Differences in the Concentration and Composition of Low-Density Lipoprotein Subfraction Particles Between Sedentary and Trained Hypercholesterolemic Men

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There is evidence that a low-density lipoprotein (LDL) subfraction profile of increased concentrations of small, dense LDL particles is less common among trained than among sedentary normocholesterolemic men, but it is still uncertain whether there is a similar association in hypercholesterolemia also. Therefore, we determined the lipid and apolipoprotein concentration and composition of six LDL subfractions (density gradient ultracentrifugation) in 20 physically fit, regularly exercising (>three times per week) hypercholesterolemic men and 20 sedentary hypercholesterolemic controls. Trained (maximal oxygen consumption [$\dot{V}O_2max$], 57.3 ± 7.4 mL/kg/min) and sedentary ($\dot{V}O_2max$, 37.5 ± 8.8 mL/kg/min) individuals (aged 35 ± 11 years; body mass index [BMI], 23.9 ± 2.7 kg/m^2) were matched for LDL apolipoprotein (apo) B levels (108 ± 23 and 112 ± 36 mg/dL, respectively). Trained subjects had significantly lower serum triglyceride ($P < .05$) and very-low-density lipoprotein (VLDL) cholesterol levels ($P < .05$) and higher high-density lipoprotein 2 (HDL_2) cholesterol levels ($P < .01$) than sedentary controls. LDL particle distribution showed that trained individuals had significantly less small, dense LDL (d = 1.040 to 1.063 g/mL) and more large LDL (d = 1.019 to 1.037 g/mL) subfraction particles than sedentary controls, despite equal total LDL particle number. Analysis of LDL composition showed that LDL particles of hypercholesterolemic trained men had a higher free cholesterol content than LDL of untrained hypercholesterolemic men. Small, dense LDL in hypercholesterolemic trained men were richer in phospholipids than those in sedentary controls. These data demonstrate the significant influence of aerobic fitness on lipoprotein subfraction concentration and composition, thereby emphasizing the role of exercise in the treatment and risk reduction of hypercholesterolemia.

LOW-DENSITY LIPOPROTEINS (LDLs) have been characterized as a group of heterogeneous particles that differ in size, density, lipid and apolipoprotein composition, and metabolism.[1-4] Two distinct LDL lipoprotein phenotypes have been identified, one characterized by a predominance of large, buoyant particles and the other by an excess of small, dense particles.[5,6] This latter profile is accompanied by increased triglyceride levels and reduced high-density lipoprotein (HDL) cholesterol, particularly the HDL_2 subfraction, and has been shown to be associated with premature coronary artery disease (CAD).[5-9]

Acute or regular physical exercise has been shown to change the lipoprotein profile favorably, mainly by reducing triglycerides and increasing HDL cholesterol levels, but the influence of exercise on total LDL cholesterol and LDL apolipoprotein (apo) B is less striking.[10-12] Since the recognition of different LDL subfraction profiles, it has become of interest to know whether certain LDL subfractions may be particularly influenced by physical exercise. So far, it has been shown that in normocholesterolemia, the average LDL particle size is larger[13] and LDL size increases during acute exercise[14,15] and in long-term exercise training,[16] partly due to a reduction in small, dense LDL particles. The influence of exercise on LDL subfraction phenotypes in hypercholesterolemia has not been investigated before. This is of special interest, since lower concentrations of small, dense LDL in physically active hypercholesterolemic subjects could reflect an additional beneficial effect of physical exercise on lipoprotein metabolism and cardiovascular risk besides the known increase in HDL_2 cholesterol levels. Therefore, we investigated the LDL subfraction profile in hypercholesterolemic men with different aerobic fitness and leisure time physical activity. Using preparative density gradient ultracentrifugation, a precise analysis of the concentration and composition of very-low-density lipoproteins (VLDLs), intermediate-density lipoproteins (IDLs), and LDL subfraction particles was possible.

