Influence of 4 Weeks' Intervention by Exercise and Diet on Low-Density Lipoprotein Subfractions in Obese Men With Type 2 Diabetes

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Insulin resistance is associated with dyslipoproteinemia characterized by increased serum triglycerides, reduced high-density lipoprotein 2 (HDL_2) cholesterol, and increased small, dense low-density lipoprotein (LDL) subfraction particles. Physical activity and weight reduction are known to improve insulin resistance and dyslipoproteinemia, but their influence on LDL subfractions in diabetic patients is unknown. Therefore, we investigated the effect of a 4-week intervention program of exercise (2,200 kcal/wk) and diet (1,000 kcal/d: 50% carbohydrate, 25% protein, and 25% fat; polyunsaturated/saturated fat ratio, 1.0) on glycemic control and HDL and LDL subfractions in 34 obese patients with non–insulin-dependent diabetes (age, 49 ± 9 years; body mass index [BMI], 33.1 ± 5.1 kg/m^2). Reductions in body weight ($P < .001$) and improvements in fasting blood glucose, insulin, fructosamine ($P < .001$), and free fatty acids ($P < .01$) by intervention were associated with reductions in serum cholesterol and apolipoprotein B (apo B) concentrations in very-low-density lipoprotein (VLDL) ($P < .01$), intermediate-density lipoprotein (IDL), and small, dense (>1.040 g/mL) LDL particles ($P < .001$). These data underlie the positive influence of weight reduction induced by exercise and diet on insulin resistance and lipoprotein metabolism in obese diabetic patients, particularly showing improvements of the LDL subfraction profile with a decrease of small, dense LDL particles. This is of particular importance, as these particles have been shown to be associated with coronary artery disease.

HYPERINSULINEMIA and peripheral insulin resistance are associated with an unfavorable lipoprotein profile,[1] which has been addressed as one of the reasons for an increased incidence of atherosclerosis in diabetic patients. This lipoprotein profile is characterized by increased concentrations of serum triglycerides and low concentrations of high-density lipoprotein (HDL) cholesterol. In addition a low-density lipoprotein (LDL) subfraction profile of elevated concentrations of small, dense LDL particles has been shown to be prevalent in these patients.[2-4] This profile is closely related to the incidence of cardiovascular disease.[5,6]

Besides the impairment of increased insulin resistance on lipoprotein metabolism, obesity, low physical activity, and low physical fitness have an additional negative effect.[7,8] In contrast, intervention measures to increase physical activity and reduce body weight are known to improve insulin resistance and lipoprotein metabolism.[9-12] Physical exercise and weight reduction have also been shown to improve the LDL subfraction profile in healthy obese subjects,[13] but the influence in patients with type 2 diabetes mellitus has not been previously investigated.

Therefore, we assessed the influence of a 4-week intervention program including daily physical exercise and changes in diet on body weight, insulin resistance, and lipoprotein metabolism in obese subjects with type 2 diabetes. Using preparative density-gradient ultracentrifugation, a precise analysis of the concentration and distribution of lipoprotein subfraction particles was possible.

SUBJECTS AND METHODS

Patients with diabetes mellitus were referred by their general practitioner or diabetes specialist to the Kandertal Rehabilitation Clinic for Metabolic Disorders in Malsburg-Marzell for improvement of metabolic control. Upon reporting to the hospital, these patients were asked whether they were willing to participate in an intervention study. The patients had to fulfill the following criteria: age between 30 and 60 years, obesity with a body mass index (BMI) more than 27.0 kg/m^2, diagnosis of diabetes mellitus adequately treated with dietary measurements, and urine samples negative for ketones. Exclusion criteria were medication with insulin or lipid-lowering agents, hypertension resistant to pharmacological treatment, history of proliferative retinopathy, ischemic heart disease, peripheral vascular disease, orthopedic problems limiting exercise training, or any other disease constituting a contraindication for exercise training. These metabolic data were provided by the patients' referring physicians. Prior to the intervention program, an exercise stress test was performed to detect possible exclusion criteria for the study such as signs of ischemic heart disease, a low exercise capacity of less than 100 W, and hypertension or arrhythmias during exercise. Laboratory screening parameters were used to exclude electrolyte abnormalities (potassium and sodium) and impairment of liver (ALT and AST) and kidney (serum creatinine) function. The study was approved by an institutional review committee, and all subjects provided informed consent before participation.

