

Impact of exercise training on insulin sensitivity, physical fitness, and muscle oxidative capacity in first-degree relatives of type 2 diabetic patients

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Østergård, Torben, Jesper L. Andersen, Birgit Nyholm, Sten Lund, K. Sreekumaran Nair, Bengt Saltin, and Ole Schmitz. Impact of exercise training on insulin sensitivity, physical fitness, and muscle oxidative capacity in first-degree relatives of type 2 diabetic patients. *Am J Physiol Endocrinol Metab* 290: E998–E1005, 2006. First published December 13, 2005; doi:10.1152/ajpendo.00012.2005.—First-degree relatives of type 2 diabetic patients (offspring) are often characterized by insulin resistance and reduced physical fitness ($\dot{V}O_{2\max}$). We determined the response of healthy first-degree relatives to a standardized 10-wk exercise program compared with an age-, sex-, and body mass index-matched control group. Improvements in $\dot{V}O_{2\max}$ (14.1 ± 11.3 and $16.1 \pm 14.2\%$; both $P < 0.001$) and insulin sensitivity (0.6 ± 1.4 and $1.0 \pm 2.1 \text{ mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$; both $P < 0.05$) were comparable in offspring and control subjects. However, $\dot{V}O_{2\max}$ and insulin sensitivity in offspring were not related at baseline as in the controls ($r = 0.009$, $P = 0.96$ vs. $r = 0.67$, $P = 0.002$). Likewise, in offspring, exercise-induced changes in $\dot{V}O_{2\max}$ did not correlate with changes in insulin sensitivity as opposed to controls ($r = 0.06$, $P = 0.76$ vs. $r = 0.57$, $P = 0.01$). Skeletal muscle oxidative capacity tended to be lower in offspring at baseline but improved equally in both offspring and controls in response to exercise training (Δ citrate synthase enzyme activity 26 vs. 20%, and Δ cyclooxygenase enzyme activity 25 vs. 23%). Skeletal muscle fiber morphology and capillary density were comparable between groups at baseline and did not change significantly with exercise training. In conclusion, this study shows that first-degree relatives of type 2 diabetic patients respond normally to endurance exercise in terms of changes in $\dot{V}O_{2\max}$ and insulin sensitivity. However, the lack of a correlation between the $\dot{V}O_{2\max}$ and insulin sensitivity in the first-degree relatives of type 2 diabetic patients indicates that skeletal muscle adaptations are dissociated from the improvement in $\dot{V}O_{2\max}$. This could indicate that, in first-degree relatives, improvement of insulin sensitivity is dissociated from muscle mitochondrial functions.

insulin resistance; exercise; skeletal muscle; oxidative capacity

THE ROLE OF LIFESTYLE INTERVENTION in preventing development of type 2 diabetes has been explored in several trials during recent years, and there is now a vast body of evidence demonstrating that physical activity often in combination with weight loss causes a considerable reduction in the risk of developing type 2 diabetes (16, 24, 40). First-degree relatives of patients with type 2 diabetes (offspring) are one group of subjects who have a significant increased risk of developing the disease. Dependent on the burden of type 2 diabetic relatives, the lifetime risk of developing type 2 diabetes is 40–80% (20). Moreover, it has repeatedly been shown that apparently healthy

and normal glucose-tolerant first-degree relatives show a range of metabolic abnormalities (25, 34, 41). Because metabolic derangement resulting from glucose toxicity is negligible in these subjects, they are often employed in studies exploring potentially primary steps in the development of type 2 diabetes.

Poor physical fitness is a strong indicator of an increased risk of developing diabetes (9, 42). Similar to subjects diagnosed with type 2 diabetes and impaired glucose tolerance (32, 35, 36), healthy and glucose-tolerant offspring have been characterized by decreased physical capacity [maximal oxygen uptake ($\dot{V}O_{2\max}$)] even though the level of habitual physical activity was comparable in these groups (6, 22). The cause of decreased $\dot{V}O_{2\max}$ in type 2 diabetic subjects and their relatives has not yet been fully determined. We have previously shown that offspring have a higher proportion of fast, glycolytic type IIb fibers (23). This is important, since low physical fitness is associated with skeletal muscle features influencing insulin sensitivity, e.g., decreased capillary density, a low proportion of type I muscle fibers (19), and a higher glycolytic-to-oxidative enzyme ratio (37).

Thus, since there is evidence that offspring have a lower $\dot{V}O_{2\max}$ compared with subjects without diabetic predisposition, we found it of interest to ascertain whether offspring differ in their ability to respond to exercise training (ET) in terms of improvement in aerobic capacity and insulin sensitivity. At the same time, we assessed metabolic components influencing insulin sensitivity and physical fitness, e.g., fiber type distribution and oxidative enzyme activities.

METHODS

Subjects

Twenty-nine first-degree relatives of patients diagnosed with type 2 diabetes (offspring) were compared with a group of 19 control subjects without any predisposition to type 2 diabetes matched by age and body mass index (BMI). The groups were composed of a comparable distribution of gender. Offspring were recruited either through their parents (diagnosed with type 2 diabetes) consulting the outpatient clinic at Medical Department M, Aarhus University Hospital, Denmark or, like control subjects, through advertisements in local newspapers. If recruited through advertisements, the prediabetic status of the volunteers was verified by meticulous questioning regarding diabetes duration, treatment, and possible late diabetic complications of their parents. Moreover, all subjects had to meet the following inclusion criteria: Caucasian inheritance; age 20–50 yr;

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BMI <30 kg/m²; normal glucose tolerance; being sedentary (not engaged in regular physical activity), and $\dot{V}O_{2\max}$ <50 ml·kg⁻¹·min⁻¹. A questionnaire by Baecke et al. (5) was used to assess the level of habitual physical activity during work and leisure time. Finally, subjects were required to be healthy and not taking any form of prescribed medication on a regular basis. None (offspring and controls) were related. All subjects gave written consent to participate in the study, which was approved by the local ethics committee of the county of Aarhus, Denmark. The study complies with the guidelines proposed in The Declaration of Helsinki.

