pm$ 1.3	22.0	62.4
%Type IIb fibers	30.4 $\pm$ 2.0	6.2	65.0
CS activity, units/g	12.7 $\pm$ 0.6	4.5	21.2
HK activity, units/g	1.25 $\pm$ 0.1	0.58	2.86
Elongase activity, 18:0/16:0	0.88 $\pm$ 0.2	0.67	1.11
Total n-3 fatty acids	1.95 $\pm$ 0.8	1.09	3.03
$\Delta^5$ -Desaturase activity, 20:4(n-6)/20:3(n-6)	8.52 $\pm$ 0.2	6.05	11.91
$\Delta^9$ -Desaturase activity, 18:1(n-9)/18:0	0.61 $\pm$ 0.2	0.36	0.90

BMI, body mass index; M and MZ, low- and high-dose in vivo insulin-mediated glucose disposal rate; CS, citrate synthase; HK, hexokinase; FFM, fat-free mass.

in these studies, because comparisons are made between individuals and not the calculation of glucose turnover rates or hepatic glucose production, this is not critical. The low-dose or physiological insulin stimulation level (M) and high-dose or supraphysiological stimulation level (MZ) use the units of mg $\cdot$ min $^{-1}\cdot$ kg fat-free mass $^{-1}$  + 17.7 (19). Plasma glucose was measured by the glucose oxidase method with a glucose analyzer (Beckman Instruments, Fullerton, CA) and insulin concentrations by radioimmunoassay with a radioassay analyzer (Concept 4, ICN, Horsham, PA).

### Muscle Biopsy

One day after the euglycemic-hyperinsulinemic clamp, a percutaneous biopsy of the vastus lateralis muscle was obtained. After a 12-h overnight fast, the skin of the quadriceps was anesthetized with a local anesthetic, and the biopsy was performed using a Bergström needle (Depuy, Phoenix, AZ). One part of the specimen was immediately frozen in liquid nitrogen and stored at  $-70^\circ\text{C}$  for later analysis. The remaining muscle specimen was examined under a low-power microscope to determine muscle fiber orientation. The specimen was then mounted in an embedding matrix (Tissue Tek, Miles Laboratories, Elkhart, IL) and frozen in isopentane (Aldrich Chemical, Milwaukee, WI) cooled in liquid nitrogen and stored at  $-70^\circ\text{C}$  for further analysis. All muscle analyses were performed at the Royal Prince Alfred Hospital (Sydney, Australia).

### Muscle Morphology Determinations

Serial muscle sections 20  $\mu$ m thick for capillary density staining and 10  $\mu$ m thick for histochemical and immunocyto-

chemical staining were cut using a cryostat microtome (Leica, Nussloch, Germany) set at  $-20^{\circ}\text{C}$ .

**Capillary density.** The periodic acid-Schiff staining method (2) was applied to sections to visualize capillaries. The analysis of capillary density was performed on a Macintosh Centris 660AV computer with use of the public domain NIH Image analysis program.<sup>1</sup> The magnification was  $\times 252$ . The number of capillaries and fibers within the known area were counted, and capillary density (capillaries/ $\text{mm}^2$ ) and capillaries per fiber were determined. In addition, the mean fiber area was determined.

**Fiber type analysis.** To determine fiber type proportions, the standard histochemical myosin adenosinetriphosphatase (ATPase) method (6) was used. The sections were preincubated at pH 4.3, 4.6, and 10.3, and fibers were classified as type I, IIa, or IIb according to Brooke and Kaiser (6). The use of antibodies against specific muscle myosin, immunocytochemistry, was also used to validate the fiber type proportions estimated from the histochemical technique. The monoclonal anti-fast-twitch antibody MY-32 (Sigma Chemical, St. Louis, MO) reacts specifically with type II myosin in many species, including humans. No antibodies are commercially available to distinguish type IIa and IIb fibers in human skeletal muscle. Sections were incubated overnight at  $4^{\circ}\text{C}$  with the monoclonal antibody MY-32 as the primary antibody diluted 1:2,000 with phosphate-buffered saline (PBS). The sections were then washed with PBS and incubated for 1.5 h at  $37^{\circ}\text{C}$  with a peroxidase-labeled secondary anti-mouse immunoglobulin G antibody (Dako Immunoglobulins, Carpinteria, CA) diluted 1:40 with PBS to conjugate and form a complex with the primary antibody. After incubation, the sections were washed with PBS, incubated for 15 min at room temperature with 0.06% diaminobenzidine (BDH, Sydney, Australia) and 0.03% hydrogen peroxide in 0.05% tris(hydroxymethyl)aminomethane buffer (pH 7.6), and then washed and mounted in Aquamount (BDH).

