Increased Peripheral Insulin Sensitivity and Muscle Mitochondrial Enzymes but Unchanged Blood Glucose Control in Type I Diabetics After Physical Training

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SUMMARY

Nine male, insulin-dependent diabetic patients participated in a 16-wk training program consisting of 1 h of jogging, running, ball games, and gymnastics, performed 2–3 times/wk. The training resulted in an 8% increase of maximal oxygen uptake ($P < 0.01$). Insulin sensitivity as determined by the insulin clamp technique increased 20% ($P < 0.05$). Glycosylated hemoglobin showed no change (10.4 ± 0.7% versus 11.3 ± 0.5%), 24-h urinary glucose excretion was not reduced, and home-monitored urine tests were unchanged. The frequency of hypoglycemic attacks did not change during the training period and body weight remained constant. There was a 14% fall in plasma cholesterol ($P < 0.01$) and a rise in the proportion of HDL-cholesterol from 24 ± 2% to 30 ± 3% ($P < 0.01$).

Thigh muscle oxidative capacity increased, as indicated by a 24% increase in succinate dehydrogenase activity ($P < 0.05$). The number of capillaries/muscle fiber increased 15% ($P < 0.01$). However, as the mean muscle fiber cross-sectional area increased to a similar extent (11%, $P < 0.05$), capillary density (cap × mm^{-2}) was unchanged.

In conclusion, this study demonstrates that physical training in insulin-dependent diabetics results in increased peripheral insulin sensitivity, a rise in muscle mitochondrial enzyme activities, decreased total plasma cholesterol levels, and unchanged blood glucose control. The findings suggest that in the absence of efforts to alter dietary regulation and insulin administration, physical training consisting of 2–3 weekly bouts of moderate exercise may not of itself improve blood glucose control in type I diabetes. DIABETES 31:1044–1050, December 1982.

Physical training has generally been recommended for patients with diabetes mellitus as an important part of their treatment.[1] Since blood glucose often decreases during acute physical exercise in insulin-dependent diabetics,[2–4] it has been thought that regular exercise on a long-term basis might improve metabolic control[1] and thereby avoid or postpone diabetic angiopathy.[5,6] So far, however, the effect of such training on metabolic control in these patients has not been clarified. In normal-weight and obese subjects, an increase in insulin sensitivity after regular physical training has been suggested on the basis of lower insulin levels in serum in association with unchanged glucose tolerance.[7–10] Whether physical training alters insulin sensitivity and/or blood glucose regulation in patients with type I (insulin-dependent) diabetes has not been investigated.

Skeletal muscle, as the largest tissue in the body, is an important target organ for insulin. Furthermore, it is well known that skeletal muscle adapts profoundly to physical training (see Salmons and Henriksson[11]). In the rat, physical training increases the sensitivity of muscle tissue to insulin.[12,13] Therefore it is conceivable that part of this reported increase in insulin sensitivity with training might be related to the metabolic or microvascular adaptation in skeletal muscle. At present, however, little information is available on the adaptation of skeletal muscle to physical training in diabetes.

The purpose of the present study was to examine how physical training in insulin-dependent diabetics affects insulin sensitivity and blood glucose control as well as some biochemical and structural characteristics of skeletal muscle.

MATERIALS AND METHODS

SUBJECTS

Nine male, insulin-dependent type I diabetic patients regularly attending the outpatient clinic at the Department of Medicine, Huddinge Hospital, participated in the study. Clinical and laboratory data for the patients are presented in

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Received for publication 28 April 1982.

Table 1. None of the patients had clinical evidence of diabetic neuropathy, nephropathy, or hypertension. Background retinopathy was found in two patients. Two had detectable C-peptide levels (≥0.03 nmol/L) in plasma in the basal state. None of the patients received any medication other than insulin. Five patients were cigarette smokers and did not change their smoking habits during the study. The nature, purpose, and possible risks of the study were carefully explained to the patients before they gave their consent to participate. The protocol was reviewed and approved by the institutional ethics committee.

PROCEDURE

Pretraining values for clinical control of diabetes (including blood glucose, urine glucose, and glycosylated hemoglobin), insulin sensitivity, maximal oxygen uptake, muscle enzyme activities, and capillarization were obtained over a 4-wk control period.

Physical training was performed for 16 wk, with three 60-min sessions each week, at Huddinge Hospital in Stockholm, under the guidance of a qualified instructor. The training, consisting of jogging, running, ball games, and gymnastics, was carried out indoors during the first half of the training period and outdoors during the second half. All patients ingested a snack (milk, bread and butter) containing approximately 1600 kJ after the exercise. The participants were instructed not to change their daily routine for physical activity, apart from the training. No dietary advice was provided during the training period. The patients were instructed to continue their usual insulin dose, with a reduction on training days in accordance with their own experience prior to starting the program. 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; grade IV, coma. Once a month during the training period measurements were obtained for plasma cholesterol, glycosylated hemoglobin, and 24-h urine glucose excretion. Blood samples were drawn immediately before a training session (6 p.m.). Home-monitored urine tests were performed 4 times/day, 2 days/wk.

