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Hyperlipoproteinemia Impairs Endothelium-Dependent Vasodilation

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Summary

Atherogenic lipoproteins can cause endothelial dysfunction in the initial stage of atherogenesis. In our study we examined 134 patients with defined hyperlipoproteinemia (non-HDL cholesterol > 4.1 mmol/l or triglycerides > 2.5 mmol/l or taking any of lipid lowering drugs) – 94 men and 40 women. The subgroup of controls of comparable age contained 54 normolipidemic individuals – 30 men and 24 women. Patients with hyperlipoproteinemia revealed significantly lower ability of endothelium-dependent flow-mediated vasodilation (EDV) measured on brachial artery (4.13±3.07 vs. 5.41±3.82 %; p=0.032) and higher carotid intima media thickness than normolipidemic controls (0.68±0.22 vs. 0.58±0.15 mm; p=0.005). In regression analysis, EDV correlated significantly with plasma concentrations of oxLDL ($p<0.05$) HDL-cholesterol ($p<0.05$), Apo A1 ($p<0.05$), ATI ($p<0.01$) and non-HDL cholesterol ($p<0.05$). Patients with hyperlipoproteinemia showed higher plasma levels of oxLDL (65.77±9.54 vs. 56.49±7.80 U/l; p=0.015), malondialdehyde (0.89±0.09 vs. 0.73±0.08 μmol/l; p=0.010) and nitrites/nitrates (20.42±4.88 vs. 16.37±4.44 μmol/l; p=0.018) indicating possible higher long-term oxidative stress in these patients.

Key words

Endothelium-dependent vasodilation • Hyperlipoproteinemia • Oxidative stress • Oxidized LDL • Atherosclerosis

Introduction

Oxidative modification of lipoproteins represents a key event of the atherosclerotic process. This has been proved by a number of epidemiological studies as well as by experiments in animal model (Aviram 1993). Oxidized atherogenic lipoproteins, namely oxidized LDL (oxLDL), penetrate into the subendothelial space of the arterial intima where they are readily absorbed by monocytes through scavenger receptors. Monocytes thus become overfilled with lipid particles and transform into foam cells. OxLDL stimulate chemotaxis of circulating leukocytes and thrombocytes, they modulate the gene expression of various growth factors, cytokines and adhesive molecules on the surface of the future atherosclerotic plaque and directly as well as indirectly inhibit arterial vasodilation, namely the endothelium-dependent vasodilation (flow-mediated

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vasodilation, EDV) (Abdalla *et al.* 1992, 1994, Parthasarathy *et al.* 1992, Timothy and Aust 1992, Ross 1993). Endothelium regulates the vasomotor tone by production of various molecules among which nitric oxide (NO) seems to play the most important role (Bauersachs *et al.* 1997, Irvine *et al.* 2003). This molecule not only causes vasodilation but also inhibits thrombocyte aggregation and expression of adhesive molecules on the endothelial surface as well as inhibits proliferation of the smooth muscle cells (SMC). Oxidative stress substantially inhibits the biological availability of NO. In the presence of serious risk factors (e.g. hypertension, hyperlipoproteinemia, hyperinsulinemia, hyperglycemia, obesity or smoking), endothelial dysfunction increases the risk of acute myocardial infarction or cerebrovascular stroke and contributes to worsening of existing myocardial or cerebral ischemia (Anderson *et al.* 1995, Britten and Schachinger 1998, Liao 1998, Tousoulis *et al.* 1997).

It can be anticipated that patients with hyperlipoproteinemia (hypercholesterolemia, hypertriglyceridemia) may show impaired endothelium-dependent vasodilation even before hemodynamically significant arterial stenosis develops. The aim of our study was to assess the impact of each of the basic plasma lipid parameters (total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides (TAG), Lp(a), apo A1, apo B) on the endothelium-dependent vasodilation, measured in the brachial artery.

