Strength Training Increases Insulin-Mediated Glucose Uptake, GLUT4 Content, and Insulin Signaling in Skeletal Muscle in Patients With Type 2 Diabetes

Mads K. Holten,[1,2] Morten Zacho,[2] Michael Gaster,[3] Carsten Juel,[2,4] Jørgen F.P. Wojtaszewski,[2,5] and Flemming Dela[1,2]

Strength training represents an alternative to endurance training for patients with type 2 diabetes. Little is known about the effect on insulin action and key proteins in skeletal muscle, and the necessary volume of strength training is unknown. A total of 10 type 2 diabetic subjects and 7 healthy men (control subjects) strength-trained one leg three times per week for 6 weeks while the other leg remained untrained. Each session lasted no more than 30 min. After strength training, muscle biopsies were obtained, and an isoglycemic-hyperinsulinemic clamp combined with arterio-femoral venous catheterization of both legs was carried out. In general, qualitatively similar responses were obtained in both groups. During the clamp, leg blood flow was higher ($P < 0.05$) in trained versus untrained legs, but despite this, arterio-venous extraction glucose did not decrease in trained legs. Thus, leg glucose clearance was increased in trained legs ($P < 0.05$) and more than explained by increases in muscle mass. Strength training increased protein content of GLUT4, insulin receptor, protein kinase B-α/β, glycogen synthase (GS), and GS total activity. In conclusion, we found that strength training for 30 min three times per week increases insulin action in skeletal muscle in both groups. The adaptation is attributable to local contraction-mediated mechanisms involving key proteins in the insulin signaling cascade. *Diabetes* 53:294–305, 2004

From the [1]Department of Medical Physiology, the Panum Institute, Faculty of Health Sciences, University of Copenhagen, Copenhagen, Denmark; the [2]Copenhagen Muscle Research Centre, Rigshospitalet, Copenhagen, Denmark; the [3]Departments of Endocrinology and of Pathology, Odense University Hospital, Odense, Denmark; the [4]August Krogh Institute, Copenhagen, Denmark; and the [5]Institute of Exercise and Sport Sciences, Faculty of Natural Sciences, University of Copenhagen, Copenhagen, Denmark.

Address correspondence and reprint requests to Flemming Dela, MD, Department of Medical Physiology, the Panum Institute, University of Copenhagen, Blegdamsvej 3, DK 2200 Copenhagen N, Denmark. E-mail: f.dela@mfi.ku.dk.

Received for publication 7 July 2003 and accepted in revised form 17 October 2003.

CS, citrate synthase; FFA, free fatty acid; G6P, glucose-6-phosphate; GS, glycogen synthase; HAD, hydroxyacyl-3-dehydrogenase; HRP, horseradish peroxidase; IRS-1, insulin receptor substrate-1; LDH, lactate dehydrogenase; PI, phosphatidylinositol; PKB, protein kinase B; PMSF, phenylmethylsulfonyl fluoride; PVDF, polyvinylidiene diflouride; RM, repetition maximum.

It is an established finding that aerobic endurance training increases insulin action in patients with type 2 diabetes (1–9), and also that the effect of training is predominantly located to the skeletal muscle (10). Glycemic control also improves along with training (11). Furthermore, with the increased insulin action, the need for insulin to mediate the clearance of a given amount of glucose is lessened. Thus, the need for exogenous insulin or oral hypoglycemic agents is decreased (12). Apart from the beneficial effects on glucose metabolism, physical training also exerts marked improvement on most of the components of the metabolic syndrome (13).

Despite the scientific evidence of the therapeutic effect of exercise training, it is a well-known clinical experience that it is often very difficult to engage the patients into taking exercise on a regular basis, and even if one succeeds, the adherence is disappointing. The majority of patients with type 2 diabetes are overweight and have usually been sedentary for the major part of their lives. For many reasons, both psychological and sociological, they are not likely to take up endurance training. Obesity may even be a physical problem in the performance of exercise, especially endurance-type exercises.

For patients with type 2 diabetes, resistance training probably represents an attractive exercise modality, but little is known about the overall effect, and the effect in muscle has not been studied. Furthermore, dose-response studies on resistance training effects have not been carried out. To provide support for the recommendations about the type and intensity of effective exercise, we have now carried out a study where we investigated the effect of a very low amount of strength training on insulin action in the skeletal muscle in patients with type 2 diabetes. Based on the sparse literature available on strength training regimens in these patients (14–19), we used a training program that we a priori considered to be minimally effective. We used a one-legged training protocol, a model that is robust against biological variation and that has previously been used to demonstrate the effect of endurance training on skeletal muscle insulin sensitivity (10).

Second, we obtained muscle biopsies from both legs and analyzed these for differences in content and activities of proteins and enzymes that could explain a possible effect of strength training.

TABLE 1
Subject characteristics

	Normal reference interval	Type 2 diabetes (n = 10)		Control (n = 7)	
		Before training	After training	Before training	After training
Blood glucose (mmol/l)	3.5–5.5	8.2 ± 0.7	7.9 ± 0.9	4.7 ± 0.2†	4.7 ± 0.3†
HbA_{1c}	4.1–6.4	7.6 ± 0.3	7.4 ± 0.4	6.0 ± 0.2†	6.0 ± 0.2†
Serum insulin (pmol/l)	5–69	48 ± 7	72 ± 17	28 ± 5	39 ± 5†
Serum C-peptide (pmol/l)	200–700	876 ± 96	1,019 ± 115	536 ± 65†	664 ± 63†
Cholesterol (mmol/l)	4.3–7.7	5.6 ± 0.3	5.4 ± 0.3	5.6 ± 0.4	5.4 ± 0.5
HDL cholesterol (mmol/l)	1.1–1.8	1.5 ± 0.1	1.4 ± 0.1	1.4 ± 0.1	1.3 ± 0.1
LDL cholesterol (mmol/l)	2.7–4.1	3.4 ± 0.2	3.3 ± 0.2	3.7 ± 0.4	3.6 ± 0.4
VLDL cholesterol (mmol/l)	5–10%*	0.59 ± 0.04	0.61 ± 0.07	0.46 ± 0.05†	0.51 ± 0.11
Triglycerides (mmol/l)	0.5–2.2	1.5 ± 0.3	1.4 ± 0.1	0.9 ± 0.1†	1.0 ± 0.3
Aspartate aminotransferases (U/l)	≤50	31 ± 5	36 ± 6	25 ± 1	26 ± 1
Alkaline phosphatase (U/l)	80–275	160 ± 14	157 ± 9	153 ± 16	154 ± 12
γ-Glutamyltransferase (U/l)	≤90	124 ± 48	125 ± 43	37 ± 12†	30 ± 7†
Pancreatic amylase (U/l)	30–190	123 ± 27	124 ± 29	85 ± 7	81 ± 7
Albumin (g/l)	36.6–48.2	42 ± 1	42 ± 1	42 ± 1	42 ± 1
Carbamide (mmol/l)	2.5–7.5	5.3 ± 0.4	5.9 ± 0.4	5.6 ± 0.6	4.8 ± 0.5
Creatinine (μmol/l)	60–130	93 ± 4	94 ± 4	94 ± 4	95 ± 3

Blood biochemistry is fasting values measured in plasma, except glucose, which is measured in whole blood, and insulin and C-peptide, which were measured in serum. *Of cholesterol; †$P < 0.05$ (type 2 diabetic vs. control subjects at corresponding time).

RESEARCH DESIGN AND METHODS

Subjects and experimental protocol. We recruited 10 Caucasian patients with type 2 diabetes with fasting plasma glucose concentrations (means ± SE) of 8.4 ± 0.6 mmol/l and 7 Caucasian healthy control subjects with fasting plasma glucose concentrations of 5.6 ± 0.1 mmol/l and without a family history of type 2 diabetes. The study was approved by the ethical committee of Copenhagen and Frederiksberg (reference no. KF 01-204/99). Time since diagnosis of type 2 diabetes ranged from 2 to 11 years. All of the patients were treated with diet recommendations, and in addition some patients were treated with tolbutamide 1,000 mg/day (n = 2), glibenclamide 7 mg/day (n = 1), metformin 1,700 mg/day (n = 1), amlodipin 5 mg/day (n = 1), and cerivastatin 200 μg/day (n = 1). On the experimental day, no medication was taken. None of the control subjects took any medication. The diabetic patients were similar to the control subjects with respect to age (62 ± 2 vs. 61 ± 2 years) and body weight (85 ± 5 vs. 78 ± 3 kg), but height (172 ± 1 vs. 178 ± 2 cm, $P < 0.05$) was higher in control subjects compared with type 2 diabetic subjects. Thus, BMI was different ($P < 0.05$) between control (24.5 ± 0.8 kg/m^2) and type 2 diabetic (28.3 ± 1.2 kg/m^2) subjects. Resting arterial blood pressure covered a wide range and is therefore given as 25% quartile, median, and 75% quartile as follows: for control subjects, values were 133, 146, and 159 mmHg for systolic blood pressure and 69, 73, and 76 mmHg for diastolic blood pressure; for type 2 diabetic subjects, values were 136, 154, and 171 mmHg for systolic pressure and 70, 79, and 92 mmHg for diastolic pressure. Thus, both groups included normo- and hypertensive subjects. Other characteristics of the subjects are given in Table 1.

In one of the healthy control subjects, femoral venous catheterization in one leg proved to be very difficult, and for ethical reasons it had to be given up. Nevertheless, the experiment was continued without a venous catheter in one leg. Thus, paired comparisons between the legs could not be performed and therefore n = 6 for all leg balance data.

