Muscle Fiber Composition and Capillary Density in Women and Men With NIDDM

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OBJECTIVE — To determine whether muscle fiber composition and capillary density differed between diabetic and nondiabetic subjects.

RESEARCH DESIGN AND METHODS — Muscle fiber composition and capillary density were determined in biopsies from women and men with non-insulin-dependent diabetes mellitus (NIDDM) and compared with those of control subjects matched for gender, age, obesity, and the waist-to-hip ratio, which are all factors known to influence muscle morphology.

RESULTS — Patients with NIDDM, as well as control subjects with abdominal obesity and insulin resistance, showed the same abnormalities in muscle morphology, namely, a low percentage of type I fibers, elevated type II (particularly type IIB) fibers, and a low capillary density. These changes correlated closely with insulin concentrations in both diabetic and nondiabetic groups.

CONCLUSIONS — Recent information suggests that insulin may regulate myosin synthesis in muscle in the direction of the changes observed. Therefore, it is possible that muscle fiber composition abnormalities in insulin-resistant conditions are secondary to hyperinsulinemia. However, the low capillary density, hypothetically, may contribute to insulin resistance.

Insulin resistance is considered a cornerstone in the development of non-insulin-dependent diabetes mellitus (NIDDM) (1), and a major site for insulin resistance is muscle tissue (2). Insulin sensitivity in muscle is regulated by a complex interplay between the insulin receptor (which signals postreceptor events, such as the glucose transporter system), glycolysis, and glycogen synthesis. Results of studies that were performed mainly in rats have indicated that insulin receptor density varies in different muscles. A high density has been found in muscles with predominantly oxidative, slow twitch, red (type I) fibers, such as the soleus muscle; muscles with mainly glucolytic, fast twitch, white (type II) fibers, such as the white part of the gastrocnemius head, have a low insulin receptor density (3). Insulin sensitivity of the muscles is apparently parallel to the density of insulin receptors (4).

In the human, muscle fiber composition is more mixed than in rats (5). However, in conditions with insulin resistance in muscle, a shift in muscle fiber composition has been demonstrated, with a higher proportion of white fibers at the expense of red fibers (5–7). Therefore, in analogy with the data obtained in rats, it has been considered that the insulin resistance in muscle may be due to a low density of insulin receptors.

Another morphological characteristic of insulin-resistant muscles in humans and rats is a low density of capillaries, often in parallel with the fiber composition abnormalities (5–7). Capillary endothelium has been shown to bind insulin (8), and insulin uptake and function has been found to be dependent on capillary density (9) and function (10) in muscles. Furthermore, lymph concentrations of insulin, presumably equivalent to extravascular, extracellular concentrations, are more tightly correlated to glucose uptake in muscle than are the concentrations in circulation (11). Taken together, these observations might mean that capillary endothelium provides a barrier for the interaction of insulin with its cellular receptor.

NIDDM in humans is often associated with muscular insulin resistance (2). The morphological characteristics of muscle in those with NIDDM, however, have not been examined. Considering the background above, we decided it would be of interest to perform such a study.

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NIDDM, non-insulin-dependent diabetes mellitus; BMI, body mass index; WHR, waist-to-hip ratio; ANOVA, analysis of variance.

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Table 1—*Age, anthropometric variables, and fasting plasma insulin and blood glucose values in diabetic and nondiabetic women*

	Women with low WHR	Women with high WHR	Women with diabetes
n	22	19	14
Age (years)	48 ± 4	48 ± 4	51 ± 4
BMI (kg/m^2)	32.7 ± 0.4	33.1 ± 0.6	30.5 ± 0.8
WHR	0.80 ± 0.02	0.97 ± 0.02	0.95 ± 0.02
Insulin (mU/L)	10.1 ± 1.1	16.1 ± 2.3	17.0 ± 3.1
Glucose (mM)	5.9 ± 0.9	6.0 ± 0.7	11.0 ± 0.8

Data are means ± SE. ANOVA: Age and BMI: NS; WHR and insulin: $P < 0.05$, low WHR < high WHR and diabetes; Glucose: $P < 0.01$, diabetes > low and high WHR.

