

Altered Fiber Distribution and Fiber-Specific Glycolytic and Oxidative Enzyme Activity in Skeletal Muscle of Patients With Type 2 Diabetes

ANDREAS OBERBACH^{1,2}
YVONNE BOSSENZ²
STEFANIE LEHMANN,²
JOSEF NIEBAUER, MD, PHD³
VOLKER ADAMS, PHD⁴

RALF PASCHKE, MD⁵
MICHAEL R. SCHÖN, MD⁶
MATTHIAS BLÜHER, MD^{2,5}
KARLA PUNKT, PHD¹

OBJECTIVE — We investigated whether alterations of glycolytic and oxidative enzyme capacity in skeletal muscle of patients with type 2 diabetes pertain to specific muscle fibers and are associated with changes in muscle fiber composition.

RESEARCH DESIGN AND METHODS — Vastus lateralis muscle was obtained by percutaneous biopsy from 10 patients with type 2 diabetes and 15 age- and BMI-matched healthy volunteers. Using cytophotometry, muscle fiber composition and fiber type-specific glycolytic and oxidative enzyme activities were measured in slow oxidative, fast oxidative glycolytic, and fast glycolytic fibers.

RESULTS — In the whole muscle, oxidative activity was decreased in patients with type 2 diabetes. The slow oxidative fiber fraction was reduced by 16%, whereas the fast glycolytic fiber fraction was increased by 49% in skeletal muscle from the diabetic patients. Both oxidative and glycolytic enzyme activities were significantly increased in fast glycolytic and fast oxidative glycolytic fibers of type 2 diabetic patients. However, the fiber-specific ratio of glycolytic enzyme activity relative to oxidative activity was not different between type 2 diabetic patients and the control subjects. The myofibrillar ATP activity was significantly lower in all fiber types of patients with type 2 diabetes and correlates with glucose infusion rate during the steady state of a euglycemic-hyperinsulinemic clamp and maximal aerobic capacity and negatively with HbA_{1c} values.

CONCLUSIONS — Reduced oxidative enzyme activity in muscle of type 2 diabetic patients is most likely due to a reduction in slow oxidative fibers. Increased glycolytic and oxidative enzyme activities in individual muscle fibers are closely related to measures of long-term glycaemic control and whole-body insulin sensitivity and could therefore represent a compensatory mechanism of the muscle in function of the altered glucose metabolism.

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Type 2 diabetes is characterized by severe insulin resistance of skeletal muscle associated with an impaired insulin-stimulated glucose disposal rate (1,2). Insulin resistance correlates with skeletal muscle fiber type distribution (3), a reduced percentage of slow oxidative type I fibers (4), and reduced oxidative

From the ¹Institute of Anatomy, University of Leipzig, Germany; the ²Junior Research Group N03, Interdisciplinary Centre for Clinical Research (IZKF) Leipzig, University of Leipzig, Leipzig, Germany; the ³Institute of Sports Medicine, Paracelsus Private Medical University, Salzburg, Austria; the ⁴Heart Center, Department of Cardiology, University of Leipzig, Leipzig, Germany; the ⁵Department of Medicine III, University of Leipzig, Germany; and the ⁶Department of Surgery, University of Leipzig, Germany.

Address correspondence and reprint requests to Prof. Dr. med. M. Blüher, University of Leipzig, IZKF Leipzig, Junior Research Group N03, Inselstr. 22, D-04103 Leipzig, Germany. E-mail: bluma@medizin.uni-leipzig.de.

