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Acute and delayed effects of prolonged exercise on serum lipoproteins

II. Concentration and composition of low-density lipoprotein subfractions and very low-density lipoproteins

Manfred W. Baumstark, Ingrid Frey, and Aloys Berg

Department of Physical Performance Medicine, Center of Internal Medicine, University of Freiburg, Hugstetter Strasse 55, W-7800 Freiburg, Germany

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Summary. To investigate the effects of a single period of prolonged exercise on lipoprotein concentration and composition, 13 healthy endurance-trained men were examined before and after (1 h, 20 h) a cross-country run [30 km, time: 130 (SD 7.4) min]. The data show that following acute exercise, serum triglyceride (TG) concentration were reduced (36%) as a consequence of a reduced number (31%) of very low density lipoprotein (VLDL) particles. Changes in composition of VLDL were present but less evident. In contrast to this, acute exercise did not induce significant changes in the average concentration of individual low-density lipoprotein (LDL) subfractions. However, changes in dense LDL [density (d) > 1.044 $g \cdot ml^{-1}$] concentration were significantly correlated to changes in serum TG: a reduction of dense LDL occurred in subjects with large reductions in serum TG. In addition, LDL composition changed significantly. Immediately (1 h) after exercise the TG content of all LDL subfractions was reduced. These reductions were significant in large (d = 1.006–1.037 $g \cdot ml^{-1}$) and small LDL (1.044–1.063 $g \cdot ml^{-1}$). It can be concluded therefore from our study that acute exercise primarily altered the composition of LDL subfractions while their concentration remained stable.

Key words: Exertion – Sports – Lipoproteins – Low density lipoprotein – Very low density lipoproteins

Introduction

Most studies concerning the influence of exercise on lipoprotein metabolism have concentrated on high density lipoproteins (HDL). Only very few studies have investigated in detail the influence of exercise on the metabolism of low density lipoprotein (LDL) and its subfractions. From these studies there is evidence that exercise specifically influences the concentration of certain LDL subfractions. Cross-sectional studies have shown that trained subjects have an increased average LDL particle size (Lamon-Fava et al. 1989a), mainly due to a reduced concentration of small dense LDL (Williams et al. 1986; Berg et al. 1992). One longitudinal study (Williams et al. 1989) has shown that during exercise-induced loss of body mass there is a decrease in small LDL concentrations and an increase in LDL peak flotation rate. After an endurance triathlon, in 7 out of 40 subjects an increased LDL particle size was documented by (Lamon-Fava et al. 1989b) using gradient gel electrophoresis. The effect of a single period of exercise on the composition and concentration of individual LDL subfractions has not yet been studied.

The present study was undertaken to examine the effects of a single period of prolonged exercise on serum lipid- and lipoprotein concentrations and on the composition of lipoprotein subclasses, in this case very low density lipoprotein (VLDL) and LDL subfractions. The importance of LDL subfractions arises from the fact that coronary angiographic studies have demonstrated a direct correlation between levels of small dense LDL particles and the extent (Austin et al. 1990; Krauss 1991) and progression (Krauss et al. 1987) of coronary atherosclerosis. It has to be mentioned that in contrast to previous studies, we used preparative density gradient ultracentrifugation to separate LDL subfractions. Using this approach, it is possible to measure precisely both concentration and composition of LDL subfraction particles. With gradient gel electrophoresis, as used by most other authors, only an estimation of the size distribution of the LDL particles can be made.

Methods

Subjects and procedure. As detailed in the accompanying paper (Frey et al. 1993), 13 male, clinically healthy, endurance-trained

This paper is dedicated to Prof. Dr. J. Keul, Medical Director of our Department, on the occasion of his 60th birthday