The intensity of the training was evaluated in all subjects, using a portable heart-rate recorder (Swedish Radio AB, Stockholm, Sweden), during one typical 60-min session in the last week of training. In two of the subjects, EMG activity from the lateral portion of the quadriceps femoris muscle (corresponding to the muscle biopsy site) and from the gastrocnemius muscle was recorded by surface electrodes, telemetrically transmitted to a receiver (Medinik model IC-600, Medinik AB, Örbyhus, Sweden) and transcribed on an ink recorder (Mingograph 34, Siemens-Elema, Stockholm, Sweden). The EMG recordings confirmed that the muscle biopsies had been obtained from a muscle that was thoroughly activated during the training sessions. Furthermore, the average EMG activity of the lateral portion of the quadriceps muscle was not different from that of the gastrocnemius muscle.

Eleven subjects had agreed to participate in the study, but two failed to carry out the training program. The nine who completed the training period participated an average of 2.4 times/week.

Posttraining studies (insulin sensitivity and muscle sampling) were performed 60–70 h after the last training session. Blood samples (for glycosylated hemoglobin and cholesterol) were obtained during the last week of training.

MAXIMAL OXYGEN UPTAKE DETERMINATIONS

Before and after the training period, maximal oxygen uptake ($\dot{V}O_2$ max) was determined on a bicycle ergometer. The exercise was started at 50 W and the work load was increased by 50 W every 6 min until exhaustion. Expired air was collected in Douglas bags and analyzed by the Scholander microtechnique. At least two $\dot{V}O_2$ max determinations were made for each subject, before as well as after the 4-mo training period (for criteria of $\dot{V}O_2$ max see Åstrand and Rodahl[14]). In addition, submaximal heart rate, at 150 W, was registered before training, after 6 wk of training, and at the end of the training period.

INSULIN SENSITIVITY

The sensitivity of peripheral tissues to the in vivo action of insulin was evaluated before and after the training period at one dose level of insulin, using the insulin clamp technique.[15] The patients were studied in the basal state after an overnight fast. After a control period, a continuous infusion of insulin (1 mU/kg/min) was administered for 100 min to obtain physiologic hyperinsulinemia. In the pretraining study, the plasma glucose concentration was maintained during

TABLE 1
Clinical data for diabetic patients

Patient	Age (yr)	Duration of diabetes mellitus (yr)	Height (cm)	Weight (kg)	Weight (% of ideal)	Blood pressure (mm Hg)	Daily insulin dose (U)	Hemoglobin A_1 (%)*
1	46	16	176	70.2	104	105/60	56	9.7
2	30	11	183	73.0	99	125/75	32	9.5
3	36	13	186	84.3	112	150/80	56	15.3
4	34	15	185	67.9	90	135/70	32	9.9
5	34	11	176	72.8	108	135/75	50	9.8
6	39	8	178	74.3	109	110/60	36	8.0
7	37	7	175	82.2	122	130/75	52	11.7
8	37	15	173	80.0	122	150/75	68	10.1
9	25	11	190	88.4	111	115/85	56	9.4
Mean ± SEM	35 ± 2	12 ± 1	180 ± 2	77.0 ± 2	108 ± 3	128 ± 5/73 ± 3	49 ± 4	10.4 ± 0.7

* Normal value < 8.0%.

the insulin infusion at the ambient fasting level by means of a variable glucose infusion and blood glucose determination every 5 min. However, in five of the patients, in whom the plasma glucose levels were initially 12.1–18.5 mmol/L, the values were allowed to decline to 8.3–10.0 mmol/L before the glucose infusion was started. In the posttraining insulin clamp study the plasma glucose level was maintained at the same levels as in the pretraining study. The rate of glucose infusion required to maintain a constant glucose level during administration of insulin at a rate of 1 mU/kg/min equals the total body glucose turnover, since insulin in this dose level has been shown to inhibit hepatic glucose production.[16]

BIOCHEMICAL DETERMINATIONS

All blood samples (except those obtained during the insulin clamp study) were obtained in the nonfasting state (6 p.m.). Venous plasma glucose was measured by the hexokinase method and 24-h urinary glucose by a glucose-oxidase method. Glycosuria was monitored at home with Keto-Diastix (Ames Co., Elkhart, Indiana). C-peptide in plasma was determined by radioimmunoassay using antibody M 1230.[18] Synthetic human C-peptide was used as standard and ^{125}I-Tyr-C-peptide as tracer. Glycosylated hemoglobin (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 less than 8%. Total cholesterol was measured as described by Allain et al.,[18] using a commercial kit (Nyegaard, Oslo, Norway). The HDL fraction was determined as the cholesterol remaining after precipitation with magnesium and dextran sulphate.[19] All samples for total cholesterol and HDL cholesterol were analyzed in a single assay. Plasma free insulin was determined by the method of Kuzuya et al.[20]

MUSCLE BIOPSIES

Muscle biopsy samples were taken from the middle portion of the lateral head of the quadriceps femoris muscle using a biopsy needle.[21] Approximately 60–100 mg of muscle tissue (two samples) were obtained from each leg on each sampling occasion. The posttraining samples were obtained in half of the subjects 2.5–3 cm above and in the other half the same distance below the initial sampling site. The muscle samples obtained from one of the legs (alternately right or left) were immediately freed from blood and visible connective tissue, rapidly frozen in liquid nitrogen, and stored at −80°C for subsequent biochemical analysis (enzyme assays, protein and glycogen 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.

