Influence of Physical Training on Formation of Muscle Capillaries in Type I Diabetes

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SUMMARY

The effects of physical training on skeletal muscle morphology and enzyme activities were compared in 10 male, type I diabetic subjects and 10 healthy, male, control subjects. The training program consisted of running for 45 min, three times per week for 8 wk. Muscle biopsies were obtained before and after the training period from the lateral portion of the gastrocnemius muscle. Pretraining maximal oxygen uptake was similar in the two groups (diabetic subjects 42 ± 1 versus control subjects 43 ± 2 ml × kg^{-1} × min^{-1}), and the training resulted in an identical increase (+13%, $P < 0.01$). Muscle capillarization (number of capillaries per muscle fiber) increased on the average in the control group (+14 ± 4%, $P < 0.01$), but was unchanged in the diabetic group (0 ± 4%). Capillary density, expressed as number of capillaries per unit muscle cross sectional area, also increased on the average in controls (8 ± 4%, $P < 0.05$) but failed to do so in the diabetic patients (−8 ± 6%, NS). The activities of the mitochondrial enzymes citrate synthase (+26–27%, $P < 0.01$–0.05) and succinate dehydrogenase (+24–25%, $P < 0.05$) increased significantly and similarly in the two groups, whereas training did not result in significant changes in the activities of the glycolytic enzymes 6-phosphofructokinase and glyceraldehyde-phosphate dehydrogenase. Glycemic control in the diabetic group did not improve with the training, as evaluated from hemoglobin A_1 and home-monitored blood glucose.

The findings suggest that, compared with controls, the ability to form new skeletal muscle capillaries in response to physical training may be deficient in patients with type I diabetes mellitus of long standing, while the increase in mitochondrial enzyme activities is normal. A deficient formation of new capillaries may be an expression of the microangiopathy of this disorder.

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Since skeletal muscle is an important target organ for insulin, it is of interest to study the biochemical and morphologic adaptation of muscle to regular physical training in patients with diabetes mellitus. Previous studies have established that patients with type I diabetes are able to increase their maximal oxygen uptake in a normal fashion.[1,2] Moreover, in keeping with the response in healthy controls, the rise in aerobic capacity in these patients is accompanied by increased activities of mitochondrial enzymes in skeletal muscle but no significant changes in the activity of glycolytic enzymes.[1,2]

It has been reported that muscle capillarization is lowered in patients with type I diabetes.[3] Little information is available on the morphologic adaptation of skeletal muscle during training in type I diabetes; a recent study, involving physical training for 4 mo, showed no increase in capillary density.[1] This suggested that the microvascular adaptation to training may be altered in patients with type I diabetes. Consequently, the aim of the present study was to further investigate the adaptation of skeletal muscle to physical training in type I diabetic subjects and in age-matched healthy subjects, particularly with regard to capillarization and enzyme activities. Furthermore, the effect of training on glycemic control was followed in the diabetic group.

MATERIALS AND METHODS

Participants. Ten male, insulin-dependent, type I, diabetic patients and ten male, healthy, control subjects participated in the study. The diabetic patients were recruited among patients regularly attending the outpatient clinic at the Department of Medicine, Huddinge Hospital. The control subjects were recruited among hospital employees and among friends of the diabetic patients. The nature, purpose, and possible risks of the study were carefully explained to the patients and the control subjects before they gave their consent to participate. The study protocol was reviewed and approved by the institutional ethical committee. Eleven pa-

TABLE 1
Pretraining data for the diabetic patients and control subjects

Patient no.	Age (yr)	Height (cm)	Weight (kg)	Ideal weight (%)	BP (mm Hg)	Duration of diabetes (yr)	Insulin dose (U/day)	HbA_1 (%)	Background retinopathy
1	25	171	67.8	98	140/85	13	36	13.2	+
2	25	185	78.9	98	140/90	6	36	10.8	–
3	29	175	73.2	102	140/80	18	48	9.0	–
4	30	178	80.7	105	160/95	16	44	10.6	+
5	33	179	60.0	77	115/80	22	32	7.4	–
6	34	176	74.4	100	135/80	12	46	8.5	+
7	35	172	64.0	88	140/80	21	30	10.7	–
8	35	174	71.4	97	135/85	18	48	7.4	–
9	36	179	66.7	86	130/70	6	56	10.7	–
10	36	190	79.7	88	160/90	10	60	10.3	–
Mean ± SEM	32 ± 1	178 ± 2	71.7 ± 2.2	94 ± 3	140 ± 4/84 ± 2	14 ± 2	44 ± 3	9.8 ± 0.6	3/10
Controls N = 10									
Mean ± SEM	30 ± 2	178 ± 1	77.0 ± 4.1	102 ± 5	134 ± 4/81 ± 2	—	—	6.2 ± 0.1	—
P-value	NS	NS	NS	NS	NS/NS	—	—	$P < 0.001$	—