Methods

Patients

In this study we examined 188 subjects aged 45-65 years. The basic characteristics are shown in Table 1. The definition of patients with severe hyperlipidemia was based on the NCEP III guidelines for treatment and prevention of patients at risk for cardiovascular diseases (Brewer 2003, Third Report NCEP 2002), i.e. with non-HDL cholesterol concentration above 4.1 mmol/l and TAG above 2.5 mmol/l. Furthermore, all subjects taking hypolipidemic drugs (statins, fibrates, resins) were included in the patient cohort. The hyperlipidemic group was thus represented by 134 subjects: 94 men (mean age 54.4±4.8 years) and 40 women (53.4±5.1 years). In the control group, there were 54 subjects: 30 men (mean age 54.2±4.5 years) and 24 women (53.0±3.9 years) with plasma non-HDL cholesterol concentrations below 4.1 mmol/l, TAG below 2.5 mmol/l and no history of hypolipidemic treatment at all.

Table 1. Basic characteristics of examined subjects.

	Control group		HL Patients		p
	n	Mean ± SD	n	Mean ± SD	
Age (years)	54	53.69±4.257	134	54.69±4.859	0.1847
BMI (kg/m^2)	54	27.44±4.093	131	28.62±3.822	0.0599
Waist hip ratio	52	0.89±0.115	121	0.94±0.091	0.0127
SBP (mm Hg)	53	132.3±15.85	134	136.8±19.71	0.1339
DBP (mm Hg)	53	80.64±6.711	134	81.81±9.196	0.5137
OxLDL (U/l)	42	56.49±7.796	91	65.77±9.536	0.0147
Anti oxLDL (mU/ml)	49	273.5±93.55	130	238.2±83.62	0.3106
Total cholesterol (mmol/l)	54	4.95±0.258	134	5.75±0.489	0.0000
Triglycerides (mmol/l)	54	1.12±0.214	133	1.88±0.382	0.0000
HDL-cholesterol (mmol/l)	54	1.46±0.158	133	1.23±0.133	0.0001
LDL-cholesterol (mmol/l)	54	2.89±0.228	128	3.55±0.440	0.0000
Non-HDL cholesterol	54	3.44±0.241	133	4.45±0.485	0.0000
Apo A1 (g/l)	54	1.30±0.108	131	1.25±0.112	0.2415
Apo B (g/l)	54	0.95±0.070	131	1.21±0.117	0.0000
Lp(a) (g/l)	52	0.13±0.017	124	0.19±0.034	0.0000
Glycemia (mmol/l)	52	5.50±0.401	134	6.07±0.686	0.0012
Uric acid (μmol/l)	53	290.4±32.24	133	319.2±41.17	0.0382

Table 2. Presence of atherosclerotic diseases and major cardiovascular risk factors.

	Controls	Patients	X^2/p
Sex			
Men	30	94	III.65
Women	24	40	0.0560
Coronary heart disease			
NO	47	60	28.III
YES	7	74	<0.0001
Hypertension			
NO	36	71	II.94
YES	18	63	0.0865
Peripheral vascular disease			
NO	54	128	II.50
YES	0	6	0.1140
Diabetes mellitus			
NO	49	110	II.21
YES	5	24	0.1373
Stroke			
NO	54	132	0.81
YES	0	2	0.3668
Chronic inflammatory diseases			
NO	45	118	0.75
YES	9	16	0.3878
Smoking			
NO	35	94	0.51
YES	19	40	0.4757
Menopausal status in women			
NO	7	6	I.86
YES	17	34	0.1486

Table 2 shows the incidence of atherosclerotic diseases (coronary heart disease, cerebrovascular stroke, lower limb ischemia) as well as major cardiovascular risk factors in both patient and control groups. At time of the examination, there were 51 subjects in the patient group on statin monotherapy, 7 patients on fibrate monotherapy and 5 patients on a combination of statin and fibrate treatment. None of the patients was taking resins. Distribution of hypolipidemic therapy as well as other cardiovascular drug therapy is shown in Table 3.