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Abbreviations: GPDH, glycerol-3-phosphate dehydrogenase; LDH, lactate dehydrogenase; NGT, normal glucose tolerant; SDH, succinate dehydrogenase.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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enzyme capacity (5–7). Aging and physical inactivity, which are both associated with insulin resistance, also lead to diminished oxidative capacity of skeletal muscle (6). Simoneau and Kelley (6) showed that an increased ratio of glycolytic to oxidative enzymes contributes to insulin resistance in skeletal muscle of patients with type 2 diabetes. Interestingly, with single-fiber analysis, no differences have been found in the fiber type composition between lean, obese, and type 2 diabetic subjects (7). In this study, significant differences in oxidative but not in glycolytic enzyme activity and in the glycolytic-to-oxidative ratio were reported for the comparison of lean and obese subjects and patients with type 2 diabetes (7). However, it is still not entirely understood whether metabolic changes in skeletal muscle of patients with type 2 diabetes are related to chronic hyperglycemia and/or decreased insulin sensitivity and whether an increased glycolytic-to-oxidative ratio is primarily due to a reduced number of oxidative fibers or metabolic changes of one or more fiber types or both. We therefore investigated whether alterations of glycolytic and oxidative enzyme capacity in skeletal muscle of patients with type 2 diabetes are associated with changes in muscle fiber composition and pertain to specific fiber types. We further asked whether fiber composition and fiber type-specific metabolic alterations in skeletal muscle of type 2 diabetic patients are correlated with parameters of hyperglycemia and insulin resistance as determined by euglycemic-hyperinsulinemic clamps.

RESEARCH DESIGN AND METHODS

Ten patients with type 2 diabetes (4 male and 6 female) and 15 (8 male and 7 female) age- and BMI-matched subjects participated in the study. Subjects with normal glucose tolerance were defined by fasting plasma glucose <6.0 mmol/l and a 120-min plasma glucose <7.8 mmol/l after a 75-g oral glucose load (8). Patients with type 2 diabetes were defined by fasting plasma

Table 1—Anthropometric and metabolic parameters of the subjects

Parameter	NGT	Diabetes
n	15	10
Sex (male/female)	8/7	4/6
Age (years)	56.8 ± 7.9	58.7 ± 6.4
BMI (kg/m ²)	31.4 ± 3.2	32.1 ± 3.4
Body fat content (%)	33.6 ± 6.6	35.1 ± 8.1
Waist-to-hip ratio	1.24 ± 0.12	1.27 ± 0.17
V _{O₂max} (ml · kg ⁻¹ · min ⁻¹)	23.5 ± 2.7	17.4 ± 3.8*
Fasting plasma glucose (mmol/l)	5.34 ± 0.4	6.55 ± 0.9*
A1C (%)	5.15 ± 0.31	6.6 ± 0.4*
Fasting plasma insulin (pmol/l)	91.9 ± 42	157 ± 51*
Glucose infusion rate, clamp (ml/min)	87.5 ± 13	29 ± 12*

Data are means ± SD. *P < 0.05 for NGT vs. type 2 diabetic subjects.

glucose >7.0 mmol/l and/or glucose >11.1 mmol/l on an oral glucose tolerance test (8). All subjects fulfilled the previously described inclusion criteria (9).

The study was approved by the ethics committee of the University of Leipzig. All subjects gave written informed consent before participation in the study.

Measures of body fat content, glucose metabolism, and insulin sensitivity

BMI was calculated as weight in kilograms divided by the square of height in meters. Waist and hip circumferences were measured and waist-to-hip ratio was calculated. Percent body fat was measured by dual X-ray absorptiometry. The oral glucose tolerance test was performed according to the criteria of the American Diabetes Association (8). Insulin sensitivity was assessed with the euglycemic-hyperinsulinemic clamp method (10) as described previously (9).

Maximal exercise test

All subjects completed a graded bicycle ergometer test to volitional exhaustion by measuring maximal oxygen uptake with an automated open-circuit gas analysis system at baseline (Oxycon Alpha; Jäger). The highest oxygen uptake per minute reached was defined as the maximal oxygen uptake (V_{O₂max}).