tients and 11 controls started the study, but two participants (one patient and one control) failed to carry out the training program. Of the 20 subjects completing the training period, 8 had sedentary occupations and 12 (8 patients and 4 controls) had occupations demanding a moderate degree of physical activity. Nine subjects (4 patients and 5 controls) had performed regular physical training during the preceding year (ball games or jogging approximately once a week). One patient and 2 control subjects were cigarette smokers and did not change their smoking habits during the study.

Clinical and laboratory data for the patients and the controls are given in Table 1. None of the patients had clinical evidence of diabetic neuropathy, nephropathy, or hypertension. Background retinopathy was found in 3 patients. Two had detectable C-peptide levels in plasma (≥ 0.03 nmol/L) in the basal state. None of the patients received any medication other than insulin.

Procedure. Pretraining values for physical work capacity (two measurements of maximal oxygen uptake and submaximal heart rate), as well as muscle biopsies for determination of skeletal muscle enzyme activities and capillarization, were obtained over a 3-wk period.

Physical training was performed for 8 wk with three 45-min sessions each week. The training consisted of jogging and running, with the main emphasis on endurance. Twice a week the training took the form of long-distance running (5–10 km) and once a week of intermittent running (5–7 × 1 km or 3–5 × 1.5 km). Each subject's running pace was monitored once a week, and the averages for the two groups did not differ in any week during the training period. The average number of sessions per week in the diabetic and control group was 2.7 ± 0.4 and 2.8 ± 0.5, respectively (NS). All training sessions were performed under the guidance of a qualified instructor. The participants were instructed not to change their daily routine for physical activity, apart from the training.

Posttraining measurements included two determinations of maximal oxygen uptake, one during the last week of training and one during the third or fourth day after the training period. Submaximal heart rate was also determined on these occasions. Muscle biopsies were obtained on the third day (60–70 h) after the last training session.

Maximal oxygen uptake determinations. Maximal oxygen uptake ($\dot{V}_{O_{2max}}$) was determined on a bicycle ergometer (Elema Schönander, Sweden) at 60 rpm on two occasions (or three, if the difference between the first and second determination exceeded 5%) before as well as after the training period. The mean value of the two measurements was used as the pretraining and the posttraining value, respectively. When three determinations were performed, the mean of the two closest measurements was used. The exercise was started at 50 W and the work load was increased by 50 W every 5 min until exhaustion. Expired air was collected in Douglas bags and analyzed by the Scholander microtechnique. For criteria of $\dot{V}_{O_{2max}}$, see Åstrand and Rodahl.[4] In addition, submaximal heart rate, at 150 W, was recorded electrocardiographically at steady-state conditions (6 min of exercise) before training and at the end of the training period.

Muscle biopsies. Muscle biopsy samples were obtained from the lateral part of the gastrocnemius muscle using Weil-Blakesley's conchotome.[5] After local anesthesia, an incision in the skin and fascia was made over the medial part of the lateral head of the muscle at a level where the gastrocnemius muscle had its largest bulk. From the incision, the conchotome was inserted laterally into the muscle. Approximately 100–150 mg of muscle tissue (two samples) were obtained from both the right and the left leg on each occasion. In half of the subjects, the posttraining samples were obtained 2.5–3 cm above, and, in the other half, the same distance below, the initial sampling site. One set of muscle samples (alternately from right or left leg) was immediately freed from blood and visible connective tissue, rapidly frozen in liquid nitrogen and stored at −80°C for subsequent biochemical analysis (enzyme assays and protein determinations). The biopsies taken from the contralateral leg were mounted in an embedding medium (Tissue-Tek II O.C.T. Compound, Lab-Tek

Products, Naperville, Illinois), frozen in isopentane cooled to its freezing point with liquid nitrogen, and then stored at −80°C for subsequent histochemical analysis on serial transverse sections. All analyses were performed after the training period. For the enzyme assays as well as histochemical analysis, all samples were randomized with respect to experimental group and sampling occasion (pre- or posttraining).