Correspondence to: M. W. Baumstark

volunteers [age, 31.0 (SD 7.5) years; maximal oxygen uptake ($\dot{V}O_{2max}$), 59.5 (SD 2.1) $ml \cdot kg^{-1} \cdot min^{-1}$] participated in a standardized endurance stress test (30 km cross-country running). All methods except LDL-subfraction preparation are described in (Frey et al. 1993). In brief, VLDL, LDL, and HDL were prepared by sequential flotation according to Lindgreen (1975) and Lindgren et al. (1972). Total LDL ($d = 1.006–1.063\ g \cdot ml^{-1}$) was fractionated into six density classes by equilibrium density gradient centrifugation as has been described previously (Baumstark et al. 1990). The d ranges of the subfractions as determined by precision refractometry (Lindgren 1975) of blank gradients were: LDL-1, <1.031; LDL-2, 1.031–1.034; LDL-3, 1.034–1.037; LDL-4, 1.037–1.040; LDL-5, 1.040–1.044; LDL-6, >1.044 $g \cdot ml^{-1}$. All centrifugation steps were performed at a temperature of 18° C using polycarbonate bottles (Kontron, 4 ml) in a 50.4Ti (Beckman) rotor. The LDL subfractions were recovered by means of an Auto Densi-Flow IIC (Buchler Instruments) device. Phospholipid (PL), free cholesterol (FC), total cholesterol (TC), and triglycerides (TG) were measured by automated (EPOS, Eppendorf, Hamburg) enzymatic methods. Cholesterol ester (CE) was calculated as molar difference of TC and FC. The apolipoproteins apoA-I and apoB were measured with kinetic nephelometry (Beckman, Munich), apoA-II with endpoint nephelometry (Behring, Marburg).

Values before and after the field test (1 h, 20 h) were first compared by nonparametric Friedman two-way ANOVA. Univariate comparisons (before vs 1 h after, and before vs 20 h after) were performed by a Wilcoxon matched-pairs signed ranks test. All calculations were performed using the software package SPSS PC (SPSS Inc., Chicago).

Results

In serum we found significant reductions ($P < 0.01$) of TC, TG and apoB 20 h after exercise. The values were (before exercise, 1 h after, 20 h after): TC, 4.53 (SD 0.78), 4.64 (SD 0.80), 4.21 (SD 0.64); TG, 1.10 (SD 0.31), 0.92 (SD 0.30), 0.76 (SD 0.20) $mmol \cdot l^{-1}$; apoB, 68.6 (SD 13.0), 69.6 (SD 16.0), 63.6 (SD 12.2) $mg \cdot dl^{-1}$. In VLDL (Table 1) there was a significant reduction of all components after exercise. As each VLDL particle contains exactly one molecule of apoB, this would indicate a reduced number of VLDL particles 24 h after exercise. Changes in VLDL composition (Table 2) occurred 1 h after exercise, where a reduced size and lipid content (PL, FC, TG) of VLDL was observed. The 20 h after exercise measurements showed that only the FC content remained significantly reduced.

The particle concentration in LDL subfractions of the group examined was as expected for physically active subjects with normal serum cholesterol concentrations (Berg et al. 1992). The LDL distribution profile was not changed following acute exercise, as indicated by the unchanged apoB concentrations (1 h and 20 h) after running (Table 1). To assess whether changes in individuals in dense LDL (LDL-6) were purely random or associated with other changes, Spearman's

Table 1. Concentration of lipids and apolipoprotein B (apoB) in serum, very low density lipoprotein (VLDL), low density lipoprotein (LDL) and subfractions of LDL