Assays and muscle biopsies

Basal, fasting blood samples were taken after an overnight fast to determine glucose, insulin, HbA_{1c} (A1C), and standard laboratory parameters. Plasma concentrations of insulin, C-peptide, free fatty acids, and leptin were measured as described previously (9). Participants were admitted into the study at 0900 after

14 h of fasting. They had been asked to avoid vigorous exercise for at least 3 days before the muscle biopsy. After administration of local anesthesia, muscle biopsy specimens were obtained from the musculus vastus lateralis using a biopsy device (2.1; SOMATEX, Teltow, Germany). Samples were powdered with talcum and frozen immediately in liquid nitrogen. Muscle samples paired for the type 2 diabetic and normal glucose tolerant (NGT) groups were mounted together on a cryostat chuck to avoid differences in section thickness and incubation conditions. Cryosections (10 μm) were used for morphometric and immunohistochemical analysis.

Enzyme activity, histochemistry, and cytophotometry

The remaining muscle samples after the cryostat sections were taken were pooled within the groups to obtain enough protein for biochemical measurements. The frozen muscle samples were homogenized in 50 mmol/l Tris-HCl buffer, pH 7.4, containing 1 mmol/l EDTA, 1 mmol/l dithiothreitol, 100 μg/ml phenylmethylsulfonyl fluoride, 10 μg/ml trypsin inhibitor, 2 μg/ml aprotinin, and 10 μg/ml leupeptin, and the activities of succinate dehydrogenase (SDH) and lactate dehydrogenase (LDH) were measured. Activities of SDH (EC 1.3.5.1) and mitochondrial glycerol-3-phosphate dehydrogenase (GPDH) (EC 1.1.99.5) and myofibrillar ATPase (EC 3.6.1.32) were measured as described previously (11). Measurements were performed with a computer-controlled microscope photometer (MPM 200; Carl Zeiss, Oberkochen, Germany). The mean absorbance of the final reaction product of the respective enzyme reactions was measured in defined fibers and was taken as a measure of relative enzyme activity.

The correlation of absorbance of the final reaction product with the respective enzyme activity was shown (12). Moreover, the GPDH/SDH activity quotient was calculated, characterizing each fiber type. The cytophotometrical method was established and described as a tool in metabolic fiber differentiation (11,12). In each muscle section, ~30 fibers, containing all fiber types, were measured. Three sections were analyzed per muscle and enzyme reaction. In this way, ~6,755 fiber cross sections per group of patients were cytophotometrically measured.

Fiber typing

The methods of fiber typing have been described previously (12). In brief, the physiological metabolic fiber typing into slow oxidative, fast oxidative glycolytic, and fast glycolytic is based on cytophotometrically quantified activities of myofibrillar ATPase (marker of contractile activity), SDH (marker of oxidative activity), and GPDH (marker of glycolytic activity) in serial cross sections of the same fiber. These enzyme activities characterize the metabolic profile of muscle fibers. By means of the GPDH/SDH activity quotient, fast oxidative glycolytic fibers were subdivided into type I fibers with moderate SDH and moderate GPDH activity and type II fibers with high SDH and moderate or high GPDH activity. Counting of fibers was performed with Imaging System KS 100 (Kontron, Eching, Germany). Serial sections with GPDH, SDH, and alkaline ATPase activities were analyzed.

Statistical analyses

Data are shown as means ± SD unless stated otherwise. The following statistical tests were used: paired Student's *t* test and Pearson's simple correlation. Linear relationships were assessed by least-squares regression analysis. Multivariate linear relationships were assessed by a general linear model. Statistically analysis was performed using SPSS Version 12.0 (Chicago, IL). *P* < 0.05 was considered to be statistically significant.