Furthermore, we performed an additional distribution of patients in the whole cohort of examined subjects and the controls based on levels of the so-called atherogenic index (ATI, i.e. regardless of the basic plasma lipid parameters). The level of ATI = 0.2 was assessed as limit value so that all subjects with HDL cholesterol/total cholesterol ratio below this value were included in the high risk group. According to several studies, ATI is the most important predictor of atherosclerosis progression (Hausmann *et al.* 1996).

All respondents were examined in the morning after at least 10 h of fasting. Patients on medication were asked not to take their drugs before the examination was completed. First, every participant underwent duplex sonography of the carotid arteries and FMV measurement on a brachial artery. Then, 20 ml of venous blood was withdrawn from cubital vein for various laboratory analyses. Patient´s personal, family, and social history was registered, with special attention to cardiovascular diseases and their risk factors, including dietary habits. Blood pressure (BP) was measured in the sitting position on the right arm after 10 min rest. The mean of two measurements was used for further analysis. Anthropometric measurements included assessment of body height, weight and circumference of the waist and hips.

Measurement of endothelial function and intima-media thickness

Measurement of endothelium-dependent vasodilation (EDV) was performed using the method described by Celermajer (1998a,b) and Sorensen *et al.* (1995). This method is based on the release of the vasodilation factor (NO) from endothelial cells after blood flow reestablishment in the examined artery. After 10 min at rest in the horizontal position, patient's blood pressure was measured on the left arm. The value of systolic BP was essential for constriction pressure assessment (systolic BP + 60 mmHg) during the FMV measurement on the right brachial artery. Using the Siemens Sonoline Versa Pro sonograph equipped with 7.5 MHz linear transducer, an optimal position on the brachial artery was localized. In this position, a 60-s long video was recorded. Then the cuff of the sphygmomanometer, placed on the right forearm, was inflated to the desired pressure for 4 min, video was recorded all the time, keeping the transducer still in the same position. The cuff was deflated and video was recorded for one additional minute.

Evaluation of EDV was performed under standard conditions from the video record using special software ImagePro Plus. Ten snaps in the diastole were taken in the basal phase before constriction and other

10 snaps (again in diastole) were taken between 30-45 s after the deflation. The diameter of the artery was exactly measured in the same localization in all snaps with accuracy of 0.01 mm. Means of every 10 measurements before and after arterial constriction were calculated and the rate of dilation (EDV) after the flow reestablishment was expressed in percentage of basal diameter before the constriction.

Table 3. Drug therapy in patient and control groups.

	Controls	Patients	X^2/p
Beta Blockers			
NO	39	58	XII.60
YES	15	75	0.0004
Nitrates			
NO	52	120	I.92
YES	2	13	0.1660
ACE Inhibitors			
NO	47	99	III.56
YES	7	34	0.0591
Acetylosalicylic Acid			
NO	45	71	14.63
YES	9	62	0.0001
Calcium Channel Blockers			
NO	51	120	0.87
YES	3	13	0.3499
Diuretics			
NO	47	113	0.13
YES	7	20	0.7145
Statins			
NO	54	77	32.46
YES	0	56	0.0001
Fibrates			
NO	54	121	V.21
YES	0	12	0.0144

Intima-media thickness (IMT) was measured again with Siemens Sonoline Versa Pro ultrasound, using a 7.5 MHz linear transducer (Gnasso *et al.* 1996). Patient was examined in horizontal position. IMT of the left and right common carotid arteries was measured on the distant arterial wall from the transducer, always in the section 10-20 mm below the carotid bifurcation. Ten measurements were performed on each side and the final value represented mean of 20 measurements.

Nitrites/nitrates are oxidative products of NO and their plasma levels may thus reflect the function of endothelium. The estimation method for nitrites/nitrates is based on enzymatic reduction of nitrates by nitrate reductase and following assessment of nitrites by Griess reaction with sulphonamide and naphtyldiamine and final samples deproteination by ultrafiltration (Crkovská and Štípek 1998).

