Effect of Physical Exercise on Lipoprotein(a) and Low-Density Lipoprotein Modifications in Type 1 and Type 2 Diabetic Patients

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To evaluate the effect of physical exercise on blood pressure, the lipid profile, lipoprotein(a) (Lp(a)), and low-density lipoprotein (LDL) modifications in untrained diabetics, 27 diabetic patients (14 type 1 and 13 type 2) under acceptable and stable glycemic control were studied before and after a supervised 3-month physical exercise program. Anthropometric parameters, insulin requirements, blood pressure, the lipid profile, Lp(a), LDL composition, size, and susceptibility to oxidation, and the proportion of electronegative LDL (LDL(−)) were measured. After 3 months of physical exercise, physical fitness improved (maximal O_2 consumption [Vo_{2max}], 29.6 ± 6.8 *v* 33.0 ± 8.4 mL/kg/min, $P < .01$). The body mass index (BMI) did not change, but the waist circumference (83.2 ± 11.8 to 81.4 ± 11.2 cm, $P < .05$) decreased significantly. An increase in the subscapular to triceps skinfold ratio (0.91 ± 0.37 *v* 1.12 ± 0.47 cm, $P < .01$) and midarm muscle circumference ([MMC], 23.1 ± 3.4 *v* 24.4 ± 3.7 cm, $P < .001$) were observed after exercise. Insulin requirements (0.40 ± 0.18 *v* 0.31 ± 0.19 U/kg/d, $P < .05$) and diastolic blood pressure (80.2 ± 10 *v* 73.8 ± 5 mm Hg, $P < .01$) decreased in type 2 diabetic patients. High-density lipoprotein cholesterol (HDL-C) increased in type 1 patients (1.48 ± 0.45 *v* 1.66 ± 0.6 mmol/L, $P < .05$), while LDL cholesterol (LDL-C) decreased in type 2 patients (3.6 ± 1.0 *v* 3.4 ± 0.9 mmol/L, $P < .01$). Although Lp(a) levels did not vary in the whole group, a significant decrease was noted in patients with baseline Lp(a) above 300 mg/L (mean decrease, −13%). A relationship between baseline Lp(a) and the change in Lp(a) ($r = -.718$, $P < .0001$) was also observed. After the exercise program, 3 of 4 patients with LDL phenotype B changed to LDL phenotype A, and the proportion of LDL(−) tended to decrease (16.5% ± 7.4% *v* 14.0% ± 5.1%, $P = .06$). No changes were observed for LDL composition or susceptibility to oxidation. In addition to its known beneficial effects on the classic cardiovascular risk factors, regular physical exercise may reduce the risk of cardiovascular disease in diabetic patients by reducing Lp(a) levels in those with elevated Lp(a) and producing favorable qualitative LDL modifications.

TYPE 1 AND TYPE 2 diabetes mellitus are well-known predisposing factors for cardiovascular disease.[1] The increased cardiovascular risk in diabetic patients cannot be attributed only to the classic factors such as hypertension or hyperlipidemia to which these patients are prone. An increment in lipoprotein(a) (Lp(a)) levels and the presence of qualitative modifications in low-density lipoprotein (LDL) particles[2-4] have recently been proposed as contributing factors to the accelerated development of macrovascular complications in diabetes mellitus.

Although the mechanisms by which Lp(a) promotes atherosclerosis are not well defined, individuals with Lp(a) levels above 300 mg/L (hyperLp(a)) have an increased risk of coronary heart disease even after other known predisposing factors are considered.[5] However, studies that focused on Lp(a) have not consistently found higher levels in diabetic patients,[6,7] and Lp(a) concentrations do not appear to be related to the degree of glycemic control.[8-11] LDL modifications such as nonenzymatic glycation, oxidation and enhanced electronegative charge, and the proportion of small, dense LDL particles and modification are increased in patients with diabetes mellitus.[3,12,13]

Physical exercise is a protective factor against atherosclerosis, and is recommended as a therapeutic tool in the management of diabetic patients.[14] The results of studies analyzing the effect of physical exercise on Lp(a) concentrations in diabetic and nondiabetic subjects are controversial and show a decrease,[15-17] no variation,[18,19] and even an increase[20,21] in Lp(a). Although an increase in the LDL susceptibility to oxidation and the plasma electronegative LDL proportion has been observed after acute heavy aerobic exercise,[22] physical training seems to exert favorable effects on LDL size, composition, and susceptibility to oxidation in healthy subjects.[23-26] To our knowledge, no data are available on the effect of physical exercise on LDL modifications in diabetic patients.

The purpose of this study was to assess the influence of a 3-month physical exercise program on blood pressure, the lipid profile, Lp(a) levels, and LDL modifications (composition, size, susceptibility to oxidation, and LDL(−) proportion) in a group of untrained type 1 and type 2 diabetic patients under stable glycemic control.

SUBJECTS AND METHODS

Patients

Thirty diabetic patients (15 type 1 and 15 type 2) were recruited from the diabetic outpatient clinic on the basis of the following criteria: body mass index (BMI) less than 30 kg/m^2, hemoglobin A_{1c} (HbA_{1c}) less than 8.5%, absence of kidney failure defined as plasma creatinine less than 94 μmol/L for women and 114 μmol/L for men, absence of liver or thyroid disease, and absence of regular exercise. Two type 2 diabetic men and one type 1 diabetic woman were excluded once the program started because of inadequate compliance (2 cases) and an episode of acute hepatitis A (1 case), respectively.

The main features of the 27 eligible patients are as follows: 14 type 1 diabetic patients (7 men and 7 women; mean age, 25.5 ± 6 years; range, 17 to 42) with a mean diabetes duration of 6 ± 5 years (0.7 to 16.2), and 13 type 2 diabetic patients (9 men and 4 postmenopausal women; mean age, 55.8 ± 5 years; range, 48 to 64) with a mean diabetes duration of

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8.2 ± 5 years (2.1 to 19.8). All were treated with diet plus insulin except two type 2 diabetic patients, who were on diet therapy alone. Ten patients smoked and 6 were regular alcohol consumers (< 200 g/wk). Two patients had background retinopathy and 2 had microalbuminuria. No patients had a history or clinical manifestations of peripheral vascular disease or stroke. The only hypertensive patient had an acute myocardial infarction 2 years previously. All patients attended our outpatient clinic for at least 1 year prior to the study. Furthermore, they were previously instructed in diabetes care (diet, blood glucose monitoring, insulin administration and self-adjustment, etc.) and presented stable body weight and glycemic control.

