VASA 2002; 31:75–80

University Hospital Zurich, Department of Internal Medicine, Division of Hypertension, Switzerland[1]
University Hospital Regensburg, Institute for Clinical Chemistry and Laboratory Medicine, Germany[2]

LDL-oxidation and -subgroups in normo- and hypertensive patients with and without a family history of hypertension

C. Brockes[1], R. Locher[1], A. Böttcher[2], G. Schmitz[2] and W. Vetter[1]

Summary

Objectives: Oxidative modification of low-density-lipoprotein (LDL) increases its atherogenic potential to induce the accumulation of lipids and cells in the vascular wall. Patients have different lipoprotein profiles according to their LDL-subgroup pattern. The subgroup of LDL, which is most susceptible to oxidation, is most likely the dense LDL_3 subfraction. In order to study an assumed association between hypertension, LDL subgroup distribution and the susceptibility of LDL to oxidation, 14 normotensive patients without family histories of hypertension (NT), 13 normotensive patients with family histories of hypertension (NT-FH), 10 hypertensive patients without family histories of (HT) and 11 hypertensive patients with family histories of hypertension (HT-FH) were evaluated.

Patients and methods: LDL was oxidatively modified by incubation with copper ions (1.6 µM/L). The course of LDL-oxidation was measured in vitro by continuous photometric monitoring and the quantitative distribution of 3 LDL-subgroups by capillary isotachophoresis (ITP).

Results: The lag-phases of NT-FH and hypertensive patients were shorter than those of the control group (NT: 116 ± 36 minutes; NT-FH 92 ± 32 minutes, $p < 0.05$; HT: 95 ± 41 minutes; HT-FH: 76 ± 33 minutes, $p < 0.05$). Compared to NT a significant difference in the relative preponderance of LDL_3 subgroup was observed for HT-FH (23.5 ± 4.6% versus NT: 19.3 ± 6.6%), additionally, statistical analysis showed a similar trend amongst the other patient groups (NT-FH: 20.4 ± 7.4%, HT: 21.4 ± 4.6%).

Conclusions: The increased occurence of the LDL_3 subgroup might contribute to a higher susceptibility to LDL oxidation and therefore create an increased risk of vascular disease in the genotypic and phenotypic hypertensive patient population.

Key words

Hypertension, cholesterol, LDL oxidation, LDL subgroups

Zusammenfassung

LDL-Oxidation und -Subgruppenverteilung bei normo- und hypertensiven Patienten mit und ohne familiäre hypertonische Belastung

Hintergrund: Oxidative Modifikation von Low-Density-Lipoproteinen (LDL) verursacht eine vermehrte Einlagerung von Lipiden in die Arterienwand und steigert somit das atherogene Potential. Patienten haben verschiedene Lipoprotein-Profile, die von der Verteilung der LDL-Subgruppen beeinflusst werden. Die kleine dichte LDL_3-Fraktion ist durch eine schnelle Oxidationsbereitschaft geprägt. Um festzustellen, ob eine Verbindung zwischen Hypertonie, LDL-Oxidation und -Subgruppenverteilung besteht, haben wir bei 14 normotensiven Probanden ohne familiäre hypertonische Belastung (NT), bei 13 Normotensiven mit familiärer Belastung (NT-FH) und bei 10 Hypertensiven ohne (HT) und 11 mit familiärer Belastung (HT-FH) die LDL-Oxidationsbereitschaft und -Subfraktionen bestimmt.

Patienten und Methoden: LDL wurde mit Kupfer (1.6 µmol/L) oxidiert und der Verlauf der Oxidation in vitro mit der kontinuierlichen spektrophotometrischen Absorption gemessen. Die quantitaive Verteilung der 3 LDL-Subgruppen wurde mit der Kapillarelektrophorese bestimmt.