Histochemical methods. Serial transverse sections (10 μm) were cut with a microtome at −20°C and stained for myofibrillar ATPase activity[6,7] and with the amylase-PAS method to visualize capillaries.[8] Based on the ATPase stainings after preincubation at different pHs, fibers were classified into two main groups, type I and type II,[9] and type II fibers were subdivided into groups IIA and IIB.[10]

The muscle cross-sections stained with the amylase-PAS method were photographed and copied (magnification ×150) and used for the determination of the following variables: capillaries per fiber, the number of capillaries divided by the number of fibers within a given cross-sectional area; capillary density, the number of capillaries within a given cross-sectional area divided by this area in mm^2; and mean fiber cross-sectional area, a given cross-sectional area in mm^2 divided by the number of fibers within the area.

All of the above parameters were determined from an average muscle cross-sectional area of 2.4 ± 0.3 mm^2 before and 2.0 ± 0.2 mm^2 after training, containing 497 ± 60 and 402 ± 47 fiber cross-sections, respectively. There were no significant differences between the diabetic and control group regarding the size of the analyzed cross-sectional areas or the number of fiber cross-sections included in the analysis. All fiber counting and area measurements were performed by the same person, without knowledge of the origin of the sample. The coefficient of variation for the determination of capillary density (capillaries/mm^2) estimated from measurements on two different parts of the same biopsy specimen was 4% (N = 6); details about the staining procedure for myofibrillar ATPase activity and capillarization have been published elsewhere.[11]

Enzyme assays. Pieces of muscle tissue weighing approximately 10–20 mg were weighed at −20°C. The frozen muscle samples were then transferred to small, all-glass, Potter-Elvehjem homogenizers, containing a 100-fold volume of ice-cold, 0.1 M potassium phosphate buffer, pH 7.3, with 0.05% bovine serum albumin, and homogenized by hand. The resulting crude homogenate, which was kept ice-cooled, was used for the following enzyme activity determinations: citrate synthase (CS, EC 4.1.3.7), succinate dehydrogenase (SDH, EC 1.3.99.1), 3-hydroxyacyl-CoA dehydrogenase (HAD, EC 1.1.1.35), hexokinase (HK, EC 2.7.1.1), 6-phosphofructokinase (PFK, EC 2.7.1.11), and lactate dehydrogenase (LDH, EC 1.1.1.27) determined fluorometrically, and malate dehydrogenase (MDH, EC 1.1.1.37) and glyceraldehydephosphate dehydrogenase (GAPDH, EC 1.2.1.12) determined spectrophotometrically. The various enzymatic assays have been described in detail previously.[1] Enzyme activities are expressed as μkatal/kg wet weight of muscle (1 katal = 1 mol/s, for conversion of μkatal/kg wet weight to μmol × g^{-1} wet weight × min^{-1}, multiply by 0.06). Muscle protein content was determined according to Lowry et al.[12] with bovine serum albumin as a standard.

Glucose control. Pretraining glycemic control was assessed from home-monitored blood glucose 2 days/wk for 3 wk before the training period and from two measurements of hemoglobin A (HbA_1). During the training period, the diabetic subjects were asked to monitor blood glucose at home 5–7 times a day 2 days/wk. HbA_1 was measured in the middle of the training period, immediately after the training period, and 4 wk after the training was finished.

HbA_1 as a percentage of total hemoglobin in blood was determined at constant room temperature (23°C) by a microchromatographic technique, using a commercial kit (Bio-Rad Laboratories, Richmond, California). The normal value for HbA_1 in our laboratory is < 8%.

