# Skeletal Muscle Lipid Content and Oxidative Enzyme Activity in Relation to Muscle Fiber Type in Type 2 Diabetes and Obesity

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In obesity and type 2 diabetes, skeletal muscle has been observed to have a reduced oxidative enzyme activity, increased glycolytic activity, and increased lipid content. These metabolic characteristics are related to insulin resistance of skeletal muscle and are factors potentially related to muscle fiber type. The current study was undertaken to examine the interactions of muscle fiber type in relation to oxidative enzyme activity, glycolytic enzyme activity, and muscle lipid content in obese and type 2 diabetic subjects compared with lean healthy volunteers. The method of single-fiber analysis was used on vastus lateralis muscle obtained by percutaneous biopsy from 22 lean, 20 obese, and 20 type 2 diabetic subjects (ages $35 \pm 1$, $42 \pm 2$, and $52 \pm 2$ years, respectively), with values for BMI that were similar in obese and diabetic subjects ($23.7 \pm 0.7$, $33.2 \pm 0.8$, and $31.8 \pm 0.8$ kg/m$^2$, respectively). Oxidative enzyme activity followed the order of type I > type IIa > type IIb, but within each fiber type, skeletal muscle from obese and type 2 diabetic subjects had lower oxidative enzyme activity than muscle from lean subjects ($P < 0.01$). Muscle lipid content followed a similar pattern in relation to fiber type, and within each fiber type, muscle from obese and type 2 diabetic subjects had greater lipid content ($P < 0.01$). In summary, based on single-fiber analysis, skeletal muscle in obese and type 2 diabetic subjects mani-fests disturbances of oxidative enzyme activity and increased lipid content that are independent of the effect of fiber type. *Diabetes* **50:** 817–823, 2001

A reduced oxidative enzyme capacity of skeletal muscle has been found in type 2 diabetes as well as in obesity that is not complicated by diabetes. This metabolic characteristic is associated with insulin-resistant glucose metabolism (1–3) and with impaired lipid oxidation by skeletal muscle during fasting conditions (4,5). In these studies, there was strong within-subject correlation in the activities of oxidative enzyme citrate synthase, cytochrome C oxidase, and hy-droxyacyl dehydrogenase, indicative of a coordinated regulation of enzyme activity within oxidative pathway (4). The lower oxidative enzyme activity in type 2 diabetes and obesity suggests either a reduced mitochondria function or a reduced mitochondria content in skeletal muscle in these disorders. Activities of skeletal muscle glycolytic enzymes (phosphofructokinase activities) and α-glycerol-phosphate dehydrogenase (GPDH) have been found to be increased in obesity and type 2 diabetes (1–3). Although a full explanation for these differences in glycolytic and oxidative enzyme activities in skeletal muscle in obesity and type 2 diabetes compared with lean individuals has not been determined, one possibility is an altered proportion of muscle fiber types.

An increased proportion of type IIb muscle fibers, also termed glycolytic fast-twitch fibers, has been reported in type 2 diabetes in several studies (6–9), though not in all studies (10,11). In general, the metabolic characteristics of type IIb fibers include a reduced oxidative enzyme activity and an increased glycolytic enzyme activity in comparison with type I muscle fibers (oxidative slow-twitch) or type IIa (oxidative fast-twitch) (12). Moreover, it has been postulated, mostly based on animal studies, that muscle fibers follow an order of type I > type IIa > type IIb for insulin sensitivity (13–15). Thus, the reductions in oxidative enzyme activities, found within muscle homogenate, may be attributable to an increased proportion of type IIb and a decreased proportion of type I muscle fibers. The current study was designed to test individual muscle fiber metabolic capacity across serial sections of vastus lateralis muscle based on identifying and tracking oxidative and glycolytic enzyme activities in relation to fiber type.