Training program. All subjects participated in a 6-week strength training program. The focus of the program was to have one leg perform strength training exercises while the other leg remained sedentary. The leg to be trained was chosen by drawing lots.

Training sessions were all supervised and took place three times a week, with each training session lasting no more than 30 min. This included time for warm-up, which were light exercises for the upper body plus a warm-up set of 10–12 repetitions with a light load in each leg followed by a 2-min rest period. During the first and the last training session, the subject's 3 repetition maximum (3-RM) were measured. We calculated 1-RM as 106% of the measured 3-RM for each leg exercise (leg press, knee extension, and hamstring curl).

During the first 2 weeks of the exercise, the subjects performed three sets of 10 repetitions, utilizing a load equivalent to 50% of 1-RM. During weeks 3–6, the subjects performed four sets of 8–12 repetitions utilizing 70–80% of 1-RM. During the last 2 weeks of the 6-week period, the load was adjusted so that all sets were exhaustive within 8–12 repetitions. The subjects rested for >90 s between sets and for >2 min between lifting stations. For further details of the leg resistance training program, see Table 2.

Experimental procedures. At 16 h after the last training session (i.e., in the trained state, "between" sessions), an isoglycemic-hyperinsulinemic clamp combined with arterio-venous catheterization of both legs was performed. Having fasted since midnight, the subjects arrived in the laboratory in the morning. Leg volume was measured by water displacement, and thigh circumference (20 cm proximal to the patella) was measured. The subjects were weighed and had their height measured, and then they were placed in bed. Electrocardiogram and heart rate were monitored by precordial electrodes. A catheter was inserted in a medial cubital vein for infusions of insulin and glucose (20%), and an arterial cannula was inserted in the radial or brachial artery for sampling of blood and continuous monitoring of blood pressure. In both femoral veins, Teflon catheters were inserted for sampling of blood and measurements of leg blood flow (thermodilution technique) as previously described (20).

After basal measurements, a two-step sequential euglycemic-hyperinsulinemic clamp was started. For each subject, a 50-ml insulin infusate had been prepared for each clamp step from insulin (100 IU/ml Actrapid; Novo Nordisk, Copenhagen, Denmark), saline, and 2.5 ml of the subject's own plasma. At each clamp step, insulin was given as a 2-ml bolus followed by constant infusion (rates of 28 and 480 mU · min^{-1} · m^{-2}, respectively) for 120 min each. Plasma glucose was maintained at isoglycemia, i.e., the glucose concentration was kept at individual fasting plasma glucose concentrations throughout the clamp by frequent arterial blood samples analyzed on an automatic glucose analyzer (YSI 2300; Yellow Springs Instruments, Yellow Springs, OH), with

TABLE 2
One-legged training program

	Week				
Exercise	1–2	3	4	5	6
Leg press					
Sets	3	4	4	4	4
Repetitions	10	10	10	12	10
Working loads (RM)	20	12	12	12	10
Knee extension					
Sets	3	4	4	4	4
Repetitions	10	8	8	10	8
Working loads (RM)	20	12	10	10	8
Hamstring curl					
Sets	3	4	4	4	4
Repetitions	10	8	8	10	8
Working loads (RM)	20	12	10	10	8

Working loads refer to the weight used. A working load of, for example, 12 repetition maximum (RM) means that the load (weight used on the equipment) is so high that 12—but not 13—repetitions can be performed correctly.

subsequent adjustment of the glucose infusion rate. Arterial and femoral venous blood samples were drawn, and blood flow measurement (3–5 single measurements in each leg at each time point) were performed at $t = -30$ and -15 min and then at 90, 105, and 120 min in each clamp step. In the basal state, before initiation of the clamp, muscle biopsies were obtained from both legs.

Calculations and analytical procedures. Leg uptake and release of glucose, D-β-hydroxybutyrate, glycerol, and free fatty acids (FFAs) were calculated as arterio-venous concentrations in whole blood or plasma (FFA) difference multiplied by blood flow. Plasma concentrations of FFAs were converted to whole-blood concentrations by multiplying with (1 − hematocrit). Leg balance data are expressed relative to leg mass, assuming that the volume of 1 l corresponds to 1 kg of leg. All samples were stored at −20°C until analysis, except for C-peptide, FFA, and muscle biopsies, which were stored at −80°C. A detailed description of the stabilization of blood samples and analysis of hormones, metabolites, and gasses was previously described by us (21). Glucose uptake rates in the whole body (*M*-values, calculated as the averaged glucose infusion rate during the final 30 min of each clamp step) and specifically in the legs were transformed to clearance rates (glucose uptake divided by the prevailing arterial glucose concentration), allowing for comparisons between the groups who were clamped at their prevailing glucose concentration (isoglycemic clamp).

After excision, the muscle biopsies were quickly cleaned from visible blood and fat and were then frozen immediately in liquid nitrogen. Biopsies were stored at −80°C until analyzed. Before fluorometric measurements of citrate synthase (CS), hydroxyacyl-3-dehydrogenase (HAD), and lactate dehydrogenase (LDH) activity, biopsies were freeze dried, and connective tissue and fat tissue were removed.

GLUT4 detection in total crude membranes. We homogenized 30 mg of each muscle sample in sucrose buffer (250 mmol/l sucrose, 30 mmol/l HEPES, 2 mmol/l EGTA, 40 mmol/l NaCl, and 2 mmol/l phenylmethylsulfonyl fluoride [PMSF], pH 7.4) using a Polytron 2100 and centrifuged it at 1,000*g* for 5 min. The supernatant was spun at 190,000*g* for 90 min at 4°C in a high-speed centrifuge equipped with a swing-out bucket rotor. The resulting pellet (corresponding to the membrane fraction) was resuspended in Tris-SDS (10 mmol/l Tris, 4% SDS, 1 mmol/l EDTA, and 2 mmol/l PMSF, pH 7.4), and protein content was determined with a BSA standard (DC protein assay; Bio-Rad). Samples were mixed 1:1 with sample buffer containing 10% SDS, 5% glycerol, 10 mmol/l Tris-HCl, 1 mmol/l EDTA, and 10 mmol/l dithiothreitol, and then they were subjected to SDS-PAGE (ExcelGel 7.5% or 8–18% gradient gel). The amount of protein per lane was 6 μg. The separated proteins were electroblotted to a Millipore Immobilon-P polyvinylidiene difluoride (PVDF) membrane (Millipore, Glostrup, Denmark). This membrane was blocked by 1% BSA, 0.5% low-fat dry milk, and 0.1% Tween-20 and then incubated with rabbit anti-GLUT4 antibodies (AB1346; AH Diagnostics, Aarhus, Denmark) diluted in a BSA-containing buffer. After treatment with the horseradish peroxidase (HRP)-coupled secondary antibody, it was repeatedly washed with distilled water, 0.05% Tween-20, and 1 mol/l NaCl. The membrane was then incubated with ECL reagent (Amersham) and visualized on a film. Scanning the film and analyzing band intensities with SigmaGel software yielded quantities of protein. Samples to be compared were loaded on the same gel.

Detection of insulin signaling molecules in muscle lysates. Muscle lysate proteins were separated using 7.5% Bis-Tris gels (Invitrogen, Glostrup, Denmark), and transferred (semi-dry) to PVDF membranes (Immobilion transfer membrane; Millipore). After blocking (TBST + 1% skim milk overnight at 4°C), the membranes were incubated with primary antibodies (TBST + 1% skim milk, 2 h at room temperature) followed by incubation in HRP-conjugated secondary antibody (TBST + 1% skim milk, 1 h at room temperature). After detection and quantification using a charge-coupled device image sensor and 1D software (Kodak Image Station, E440CF; Kodak, Ballerup, Denmark), the protein content was expressed in arbitrary units relative to a human skeletal muscle standard.

The primary antibodies used were anti–insulin receptor substrate-1 (IRS-1), anti-p85, and anti–Akt-1/protein kinase B (PKB) (no. 06-248, 06-497, and 06-558, respectively; Upstate Biotechnology, Lake Placid, NY). Rabbit anti–glycogen synthase (GS) (22) was provided by Dr. Oluf Pedersen (Steno Diabetes Center, Copenhagen, Denmark). The insulin receptor monoclonal CT3 antibody was raised against the COOH terminus of the insulin receptor-β subunit and was a gift from Dr. Ken Siddle (Cambridge University, Cambridge, U.K.). The secondary antibodies used were goat anti-rabbit HRP, goat anti-mouse HRP, and rabbit anti-sheep HRP (no. P0448, P0447, and P0163, respectively; Dako, Glostrup, Denmark).

A microtiter plate assay was used for the measurement of GS activity in muscle homogenates using Unifilter 350 plates (Whatman, Frisenette, Ebeltoft, Denmark). The assay ran in triplicate based on the original protocol described by Thomas et al. (23). GS activity was determined in the presence of 8 or 0.02 mmol/l glucose-6-phosphate (G6P), representing the total GS activity and the percent G6P independent activity (referred to as the % I form [100 times the activity in the presence of 0.02 mmol/l G6P divided by the activity at 8.0 mmol/l G6P {saturated}]), respectively.

Muscle fiber types and capillary density. All procedures were performed on 5-μm unfixed cryosections of human skeletal muscle specimens, essentially as described previously (24). Primary antibodies were mouse anti-skeletal fast myosin antibody (clone MY32; Sigma, St. Louis, MO) and mouse anti-skeletal slow antibody (clone WB-MHCs; Novocastra, Newcastle, U.K.). Capillaries were localized by immunostaining for the endothelial surface marker CD31, using mouse anti-CD31 (Dako, Glostrup, Denmark). Immunohistochemically stained sections from each biopsy specimen were analyzed in a Cast Grid system (Olympus, Copenhagen, Denmark). In each section, >20 fields (each 0.1134 mm^2 in size) including >100 muscle fibers were automatically randomly sampled for analysis. In each field the fibers were counted and their diameter determined as the lesser diameter. The mean fiber diameter for each fiber type was calculated as the average of measured fiber diameters for this fiber type in each section. The fractions of slow and fast fibers were calculated as the number of counted, typed fibers divided by the total number of fibers. The number of capillaries were counted per field and expressed as capillaries per square millimeter.