Home monitoring of blood glucose was performed with a strip for visual reading (BM Test Glycemie 1–44, Boehringer-Mannheim). On 5–15 occasions, each patient collected extra blood in a capillary tube containing fluoride and heparin (Sarstedt, Model 16.446, FRG). The tubes were sent on the same day to the hospital together with the visually read blood glucose value. Linear regression analysis of the values obtained in the hospital versus the home monitored blood glucose values showed an acceptable correlation ($r = 0.71$, N = 95). The strip underestimated blood glucose values in the lower range and the regression line obtained was: laboratory value = strip value × 0.86 + 2.28. For further cal-

TABLE 2
Skeletal muscle capillarization, fiber areas, and fiber type distribution in diabetic and healthy subjects before and after 8 wk of physical training

	Diabetic subjects				Control subjects			
	Before	After	Change	P-value	Before	After	Change	P-value
Capillaries/fiber	1.69 ± 0.05	1.68 ± 0.08	0 ± 4%*	NS	1.59 ± 0.09	1.79 ± 0.08	+14 ± 4%	< 0.01
Mean fiber area (μm²)	4645 ± 234	5050 ± 272	+10 ± 5%	NS	5005 ± 235	5235 ± 243	+5 ± 4%	NS
Capillaries/mm²	366 ± 10†	333 ± 21	−8 ± 6%*	NS	323 ± 12	349 ± 14	+8 ± 4%	< 0.05
Fiber type distribution (%)								
I	57 ± 3	56 ± 3		NS	56 ± 3	56 ± 3		NS
IIa	22 ± 3	23 ± 2*		NS	25 ± 1	29 ± 2		< 0.05
IIb	21 ± 2	21 ± 2		NS	19 ± 2	15 ± 2		< 0.05

*P < 0.05 in comparison with control subjects.
†P < 0.01 in comparison with control subjects.

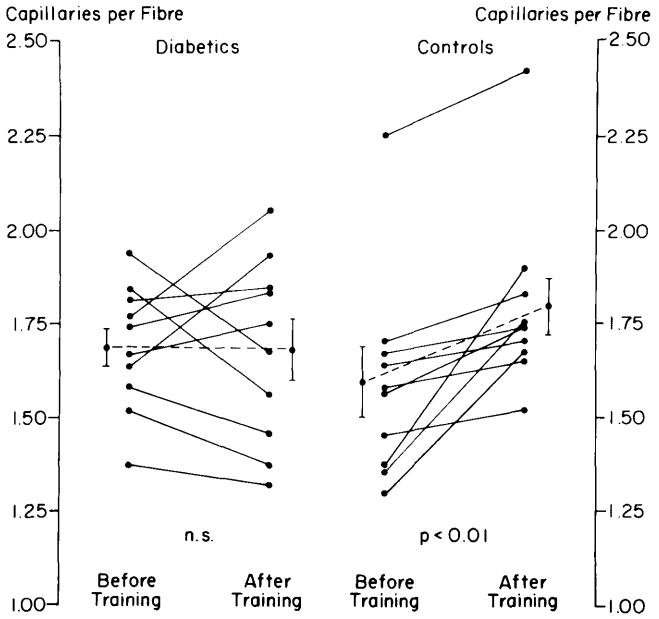

FIGURE 1. Individual changes in the number of capillaries/fiber of the gastrocnemius muscle of diabetic patients and healthy controls in response to 8 wk of physical training. The dashed lines unite mean ± SEM before and after training.

culations of blood glucose control, home-monitored blood glucose values were corrected according to the equation obtained. In the entire period, a total of 946 blood glucose measurements were made at home. Seven of the patients each did 90–163 measurements, whereas 3 performed only 30, 66, and 76 tests, respectively. The home-monitored blood glucose values were used for calculation of mean blood glucose and a glucose control index. After correction of each blood glucose value (see above), the values were transformed to M-values as described by Schlichtkrull et al.[13] The weekly control index for each individual is given as the mean of all his M-values for the week.

No dietary advice was given during the training period. All participants ingested an extra snack (milk or coffee, bread and butter), containing approximately 1100 kJ, after the exercise. The patients were instructed to continue their usual insulin dose, with a reduction on training days in accordance with their own experience before starting the program. Five patients reduced their insulin dose by 2–6 U on training days. The patients and their families were requested to record all episodes of symptomatic hypoglycemia, using the following scale: grade I, symptoms that subsided with no treatment; grade II, symptoms necessitating carbohydrate intake administered by the patient; grade III, symptoms necessitating assistance with carbohydrate administration; and grade IV, coma.

C-peptide in plasma was determined by radioimmunoassay using antibody M 1230.[14] Synthetic human C-peptide was used as standard and ^{125}I-Tyr-C-peptide as tracer.

Statistical methods. Data are given as mean ± SEM in the text, tables, and figures. Intra- and interindividual comparisons were performed with Student's paired and unpaired *t* tests, respectively. Percentage changes were calculated as means of individual values.