**Oxidative capacity.** The oxidative capacity of muscle fiber was assessed histochemically using the NADH tetrazolium reductase technique (23). Transverse serial sections were incubated in a solution of NADH (Boehringer Mannheim, Sydney, Australia) and *p*-nitro blue tetrazolium chloride (Boehringer Mannheim) in 2 mmol/l 3-(*N*-morpholino)propanesulfonic acid for 30 min at  $37^{\circ}\text{C}$  and mounted in Aquamount. Fibers that were subjectively determined by visual inspection to be intensely stained and therefore dark were considered to be oxidative (9).

The oxidative capacity of muscle was assessed enzymatically by measuring the activities of CS and HK. Samples of vastus lateralis muscle ( $\sim 40$  mg) were homogenized (Polytron Kinematica, Lucerne, Switzerland) in 300  $\mu\text{l}$  of extraction buffer [50 mmol/l tris(hydroxymethyl)aminomethane-HCl (pH 7.5) containing 2 mmol/l dithiothreitol, 1 mmol/l EDTA, and 5 mmol/l magnesium chloride; BDH]. The tissue homogenates were used for the analysis of CS and HK activities.

**CS.** The activity of CS was measured by the method of Srere (33). CS catalyzes the condensation of acetyl-CoA with oxaloacetate to form citrate. The rate of CoA release from acetyl-CoA during this condensation was followed by measurement of the rate of reaction of CoA (Sigma Chemical) with 5,5'-dithiobis(2-nitrobenzoic acid) (Boehringer Mannheim)

spectrophotometrically (Cary 3, Varian, Mulgrave, Victoria, Australia) at 412 nm (8).

**HK.** The activity of HK was determined by coupling the HK reaction to that catalyzed by glucose-6-phosphate dehydrogenase (3). Glucose-6-phosphate dehydrogenase, glucose, ATP, magnesium ions, and NADP<sup>+</sup> (BDH) were added in excess. The rate of formation of NADPH (the rate of the overall coupled reaction) was followed spectrophotometrically (Cary 3) at 340 nm to determine the total activity of HK present.

#### Phospholipid FA Analysis

Extraction and derivatization of the FA components of muscle phospholipids have been described elsewhere (26). Briefly, muscle tissue was homogenized in 2:1 (vol/vol) chloroform-methanol, and total lipid extracts were prepared according to Folch et al. (13). Phospholipids were isolated from less polar lipids by solid-phase extraction on Sep-Pak silica cartridges (Waters, Milford, MA). The phospholipids were trimethylated, and the methyl FAs were separated, identified, and quantitated by gas chromatography. The results of the FA analysis in relation to obesity and insulin action have been published elsewhere (25). The content of individual FAs in the skeletal muscle phospholipids was expressed as a percentage of the total FAs identified, and the activity of a number of the enzymes of FA biosynthesis was estimated according to the product-to-precursor ratios of individual FAs. The estimated enzyme activities include the ubiquitous elongase, calculated from the ratio of the percentage of 18:0 to 16:0, and the ratios of 18:1(n-9) to 18:0 and 20:4(n-6) to 20:3(n-6), which are desaturations catalyzed by the  $\Delta^9$ - and  $\Delta^5$ -desaturase, respectively.

#### Statistics

All statistical calculations, including calculations of means and simple regression analyses, were performed using Statview II (Abacus Concepts, Berkeley, CA). The relationships between variables were analyzed by simple and multiple regressions.