A corollary aim was to examine the characteristics of fiber type and oxidative and glycolytic enzyme activities in relation to muscle lipid content. There has been renewed interest in the association of muscle lipid content with skeletal muscle insulin resistance (11,16–18). Recent microscopy studies from our laboratory (19) have found an elevated muscle lipid content in obesity. The relation of lipid content in human skeletal muscle to fiber type and oxidative enzyme capacity is not certain. In general, muscle fibers endowed with higher oxidative enzyme activity tend to have greater lipid content than fast-twitch glycolytic fibers (20–23). How these findings may relate to a greater lipid content in muscle fibers from obese individuals is unclear, particularly because lower oxidative enzyme activity has been described for obesity (24,25). Therefore, using a quantitative histochemical method based

on Oil red O staining (26), the current study also addressed the relation between oxidative enzyme activity and lipid content in skeletal muscle in obesity and type 2 diabetes.

## RESEARCH DESIGN AND METHODS

The research participants in this investigation were 22 lean nondiabetic individuals (11 women and 11 men, mean age $35 \pm 1$ years), 20 obese nondiabetic individuals (6 women and 14 men, mean age $42 \pm 2$ years), and 20 type 2 diabetic individuals (11 women and 9 men, mean age $52 \pm 2$ years). The groups differed by age ($P < 0.01$) and BMI ($23.7 \pm 0.7$, $33.2 \pm 0.8$, and $31.8 \pm 0.8$ kg/m$^2$ for lean, obese, and diabetic subjects, respectively) ($P < 0.01$ for lean vs. obese and lean vs. diabetic subjects). Fasting plasma glucose and HbA$_{1c}$ were normal in the lean ($85 \pm 3$ mg/dl and $5.2 \pm 0.3\%$) and the obese subjects ($90 \pm 4$ mg/dl and $5.3 \pm 0.3\%$), but were significantly increased (both $P < 0.001$) among the volunteers with type 2 diabetes ($198 \pm 10$ mg/dl and $8.0 \pm 0.2\%$). Participants were recruited by public advertisement, and all subjects had a screening medical examination before participating. Muscle biopsy samples of vastus lateralis muscle used for this study were obtained from research volunteers who participated in one of three clinical investigations. Each of these studies had the same procedure for the preparation of the research volunteers before the muscle biopsy procedure (e.g., overnight fast), as described below. Also, in each of the three investigations, a similar inclusion criterion was that volunteers were sedentary, which was defined as not participating in more than two exercise sessions weekly on a regular basis. Each of the research studies was reviewed and approved annually by the University of Pittsburgh Institutional Review Board, and written informed consent was obtained from all volunteers.

At the time of the screening examination, the volunteers with type 2 diabetes were treated either by diet alone ($n = 5$) or by oral agents, but none of these volunteers were receiving insulin. Diabetic medication was discontinued 4 weeks before this study. All subjects were free from any known cardiovascular or neuromuscular disease.

**Study design.** This investigation was carried out at the University of Pittsburgh General Clinical Research Center. Subjects were instructed to fast overnight and not to exercise the day before the study. Fasting samples of blood were obtained for glucose, HbA$_{1c}$, and insulin levels, and height and weight were determined. Before the muscle biopsy procedure, subjects rested supine for at least 30 min. A percutaneous muscle biopsy was performed 12–16 cm above the patella, in the vastus lateralis muscle, as previously described (19).

**Skeletal muscle analysis.** Muscle biopsy samples were immediately dissected free of adipose and connective tissue before being mounted in tissue freezing compound (Triangle Biomedical Sciences, Durham, NC) and frozen directly in isopentane cooled in liquid nitrogen. Biopsy samples were maintained within sealed containers at –70°C until microscopy studies. On the day of the preparation of samples for microscopy, tissue blocks from each of the three different study groups (lean, obese, and diabetic) were mounted and prepared in parallel. From each tissue block, serial transverse sections (10 μm) were cut using a cryostat at −20°C and then mounted on Superfrost/Plus slides (Fisher Scientific, Pittsburgh, PA).

**Histochemical reaction.** To determine muscle fiber type (types I, IIa, and IIb) and to calculate proportions for each fiber type, myofibrillar ATPase (mATPase) histochemical staining procedure was performed according to an established technique (27). At least 300–450 fibers were classified for each specimen. The adjacent sections were incubated in different media for succinate dehydrogenase (SDH), α-GPDH (28,29), and Oil red O staining solution (26) to assess oxidative enzyme activity, glycolytic enzyme activity, and lipid content in muscle. To assess background staining, as negative control slides for these reactions, sections were incubated in media without enzyme substrates; the omissions were succinate for the SDH reaction and glycerophosphate for the α-GPDH reaction. For a control slide for Oil red O staining, slides were incubated in 100% acetone for 10 min before immersing into oil red O staining solution.