Study Protocol

The training program lasted 3 months and was held at the same fitness center in all cases. Attendance at the center was recorded on a personal chipcard. Patients were instructed to attend the fitness center at least 3 times per week and not to modify their other usual daily activities. An individualized aerobic exercise program was designed according to the patient's characteristics and degree of physical fitness, which was determined by a treadmill exercise test at the beginning of the study and repeated after 3 months of training. All exercise sessions were supervised by a coach specifically trained by the investigators for that purpose. Each session included 10 minutes of warm-up, 30 to 40 minutes of aerobic activity, and 10 minutes of cool-down. The exercise consisted mainly of walking or running on a treadmill, cycling, or a combination of both. Initially, subjects exercised at an intensity corresponding to 60% to 65% maximal O_2 consumption ($\dot{V}O_{2max}$) 1 to 2 weeks and 65% to 75% $\dot{V}O_{2max}$ thereafter to improve the aerobic capacity. The intensity of each session was monitored by heart rate. The exercises performed and the intensity and duration of each training session were recorded in an individual logbook to determine the energy expenditure as kilocalories per week using the method proposed by Ainsworth et al.[27]

The exercise test was performed in the test laboratory and supervised by a cardiologist. A continuous electrocardiogram with the standard 12 leads (Quinton Q 5000; Quinton Instrument Co, Seattle, WA) was obtained at rest and during and after exercise. Blood pressure was recorded every 3 minutes using a standard mercury sphygmomanometer. All subjects completed an exercise test on a treadmill until exhaustion according to the Bruce protocol. Pulmonary gas exchange was measured using a breath-by-breath automated gas analysis system (Collins CPX/PLUS; Collins, Braintree, MA). The following parameters were recorded: pulmonary ventilation, tidal volume, respiratory frequency, end-tidal P_{CO_2}, end-tidal P_{O_2}, $\dot{V}O_2$ (mL/min/STPD), and expired CO_2. The following data were automatically calculated: respiratory quotient, oxygen uptake relative to body mass, respiratory equivalent for oxygen and carbon dioxide, and energy expenditure expressed in metabolic units. The respiratory values for each step were calculated as the average of the values measured during the last 30 seconds. The same calculation was made during exhaustion, with the maximum obtained peak values rejected. Oxygen pulse was calculated as $\dot{V}O_2$/heart rate (mL/bpm).

All patients were taught to modify their insulin dose or diet to avoid exercise-related hypoglycemia and instructed in self-monitoring of blood glucose 3 or more times per day. All patients were instructed in recording the insulin dose immediately after each administration. Insulin doses were obtained from the average daily insulin dose administered during the week prior to initiation and during the last week of the training program.

Patients were evaluated in the outpatient unit every 4 weeks to reinforce the program compliance and receive instructions on insulin dose modifications. The aerobic capacity, anthropometric parameters, ambulatory blood pressure, glycemic profile, and laboratory analyses were evaluated before and after the planned exercise program.

Anthropometric Parameters and Blood Pressure

Height and weight were measured with the patient in light clothing without shoes on a standard physician's scale, and the BMI was calculated. The waist and hip circumferences were measured and the waist to hip ratio (WHR) was calculated. Waist circumference was taken as the smallest horizontal circumference between the 12th rib and iliac crest, and hip circumference as the maximum posterior extension of the buttocks. Subscapular and tricipital skinfolds were obtained in triplicate by the same investigator from the nondominant side using a skinfold caliper (Holtain, Crymych, UK). Midarm muscle circumference (MMC) was calculated as arm perimeter in the midarm-(tricipital skinfold × π). The mean subscapular to tricipital skinfold ratio, an indicator of body fat distribution, was also calculated.

Twenty-four-hour ambulatory blood pressure was registered using a Spacelab 90207 device (Redmond, WA). During the estimated waking hours (7 AM to 11 PM), blood pressure was recorded every 20 minutes, and every 30 minutes during the overnight period.

Laboratory Procedures

Blood samples were obtained with the patient sitting after at least a 10-hour overnight fast and a minimum of 24 hours from the last exercise session. Glucose was determined by an automated enzymatic method, and fructosamine by a colorimetric method (Boehringer, Mannheim, Germany; reference range, 205 to 285 μmol/L). HbA_{1c} was measured by high-performance liquid chromatography (BioRad, Richmond, CA; reference range, 4.6% to 5.7%). Cholesterol and triglyceride concentrations were determined by standardized enzymatic methods (Boehringer). Cholesterol fractions were measured by a combined ultracentrifugation plus precipitation method as recommended by the Lipid Research Clinics.[28] High-density lipoprotein cholesterol (HDL-C) was measured in the d > 1.006 kg/L fraction by a commercial precipitation method with phosphotungstate and magnesium chloride (Boehringer), very–low-density cholesterol (VLDL-C) was measured in the d < 1.006 kg/L fraction, and LDL-C was obtained by calculation from total cholesterol, VLDL-C, and HDL-C concentrations. Interassay coefficients of imprecision were less than 3% for total cholesterol and triglyceride, less than 5% for HDL-C, and less than 10% for LDL-C and VLDL-C, respectively, according to internal and external quality-control programs.

Lp(a) was measured by the Apo-Tek Lp(a) kit (Organon Teknika, Turnhout, Belgium). The assay detects all Lp(a) isoforms on an equimolar basis[29] using a sandwich ELISA with 2 antibodies against apolipoprotein B (apo B) and apo(a). All samples from the same patient were processed in the same batch after storage of the basal samples for 3 months at −80°C. In these conditions, we observed a decrease in Lp(a) of 1.9%. Similar results have been obtained by others at −80°C.[30] Within- and between-assay imprecision at concentrations near the accepted cutoff values (300 mg/L) was 7.5% and 5%, respectively, with pooled sera.