	Time	PL		FC		CE		TG		apoB		Total mass	
		($\mu mol \cdot l^{-1}$)								($mg \cdot dl^{-1}$)			
		mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
VLDL	Before exercise	201.5	96.8	108.6	67.5	183.2	123.8	590.2	245.1	4.35	2.20	87.84	38.15
	1 h after	158.4	101.8*	85.5	69.9*	159.5	116.3	449.0	243.6	4.01	2.00	69.35	40.58
	20 h after	126.5	59.1**	55.7	44.6**	99.0	58.7*	380.6	146.2**	2.97	0.84*	54.75	21.86**
LDL	Before exercise	856.5	195.2	647.0	179.2	1875.9	427.1	235.3	85.0	66.04	13.11	299.96	65.76
	1 h after	849.6	190.9	680.9	182.5*	1905.4	425.2	232.5	54.6	66.24	18.94	302.61	69.66
	20 h after	799.3	136.5	641.0	129.5	1731.4	314.3*	212.2	51.6	61.82	16.08	279.68	52.45[a]
LDL-1	Before exercise	206.4	89.2	159.4	77.0	444.0	190.5	99.9	42.4	12.69	5.79	72.46	30.81
	1 h after	202.5	73.5	173.4	61.0	453.9	153.9	74.3	29.4[a]	12.58	4.92	71.00	24.20
	20 h after	188.6	62.5	165.6	63.7	411.1	177.1	82.0	24.3	12.37	5.80	67.29	25.47
LDL-2	Before exercise	136.0	57.7	105.0	52.8	324.0	132.1	30.5	15.8	10.09	4.71	48.42	20.36
	1 h after	129.6	59.0	109.2	59.9	311.9	142.9	24.4	12.0*	10.25	4.07	46.93	19.87
	20 h after	129.0	41.9	106.4	37.1	303.2	100.2	23.9	6.0	10.09	5.21	46.01	16.34
LDL-3	Before exercise	133,3	52.9	109.0	36.8	347.9	103.9	24.5	12.8	10.66	3.25	49.96	15.37
	1 h after	140.0	48.0	115.4	46.2	347.0	115.7	22.0	10.4	10.96	3.03	50.75	15.87
	20 h after	132.1	24.4	109.2	24.4	329.5	66.2	21.1	6.3	10.71	3.44	48.43	9.82
LDL-4	Before exercise	122.9	28.8	92.3	24.1	302.8	79.7	19.4	8.8	10.83	2.89	45.29	11.13
	1 h after	127.4	31.3	101.4	31.0	319.7	78.6	19.4	8.1	11.04	2.22	47.31	10.52
	20 h after	113.9	29.7	90.4	27.4	281.5	73.4	19.0	6.6	10.43	3.14	42.71	10.74
LDL-5	Before exercise	99.7	30.5	67.0	26.4	263.7	94.6	17.3	6.1	9.12	2.70	38.09	12.01
	1 h after	99.1	28.0	69.3	31.5	255.8	72.7	16.0	6.4	9.24	2.24	37.61	9.81
	20 h after	93.0	34.3	65.0	29.7	226.5	85.9	18.3	8.8	9.02	3.84	35.06	12.76
LDL-6	Before exercise	106.4	36.9	65.4	27.4	242.6	88.2	26.4	5.5	10.47	3.43	39.33	12.73
	1 h after	109.1	29.1	90.3	76.6[a]	239.0	51.1	25.1	7.3	11.36	3.22	41.04	10.14
	20 h after	95.8	35.4	65.8	26.0	218.4	90.5[a]	24.4	8.8	10.62	3.75	36.91	12.74

PL, Phospholipid; FC, free cholesterol; CE, cholesterol ester; TG, triglyceride.
The total mass has been calculated as the sum of the components in $mg \cdot dl^{-1}$. $n = 13$.

* $P < 0.05$, ** $P < 0.01$ versus before exercise by Wilcoxon test. Preceding Friedman ANOVA test, $P < 0.05$.
[a] $P < 0.05$ versus before exercise by Wilcoxon test. Preceding Friedman ANOVA test NS.

Table 2. Number of lipid molecules per particle, calculated as molar ratio of lipid to apolipoprotein B

	Time	PL		FC		CE		TG		Radius [nm]	
		mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
VLDL	Before exercise	2395	567	1230	286	2019	476	7444	2929	16.25	1.56
	1 h after	1905	437[a]	971	337*	1850	478	5671	1299[a]	15.12	1.00[a]
	20 h after	2158	649	898	516*	1673	630	6752	2343	15.62	1.64
LDL	Before exercise	607	74	454	55	1327	158	169	53	9.53	0.24
	1 h after	679	116	540	94	1521	264	189	46	9.63	0.45
	20 h after	682	96	546	84[a]	1470	184	182	42	9.58	0.35
LDL-1	Before exercise	842	67	645	101	1809	144	423	120	10.40	0.26
	1 h after	850	209	723	122*	1883	231	315	82**	10.36	0.46[a]
	20 h after	807	86	700	76	1712	152*	368	90	10.25	0.30
LDL-2	Before exercise	699	75	535	121	1675	213	159	49	9.76	0.28
	1 h after	666	158	553	156	1612	411	124	42[a]	9.57	0.82
	20 h after	688	94	566	97	1616	262	134	39[a]	9.67	0.40
LDL-3	Before exercise	630	168	528	84	1681	205	119	43	9.63	0.30
	1 h after	665	134	547	134	1648	333	103	37[a]	9.59	0.66
	20 h after	661	102	541	81	1626	206	107	37	9.60	0.40
LDL-4	Before exercise	590	58	442	52	1444	152	94	34	9.27	0.21
	1 h after	597	104	474	104	1499	277	89	26	9.33	0.49
	20 h after	582	112	461	102	1436	287	95	20	9.25	0.53
LDL-5	Before exercise	566	57	382	84	1478	211	102	35	9.25	0.30
	1 h after	557	95	387	146	1438	269	90	27	9.17	0.49
	20 h after	550	90	395	103	1340	229*	105	36	9.09	0.47
LDL-6	Before exercise	535	161	325	81	1192	196	136	30	8.92	0.44
	1 h after	501	64	385	176	1134	277	116	29*	8.82	0.44
	20 h after	477	99	328	72	1085	248	128	46	8.69	0.63