RESEARCH DESIGN AND METHODS

— The women examined were recruited from outpatient clinics and by advertisements in a local newspaper. Fourteen women with diabetes were included, as were 41 women without diabetes but with a comparable degree of obesity as defined by the body mass index (BMI, weight/height squared, kg/m^2). The latter were divided into smaller groups with low and high waist-to-hip ratio (WHR) by dividing the large group after the median WHR value, which resulted in groups with WHR of 0.80 ± 0.02 and 0.97 ± 0.02, respectively.

Men were recruited similarly, and 15 men with NIDDM and 48 control men were included. The men with diabetes are a subgroup of those reported on in another study (this issue, B. Andersson et al., p. 405–411) and constitute those who volunteered for muscle biopsy.

Patients had NIDDM diagnosed at least 6 months before examinations and were recommended a diet with a 20:40:40% distribution of macronutrients of proteins, fat, and carbohydrate, respectively. The diet was prescribed by a dietitian. The men were examined every third month by a physician, who considered their diabetes to be under control.

All subjects had a stable weight (<3 kg change) within the preceding 6 months as judged by body weight recordings performed by the participants and at the laboratory. Nobody took any drugs of known importance to the study. Other characteristics are found in Tables 1 and 2.

Anthropometry, blood glucose, and plasma insulin concentrations were determined as described in the adjacent study (this issue, B. Andersson et al., p. 405-411).

A muscle biopsy was performed from the lateral part of the vastus lateralis muscle with a conchotome under local anesthesia. The sample was trimmed, mounted, and frozen in isopentane, which was cooled by liquid nitrogen, and then kept at −80°C. Several cross-sections of 10 μm thick were cut with a cryotome at −20°C and stained for morphological examinations of muscle fibers and capillaries. Myofibrillar ATPase activity was identified by alkaline preincubation at pH 10.3, after which the reactions were performed at pH 9.4 (12). Fibers were classified into slow twitch (type I) and fast twitch (type II) fibers. The fast twitch fibers were then further classified into fast twitch A and fast twitch B fibers by preincubation at pH 4.6 and 4.4, respectively (13). In each sample, ~500 fibers were counted. Amylase periodic acid Schiff staining was used for capillary visualization (14). A total of ~100 capillaries was counted in each sample. Analyses were performed blindly.

Statistical analysis

Analysis of variance (ANOVA) was used for comparisons between more than two groups. For other statistics, Student's *t* test and conventional regression analyses were performed using the Statview program of the Macintosh system. $P < 0.05$ was considered significant.

RESULTS

— Characteristics of the women are found in Table 1. Age was not different between diabetic and control women. WHR was, by definition, lower in the low WHR group than in the high WHR group. The diabetic women were not different from the high WHR group but had a higher WHR than women with low WHR. The same differences were found for insulin. Glucose was higher in the diabetic women than in the two other groups, which were not different. Similar data of the men are found in Table 2. Age, BMI, and WHR did not differ between nondiabetic and diabetic men, but insulin

Table 2—*Age, anthropometric variables, and fasting plasma insulin and blood glucose values in nondiabetic and diabetic men*

	Nondiabetic men	Diabetic men	*P* value
n	48	15	—
Age (years)	50.4 ± 2.0	54.2 ± 2.0	NS
BMI (kg/m^2)	28.9 ± 0.6	27.9 ± 0.7	NS
WHR	0.96 ± 0.01	0.98 ± 0.02	NS
Insulin (mU/L)	10.6 ± 1.1	18.0 ± 0.6	<0.01
Glucose (mM)	5.3 ± 0.9	10.1 ± 0.7	<0.001

Data are means ± SE.

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Table 3—*Muscle morphology data in women*