SUBJECTS AND METHODS

Study Subjects

Forty healthy hypercholesterolemic men were recruited for the study. All men had type II hypercholesterolemia and fulfilled the high-risk criteria for CAD according to the National Cholesterol Education Program,[17] with serum cholesterol greater than 240 mg/dL and LDL cholesterol greater than 160 mg/dL. These subjects either were patients of the lipid clinic of Freiburg University Hospital or were randomly selected from the outpatient clinic of the Department of Rehabilitation, Prevention, and Sports Medicine, where they had attended for an elective cardiopulmonary assessment. All participants were instructed by a dietician regarding a low-fat, low-cholesterol diet at least 2 weeks before blood was drawn for lipoprotein analysis. Exclusion criteria were age greater than 50 years, medication of any kind, particularly lipid-lowering drugs, triglycerides greater than 300 mg/dL, body mass index (BMI) greater than 30 kg/m^2, diabetes mellitus, a history of gastrointestinal, hepatic, or endocrine disease, symptoms of CAD, a pathological result on exercise testing, or an abnormal physical examination.

Anthropometric Data, Exercise Testing, and Questionnaire

Weight and height were measured after blood was drawn. BMI was calculated as the weight in kilograms divided by the square of the height in meters. A bicycle exercise stress test in the sitting position was performed with a stepwise increment in the work rate of 50W every 3 minutes with continuous electrocardiographic recording. Maximal oxygen consumption ($\dot{V}O_2max$) was estimated according to a nomo-

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gram.[18] All individuals answered a questionnaire on the weekly amount of leisure time physical exercise. The individuals were defined on the basis of physical fitness as trained ($\dot{V}O_2max < 45$ mL/kg/min) or untrained ($\dot{V}O_2max > 50$ mL/kg/min). In addition, those reaching a $\dot{V}O_2max$ greater than 50 mL/kg/min during ergometry had to exercise at least three times per week for more than 30 minutes in vigorous sports (> five METs), and untrained individuals not more than once per week. Individuals not meeting these criteria were not included in the analysis.

Density Gradient Ultracentrifugation and Chemical Analysis

EDTA plasma was obtained after an overnight fast. VLDL (d < 1.006 g/mL), 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.[19] Total LDLs were separated into six classes and HDLs into two density classes by density gradient centrifugation as described previously.[4,20] The density ranges of LDL subfractions as determined by precision refractometry[19] of blank gradients were 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_2, 1.063 to 1.125 g/mL; and HDL_3, 1.125 to 1.210 g/mL.

In all HDL and LDL subfractions, total cholesterol, free cholesterol, triglycerides, and phospholipids were determined by automated (EPOS; Eppendorf, Hamburg, Germany) enzymatic methods (Boehringer, Mannheim, Germany, and bioMérieux, Nürtingen, Germany). Esterified cholesterol was calculated as the molar difference between total and free cholesterol. The apolipoproteins apo A-I, apo B, and apo A-II were assayed by end-point nephelometry (Behring, Marburg, Germany).

The within-assay coefficient of variation for the determination of LDL subfraction concentrations was 2.2% to 4.5% for cholesterol and 3.0% to 5.8% for apo B, depending on the subfraction.

Statistical Analysis

For univariate comparison between trained and sedentary hypercholesterolemic men, the nonparametric Mann-Whitney test was applied.

The composition of apo B–containing particles (VLDL, IDL, and LDL) is expressed as lipid molecules per apo B (lipids/apo B), since one VLDL, IDL, or LDL particle contains exactly one apo B molecule.[21,22] The calculation of lipid molecules per LDL particle apo B is superior to percentage calculations, because with the latter, differences in the concentration of only one component influence the percentage values of all other components of that particle.

In addition, Pearson correlation analysis was performed between BMI and $\dot{V}O_2max$. To investigate which of the two factors ($\dot{V}O_2max$ or BMI) were primarily influencing the HDL and LDL subfraction profile (HDL_2 and LDL_6), a stepwise multiple regression analysis was performed with the lipoprotein subfractions as the dependent variable. 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$^+$). Values are expressed as the mean ± SD. *P* values less than .05 were considered to indicate statistical significance.