Statistics. Results are presented as the means ± SE. ANOVA for repeated measures was used for detection of differences between the trained and the untrained legs in the two groups. When a significant main effect was observed, the Student-Newman-Keuls test was used post hoc. In comparisons with a single measurement (e.g., glycogen content in the untrained and in the trained leg), Wilcoxon's signed-rank test was used. The SigmaStat version 2.03 software package was used for all statistical calculations. $P < 0.05$ was considered significant in two-tailed testing.

RESULTS

Leg size and strength. After the strength training program, leg volume was 12.9 ± 1.0 vs. 13.4 ± 1.0 l for untrained versus trained legs ($P < 0.1$) and 12.5 ± 0.7 vs. 12.7 ± 0.6 l for untrained versus trained legs (NS) in control and type 2 diabetic subjects, respectively. Percent difference between untrained and trained legs was 4.1 ± 1.5 and 2.3 ± 2.2% in control and type 2 diabetic subjects, respectively. Thigh circumference was 52.8 ± 3.1 vs. 53.4 ± 2.7 cm for untrained versus trained legs (NS) and 52.5 ± 1.5 vs. 54.3 ± 1.9 cm for untrained versus trained legs ($P < 0.05$) in control subjects ($n = 4$) and type 2 diabetic subjects ($n = 8$), respectively. Percent difference between untrained and trained legs was 1.5 ± 1.6 and 3.4 ± 1.1% in control and type 2 diabetic subjects, respectively. No differences in these parameters were present between control and type 2 diabetic subjects.

Muscle strength increased in all subjects with training. Thus, in type 2 diabetic subjects, knee extension and leg press increased by 42 ± 8 and 75 ± 7% (both $P < 0.05$), respectively, and in control subjects they increased by 29 ± 1 and 77 ± 15% (both $P < 0.05$), respectively (Fig. 1). Muscle strength and the training-induced improvements were similar in the two groups.

Whole-body glucose metabolism. Type 2 diabetic subjects exhibited considerable insulin resistance, with whole-body glucose clearance rates approximately half the rates measured in control subjects (2.5 ± 0.6 and 8.9 ± 0.7 vs. 5.7 ± 0.9 and 14.4 ± 0.7 ml · min^{-1} · kg body wt^{-1} in the two clamp steps, respectively; both $P < 0.05$). In absolute values, whole-body glucose uptake rates were not significantly different between type 2 diabetic and control subjects (clamp step I: 3.5 ± 0.7 vs. 5.8 ± 0.9 mg · min^{-1} · kg body wt^{-1}, $P = 0.06$; clamp step II: 13.8 ± 1.5 vs. 15.4 ± 0.5 mg · min^{-1} · kg body wt^{-1}, $P = 0.40$).

Leg glucose clearance. During the experiment, arterial blood glucose concentrations remained quite constant (Fig. 2), with a coefficient of variation of 6.1 ± 0.9 and

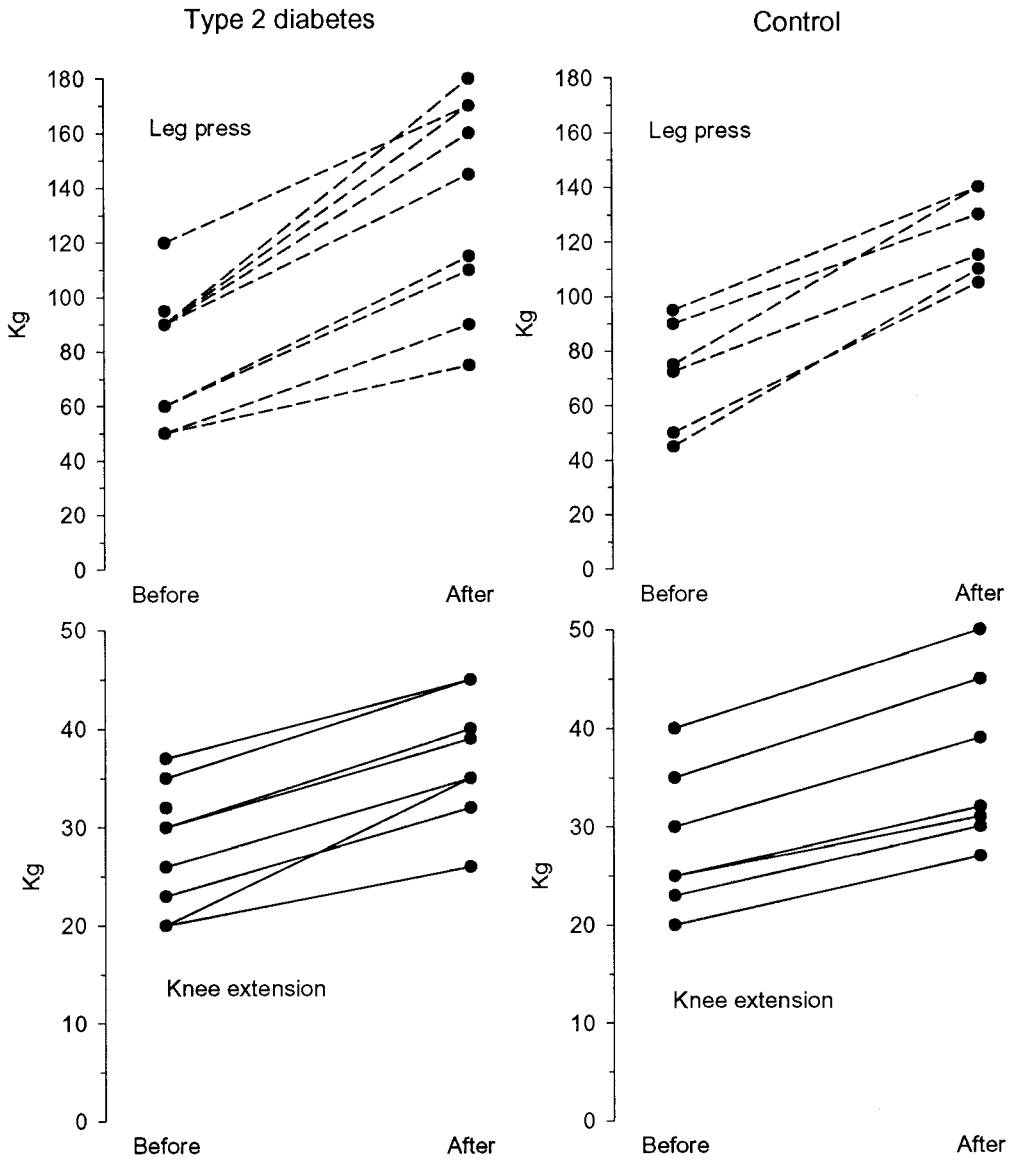

FIG. 1. Maximal muscle strength in 10 patients with type 2 diabetes and in 6 healthy control subjects before and after completion of a 6-week strength training program. Individual values for leg press (top) and knee extension (below) are shown. For one patient measurement after training was not achieved.

4.5 ± 0.9% in control and type 2 diabetic subjects, respectively. The arterio-venous glucose concentration difference was not different between trained and untrained legs in any of the groups. A difference between type 2 diabetes and control subjects approached statistical significance at clamp step II ($P < 0.1$) (Fig. 2).

Glucose clearance rates in the legs were not significantly different in the basal state between type 2 diabetic and control subjects. However, during clamp step I and II, clearance rates in the untrained legs ($P = 0.041$ and $P = 0.002$, respectively) as well as in the trained legs ($P = 0.002$ and $P < 0.001$, respectively) were decreased in type 2 diabetic subjects compared with control subjects (Fig. 3). In both groups a significant training effect was seen during clamp step II, whereas the difference between untrained and trained legs during clamp step I only approached significance (control subjects: $P = 0.062$) or was not different (type 2 diabetic subjects: $P > 0.05$) (Fig. 3).

Insulin infusion increased leg blood flow in both groups (Fig. 3), and leg blood flow was higher in control subjects compared with type 2 diabetic subjects (main effect: $P = 0.034$). Among type 2 diabetic subjects, leg blood flow was increased in trained versus untrained legs during clamp steps I ($P = 0.012$) and II ($P = 0.005$), but in control subjects a difference was seen between untrained and trained legs only at clamp step II ($P = 0.016$).

Despite higher insulin-mediated leg blood flow in trained versus untrained legs, glucose extraction was not different in trained and untrained legs (Fig. 3). However, glucose extraction was decreased in type 2 diabetic subjects compared with control subjects (main effect: $P = 0.047$), and this was primarily attributable to differences between the groups at clamp step I, for both trained ($P = 0.004$) and untrained ($P = 0.006$) legs, because no significant difference between control subjects and type 2 diabetic subjects in glucose extraction was detected at clamp step II.