LDL particle size was analyzed from frozen plasma stored at −80°C by 2% to 16% gradient polyacrylamide electrophoresis as described by Nichols et al[31] with minor modifications. Bands corresponding to different classes of LDL were scanned by densitometry at 595 nm (Rep; Helena Instruments, Beaumont, CA). LDL size was determined by comparison of majority band(s) against a standard pool of plasma containing 4 LDL fractions whose diameter was previously assessed by electron microscopy (22.9 ± 0.7, 24.5 ± 0.6, 26.2 ± 0.5, and 28.4 ± 0.9 nm, respectively). Patients were distributed into 2 groups according to LDL size[32]: phenotype A, at least 25.5 nm; phenotype B, less than 25.5 nm.

LDL lipoproteins (density 1.025 to 1.050 mg/L) were isolated by sequential flotation ultracentrifugation.[33] The composition of total LDL was evaluated on phosphate-buffered saline (PBS)-dialyzed LDL. Total and free cholesterol, triglyceride (Boehringer), phospholipid (Wako

Chemicals, Neuss, Germany), and the protein (Protein Assay; BioRad) content of LDL were determined by commercially available methods, and the results are expressed as a percentage of LDL mass.

Electronegative LDL was isolated from total LDL by anion-exchange chromatography in a Mono Q 5/5 column using a fast protein liquid chromatography system (Pharmacia, Uppsala, Sweden) as previously described.[13,34] Two LDL forms, LDL(+) and LDL(−), differing in electronegativity were identified at 280 nm and their relative proportion was quantified by peak integration. All samples were analyzed within 2 days after LDL isolation.

To analyze LDL susceptibility to in vitro–induced oxidation, LDL was dialyzed against PBS, pH 7.4, by gel filtration chromatography in a G-25M Sephadex column (Pharmacia) diluted to a concentration of 50 mg protein/L and incubated with 2.5 μmol/L $CuSO_4$ at 30°C. Conjugated diene formation was determined by continuous monitoring at 234 nm for 4 hours in a Biochrom 4060 spectrophotometer (Pharmacia) as previously described.[26,35]

Statistical Methods

Statistical analyses were performed using SPSS for Windows 6.0.1 (SPSS, Chicago, IL). All data are expressed as the mean ± SD. Lp(a), triglyceride, and VLDL-C were logarithmically transformed (to reduce skewness). Statistical differences between the 2 study periods were analyzed using a nonparametric test (Wilcoxon's matched-pairs *T* test) for variables showing non-Gaussian distribution and a paired Student's *t* test for normally distributed variables. Chi-square and Mann-Whitney tests were used to examine differences between groups. Pearson's correlation coefficient was used to analyze the relationship between normally distributed variables. Spearman's rank-order correlation was applied for the same purpose in non–Gaussian distribution variables. A probability value less than .05 was considered statistically significant.

RESULTS

Physical Activity and Fitness

The average attendance at the sports center was 3.1 ± 0.8 days per week. The mean caloric expenditure per week derived from exercise was 27.2 ± 6.3 kcal/kg/wk. An improvement in $\dot{V}O_{2max}$ (29.6 ± 6.8 *v* 33.0 ± 8.4 mL/kg/min, $P < .05$) was observed when all patients were considered at the end of the study period. The oxygen pulse increased from 11.8 ± 2.5 to 12.7 ± 3.1 mL/beat, which represents a mean 11.7% ± 18.4% improvement. This improvement correlated with the calculated caloric expenditure per week ($r = .68$, $P < .0001$). The maximum heart rate was similar in the 2 tests (174.6 ± 15 *v* 174.9 ± 14 bpm) and the maximum treadmill time improved from 12.0 ± 2.5 to 12.8 ± 2.2 minutes ($P < .05$). Physical fitness parameters for type 1 and type 2 diabetic subsets are shown in Table 1.

Anthropometric Data, Blood Pressure, and Glycemic Control

In the whole group of patients, waist and hip circumference decreased significantly (83.2 ± 11.8 to 81.4 ± 11.2 cm, $P < .05$, and 99.7 ± 6.9 to 96.9 ± 6.5 cm, $P < .005$, respectively) after the exercise program, while the MMC and subscapular to tricipital skinfold ratio increased (22.8 ± 3.4 to 24.3 ± 3.7 cm, $P < .001$, and 0.93 ± 0.37 to 1.12 ± 0.47 cm, $P < .01$, respectively). The WHR, weight, and BMI did not change. Variations in anthropometric parameters according to the type of diabetes are summarized in Table 2. Regarding the average of the 24-hour measurement of systolic, diastolic, and mean blood pressure, no changes were observed when all patients were studied together. Nevertheless, for the subgroup of type 2 diabetic patients, a significant decrease in diastolic blood pressure was observed (80.2 ± 10 *v* 73.8 ± 5 mm Hg, $P < .01$; Table 2). HbA_{1c}, fructosamine, and fasting blood glucose remained unchanged in both diabetic groups. The insulin dose decreased (0.40 ± 0.18 *v* 0.31 ± 0.19 U/kg/d, $P < .05$) in type 2 patients but remained stable in type 1 diabetic patients. No patients suffered from any severe hypoglycemic event.

Lipid and Lipoprotein Parameters

In the whole group, HDL-C increased from 1.37 ± 0.42 to 1.49 ± 0.6 mmol/L ($P < .05$), while triglycerides, total cholesterol, VLDL-C, and LDL-C did not vary after the 3-month exercise program. Lipid and lipoprotein levels in type 1 and type 2 diabetic patients are shown in Table 3. While HDL-C increased in type 1 diabetics (10.7% ± 17.3%), the main change in type 2 diabetic subjects was a reduction in total cholesterol and LDL-C levels (−7.47% ± 7.2%).