Intervention Program

Thirty-four of 70 patients (age, 49 ± 9 years; body weight, 97.2 ± 17.9 kg; height, 172 ± 7 cm) fulfilled all criteria already listed and were included in this prospective longitudinal intervention study. For the duration of the study, the patients were closely evaluated medically by the hospital staff. The intervention program included daily exercise, a low-calorie diabetic diet, and an education program. Medication remained unchanged during the study.

Exercise program. The exercise program included individual exercise on a bicycle ergometer for 30 minutes 5 days per week at an individual intensity of 70% maximal heart rate (1,100 kcal/wk). The maximal heart rate for calculating the individual heart rate during the exercise program was assessed by a stepwise exercise stress test performed before the intervention study. The heart rate during the program was monitored by telemetry (Polar, Pacer, Kempele, Finland). An additional energy expenditure of 1,100 kcal/wk was achieved by exercise in groups (2-hour hiking tours once per week [800 kcal], and

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swimming, water games, and stretching twice per week for 30 minutes [300 kcal]). A total energy expenditure of 2,200 kcal/wk was achieved by physical exercise. Adherence to the exercise program was reinforced and monitored daily by the exercise staff of the hospital.

Diet. The diet consisted of a 1,000-kcal diabetic diet with a carbohydrate content of approximately 50%, fat content 25%, and protein content 25%. The ratio of polyunsaturated to saturated fatty acids was 1.0. The amount of fiber in the diet was approximately 10 g/d. Nutrition was monitored by dieticians.

Education program. To form the basis for a long-term change in lifestyle, all patients participated in a structured education program for the first 2 weeks of the intervention program. This part consisted of lectures and practical applications. Topics were the importance of measuring glucose in blood and urine, the pathophysiology of the development of atherosclerosis and microvascular disease, and the importance of nutrition for diabetics. This was accompanied by individually supervised shopping in a local supermarket, cooking sessions, and individual dietary counseling.

Clinical Chemistry

After an overnight fast of 12 hours, blood was drawn in the morning on the second day after admission to the hospital for measurement of routine parameters (glucose and fructosamine) performed directly in the Rehabilitation Clinic Malsburg-Marzell and for analysis of additional risk factors (lipoprotein subfraction analysis, insulin, and FFA) performed in the biochemical laboratory of the Department of Rehabilitation, Prevention and Sports Medicine of Freiburg University Hospital. This procedure was repeated 28 days afterwards for comparison of the laboratory values.

EDTA plasma (20 mL) was sent directly to the Department of Rehabilitation, Prevention and Sports Medicine, where density-gradient ultracentrifugation was started within a maximum of 3 days.

Density-Gradient Ultracentrifugation

Very–low-density lipoprotein ([VLDL]d < 1.006 g/mL), intermediate-density lipoprotein ([IDL], d 1.006 to 1.019 g/mL), LDL (d 1.019 to 1.063 g/mL), and HDL (d 1.063 to 1.210 g/mL) were prepared by sequential flotation according to the method of Lindgren.[14] Total LDLs were separated into six density classes and HDLs into three density classes by equilibrium density-gradient centrifugation as previously described.[15,16] The density range for LDL subfractions as determined by precision refractometry[14] of blank gradients is as follows: LDL-1, 1.019 to 1.031 g/mL; LDL-2, 1.031 to 1.034 g/mL; LDL-3, 1.034 to 1.037 g/mL; LDL-4, 1.037 to 1.040 g/mL; LDL-5, 1.040 to 1.044 g/mL; LDL-6, 1.044 to 1.063 g/mL; HDL_{2b}, 1.063 to 1.100 g/mL; HDL_{2a}, 1.100 to 1.125 g/mL; and HDL_3, 1.125 to 1.210 g/mL.

Chemical Analysis

Lipids and apolipoproteins. In all HDL and LDL subfractions, cholesterol and triglyceride levels were measured by automated (EPOS: Eppendorf, Hamburg, Germany) enzymatic methods (Boehringer, Mannheim, Germany; and bioMérieux, Nürtingen, Germany). Apolipoprotein (apo) A-I, B, and A-II levels were measured by endpoint nephelometry (Behring, Marburg, Germany). The within-assay coefficient of variation for the determination of LDL subfraction concentrations was between 2.2% and 4.5% for cholesterol and between 3.0% and 5.8% for apo B, depending on the subfraction.