## RESULTS

Mean values and ranges obtained for BMI (wt/ht<sup>2</sup>), pFAT, waist circumference, waist-to-thigh ratio, fasting plasma glucose concentration, fasting plasma insulin concentration, M, and MZ derived from glucose-clamp studies along with the subject characteristics and a number of metabolic variables are shown in Table 1. The study population was generally overweight, with mean BMI of 32 kg/m<sup>2</sup> and pFAT of 28%.

#### Muscle Fiber Type, Capillary Density, Adiposity, and Insulin Action

Muscle fiber type analysis was performed histochemically and immunocytochemically. The former method allowed the classification of type I, IIa, and IIb fibers, whereas the antibody staining allowed only the distinction of type I and II fibers. There was a tight homology ( $r = 0.97$ ,  $P = 0.0001$ ) between the two methods of classifying type I or II muscle fibers. Capillary density was significantly related to the percentage of type I fibers determined histochemically ( $r = 0.31$ ,  $P = 0.03$ ) and immunocytochemically ( $r = 0.29$ ,  $P = 0.05$ ). However, capillary density was unrelated to pFAT or insulin action in this study population (data not shown), and

<sup>1</sup> The NIH Image analysis program, written by Wayne Rosband at the NIH, is available from the Internet by anonymous ftp from zippy.nimh.nih.gov or on floppy disk from NTIS, 5285 Port Royal Rd., Springfield, VA 22161 (PB93-504868).

its inclusion in multiple regressions did not alter any of the observed relationships.

There were significant relationships between pFAT and the percentage of type I and IIb fibers, respectively (see correlation matrix in Table 2). The degree of obesity (pFAT) correlated negatively with the percentage of type I fibers ( $r = -0.44$ ,  $P = 0.002$ ) and positively with the percentage of type IIb fibers ( $r = 0.40$ ,  $P = 0.005$ ). pFAT was also negatively correlated with the percentage of type I fibers assessed immunocytochemically ( $r = -0.36$ ,  $P = 0.015$ ). Furthermore, waist circumference, a measure of visceral adiposity, was also significantly related to fiber type (Table 2). Thus obesity is associated with fewer type I fibers and more type IIb fibers. A weak relationship was also found between insulin action (log M) and the percentage of type IIb fibers ( $r = -0.29$ ,  $P = 0.05$ ).

#### Enzyme Activity, Muscle Oxidative Capacity, Adiposity, and Insulin Action

The activity of CS (units/g tissue wet wt) was significantly associated with pFAT ( $r = -0.43$ ,  $P = 0.008$ ),

waist circumference ( $r = -0.37$ ,  $P = 0.02$ ), and measures of insulin action as indexed by fasting insulin ( $r = -0.33$ ,  $P = 0.04$ ) and log M ( $r = 0.41$ ,  $P = 0.01$ ). Multiple regression analysis showed that CS did not relate to log M or fasting insulin independently of pFAT.

The percentage of type I muscle fibers determined histochemically and immunocytochemically was significantly related to the oxidative capacity of muscle (intense NADH-stained fibers;  $r = 0.95$  and  $0.88$ , respectively,  $P = 0.0001$  for both). Figure 1 shows the negative relationship between the oxidative capacity of muscle (NADH stain) and pFAT ( $r = -0.47$ ,  $P = 0.0007$ ). No relationships between oxidative capacity assessed by NADH staining and any measure of insulin action were observed (Table 2), nor did including measures of insulin action in a multiple regression alter the relationship between assessment of NADH staining and pFAT.

Interrelationships were found between oxidative capacity of muscle, assessed by NADH staining, and CS ( $r = 0.41$ ,  $P = 0.01$ ) and HK activity (units/g tissue wet wt;  $r = 0.32$ ,  $P = 0.05$ ).