RESULTS

Physical work capacity. The pretraining value for maximal oxygen uptake was similar in the diabetic and the control groups, 42.2 ± 1.3 and 42.9 ± 2.2 ml × kg^{-1} × min^{-1}, respectively, and in the normal range for healthy subjects in the corresponding age group.[15] Training resulted in a 13% increase in $\dot{V}_{O_{2max}}$ in both groups, for the diabetic subjects to 47.5 ± 1.4 ml × kg^{-1} × min^{-1} ($P < 0.001$), and for the controls to 48.4 ± 2.2 ml × kg^{-1} × min^{-1} ($P < 0.001$). The individual increases in $\dot{V}_{O_{2max}}$ varied between 2% and 38%. Submaximal heart rate at 150 W decreased from 158 ± 4 to 144 ± 4 beats/min ($P < 0.01$) and from 154 ± 5 to 144 ± 4 beats/min, ($P < 0.05$) in the diabetic and the control groups, respectively, further indicating improved physical work capacity. Maximal heart rate decreased from 193 ± 2 to 182 ± 1 beats/min ($P < 0.01$) in the diabetic group and from 194 ± 2 to 187 ± 2 beats/min ($P < 0.05$) in the control group.

Muscle morphology. Table 2 presents data on muscle fiber type distribution, fiber areas, and capillarization. The number of capillaries per fiber was similar in the patients and the controls before training, but, whereas this number increased by 14 ± 4% ($P < 0.01$) in the controls in response to training, no corresponding augmentation was observed in the diabetic patients. This difference in response between the two groups was statistically significant ($P < 0.05$). However, as evident from Figure 1, there were variable individual responses. Whereas all the control subjects displayed an increase in capillaries per fiber, only 5 of the 10 diabetic subjects showed an increase. It is noteworthy that the diabetic subjects who showed an increase in capillarization had significantly shorter disease duration than those lacking a response; 11 ± 2 yr versus 18 ± 2 yr ($P < 0.05$). There were no significant differences between the two subsets of dia-

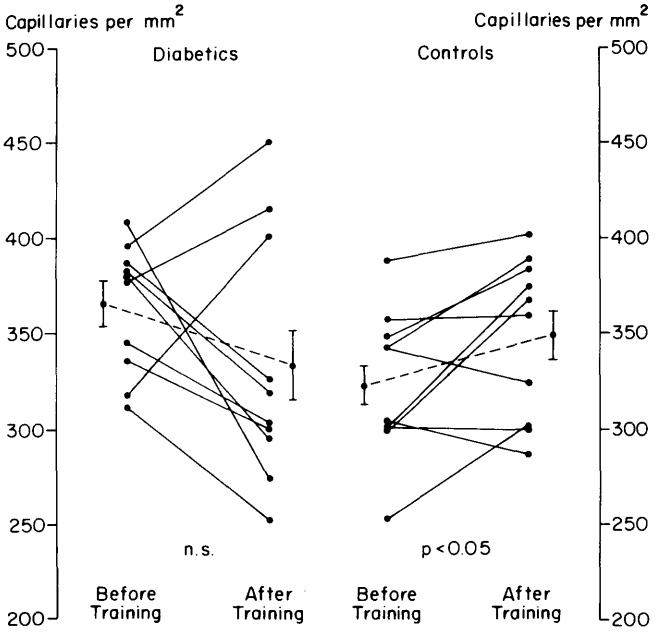

FIGURE 2. Individual changes in the number of capillaries/mm^2 cross-sectional area of the gastrocnemius muscle of diabetic patients and healthy controls in response to 8 wk of physical training. The dashed lines unite mean ± SEM before and after training.

TABLE 3
Skeletal muscle enzyme activities (μkatal/kg wet wt)* and protein content (g protein/g muscle wet wt) in diabetic and healthy control subjects before and after 8 wk of physical training