Insulin, fructosamine, and FFA. Fasting insulin was determined by an enzyme-linked immunosorbent assay (Boehringer) and FFA by an enzymatic colorimetric method (Wako Chemicals, Neuss, Germany). Fructosamine was also determined by a commercial enzymatic test (Enzymotest; Hoffman-La Roche, Basel, Switzerland). Glucose was measured during the day before and 2 hours after meals, and the "glucose profile" is equivalent to the mean of these six glucose measurements.

Statistical Analysis

Data from all 34 patients obtained before the intervention program and 4 weeks afterward were compared by Wilcoxon's matched-pair signed-ranks test. Pearson's correlation coefficient was determined between changes in the BMI, metabolic parameters (serum triglycerides, fructosamine, and glucose profile), and LDL subfraction profile during intervention. This analysis was followed by a stepwise multiple regression analysis in which changes in metabolic control during intervention were entered with lipoprotein subfractions as dependent variables. For this procedure, all variables were logarithmically transformed to reduce the skew of the distribution. Data were analyzed using the Statistical Package for the Social Sciences (SPSS/PC+; SPSS, Chicago, IL). Data are expressed as the mean ± SD. A P value less than .05 indicates statistical significance.

RESULTS

During the 4 weeks, none of the subjects had to be removed from the study or withdrew because of other reasons. No serious complications or injuries were observed during exercise sessions. Daily adherence to the exercise program was greater than 90%.

The 4-week intervention program induced a significant improvement in the obesity and metabolic cardiovascular risk factors in the study population of obese diabetic patients. The combination of diet and exercise reduced body weight from 97.2 ± 17.9 kg to 94.4 ± 18.2 kg ($P < .001$). Significant improvements in carbohydrate metabolism were observed for the glucose profile and fasting insulin and fructosamine levels (Table 1).

In addition, significant improvements during the program were also observed for lipoprotein metabolism. Serum cholesterol and triglyceride levels were reduced substantially. Preparative density-gradient ultracentrifugation demonstrated a reduction of cholesterol in VLDL, IDL, and LDL fractions. Dividing LDL into subfractions showed that cholesterol concentrations were particularly reduced in LDL subfractions with a density more than 1.040 g/mL (LDL-5 and LDL-6). In contrast, cholesterol concentrations of medium, dense LDL particles even increased (LDL-2 and LDL-3, d 1.031 to 1.037 g/mL). No changes were observed for cholesterol levels in HDL or even HDL subfractions (Table 2).

Also, apolipoprotein concentrations changed significantly during 4 weeks of intervention. Serum apoB significantly

Table 1. Influence of a 4-Week Intervention Program (exercise and diet) on Factors of Insulin Resistance in 34 Obese Patients With Type 2 Diabetes Mellitus

Factor	Before Intervention	After Intervention	P
Body weight (kg)	97.2 ± 17.9	94.4 ± 18.2	<.001
BMI (kg/m²)	33.1 ± 5.1	31.3 ± 4.3	<.001
Glucose profile (mmol/L)*	9.7 ± 3.1	6.8 ± 1.9	<.001
Insulin (pmol/L)	103 ± 57	84 ± 60	<.05
Fructosamine (μmol/L)	325 ± 79	272 ± 55	<.001
FFA (mmol/L)	1.05 ± 0.43	0.93 ± 0.24	<.05

*Equivalent to the mean of 6 glucose measurements per day. Other measurements are fasting values.