RESULTS— Twenty-five individuals, 10 patients with type 2 diabetes and 15 age- and BMI-matched control subjects with normal glucose tolerance, were studied. Age, sex distribution, and anthropometric parameters were not significantly different between the groups (Table 1). In parallel with the altered parameters of glucose metabolism and insulin sensitivity, maximal aerobic capacity

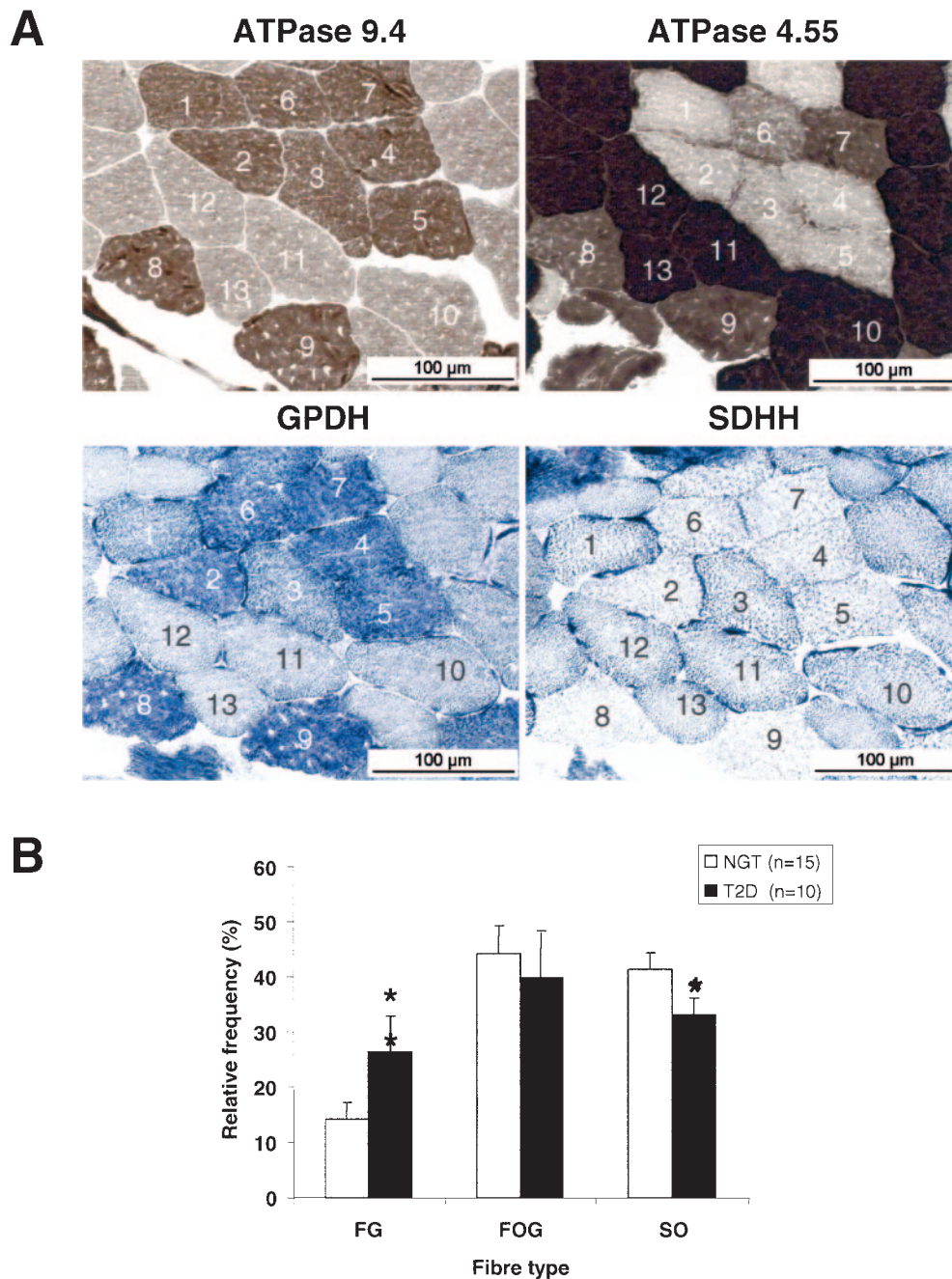


Figure 1— Skeletal muscle fiber typing in serial sections of biopsies from the vastus lateralis muscle in a NGT healthy volunteer. A: Activity of ATPase, ATPase after alkaline (ATPase 9.4) or acid preincubation (ATPase 4.55), GPDH, and SDH. As an example, 13 fibers were identified and typed in each serial section: fibers 1–9 are fast fibers (type II), and fibers 10–13 are slow fibers (type I, slow oxidative [SO]). Subtypes: fibers 1–5 are type IIA (fast oxidative glycolytic [FOG] I or II) fast glycolytic (FG); fiber 6 is IIX (FG), 7–9 = IIB (FG). B: Different skeletal muscle fiber type distribution in the NGT and type 2 diabetic groups. * $P < 0.05$ for NGT vs. type 2 diabetic subjects.

($\text{VO}_{2\text{max}}$) was significantly lower in the type 2 diabetic group (Table 1).

Glycolytic and oxidative enzyme activities in the muscle homogenate

SDH and LDH activities were measured in muscle homogenates to assess oxidative (SDH) and glycolytic (LDH) activity in the whole muscle sample. The same samples were used for measurements of enzyme