The particle radius is calculated from the number of lipid molecules per particle and molecular volumes of lipids and apolipoprotein B. For details see (Baumstark et al. 1990). $n=13$.

* $P<0.05$, ** $P<0.01$ versus before exercise by Wilcoxon test. Preceding Friedman ANOVA test, $P<0.05$.

[a] $P<0.05$ versus before exercise by Wilcoxon test. Preceding Friedman test NS. For definitions see Table 1

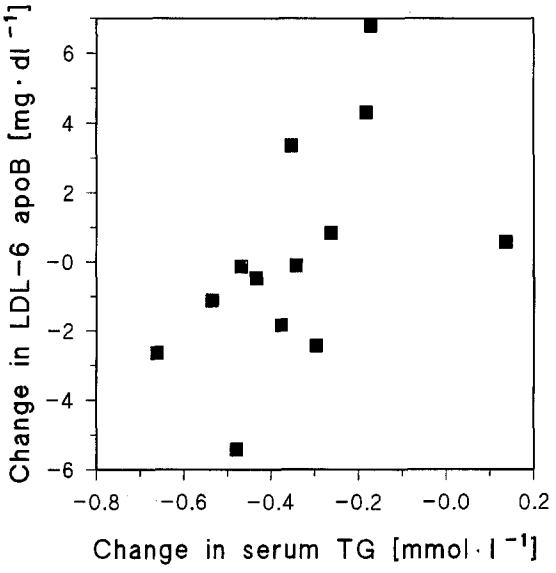

Fig. 1. Change in concentration of dense *LDL* versus change in serum *TG* concentration. Spearman's rank correlation coefficient: $r=0.73$, significance $P=0.0045$. For definitions see Table 1

rank correlation coefficients were calculated. As shown in Fig. 1 changes in LDL-6 (before exercise versus 20 h after) correlated significantly ($P=0.0045$) with changes in serum TG (Fig. 1).

In addition, the composition of LDL subfractions was significantly different after the field test. Table 2 illustrates the exercise-induced changes in the composition of LDL subfractions. Immediately (1 h) after exercise the TG content of all LDL subfractions was reduced (Fig. 2). The reduction was significant in large ($d=1.006$–$1.037\ g\cdot ml^{-1}$) and small LDL (1.044–$1.063\ g\cdot ml^{-1}$). From 1 h to 20 h after exercise TG content increased, but did not reach values measured before running. It is noteworthy that FC and cholesterol ester (CE) content (Table 2) changed in different directions – FC content of LDL-1 was significantly increased 1 h after the run, while CE of LDL-1 and LDL-5 was reduced 24 h after exercise. The same tendency was found in the other LDL subfractions.

Discussion

It has been established by longitudinal and cross-sectional studies that habitual endurance training lowers LDL cholesterol concentrations (Wood et al. 1991; Williams et al. 1989) and increases average LDL particle size (Lamon-Fava et al. 1989a; Williams et al. 1986). In the present study, the influence of a single period of prolonged exercise on VLDL and LDL subfraction concentration and composition was analysed. We showed that the well-known reduction of serum