Hormones, substrates, and metabolites. Fasting glu-

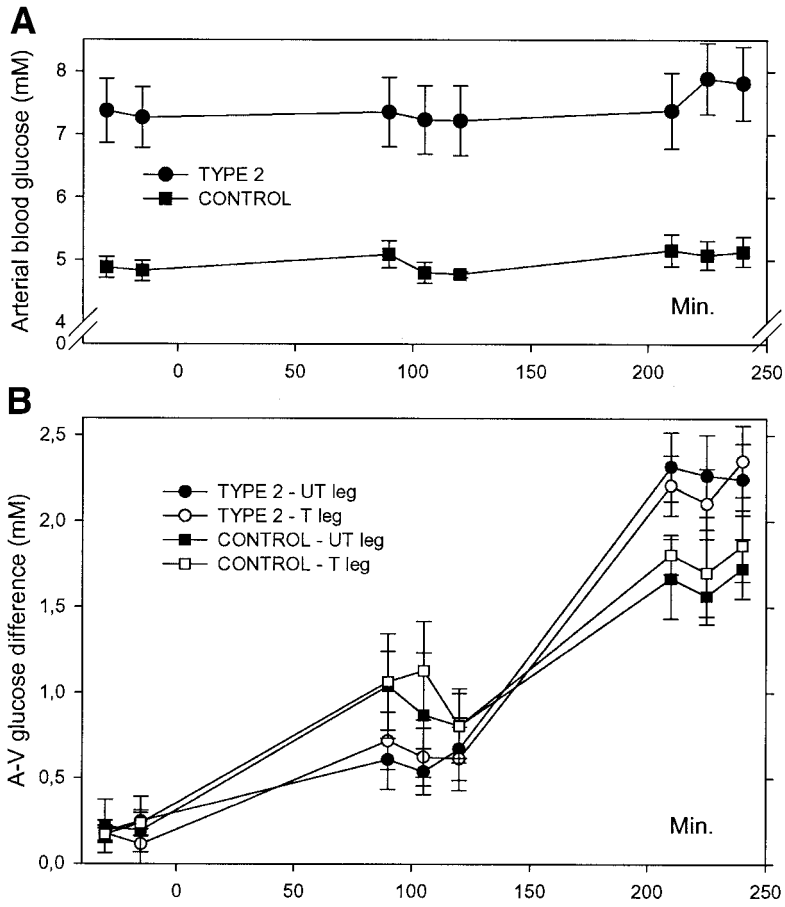

FIG. 2. A total of 10 patients with type 2 diabetes and 6 healthy control subjects strength-trained one leg (T leg) three times per week for 6 weeks while the other leg (UT leg) remained untrained. At 16 h after the last training bout, an isoglycemic-hyperinsulinemic clamp with two insulin infusion steps (0–120 min: step I [28 mU · min^{-1} · m^{-2}]; and 120–240 min: step II [480 mU · min^{-1} · m^{-2}]) was carried out. Arterial blood glucose concentration (*A*) and arterio-femoral venous (A-V) blood glucose concentration difference (*B*) are shown. Blood samples were drawn at basal (t = −30 and −15 min), during step I (t = 90, 105, and 120 min), and during step II (t = 210, 225, and 240 min). Data are means ± SE.

cose, insulin, and C-peptide concentrations in plasma were higher in type 2 diabetic compared with control subjects, but in both groups these parameters were unaltered by training (Table 1).

On the experimental day, fasting plasma insulin concentration was higher in type 2 diabetic subjects (60 ± 12 pmol/l) compared with control subjects (29 ± 4 pmol/l) ($P < 0.05$). Probably because of this, a higher plasma insulin concentration at clamp step I was achieved in type 2 diabetic compared with control subjects (377 ± 26 vs. 270 ± 20 pmol/l, respectively; $P < 0.05$), whereas similar insulin concentrations were seen during clamp step II (12,453 ± 856 vs. 11,066 ± 874 pmol/l, respectively). In clamp steps I and II, plasma C-peptide concentrations were 735 ± 102 and 563 ± 62 pmol/l, respectively, in type 2 diabetic subjects and 413 ± 86 and 344 ± 66 pmol/l, respectively, in control subjects.

Arterial concentrations of glycerol, FFAs, and D-β-hydroxybutyrate always decreased with insulin infusion (Fig. 4). Basal arterial concentrations of FFAs ($P < 0.05$) and glycerol ($P < 0.1$) were slightly higher in type 2 diabetic compared with control subjects. At the lowest insulin infusion (clamp step I), arterial glycerol and FFA concentrations were significantly higher ($P < 0.05$) in type 2 diabetic compared with control subjects, but at clamp step II, concentrations were similar in the two groups. Arterial concentrations of D-β-hydroxybutyrate were, especially in the basal state, quite variable, and no statistical difference between the groups could be detected. Leg balance data (net uptake and/or release in/from the leg) for glycerol and FFAs showed, in general, the same pattern in the two groups, with no difference between the trained and the untrained legs. Thus, the release was considerably reduced with insulin infusion, and as regards FFAs, sometimes not distinguishable from 0 (Fig. 4). Because of substantial variation, no apparent pattern for D-β-hydroxybutyrate leg balance was seen in control subjects, whereas in type 2 diabetes, the release was significantly reduced with increasing insulin concentrations (Fig. 4).

Biopsy data

Glycogen. Glycogen content in skeletal muscle was similar in type 2 diabetic and control subjects, and when data were analyzed separately, there was a tendency ($P < 0.1$) to an increase in trained (366 ± 27 and 358 ± 39 nmol · mg^{-1} · dry wt^{-1}, respectively) compared with untrained (316 ± 19 and 285 ± 19 nmol · mg^{-1} · dry wt^{-1}, respectively) muscle. When data from the two groups were pooled, a significant ($P < 0.05$) effect of training was seen.

Muscle fiber types, diameter, and capillary density. The fraction of slow type I to fast type II fibers was similar in untrained and trained legs in control subjects (n = 6; 0.52 ± 0.02 and 0.63 ± 0.05, respectively) and in type 2

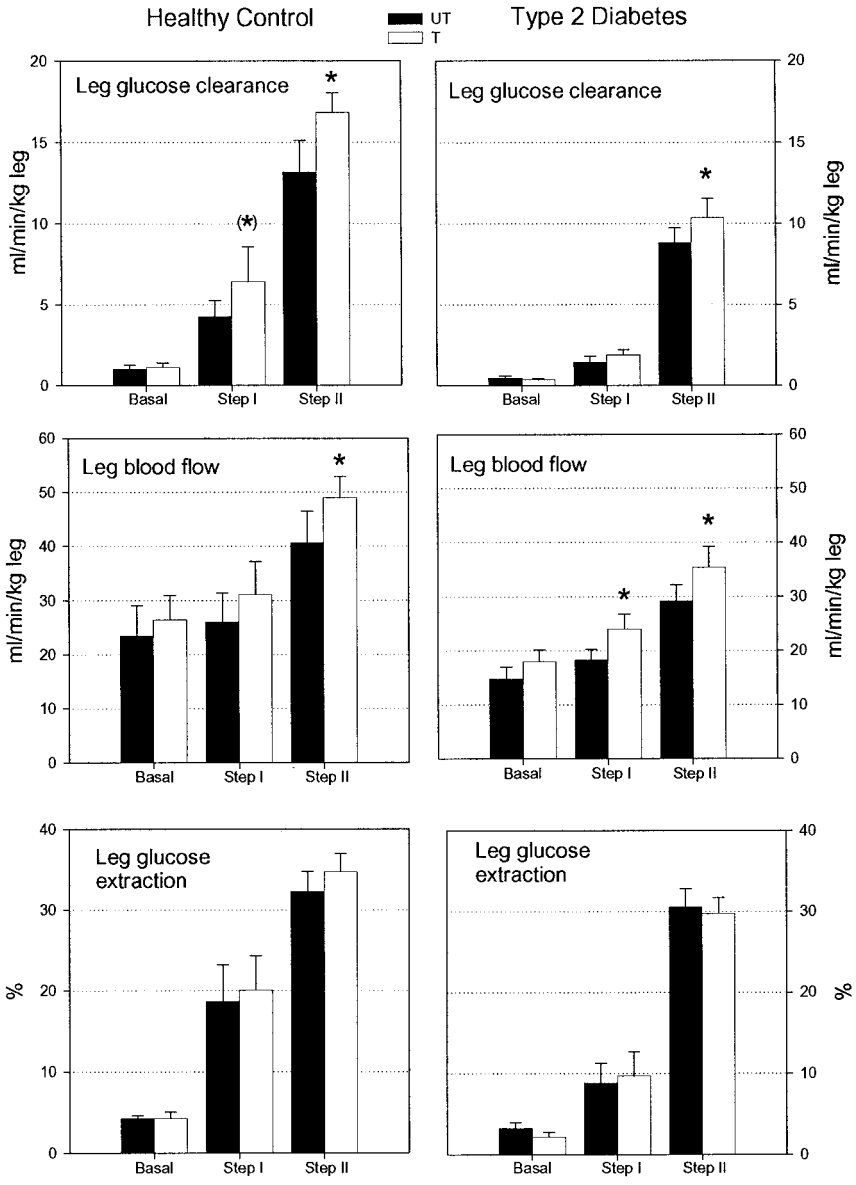

FIG. 3. Basal and insulin stimulated glucose clearance, leg blood flow, and glucose extraction rates in strength-trained and untrained legs in 10 patients with type 2 diabetes and 6 healthy control subjects. Data are means ± SE. *$P < 0.05$, (*)$P = 0.06$ for difference between trained and untrained leg. See text for details.

diabetic subjects ($n = 10$; 0.56 ± 0.05 and 0.51 ± 0.04, respectively), with no significant difference between control and type 2 diabetic subjects. The diameter of the fast fibers was slightly increased in trained versus untrained muscle in both control subjects (64.6 ± 1.1 vs. 62.9 ± 0.9 μm, respectively; $P < 0.05$) and type 2 diabetic subjects (66.2 ± 1.2 vs. 62.5 ± 1.8 μm, respectively; $P < 0.05$). In contrast, the diameter of the slow fibers was similar in trained versus untrained muscle in control subjects (61.8 ± 1.0 vs. 60.3 ± 0.7 μm, respectively) and type 2 diabetic subjects (61.7 ± 1.1 vs. 59.3 ± 1.7 μm, respectively). Thus, the average diameter of the muscle fibers (i.e., including both slow and fast fibers, corrected for fiber type ratio) was 61.5 ± 0.8 μm (untrained) and 63.0 ± 0.7 μm (trained) in control subjects ($P < 0.1$) and 60.9 ± 1.6 μm (untrained) and 64.1 ± 1.2 μm (trained) in type 2 diabetic subjects ($P < 0.05$). The percentage difference in fiber diameter was 2.4 ± 0.9 and 5.4 ± 1.7% in control and type 2 diabetic subjects, respectively. A representative cross-sectional "picture" of fiber types is seen in Fig. 5.