	Women with low WHR	Women with high WHR	Women with diabetes
n	22	19	14
Fiber composition (%)			
Type I	50.8 ± 3.4	41.4 ± 3.2*	42.3 ± 4.1†
Type II	43.2 ± 2.3	58.6 ± 3.4*	57.7 ± 4.1‡
Type IIA	34.4 ± 1.9	31.7 ± 1.6	29.3 ± 2.7
Type IIB	8.8 ± 2.4	26.9 ± 1.9§	28.0 ± 3.0‖
Capillaries			
Fiber area supplied by one capillary (μm^2)			
Type I	0.87 ± 0.06	0.95 ± 0.07¶	0.99 ± 0.07‡
Type II	0.74 ± 0.08	0.80 ± 0.06¶	0.88 ± 0.05‡
Type IIA	0.79 ± 0.07	0.84 ± 0.04	0.87 ± 0.04‡
Type IIB	0.80 ± 0.05	0.85 ± 0.05¶	0.96 ± 0.06#
Number of capillaries around muscle			
Type I	4.88 ± 0.20	4.42 ± 0.21	4.10 ± 0.21‡
Type II	4.20 ± 0.22	3.59 ± 0.22[a]	3.40 ± 0.22‡
Type IIA	4.56 ± 3.90	3.90 ± 0.23¶	3.18 ± 0.22‡
Type IIB	3.94 ± 0.20	3.40 ± 0.21¶	2.97 ± 0.23#

Data are means ± SE. Capillaries were examined in 14 women in the low-WHR group, 14 women in the high-WHR group, and 12 women in the diabetic group. ANOVA: *$0.05 < P < 0.10$ compared between low and high WHR. †$0.05 < P < 0.10$ compared between low WHR and diabetes. ‡$P < 0.05$ compared between low WHR and diabetes. §$P < 0.001$ compared between low and high WHR. ‖$P < 0.001$ compared between low WHR and diabetes. ¶$P < 0.05$ compared between low and high WHR. #$P < 0.01$ compared between low WHR and diabetes. [a] $P < 0.01$ compared between low and high WHR.

and glucose values were higher in the latter.

Muscle morphology data for the women are found in Table 3. In comparisons between women with low and high WHR, the latter showed trends ($P < 0.1 > 0.05$) toward having lower type I fibers and higher type II fibers, fully significant for type IIB fibers. Fiber area supplied by one capillary was significantly larger for fibers type I, II, and IIB in women with high WHR, and the number of capillaries around each fiber was lower for all except type I fibers. In comparisons between the data of the diabetic women and women with a low WHR, the same significant differences were found, but they appeared to be more pronounced; in addition, there was a significantly larger fiber area for supply by one capillary for fiber type IIA, and the number of capillaries around type I fibers.

Table 4 shows the muscle morphology results in the men. Diabetic men had significantly fewer type I fibers but had more type II and type IIB fibers than did control subjects. The number of capillaries around fibers was significantly lower in diabetic men for each of the fiber types.

Correlation analyses between muscle morphology and insulin values revealed significant negative relationships between insulin and type I fibers and positive relationships between type II, particularly type IIB fiber (*r* values: 0.40–0.60, $P < 0.05$–0.01) in both men and women. No significant correlations with blood glucose were noted. Furthermore, in women, the number of capillaries around each type of muscle fiber correlated negatively with insulin and blood glucose (*r* values: −0.45, $P < 0.01$). Similar correlations were found when examining the fiber area supplied by one capillary, although the directions of the correlations were positive (not shown). In men, significant negative correlations were observed between insulin and capillarization (*r* values: −0.50–0.74, $P < 0.05$–0.001).

CONCLUSIONS— In this study, muscle morphology in both men and women was compared between NIDDM and control subjects. To obtain muscle morphology characteristic for NIDDM, it was considered necessary to make comparisons between groups matched not only for gender but also for age, obesity, and body fat distribution, which influence muscle morphology (5–7). NIDDM women had less red and more white fibers and a low capillarization in comparison with nondiabetic women matched for BMI, but who have lower WHR. Women with a low WHR had similar muscle histochemistry to nonobese control women (5). The same findings, although not as pronounced, applied to obese women with elevated WHR, as described previously (6,7). Men with NIDDM showed the same muscle morphology abnormalities compared with control men who had similar BMI and WHR values.