Table 2. Correlation matrix of metabolic determinants, muscle characteristics, and derived FA indexes

	pFAT	W/T Ratio	Waist, cm	Fasting Plasma Insulin, log <sub>10</sub>	Log <sub>10</sub> Total M	Log <sub>10</sub> Total MZ	%Type I	%Type IIa	%Intense NADH Fibers	%Type IIb	CS, U/g	HK, U/g
pFAT	1											
W/T ratio	0.58 (0.0001)	1										
Waist, cm	0.90 (0.0001)	0.70 (0.0001)	1									
Fasting plasma insulin, log <sub>10</sub>	0.64 (0.0001)	0.48 (0.0004)	0.60 (0.0001)	1								
Log <sub>10</sub> total M	-0.60 (0.0001)	-0.45 (0.001)	-0.56 (0.0001)	-0.79 (0.0001)	1							
Log <sub>10</sub> total MZ	-0.44 (0.002)	-0.44 (0.002)	-0.42 (0.003)	-0.84 (0.0001)	0.78 (0.0001)	1						
%Type I	-0.44 (0.002)	-0.17 (0.25)	-0.30 (0.04)	-0.22 (0.13)	0.07 (0.65)	0.09 (0.55)	1					
%Type IIa	-0.05 (0.74)	-0.05 (0.72)	-0.07 (0.64)	-0.08 (0.59)	0.27 (0.07)	0.13 (0.41)	-0.19 (0.19)	1				
%Intense NADH	-0.47 (0.0007)	-0.20 (0.18)	-0.35 (0.015)	-0.25 (0.08)	0.08 (0.58)	0.11 (0.47)	0.95 (0.0001)	-0.26 (0.07)	1			
%Type IIb	0.40 (0.005)	0.19 (0.19)	0.30 (0.04)	0.25 (0.09)	-0.29 (0.05)	-0.18 (0.23)	-0.61 (0.0001)	-0.64 (0.0001)	-0.53 (0.0001)	1		
CS, U/g	-0.43 (0.008)	-0.25 (0.12)	-0.37 (0.02)	-0.33 (0.04)	0.41 (0.01)	0.30 (0.08)	0.35 (0.03)	0.10 (0.55)	0.41 (0.01)	-0.40 (0.014)	1	
HK, U/g	-0.20 (0.24)	0.05 (0.78)	-0.03 (0.88)	-0.21 (0.20)	0.21 (0.23)	0.25 (0.14)	0.30 (0.70)	0.16 (0.35)	0.32 (0.05)	-0.35 (0.04)	0.63 (0.0001)	1
Elongase activity (18:0/16:0)	-0.41 (0.004)	-0.14 (0.36)	-0.34 (0.02)	-0.13 (0.37)	0.19 (0.21)	0.13 (0.39)	0.29 (0.05)	0.14 (0.35)	0.24 (0.11)	-0.39 (0.007)	0.41 (0.015)	0.22 (0.19)
Total n-3 FA	-0.30 (0.04)	-0.28 (0.05)	-0.10 (0.48)	-0.21 (0.15)	0.22 (0.15)	0.25 (0.10)	0.37 (0.01)	0.09 (0.55)	0.38 (0.01)	-0.33 (0.03)	0.03 (0.86)	0.12 (0.50)
Δ <sup>5</sup> Activity [(20:4)n-6/(20:3)n-6]	-0.52 (0.0001)	-0.28 (0.05)	-0.45 (0.001)	-0.57 (0.0001)	0.58 (0.0001)	0.42 (0.003)	0.24 (0.11)	0.10 (0.49)	0.26 (0.08)	-0.28 (0.06)	0.57 (0.0002)	0.30 (0.07)
Δ <sup>9</sup> Activity [(18:1)n-9/18:0]	0.30 (0.04)	0.10 (0.50)	0.23 (0.11)	0.02 (0.88)	-0.18 (0.22)	0.06 (0.70)	-0.34 (0.02)	-0.10 (0.52)	-0.35 (0.015)	0.40 (0.006)	-0.16 (0.33)	-0.06 (0.71)

Values in parentheses represent correlation  $P$  values. pFAT, percent body fat; W/T ratio, waist-to-thigh ratio; FA, fatty acid; see Table 1 footnote for definition of other abbreviations.