The capillary density, measured as the number of capillaries per millimeter squared of muscle, was unchanged with strength training in both control subjects (untrained: 238 ± 29; trained: 241 ± 36) and type 2 diabetic subjects (untrained: 228 ± 27; trained: 225 ± 29). No significant difference existed in capillary density between the two groups.

GLUT4. The density of GLUT4 protein in the muscle homogenate was similar in type 2 diabetic and control subjects, i.e., the difference between GLUT4 protein content in the untrained ($P = 0.209$) and the trained ($P = 0.591$) legs was not significantly different between the two groups (Fig. 6). In type 2 diabetic subjects, a 40% increase of GLUT4 density in the trained muscle was seen ($P < 0.05$), but in control subjects the 13% increase did not achieve statistical significance (Fig. 6).

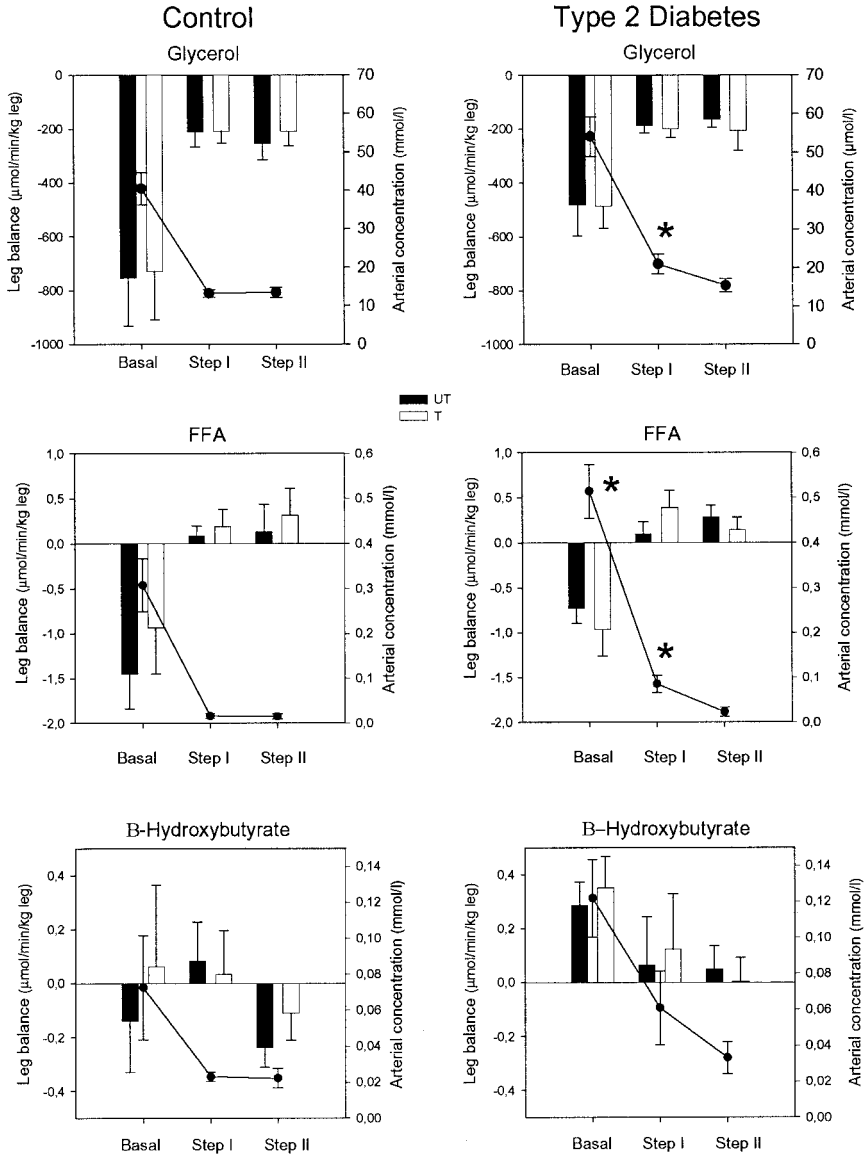

FIG. 4. Arterial concentrations (right *y*-axis) and net leg uptake (positive values) and release (negative values) (left *y*-axis) of glycerol, FFAs, and D-β-hydroxybutyrate in 10 patients with type 2 diabetes and in 6 healthy control subjects before (basal) and during an isoglycemic-hyperinsulinemic clamp with two insulin infusion rates (steps I and II). Each subject had strength trained only one leg (T) while the other was untrained (UT). Leg balance data are represented by bars and arterial concentrations by a line with symbols. Data are means ± SE. *Difference between the two groups at corresponding time points ($P < 0.05$). No significant effect of training was detected.

Insulin signaling. No significant differences existed between control and type 2 diabetic subjects in regard to protein content of the insulin receptor, IRS-1, the p85 subunit of phosphatidylinositol (PI) 3-kinase, PKB α/β (Akt 1/2), GS, GS total activity, or GS activity measured as % I-form in comparisons within either untrained or trained legs (data not shown). However, in response to training, significant increases were found in protein content of the insulin receptor (control subjects: 19 ± 7%; type 2 diabetic subjects: 21 ± 6%; both $P < 0.05$), PKB-α/β (Akt 1/2) (control subjects: 22 ± 9%; type 2 diabetic subjects: 12 ± 7%; main effect $P < 0.05$), GS protein content (control subjects: 12 ± 9%; type 2 diabetic subjects: 13 ± 5%; main effect $P < 0.05$), and GS total activity (control subjects: 9 ± 3%; type 2 diabetic subjects: 21 ± 4%; both $P < 0.05$) (Fig. 7). No effect of training was seen for the protein content of IRS-1, the p85 subunit of PI3-kinase, or percent GS activity in the % I-form in either group (Fig. 7).

Markers of oxidative capacity. The enzyme activities of CS, LDH, and HAD were similar in control and type 2 diabetic subjects, and no effect of the resistance training program was seen (Table 3).

DISCUSSION

There are several novel findings in the present study. First, we have shown that the resistance type of exercise training increases insulin action in skeletal muscle in patients with type 2 diabetes, an effect that was likely not caused solely by an increase in muscle mass. Thus, skeletal muscle also adapts qualitatively to a strength training program. Secondly, the study has revealed possible mechanisms behind the effect of training, i.e., changes in protein