Table 2. Influence of a 4-Week Intervention Program (exercise and diet) on Lipoprotein Metabolism in 34 Obese Patients With Type 2 Diabetes Mellitus

Parameter	Before Intervention	After Intervention	P
Serum cholesterol	6.03 ± 1.32	5.02 ± 0.88	<.05
Serum triglycerides	2.49 ± 1.9	1.62 ± 1.25	<.05
VLDL cholesterol	1.03 ± 0.8	0.72 ± 0.46	<.05
IDL cholesterol	0.31 ± 0.13	0.21 ± 0.1	<.001
LDL cholesterol	2.95 ± 0.85	2.69 ± 0.67	<.05
LDL-1	0.44 ± 0.18	0.47 ± 0.18	NS
LDL-2	0.26 ± 0.16	0.34 ± 0.16	<.01
LDL-3	0.31 ± 0.18	0.41 ± 0.18	<.01
LDL-4	0.47 ± 0.21	0.52 ± 0.21	NS
LDL-5	0.67 ± 0.31	0.49 ± 0.21	=.001
LDL-6	0.75 ± 0.41	0.44 ± 0.21	<.001
HDL cholesterol	0.91 ± 0.29	0.93 ± 0.34	NS
HDL_{2b}	0.23 ± 0.16	0.26 ± 0.16	NS
HDL_{2a}	0.29 ± 0.13	0.31 ± 0.13	NS
HDL_3	0.39 ± 0.1	0.39 ± 0.08	NS
VLDL apoB	13 ± 8	9 ± 4	<.01
IDL apoB	6 ± 2	4 ± 1	<.001
LDL apoB	77 ± 21	64 ± 15	<.01
LDL-1	9 ± 3	9 ± 4	NS
LDL-2	6 ± 3	7 ± 3	<.05
LDL-3	8 ± 4	9 ± 4	<.05
LDL-4	12 ± 5	12 ± 5	NS
LDL-5	18 ± 8	12 ± 6	<.001
LDL-6	23 ± 13	12 ± 7	<.001
HDL apoA-I	89 ± 24	88 ± 26	NS
HDL_{2b}	8 ± 10	10 ± 9	<.01
HDL_{2a}	24 ± 11	25 ± 12	NS
HDL_3	54 ± 13	51 ± 8	NS
HDL apoA-II	34 ± 10	31 ± 6	NS
HDL_{2b}	3 ± 3	3 ± 1	NS
HDL_{2a}	10 ± 4	9 ± 4	NS
HDL_3	22 ± 6	20 ± 3	<.01

NOTE. Cholesterol values are mmol/L; apolipoprotein values are mg/dL.

Abbreviation: NS, nonsignificant.

decreased from 107 ± 24 mg/dL to 89 ± 18 mg/dL, whereas serum apoA-I and apoA-II levels did not change significantly (apoA-I: before, 136 ± 25 mg/dL; after, 130 ± 20 mg/dL; apoA-II: before, 44 ± 10 mg/dL; after, 37 ± 6 mg/dL). The reductions in serum apoB reflected reductions of apoB in VLDL, IDL, and LDL during intervention (Table 2). LDL subfraction analysis showed that small, dense LDL particles (d > 1.044 g/mL, LDL-6) could even be significantly reduced by almost 50%. Changes in the concentration of apoB reflect changes in the number of VLDL, IDL, or LDL particles, as one VLDL, IDL, or LDL particle contains exactly one apoB molecule. Therefore, also the number of circulating small, dense LDL particles could be reduced. The reductions in small, dense LDL particles (LDL-6 apoB) were associated with reductions in serum triglycerides ($r = .62$, $P < .001$; Fig 1) and fructosamine ($r = .46$, $P = .009$). Improvements in the BMI were not directly associated with the changes in small, dense LDL particles. Multivariate regression analysis including triglycerides and fructosamine showed that both variables contributed significantly to changes in the concentration of small, dense LDL particles; however, triglycerides were included first in the regression equation ($R^2 = .60$, $P < .01$) and fructosamine second ($R^2 = .67$, $P < .05$).

Although no changes were observed for apoA-I or apoA-II concentrations in total HDL, subfraction analysis demonstrated an increase of apoA-I in the HDL_{2b} subfraction and a decrease of apoA-II in the HDL_3 subfraction (Table 2). These changes were not associated with reductions in the BMI or improvements in glycemic control.

Improvements in lipids and parameters of glycemic control were not dependent on the baseline BMI. When dividing the subjects according to BMI (BMI < 35 kg/m^2 and BMI ≥ 35 kg/m^2), no significant differences in the changes of lipoprotein subfractions during intervention were observed.