**Image analysis.** Images were captured by an optical microscope (Microphot-FXL; Nikon, Tokyo) with a connected CCD Sony video camera under the same microscope objective (10×). Optimas (Media Cyberneics, Silver Spring, MD) was used to perform image analyses. To determine enzyme activity and lipid content in individual muscle fibers, the quantitative histochemical microphotometric procedure was used. First, muscle fibers were classified as fiber types I, IIa, or IIb based on the mATPase staining. In serial sections, individual fibers identified on the slides prepared for fiber type determination were then reidentified on the sections stained for SDH, α-GPDH, and Oil red O and were assessed for oxidative and glycolytic enzyme activities and lipid content. A representative photograph of serial sections of vastus lateralis muscle from a lean, an obese, and a type 2 diabetic volunteer are shown in Fig. 1.

The mean value for each of these parameters in relation to fiber type was then calculated for each individual. At least five fields from each section were analyzed in collecting data for these determinations. The intensity of readings in the control slides was used as a background value and was subtracted. The ratios of glycolytic to oxidative enzyme activity were calculated, for each fiber type, as the ratio of mean intensities of α-GPDH to SDH. Similarly, the oxidative–to–lipid content ratio was calculated for fiber types as the ratio of staining intensity of SHD to Oil Red O. To determine a composite value for oxidative or glycolytic enzyme activity, or lipid content, the respective value for each fiber type was multiplied by the proportion of each fiber type and then summed for the three fiber types.

**Statistic analysis.** Data are means $\pm$ SE unless otherwise indicated. Two-way analysis of variance was used to examine the differences in sex and group (lean, obese, and type 2 diabetic). Multiple regression analysis was used to examine the effect of group or BMI after adjusting for age. $P < 0.05$ was considered significant.

## RESULTS

The fiber type distributions of vastus lateralis skeletal muscle in lean, obese, and type 2 diabetic volunteers are shown in Fig. 2. Type I and type IIa muscle fibers constituted the majority of the fibers. No significant group differences in fiber type proportions were observed; although in obese and type 2 diabetic men compared with lean men, there was a nonsignificant ($P = 0.054$) trend for a lower proportion of type I fibers. However, this pattern was not observed among women. Type IIb muscle fibers accounted for ~10% of muscle fibers in each group.

Plots for oxidative enzyme activity (SDH), glycolytic enzyme activity (α-GPDH), and lipid content (Oil red O) for type I, IIa, and IIb muscle fibers from lean, obese, and type 2 diabetic subjects are shown in Fig. 3A–C. In comparing muscle fiber types, type I muscle fibers had the highest SDH activity, type IIa had intermediate values, and type IIb had the lowest oxidative activity. This pattern was observed for each group. In addition, there was a significant effect of group. Within each fiber type, oxidative enzyme activity was greatest in muscle from lean subjects by ~25%, compared with muscle from obese or type 2 diabetic subjects (type I $P < 0.001$; type IIa $P < 0.001$; and type IIb $P = 0.05$). Values for SDH activity were not significantly different in obese compared with type 2 diabetic subjects in any of the three fiber types. There was not a significant effect of sex. For each biopsy sample, a composite index of SDH activity was calculated based on SDH intensity in each fiber type and fiber-type proportions. SDH activity was significantly greater in lean compared with obese or type 2 diabetic subjects ($43 \pm 2$, $32 \pm 3$, and $32 \pm 3$, respectively; $P = 0.003$), without a significant effect of sex.

Glycolytic enzyme activity in muscle fibers was assessed based on staining for α-GPDH activity. Glycolytic enzyme activity was lowest in type I muscle fibers and was of similar value in types IIa and IIb muscle fibers, although it was slightly increased in type IIb fibers. There was not a significant group effect for glycolytic enzyme activity within any of the three fiber types. The composite value for glycolytic enzyme activity intensity was not different across groups ($71 \pm 5$, $77 \pm 6$, and $79 \pm 6$ for lean, obese, and diabetic groups, respectively, $P = 0.6$).