Measurement of lipid oxidation

Malondialdehyde was measured by liquid chromatography using SUPELCOSI LC-18 column. Isoprostanes were estimated by enzyme immunoassay (kit CAYMAN CHEMICAL, USA). Antibodies against oxidized LDL (anti-oxLDL) were measured by enzyme immunoassay (kit BIOMEDICA, Austria). Oxidized LDL (oxLDL) were estimated directly from plasma using enzyme immunoassay (kit MERCODIA AB, Sweden). Lipophilic vitamins A, E were estimated by HPLC method, described by Fojtíková and Binková (1991).

Other biochemical and hematological parameters

Glycemia was estimated by the hexokinase reaction (kit KONELAB, Germany); creatinin by the kinetic method (kit KONELAB, Germany); transaminases ALT, AST by the kinetic method (standardization DGKC); uric acid, total cholesterol, and triglycerides by enzymatic method (kit KONELAB, Germany). HDL-cholesterol was determined directly by PEG modified enzymatic measurement (kit ROCHE, Switzerland), homocystein by fluorescence polarization immunoanalysis (kit ABBOTT, IMx Homocystein Reagent Pack), and ferritin by enzyme immunoassay (kit KONE QUARTUS EIA-System, Germany).

Blood count (number of leukocytes, erythrocytes, platelets, hemoglobin concentration and hematocrit) was determined with T660 Coulter Counter hematologic analyzer. Coagulation parameters (Prothrombin time and APTT) were measured from citrate containing plasma by STAGO method (STAGO COMPACT) and fibrinogen by immunoturbidimetric method (kit DAKO, Germany).

Statistical methods

Patients and control subjects were compared using Student's *t*-test for two independent samples. Parameters with lognormal distribution were transformed by a natural logarithm first. Pearson's coefficient was used for correlations of lognormal values and Spearman's coefficient was used for correlation of parameters with

unknown (non-normal and non-lognormal) distribution.

Results

Table 4 shows comparison of the hyperlipidemic and control groups in the measured parameters. Patients with hyperlipidemia had significantly lower levels of endothelium-dependent vasodilation and higher intima-media thickness of common carotid artery when compared with the controls.

Similarly, when ATI was used for the cardiovascular risk assessment, patients at higher risk showed lower EDV and higher intima-media thickness than subjects at lower risk (Table 5). Furthermore, the high risk patients had significantly higher plasma ferritin concentrations.

Table 4. Comparison of hyperlipidemic and control groups in measured parameters.

	Controls		HL Patients		p
	n	Mean ± SD	n	Mean ± SD	
EDV (%)	42	5.41±3.822	109	4.13±3.068	0.0316
IMT (mm)	53	0.58±0.148	134	0.68±0.220	0.0053
Nitrites/nitrates (μmol/l)	51	16.37±4.436	131	20.42±4.880	0.0184
Retinol (μg/ml)	53	0.65±0.083	133	0.70±0.078	0.0668
Tocopherol (μg/ml)	53	9.82±0.815	133	11.72±1.172	0.0000
Homocysteine (μmol/l)	46	10.89±1.165	118	11.35±1.317	0.6407
Isoprostanes (pg/ml)	9	143.22±54.280	39	132.13±35.543	0.7453
Malondialdehyde (μmol/l)	16	0.73±0.082	31	0.89±0.092	0.0096
C-reactive protein (mg/l)	54	7.06±1.208	134	8.15±1.678	0.0497
Ferritin (ng/ml)	53	102.09±46.146	132	126.18±49.716	0.1647
Leukocytes ($\times 10^9$/l)	50	6.14±0.681	128	6.59±0.751	0.1012
Erythrocytes ($\times 10^{12}$/l)	50	4.63±0.176	128	4.70±0.179	0.5995
Hemoglobin (g/dl)	50	14.42±0.562	128	14.62±0.512	0.6093
Hematocrit (%)	50	42.17±1.560	128	42.66±1.578	0.6275
ESR 1 h (mm/h)	52	9.40±3.233	126	8.32±3.202	0.6131

Table 5. Comparison of measured parameters in high-risk and low-risk groups according to atherogenic index (ATI).