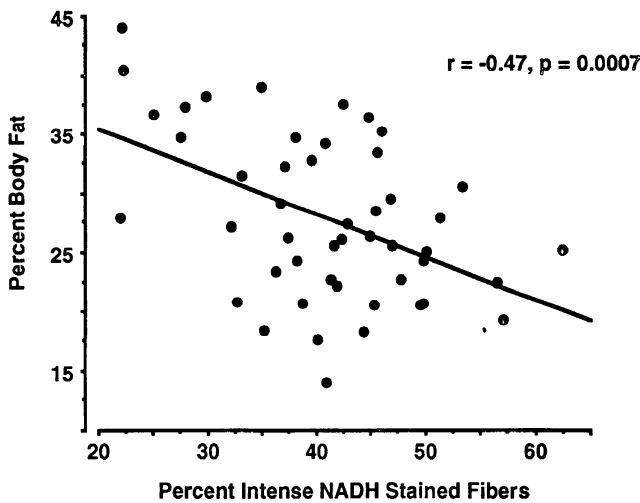


Fig. 1. Relationship between percent body fat and percentage of intensely stained NADH fibers in vastus lateralis muscle.

#### FA Variables, Muscle Fiber Type, and Oxidative Capacity

The percentage of individual FA and derived indexes such as 18:0/16:0 elongase activity and  $\Delta^5$ - and  $\Delta^9$ -desaturase activities were related to pFAT. In relation to measures of muscle morphology, the elongase activity was positively correlated with the percentage of type I fibers assessed by myosin ATPase ( $r = 0.29$ ,  $P = 0.05$ ) or MY-32 antibody ( $r = 0.30$ ,  $P = 0.05$ ) and negatively correlated with the percentage of type IIb fibers ( $r = -0.39$ ,  $P = 0.007$ ). In addition, elongase activity was positively correlated with oxidative capacity assessed by CS activity ( $r = 0.41$ ,  $P = 0.015$ ). Significant relationships were found between fiber type profiles and the amount of the individual FAs that constitutes the elongase ratio, namely, 18:0 and 16:0. The amount of 18:0 (stearic acid) was negatively correlated with the percentage of type IIb fibers ( $r = -0.34$ ,  $P = 0.02$ ), whereas the amount of 16:0 (palmitic acid) was negatively correlated with the percentage of type I fibers ( $r = -0.32$ ,  $P = 0.04$ ).

Total n-3 FAs were positively correlated with oxidative capacity [ $r = 0.38$ ,  $P = 0.01$  (NADH)] and percentage of type I fibers [ $r = 0.37$ ,  $P = 0.01$  (myosin ATPase); Fig. 2A]. Total n-3 FAs were negatively correlated with the percentage of type IIb fibers ( $r = -0.33$ ,  $P = 0.03$ ; Fig. 2B).

Relationships between pFAT and  $\Delta^5$ -desaturase activity ( $r = -0.52$ ,  $P = 0.0001$ ) and pFAT and  $\Delta^9$ -desaturase activity ( $r = 0.30$ ,  $P = 0.04$ ) have been identified in this population (25). In an extension of that data set,  $\Delta^5$ -desaturase activity was also significantly correlated with CS activity (units/g tissue wet wt;  $r = 0.57$ ,  $P = 0.0002$ ; Fig. 3).  $\Delta^9$ -Desaturase activity was negatively correlated with the percentage of type I fibers [ $r = -0.34$ ,  $P = 0.02$  (myosin ATPase);  $r = -0.38$ ,  $P = 0.011$  (MY-32)] and with the percentage of oxidative fibers [ $r = -0.35$ ,  $P = 0.015$  (NADH)].  $\Delta^9$ -Desaturase activity was positively correlated with the percentage of type IIb fibers ( $r = 0.40$ ,  $P = 0.006$ ). Finally, a

stepwise regression was performed on pFAT vs. the muscle variables significantly related by simple regression. This demonstrated a strong relationship of pFAT and oxidative capacity assessed by NADH staining followed independently by  $\Delta^9$ -desaturase activity, with the other variables no longer contributing.