All subjects were examined before (pre-ET) and after (post-ET) a 10-wk endurance exercise program, as outlined below.

Exercise Protocol

All study subjects were instructed to perform aerobic ET on a bicycle ergometer. The exercise program was three times weekly, 45 min each session at 70% of $\dot{V}O_{2\max}$. Subjects either exercised at local fitness centers or were provided an ergometer bicycle to use at home. The exercise intensity was prescribed, adjusted, and monitored by heart rate. If the bicycle ergometer did not contain a pulse measurement device, subjects were supplied with a pulse wrist watch (Polar Electro, Oy, Finland) to use during the training program. By the initial exercise test, the maximal pulse rate of each individual was assessed simultaneously with the maximal aerobic capacity ($\dot{V}O_{2\max}$). From these parameters, the pulse rate corresponding to ~70% of $\dot{V}O_{2\max}$ was estimated by the curves obtained and passed on to each subject to aim for in their training sessions. Every subject was supplied a diary in which they were instructed to enter date, actual duration of each exercise session, and pulse rate at 15, 30, and 45 min after commencing each training session. By this and by one or two visits in the research unit during the training period of 10 wk, compliance of subjects were assessed. At an intermediary visit during the training program (5 wk), $\dot{V}O_{2\max}$ was assessed, and the exercise protocol was reviewed and adjusted if necessary.

Timing

The timing of the procedures before commencement of the exercise program and after completion was planned in accordance with our attempt to evaluate the chronic effects of ET and to diminish influence from the last exercise bout (21). Accordingly, after completion of the training period (10 wk/30 sessions of exercise), evaluation of insulin sensitivity and muscle biopsies were performed 3–4 days after the last exercise bout. Earlier (1 wk), an oral glucose tolerance test (OGTT) was performed (after 3 days of exercise abstinence), and the subjects continued to exercise for another 4–5 days before stopping the program 3–4 days before the day of the clamp experiment. Moreover, women were studied in the follicular phase in the menstrual cycle both before and after the exercise period to minimize variations in insulin sensitivity resulting from hormonal influence.

Exercise Tests

To make subjects familiar with the exercise test procedures, subjects carried out two exercise tests at baseline. They were asked to report to the research unit in a postabsorptive state to perform an initial exercise test. Subsequently, another test was done 1 wk later (after the OGTT), and the best results of these were taken as baseline values. The test was carried out either as a submaximal test ad modum Astrand ($n = 5$ offspring, 5 controls) or most frequently as a maximal exhaustive incremental exercise test on a cycle ergometer ($n = 24$ offspring, 14 controls). Each individual was assessed by the same method before and after the exercise program.

Submaximal Test

Subjects were placed on a cycle ergometer, and heart rate was monitored during the test. They were instructed to pedal constantly at

50 rpm, and by increasing the exercise intensity by 25 (women) or 50 (men) watts, heart rate was aimed to reach 125 beats/min or more and kept constant during the 4 and 6 min of the test. By using the intensity and the actual heart rate at 4–6 min, $\dot{V}O_{2\max}$ can be estimated by extrapolation (1).

Maximal Incremental Test

Subjects were placed on a bicycle ergometer and were connected to a spiroergometer system by which heart rate, oxygen consumption, and carbon dioxide production were monitored continuously throughout the test (Oxycon Delta; Erich Jaeger, Würzburg, Germany). After a short warm up, the exercise intensity was increased by 20 or 30 W/min so that the total time of the test would not be more than 10–12 min. The test was considered sufficient if 1) subjects felt exhausted, 2) respiratory exchange ratio was >1.1, and/or 3) heart rate was close to the expected maximal heart rate (220 – age). Peak oxygen consumption (ml/min) was divided by body weight, and $\dot{V}O_{2\max}$ accordingly given as milliliters per kilogram per minute.

Anthropometrics

Height, weight, and waist-to-hip ratio were assessed in the standing position.

OGTT and Fasting Blood Samples

At least 5 days after the initial exercise test, subjects were admitted to the research unit after an overnight fast (10–12 h). They were placed in a semiprone position on a bed, and a polyethylene catheter was inserted in an antecubital vein for blood samples. At time (t) 0 min, 75 g of glucose were ingested, and blood samples for glucose, insulin, C-peptide, and NEFAs were obtained at $t = 0, 15, 30, 45, 60, 90,$ and 120 min. After a snack, another exercise test was performed.

Hyperinsulinemic-euglycemic Clamp

Subjects were told to abstain from physical activity and to consume a weight-maintaining diet for 3 days preceding the investigations. Subjects were admitted to the research unit in the morning (0800) after an overnight fast. They were placed on a bed, and intravenous catheters were inserted in an antecubital vein for infusions and in a heated dorsal hand vein on the opposite arm for blood samples. Subjects rested for 150 min before the start of the clamp. In the interval from 120 to 150 min [after indirect calorimetry (IC)], basal muscle biopsies were obtained (see below). From $t = 150$ min, insulin was infused at a rate of 1.0 mU·kg⁻¹·min⁻¹, and plasma glucose was kept at 5 mmol/l by a variable infusion of glucose (200 mg/l). The interval from 270 to 300 min was considered a “steady-state” situation, and the average glucose infusion rate in this period was taken as an expression of insulin-stimulated glucose uptake (ISGU), since endogenous glucose release during this level of insulinemia is negligible (22). Basally (120–150 min) and during the steady state (270–300 min), energy expenditure and oxidation rates of glucose and lipid were assessed by open-circuit ventilated hood IC (see Ref. 11; Deltatrac Metabolic Monitor; Datex, Helsinki, Finland).