Lp(a) concentrations at baseline were 62.5 to 1,582 mg/L (mean, 380.6 ± 423; median, 196). After exercise, Lp(a) concentrations did not vary in the whole group of patients. However, an interesting relationship between baseline Lp(a) and the change in Lp(a) expressed as an absolute value ($r = -.718$, $P < .0001$) and also as a percent change ($r = -.554$, $P = .003$, Fig 1) was noted, such that patients with Lp(a) levels above 300 mg/L (high Lp(a)) showed significant decreases (Fig 2). The point value of 90 mg/L proved to be the cutoff value, from which the decrease in Lp(a) reached statistical significance. Moreover, the Lp(a) variation in patients with Lp(a) levels below 300 mg/dL was 6.8%, while in patients with high Lp(a) it was −13.25% (5 of 7 patients above 7%). In this subgroup of patients, comparable to the remaining patients for all other variables tested, HDL-C increased significantly (from 1.52 ± 0.40 to 1.78 ± 0.50 mmol/L, $P < .05$) after the 3-month exercise period, while LDL-C and the total cholesterol to HDL-C ratio showed

Table 1. Physical Fitness at Baseline and After Three Months of an Exercise Program in Type 1 and Type 2 Diabetic Patients

Parameter	Type 1 Diabetics		Type 2 Diabetics	
	Baseline	After Exercise	Baseline	After Exercise
Maximal HR (bpm)	185 ± 10	185 ± 10	162 ± 7	163 ± 5
$\dot{V}O_2$max (mL/kg/min)	33.7 ± 7	38.5 ± 7.7*	25.2 ± 2.5	26.9 ± 3.8
O_2 pulse (mL/beat)	12.2 ± 2.8	13.4 ± 3.7*	11.4 ± 2.3	11.9 ± 2.3
Maximal treadmill time (min)	13.5 ± 2.2	14.0 ± 2	10.4 ± 1.2	11.4 ± 13*

Abbreviation: HR, heart rate.

*$P < .05$ *v* baseline.

Table 2. Anthropometric Data, Blood Pressure, and Glycemic Control in Type 1 and Type 2 Diabetic Patients at Baseline and After the Three-Month Exercise Program

Parameter	Type 1 Diabetics		Type 2 Diabetics	
	Baseline	After Exercise	Baseline	After Exercise
Weight (kg)	65.8 ± 11 (44-87)	66.0 ± 11 (43.5-87)	73.5 ± 13 (53-100)	73.6 ± 12 (54-101)
BMI (kg/m²)	23.8 ± 3 (20-29.6)	23.8 ± 3 (19.6-29)	26.6 ± 3.6 (23.4-33)	26.3 ± 3.6 (23.1-33)
Waist (cm)	75.6 ± 8 (61-103)	74 ± 7.4 (63-83.5)	91.4 ± 9 (75-111)	89.4 ± 9 (79-112)
Hip (cm)	97.8 ± 7 (84-109)	95.5 ± 5.7 (86-105)*	101.7 ± 6 (93-113)	98.5 ± 7 (85-111)
WHR	0.77 ± 0.6 (0.7-0.9)	0.77 ± 0.4 (0.7-0.85)	0.89 ± 0.5 (0.81-0.98)	0.9 ± 0.4 (0.86-1.01)
TC skinfold (mm)	19.7 ± 7.8 (9.5-33.5)	15.8 ± 6.9 (5.5-28)†	19.9 ± 8.4 (9.8-30)	16.9 ± 7.8 (8.5-29)*
MMC (cm)	22.1 ± 3.8 (16.2-29)	23.8 ± 4.1 (18.2-31.7)†	24.06 ± 2.6 (19-26.5)	25.2 ± 3.35 (21-31.3)
SS skinfold (mm)	13.2 ± 3.7 (9.2-24)	13.3 ± 4.6 (9-26)	20.6 ± 7.7 (12.2-34.5)	19.9 ± 9 (11.39)
SS:TC ratio	0.76 ± 0.3 (0.36-1.42)	0.97 ± 0.48 (0.46-2.2)*	1.07 ± 0.37 (0.5-1.8)	1.28 ± 0.42 (0.55-2)*
Systolic BP (mm Hg)	116 ± 9.2 (99-131)	116.9 ± 9 (104-137)	127 ± 8.7 (110-141)	123 ± 9 (107-137)
Diastolic BP (mm Hg)	69.4 ± 4.4 (61-76)	69.5 ± 5 (63-79)	80.2 ± 10 (69-105)	73.8 ± 5 (66-82)*
HbA_{1c} (%)	6.5 ± 0.8 (5.4-8.4)	6.7 ± 1 (5.4-8.5)	7.4 ± 0.8 (5.8-8.5)	7.3 ± 1 (6.5-8.3)
Fructosamine (μmol/L)	309 ± 53 (241-400)	310 ± 61 (221-423)	331 ± 62 (232-406)	301 ± 44 (244-371)
Glucose (mmol/L)	7.9 ± 3.6 (4.1-14.4)	7.7 ± 4.2 (3.7-18)	7.9 ± 3.3 (4-13.5)	8.0 ± 2.2 (5.5-13)

Abbreviations: TC, tricipital; SS, subscapular; BP, blood pressure.
*$P < .05$, †$P < .01$ *v* baseline.

significant reductions (from 3.18 ± 0.97 to 2.91 ± 0.95 mmol/L, $P < .05$, and from 3.09 ± 0.89 to 2.68 ± 0.94 mmol/L, $P < .05$, respectively). No significant correlation was found between changes in Lp(a) and variations in the anthropometric parameters, insulin requirements, glycemic control, lipoprotein profile, cardiorespiratory fitness, or blood pressure assessed after physical exercise.

The concentration of each LDL component did not change significantly after the exercise period (data not shown). In the whole group, mean LDL size remained stable throughout the study (26.1 ± 0.4 *v* 26.1 ± 0.34 nm; Table 4). When LDL size phenotype was analyzed at baseline, 23 patients (12 type 1 and 11 type 2) had phenotype A and 4 patients (two type 1 and two type 2) had phenotype B. After the exercise program, the two type 1 patients and one type 2 patient with phenotype B converted to phenotype A.

LDL susceptibility to oxidation expressed as the lag-phase time (43.33 ± 6.7 *v* 43.30 ± 7.0 minutes) and rate of conjugated diene formation (0.021 ± 0.003 *v* 0.021 ± 0.003 Δ abs/min) did not change after the exercise program in any patient. Finally, the percentage of LDL(−) tended to diminish after the exercise program in the whole group (16.5% ± 7.4% *v* 14.0% ± 5.1%, $P = .06$), with the decrease nearing statistical significance. The effect of physical exercise on LDL modifications in type 1 and type 2 diabetic patients is shown in Table 4.