Enzyme assays. For enzyme analysis, pieces from the muscle biopsies of 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-cooled 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 enzyme activity determinations. For MDH analysis, this homogenate was further diluted 1:5 prior to activity determination. The biopsy specimens from the pre- and posttraining sampling occasions were analyzed simultaneously in a randomized order within 6–8 wk after the termination of the training period. Preliminary studies had shown that the longer period of storage for the pretraining samples did not influence the activities of the studied enzymes.

The enzymatic assays were performed in duplicate at 30°C under conditions where reaction rates were proportional to enzyme concentration. 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) were determined fluorometrically. CS was determined with the method described by Shepherd and Garland[22] and HAD (at pH 7.0) and PFK with the methods described by Lowry et al.[23] SDH was determined in a two-step reaction using phenazine methosulfate as electron acceptor.[24] For HK and LDH analyses, the spectrophotometric methods of Bass et al.[25] were slightly modified for fluorometry. Malate dehydrogenase (MDH, EC 1.1.1.37) and glyceraldehyde-phosphate dehydrogenase (GAPDH, EC 1.2.1.12) were determined spectrophotometrically with the methods of Bücher et al.[26]

Muscle protein content was determined according to Lowry et al.[27] with bovine serum albumin as a standard, and glycogen was analyzed with the fluorometric method described by Lowry and Passonneau.[28]

Histochemical methods. Serial transverse sections (10 μm) were cut with a microtome at −20°C and stained for myofibrillar ATPase activity and with the amylase-PAS method to visualize capillaries. Based on the ATPase stainings after preincubation at different pHs, fibers were classified into two main groups, type I and type II; type II fibers were subclassified into groups IIa and IIb. Details about the staining procedure for myofibrillar ATPase activity and capillarization are found in ref. 29.

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 mm²; *mean number of capillaries in contact with fibers of each type (CC)* (counting all capillaries around each type I, IIa, and IIb fiber within the given area) and furthermore, CC relative to fiber-type area for all three fiber types. All of the above parameters were determined from an average muscle cross-sectional area of 0.90 ± 0.04 mm² (range 0.64–1.21), containing 164 ± 10 fiber cross-sections (range 114–242). Finally, *fiber-type cross-sectional areas* were determined by measuring the area of, on average, 46 ± 2 type I fibers (range 34–50), 46 ± 2 type IIa (range 21–60), and 31 ± 3 type IIb fibers (range 9–50) evenly distributed in the section. Fiber areas were measured by tracing the cell membranes on the photocopies with a pen. Based on magnetostrictive principle, the x- and y-coordinates passed by the pen were entered into a microprocessor and converted

into area values (MOP, Kontron Messgeräte, Munich, West Germany). All the muscle cross-sections were randomly stained, mounted, and photographed. Furthermore, the capillary counting was performed by the same person without knowledge of the origin of the sample.

STATISTICAL METHODS

Standard statistical methods were employed using Student's paired *t* test, when applicable. Data are given as mean ± SEM in the text, tables, and figures.

RESULTS

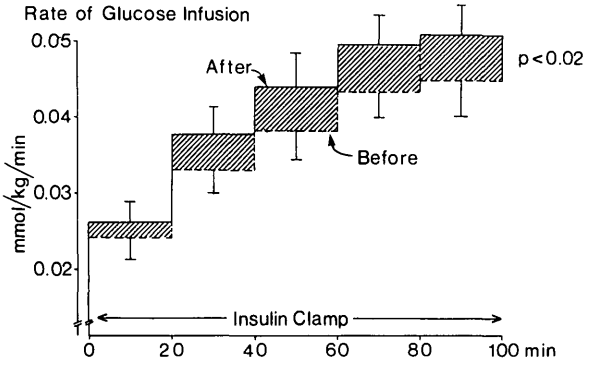
MAXIMAL OXYGEN UPTAKE

The pretraining value for maximal oxygen uptake was 3.24 ± 0.16 L/min, which is in the normal range for healthy subjects in the corresponding age group.[30] The heart rate during the training sessions ranged between 141 ± 2 and 166 ± 3 beats/min. This training intensity resulted in an 8% rise in maximal oxygen uptake to 3.49 ± 0.17 L/min ($P < 0.01$). The individual increases in $\dot{V}O_2$ max varied between 3% and 22%. The maximal heart rate decreased from 187 ± 4 beats/min before training to 181 ± 5 beats/min after training ($P < 0.01$). The heart rate response to submaximal exercise (150 W) had decreased by 7% ($P < 0.01$) after the first 6 wk of training, and by 10% ($P < 0.001$) after 16 wk of training, further indicating the improved state of physical fitness.