Oil red O staining was used to assess muscle content of triglycerides (Fig. 3C). Muscle content of neutral lipid was highest in type I muscle fibers, with intermediate levels in type IIa and the lowest values in type IIb. The effect of fiber type as a determinant of lipid content was observed
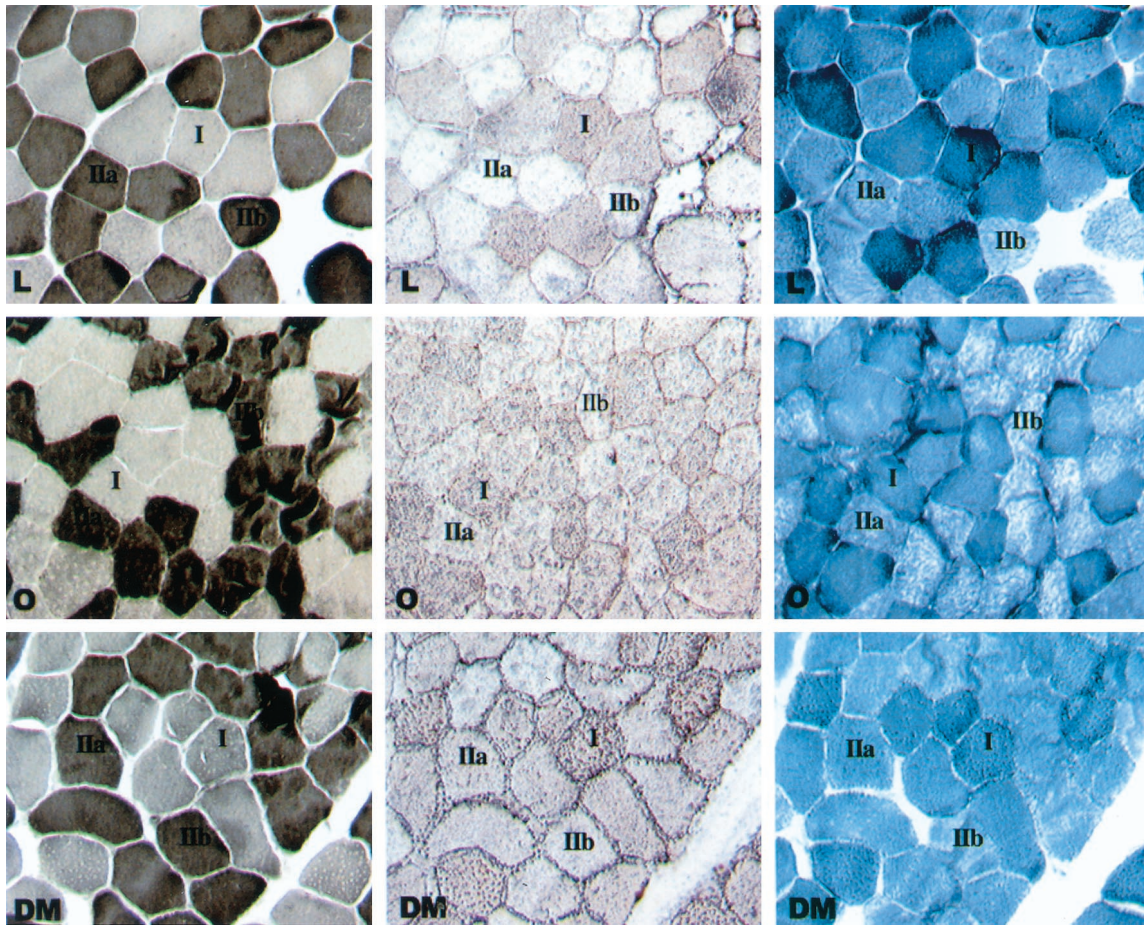
FIG. 1. Photographs of representative cross-sections of vastus lateralis muscle from lean (L), obese nondiabetic (O), and obese type 2 diabetic (D) volunteers with serial sections stained for fiber type, lipid content, and oxidative enzyme activity. The muscle fibers are labeled as type I, type IIa, and type IIb.

within each group. However, there were also significant group differences within each fiber type: lipid content was ~25–50% higher in muscle from type 2 diabetic and obese subjects compared with muscle from lean subjects ($P <$ 0.01). The composite lipid content in muscle was ~40–50% greater in skeletal muscle from obese and type 2 diabetic subjects compared with lean subjects (10.2 $\pm$ 1.1, 14.1 $\pm$

1.3, and 15.2 $\pm$ 1.2 for lean, obese, and diabetic subjects, respectively; $P <$ 0.01).