DISCUSSION

This study shows that a nonpharmacological approach in the treatment of type 2 diabetes is capable of significantly improving carbohydrate and lipid metabolism. In particular, the improvement of the LDL subfraction profile by exercise and diet alone has not been shown previously for diabetic patients. Small, dense LDL particles with a density more than 1.044 g/mL could even be reduced by almost 50% to values found in healthy nondiabetic subjects.[7] This was associated with significant improvements in glucose control, reaching values within the normal range for fructosamine and body weight. These improvements were achieved by a combination of daily exercise amounting to 2,200 kcal/wk and a diet of 1,000 kcal/d. Since this diet is very restrictive in active men, these measures may only be used in a selected group of patients.

It has been shown previously that weight reduction induced by exercise and diet can reduce the concentration of small, dense LDL particles in overweight nondiabetic subjects.[13] In these subjects, a reduction of small, dense LDL particles by exercise-induced weight loss was primarily dependent on the degree of weight reduction.[13] Moreover, a study in normoinsulinemia showed that even mild obesity with a BMI more than 25 kg/m^2 seems to favor a lipid profile of increased small, dense LDL particles.[7] However, the present study shows that reduc-

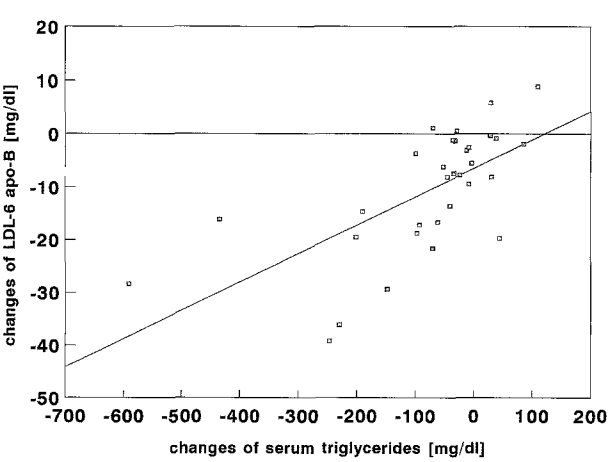

Fig 1. Relationship between changes in serum triglycerides and the number of circulating small, dense LDL particles during 4 weeks of intervention by exercise and diet in 34 men with type 2 diabetes ($r = .62$, $P < .001$).

tions in the concentration of small, dense LDL particles can only be observed when triglycerides decrease concomitantly (Fig 1). Then, these changes are associated with reductions in triglyceride-rich lipoproteins such as VLDL and IDL cholesterol.

Unfortunately, this study did not differentiate between effects of the exercise and the reduction of body fat on the LDL subfraction profile. However, both intervention measures are often closely related, so it is generally difficult to resolve which factor predominantly influences the metabolic risk factors. This is particularly so, as these factors affect insulin sensitivity and lipoprotein metabolism in a similar fashion[12,13,17] and improvements in risk factors are greatest when exercise training is accompanied by weight reduction.[13,18,19] In the present study, physical exercise was performed at least 5 days per week, amounting to approximately 2,200 kcal/wk. Studies on exercise in the prevention and rehabilitation of diabetes and atherosclerosis have shown that 2,000 kcal/wk is sufficient for a significant reduction of the incidence of type 2 diabetes or cardiovascular complications[10,20] and can even improve coronary lesions.[21] Data from a Finnish cohort show that the minimum intensity of exercise necessary for the prevention of diabetes is 5.5 metabolic units, equivalent to approximately 100 W, for at least 40 minutes per week.[22] Therefore, it seems possible that improvements in insulin resistance and the lipoprotein profile may also be observed when exercise is performed on only 2 to 3 days per week, amounting to less than 2,000 kcal/wk, as in the present study.

Overall, this study has confirmed that nonpharmacological measures are capable of improving the factors of the insulin resistance syndrome in overweight diabetic patients independently of the severity of obesity. The improvements of the LDL subfraction profile with a significant reduction of small, dense LDL particles have not been shown previously for this group of patients. They are particularly important, as small, dense LDL particles have been shown to be closely related to cardiovascular disease.[5,6] Reductions in these particles can be expected when intervention measures are capable of reducing serum triglycerides (Fig 1). This is important for clinicians without access to the method of LDL subfraction analysis, as they will receive indirect information on the concentration of small, dense LDL particles when determining serum triglycerides during intervention. Further studies must confirm the present findings by including a control group and differentiating between the effects of exercise and diet.

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