INSULIN SENSITIVITY

Insulin sensitivity was evaluated using the insulin clamp technique. The blood glucose concentrations before starting the insulin infusion were 12.5 ± 1.8 and 12.8 ± 1.5 mmol/L before and after training, respectively. During insulin infusion the blood glucose level was maintained on the average at 7.7 ± 0.8 mmol/L on both study occasions. The rates of glucose infusion needed to maintain this level of blood glucose during insulin infusion are presented in Figure 1. After training, a consistently greater (9–20%) glucose infusion was required during the entire study as compared with before training ($P < 0.02$, analysis of variance). During the final 20-min period the glucose infusion was 20% greater after training ($P < 0.05$), indicating an augmented insulin-mediated glucose uptake. The average level of free insulin during the clamp study was similar before (125 ± 10 μU/ml) and after (119 ± 10 μU/ml; NS) training.

FIGURE 1. Glucose infusion rate required to maintain the same level of glycemia before (----) and after (——) physical training in diabetic subjects. Mean values ± SEM are given for each 20-min period. The hatched area denotes the difference in glucose infusion rate before and after training, respectively.



CLINICAL PARAMETERS OF DIABETES CONTROL (TABLE 2)

Glycosylated hemoglobin concentration, 24-h urinary glucose excretion, and glycosuria as determined by home monitoring of urine glucose showed no decline during the training period. No changes were observed in the frequency or intensity of hypoglycemic episodes during the training program. Five patients, following their usual regimen, reduced their morning or evening dose of intermediate-acting insulin by 4–8 U on days on which exercise sessions were held. No other changes in insulin dose were made during the course of the training program.

Total plasma cholesterol fell by 14% during the training period ($P < 0.01$) (Table 2), while the proportion of HDL cholesterol rose from 24% to 30% ($P < 0.01$). Body weight remained unchanged throughout the study (Table 2).

MUSCLE ENZYME ACTIVITIES AND MORPHOLOGY

Table 3 summarizes the results of enzyme activity measurements in the quadriceps femoris muscle before and after training. Muscle oxidative capacity increased, as evidenced by the 24% increase in SDH activity ($P < 0.05$) and the tendency toward an increase in CS activity (16%, $0.05 < P < 0.1$). Total MDH activity (intra- and extra-mitochondrial) and HAD activity (marker of fatty acid β-oxidation) did not change significantly with training. Neither did any change occur in glycolytic enzyme activities (PFK, GAPDH, LDH). Muscle glycogen content was 82 ± 4 before and 97 ± 3 mmol/kg wet weight after the training period ($0.05 < P < 0.1$).

Table 4 gives the data on percentage muscle fiber type distribution, fiber area, and capillarization. No significant change was found in the relative occurrence of type I and type II fibers in the quadriceps femoris muscle as a result of training. However, the percentage of type IIa fibers increased significantly (35 ± 3% to 41 ± 2%, $P < 0.05$), while type IIb fibers tended to decrease.

The mean cross-sectional fiber area was increased by 11 ± 4% after training ($P < 0.05$). This increase was due to a hypertrophy of all three fiber types, although the increase was least marked in type IIb fibers. The number of capillaries per fiber increased by 15% ($P < 0.01$), indicating formation of new capillaries, but, since the mean fiber area also increased to a similar extent, the number of capillaries/unit cross-sectional area did not change significantly. The mean number of capillaries in contact with fibers of each type increased with training for all three fiber types, although to a lesser extent for type IIb (Table 3). However, when this variable was related to the mean area of the different fiber types, no change with training was observed.

DISCUSSION

The present study demonstrates by direct measurements that physical training in patients with type I diabetes results in an increase in insulin-mediated glucose uptake. Increased insulin sensitivity with training has been suggested in several previous reports; nondiabetic rats undergoing physical training show increased insulin-mediated glucose uptake by the perfused hind limb,[12,13] athletes have lower plasma insulin levels during a glucose tolerance test com-

TABLE 2
Glucose control, cholesterol levels, and body weight during the 16-wk training period