Table 3. Lipid and Lipoprotein Concentrations Before (baseline) and After the Three-Month Exercise Program in Type 1 and Type 2 Diabetic Patients

Parameter	Type 1 Diabetics		Type 2 Diabetics	
	Baseline	After Exercise	Baseline	After Exercise
Cholesterol (mmol/L)	4.44 ± 0.81	4.58 ± 0.87	5.57 ± 1.11	5.20 ± 1.05*
HDL-C (mmol/L)	1.48 ± 0.45	1.66 ± 0.66*	1.26 ± 0.37	1.23 ± 0.39
LDL-C (mmol/L)	2.70 ± 0.74	2.56 ± 0.65	3.65 ± 0.97	3.37 ± 0.87†
VLDL-C (mmol/L)	0.35 ± 0.27	0.33 ± 0.15	0.66 ± 0.26	0.60 ± 0.31
Triglycerides (mmol/L)	0.76 ± 0.57	0.92 ± 0.83	1.46 ± 0.57	1.32 ± 0.68
Cholesterol/HDL-C	3.21 ± 0.87	3.07 ± 1.14	4.80 ± 1.36	4.74 ± 1.24
Lp(a) (mg/L)	344 ± 430	295 ± 327	423 ± 429	387 ± 369

*$P < .05$.
†$P < .01$.

DISCUSSION

Exercise has long been accepted as a useful tool in the management of diabetes owing to its favorable effects on central obesity, hypertension, hyperinsulinemia, glycemic control, and dyslipoproteinemia. Epidemiological evidence suggests that physical activity is associated with a lower incidence of macrovascular disease and higher life expectancy in type 1 patients, despite the fact that exercise has no effect on long-term blood glucose control.[36-38] In type 2 diabetes, regular exercise can delay and even prevent overt disease onset in individuals at risk[39] and can diminish metabolic alterations related to insulin resistance.[40]

In the present study, after a 3-month exercise program, physical fitness improved significantly as assessed by changes in $\dot{V}O_{2max}$, O_2 pulse, and maximal treadmill time. In addition to the physical fitness improvement, beneficial effects were observed on body fat content, insulin requirements, blood pressure, and the lipoprotein profile. However, one of the most interesting findings in the current study was the decrease in Lp(a) in patients with higher Lp(a) concentrations, as well as the tendency of LDL modifications to improve. In the absence of variations in BMI, blood glucose control, diet composition, and tobacco and alcohol consumption, the observed changes may be attributed mainly to physical training. Blood pressure after an exercise program is more likely to improve in older and insulin-resistant patients.[41,42] In this respect, a mild descent in diastolic blood pressure was observed among our type 2 diabetic patients. As previously stated for nondiabetic[43] and diabetic[44] subjects, lipid and lipoprotein parameters improved after physical exercise, but the increase in HDL-C in type 1 and the decrease in cholesterol and LDL-C in type 2 diabetics were the only variables reaching statistical significance. A plausible

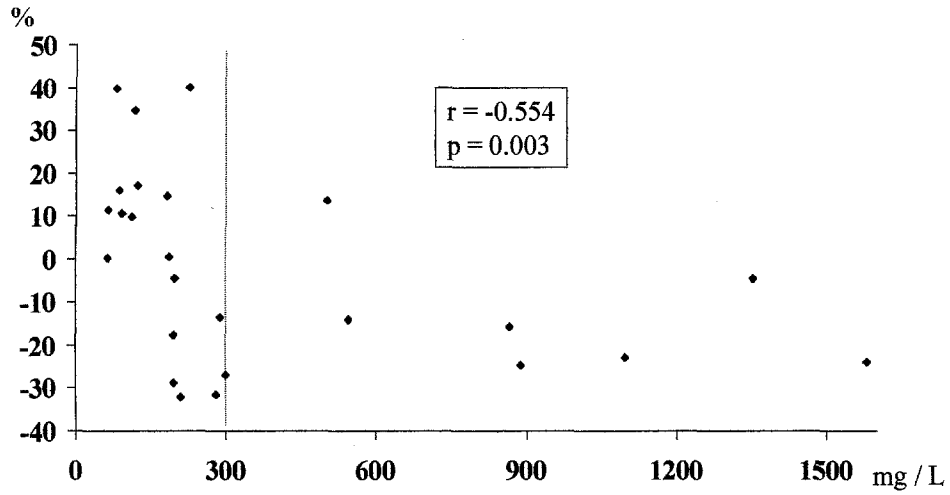

Fig 1. Lp(a) percent change observed between both periods of study (baseline and after a 3-month physical exercise program) in a group of type 1 and type 2 diabetic patients.

explanation for the limited lipoprotein improvement may be related to the favorable lipoprotein profile observed at baseline, particularly in type 1 diabetic patients. It is known that the more adverse the lipid profile at the beginning, the greater the improvement derived from physical training.[45,46] Reductions in LDL-C after exercise have been observed by some groups,[47,48] and increments in HDL-C are also found, but only following intense exercise during a prolonged period or a coexisting dietary intervention.[49]

In the general population, plasma Lp(a) levels above 300 mg/L have been associated with an increased incidence of atherosclerotic cardiovascular disease.[5,50] Although not demonstrated, it has been assumed that the reduction in these high levels is likely to reduce cardiovascular risk. On the other hand, Lp(a) concentrations are primarily determined by genetic factors, and thus pharmacological therapy is not recommended for the general population or for diabetic patients. Dietary manipulations[51] and glycemic control[8-10] do not appear to reduce Lp(a) levels. The relationship between physical exercise and Lp(a) levels has been investigated in nondiabetic patients in some cross-sectional[17,52,53] and few prospective[20,54] studies. The

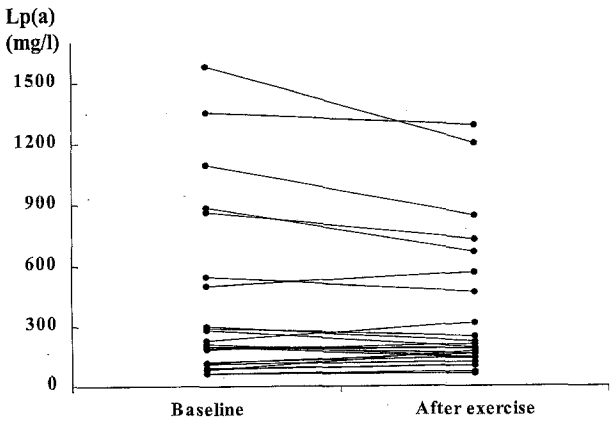

Fig 2. Individual changes observed in absolute values for plasma Lp(a) between both periods of study (baseline and after a 3-month physical exercise program) in a group of type 1 and type 2 diabetic patients.