#### DISCUSSION

Because muscle morphology relates to energy production and glucose disposal, alterations in muscle morphology may play a central role in the development of the syndromes of insulin resistance, including obesity (21, 25). This study has identified relationships between pFAT, muscle fiber type proportions, oxidative capacity, and structural lipid FA composition. Stepwise regression of pFAT with the other variables indicated that reduction in oxidative capacity and  $\Delta^5$ -desaturase activity were independently related to increased total body adiposity. Increased oxidative capacity (assessed by NADH staining and CS activity), which is associated

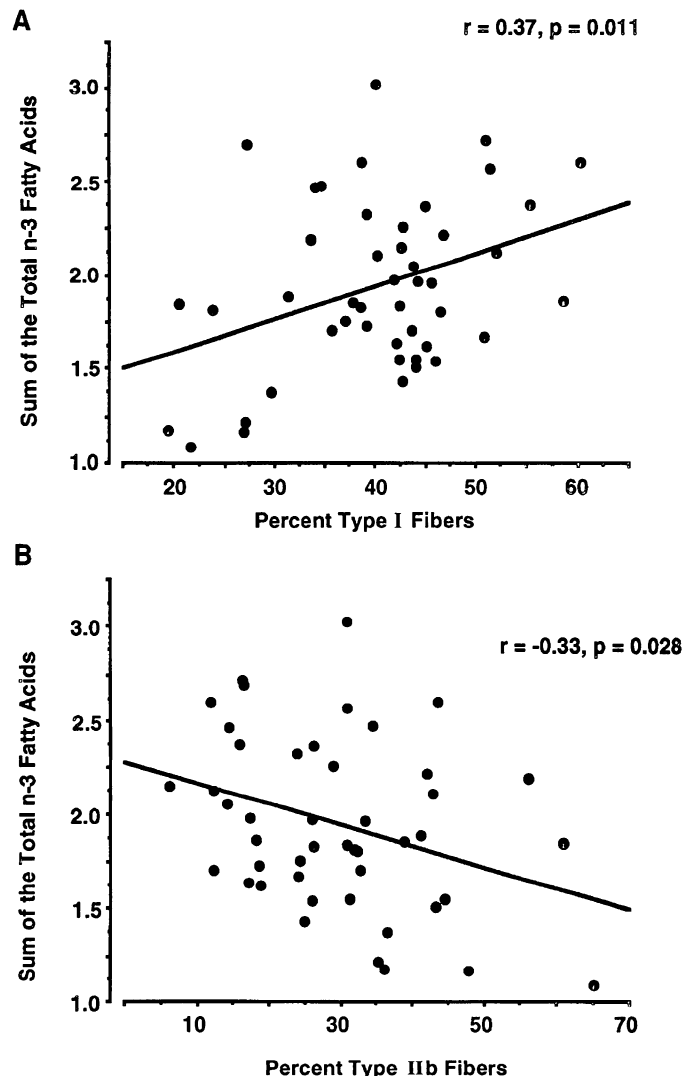


Fig. 2. Relationship between total percentage of n-3 fatty acids and the percentage of type I (A) and type IIb fibers (B) in vastus lateralis muscle.

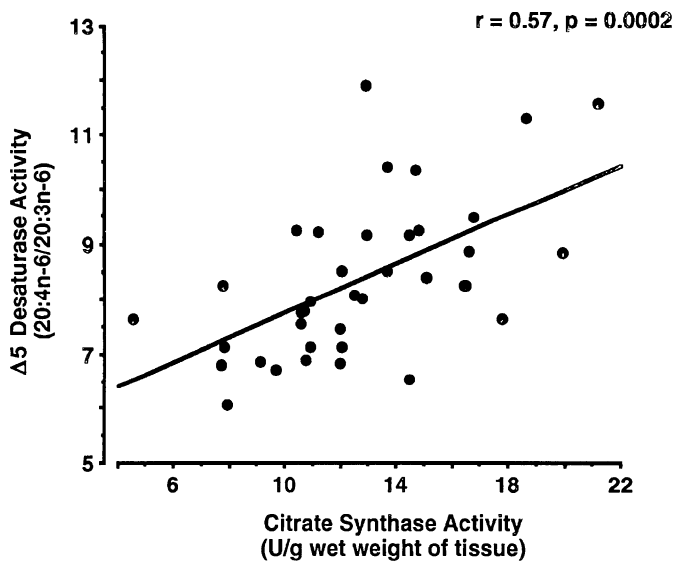


Fig. 3. Relationship between citrate synthase activity and  $\Delta^5$ -desaturase activity in vastus lateralis muscle.