	Low risk (ATI > 0.2)		High risk (ATI < 0.2)		p
	n	Mean ± SD	n	Mean ± SD	
EDV (%)	99	4.96±3.457	51	3.58±2.929	0.0151
IMT (mm)	129	0.62±0.202	57	0.71±0.208	0.0110
Nitrites/nitrates (μmol/l)	124	18.97±5.083	57	19.41±4.037	0.7450
Retinol (μg/ml)	129	0.68±0.079	56	0.70±0.082	0.5295
Tocopherol (μg/ml)	129	10.54±0.928	56	12.65±1.404	0.0000
Homocysteine (μmol/l)	111	11.35±1.214	53	10.94±1.389	0.5845
Isoprostanes (pg/ml)	28	130.3±42.74	20	139.6±31.98	0.7239
Malondialdehyde (μmol/l)	34	0.83±0.097	13	0.86±0.091	0.6926
C-reactive protein (mg/l)	130	7.06±1.109	57	9.84±2.401	0.0003
Ferritin (ng/ml)	129	105.9±45.76	55	158.9±52.58	0.0167
Leukocytes ($\times 10^9$/l)	123	6.38±0.708	54	6.64±0.803	0.6201
Erythrocytes ($\times 10^{12}$/l)	123	4.65±0.181	54	4.75±0.157	0.1177
Hemoglobin (g/dl)	123	14.45±0.549	54	14.83±4.448	0.0887
Hematocrit (%)	123	42.27±1.564	54	43.55±1.219	0.0360
ESR 1 h (mm/h)	122	8.73±3.160	55	8.53±3.395	0.8630

Table 6. Endothelium-dependent vasodilation in the whole cohort of investigated subjects according to drug therapy.

	No		Yes		p
	n	Mean ± SD	n	Mean ± SD	
Beta Blockers	74	4.967±3.697	76	4.076±3.697	0.1754
Nitrates	144	4.319±3.474	13	3.575±3.512	0.5323
ACE Inhibitors	123	4.462±3.428	34	3.520±3.579	0.1585
Acetylosalicylic Acid	91	3.984±3.417	66	4.636±3.537	0.2453
Calcium Channel Blockers	142	4.364±3.549	15	3.248±2.499	0.2357
Diuretics	133	4.212±3.557	24	4.510±3.007	0.7028
Statins	107	4.467±3.526	50	3.810±3.343	0.2700
Fibrates	145	4.191±3.488	12	5.065±3.297	0.5912

Table 7. Correlation of EDV with other measured parameters in the whole cohort of investigated subjects.

	Correlation		
	n	r	p
Age	152	–0.04	
BMI	150	–0.13	
WHR	139	–0.16	
SBP	152	–0.04	
DBP	152	–0.12	
IMT	152	–0.08	
Ferritin	149	–0.06	
OxLDL	106	–0.19	< 0.05
Anti oxLDL	143	–0.11	
Nitrites/nitrates	146	0.04	
Retinol	151	–0.07	
Tocopherol	151	–0.05	
Total cholesterol	152	–0.11	
Triglycerides	152	–0.13	
HDL cholesterol	150	0.18	< 0.05
LDL cholesterol	146	–0.09	
Lp(a)	148	–0.07	
Apo A1	150	0.17	< 0.05
Apo B	150	–0.06	
Non-HDL chol.	150	–0.16	< 0.05
ATI	150	0.21	< 0.01
Leukocytes	144	–0.02	
Erythrocytes	144	–0.24	< 0.01
Hemoglobin	144	–0.28	< 0.01
Hematocrit	144	–0.25	< 0.01
C-reactive protein	152	0.01	
Glycemia	150	–0.02	
Uric acid	150	–0.11	
ALT	152	–0.05	
AST	152	–0.05	
ESR 1 h	146	0.08	

In further analysis, the whole cohort of investigated subjects was divided into subgroups according to the medication with cardiovascular or hypolipidemic drugs (Table 6). There was no significant difference in EDV between treated vs. untreated subjects in any defined subgroup.