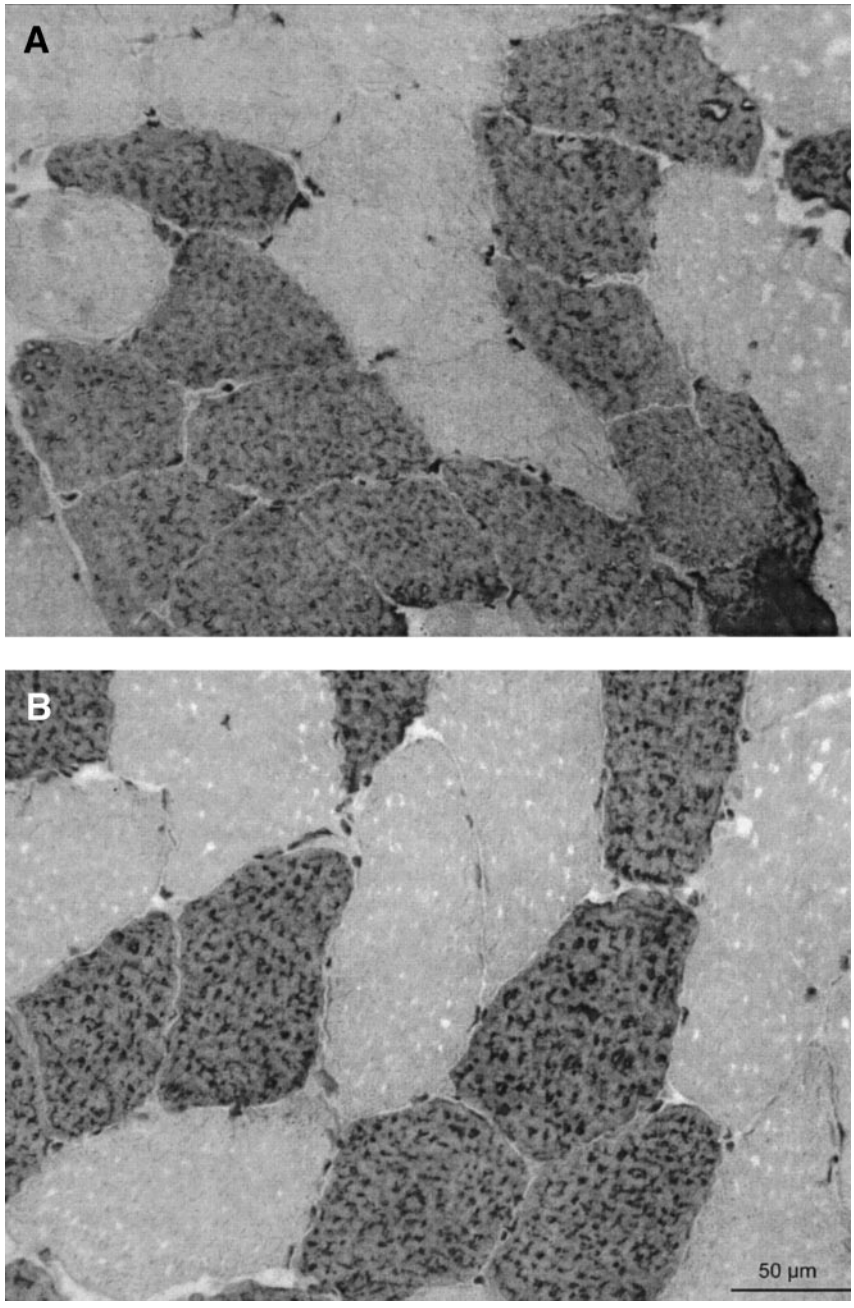

FIG. 5. Representative light micrographs of portions of muscle bundles in transverse section of m. vastus lateralis in a patient with type 2 diabetes (*A*) and a healthy control subject (*B*). Cryosections of muscle biopsy tissue were immunostained for slow myosin as described in RESEARCH DESIGN AND METHODS. Immunoreactive muscle fibers appear dark.

contents of GLUT4, PKB, and GS. Finally, we have shown that the adaptations occurred in response to a minimal training effort, which could easily be carried out on a larger scale.

The finding that the strength training–induced increase of insulin-mediated glucose clearance in the legs is independent of an increase in muscle mass is in line with our recent data in young, healthy men who abstained from strength training for 90 days, where accurate measures of changes in leg muscle mass was obtained by computed tomography scans (25). Strength training has previously been used in prospective studies in healthy subjects (26), subjects with impaired glucose tolerance (19), and subjects with type 2 diabetes (17), and in these studies 22–48% improvement in the *M*-value was found, generally attributable to increases in nonoxidative glucose metabolism. Data in two (17,26) of these studies were expressed relative to the fat-free mass, thus attempting to correct for the effect of a larger muscle mass in resistance-trained individuals. In two cross-sectional studies, no effect of resistance training on insulin-stimulated glucose uptake per kilogram muscle mass was found, and the positive effect of resistance training on the whole body were attributed solely the larger muscle mass (27,28). The data in the present study are in disagreement with these two latter studies, but our data are supported by the muscle biopsy data, speaking in favor of strength training–induced qualitative changes of the skeletal muscle. A very minor

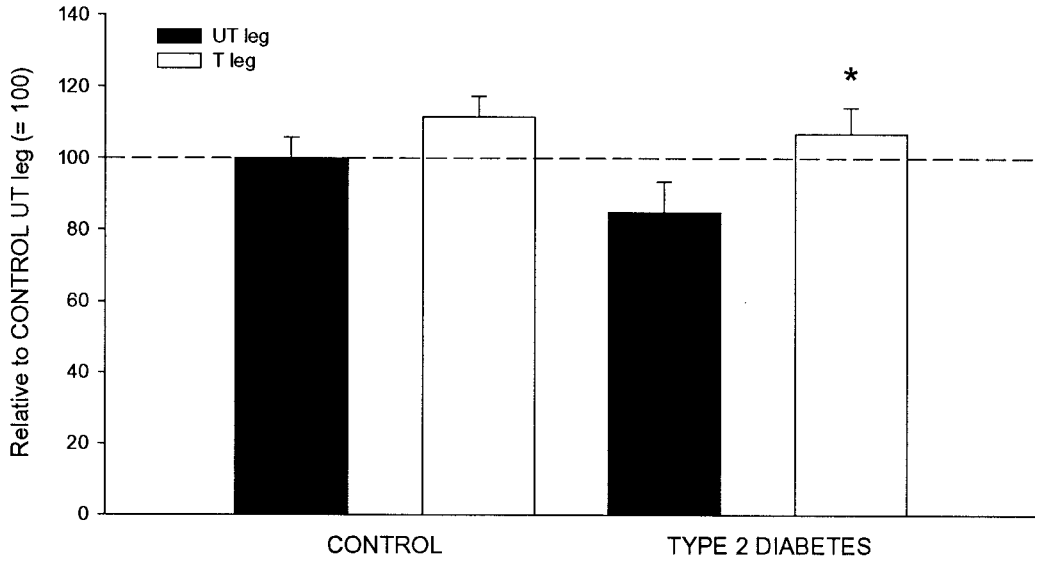

FIG. 6. GLUT4 protein content in untrained (UT) and strength-trained (T) legs in 10 patients with type 2 diabetes and in 7 healthy control subjects. GLUT4 content was similar in type 2 diabetic and control subjects. Data are means ± SE. *Difference between trained and untrained legs ($P < 0.05$).

contribution from an increase in muscle mass that we could not detect by water displacement is, however, possible. We did detect a 2–4% increase in leg volume by water displacement, and the more sensitive measure of muscle fiber diameter revealed only a marginally larger increase.

The present study is by design very different from all previous strength-training studies. We have used a one-legged training model and extended the traditional clamp experiments with arterio-femoral venous catheterization of both the trained and the untrained leg. By this model we are able to investigate both trained and untrained muscle in the same subject at the same time. The muscles are exposed to the same concentration of circulating humeral factors, and we are able to conclude that any measured difference between the two legs are due to local contraction-mediated mechanisms.

It is difficult for many patients with type 2 diabetes to engage in endurance-type training programs. With a previous sedentary lifestyle, strength training represents an attractive alternative, and the present study has provided proof for the effect. Furthermore, we have shown that the necessary training volume is of a magnitude that should present no major obstacle for putting the training into practice in the everyday life of most people. Altogether, each training session lasted no more than 30 min, and each session was only carried out three times per week. This training volume was sufficient and is in line with, but not

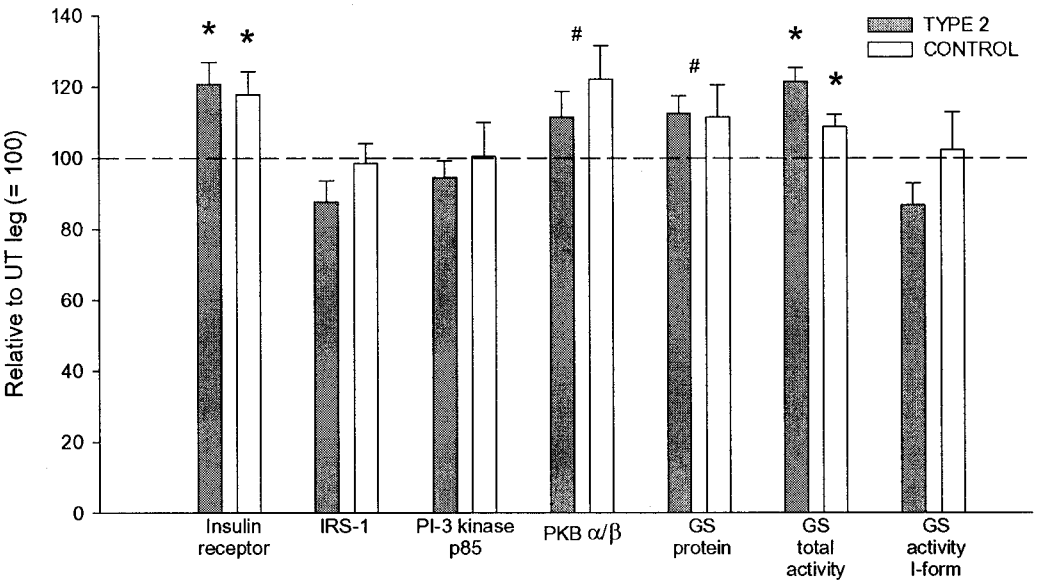

FIG. 7. Protein content of the insulin receptor, IRS-1, PKBα/β (Akt1/2), the p85 subunit of the PI-3 kinase, GS protein, total activity of GS protein (GS total activity), and activity of GS protein in the % I-form measured in skeletal muscle biopsies in untrained and strength-trained legs in 10 patients with type 2 diabetes and in 7 healthy control subjects. Data are means ± SE and for each measure expressed relative to the corresponding value in the untrained leg. #Main effect of training, *effect of training within the study group, ($P < 0.05$).

TABLE 3
Muscle enzyme activities

	Control		Type 2 diabetes	
	Untrained	Trained	Untrained	Trained
CS	84 ± 7	90 ± 7*	70 ± 5	70 ± 5
HAD	124 ± 7	127 ± 6	119 ± 6	120 ± 9
LDH	840 ± 62	922 ± 75	966 ± 93	1,069 ± 185

All units are $\mu mol \cdot min^{-1} \cdot g^{-1}$ dry weight of muscle tissue. Muscle biopsies were obtained from the trained and the untrained leg after 6 weeks of strength training with one leg in 10 patients with type 2 diabetes and in 7 matched healthy control subjects. *Different from corresponding leg in type 2 subjects ($P < 0.05$).

identical to, the general advice of accumulating 30 min of moderate physical activity on most days of the week (29). We do not know whether accumulation of, for example, 3 × 10 min 3 days per week would have resulted in the same beneficial effects.