with increased proportions of type I fibers, was also related to leanness. Likewise increased proportions of glycolytic type IIb fibers are associated with obesity and weakly associated with insulin resistance. The relationship between the oxidative capacity of skeletal muscle and adiposity (Fig. 1) extends and reinforces other recent reports (7, 16, 32). Other findings of importance include the significant associations between muscle fiber type and structural lipid FA variables and indexes, indicating that increased levels of n-3 FAs are associated with increased oxidative capacity as well as increased proportions of type I fibers (Fig. 2A) and reduced proportions of type IIb glycolytic fibers (Fig. 2B).

Type I fibers are known to be rich in mitochondria, with a higher oxidative capacity for carbohydrate and lipid fuel, and have greater insulin sensitivity (17). It makes intrinsic sense then that measures of oxidative capacity are positively related to the percentage of type I fibers (and negatively to the percentage of type IIb fibers) and, in turn, that oxidative capacity is related to insulin action and adiposity. As such, the results obtained in this study support and extend previous findings by Lillioja et al. (21), Wade et al. (36), and Hickey et al. (15). In the study by Lillioja et al., increased percentage of type IIb fibers was shown to be related to insulin resistance in a combined population of Caucasians and Pima Indians. Whereas this combined population included subjects over a large range of pFAT, from very lean to very obese, muscle fiber type composition was also shown to be related to waist-to-thigh ratio (as a measure of adiposity) and to pFAT. Wade et al. provided evidence for a direct relationship between the percentage of type I fibers and adiposity in a Caucasian population. Whereas the previous two studies examined percutaneous muscle biopsy samples of vastus lateralis, Hickey et al. showed a negative correlation between percentage of type I fibers and BMI and a positive relationship between the percentage of

type I fibers and in vitro glucose uptake in rectus abdominis muscle. Notably, this latter relationship was no longer significant after adjustment for BMI. This is consistent with the present results where the strongest relationships were between muscle morphology and pFAT. Interestingly, waist circumference was also significantly related to fiber type, oxidative capacity, and derived FA indexes. Pouliot and co-workers (27) clearly demonstrated waist circumference as an important variable in relation to cardiovascular risk factors.

The cross-sectional nature of the present study does not allow insight into the causality of the interrelationships between muscle morphology, body fatness, and insulin resistance. However, it is an area of importance for future work.

The present study demonstrated that reduced oxidative capacity of vastus lateralis muscle was related to whole body and visceral adiposity in humans, as well as to reduced insulin sensitivity (Table 2). Similar findings between skeletal muscle enzyme activity and adiposity and/or insulin action in humans have been demonstrated in recent studies (7, 16, 32). Simoneau and Bouchard (31) found a weak relationship between aerobic-oxidative capacity of skeletal muscle (MDH activity) and subcutaneous fat content. Zurlo et al. (37) showed a negative relationship between pFAT and activity of creatine kinase, an enzyme involved in high-energy phosphate metabolism. Collaborative work from the laboratories of Simoneau and Kelley have been successful in demonstrating relationships between insulin action and enzyme activities of glycolytic, anaerobic, and aerobic-oxidative pathways (7, 32), such that increased insulin sensitivity was positively related to the activity of CS and inversely to the activities of enzymes of glycolytic and anaerobic pathways.

Reduced nutrient delivery has been considered to play a role in insulin resistance, and previous work has related reduced capillary density to insulin resistance (21). No such relationships were found in this study, possibly because of the more limited range of insulin sensitivity. However, the fact that insulin action, fiber type, and oxidative capacity interrelated independently of capillary density suggests that this latter variable does not account for the current observations.

In the current study, CS activity was used as a measure of overall capacity for fuel utilization in muscle and was found to be significantly related to pFAT and insulin action: the lower the activity of CS, the more obese and insulin resistant the individual.