Muscular Assessments

At baseline (120–150 min after IC), percutaneous muscle biopsies from the quadriceps muscle were performed after injecting local anesthesia (10% lidocaine) in the skin and percutaneous regions. A small incision was made at the lateral aspect of the thigh in the midbelly of the vastus lateralis of the quadriceps muscle. Using a modified Bergstrom needle (5 mm), muscle tissue was obtained by suction. The biopsy was immediately divided, and one part was put in liquid nitrogen and kept at –80°C. Another part was mounted with Tissue-Tek (Sakura Finetek, Zoeterwoude, The Netherlands), frozen in isopentane cooled by liquid nitrogen, and kept at –80°C until analysis. Serial sections (10 μm) of the muscle biopsy samples were

Table 1. Characteristics of the participants at baseline

Variable	Offspring (n = 29)	Controls (n = 19)
Gender (M/F)	19/10	14/5
Age, yrs	33±5	31±5
Body weight, kg	83.5±7.5	83.7±13.5
BMI, kg/m ²	26.3±1.6	25.8±3.0
Waist-to-hip ratio	0.90±0.05	0.89±0.06
Fasting glucose, mmol/l	5.3±0.5	5.1±0.3
2-Hour glucose, mmol/l	5.6±1.0	5.1±0.9
Fasting C-peptide, pmol/l	646±207	531±149*
Fasting insulin, pmol/l	54±29	55±30
Fasting NEFA, mmol/l	0.48±0.16	0.46±0.21
OGTT AUC glucose, mmol·l ⁻¹ ·2 h ⁻¹	823±126	748±130*
OGTT AUC insulin, mmol·l ⁻¹ ·2 h ⁻¹	30,414±11,854	24,941±11,315
OGTT AUC C-peptide, mmol·l ⁻¹ ·2 h ⁻¹	263,238±82,038	205,162±63,522*
OGTT AUC NEFA, mmol·l ⁻¹ ·2 h ⁻¹	22±8	22±9
Hb A _{1c} , %	5.2±0.4	5.1±0.3
ISGU, mg·kg ⁻¹ ·min ⁻¹	5.5±1.9	7.2±2.6*
VO _{2max} , ml·kg ⁻¹ ·min ⁻¹	38±6.5	40.8±6.1

Values are means ± SD; n, no. of subjects. M, male; F, female; BMI, body mass index; NEFA, nonesterified fatty acid; OGTT, oral glucose tolerance test; AUC, area under the curve; Hb A_{1c}, hemoglobin A_{1c}; ISGU, insulin-stimulated glucose uptake; VO_{2max}, maximal oxygen uptake. *P < 0.05 (Student's *t*-test).

cut in a cryostat at -20°C. Staining of capillaries was performed using the double-staining method (30). Myofibrillar ATPase histochemistry was performed at pH 9.40 after preincubation at pH 4.37, 4.60, and 10.30 and was used to identify muscle fiber types (8). Computer image analysis was performed using an image analysis system (TEMA; Scan Beam ApS, Hadsund, Denmark). Fibers were divided into the following three different types: type I, type IIa, and type IIb (8). All biopsy specimens were evaluated blinded before analysis with respect to the quality and accordingly the validity of the following analyses. Only biopsies expected to give valid results both before and after the training program were included in the evaluation. Thus, for the analyses of muscle morphology, eight offspring and seven controls had to be excluded because either their pre- or post-biopsies did not meet the criteria for a reliable analysis (the no. of fibers was too small or the biopsy showed signs of freeze damage). ISGU in the two groups after exclusion of these subjects was 5.4 ± 2.0 vs. 7.3 ± 2.6 mg·kg⁻¹·min⁻¹; P < 0.05 (offspring vs. controls).

Analytical Methods

Plasma glucose was measured in duplicate immediately after sampling (Beckman Instruments, Palo Alto, CA). Serum insulin was measured with an immunoassay specific for mature insulin and with no significant cross-reactivity for pro-insulin (Dako Diagnostics, Cambridgeshire, UK; see Ref. 4). C-peptide was measured by a commercial immunoassay (Dako Diagnostics). Serum nonesterified fatty acids (NEFA) were determined by a colorimetric method using a commercial kit (Wako Pure Chemical Industries, Neuss, Germany). HbA_{1c} was determined by high-pressure liquid chromatography (normal range: 4.8–6.4%). Activities of the two mitochondrial enzymes, citrate synthase (CS) and cytochrome *c* oxidase (COX), were measured in muscle homogenates as previously described (33).

Statistical Analyses

Data are given as means ± SD. Comparisons between and within groups were performed by Student's two-tailed *t*-test on paired and unpaired data, respectively. Areas under the curve (AUCs) were calculated by the trapezoidal model. Correlation between variables was done by the Pearson product-moment correlation test and linear

regression, and an approximate test for $\beta_1 = \beta_2$ (slope regression coefficients) was used to compare slopes. P < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS for Windows version 11.0 (SPSS).

RESULTS

Subject Characteristics

Thirty-two offspring and 22 control subjects fulfilled the inclusion criteria and were initiated in the exercise program. Three subjects in the offspring group and three subjects in the control group were excluded from the study because of unwillingness to complete the exercise program and the investigational procedures. Thus 29 offspring and 19 control subjects completed the study program and are thus the basis of our analyses. In the offspring group 3 subjects had one known family member with type 2 diabetes (a parent), and 26 subjects had two or more family members (at least one parent) known to be diagnosed with type 2 diabetes. Eight had a maternal and 21 had a paternal family history of type 2 diabetes.