The ratio of glycolytic to oxidative enzyme activities was calculated for each fiber type. The glycolytic-to-oxidative ratio was lowest for type I muscle fibers and highest for type IIb, with an intermediate value in type IIa, as shown in Fig. 4A. There was not a significant group
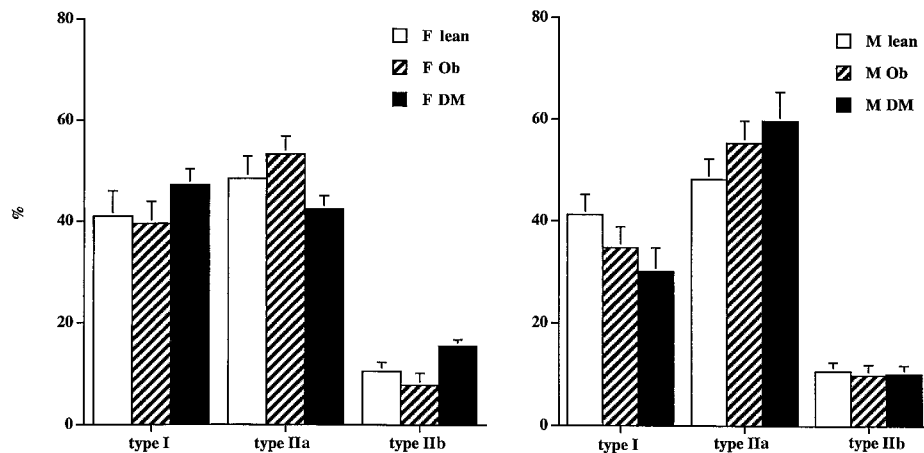


FIG. 2. The percentage of type I, type IIa, and type IIb muscle fibers in female (F) and male (M) lean, obese, and type 2 diabetic (DM) volunteers. There was no significant difference in the percentage of fiber types in lean, obese, and type 2 diabetic women or men, although the differences in men approached statistical significance ($P = 0.054$).
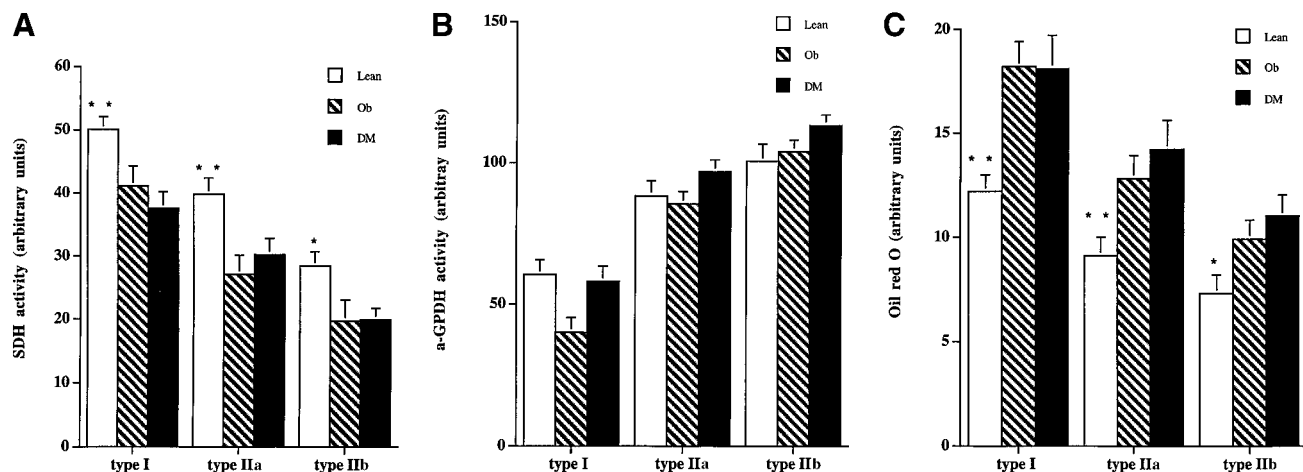
FIG. 3. *A*: Skeletal muscle oxidative enzyme activity assessed by SDH staining in each of the three fiber types. Oxidative enzyme activity was greater in lean subjects than in obese or type 2 diabetic subjects for all three fiber types. **$P < 0.01$; *$P < 0.05$. *B*: Skeletal muscle glycolytic enzyme activity assessed by $\alpha$-GPDH staining for all three fiber types. There was not a significant difference in skeletal muscle glycolytic enzyme activity in lean, obese, or type 2 diabetic subjects within each fiber type. *C*: Skeletal muscle lipid content assessed by Oil red O staining for all three fiber types. Lean subjects had less muscle-lipid content than obese or type 2 diabetic subjects in each of the three fiber types. **$P < 0.01$; *$P < 0.05$.