	Diabetic subjects				Control subjects			
	Before	After	Change	P-value	Before	After	Change	P-value
Hexokinase	24.8 ± 1.5†	31.7 ± 1.7	+28%	< 0.05	30.8 ± 2.6	32.5 ± 2.3	+5%	NS
6-Phosphofructokinase	332 ± 16	360 ± 21	+9%	NS	315 ± 17	332 ± 16	+6%	NS
Glyceraldehydephosphate-dehydrogenase	3940 ± 150	4050 ± 195	+3%	NS	3620 ± 155	3700 ± 240	+2%	NS
Lactate dehydrogenase	3420 ± 230‡	3100 ± 200	−9%	< 0.1	2440 ± 260	2390 ± 330	−4%	NS
Citrate synthase	175 ± 16	213 ± 15	+26%	< 0.05	178 ± 14	223 ± 13	+27%	< 0.01
Succinate dehydrogenase	90.0 ± 8.4	111.7 ± 12.6	+25%	< 0.05	98.2 ± 9.8	116.7 ± 9.5	+24%	< 0.05
Malate dehydrogenase	4850 ± 180	4970 ± 180	+3%	NS	5450 ± 270	5620 ± 260	+4%	NS
3-Hydroxyacyl-CoA dehydrogenase	278 ± 19	307 ± 22	+12%	NS	287 ± 23	293 ± 14	+6%	NS
Protein	0.197 ± 0.02	0.188 ± 0.02	−4%	NS	0.194 ± 0.03	0.192 ± 0.02	−1%	NS

*1 μkatal = 1 μmol/s.
†$P < 0.1$ in comparison with control subjects.
‡$P < 0.05$ in comparison with control subjects.

betic subjects with regard to age, glycemic control, or change in $\dot{V}_{O_{2max}}$ with training.

The average cross-sectional fiber area was similar in the two groups before training and did not increase significantly in either group during training (+10 ± 5% NS, diabetic subjects; +5 ± 4%, NS, controls) (Table 2). Consequently, the capillary density (capillaries/mm^2)—which was higher in the diabetic patients before training (+13%, $P < 0.01$)—was unaltered during training in the diabetic patients (−8 ± 6%, NS) while a significant rise was observed in the controls (+8 ± 4%, $P < 0.05$) (Table 2). The differences in response in this regard between the two groups was significant ($P < 0.05$). Again, the individual responses were highly variable (Figure 2). No relationships were found between changes in capillary density and age, duration of diabetes, glycemic control, or change in $\dot{V}_{O_{2max}}$.

The relative occurrence of type I and type II fibers in the gastrocnemius muscle before training did not differ between the diabetic and control groups. In the diabetic group, no significant changes took place during training with regard to fiber type distribution. In the control group, however, the percentage of type IIa fibers increased from 25 ± 1 to 29 ± 2% ($P < 0.05$), while type IIb fibers decreased from 19 ± 2 to 15 ± 2% ($P < 0.05$).

Muscle enzyme activities. The results of enzyme activity determinations on the samples from the gastrocnemius muscle before and after the training period are given in Table 3. Skeletal muscle oxidative capacity increased similarly with training in the diabetic and control groups, as shown by the 26% ($P < 0.05$) and 27% ($P < 0.01$) increase in CS activity and the 25% ($P < 0.05$) and 24% ($P < 0.05$) increase in SDH activity, respectively.

There were no significant changes with training in either group with respect to the fatty acid beta-oxidation enzyme HAD or the glycolytic enzymes PFK and GAPDH; nor were there any differences in the pretraining values for these enzyme activities between the groups. However, the pretraining activity of HK was somewhat lower in the diabetic group ($P < 0.1$) and it increased significantly by 28% with training ($P < 0.05$), while no change occurred in the control group (+5%, NS). The activity of LDH before training was higher in the diabetic group ($P < 0.05$) and tended to decrease with training (−9%, $P < 0.1$), while it was unchanged in the control group (−4%, NS).

Glucose control. Blood glucose control assessed by HbA_1 did not change during the training period (Figure 3). Before the training, the mean HbA_1 was 9.8 ± 0.6% and at 4 and 8