	Before training	Physical training			
		4 wk	8 wk	12 wk	16 wk
HbA_1 (%)	10.4 ± 0.7	10.1 ± 0.3	9.1 ± 0.3	10.8 ± 0.5	11.3 ± 0.5
Urine glucose (mmol/24 h)	162 ± 41	237 ± 56	154 ± 49	86 ± 24	215 ± 55
% of home test > 0.1%*	—	48 ± 9	45 ± 8	49 ± 6	46 ± 7†
Hypoglycemic attacks*	—	1.8 ± 0.8	1.0 ± 0.5	1.7 ± 0.7	1.9 ± 0.7†
Cholesterol (mmol/L)	5.6 ± 0.3	5.2 ± 0.3	5.2 ± 0.3	5.1 ± 0.4	4.8 ± 0.3§
HDL cholesterol					
mmol/L	1.4 ± 0.2	1.3 ± 0.2	1.3 ± 0.2	1.4 ± 0.1	1.4 ± 0.2
% of total	24.2 ± 2.1	25.0 ± 2.1	24.9 ± 2.4	27.0 ± 1.8	29.5 ± 2.5‡
Body weight (kg)	77.0 ± 2.4	77.4 ± 2.3	77.9 ± 2.5	77.8 ± 2.3	77.0 ± 2.4

—Data not available before training.
* Data for 4-wk periods.
† Data for 8 participants in the last 4-wk period.
‡ $P < 0.01$ in comparison with before training.
§ $P < 0.001$ in comparison with before training.

pared to untrained individuals,[7–9] and obese subjects show a decreased insulin response during an oral glucose tolerance test following physical training.[10]

The insulin clamp technique was used to evaluate tissue insulin sensitivity.[15] It has previously been shown that the net uptake of glucose by the splanchnic bed is negligible during this procedure.[16] Accordingly, the observed increase in glucose utilization after training is likely to be due to increased insulin sensitivity in tissues other than the splanchnic organs. The two main tissues responsible for extrasplanchnic insulin-mediated glucose uptake are skeletal muscle and adipose tissue. However, it has been demonstrated that adipose tissue accounts for only a small part of total glucose consumption.[31] In addition, improvement of i.v. glucose tolerance and lower basal and glucose-stimulated plasma insulin levels in trained rats have been shown to be a consequence of increased glucose uptake by perfused hind limb muscle.[12,13] Thus, at present, most evidence points to an increased insulin effect on skeletal muscle as the major cause of the enhanced insulin sensitivity in response to training. It is unlikely that an increase in skeletal muscle mass contributed to the augmented glucose utilization, since training-induced increases in insulin sensitivity have been demonstrated without changes in body composition in rats[12] and in obese subjects.[10]

The cellular mechanism responsible for the training-induced increase in insulin sensitivity is not, however, completely understood. It appears conceivable that changes in insulin sensitivity may result from changes at both prereceptor and receptor as well as postreceptor levels. In our diabetic group, capillary supply/unit muscle cross-sectional area did not change significantly, while insulin sensitivity increased, indicating that skeletal muscle capillarization could not explain the observed training-induced change in insulin sensitivity. However, one cannot exclude a possible role for the muscle enzymatic changes following training.

The goal of therapy in the diabetic patient is to obtain normoglycemia, as it is believed that diabetic angiopathy can be avoided or delayed by good metabolic control.[5,6] Thus, regular exercise has generally been recommended to diabetic patients for obtaining good metabolic control,[1] but so far evidence has not been presented to support the beneficial effect on glucose control. Recently Peterson et al.[32] reported improved glucose control in a group of 10 type I diabetics after an 8–10-mo program of physical training. However, in that study home monitoring of blood glucose and split-dose insulin administration were initiated simultaneously with the training, making it difficult to isolate the effect of training per se.

In the present study, we found no improvement in blood glucose control, as measured by HbA_1, or in glycosuria as determined by home-monitored glucose tests and 24-h urinary glucose excretion. Since we noted a significant increase in peripheral sensitivity to insulin, one may speculate that the unchanged glucose control in our study was due to erroneously unaltered insulin doses. However, since the frequency of symptomatic hypoglycemic attacks did not change during the training period, this explanation seems

TABLE 3
Thigh muscle enzyme activities (μkatal/kg wet weight) and protein content (g protein/g muscle) in diabetic patients before and after 16 wk of physical training

	Before	After	Change	Significance
Citrate synthase	88.5 ± 6.5	102.5 ± 6.2	+ 16%	$P < 0.1$
Succinate dehydrogenase	80.0 ± 5.8	99.3 ± 5.2	+ 24%	$P < 0.05$
Malate dehydrogenase	4230 ± 200	4670 ± 220	+ 10%	NS
3- hydroxyacyl-CoA dehydrogenase	280 ± 28	315 ± 33	+ 12%	NS
Hexokinase	43.3 ± 2.3	46.8 ± 2.7	+ 8%	NS
6-phosphofructokinase	189 ± 18	185 ± 16	− 2%	NS
Glyceraldehyde-phosphate dehydrogenase	5050 ± 250	5110 ± 400	+ 1%	NS
Lactate dehydrogenase	4240 ± 320	4160 ± 310	− 2%	NS
Protein	0.193 ± 0.007	0.196 ± 0.006	+ 2%	NS

TABLE 4
Thigh muscle fiber type distribution, fiber areas, and capillarization in diabetic patients before and after 16 wk of physical training