difference for the glycolytic-to-oxidative ratio in type I muscle fibers, but significant group differences were observed for types IIa and IIb, with skeletal muscle from lean individuals having a lower glycolytic-to-oxidative ratio than skeletal muscle from obese and type 2 diabetic subjects. The composite value for the glycolytic-to-oxidative ratio was lowest in lean subjects ($1.9 \pm 0.3$, $3.0 \pm 0.3$, and $2.9 \pm 0.3$ for lean, obese, and type 2 diabetic subjects, respectively; $P = 0.02$).

The ratios of oxidative enzyme activity to lipid content are shown in Fig. 4*B*. There were group differences; values were substantially lower in obesity and type 2 diabetes, regardless of fiber type. Despite differences between fiber types in oxidative enzyme activity and lipid content, the ratio of oxidative activity to lipid content was similar across fiber types in lean subjects. A similar pattern, albeit at a lower value, was found across fiber types in obese and type 2 diabetic subjects.

**Relation of single-fiber characteristics to obesity.** In addition to analyzing potential group differences in single-fiber metabolic characteristics, the data were analyzed by regression analysis, using obesity as the dependent variable. Single-fiber glycolytic enzyme activity did not correlate significantly with BMI for any of the three fiber types. However, single-fiber oxidative enzyme activity did correlate significantly (negatively) with BMI (type I $r = -0.42$, type IIa $r = -0.40$, and type IIb $r = -0.39$; $P < 0.01$). Single-fiber Oil red O staining was positively correlated with BMI, but this association was significant only for type I fibers (type I $r = 0.32$, $P < 0.05$, type IIa $r = 0.18$, and type IIb $r = 0.13$). Age was significantly associated with muscle SDH activity for type I muscle fibers ($r = -0.29$, $P = 0.03$), but not for types IIa and IIb. Because there were age and weight differences across the groups, the correlation between age and type I fiber SDH activity could be confounded by the effects of group or weight. In a multiple regression analysis, type I fiber SDH activity was significantly related to BMI ($t = -2.3$, $P = 0.03$), but was not independently correlated with age ($P = 0.11$). A similar pattern was found if multiple regression analysis was done

using group and age, with group as a significant predicator of type I SDH ($t = -2.2$, $P = 0.03$), but age was not independently correlated ($P = 0.28$).