Physical Activity Index and Compliance

By the Baecke questionnaire (5) all subjects were assessed with respect to habitual level of physical activity preceding the initiation of the study, and the score did not differ between the two groups (7.6 ± 1.8 vs. 7.9 ± 2.4; P = 0.59). Compliance with the exercise protocol was assessed by use of individual diaries, and by this both groups were equally compliant with the exercise program with respect to number of training sessions relative to the prescribed amount of exercise (97 ± 6 vs. 97 ± 5%; P = 0.92).

Anthropometrics

At baseline, BMI and waist-hip ratio were comparable in the two groups. In response to the exercise program, offspring showed a reduction in BMI, and a tendency toward the same was apparent in controls. These changes were comparable between groups (Tables 1 and 2).

OGTT

All were normal glucose tolerant at baseline. However, fasting plasma glucose (P = 0.07) and the 2-h plasma glucose

Table 2. Exercise effects given as delta (Δ), post-ET - pre-ET values

Variable	Offspring	Controls
Δ Weight, kg	-0.7±1.6*	-0.9±2.6
Δ BMI, kg/m ²	-0.2±0.5*	-0.3±0.8
Δ Fasting glucose, mmol/l	-0.02±0.36	0.08±0.49
Δ Fasting insulin, pmol/l	4±22	-3±15
Δ Fasting C-peptide, pmol/l	39±260	34±223
Δ Fasting NEFA, mmol/l	-0.07±0.21	0.03±0.30
Δ 2-Hour glucose, mmol/l	-0.3±1.3	0.2±1.2
Δ OGTT AUC glucose, mmol·l ⁻¹ ·2 h ⁻¹	-9±100	38±149
Δ OGTT AUC insulin, mmol·l ⁻¹ ·2 h ⁻¹	-722±9408	-1,246±7,355
Δ OGTT AUC C-peptide, mmol·l ⁻¹ ·2 h ⁻¹	1,566±61,597	8,223±62,402
Δ OGTT AUC NEFA, mmol·l ⁻¹ ·2 h ⁻¹	-1.1±7.8	-0.4±11.4
Δ ISGU, mg·kg ⁻¹ ·min ⁻¹	0.6±1.4*	1.0±2.1*
Δ VO _{2max} , ml·kg ⁻¹ ·min ⁻¹	5.8±4.7†	7.3±6.7†

Values are means ± SD. ET, exercise training. *P < 0.05 and †P < 0.001, pre-ET vs. post-ET. P between groups, all not significant (NS).

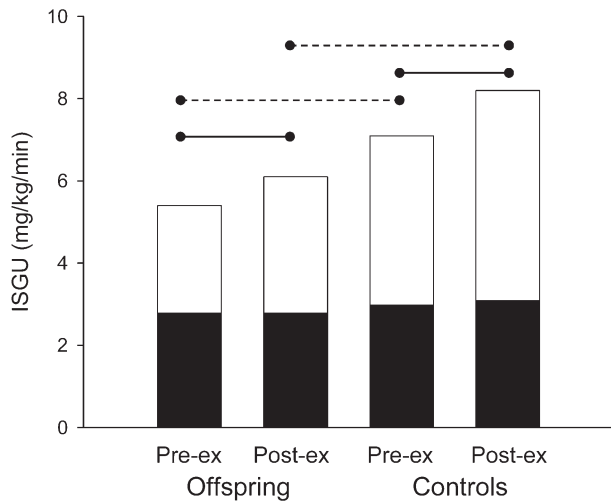


Fig. 1. Insulin-stimulated glucose uptake (ISGU) in offspring and control subjects before and after an exercise program during 10 wk. Total ISGU are divided in oxidative glucose disposal (filled bars) and nonoxidative glucose disposal (open bars) as estimated by the hyperinsulinemic-euglycemic clamp and indirect calorimetry. Solid lines: $P < 0.05$; broken lines: $P < 0.01$.

from the OGTT ($P = 0.08$) tended to be higher in offspring. Basal concentration of circulating C-peptide was increased in offspring, whereas serum insulin and NEFAs were comparable. Offspring had higher glucose levels calculated as AUC. C-peptide was clearly enhanced in offspring during the OGTT, and insulin levels tended to be higher ($P = 0.13$). AUC for NEFAs were comparable in the two groups at baseline (Table 1).

Fasting plasma glucose, fasting serum insulin, and C-peptide did not change significantly in any of the groups after ET. In offspring, fasting concentrations of NEFAs tended to be lower post-ET ($P = 0.06$), but in controls no change in this parameter could be detected. Post-ET, all subjects were still normal glucose tolerant, and 2-h plasma glucose concentrations did not change significantly. Likewise, we were not able to detect any changes post-ET in AUCs during the OGTT with respect to plasma glucose, serum insulin, C-peptide, or NEFAs (Table 2).

ISGU and Substrate Oxidation

As shown by the hyperinsulinemic-euglycemic clamp, offspring were insulin resistant pre-ET (ISGU: 5.5 ± 1.9 vs. 7.2 ± 2.6 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$; $P = 0.01$; Table 1 and Fig. 1). Basal energy expenditure at baseline was comparable between groups (20.2 ± 1.8 vs. 20.7 ± 1.7 $\text{kcal}\cdot\text{kg}^{-1}\cdot 24 \text{ h}^{-1}$; $P = 0.35$). Moreover, during hyperinsulinemia, oxidative glucose disposal was similar (2.8 ± 0.6 vs. 2.9 ± 0.7 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$; $P = 0.52$). In contrast, nonoxidative glucose disposal was lower in offspring than in the controls (2.6 ± 1.7 vs. 4.1 ± 2.2 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$; $P = 0.02$; Fig. 1). Lipid oxidation rates were not different in the two groups basally (0.53 ± 0.30 vs. 0.48 ± 0.52 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$; $P = 0.53$) or during hyperinsulinemia (0.08 ± 0.23 vs. 0.01 ± 0.20 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$; $P = 0.24$).