RESULTS

Both groups significantly differed with regard to $\dot{V}O_2max$ (sedentary *v* trained, 37.5 ± 8.8 *v* 59.3 ± 7.4 mL/kg/min, $P < .001$). Trained subjects were slightly younger (37 ± 11 *v* 33 ± 12 years, NS) and had a lower BMI (24.8 ± 2.9 *v* 23.0 ± 2.1 kg/m^2, $P = .054$) than the sedentary controls, although this did not reach statistical significance. BMI correlated inversely with absolute $\dot{V}O_2max$ ($r = -.53$, $P < .05$) and $\dot{V}O_2max$ per kilogram body weight ($r = -.81$, $P < .001$).

Lipoprotein analysis showed that serum cholesterol and LDL cholesterol were not statistically different between trained and sedentary hypercholesterolemic men (Table 1). Also, LDL apo B levels (sedentary *v* trained, 108 ± 23 *v* 112 ± 36 mg/dL) were not statistically different between the groups. Trained individuals had significantly lower serum triglycerides, VLDL cholesterol, VLDL apo B, and small, dense LDL cholesterol and higher HDL_2 cholesterol (Table 1 and Fig 1) and HDL_2 apo A-I (30 ± 15 *v* 45 ± 19 mg/dL, $P < .01$) than the sedentary controls.

LDL Particle Concentrations

Since one LDL particle contains one apo B molecule,[21,22] the apo B concentration of each LDL subfraction gives an estimation of the number of circulating apo B particles. Although sedentary and trained hypercholesterolemic men had equally elevated serum and LDL apo B levels, sedentary men had lower LDL_1 to LDL_3 (d = 1.019 to 1.037 g/mL) apo B levels and more small, dense LDL (LDL_5 and LDL_6) apo B (Fig 1). Multiple regression analysis showed that LDL_6 apo B was primarily influenced by $\dot{V}O_2max$ per kilogram body weight ($R^2 = .44$, $P < .01$).

LDL Particle Composition

Composition of apo B–containing particles (VLDL, IDL, and LDL) is expressed as the relative lipid content per apo B molecule.[4] Composition analysis showed that the lipid content of VLDL and IDL particles was similar between trained and sedentary hypercholesterolemic men, except for a lower triglyceride content of IDL particles in trained subjects (Table 2). In contrast, the lipid content of LDL particles (cholesterol and phospholipids) was significantly higher in trained men (Table 2). When dividing LDL particles into subfractions, it became evident that the higher free cholesterol content of total LDL was present in all six LDL subfractions, but that the higher cholesterol ester and phospholipid content in LDL of trained subjects was confined to LDL-5 and LDL-6 (d > 1.040 g/mL). No difference regarding the triglyceride content of LDL par-

Table 1. Serum Cholesterol, Triglyceride, and Cholesterol Concentrations of Lipoproteins and Lipoprotein Subfractions (mg/dL) of Sedentary and Trained Hypercholesterolemic Men (mean ± SD)

Variable	Untrained (n = 20)	Trained (n = 20)
Serum cholesterol	300 ± 51	321 ± 70
Triglycerides	153 ± 63	108 ± 32*
VLDL cholesterol	29 ± 15	19 ± 11*
IDL cholesterol	18 ± 8	16 ± 7
LDL cholesterol	177 ± 47	201 ± 65
LDL-1	31 ± 12	43 ± 14†
LDL-2	18 ± 8	34 ± 17‡
LDL-3	22 ± 11	41 ± 20‡
LDL-4	33 ± 16	39 ± 16
LDL-5	37 ± 15	26 ± 12*
LDL-6	36 ± 15	19 ± 6‡
HDL cholesterol	43 ± 10	56 ± 11‡
HDL_2	22 ± 8	35 ± 12‡
HDL_3	20 ± 4	20 ± 5

*$P < .05$, †$P < .01$, ‡$P < .001$: differences between groups.