results are controversial: some show a relationship[17,20,52] and others do not.[53,54]

The results of studies that focused on diabetic patients have also been controversial. One cross-sectional study described an inverse correlation between Lp(a) concentrations and physical fitness.[15] Similarly, physical exercise was associated with a 22% reduction in Lp(a) levels in a small group of diabetic patients.[16] In contrast, Lehmann et al[18] observed no Lp(a) modifications after 3 months of exercise in a group of type 1 diabetic patients. In our study, the Lp(a) concentration did not vary during the study period when the whole group was considered. However, after patients were subgrouped according to Lp(a) levels at baseline, a significant decrease in Lp(a) was observed for patients with values above 90 mg/L, particularly those with Lp(a) above 300 mg/L. In addition, a positive correlation was observed between the baseline Lp(a) concentration and the magnitude of the Lp(a) decrease after the training period. This finding concurs with modifications in other lipid parameters after exercise[46,55] and with the Lp(a) modifications after weight loss.[56] Lp(a) intraindividual biological variability has been described as inversely related to the concentration, being 7.5% in a group of patients with Lp(a) higher than the cutoff point of 300 mg/L.[57] In our patients, the mean variation was −13.2% (beyond the expected biological variability) for those with hyperLp(a) and +6.8% for patients with Lp(a) values below 300 mg/L (within the expected biological variability). This observation supports the hypothesis that the changes may result from physical exercise. However, to conclusively demonstrate this hypothesis, a further prospective study analyzing the effect of exercise, including a larger number of diabetic patients with hyperLp(a)emia, should be performed.

The mechanisms by which physical exercise decreases Lp(a) are unknown. In our study, variables that could influence Lp(a) levels (weight, alcohol intake, diet, and renal function) remained stable throughout the study period. Exercise improves insulin sensitivity,[37] and a relationship between Lp(a) concentrations and insulin levels[58] or the daily insulin dose[15] has been found by some groups, but not all.[59] Although the physical exercise program caused a reduction in the insulin doses, no significant correlation was found in our study between changes

Table 4. LDL Size, Susceptibility to Oxidation (lag-phase time and diene formation rate), and Electronegative LDL Proportion in Type 1 and Type 2 Diabetic Patients at Baseline and After the Three-Month Exercise Program

Parameter	Type 1 Diabetic		Type 2 Diabetic	
	Baseline	After Exercise	Baseline	After Exercise
LDL size (nm)	26.28 ± 0.43	26.35 ± 0.30	25.86 ± 0.32	25.86 ± 0.31
Lag-phase time (min)	41.78 ± 4.7	42.71 ± 6.3	45.00 ± 8.3	44.00 ± 8.0
Diene formation rate (Δabs/min)	0.021 ± 0.003	0.022 ± 0.002	0.020 ± 0.003	0.020 ± 0.003
LDL(−) (%)	16.12 ± 6.1	13.32 ± 5.4	17.03 ± 9.0	14.88 ± 4.8

in Lp(a) levels and changes in the insulin requirements. Thus, we cannot assume that the reduction in Lp(a) in our patients was due to the improvement in insulin sensitivity.

To our knowledge, no data exist on the effect of physical exercise on the LDL size, LDL(−) proportion, and LDL susceptibility to oxidation in diabetic patients. A predominance of large, light LDL particles has been identified as an adaptation to exercise,[60] and prolonged, very strenuous exercise increased LDL size in men but not in women.[23] In our study, the mean LDL size did not increase after exercise, but the LDL phenotype changed to phenotype A in 3 of 4 patients with phenotype B. A possible explanation for the lack of improvement is that there were few patients with phenotype B in the present study, as supported by previous results obtained by our group in well-controlled diabetic patients with similar clinical characteristics.[16] Furthermore, exercise intensity was low to moderate.

Electronegative LDL corresponds to a minor circulating LDL form with an increased negative charge that has some atherogenic characteristics.[61] We previously reported that LDL(−) is abnormally high in diabetic patients[13,62] and is related to glycemic control in type 1, but not type 2, diabetic patients.[62] In the present study, the proportion of LDL(−) was high compared with previous results obtained by our group in nondiabetic subjects[13,26] and tended to decrease after physical exercise. Finally, in this study, we observed no effect of long-term exercise on LDL susceptibility to oxidation. Previously, we observed an increase in LDL susceptibility to oxidation after an acute bout of very intense exercise,[22] which can be prevented by ascorbic acid.[63] However, it has been stated that LDL in athletes shows lower oxidizability,[26] and a decrease in LDL oxidation was obtained in studies that combined a low-fat diet with exercise.[64] This lack of effect of physical conditioning on LDL susceptibility to oxidation in our study may be related to the absence of changes in glycemic control and LDL composition, the minimal change in LDL size achieved, and possibly the short duration of the exercise program. Future studies on the effect of acute exercise after a period of physical training on LDL oxidizability may permit us to clarify whether physical conditioning improves the resistance to LDL oxidation after an acute bout of exercise.

In conclusion, our study shows that in addition to the known beneficial effects on the body fat content, blood pressure, insulin requirements, and lipoprotein profile, regular physical exercise may reduce cardiovascular risk in diabetic patients by reducing high plasma Lp(a) concentrations. Furthermore, exercise produced favorable changes in the LDL phenotype and a tendency to reduce the proportion of electronegative LDL.