After the exercise program, insulin sensitivity was improved significantly and comparable in offspring and control subjects (Table 2 and Fig. 1). Oxidative glucose disposal did not change after ET in any of the groups: $\Delta\text{ISGU}_{\text{ox}}$ (offspring) = -0.02 ± 0.9 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$; ($P = 0.93$) and $\Delta\text{ISGU}_{\text{ox}}$ (controls) =

0.2 ± 0.5 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ($P = 0.20$). In contrast, nonoxidative glucose disposal tended to increase in both offspring and controls: $\Delta\text{ISGU}_{\text{non-ox}}$ (offspring) = 0.6 ± 1.6 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ($P = 0.06$) and $\Delta\text{ISGU}_{\text{non-ox}}$ (controls) = 0.8 ± 1.9 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{min}^{-1}$ ($P = 0.09$). These changes were comparable ($P = 0.88$). Energy expenditure post-ET was still comparable between groups and did not change significantly after ET ($P = 0.46$ and $P = 0.44$; offspring and controls). Lipid oxidation (basal and insulin stimulated) did not change from pre-ET values in any group and did not differ between groups post-ET (data not shown).

Aerobic Work Capacity

$\dot{V}\text{O}_{2\text{max}}$ in offspring tended to be lower at baseline and post-ET compared with controls ($P = 0.14$ and 0.07 , respectively; Fig. 2). Maximal exercise intensity in the exercise test (260 ± 64 vs. 262 ± 44 watts; $P = 0.93$) was comparable in the two groups at baseline. Both groups were able to demonstrate a substantial improvement in aerobic work capacity in response to the exercise program (offspring: $14.1 \pm 11.3\%$, $P < 0.001$ and controls $16.1 \pm 14.2\%$, $P < 0.001$; Fig. 2). The improvements were of comparable magnitude in offspring and control subjects ($P = 0.35$). Maximal exercise intensity in the exercise test was also improved equally in both groups (offspring: 260 ± 64 to 290 ± 60 watts, $P < 0.01$ and controls: 262 ± 44 to 297 ± 69 watts, $P < 0.01$).

Muscle Morphology

At baseline, fiber-type distribution was comparable in the two groups. Furthermore, average fiber size and capillary density did not differ (Table 3). After ET, we were not able to detect any significant shift in either fiber-type distribution or fiber size in any of the groups. In offspring, capillary density increased, and this was most prominent in type II fibers. In controls, no significant changes could be detected (Table 3).

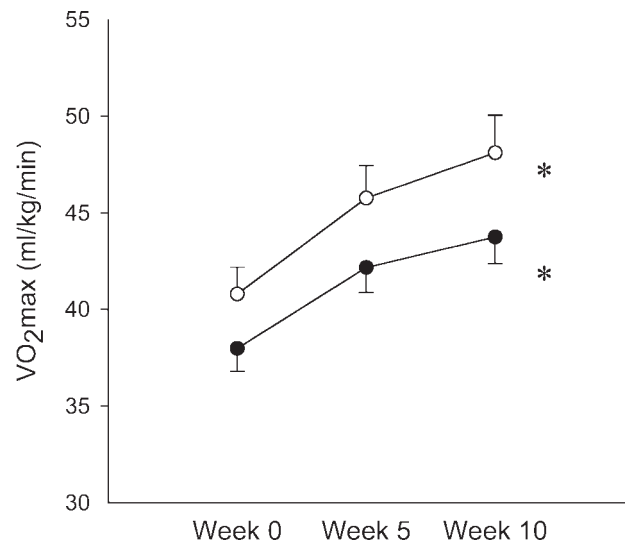


Fig. 2. Maximal oxygen uptake ($\dot{V}\text{O}_{2\text{max}}$) in offspring (●) and controls (○) during a 10-wk endurance training program. $\dot{V}\text{O}_{2\text{max}}$ tended to be lower in the offspring group preexercise ($P = 0.14$) and postexercise ($P = 0.07$). Both groups showed a clear improvement in $\dot{V}\text{O}_{2\text{max}}$ with exercise (* $P < 0.001$).

Table 3. Muscle fiber and capillary data in both groups before and after ET

	Pre-ET		Post-ET	
	Offspring	Controls	Offspring	Controls
<i>Muscle fiber type and size</i>				
<i>n</i>	21	12	21	12
Type I no, %	47±15	44±11	45±16	46±12
Type IIa no, %	36±11	39±9	38±10	37±10
Type IIb no, %	17±13	17±8	15±10	17±10
Type I size	4,890±1,335	5,181±1,391	4,557±1,941	5,117±1,433
Type IIa size	5,149±1,597	5,299±1,420	4,917±2,006	5,097±1,303
Type IIb size	4,422±1,573	4,409±1,468	4,011±1,686	4,361±1,475
<i>Capillary density</i>				
<i>n</i>	19	12	19	12
Cap/type I	4.9±1.2	5.3±0.9	5.2±1.1	5.1±1.1
Cap/type II	4.4±1.2	4.5±0.9	5.0±1.1*	4.4±0.8
Cap/type IIa	4.7±1.3	4.8±0.9	5.2±1.1	4.7±0.7
Cap/type IIb	3.9±1.1	3.8±0.9	4.2±1.1	3.7±0.8
Cap/fiber	2.3±0.6	2.4±0.6	2.6±0.6*	2.4±0.6
Cap/mm ²	482±78	473±80	492±62	478±49

Values are means ± SD; *n*, no. of subjects. Cap, capillary. *P* = NS, offspring vs. controls. Exercise changes were only significant as shown: **P* < 0.05 post-ET vs. pre-ET.