activities in individual fibers. SDH activity was diminished by 16.3% and LDH activity was increased by 47.5% in homogenates from skeletal muscles of patients with type 2 diabetes compared with control subjects.

Fiber type composition

Needle biopsy specimens of vastus lateralis muscle were analyzed for fiber type

composition. Two different characterization systems were used to classify slow oxidative, fast oxidative glycolytic, and fast glycolytic fibers (Fig. 1). For subsequent analyses, we used the physiological-metabolic classification of slow oxidative, fast oxidative glycolytic (I + II), and fast glycolytic fiber types. Vastus lateralis muscle is a mixed muscle composed of slow oxidative, fast oxidative gly-

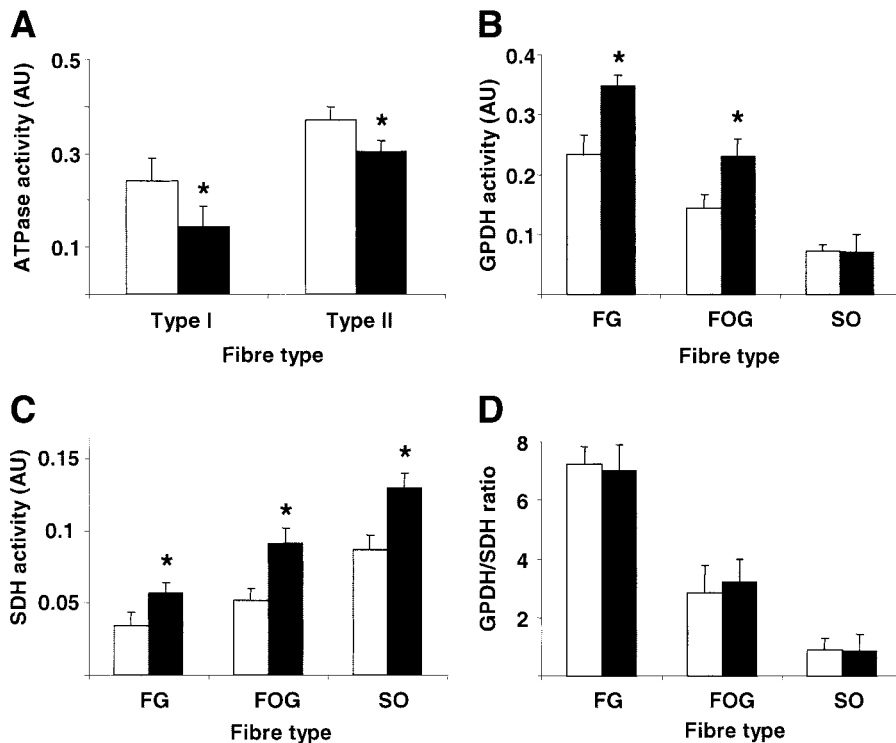


Figure 2— Skeletal muscle fiber type-associated enzyme activities for ATPase (A), GPDH (B), SDH (C), and ratio of GPDH to SDH activities in healthy volunteers with normal glucose tolerance (\square ; $n = 15$) and patients with type 2 diabetes (T2D; \blacksquare ; $n = 10$) (D). * $P < 0.05$ for NGT vs. type 2 diabetic subjects. AU, arbitrary units; FG, fast glycolytic; FOG, fast oxidative glycolytic; SO, slow oxidative.

colytic, and fast glycolytic fibers. In patients with type 2 diabetes, the slow oxidative fiber fraction was reduced by 16%, whereas the fast glycolytic fiber fraction was increased by 49% (Fig. 1B).