Plasma insulin values were higher in NIDDM patients and women with ab-

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Table 4—*Muscle morphological variables in nondiabetic and diabetic men*

	Nondiabetic men	Diabetic men
n	48	15
Fiber composition (%)		
Type I	48.3 ± 3.4	33.5 ± 4.0*
Type II	51.7 ± 4.2	66.4 ± 4.0*
Type IIA	32.5 ± 2.7	35.0 ± 4.1
Type IIB	19.2 ± 2.8	29.4 ± 5.7*
Number of capillaries around muscle fibers		
Type I	4.92 ± 0.19	4.12 ± 0.20*
Type II	4.40 ± 0.20	3.50 ± 0.20*
Type IIA	4.60 ± 0.19	3.20 ± 0.22*
Type IIB	3.90 ± 0.20	2.96 ± 0.22*

Data are means ± SE. *$P < 0.05$.

dominal obesity than in obese women with low WHR and control men. The muscle morphology correlated with the insulin values, which suggests that the abnormalities seen in NIDDM are related to the insulin resistance prevailing in these conditions.

As mentioned in the introduction, muscle fiber composition has been considered to be indicative of the insulin sensitivity of the muscle in question because, in the rat, muscle fiber composition and insulin receptor density are closely coupled statistically (3,4). Statistical associations, however, do not provide any information of causality, and the cause-effect relationship may well be the reverse, with insulin concentrations being the primary factor. This chain of events appears more likely because it does not seem logical that insulin receptor density should be tightly coupled to the contractile elements in muscle, the myosins that provide the basis for fiber typing. Furthermore, several unrelated conditions are followed by a similarly low type I:type II fiber ratio, such as excess of cortisol (15), hyperandrogenicity in women (7), physical inactivity (5), and low testosterone in male rats (16). All of these conditions, however, are characterized by hyperinsulinemia. We have recently studied the effects of chronic hyperinsulinemia on muscle morphology and insulin sensitivity in rats. Exposure to moderately elevated insulin levels for 7 days was followed by a diminution of the number of type I fibers, while the proportion of type II fibers increased. This change was associated with an increased insulin sensitivity, systemically and in muscles (17). These data demonstrate, first, that hyperinsulinemia is followed by a decrease of the type I:type II ratio of muscle fibers, and second, that this is not always followed by insulin resistance. It thus appears likely that insulin resistance with accompanying hyperinsulinemia is followed by muscle fiber changes. This idea would explain the tight correlations between a low type I:type II fiber ratio and hyperinsulinemia in several unrelated conditions and is in good agreement with the close correlations reported in this study between plasma insulin values and type I fibers (negative) and type II fibers (positive) in diabetic and nondiabetic women and men.

As briefly reviewed in the introduction, considerable evidence now suggests that the capillary bed of muscles is involved in the regulation of insulin sensitivity of that tissue by providing an intermediary pool of bound insulin. A low capillarization density, therefore, presumably would diminish the capacity of such a pool and might contribute to an apparent insulin resistance. The statistical correlations between insulin and capillarization found among diabetic subjects in this study, as well as in other insulin-resistant conditions previously (6,7), is in accord with such a possibility. This chain of events is further supported by the increased insulin sensitivity found in conditions with an elevated number of muscle capillaries, such as in physically trained subjects (5) and in subjects with early hyperinsulinemia (17).

Correlation analyses were also performed with the values of testosterone and sex hormone binding globulins, which is reported in the adjacent paper (this issue, B. Andersson et al., p. 405–411), for the subjects participating in both studies. Testosterone was directly and sex hormone binding globulins indirectly related to a low type I:II fiber ratio (not shown). This relationship may be mediated, however, via the association between androgen status and insulin resistance.

Hyperandrogenicity in the women was also indirectly related to capillary density (not shown). Androgen administration to female rats is followed by a diminished capillary density with closely associated insulin resistance (18). Furthermore, recent experiments have shown a diminished capillary blood flow in such rats (A. Holmäng, P.B., unpublished observations). Because hyperinsulinemia does not seem to reduce capillarization of muscle (17), these data, taken together, suggest the possibility that androgens might be involved in the regulation of synthesis of capillary endothelium and, therefore, of blood flow and insulin sensitivity of muscles.

In summary, women and men with NIDDM have an abnormal muscle morphology, with a low number of type I fibers and an elevated number of type II fibers, as well as a low capillary density that closely correlates with plasma insulin concentrations. Based on recently obtained information in other studies, we suggest that the fiber composition changes, equivalent with shifts in myosin composition, may follow hyperinsulinemia, while the low capillarization might

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contribute to the insulin resistance of NIDDM patients.

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