Oxidative Enzyme Activities

At baseline, activities of the oxidative muscular enzymes COX and CS tended to be lower in offspring compared with controls. In response to the exercise program, both groups demonstrated a significant increase in both COX and CS enzyme activities, and the magnitude of these changes did not differ between the two groups (Table 4). After ET, differences in enzyme activities were smaller between the two groups.

Correlative Analyses

ISGU and $\dot{V}_{O_2 \max}$. At baseline, ISGU did not show any association with maximal aerobic capacity in offspring ($r = 0.009$; $P = 0.96$). In contrast, in control subjects, ISGU and $\dot{V}_{O_2 \max}$ were clearly positively associated ($r = 0.67$; $P = 0.002$; Fig. 3A). By linear regression and an approximate test for $\beta_1 = \beta_2$ (slope regression coefficients), the slopes were significantly different in the two groups ($P = 0.003$). After the 10 wk of ET, $\dot{V}_{O_2 \max}$ and ISGU were still correlated in controls ($r = 0.70$; $P = 0.001$) and not in offspring ($r = 0.09$; $P = 0.63$). Furthermore, in control subjects, the increments in ISGU after ET correlated strongly with improvements in $\dot{V}_{O_2 \max}$ ($r = 0.57$, $P = 0.01$). This association could, however, not be demonstrated in offspring ($r = 0.06$, $P = 0.76$). The slopes of the two regression lines were again tested by use of linear regression and likewise showed a strong tendency to be different in the two groups ($P = 0.06$; Fig. 3B).

Table 4. Pre- and post-ET data of muscle mitochondrial enzyme activity in offspring and control subjects

	Pre-ET			Post-ET		
	Offspring	Controls	<i>P</i>	Offspring	Controls	<i>P</i>
COX activity, $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g wet wt}^{-1}$	43.4±11.6	49.4±11.6	0.12	55.5±12.1*	59.2±10.2†	0.32
CS activity, $\mu\text{mol}\cdot\text{min}^{-1}\cdot\text{g wt}^{-1}$	28.0±6.0	31.7±6.0	0.07	35.0±6.8*	39.5±5.8*	0.10

Values are means ± SD; *n* = 22 offspring and 16 control subjects. COX, cytochrome C oxidase; CS, citrate synthase. Exercise-effects. * $P < 0.001$. and † $P < 0.01$.

ISGU and muscle composition. At baseline, there were no statistically significant correlations between fiber-type distribution (%no. of type I, type II, or type IIb) and ISGU in any of the groups. Moreover, fiber size or capillary density did not correlate with ISGU. Number of capillaries per square milliliter correlated with ISGU when both groups were analyzed together ($r = 0.38$; $P = 0.04$). Moreover, a correlation was found between capillaries related to type IIb fibers and ISGU in control subjects ($r = 0.59$; $P = 0.04$). We did not find that changes in fiber-type proportions, fiber size, or capillary density correlated with changes in insulin sensitivity in any of the groups.

$\dot{V}_{O_2 \max}$ and muscle composition. In control subjects, $\dot{V}_{O_2 \max}$ tended to correlate positively with the number of type I fibers (%; $r = 0.51$; $P = 0.10$) and correlated negatively with the number of type IIa fibers ($r = -0.61$; $P = 0.04$). $\dot{V}_{O_2 \max}$ correlated positively with capillary density, e.g., expressed as capillary/fiber ($r = 0.45$; $P = 0.01$). This was consistent with respect to every fiber type. When groups were analyzed separately, the same overall pattern with respect to $\dot{V}_{O_2 \max}$ and capillary density was present. When analyzing exercise effects, we did not find that fiber-type changes or changes in capillary density influenced changes in $\dot{V}_{O_2 \max}$.

Oxidative enzymes. At baseline, COX enzyme activity tended to correlate with insulin sensitivity in control subjects only ($r = 0.48$; $P = 0.06$ vs. $r = 0.07$; $P = 0.78$). CS enzyme activity did not correlate significantly with insulin sensitivity in any group (offspring: $r = -0.04$; $P = 0.85$ and controls: $r = 0.31$; $P = 0.24$). Changes in insulin sensitivity did not significantly correlate with changes in enzyme activities in offspring (COX: $r = -0.10$; $P = 0.68$ and CS: $r = 0.09$; $P = 0.68$) or in control subjects (COX: $r = 0.28$; $P = 0.29$ and CS: $r = 0.36$; $P = 0.17$).

DISCUSSION

The main finding in this study is that first-degree relatives of type 2 diabetic subjects are able to respond adequately to ET in terms of improvement in $\dot{V}_{O_2 \max}$ and insulin sensitivity. Our results are in accordance with a previous study in which first-degree relatives and control subjects increased insulin sensitivity equally in response to ET (26). However, the finding in the current study of a clear and strong association between insulin sensitivity and $\dot{V}_{O_2 \max}$ in control subjects but not in offspring is quite intriguing. Not only did we find these differences between offspring and healthy controls at baseline but also when the changes in insulin sensitivity and $\dot{V}_{O_2 \max}$ after ET were analyzed. This is in contrast with previous data showing a positive correlation between insulin sensitivity and $\dot{V}_{O_2 \max}$ in both offspring and control subjects (22). In the current study, assessment of $\dot{V}_{O_2 \max}$ in the vast majority of subjects was performed by the maximal incremental exercise