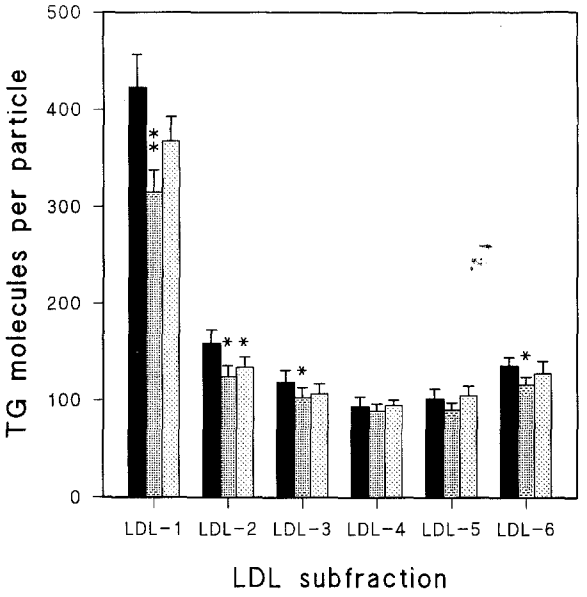

Fig. 2. The *TG* content of *LDL* subfraction particles before, 1 h after, and 20 h after a 30 km cross-country run. Data are means and SEM. * $P<0.05$, ** $P<0.01$ versus before exercise by Wilcoxon test. For definitions see Table 1. ■ before exercise; ▨ 1 h after; ⊡ 20 h after exercise

TG concentrations following acute exercise was a consequence of a reduced number of VLDL particles. It is assumed that physical activity reduces TG by enhancing lipoprotein lipase (LPL) activity, and thus increasing the peripheral clearance of TG-rich particles (Sady et al. 1986). This change is related to the increased demand of the working muscle for fatty acids as energy-yielding substrate and the significant depletion of muscle TG stores (Ren et al. 1988; Jansson and Kaijser 1987). If increased peripheral lipolysis were the only factor leading to reduced TG, one would expect a reduced lipid content of VLDL, and not primarily a reduced number of VLDL.

A new aspect arises from recent data which has shown that LPL modulates the net secretory output of apoB in vitro (Williams et al. 1991). According to that data it can be hypothesized that an increase in LPL leads to reduced VLDL apolipoprotein B (apoB) secretion, readily explaining the reduced VLDL particle concentrations found in this study. Further support comes from data showing that fasting TG concentrations are determined to a large extent by apoB synthesis rates (Cortner et al. 1992). A reduced apoB synthesis rate would also be consistent with the measured reduced serum concentration of TC, apoB, and LDL mass 20 h after the field test. A significant influence of acute exercise on the average concentration of small LDL particles was not observed in this study. However, correlation analysis showed that changes in LDL-6 were not random, but highly correlated with changes in serum TG. We were able to show that a reduction of dense LDL occurred in subjects with high reductions in serum TG. This corresponds to results in a paper by Lamon-Fava et al. (1989b) where in 7 out of 40 subjects an increased LDL particle size was found after a triathlon. These subjects had extreme reductions in plasma concentrations of TG of approximately 1.8 $mmol \cdot l^{-1}$ (160 $mg \cdot dl^{-1}$) on average. Despite lower initial serum TG values and lower TG changes in this study, correlated changes of serum TG and dense LDL were still highly significant. This would indicate that physical activity affects the regulation of LDL subfraction concentrations by influencing TG and fatty acid metabolism. The exact mechanism remains to be determined.

Furthermore, it was obvious that physical activity induced changes in the composition of LDL subfractions. The TG-rich ($d<1.034\ g \cdot ml^{-1}$) and dense LDL subfractions ($d>1.040\ g \cdot ml^{-1}$) showed pronounced changes in composition, which leads to the assumption that their metabolism especially would seem to be related to changes in energy expediture and muscle metabolism. A reduced TG content of LDL particles may be of special interest as this parameter has been shown to influence apoB conformation (Kunitake et al. 1990) and LDL fractional catabolic rate (Vega and Grundy 1986; Thompson et al. 1987), as well as the fluidity (transition temperature) of LDL particles (Deckelbaum et al. 1977). It can be concluded that with regard to the beneficial effect of endurance training on cardiovascular morbidity and mortality (see review Chandrashekhar and Anand 1991), not only does a close connection between energy metabolism and HDL metabolism appear to exist, but also an interesting relationship to LDL metabolism.

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