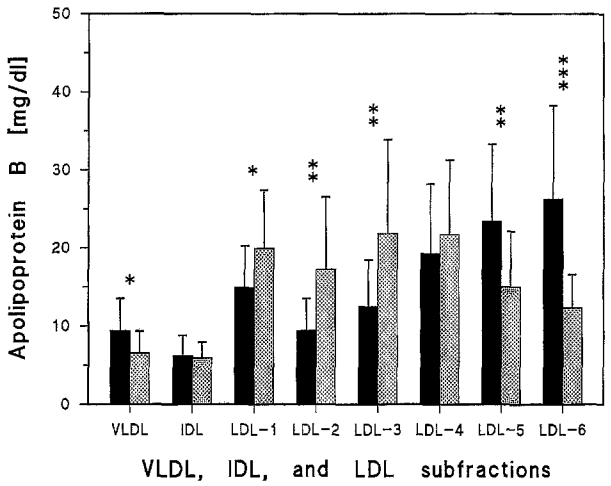

Fig 1. Apo B concentrations (mg/dL) of serum lipoproteins and lipoprotein subfractions of 20 sedentary (■) and 20 trained (▨) hypercholesterolemic men (mean ± SD). Significance values refer to differences between groups: *$P < .05$, **$P < .01$, and ***$P < .001$.

ticles was observed between trained and sedentary men (Table 2).

DISCUSSION

This study showed that hypercholesterolemic men with good aerobic fitness have a more favorable lipoprotein profile with lower triglyceride levels and elevated HDL_2 cholesterol than men with low fitness. Although both groups had the same concentrations of LDL apo B and therefore the same number of circulating LDL particles, the LDL subfraction profile differed significantly. Trained hypercholesterolemic individuals had significantly less small, dense particles ($d > 1.040$ g/mL) and instead had higher concentrations of large LDL subfraction particles ($d < 1.037$ g/mL) than untrained hypercholesterolemic controls (Fig 1). These findings are in accordance with a study in normocholesterolemia that demonstrated a predominance of large LDL particles in long-distance runners, although differences in small, dense LDL particles were not present.[13] Regular exercise training by running 16 km/wk also induces a reduction in the concentration of small, dense LDL particles in moderately overweight men.[16]

LDL subfraction phenotype expression has been shown to be associated with several factors such as genetic disposition,[6,23] nutrition, sex hormone levels, lipid enzyme activities, insulin resistance, magnitude of postprandial lipemia, and obesity.[12,20,24-29] The influence of exercise on the concentration and composition of LDL subfraction particles is thought to be primarily determined by differences in lipase activities. A low activity of hepatic lipase[27,30-33] and an increased activity of lipoprotein lipase (LPL)[31,32] have shown to be associated with an LDL pattern of increased large, buoyant particles and reduced concentrations of small, dense LDL subfraction particles, although the data are partly inconsistent.[30,33,34] An influence of cholesterol ester transfer protein (CETP) on LDL distribution has been demonstrated in vitro[35-37] and in CETP-deficient patients.[38] According to these studies, CETP activity seems to play an important role in determining the dispersion of the plasma LDL pattern. However, a shift of large LDL particles toward subpopulations of smaller size seems to be a combined action of both LPL and CETP.[37] Trained individuals have lower hepatic lipase and CETP activities and higher LPL activities at rest[13,39] that can increase during a single session of exercise.[40] Differences in these enzyme activities are one possible explanation for our finding that hypercholesterolemic trained men have lower concentrations of small, dense LDL particles than sedentary controls (Fig 1).