		Before	After	Change	Significance
Fiber type distribution (%)	I	39 ± 3	37 ± 3	− 5%	NS
	IIa	35 ± 3	41 ± 2	+ 17%	$P < 0.05$
	IIb	25 ± 2	21 ± 2	− 16%	NS
Fiber area (μm^2)	Mean	5310 ± 290	5910 ± 370	+ 11%	$P < 0.05$
	I	4780 ± 300	5380 ± 280	+ 13%	$P < 0.01$
	IIa	5480 ± 340	6210 ± 370	+ 13%	$P < 0.05$
	IIb	5100 ± 390	5360 ± 390	+ 5%	NS
Capillaries/fiber		1.5 ± 0.1	1.7 ± 0.1	+ 15%	$P < 0.01$
Capillaries/mm^2		286 ± 7	303 ± 19	+ 6%	NS
Capillaries in contact with fibers	I	4.4 ± 0.2	5.0 ± 0.2	+ 12%	$P < 0.05$
	IIa	4.2 ± 0.2	4.8 ± 0.2	+ 14%	$P < 0.05$
	IIb	3.6 ± 0.2	3.9 ± 0.2	+ 8%	NS
Capillaries in contact with fibers per 1000 μm^2 cross-sectional area	I	0.94 ± 0.04	0.95 ± 0.05	+ 1%	NS
	IIa	0.79 ± 0.04	0.80 ± 0.04	+ 1%	NS
	IIb	0.73 ± 0.05	0.76 ± 0.04	+ 4%	NS

unlikely. One explanation for the seeming discrepancy between improved insulin sensitivity and unchanged insulin dosage in our study might be an increased dietary intake (not determined). Another factor is that while muscle sensitivity to insulin is increased after physical training, liver sensitivity may be reduced.[33] Since the liver is quantitatively more important than muscle in the disposal of orally ingested carbohydrate,[34] the glycemic response after ingestion may remain unchanged despite enhanced muscle sensitivity to insulin. Regardless of the mechanism involved, the current data clearly indicate that in the absence of changes in dietary management or insulin administration, a 4-mo training program of moderate intensity fails to improve chemical control of type I diabetes.

High plasma cholesterol and low HDL-cholesterol levels have been considered risk factors in the development of cardiovascular disease.[35] Since diabetic patients are predisposed to macroangiopathy,[36] it may be of particular importance for this group to lower their plasma cholesterol level. Physically well-trained people generally have lower plasma cholesterol levels[37,38] and higher HDL-cholesterol levels[38,39] than untrained people. In type I diabetics, a tendency toward a reduction in total cholesterol has been reported after physical training.[40] Our data in fact demonstrated a significant fall in the total plasma cholesterol level, accompanied by an increase in the relative component of HDL cholesterol. The fall in the total level is presumably an effect of the physical training per se, since we could not demonstrate any improvement in glucose control, which in other studies has been shown to affect plasma cholesterol levels.[41,42]

In healthy subjects undergoing physical training with the main emphasis on development of endurance, the engaged skeletal muscles respond with increased mitochondrial enzyme activities as well as an increased capillarization, measured both as capillaries/muscle fiber and as capillaries/mm^2 of cross-sectional muscle area.[29] In our diabetic group, we found a normal rise in muscle mitochondrial enzymes and a small but significant new formation of capillaries (as shown by the rise from 1.5 to 1.7 capillaries/fiber) with training but no significant increase in capillary density.

The question arises of whether the essentially unchanged muscle capillary density after training reflects an altered ability to form new capillaries in patients with diabetes mellitus rather than an effect of the type of training employed. Regarding the former possibility, it is noteworthy that diabetic angiopathy has been demonstrated to occur in diabetic skeletal muscle.[43,44] Thus the basement membrane thickening of the capillaries may possibly limit the formation of new capillaries. However, the type of training may also have influenced the development of capillaries. Thus it may be that the periods of endurance training included in the training program were not sufficient for maximal stimulation of capillary formation.

In conclusion, this study demonstrates that physical training in insulin-dependent diabetics results in increased peripheral insulin sensitivity, a rise in muscle mitochondrial enzyme activities, decreased total plasma cholesterol levels, and unchanged blood glucose control. The findings suggest that in the absence of efforts to alter dietary regulation and insulin administration, physical training consisting of 2–3 weekly bouts of moderate exercise may not of itself improve blood glucose control in type I diabetes.

ACKNOWLEDGMENTS

The authors wish to thank Ann-Chatrine Samuelsson and Berit Sjöberg for excellent technical assistance and Agneta Lindahl for expert secretarial assistance. This study was supported by grants from the Swedish Medical Research Council (No. 3108), the Swedish Diabetes Association, the Petrus and Augusta Hedlund Foundation, and the Karolinska Institute.