## DISCUSSION

Human skeletal muscle is of mixed fiber-type composition (30–33). This is in contrast to other species, such as the rat, in which some skeletal muscles are largely comprised of a single fiber type (34). Whereas this characteristic in rodent skeletal muscle has facilitated investigations into the metabolic capacities associated with fiber type (12), the lack of homogenous fiber-type composition in human muscles has hindered a similar undertaking. Thus, it has been difficult to understand what impact, if any, fiber type distributions might have in determining metabolic characteristics, such as enzyme activities or substrate concentrations, when these are determined in muscle homogenate. There is a wealth of data indicating that skeletal muscle in obesity and type 2 diabetes manifests insulin resistance and abundant data that relate insulin resistance to altered patterns of enzyme activity, as determined within muscle homogenates (1,2,4,32,35–43). Among these findings are reduced oxidative enzyme activity and increased glycolytic to oxidative enzyme activity in obesity and type 2 diabetes (1–3). There are some data that suggest muscle fiber-type proportions differ in relation to insulin resistance (4–7), specifically that the proportion of type I fibers is lower and that of type IIb fibers is higher. This kind of fiber-type difference could conceivably account for the differences in muscle oxidative enzyme activity found in muscle homogenates. Therefore, the current study was undertaken using the approach of single-fiber analysis and histochemical staining to determine whether the differences in fiber-type proportion account for the effect of obesity and type 2 diabetes on muscle oxidative enzyme capacity and muscle lipid content.

In the current study, in vastus lateralis from lean volunteers, type I fibers comprised ~40% of the muscle fibers, type IIa fibers comprised ~50%, and type IIb fibers com-
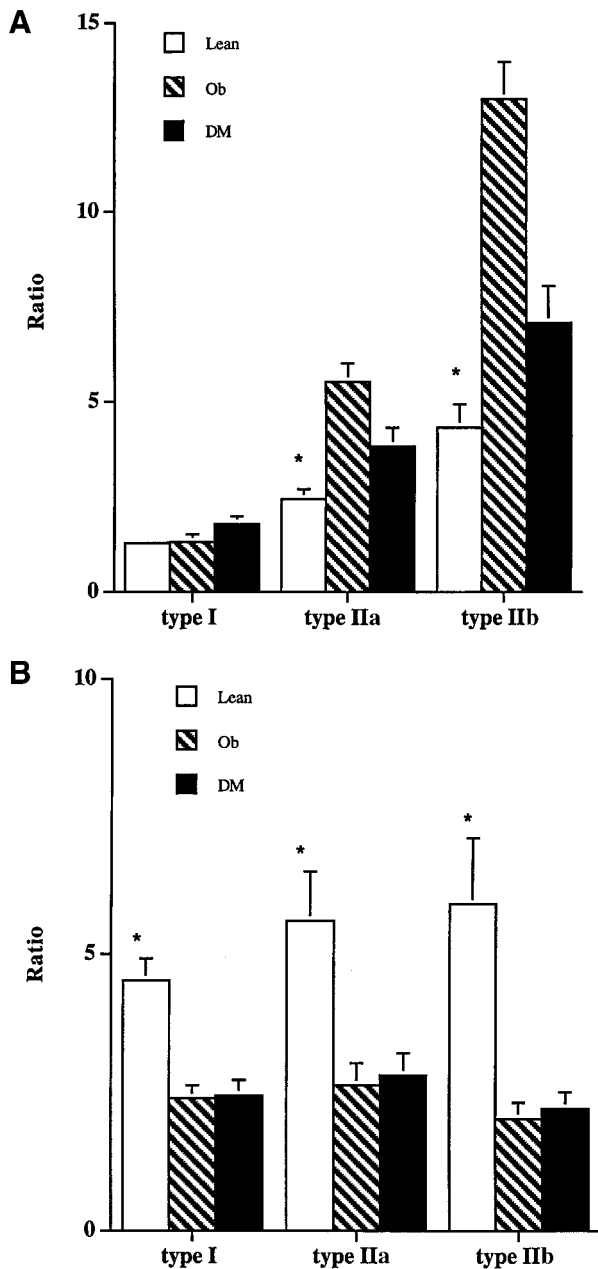
FIG. 4. *A*: The glycolytic–to–oxidative enzyme activity ratio for each of the three fiber types (I, IIa, and IIb). A significant difference of α-GPDH to SDH ratio was observed in fiber types IIa and IIb across groups with lower values in skeletal muscle from lean subjects. *$P <$ 0.05. *B*: The ratio of oxidative enzyme activity to fiber lipid content (i.e., SDH–to–Oil red O ratio). Skeletal muscle fibers, in all three fiber types, from obese and type 2 diabetic volunteers had a significantly lower ratio of SDH to Oil red O than muscle fibers from lean subjects, but within each of the three groups of subjects, there was no difference in the SDH–to–Oil red O ratio across fiber types. *$P < 0.05$.

ously established patterns recognized in relation to fiber type (33).