By increasing LPL activity, exercise also increases the peripheral clearance of triglyceride-rich particles.[41] This is in accord with our findings of reduced triglycerides and VLDL particle concentration in trained hypercholesterolemic individuals. If increased peripheral lipolysis, though, was the single factor leading to reduced triglycerides, a reduced lipid content of VLDL, IDL, and LDL and not primarily a reduced number of VLDL should be expected, a finding not observed in this study. Indeed, it is known that LPL also modulates the net secretory output of apo B,[42] which may lead to a reduced number of VLDL particles (Fig 1). Reductions in VLDL particles by

Table 2. Composition of VLDL, IDL, LDL, and Six LDL Subfraction Particles in Sedentary and Trained Hypercholesterolemic Men (mean ± SD)

	Untrained (n = 20)				Trained (n = 20)			
	FC/Apo B	CE/Apo B	PL/Apo B	TG/Apo B	FC/Apo B	CE/Apo B	PL/Apo B	TG/Apo B
VLDL	1,664 ± 285	2,304 ± 526	2,421 ± 463	6,512 ± 1,673	1,508 ± 330	2,183 ± 597	2,289 ± 448	5,888 ± 1,241
IDL	1,048 ± 131	2,637 ± 397	1,357 ± 253	1,299 ± 680	1,014 ± 139	2,478 ± 460	1,255 ± 225	856 ± 194*
LDL	540 ± 83	1,620 ± 115	657 ± 70	117 ± 42	640 ± 65‡	1,751 ± 127†	730 ± 96*	115 ± 40
LDL-1	695 ± 201	2,115 ± 477	875 ± 124	198 ± 66	817 ± 96†	2,111 ± 282	912 ± 142	174 ± 55
LDL-2	641 ± 94	1,893 ± 195	809 ± 91	149 ± 56	723 ± 104*	1,979 ± 333	840 ± 153	126 ± 54
LDL-3	591 ± 78	1,764 ± 174	743 ± 90	129 ± 55	697 ± 169‡	1,929 ± 539	806 ± 206	110 ± 50
LDL-4	556 ± 78	1,675 ± 155	685 ± 86	110 ± 50	645 ± 188*	1,837 ± 541	760 ± 202	100 ± 43
LDL-5	504 ± 82	1,591 ± 167	634 ± 112	100 ± 50	574 ± 121*	1,736 ± 347*	726 ± 128†	97 ± 35
LDL-6	425 ± 80	1,442 ± 124	565 ± 77	98 ± 28	497 ± 61†	1,567 ± 127†	676 ± 95‡	115 ± 35

NOTE. The composition is expressed as lipid molecules per apo B particle (lipids/apo B) and was calculated according to the method used by Baumstark et al.[4]

Abbreviations: FC, free cholesterol; CE, esterified cholesterol; PL, phospholipids; TG, triglycerides.

*$P < .05$.

†$P < .01$.

‡$P < .0001$.

exercise might also explain differences in the LDL subfraction pattern, since the metabolism of VLDL and small, dense LDL particles has been shown to be closely related.[43-45] Elevated VLDL particle concentrations are also often associated with postprandial hyperlipidemia, which itself is associated with a pattern of increased concentrations of small, dense LDL particles.[28,46] Physically active subjects are known to have an improved triglyceride clearance,[41] reducing postprandial hypertriglyceridemia and thereby improving the LDL subfraction profile. An increased triglyceride clearance can also lead to a lower triglyceride content of apo B–containing lipoprotein particles.[41] This was evident in our population of hypercholesterolemic trained individuals showing a lower triglyceride content of VLDL, IDL, and large, buoyant LDL subfractions, although these differences did not reach statistical significance.

A pattern of increased concentrations of small, dense LDL particles is closely related to insulin resistance.[25,47] The small, dense LDL phenotype has been shown as a prognostic factor for the future development of non–insulin-dependent diabetes mellitus[48] and is prevalent in diabetic patients.[25,31,46,49] Exercise training is known to increase the sensitivity and responsiveness of insulin-mediated glucose uptake of human skeletal muscle cells.[50] Although we did not measure parameters of insulin resistance, it may nonetheless be postulated that by this mechanism lipoprotein metabolism may also be altered, leading to a reduction of the number of small, dense LDL particles in regularly exercising hypercholesterolemic men.