Fiber type-related enzyme activities

In skeletal muscle from patients with type 2 diabetes, ATPase activity was significantly reduced by 18.4% in type I and 9.2% in type II fibers (Fig. 2A). Increased GPDH activity in fast glycolytic and fast oxidative glycolytic fibers in skeletal muscle from type 2 diabetic patients indicates increased glycolytic activity, pertaining specifically to fast fibers (Fig. 2B). There were significant correlations of GPDH activity with A1C values ($r^2 = 0.1$, $P < 0.05$) and glucose infusion rate during the steady state of euglycemic-hyperinsulinemic clamps ($r^2 = 0.1$, $P < 0.05$). SDH activity, as a measure of oxidative capacity, was significantly increased in all fiber types in the type 2 diabetic group compared with the control group (Fig. 2C). In addition, SDH activity was significantly correlated with A1C values ($r^2 = 0.2$, $P < 0.001$) and glucose infusion rate during the steady state of euglycemic-hyperinsulinemic clamps ($r^2 = 0.15$, $P < 0.01$). The

fiber-specific glycolytic-to-oxidative ratio was determined by the ratio of the GPDH to SDH activity. In all fiber types, there was no significant difference in the GPDH-to-SDH ratio between the type 2 diabetic and NGT groups (Fig. 2D).

Correlation of contractility with measures of glucose metabolism and insulin sensitivity

Using ATPase activity as a marker for contractility, we investigated whether chronic hyperglycemia and insulin resistance in patients with type 2 diabetes is associated with alterations in contractility. We found a significantly negative correlation between A1C values and ATPase activity in type I fibers (Fig. 3A) and type II fibers ($r^2 = 0.19$, $P < 0.01$). In addition, ATPase activity in type I fibers significantly correlates with glucose infusion rate during the steady state of euglycemic-hyperinsulinemic clamps (Fig. 3B) and the maximal aerobic capacity (VO_{2max}) (Fig. 3C). Similar results were obtained for the relationships between ATPase activity in type II fibers and insulin sensitivity and VO_{2max} .

Multivariate linear regression analysis of percent body fat, A1C values, and glu-

ucose infusion rate during the steady state of the euglycemic-hyperinsulinemic clamp as predictors for ATPase activity in type I fibers revealed A1C values and glucose infusion rate during the clamp as significant determinants of ATPase activity.

CONCLUSIONS— In the present study, we extend previous findings that glycolytic capacity is higher and oxidative capacity is reduced in skeletal muscle of patients with type 2 diabetes (5,6,13,14) by demonstrating that metabolic alterations in skeletal muscle of type 2 diabetic patients are a consequence of both changes in fiber composition and in fiber-specific metabolism. Moreover, chronic hyperglycemia and insulin resistance were identified as significant determinants of diminished contractility of skeletal muscle in type 2 diabetes.

Human skeletal muscle consists of a mixed fiber-type composition (15), and therefore altered enzyme activity patterns cannot be related to fiber-specific changes when determined in muscle homogenates. In accordance with our data from muscle homogenates, reduced oxidative enzyme activity and increased glycolytic-to-oxidative enzyme activity has been a consistent finding in skeletal muscle from patients with type 2 diabetes (14,16). As a potential explanation for the decreased oxidative activity, a reduction in the proportion of type I fibers has been suggested (17,18). In accordance with these studies, we found a significant reduction in the proportion of slow oxidative fibers and a significant increase in the proportion of fast glycolytic fibers in muscle from type 2 diabetic patients. Thus, our results further suggest that changes in fiber type composition contribute to the increased glycolytic capacity and reduced oxidative capacity of skeletal muscles of type 2 diabetic patients. This is in contrast to the results of He et al. (7), who could not find significant differences in fiber composition between lean, obese, and type 2 diabetic subjects.