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REFERENCES

1. Kannel VYS, McGee DL: Diabetes and glucose tolerance as a risk factor for cardiovascular disease: The Framingham Study. Diabetes Care 2:120-126, 1979

2. Haffner SM, Morales PA, Stern MP, et al: Lp(a) concentration in NIDDM. Diabetes 41:267-272, 1992

3. Sobenin IA, Tertov VV, Orekhov N: Atherogenic modified LDL in diabetes. Diabetes 45:s35-s39, 1996 (suppl 3)

4. Kennedy AL, Lyons TJ: Glycation, oxidation, and lipoxidation in the development of diabetic complications. Metabolism 46:14-21, 1997 (suppl 1)

5. Maher VMG, Brown BG: Lipoprotein (a) and coronary heart disease. Curr Opin Lipidol 6:229-235, 1995

6. Joven J, Vilella E: Serum levels of lipoprotein (a) in patients with well controlled non–insulin dependent diabetes mellitus. JAMA 265: 1113-1114, 1991

7. Haffner SM, Morales PA, Stern MP, et al: Lp(a) concentration in NIDDM. Diabetes 41:267-272, 1992

8. Haffner SM, Tuttle KR, Rainwater DL: Lack of change of lipoprotein (a) concentration with improved glycemic control in subjects with type II diabetes. Metabolism 41:116-120, 1992

9. Perez A, Carreras G, Caixas A, et al: Plasma lipoprotein (a) levels are not influenced by glycemic control in type 1 diabetes. Diabetes Care 21:1517-1520, 1998

10. Caixàs A, Pérez A, Ordóñez-Llanos J, et al: Lack of change of lipoprotein (a) levels by the optimization of glycemic control with insulin therapy in NIDDM patients. Diabetes Care 20:1459-1461, 1997

11. Emanuele N, Azad N, Abraira C, et al: Effect of intensive glycemic control on fibrinogen, lipids, and lipoproteins. Veterans Affairs Cooperative Study in Type II Diabetes Mellitus. Arch Intern Med 158:2485-2490, 1998

12. Caixàs A, Ordonez-Llanos J, de Leiva A, et al: Optimization of glycemic control by insulin therapy decreases the proportion of small dense LDL particles in diabetic patients. Diabetes 46:1207-1213, 1997

13. Sánchez-Quesada JL, Pérez A, Caixàs A, et al: Electronegative low density lipoprotein subform is increased in patients with short-duration IDDM and is closely related to glycaemic control. Diabetologia 39:1469-1476, 1996

14. American Diabetes Association: Diabetes mellitus and exercise. Diabetes Care 21:s40-s44, 1998 (suppl)

15. Austin A, Warty V, Janosky J, et al: The relationship of physical fitness to lipid and lipoprotein (a) levels in adolescents with IDDM. Diabetes Care 16:421-425, 1993

16. Hellsten G, Bomank K, Hallmans G, et al: Lipids and endurance physical activity. Atherosclerosis 75:93-94, 1989

17. Taimela S, Viikari JSA, Porkka KVK, et al: Lipoprotein(a) levels in children and young adults: The influence of physical activity. The Cardiovascular Risk in Young Finns Study. Acta Paediatr 83:1258-1263, 1994

18. Lehmann R, Kaplan V, Bingisser R, et al: Impact of physical activity on cardiovascular risk factors in IDDM. Diabetes Care 20:1603-1611, 1997

19. Hubinger L, Mackinon LT: The effect of endurance training on lipoprotein(a) [Lp(a)] levels in middle aged males. Med Sci Sports Exerc 28:757-764, 1996

20. Holme I, Urdal P, Anderssen S, et al: Exercise-induced increase in lipoprotein(a). Atherosclerosis 122:97-104, 1996

21. Ponjee GAE, Janssen EME, van Wersch JWJ: Long-term physical exercise and lipoprotein(a) levels in a previously sedentary male and female population. Ann Clin Biochem 32:181-185, 1995

22. Sánchez-Quesada JL, Homs-Serradesanferm R, Serrat-Serrat J, et al: Increase of LDL susceptibility to oxidation occurring after intense, long duration aerobic exercise. Atherosclerosis 118:297-305, 1995

23. Lamon-Fava S, Fisher EC, Nelson ME, et al: Effect of exercise and menstrual cycle status on plasma lipids, low density lipoprotein particle size, and apolipoproteins. J Clin Endocrinol Metab 68:17-21, 1989

24. Williams PT, Krauss RM, Wood PD, et al: Lipoprotein subfractions of runners and sedentary men. Metabolism 35:45-52, 1986

25. Houmard JA, Bruno NJ, Bruner RK, et al: Effects of exercise training on the chemical composition of plasma LDL. Arterioscler Thromb 14:325-330, 1994

26. Sánchez-Quesada JL, Ortega H, Payés-Romero A, et al: LDL from aerobically-trained subjects shows higher resistance to oxidative modification than LDL from sedentary subjects. Atherosclerosis 132:207-213, 1997

27. Ainsworth BE, Haskell WL, Leon AS, et al: Compendium of physical activities: Classification of energy cost of human physical activities. Med Sci Sports Exerc 1:71-80, 1993

28. Lipid Research Clinics Program: Manual of Laboratory Operations, Lipid and Lipoprotein Analysis. Bethesda, MD, National Heart, Lung, and Blood Institute, US Government Printing Office, 1982, No. 361-132:678, DHEW No. NIH 75-628 (revised)

29. Taddei-Peters WC, Butman BT, Jones GR, et al: Quantification of lipoprotein(a) particles containing various apolipoprotein(a) isoforms by a monoclonal anti-apo(a) capture antibody and a polyclonal anti-apoB detection antibody sandwich ELISA. Clin Chem 39:1382-1389, 1993

30. Fless GM, Snyder ML, Scanu AM: Enzyme-linked immunoassay for Lp(a). J Lipid Res 30:651-652, 1989

31. Nichols AV, Krauss RM, Musliner TA: Nondenaturing polyacrylamide gradient gel electrophoresis, in Segrest JP, Alberts JJ (eds): Methods in Enzymology: Plasma Lipoproteins. New York, NY, Academic, 1986, pp 417-431

32. Austin MA, Krauss RM: Genetic control of low-density-lipoprotein subclasses. Lancet 13:592-595, 1986

33. Havel RJ, Eder HA, Bragdon JH: The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. J Clin Invest 34:1345-1353, 1995

34. Vedie B, Myara I, Pech MA, et al: Fractionation of charge-modified low density lipoprotein by fast protein liquid chromatography. J Lipid Res 32:1359-1369, 1991