CS activity was also related to a number of FA variables. The strongest relationship was between CS activity and  $\Delta^5$ -desaturase activity. In other work (5, 25),  $\Delta^5$ -desaturase activity has been shown to be a powerful predictor of insulin action and adiposity. The important question that then arises is whether the FA variables determine fuel metabolic capacity of the cell or whether the metabolic capacity somehow regulates membrane composition. Work in rodents (18) indicates that voluntary exercise training can markedly increase the activity of CS with only a marginal alteration in FA composition. Such data suggest that the enzyme capac-

ity of the muscle and the FA composition of the membrane may work in synergy to affect insulin action.

In this study, relationships were shown to exist between muscle fiber type and FA composition. Increased levels of n-3 unsaturated FAs were significantly correlated with increased proportions of type I fibers and decreased proportions of type IIb fibers (Table 2). Also, increased levels of n-3 FAs were significantly related to the oxidative capacity of muscle. This is consistent with a recent study in animals, where the phospholipid FA composition was related to oxidative capacity of muscle (18). Soleus (type I) and red quadriceps (type I and IIa) muscles were shown to have increased levels of PUFAs, especially n-3 FAs, compared with the white quadriceps (type IIb) muscle (18). The metabolic significance of these relationships is not clear, although links between FA composition and functional aspects of metabolism have been demonstrated. Heart rate (and thus its metabolic activity) is directly related to the relative amount of the docosahexaenoic acid (long-chain n-3 PUFA) in heart phospholipid (14).

It remains to be determined whether obesity might affect fiber type or fiber type affects obesity or whether both are regulated by the same underlying genetic predisposition. Interestingly, in a recent study in human infants, the fiber type distribution was toward a much higher proportion of type IIa fibers and very few type IIb fibers (A. D. Kriketos, J. O'Connor, D. A. Pan, G. J. Cooney, L. A. Baur, and L. H. Storlien, unpublished observations) than in adults. This would suggest that life-style factors such as diet and level of habitual activity may play a major role in the transition of type IIa to IIb fibers. However, only studies involving intense physical training or resistance training over prolonged periods of time have been successful in demonstrating changes in fiber type composition in humans (11). In the present study population of obesity- and diabetes-prone Pima Indians, the subjects were not trained. Whether the normal range of habitual activity in relatively untrained individuals can influence fiber composition or overall oxidative capacity of muscle is unknown but important in the current setting.

As Flatt et al. (12) have elegantly argued, obesity must be derived from a long-term imbalance of the theoretical respiratory quotient (RQ) of the diet (which can be defined as the food quotient, which is calculated from the absorbed proportions of dietary fat, carbohydrate, and protein) and the RQ of the expired gases, such that the food quotient is lower than the RQ (12). Changes in metabolism that favor increased oxidation of fat (i.e., decreased RQ) without dietary change will lead to a reduction in adiposity and eventually leanness; conversely, a reduced tendency to fat oxidation (i.e., high RQ) will lead to obesity. Such a relationship has been demonstrated in a prospective study in Pima Indians (28). Type I fibers have a relatively large capacity for fat oxidation in keeping with their mitochondrial content; conversely, type IIb fibers have only a very limited ability to use fat for fuel. Therefore, the

association of a higher proportion of type I fibers (a high muscle capacity for fat utilization, e.g., low RQ) and leanness is consistent with the thesis of Flatt et al. Whereas it is unlikely that a relationship would be observed between fiber type and lipid oxidation at rest (and indeed was not seen in this study), such a relationship may be exposed under conditions of low-intensity aerobic exercise.

### Perspectives

The present study substantially extends the evidence in humans that a number of aspects of muscle morphology (fiber type distribution, oxidative capacity, and membrane structural membrane FA lipid profile) are associated with obesity and insulin resistance. It now remains to be established whether the observed diversity of muscle morphology causes or is caused by obesity and insulin resistance.

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Present address of S. Lillioja: Dept. of Medicine, University of New South Wales, South Western Clinical School, PO Box 103, Liverpool, NSW 2170, Australia.

Address for reprint requests: A. D. Kriketos, Dept. of Medicine (Endocrinology), University of Sydney, Sydney, NSW 2006, Australia.

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