Compositional variations of LDL particles could also be responsible for different LDL subfraction profiles between hypercholesterolemic men with high versus low physical fitness. Differences in the lipid content have been speculated to induce conformational changes of apo B and decrease its binding specificity for the apo B/E receptor.[51-54] Particularly, small, dense LDL particles of hypertriglyceridemic individuals have an approximately 50% reduced binding affinity to the LDL receptors of human fibroblasts.[54] Our study has shown that differences in physical fitness significantly influence the composition of LDL particles. Trained hypercholesterolemic men have LDL with a significantly higher content of free cholesterol in all subfractions. Small, dense LDL in trained hypercholesterolemic men showed a characteristic composition of a higher content of core lipids (cholesterol ester) and surface lipids (free cholesterol and phospholipids) compared with those in sedentary hypercholesterolemic controls. These findings are similar to differences between obese men (BMI $>$ 27 kg/m^2) and lean men (BMI $<$ 25 kg/m^2).[29] Lean normocholesterolemic men have small, dense LDL particles with a significantly higher free cholesterol and phospholipid content than obese men.[29] However, mean values for free cholesterol and phospholipids were higher in trained hypercholesterolemic men, and therefore seem to be induced by physical exercise. This is supported by a study showing that a single exercise session such as a 30-km cross-country run does increase the free cholesterol content of LDL particles in endurance-trained individuals; the phospholipid content, though, remains unchanged.[14] Acute exercise also induces a short-term reduction of the triglyceride content of LDL subfraction particles.[14] However, we observed no significant difference in the composition of LDL particles with regard to triglyceride content. These acute triglyceride reductions are only transient and reach pre-exercise values within a day, which might explain why only minor differences in the triglyceride content of apo B particles could be observed between trained and sedentary hypercholesterolemic subjects. A lower triglyceride content of LDL particles was nonetheless evident in trained hypercholesterolemic subjects, but did not reach statistical significance.

Exercise-induced reductions in LDL subfraction concentrations are greatest when concomitant weight loss occurs.[16] The significant improvement of the LDL subfraction profile by exercise-induced weight loss over 1 year is eliminated when adjusting for BMI.[16] Hypercholesterolemic men with high versus low aerobic fitness also differed in BMI (P = .054) in our study, so it can be argued that it is the BMI and not physical fitness that is responsible for the observed difference in the LDL subfraction profile of trained and untrained individuals. However, a multivariate regression analysis showed that small, dense LDL particles were primarily influenced by $\dot{V}O_2max$ and not by BMI, at least for this hypercholesterolemic, young male population. However, aerobic fitness and lean body mass are often closely related, so it is difficult to resolve which of the two factors is predominantly responsible for differences in lipoprotein subfraction phenotypes. This is particularly so since both factors affect the peripheral lipases in a similar fashion.[40,55] Nonetheless, from our data, there seems to be an independent effect of physical fitness on the LDL subfraction phenotype in hypercholesterolemia. This association might partly be determined by genetic factors, since up to 40% of the individual variation for $\dot{V}O_2max$ and 20% to 30% of the variation for LDL subfraction phenotype are due to genetic influences.[23,56,57] Therefore, it cannot be resolved from our study whether differences between the LDL subfraction phenotype of trained and sedentary hypercholesterolemic men are primarily determined by genetic factors or by habitual endurance exercise. To minimize the genetic influence on $\dot{V}O_2max$, men with good physical fitness who were not regularly exercising were not included in our study population. In addition, it is possible that $\dot{V}O_2max$ is more closely related to small, dense LDL particles than BMI in our study, since BMI is not a precise measure of adiposity in non-obese individuals.

Overall, the study has shown that hypercholesterolemic men with good aerobic fitness ($\dot{V}O_2max$ $>$ 50 mL/kg/min) and who exercise on a regular basis ($\geq$ three times per week and $\geq$ 30 minutes per exercise session) have a more favorable LDL subfraction profile than sedentary hypercholesterolemic men. Since the progression of CAD is related closely to the concentration of dense LDL particles,[58,59] it might be assumed that lower concentrations of small, dense LDL particles in the more fit hypercholesterolemic men will lead to less cholesterol deposition in the arterial wall and to a reduced incidence of cardiovascular events.

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