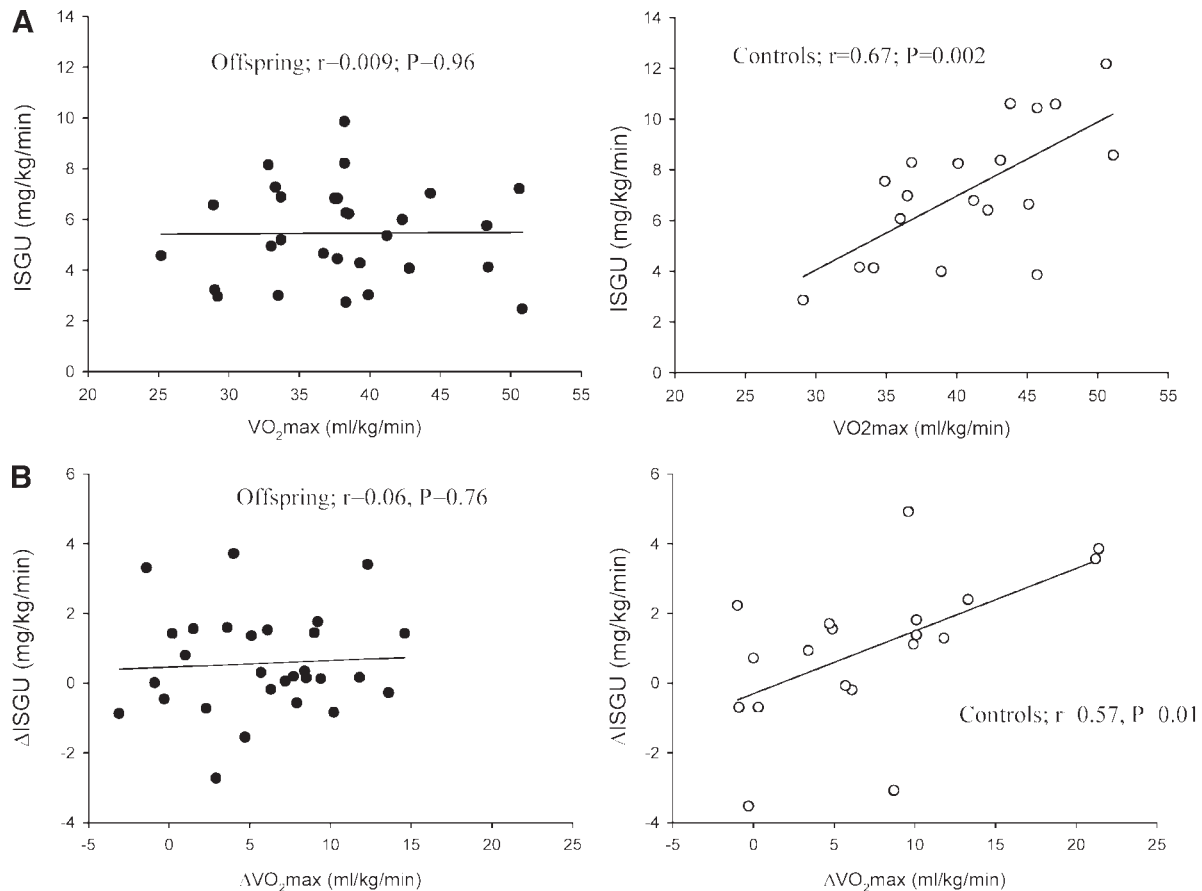


Fig. 3. A: regression at baseline between $\dot{V}O_{2\max}$ and insulin sensitivity (ISGU) in offspring (●) and controls (○). B: regression between changes in $\dot{V}O_{2\max}$ ($\Delta\dot{V}O_{2\max}$) and changes in insulin sensitivity (Δ ISGU) in offspring (●) and controls (○).

test, whereas, previously, the submaximal exercise test was used exclusively. The more accurate measurement of $\dot{V}O_{2\max}$ applied in this study might in part explain this discrepancy regarding the relationship between insulin sensitivity and $\dot{V}O_{2\max}$ among offspring and controls. Additionally, differences in population characteristics may be influential as well. In this study, only subjects with $\dot{V}O_{2\max} < 50 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ were included; furthermore, the genetic burden in the offspring group appears to be stronger.

Abnormalities in oxygen uptake and oxygen kinetics in type 2 diabetic subjects have been demonstrated by others. Regensteiner et al. (31) showed not only reduced physical fitness in subjects with type 2 diabetes but also an abnormal oxygen kinetic response during a maximal incremental exercise test, i.e., the change in the rate of oxygen uptake in response to increasing exercise intensity was compromised in diabetic individuals. The pathophysiological mechanisms could be general cardiovascular attenuation (e.g., endothelial dysfunction) or local perturbations in skeletal muscle. Interestingly, impaired mitochondrial function has recently been proposed as being contributable to the development of insulin resistance (28) and has been found in type 2 diabetic subjects (15) and in healthy offspring, comparable to those included in the present study (29). A reduced oxidative capacity in skeletal muscle has been linked to accumulation of intramyocellular triglyceride (29) which, in turn, is an important correlate of in vivo insulin resistance (17, 27). Whether reduced mitochondrial function in

prediabetic individuals is a genetic trait or caused by unfavorable lifestyle factors (2) has not yet been determined. In a study by Stump et al. (39), it was demonstrated that mitochondrial ATP production induced by insulin is compromised in type 2 diabetic subjects compared with nondiabetic subjects. Accordingly, whether insulin resistance at the mitochondrial level is causative of reduced mitochondrial function or whether primary mitochondrial dysfunction gives rise to insulin resistance in type 2 diabetic subjects and their offspring at this point still is elusive.