Some of the subjects in both groups had hypertension. High-resistance strength training is not recommended in these cases (30). However, it is important to recognize that the present training program was very far from high-resistance training, because light weights and a high number of repetitions were used. Whether the results from the present study pertain to all type 2 diabetic patients is uncertain. The type 2 diabetic population is not homogenous. The age of the patients, duration of the disease, treatment, BMI, and presence and degree of micro- and macrovascular complications and dyslipidemia varies substantially among patients. For this reason we have chosen to describe the subjects in the present study thoroughly (see Table 1).

The mechanisms behind the effect of strength training seem similar to those seen with endurance training. Thus, in a previous study using one-legged endurance training, we found that both increased blood flow and glucose extraction contributed to the training effect (10). Also, in the present study we found that insulin-mediated blood flow was increased in the trained leg (Fig. 3). Although the glucose extraction was not increased in the trained compared with the untrained leg (Fig. 3), this does not mean that factors facilitating an increased capacity for the trained muscle to extract glucose from the blood were not operating. With the increased glucose delivery to the muscles in the trained leg, glucose extraction would in fact have decreased if such mechanisms were not upgraded. This view is supported by the fact that glucose extraction was not reduced in the trained leg, although muscle glycogen concentrations, which generally are inversely related to insulin sensitivity (31), were higher in the trained compared with the untrained leg. However, if the increased flow in the trained leg was directed into new capillaries (which we did not detect) or previously underperfused capillaries (nutritive), then the increased flow could be a part of the explanation for increased glucose clearance in the trained muscle. For a detailed discussion of this issue, see the recent review by Clark et al. (32).

It is interesting to note that the effect of hyperglycemia on arterio-venous glucose concentration differences is not independent of the plasma insulin concentration (Fig. 2). At physiological plasma insulin concentrations (clamp step I, 0–120 min), arterio-venous glucose concentration differences were not different in type 2 diabetic compared with control subjects. However, at the high plasma insulin concentration (clamp step II, 120–240 min) the arterio-venous glucose concentration difference tended to be higher ($P < 0.1$) in type 2 diabetic compared with control subjects (Fig. 2). Thus, at the physiological insulin level, hyperglycemia cannot compensate for a defect in glucose extraction, and at high unphysiological insulin levels, only a partial compensation takes place.

The biochemical adaptations to training in skeletal muscle are many, and in the present study, we have focused on only some of the proteins, each of which could be partly responsible for the effects on glucose homeostasis. Thus, we found a training-induced increase in insulin receptor protein expression in both groups (Fig. 7). Our finding of increased glucose clearance at the supraphysiological insulin concentrations (Fig. 3) might be attributable to an increased insulin receptor number, but it is generally believed that insulin-sensitive tissues have more insulin receptors than necessary for eliciting maximal insulin effect (spare receptors). The finding of similar insulin receptor protein content in patients with type 2 diabetes and healthy control subjects confirms a previous finding (33), but to our knowledge the influence of exercise training on insulin receptor protein has not been carried out previously in patients with type 2 diabetes. In a cross-sectional study of 29- to 39-year-old endurance-trained and untrained men, in fact a decrease was found in the trained men (34), whereas in another study the gene expression seems unaltered by short-term endurance training in young men and women (35). In these two former studies, IRS-1 gene and protein expression followed the pattern of the insulin receptor (i.e., unchanged and decreased, respectively), whereas in the present study we found no effect on IRS-1 protein expression in response to strength training (Fig. 7). Similarly, we found that the protein expression of the p85 subunit of PI 3-kinase was unaltered by training in both groups (Fig. 7). Whether these contrasting results are caused by the type of training or the differences in age in the present and the aforementioned studies cannot be answered at the present time. The finding of similar IRS-1 and p85 protein content in patients with type 2 diabetes and in healthy control subjects is in line with findings from others (33,36).

Further distal in the insulin signaling pathway, PKB protein expression increased in response to strength training (Fig. 7). PKB is involved in the insulin signaling to GS and might also be important in the regulation of insulin-mediated GLUT4 translocation and glucose uptake in skeletal muscle. Thus, the upregulation of this enzyme might be important for the improved insulin action after training. The finding of similar protein expression of PKB in the muscle of healthy subjects and patients with type 2 diabetes (36) or first-degree relatives to type 2 diabetic patients (37) has previously been observed, whereas the observation of increased expression after strength training in humans with and without type 2 diabetes is a new finding. In contrast to this are data from a study in which similar PKB expression was found in endurance-trained and untrained men (34). Many factors can be pivotal in these different responses, but the differences in age (range 29–39 vs. 52–71 years), type of training (endurance versus

strength training), and design (cross-sectional versus paired) in the aforementioned (34) and the present study are likely candidates.

GS protein expression increased with strength training (Fig. 7). Also, the gene expression increases with training (albeit endurance training) (10) in patients with type 2 diabetes and healthy control subjects. In all, this is compatible with the training-induced increase in glycogen activity also found in some (22,38,39) but not all (40) previous studies. The finding of similar levels of GS protein in the muscle of control subjects and patients with type 2 diabetes is in agreement with previous findings (33).

The strength-training induced increase in GLUT4 protein expression also found in patients with type 2 diabetes training is novel and important. It extends the findings from endurance training studies in patients with type 2 diabetes (41) and two previous strength training studies in healthy humans (42,43).

In the present study, only content of signaling molecules and proteins (GLUT4 and GS) were measured. Thus, the functional significance of the changes in insulin receptor, PKB, GLUT4, and GS content in vivo are unclear. We may anticipate that a greater potential for signaling/activation is created by the changes, but whether these factors are limiting components during insulin stimulation (as applied in the present study) is not known. Thus, the data do not allow us to conclude that the expression changes per se are mediators of the increase in insulin action.

We have assumed that the predominant insulin-sensitive tissue in the leg is skeletal muscle. Adipose tissue in the legs may, although to a much lesser extent, also have contributed to the clearance of glucose from the blood. The specific effect of training on localized adipose tissue was not measured in this study, but it is reasonable to believe that insulin sensitivity in adipose tissue also increased in response to training (44–47). However, whether such an effect is restricted to the trained leg is not known. It does not seem that strength training influenced lipolysis, as judged from the net release of glycerol and FFAs (Fig. 4), results that are similar to those obtained after endurance training (10). Of note is the finding that in contrast to the healthy control subjects, in type 2 diabetic patients, arterial concentrations of both glycerol and FFAs was higher at basal and clamp step I, despite higher plasma insulin concentrations (Fig. 4). Only when plasma insulin concentrations were well above the physiological level were similar arterial concentrations in the two groups achieved. Altogether, this may reflect a resistance to the inhibitory effect of insulin on lipolysis in the patients with type 2 diabetes.

Markers of oxidative capacity (CS, HAD, and LDH) were measured to control for improvements that could be ascribed to endurance-like exercises. The training protocol used, with the relatively high number of repetitions, might for some of the subjects have endurance-like characteristics. If endurance was a major component of the training program, some or all of these would be expected to increase. However, this was not the case, and together with the major improvement in muscle strength, this indicates that the predominant type of exercise was indeed strength training.

In summary, we have reported that strength training, like endurance training, enhances insulin action in skeletal muscle in patients with type 2 diabetes. This effect was likely independent of increases in muscle mass and could be achieved with a low weekly training volume. The increases in muscle GLUT4 content and in various insulin signaling protein expressions and/or activity are part of the mechanism behind the improvement in insulin action, but each of them should probably not be attributed a single effect, but rather be looked on in concert.

ACKNOWLEDGMENTS

Financial support from the Danish National Research Foundation (reference no. 504-14), the Danish Diabetes Association, the Novo-Nordisk Foundation, the Foundation of 1870, the Jacob and Olga Madsens Foundation, and the Danish Medical Research Council are gratefully acknowledged. J.F.P.W. was supported by a Hallas Møller fellowship from the Novo-Nordisk Foundation.

Professor Oluf Pedersen (Steno Diabetes Center) and professor Ken Siddle (Cambridge University) are greatly acknowledged for the kind donation of antibodies. Regitze Kraunsøe, Jeppe Bach, Irene Lynfort, and Jesper B. Birk are thanked for excellent technical assistance.