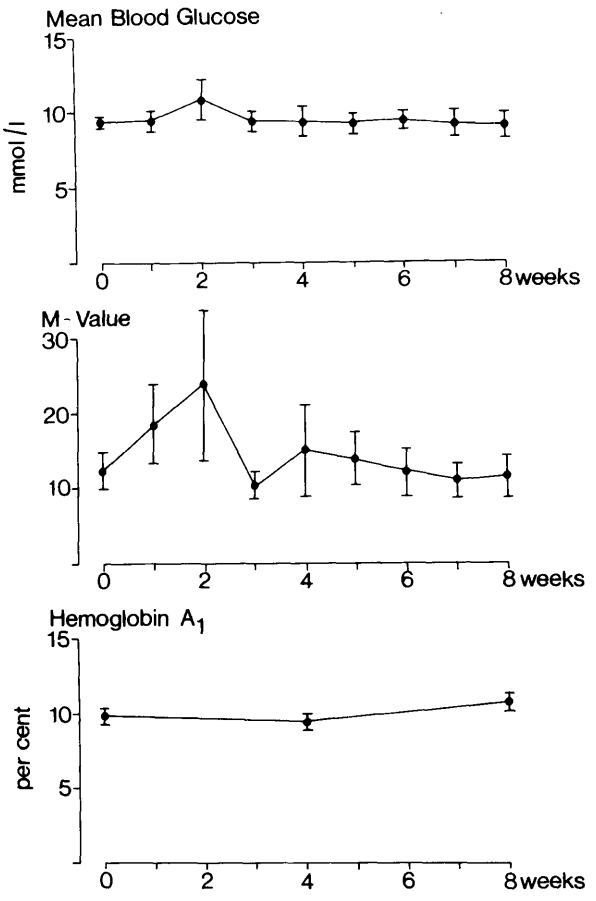

FIGURE 3. Glycemic control assessed by home-monitored blood glucose (mean blood glucose and M-value) and HbA_1, before and during 8 wk of training.

wk it was 9.4 ± 0.6 and 10.8 ± 0.6%, respectively. Any delayed effect on HbA_1 was excluded by a measurement 4 wk after the training period, showing a value of 10.4 ± 0.6%. The mean blood glucose and the M-index calculated from the home-monitored blood glucose did not change significantly during the training period, although there was a tendency toward deterioration during the early phase of the training (Figure 3). The number and severity of registered hypoglycemic attacks were unchanged during the study period and averaged 0.4 ± 0.1 (grade II–III) attacks per patient and week. There was no episode of hypoglycemic coma (grade IV). Three of the participants had to reduce their daily maintenance insulin dose by 4 U each during the latter part of the training period.

DISCUSSION

The increase in maximal oxygen uptake was virtually identical in diabetic and control subjects in the present study, demonstrating that patients with type I diabetes have a normal ability to increase their physical work capacity. This is in agreement with previous reports.[2,16,17] Likewise, the average pretraining value for maximal oxygen uptake did not differ between the diabetic and control groups, which agrees with recent studies.[2,3] Thus, contrary to earlier studies,[17,18] it appears that the disease itself does not influence the level of maximal oxygen uptake—at least not in patients with no or mild clinical signs of microangiopathy. Previously reported differences may possibly be a consequence of differences in the level of physical activity.

In healthy subjects, physical training is a powerful stimulus for formation of new capillaries in skeletal muscle[11] and it has been shown by several authors that there is a close correlation between maximal oxygen uptake and muscle capillarization.[19,20] In this study, however, the muscle capillarization in the 10 diabetic patients as a group, measured as capillaries per fiber, remained unchanged despite a 13% increase in $\dot{V}_{O_{2max}}$. This average value resulted from the fact that half of the diabetic subjects showed increases in capillaries per fiber, whereas the other half of the patients had no such response (Figure 1). In contrast, all of the control subjects showed an increase in capillaries per fiber (mean +14%, $P < 0.01$). In the measurements of capillary density (capillaries per mm^2), the diabetic group failed to respond with an increase (mean −8%, NS), whereas there was a significant increase in the control group (mean +8%, $P < 0.05$). In both groups, however, considerable interindividual variation was seen.

The question then arises as to why the skeletal muscle in some of the diabetic patients did not respond to the physical training with an increased capillarization. Differences in the frequency and intensity of the training can be ruled out as an explanation, since both these variables were similar in the two groups. The interindividual variation in capillary response in the diabetic group is interesting. It could be speculated that, in the diabetic subjects lacking a capillary response to training, microvascular lesions of the muscles impaired the capacity to form new capillaries. The observation that the diabetic subjects with no new formation of capillaries had significantly longer diabetes duration supports this assumption. It is well known that the microangiopathy of diabetes mellitus also involves skeletal muscle with thickening of the capillary basal membrane,[21,22] but information is, so far, lacking on its pathophysiologic significance. However, it may be that, as a consequence of the diabetic condition, formation of new capillaries in skeletal muscle in response to physical training takes a longer time than provided in the present study, since an increased number of capillaries per fiber has been observed after 4 mo of physical training.[1]

The present results may appear to be at variance with our own earlier findings.[1] However, our previous report involved training during a longer period (4 mo), the type of training was different, and biopsy material was taken from another muscle group (m. quadriceps femoris). Moreover, in the previous study we did observe an increase in the number of capillaries per muscle fiber, but there was also an increase in the fiber area. Thus, capillary density (capillaries per unit cross-sectional area) failed to increase in the diabetic subjects in our previous report as well as in the present study. The earlier study did not involve a control group, which made it impossible for us to draw firm conclusions in this regard.