In addition to the altered composition of skeletal muscle in type 2 diabetic patients, we detected significant changes in fiber metabolism. The results of muscle fiber analysis in our study are consistent with previous studies on the fiber type-specific enzyme activity pattern (7,15). Surprisingly, we found a parallel significant increase of oxidative (SDH) and glycolytic (GPDH) enzyme capacity in different fibers from muscle of type 2 diabetic patients. This result is in contrast

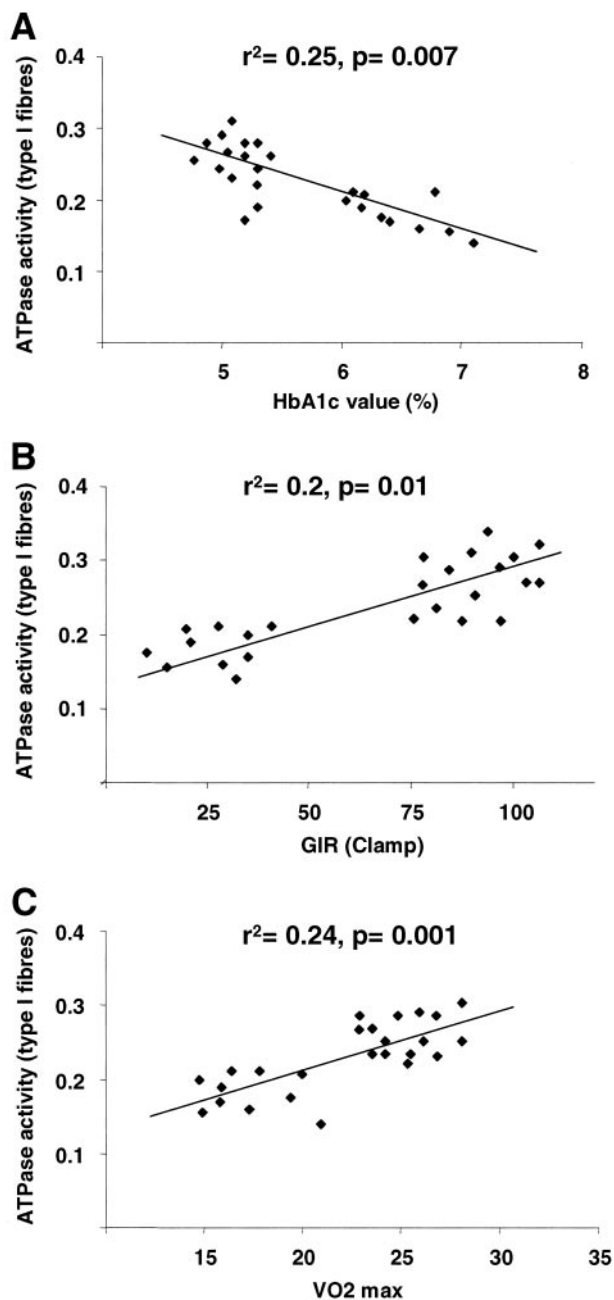


Figure 3— Correlation of ATPase activity in type I fibers with (A) A1C values, (B) glucose infusion rate (GIR) during the steady state of a euglycemic-hyperinsulinemic clamp and (C) VO_{2max} .