REFERENCES

1. Krotkiewski M, Lönnroth P, Mandroukas K, Wroblewski Z, Rebuffé-Scrive M: The effects of physical training on insulin secretion and effectiveness and on glucose metabolism in obesity and type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 28:881–890, 1985
2. Ligtenberg PC, Hoekstra JB, Bol E, Zonderland ML, Erkelens DW: Effects of physical training on metabolic control in elderly type 2 diabetes mellitus patients. *Clin Sci* 93:127–135, 1997
3. Mourier A, Gautier JF, De Kerviler E, Bigard AX, Villette JM, Garnier JP, Duvallet A, Guezennec CY, Cathelineau G: Mobilization of visceral adipose tissue related to the improvement in insulin sensitivity in response to physical training in NIDDM: effects of branched-chain amino acid supplements. *Diabetes Care* 20:385–391, 1997
4. Ruderman NB, Ganda OP, Johansen K: The effect of physical training on glucose tolerance and plasma lipids in maturity-onset diabetes. *Diabetes* 28 (Suppl. 1):89–92, 1979
5. Reitman JS, Vasquez B, Klimes I, Nagulesparan M: Improvement of glucose homeostasis after exercise training in non-insulin-dependent diabetes. *Diabetes Care* 7:434–441, 1984
6. Trovati M, Carta Q, Cavalot F, Vitali S, Banaudi C, Lucchina PG, Fiocchi F, Emanuelli G, Lenti G: Influence of physical training on blood glucose control, glucose tolerance, insulin secretion, and insulin action in non–insulin-dependent diabetic patients. *Diabetes Care* 7:416–420, 1984
7. Schneider SH, Amorosa LF, Khachadurian AK, Ruderman NB: Studies on the mechanism of improved glucose control during regular exercise in type 2 (non-insulin-dependent) diabetes. *Diabetologia* 26:355–360, 1984
8. Segal KR, Edano A, Abalos A, Albu J, Blando L, Tomas MB, Pi-Sunyer FX: Effect of exercise training on insulin sensitivity and glucose metabolism in lean, obese, and diabetic men. *J Appl Physiol* 71:2402–2411, 1991
9. Bogardus C, Ravussin E, Robbins DC, Wolfe RR, Horton ES, Sims EA: Effects of physical training and diet theraphy on carbohydrate metabolism in patients with glucose intolerance and non–insulin-dependent diabetes mellitus. *Diabetes* 33:311–318, 1984
10. Dela F, Mikines KJ, Larsen JJ, Ploug T, Petersen LN, Galbo H: Insulin-stimulated muscle glucose clearance in patients with type 2 diabetes mellitus: effects of one-legged physical training. *Diabetes* 44:1010–1020, 1995
11. Boule NG, Haddad E, Kenny GP, Wells GA, Sigal RJ: Effects of exercise on glycemic control and body mass in type 2 diabetes mellitus: a meta-analysis of controlled clinical trials. *JAMA* 286:1218–1227, 2001
12. Barnard RJ, Jung T, Inkeles SB: Diet and exercise in the treatment of NIDDM: the need for early emphasis. *Diabetes Care* 17:1469–1472, 1994
13. Yki-Jarvinen H, Dela F: Exercise in the treatment of type 2 and type 1 diabetes. In *Textbook of Sports Medicine. Basic Science and Clinical Aspects of Sports Injury and Physical Activity*. Kjær M, Krogsaard M,

Magnusson P, Engebretsen L, Roos H, Takala T, Woo SL-Y, Eds. Oxford, U.K., Blackwell Science, 2003, p. 435–450
14. Dunstan DW, Daly RM, Owen N, Jolley D, De Courten M, Shaw J, Zimmet P: High-intensity resistance training improves glycemic control in older patients with type 2 diabetes. *Diabetes Care* 25:1729–1736, 2002
15. Castaneda C, Layne JE, Munoz-Orians L, Gordon PL, Walsmith J, Foldvari M, Roubenoff R, Tucker KL, Nelson ME: A randomized controlled trial of resistance exercise training to improve glycemic control in older adults with type 2 diabetes. *Diabetes Care* 25:2335–2341, 2002
16. Maiorana A, O'Driscoll G, Goodman C, Taylor R, Green D: Combined aerobic and resistance exercise improves glycemic control and fitness in type 2 diabetes. *Diabetes Res Clin Pract* 56:115–123, 2002
17. Ishii T, Yamakita T, Sato T, Tanaka S, Fujii S: Resistance training improves insulin sensitivity in NIDDM subjects without altering maximal oxygen uptake. *Diabetes Care* 21:1353–1355, 1998
18. Honkola A, Forsen T, Eriksson J: Resistance training improves the metabolic profile in individuals with type 2 diabetes. *Acta Diabetol* 34:245–248, 1997
19. Eriksson J, Tuominen J, Valle T, Sundberg S, Sovijarvi, Lindholm H, Tuomilehto J, Koivisto V: Aerobic endurance exercise or circuit-type resistance training for individuals with impaired glucose tolerance? *Horm Metab Res* 30:37–41, 1998
20. Dela F, Larsen JJ, Mikines KJ, Galbo H: Normal effect of insulin to stimulate leg blood flow in NIDDM. *Diabetes* 44:221–226, 1995
21. Dela F: On the influence of physical training on glucose homeostasis. *Acta Physiol Scand* 158:5–41, 1996
22. Vestergaard H, Andersen PH, Lund S, Schmitz O, Junker S, Pedersen O: Pre- and posttranslational upregulation of muscle-specific glycogen synthase in athletes. *Am J Physiol* 266:E92–E101, 1994
23. Thomas JA, Schlender KK, Larner J: A rapid filter paper assay for UDPglucose-Glycogen glucosyltransferase, including an improved biosynthesis of UDP-^{14}C-glucose. *Anal Biochem* 25:486–499, 1968
24. Gaster M, Poulsen P, Handberg A, Schroder HD, Beck-Nielsen H: Direct evidence of fiber type-dependent GLUT-4 expression in human skeletal muscle. *Am J Physiol Endocrinol Metab* 278:E910–E916, 2000
25. Andersen JL, Schjerling P, Andersen LL, Dela F: Resistance training and insulin action: effects of de-training. *J Physiol* 551:1049–1058, 2003
26. Miller JP, Pratley RE, Goldberg AP, Gordon P, Rubin M, Treuth MS, Ryan AS, Hurley BF: Strength training increases insulin action in healthy 50- to 65-yr-old men. *J Appl Physiol* 77:1122–1127, 1994
27. Yki-Järvinen H, Koivisto VA: Effects of body composition on insulin sensitivity. *Diabetes* 32:965–969, 1983
28. Takala TO, Nuutila P, Knuuti J, Luotolahti M, Yki-Järvinen H: Insulin action on heart and skeletal muscle glucose uptake in weight lifters and endurance athletes. *Am J Physiol* 276:E706–E711, 1999
29. American Diabetes Association: Physical activity/exercise and diabetes mellitus (Position Statement). *Diabetes Care* 26 (Suppl. 1):S73–S77, 2003
30. American Diabetes Association: Diabetes mellitus and exercise (Position Statement). *Diabetes Care* 25 (Suppl. 1):S64–S68, 2002
31. Ivy JL, Frishberg BA, Farrell SW, Miller WJ, Sherman WM: Effects of elevated and exercise-reduced muscle glycogen levels on insulin sensitivity. *J Appl Physiol* 59:154–159, 1985
32. Clark MG, Wallis MG, Barrett EJ, Vincent MA, Richards SM, Clerk LH, Rattigan S: Blood flow and muscle metabolism: a focus on insulin action. *Am J Physiol Endocrinol Metab* 284:E241–E258, 2003
33. A Krook, M Bjornholm, D Galuska, XJ Jiang, R Fahlman, MG Myers, H Wallberg-Henriksson, JR Zierath: Characterization of signal transduction and glucose transport in skeletal muscle from type 2 diabetic patients. *Diabetes* 49:284–292, 2000
34. Yu M, Blomstrand E, Chibalin AV, Wallberg-Henriksson H, Zierath JR, Krook A: Exercise-associated differences in an array of proteins involved in signal transduction and glucose transport. *J Appl Physiol* 90:29–34, 2001
35. Wadley GD, Tunstall RJ, Sanigorski A, Collier GR, Hargreaves M, Cameron-Smith D: Differential effects of exercise on insulin-signaling gene expression in human skeletal muscle. *J Appl Physiol* 90:436–440, 2001
36. Kim YB, Nikoulina SE, Ciaraldi TP, Henry RR, Kahn BB: Normal insulin-dependent activation of Akt/protein kinase B, with diminished activation of phosphoinositide 3-kinase, in muscle in type 2 diabetes. *J Clin Invest* 104:733–741, 1999
37. Storgaard H, Song XM, Jensen CB, Madsbad S, Björnholm M, Vaag A, Zierath JR: Insulin signal transduction in skeletal muscle from glucose-intolerant relatives of type 2 diabetic patients. *Diabetes* 50:2770–2778, 2001. Erratum in *Diabetes* 51:552, 2002
38. Allenberg K, Nilsson M, Landin K, Lindgärde F: Glycogen and lactate synthetic pathways in human skeletal muscle in relation to obesity, weight reduction and physical training. *Eur J Clin Invest* 18:250–255, 1988
39. Piehl K, Adolfsson S, Nazar K: Glycogen storage and glycogen synthetase activity in trained and untrained muscle of man. *Acta Physiol Scand* 90:779–788, 1974
40. Allenberg K, Johansen K, Saltin B: Skeletal muscle adaptations to physical training in type II (non-insulin-dependent) diabetes mellitus. *Acta Med Scand* 223:365–373, 1988
41. Dela F, Ploug T, Handberg A, Petersen LN, Larsen JJ, Mikines KJ, Galbo H: Physical training increases muscle GLUT-4 protein and mRNA in patients with NIDDM. *Diabetes* 43:862–865, 1994
42. Tabata I, Suzuki Y, Fukunaga T, Yokozeki T, Akima H, Funato K: Resistance training affects GLUT-4 content in skeletal muscle of humans after 19 days of head-down bed rest. *J Appl Physiol* 86:909–914, 1999
43. Derave W, Eijnde BO, Verbessem P, Ramaekers M, Van Leemputte M, Richter EA, Hespel P: Combined creatine and protein supplementation in conjunction with resistance training promotes muscle GLUT-4 content and glucose tolerance in humans. *J Appl Physiol* 94:1910–1916, 2003
44. Stallknecht B, Larsen JJ, Mikines KJ, Simonsen L, Bülow J, Galbo H: Effect of training on insulin sensitivity of glucose uptake and lipolysis in human adipose tissue. *Am J Physiol* 279:E376–E385, 2000
45. James DE, Kraegen EW, Chisholm DJ: Effects of exercise training on in vivo insulin action in individual tissues in the rat. *J Clin Invest* 76:657–666, 1985
46. Craig BW, Treadway J: Glucose uptake and oxidation in fat cells of trained and sedentary pregnant rats. *J Appl Physiol* 60:1704–1709, 1986
47. Vinten J, Petersen LN, Sonne B, Galbo H: Effect of physical training on glucose transporters in fat cell fractions. *Biochim Biophys Acta* 841:223–227, 1985