In contrast to results from an earlier study,[3] we found a higher capillary density in the diabetic group before training. This cannot be ruled out entirely as an explanation for the unchanged capillarization after physical training in this group. However, since the two groups in the present study had a similar average physical activity level, it appears more likely that the higher pretraining capillary density in the diabetic muscle represents a compensatory mechanism for an impaired capillary function.

Physical training of endurance character induces adaptive increases in skeletal muscle respiratory capacity, as indicated by increased mitochondrial enzyme activities in healthy subjects (see ref. 23) and in type I diabetic subjects.[1,2] In the present study, the pretraining values for the two mitochondrial enzymes, CS and SDH, did not differ between diabetic patients and controls and, furthermore, in response to physical training the two groups showed the same adaptive increase in the activity of these two enzymes. The two glycolytic enzyme markers PFK and GAPDH were largely unaffected by the training regimen in both groups, which accords with results from previous studies of healthy[23] and diabetic subjects.[1] Regarding HK and LDH, however, there seem to be some differences between the two groups. The pretraining activity of HK tended to be lower in the diabetic subjects, 24.8 ± 1.5 versus 30.8 ± 2.6 μkatal/kg wet weight ($P < 0.1$). A lower HK activity in type I diabetic subjects has been reported by Saltin et al.,[3] while Costill et al. did not find any difference.[2] As HK activity has been shown to be decreased in skeletal muscle of streptozocin-diabetic rats, while normal values were restored by insulin administration,[24–26] the lower HK activity found in the muscle of type I diabetic subjects could possibly be explained by a relative insulin deficiency, resulting in diminished HK synthesis either primarily or secondarily (via a decreased entry of glucose into the muscle cell). However, regardless of the explanation, physical training restored HK activity in the diabetic group (+28%, $P < 0.05$) to a level similar to that found in the control group.

Concerning LDH, the pretraining enzyme activity was 40% higher in the diabetic group, 3420 ± 230 versus 2440 ± 260 μkatal/kg wet weight ($P < 0.05$), which is in agreement with

the 50% higher LDH activity found by Saltin et al.[3] in a type I diabetic group. Physical training tended to decrease LDH activity in the diabetic group (−9%, $P < 0.1$), while no change occurred in the control group (−4%, NS). It has been shown that, in diabetic rats, lactate release from the resting hind limb is significantly higher than in normal rats.[27] Furthermore, diabetic patients show a greater increase in blood levels and leg release of lactate during exercise in comparison with healthy controls.[28,29] This seems to fit well with the present finding of higher LDH activity in the diabetic skeletal muscle. Lower pyruvate dehydrogenase activity has been reported in diabetic rats[30,31] and, furthermore, free fatty acids, which reach high plasma levels in an insulin-deficient state,[32] have been demonstrated to decrease the activity of pyruvate dehydrogenase.[33] The physiologic implication of this could be a compensatory increase in the LDH activity.

Glycemic control did not improve during the period with regular training, which is in accordance with a previous study.[1] It was encouraging, however, that the diabetic subjects were able to perform the intermittent bouts of vigorous exercise without having an increased number of hypoglycemic attacks.

In summary, the present study demonstrates that type I diabetic subjects with no or mild clinical evidence of diabetic complications respond normally to physical training of endurance character as regards maximal oxygen uptake and skeletal muscle mitochondrial enzyme activities. However, the disease seems to alter the capillary response to training. The present results show that muscle capillarization is not a limiting factor for the training-induced increase in $\dot{V}_{O_{2max}}$ and muscle mitochondrial enzyme activities. Since no effect was observed on HbA_1 or home-monitored blood glucose, it is suggested that, in patients with type I diabetes, regular physical training of relatively short duration does not improve glycemic control.

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