The reports of reduced mitochondrial function in prediabetic subjects (29) are in accordance with the present observations of a tendency toward lower basal activities of the oxidative enzymes CS and COX in offspring. Mitochondrial function, i.e., mitochondrial oxidative capacity, is highly correlated with both insulin sensitivity and $\dot{V}O_{2\max}$ (37, 43) and could, therefore, be a potential candidate for linking the two parameters. The activity of oxidative enzymes in skeletal muscle correlates with whole body oxygen consumption during aerobic work, but it is not generally viewed as being the limiting factor in determining maximal work capacity in healthy subjects (7). On the other hand, this might be different in subjects with impaired mitochondrial function and may probably, to some extent, explain our findings.

In both offspring and control subjects, we detected a notable improvement in enzyme activities after the exercise program. This is known to reflect stimulation of mitochondrial biogen-

esis and mitochondrial function induced by ET (14). Hence, from these observations, it can be concluded that offspring and controls do not differ in the ability to improve the activities in these enzymes. Interestingly, in line with the lack of a correlation between $\dot{V}O_{2\max}$ and ISGU in offspring, we found indications of a weaker relation between the enzyme activities and insulin sensitivity in offspring. Thus it would appear that, in offspring, changes in insulin sensitivity are dissociated from changes in $\dot{V}O_{2\max}$ and changes in mitochondrial function and thus might point to different mechanisms prevailing in improving insulin sensitivity. However, with this study, we are not able to determine the precise pathophysiological mechanisms underlying our findings. Several mechanisms may be plausible. Because mitochondrial processes influence both insulin sensitivity and oxygen consumption and because we (present study) and others (29) have found indications of impaired mitochondrial function in first-degree relatives of type 2 diabetic subjects, we speculate whether an impaired regulation of mitochondrial processes might disturb the relation between insulin sensitivity and oxygen consumption as found in this study. However, we cannot exclude that a number of other factors, which we were not able to evaluate in the present study, might impose on our findings, e.g., changes in intramyocellular triglyceride content. Future studies will be needed to resolve these mechanisms.

Of notice, the observations of reduced mitochondrial enzyme activities in offspring were performed in a state of comparable fiber-type distribution between offspring and control subjects. In a previous report, we demonstrated that offspring are characterized by an increased proportion of the glycolytic type IIB fibers and that fiber type and capillary density were related to insulin sensitivity (23). We were not able to replicate these observations in the present study. This discrepancy is most likely attributable to differences in the recruitment procedure. In contrast to our previous study, only subjects with $\dot{V}O_{2\max} < 50 \text{ ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ were included in the current study. As a result of this, conditions relating to potential differences in $\dot{V}O_{2\max}$ (e.g., caused by fiber-type differences) would be harder to detect, since control subjects with a higher $\dot{V}O_{2\max}$ (and probably a more "oxidative" fiber-type composition) would not have been included. Petersen et al. (29) have suggested that compromised mitochondrial function in first-degree relatives could arise from a reduced proportion of oxidative fibers in these subjects. However, in the present study, fiber-type distribution was comparable in offspring and controls. With the limitations aforementioned and the notion that fiber-type analyses could not be performed in all individuals because of inappropriate quality of the pre- or post-ET sample, the present data could suggest that also intrinsic fiber and/or mitochondrial dysfunction may play a role in causing a reduction in the oxidative capacity in skeletal muscle of potentially prediabetic subjects.

In the current study, we failed to show any significant fiber-type shift after ET. Moreover, alterations in fiber size and capillary density were negligible. It is well known that regular ET in humans can elicit structural and biochemical alterations in skeletal muscle, e.g., hypertrophy of muscle fibers, increased capillary density, and often alterations in the proportion of the various fiber types in muscle, particularly a decrease in the amount of type IIB fibers and a corresponding increase in the type IIA fibers (10). Nevertheless, the magnitude of changes

may vary depending on the type of training performed (resistance training vs. endurance training) and the duration and intensity of the training program. Fiber-type changes in response to endurance training have previously been described in some (13), but far from all, studies (12). In general, it is believed that a switch from type IIB to type IIA fibers is relatively easier to evoke in shorter exercise courses, whereas a switch from type II to type I is probably much less feasible over a shorter time range (3).

Because this study was primarily aimed at detecting potential differences in insulin sensitivity and $\dot{V}O_{2\max}$, we are not entirely surprised that fiber-type changes did not occur by the completion of this 10-wk endurance training program. Because changes in capillary density, generally a very robust change with ET, were also minor in this study, it is conceivable that the duration of the current training program has been too short for inducing changes in these parameters. Finally, even though muscle biopsy procedures were standardized in the present study, some variance in biopsy results were to be expected because of unavoidable variation in muscle sampling and technical procedures (18, 38).

This study has examined a group of first-degree relatives and control subjects that were comparable with respect to age, gender, and BMI. In the inclusion procedure, the offspring differed only from the control subjects by the genetic background. Thus we did not select offspring according to the degree of insulin resistance, but despite this recruiting strategy the offspring as a group were insulin resistant compared with the controls. Others (29) have more specifically examined insulin-resistant offspring, e.g., to evaluate the mechanisms of insulin resistance in first-degree relatives. Whether having applied this strategy instead might have influenced the outcome is uncertain.

In conclusion, this study shows that first-degree relatives of type 2 diabetic patients are able to respond as well as the control subjects to endurance training in terms of changes in $\dot{V}O_{2\max}$ and insulin sensitivity. However, in contrast to the control group, we found a lack of a significant correlation between the increased $\dot{V}O_{2\max}$ and insulin sensitivity. In accordance with previous studies, we found indications of reduced oxidative capacity (CS and COX activities) in first-degree relatives of type 2 diabetic patients. Whether these observations are interrelated cannot be determined by this study.

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