REFERENCES

[1] Marble, A.: Insulin in the treatment of diabetes. *In* Joslin's Diabetes Mellitus. Marble, A., White, P., Bradley, R. F., and Krall, L. P., Eds. Philadelphia, Lea and Febiger, 1971, p. 291.

[2] Lawrence, R. D.: The effect of exercise on insulin action in diabetes. Br. Med. J. *1*:648–50, 1926.

[3] Zinman, B., Murray, F. T., Vranic, J., Albisser, A. M., Leibel, B. S., McClean, P. A., and Marliss, E. B.: Glucoregulation during moderate exercise in insulin treated diabetics. J. Clin. Endocrinol. Metab. *45*:641–52, 1977.

[4] Kemmer, F. W., Berchtold, P., Berger, M., Starke, A., Cüppers, H. J., Gries, F. A., and Zimmermann, H.: Exercise-induced fall of blood glucose in insulin-treated diabetics unrelated to alteration of insulin mobilization. Diabetes *28*:1131–37, 1979.

[5] Engerman, R., Bloodworth, J. M. B., and Nelson, S.: Relationship of microvascular disease in diabetes to metabolic control. Diabetes *26*:760–69, 1977.

[6] Pirart, J.: Diabete et complications dégénératives présentation d'une étude prospective portant sur 4400 cas observés entre 1947 et 1973. Diabete Metab. *3*:97–107, 173–82, 245–56, 1977.

[7] Björntorp, P., Fahlén, M., Grimby, G., Gustafson, A., Holm, J.,

Renström, P., and Scherstén, T.: Carbohydrate and lipid metabolism in middle-aged, physically well-trained men. Metabolism *21*:1037–1044, 1972.

[8] Lohmann, D., Liebold, F., Heilmann, W., Senger, H., and Pohl, A.: Diminished insulin response in highly trained athletes. Metabolism *27*:521–24, 1978.

[9] LeBlanc, J., Nadeau, A., Boulay, M., and Rousseau-Migneron, S.: Effects of physical training and adiposity on glucose metabolism and ^{125}I-insulin binding. J. Appl. Physiol. *46*:235–39, 1979.

[10] Björntorp, P., Holm, G., Jacobsson, B., Schiller-deJounge, K., Lundberg, P.-A., Sjöström, L., Smith, U., and Sullivan, L.: Physical training in human hyperplastic obesity. IV. Effects on the hormonal status. Metabolism *26*:319–28, 1977.

[11] Salmons, S., and Henriksson, J.: The adaptive response of skeletal muscle to increased use. Muscle Nerve *4*:94–105, 1981.

[12] Berger, M., Kemmer, F. W., Becker, K., Herberg, L., Schwenen, M., Gjinavci, A., and Berchtold, P.: Effect of physical training on glucose tolerance and on glucose metabolism of skeletal muscle in anaesthetized normal rats. Diabetologia *16*:179–84, 1979.

[13] Mondon, C. E., Dolkas, C. B., and Reaven, G. M.: Site of enhanced insulin sensitivity in exercise-trained rats at rest. Am. J. Physiol. *239*:E169–77, 1980.

[14] Åstrand, P.-O., and Kaare, R.: Textbook of Work Physiology. New York, McGraw-Hill Book Company, 1977, p. 297.

[15] DeFronzo, R. A., Tobin, J. D., and Andres, R.: Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am. J. Physiol. *237*:E214–23, 1979.

[16] DeFronzo, R. A., Ferrannini, E., Hendler, R., Wahren, J., and Felig, P.: Influence of hyperinsulinemia, hyperglycemia, and the route of glucose administration on splanchnic glucose exchange. Proc. Natl. Acad. Sci. USA *75*:5173–77, 1978.

[17] Heding, L.: Radioimmunological determination of human C-peptide in serum. Diabetologia *11*:541–48, 1975.

[18] Allain, C. C., Poon, L. S., Chan, C. S. G., Richmond, W., and Fu, P. C.: Enzymatic determination of total serum cholesterol. Clin. Chem. *20*:470–75, 1974.

[19] Finley, P. R., Schifman, R. B., Williams, R. J., and Lichti, D. A.: Cholesterol in high-density lipoprotein: use of Mg^{2+}/dextran sulfate in its enzymatic measurement. Clin. Chem. *24*:931–33, 1978.

[20] Kuzuya, H., Blix, P. M., Horwitz, D. L., Steiner, D. F., and Rubenstein, A. H.: Determination of free and total insulin and C-peptide in insulin-treated diabetics. Diabetes *26*:22–29, 1977.

[21] Bergström, J.: Muscle electrolytes in man. Scand. J. Clin. Lab. Invest. *14*(Suppl.):1–110, 1962.

[22] Shepherd, D., and Garland, P. B.: Citrate synthase from rat liver. Methods Enzymol. *13*:11–16, 1969.

[23] Lowry, C. V., Kimmey, J. S., Felder, S., Chi, M. M.-Y., Kaiser, K. K., Passonneau, P. N., Kirk, K. A., and Lowry, O. H.: Enzyme pattern in single human muscle fibers. J. Biol. Chem. *253*(*22*):8269–77, 1978.