When smokers and non-smokers resp. patients with manifested atherosclerotic disease and controls in the whole cohort of investigated subjects was compared, no significant difference in EDV was found (4.11±2.09 vs. 4.67±3.49 and 3.92±3.23 vs. 4.50±3.68).

Table 7 shows the correlation of endothelium-dependent vasodilation with other measured values. Among lipid parameters, non-HDL cholesterol correlated negatively with the EDV response ($p<0.05$), whereas HDL cholesterol, apo A1 and ATI correlated positively with the EDV response ($p<0.05$, $p<0.05$ and $p<0.01$, respectively). When focused on parameters of lipoperoxidation, EDV correlated negatively with plasma levels of oxLDL ($p<0.05$).

Discussion

Our results clearly show that patients with hyperlipoproteinemia (hypercholesterolemia and/or hypertriglyceridemia) have a significantly lower endothelium-derived vasodilatatory response than subjects in control group. Hyperlipidemia may thus cause endothelial dysfunction similar to hypertension (John and Schmieder 2003). Moreover, hyperlipidemic patients had higher intima-media thickness measured on the common carotid artery. It might be expected that there would be a negative correlation between EDV and IMT which was, however, not found in our study. This is not surprising since the thickness of the arterial intima-media layer as

well as the thickness of atherosclerotic plaques provide no information about the composition of the plaque, its stability or function of the endothelium covering its surface.

Out of the lipid parameters, non-HDL cholesterol, HDL-cholesterol and oxLDL showed a significant correlation with EDV. Non-HDL cholesterol contains cholesterol bound within the atherogenic lipoproteins (i.e. apo B-100 containing lipoproteins LDL, IDL and VLDL) and is thus of important prognostic value for cardiovascular risk assessment, as has recently been recommended by cardiology societies. When analyzed separately, neither total cholesterol or LDL cholesterol, nor triglycerides or apo B levels showed any correlation with EDV. Due to their direct toxic effect on endothelial cells, the plasma concentration of oxLDL might be one of the strongest predictors of endothelial dysfunction in early atherosclerotic lesions as observed in our study. There was a significant positive correlation of EDV with HDL cholesterol, apo A1 and ATI. HDL particles not only play a key role in the reverse cholesterol transport, but also possess considerable antioxidative properties. Although decreased levels of HDL cholesterol are typical for patients with insulin resistance (Hsueh and Quinones 2003) and Yki-Jarvinen (2003) even demonstrated endothelial dysfunction in patients with insulin resistance, no other markers of the metabolic syndrome (WHR, BMI, hyperglycemia, hypertension or hypertriglyceridemia) showed any correlation with EDV in our study.

It is well known that oxidative modification of lipoproteins enhances their atherogenicity. In our study, patients with hyperlipidemia showed higher levels of oxLDL and MDA. This might be simply explained by the fact that the increased plasma concentration of lipoproteins (as a substrate for oxidation) results in higher concentrations of their oxidation products. Some authors also consider elevation of nitrites/nitrates (oxidation products of NO) as a marker of oxidative stress. However, it is generally accepted that prolonged endothelial dysfunction is characterized by impaired NO synthesis, resulting in lower levels of its oxidation products. Nevertheless, in our study the plasma levels of nitrites/nitrates were significantly higher in hyperlipidemic patients than in the controls although we would expect the opposite since hyperlipidemic patients showed impaired endothelium-dependent vasodilation. Similarly, Lubrano *et al.* (2003) observed in patients with familial hypercholesterolemia that lipoperoxides stimulate endothelial NOS and thus increase generation of nitrites/nitrates. A possible explanation for impaired EDV accompanied by paradoxically elevated concentration of NO oxidation products (as seen in our study) is that in early atherosclerotic lesions the endothelial vasodilation response is impaired, although the synthesis of NO is still preserved. In our study, the plasma concentration of oxLDL correlated with impaired EDV, indicating that oxidized LDL are capable of endothelial injury at an early stage of the atherosclerotic process.