with a previous study showing decreased oxidative and unchanged glycolytic enzyme activity, subsequently leading to an increased glycolytic-to-oxidative ratio in patients with type 2 diabetes (7). In our study, the glycolytic-to-oxidative ratio was not significantly different between the NGT and type 2 diabetic subjects. One potential explanation for the divergent results could be the different fiber classification system. The results of He et al. (7) are based on the ATPase classifica-

tion system. Because of overlapping SDH activities of the ATPase fiber types, these results are difficult to compare with our metabolic fiber characterization. Moreover, the contradictory results could be due to different characteristics of the patients with type 2 diabetes in this study. In particular, differences in the antidiabetic treatment, age, degree of chronic hyperglycemia, and duration of diabetes or concomitant diseases could explain the different results in our and the previous

study (7). Therefore, the role of these potentially confounding factors of the glycolytic-to-oxidative ratio in patients with type 2 diabetes needs to be further investigated in more detail. Despite the increased oxidative capacity in individual muscle fibers, we observed, in accordance with previous studies (5,14,16), diminished oxidative enzyme activity in muscle homogenates of type 2 diabetic patients. Therefore, in our type 2 diabetic patients, a reduction in oxidative enzyme activity in the whole muscle is most likely due to the decreased percentage of oxidative fibers and is not caused by changes in the enzyme activity of individual fibers. However, the mechanisms for the reduced oxidative enzyme activity and the reduction in slow oxidative fibers in patients with type 2 diabetes need to be more clearly defined. One potential explanation for the reduced oxidative enzyme activity could be a disturbed balance between muscle lipid content and enzymatic capacity for substrate oxidation in subjects with type 2 diabetes. Muscle fibers from type 2 diabetic subjects were found to contain more intramyocyte triglycerides (2,5,16). In contrast to the consistent ratio of muscle triglycerides to oxidative enzyme activity in lean subjects, this proportionality is substantially different in obese subjects with type 2 diabetes, suggesting that a putative regulatory mechanism between muscle lipid content and substrate oxidation is impaired in muscle of patients with type 2 diabetes (7).

However, until now it has not been known whether alterations in oxidative and glycolytic enzyme activity in skeletal muscle of patients with type 2 diabetes are related to metabolic alterations including chronic hyperglycemia and insulin sensitivity. We found a significant correlation between SDH activity of slow oxidative, fast oxidative glycolytic II and I fibers, and A1C values and the degree of insulin sensitivity. We therefore postulate that altered enzyme activity is a compensatory mechanism of the diabetic muscle in response to altered glucose metabolism and/or insulin resistance. Slow oxidative fibers are characterized by high oxidative metabolism and mediate the endurance capacity of skeletal muscle, whereas the fast oxidative glycolytic fibers have higher glycolytic enzyme activity. It has been suggested that slow oxidative fibers are more insulin sensitive than fast oxidative glycolytic fibers (7). These differences in insulin sensitivity between slow oxidative and fast oxidative glycolytic fibers dem-

onstrate the clinical importance of reduced slow oxidative fibers in patients with type 2 diabetes found in our study. Thus, a reduced proportion of slow oxidative fibers could at least contribute to insulin resistance in patients with type 2 diabetes. However, further studies are required to dissect whether changes in fiber-specific enzyme activity are primarily caused by hyperglycemia or decreased insulin sensitivity.

We demonstrate reduced ATPase activity, a marker of reduced contractility (19), in both type I and type II fibers of diabetic skeletal muscle. Moreover, ATPase activity significantly correlates with the degree of insulin sensitivity and the individual fitness level as measured by the maximal aerobic capacity (VO_{2max}). The negative correlation between ATPase activity and A1C values further indicates a close relationship between altered enzyme activity in skeletal muscle of type 2 diabetes patients and long-term glycemic control.

In summary, our results suggest that decreased oxidative enzyme activity in skeletal muscle of patients with type 2 diabetes could be due to a reduced proportion of slow oxidative fibers rather than to diminished oxidative activity in individual fibers. Increased glycolytic and oxidative enzyme activities in individual muscle fibers are closely related to measures of long-term glycemic control and whole-body insulin sensitivity and could therefore represent a compensatory mechanism of the muscle in the function of the altered glucose metabolism.

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