[24] Henriksson, J., Nygaard, E., Andersson, J., and Eklöf, B.: Enzyme activities, fibre types and capillarization in calf muscles of patients with intermittent claudication. Scand. J. Clin. Lab. Invest. *40*:361–69, 1980.

[25] Bass, A., Brdiczka, D., Eyer, P., Hofer, S., and Pette, D.: Metabolic differentiations of distinct muscle types at the level of enzymatic organization. Europ. J. Biochem. *10*:198–206, 1969.

[26] Bücher, T., Luh, W., and Pette, D.: Einfache und zusammengesetzte optische Tests mit Pyridinnucleotiden. *In* Hoppe-Seyler/Thierfelder, Handbuch der physiologisch-und pathologisch-chemischen Analyse, 10th edit. Vol. VI/A. Berlin, Springer-Verlag, 1964.

[27] Lowry, O. H., Rosebrough, H. J., Farr, A. L., and Randall, R. J.: Protein measurement with the folin phenol reagent. J. Biol. Chem. *193*:265–75, 1951.

[28] Lowry, O. H., and Passonneau, J. V.: A Flexible System of Enzymatic Analysis. New York, Academic Press, 1973.

[29] Andersen, P., and Henriksson, J.: Capillary supply of the quadriceps femoris muscle of man: adaptive response to exercise. J. Physiol. *270*:677–90, 1977.

[30] Åstrand, P.-O., and Kaare, R.: Textbook of Work Physiology. New York, McGraw-Hill Book Company, 1977, pp. 318–25.

[31] Björntorp, P., Scherstén, T., and Gottfries, A.: Effects of glucose infusion on adipose tissue lipogenesis in man. Acta Med. Scand. *183*:565–71, 1968.

[32] Peterson, C. M., Jones, R. L., Esterly, J. A., Wantz, G. E., and Jackson, R. L.: Changes in basement membrane thickening and pulse volume concomitant with improved glucose control and exercise in patients with insulin-dependent diabetes mellitus. Diabetes Care 3:586–89, 1980.

[33] Zawalich, W., Maturo, S., and Felig, P.: Physical training: decreased insulin secretion and liver glucokinase in the rat. Clin. Res. *29*:683A, 1981.

[34] Felig, P., Wahren, J., and Hendler, R.: Influence of oral glucose ingestion on splanchnic glucose and gluconeogenic substrate metabolism in man. Diabetes *24*:468–75, 1975.

[35] Miller, G. J., and Miller, N. E.: Plasma-high-density-lipoprotein concentration and development of ischaemic heart-disease. Lancet *1*:16–19, 1975.

[36] Kannel, W. B., and McGee, D. L.: Diabetes and cardiovascular disease. The Framingham Study. JAMA *241*:2035–2038, 1979.

[37] Hoffman, A. A., Nelson, W. R., and Goss, F. A.: Effects of an exercise program on plasma lipids of senior Air Force officers. Am. J. Cardiol. *20*:516–24, 1967.

[38] Hartung, G. H., Foreyt, J. P., Mitchell, R. E., Vlasek, I., and Gotto, A. M.: Relation of diet to high-density-lipoprotein cholesterol in middle-aged marathon runners, joggers, and inactive men. N. Engl. J. Med. *302*:357–61, 1980.

[39] Nikkilä, E. A., Taskinen, M.-R., Rehunen, S., and Härkönen, M.: Lipoprotein lipase activity in adipose tissue and skeletal muscle of runners: relation to serum lipoproteins. Metabolism *27*:1661–71, 1978.

[40] Larsson, Y., Persson, B., Sterky, G., and Thorén, C.: Effect of exercise on blood-lipids in juvenile diabetes. Lancet *1*:350–55, 1964.

[41] Gabbay, K. H., Hasty, K., Breslow, J. L., Ellison, R. C., Bunn, H. F., and Gallop, P. M.: Glycosylated hemoglobins and long-term blood glucose control in diabetes mellitus. J. Clin. Endocrinol. Metab. *44*:859–64, 1977.

[42] Peterson, C. M., Koenig, R. J., Jones, R. L., Saudek, C. D., and Cerami, A.: Correlation of serum triglyceride levels and hemoglobin A concentrations in diabetes mellitus. Diabetes *26*:507–509, 1977.

[43] Siperstein, M. D., Unger, R. H., and Madison, L. L.: Studies of muscle capillary basement membranes in normal subjects, diabetic, and prediabetic patients. J. Clin. Invest. *47*:1973–99, 1968.

[44] Deckert, T., Egeberg, J., Frimodt-Möller, C., Sander, E., and Svejgaard, A.: Basement membrane thickness, insulin antibodies and HLA-antigens in long standing insulin dependent diabetics with and without severe retinopathy. Diabetologia *17*:91–96, 1979.