In vastus lateralis from obese and type 2 diabetic individuals, no significant differences in fiber-type proportions were observed as compared with muscle from lean subjects. In contrast to our findings, Hickey et al. (6) found a greater proportion of type IIb fibers in obese compared with lean individuals, and Nybolm et al. (8) found a greater percentage of type IIb and a lower percentage of type I fibers in type 2 diabetes. However, Simoneau et al. (33) did not find differences in fiber-type proportions. Indeed, the absence of differences in fiber-type proportions reported by Simoneau et al. (2,3,33) is noteworthy, because they consistently found lower oxidative enzyme activity in vastus lateralis muscle homogenate in relation to obesity and type 2 diabetes (46,47). Based on these findings, Simoneau et al. (2,3) proposed that metabolic differentiation of muscle, defined in terms of the glycolytic–to–oxidative enzyme activity ratio, was a more important determinant of metabolic capacity than fiber type per se. Saltin et al. (44) and Pette et al. (45) had originally advanced the concept that metabolic differentiation of muscle fibers can be characterized based on glycolytic to oxidative enzyme ratios and that this characteristic is malleable to interventions such as physical training.

The current findings reveal important new information on the interaction of fiber type and metabolic capacity and the substantial alteration that occurs in obesity and type 2 diabetes. Within each fiber type, muscle from obese and type 2 diabetic subjects had significantly lower oxidative enzyme activity than that of lean subjects. Glycolytic enzyme activity was not significantly different in obese and type 2 diabetic individuals compared with lean individuals. These current findings provide a clear indication that reduced oxidative enzyme activity in muscle in obesity and type 2 diabetes is because of reduced oxidative enzyme activity within each fiber type.

In recent years, a number of groups have addressed the issue of muscle lipid content. A general consensus has emerged that triglyceride content is increased in skeletal muscle in obesity and obesity complicated by type 2 diabetes (16,17,19). The data of the current study provide further confirmation of this hypothesis and yield new insight into the interaction of muscle fiber type and single-fiber oxidative capacity in relation to lipid content of muscle in obesity and type 2 diabetes. Muscle fibers from obese and type 2 diabetic subjects were found to contain greater intramyocyte content of triglyceride regardless of fiber type. Additional novel findings of the present study concern the relationship of triglyceride content to oxidative enzyme activity.

In muscle from lean individuals, the ratio of muscle triglycerides to oxidative enzyme activity was remarkably consistent across the three fiber types, despite clear differences in each fiber type for oxidative enzyme activity and for lipid content when each parameter was considered independently. This relatively constant proportionality suggests that some regulatory process may exist between muscle lipid and enzymatic capacity for substrate oxidation. However, this balance appears to be disturbed in obesity and type 2 diabetes. In muscle from obese and type 2 diabetic volunteers, the proportionality between lipid

prised ~10%. This is consistent with previously published data (33). Also consistent (12), type I fibers had higher oxidative enzyme activity than type IIa, and the lowest oxidative capacity was found in type IIb fibers. In addition, type I fibers from lean volunteers had lower glycolytic enzyme activity than types IIa and IIb. Thus, the muscle fiber analysis performed in the current study yielded a pattern of metabolic characterization for muscle fibers in lean volunteers that is extremely consistent with previ-

stores and oxidative enzyme activity was substantially different from that observed in muscle from lean subjects. In obesity and type 2 diabetes, much more lipid was stored in relation to oxidative capacity. The reason for this imbalance in obese and type 2 diabetic individuals remains to be more clearly defined. Our recent in vivo studies indicate that the rates of fatty acid uptake into muscle are not greater in obesity or type 2 diabetes, but that instead, rates of fat oxidation are reduced during postabsorptive (fasting) conditions (5).

In summary, reduced oxidative enzyme activity and increased lipid content in skeletal muscle in obesity and type 2 diabetes were found to be present in each of the three major fiber types. In contrast, no significant group differences were observed for fiber type distribution. Thus, the differences previously noted in skeletal muscle homogenates for oxidative enzyme activity and for lipid content appear to be derived from an effect of obesity and type 2 diabetes on each fiber type.

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