Moreover, Barron *et al.* (2001) demonstrated that even in pronounced endothelial damage (in virtually endothelium-denuded arteries) there still exists an endogenous NO production by non-endothelial cells of the arterial wall leading paradoxically to depressed contractility in these arteries.

When using the ATI for cardiovascular risk assessment, the group at higher risk (ATI < 0.2) showed significantly higher concentrations of plasma ferritin. In recent years, iron is intensively discussed as possible risk factor of atherosclerosis, mainly for its catalytic role in free oxygen radical formation and subsequent oxidative modification of atherogenic lipoproteins (namely LDL) (Kiechl *et al.* 1997, Salonen *et al.* 1992, Tuomainen *et al.* 1998). Moreover, hemoglobin is another possible pro-oxidative factor which may contribute to lipoperoxidation. In our study, EDV negatively correlated with hemoglobin concentration, number of erythrocytes and hematocrit. It is still unclear whether elevated iron stores alone or hemoglobin alone or both play a crucial role in pathogenesis of atherosclerosis.

In our study, patients with hyperlipidemia also included subjects taking hypolipidemic drugs. According to several studies, hypolipidemic therapy (namely with statins) can abolish endothelial damage and restore its vasodilative function (Langer *et al.* 2003). However, Pereira *et al.* (2003) showed that statin therapy has no effect on endothelium-dependent vasodilation in hyperlipidemic subjects. Furthermore, other drugs used in primary as well as secondary prevention (beta adrenergic blockers, calcium channel blockers, angiotensin converting enzyme inhibitors, AT_1 receptor blockers, nitrates or acetylosalicylic acid) could theoretically influence EDV measurement. Since there was no significant difference in endothelium-dependent vasodilation between treated and untreated subjects in each of the subgroups formed according to the specific drug treatment, we conclude that such therapy had no

major impact on the results of this study (Tables 3 and 6).

Other parameters theoretically affecting EDV include age, sex, menopausal status, smoking, hypertension, diabetes mellitus or chronic inflammatory diseases. As shown in Table 2, the presence of these factors did not statistically differ between the hyperlipidemic and normolipidemic groups. Moreover, when smokers and non-smokers or patients with manifested atherosclerotic diseases and controls in the whole investigated cohort was compared, no significant difference in EDV was found.

Patients with hyperlipoproteinemia showed considerably higher levels of C-reactive protein (CRP), but not in the number of leukocytes or ESR in 1 hour than the controls (Tables 4 and 5). None of the subjects showed symptoms of acute or chronic inflammatory disease at the time of investigation and there was no difference in history of chronic inflammatory diseases between patients and controls. Several recent studies demonstrated a link between elevated CRP levels and oxidative stress in atherosclerotic diseases. Plasma CRP is an early, sensitive marker of inflammatory changes within arterial wall in atherosclerotic lesions (Yasunari *et al.* 2002, Cosin-Sales *et al.* 2003, Korantzopoulos *et al.* 2003). However, CRP did not correlate independently with EDV in our study.

Patients with established hyperlipoproteinemia showed clinical symptoms of endothelial dysfunction measured by endothelium-dependent vasodilation of the brachial artery. Out of the lipid parameters, only plasma concentrations of oxLDL, HDL cholesterol, Apo A1, ATI and non-HDL cholesterol were significant predictors of endothelial dysfunction indicating that namely oxLDL and HDL lipoproteins play the crucial (toxic, resp. protective) role in early stages of atherosclerosis. Patients with hyperlipidemia had higher levels of MDA, ox LDL, nitrites/nitrates and CRP, demonstrating higher oxidative stress in hyperlipidemic subjects.

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