35. Esterbauer H, Striegl G, Puhl H, et al: Continuous monitoring of in vitro oxidation of human low density lipoprotein. Free Radic Res Commun 6:67-75, 1989

36. Moy CS, Songer TJ, LaPorte RE, et al: Insulin-dependent diabetes mellitus, physical activity, and death. Am J Epidemiol 137:74-81, 1993

37. Yki-Jarvinen H, DeFronzo RA, Koivisto VA: Normalization of insulin sensitivity in type I diabetic subjects by physical training during insulin pump therapy. Diabetes Care 7:520-527, 1984

38. Zinman B, Zuñiga-Guajardo S, Kelly D: Comparison of the acute and long-term effects of exercise on glucose control in type 1 diabetics. Diabetes Care 7:515-519, 1984

39. Helmrich SP, Ragland DR, Leung RW, et al: Physical activity and reduced occurrence of non–insulin-dependent diabetes mellitus. N Engl J Med 325:147-152, 1991

40. Koivisto VA, Yki-Jarvinen H, DeFronzo RA: Physical training and insulin sensitivity. Diabetes Metab Rev 1:445-481, 1986

41. Hagberg JM, Montain ST, Martin WH, et al: Effect of exercise training in 60- to 69-year-old persons with essential hypertension. Am J Cardiol 64:348-353, 1989

42. Krotkiewsky M, Mandroukas K, Sjostrom L: Effects of long-term physical training on body fat, metabolism, and blood pressure in obesity. Metabolism 28:650-658, 1979

43. Tran ZV, Weltman A, Glass GV, et al: The effects of exercise on blood lipids and lipoproteins: A meta-analysis of studies. Med Sci Sports Exerc 15:393-402, 1983

44. Costill DL, Cleary P, Find W, et al: Training adaptations in skeletal muscles of juvenile diabetics. Diabetes 28:818-822, 1979

45. Lamon-Fava S, McNamara JR, Farber HW, et al: Acute changes in lipid, lipoprotein, apolipoprotein and low-density lipoprotein particle-size after an endurance triathlon. Metabolism 38:921-925, 1989

46. Superko HR: Exercise training, serum lipids and lipoprotein particles: Is there a change threshold? Med Sci Sports Exerc 23:677-685, 1991

47. Manson JE, Colditz GA, Stampfer MJ, et al: A prospective study of maturity-onset diabetes mellitus and risk of coronary heart disease and stroke in women. Arch Intern Med 151:1141-1147, 1991

48. Rönnemaa T, Marniemi J, Puukka P, et al: Effects of long-term physical exercise on serum lipids, lipoproteins and lipid metabolizing enzymes in type 2 (non–insulin-dependent) diabetic patients. Diabetes Res 7:79-84, 1988

49. Vanninen E, Uusitupa M, Siitonen O, et al: Habitual physical activity, aerobic capacity and metabolic control in patients with newly-diagnosed type 2 (non–insulin-dependent) diabetes mellitus: Effect of 1-year diet and exercise intervention. Diabetologia 35:340-346, 1992

50. Armstrong VW, Harrach B, Robenek H, et al: Heterogeneity of human lipoprotein Lp(a): Cytochemical and biochemical studies on the interaction of two Lp(a) species with the LDL receptor. J Lipid Res 31:429-441, 1990

51. Brown SA, Morriset J, Patchs JR, et al: Influence of short term cholesterol and fat on human plasma Lp(a) and LDL levels. J Lipid Res 32:1281-1289, 1991

52. Cardoso GC, Posadas C, Orvafianos O, et al: Long distance runners and body-builders exhibit elevated plasma levels of lipoprotein(a). Chem Phys Lipids 67:207-221, 1994

53. Israel RG, Sullivan MJ, Marks RH, et al: Relationship between cardiorespiratory fitness and lipoprotein(a) in men and women. Med Sci Sports Exerc 26:425-431, 1994

54. Lobo RA, Notelovitz M, Bernstein L, et al: Lp(a) lipoprotein: Relationship to cardiovascular disease risk factors, exercise, and estrogen. Am J Obstet Gynecol 166:1182-1190, 1992

55. Haskell WL: The influence of exercise on the concentrations of triglyceride and cholesterol in human plasma. Exerc Sport Sci Rev 12:205-244, 1984

56. Muls E, Kempen K, Vansant G, et al: The effects of weight loss and apolipoprotein E polymorphism on serum lipids, apolipoproteins A-1 and B, and lipoprotein(a). Int J Obes 17:711-716, 1993

57. Cobbaert C, Arentsen JC, Mulder P, et al: Significance of various parameters derived from biological variability of lipoprotein(a), homocysteine, cysteine, and total antioxidant status. Clin Chem 43:1958-1964, 1997

58. Inoue K, Nago N, Matsuo H, et al: Serum insulin and lipoprotein(a) concentrations. The Jichi Medical School Cohort Study. Diabetes Care 20:1242-1247, 1997

59. Duell PB, Hagemenas F, Connor WE: The relationship between serum lipoprotein(a) and insulinemia in healthy non-diabetic adult men. Diabetes Care 17:1135-1140, 1994

60. Williams PT, Krauss RM, Wood PD, et al: Lipoprotein subfractions of runners and sedentary men. Metabolism 31:844-847, 1986

61. Demuth K, Myara I, Chappey B, et al: A cytotoxic electronegative LDL subfraction is present in human plasma. Arterioscler Thromb Vasc Biol 16:773-783, 1996

62. Sánchez-Quesada JL, Payés A, Rigla M, et al: Electronegative LDL proportion is related to nonenzymatic glycation in IDDM but not in NIDDM. Diabetologia 41:A17, 1998 (suppl 1, abstr)

63. Sanchez-Quesada JL, Jorba O, Payes A, et al: Ascorbic acid inhibits the increase in low-density lipoprotein (LDL) susceptibility to oxidation and the proportion of electronegative LDL induced by intense aerobic exercise. Coron Artery Dis 9:249-255, 1988

64. Parks EJ, German JB, Davis PA, et al: Reduced oxidative susceptibility of LDL from patients participating in an intensive atherosclerosis treatment program. Am J Clin Nutr 68:778-785, 1998