Ergebnisse: NT-FH und hypertensive Patienten haben kürzere antioxidative Phasen als die Kontrollpersonen (NT: 116 ± 36 Minuten; NT-FH 92 ± 32 Minuten, $p < 0.05$; HT: 95 ± 41 Minuten; HT-FH: 76 ± 33 Minuten, $p < 0.05$). Im Vergleich zu NT konnte bei hypertensiven Patienten mit familiärer Belastung ein signifikantes Überwiegen der LDL_3-Subgruppe festgestellt werden (23.5 ± 4.6% versus NT: 19.3 ± 6.6%). Zusätzlich zeigte die statistische Analyse einen ähnlichen Trend für die anderen Patientengruppen (NT-FH: 20.4 ± 7.4%, HT: 21.4 ± 4.6%).

Schlussfolgerung: Die Zunahme der LDL_3-Subgruppe ist vermutlich mit einer gesteigerten LDL-Oxidationsbereitschaft und einem erhöhten Risiko für vaskuläre Erkrankungen bei genetischer Prädisposition und manifester Hypertonie verbunden.

Submitted 18. 10. 2001 / Accepted after revision 10. 12. 2001

Introduction

Hypertension is a risk factor for atherogenesis [17]. The increased risk in hypertensive patients correlates not only with blood pressure but may also be related to other factors [1]. Among these factors it is suggested that the oxidative modification of low-density-lipoprotein (LDL) could promote and accelerate the development of atherosclerosis [39]. Animal experiments reveal that oxidative modification of LDL is a crucial early step in the pathogenesis of atherosclerosis [33]. When LDL is chemically modified, an uncontrolled uptake of such LDL by the scavenger receptors in macrophages can be observed [14, 33]. As a consequence macrophages dedifferentiate into foam cells, which accumulate in the arterial wall forming early atherosclerotic lesions [30, 40].

Human plasma LDL can be characterized by different subfractions in relation to their size, density, fatty acids and apolipoprotein content [21, 35]. The separation of the LDL-subgroups is mostly achieved by ultracentrifugation techniques utilizing the different physical densities of the LDL subgroups. This excessive method can hardly be integrated into the routine diagnosis of the lipid profile with patients. In comparison to conventional methods, analytical capillary isotachophoresis (ITP) has proven to be superior with respect to lipoprotein analysis. It separates LDL-particles according to their electrophoretic mobility [26, 31]. This is a simple and reproducible method with a high discrimination capability of functional subgroups. It is known that the distribution of LDL subfractions is partly genetically determined [3, 9, 24]. Studies have shown that in particular the small dense LDL subfractions are more susceptible to oxidation in vitro than the less dense particles, due to their greater amount of unsaturated fatty acids [6, 8, 32]. Therefore, a predominance of this specific small subgroup might indicate an increased risk for atherosclerosis [2, 5].

The results of a previous study carried out by our research group showed that LDL derived from hypertensive patients and from normotensive patients with a family history of hypertension had a higher susceptibility to oxidation than normotensive patients without a family history of hypertension [4]. This implies that phenotypic hypertension and also genetic predisposition for hypertension renders LDL more susceptible to oxidation. One explanation for the higher susceptibility to LDL oxidation might be a preponderance of certain LDL subgroups in hypertensive patients and in normotensive patients with a family history of hypertension. Because, both the LDL subgroups and primary hypertension may partly be dependent on genetic factors, we studied the relationship between in vitro oxidation of LDL and the distribution pattern of the LDL-subgroups isolated from normotensive and hypertensive patients with and without histories of hypertension [12, 30].

Patients and methods

48 patients were investigated with their informed consent. The protocol was approved by the ethics committee on the use of human subjects in clinical investigations of the University Hospital. Patients were divided into 4 groups depending on blood pressure according to the Joint National Committee for detection, evaluation and treatment of high blood pressure and family history of hypertensive values [25]. The groups were as follows: normotensive patients with blood pressure under 140/90 mmHg without (NT: group 1: control-group) and with family histories of hypertension (NT-FH: group 2) and hypertensive patients (blood pressure > 140/90 mmHg) without (HT: group 3) and with family histories of hypertension (HT-FH: group 4). Hypertensive patients were recruited at the ambulatory of the division of hypertension at the university hospital in Zurich. Subjects treated according to clinical practice with antihypertensive medication were included: 12 patients received angiotensin-converting enzyme inhibitors, 6 beta-blockers, 2 calcium antagonists, 1 centrally acting agents and 2 patients beta-blockers with calcium antagonists or diuretics. However, subjects with known diabetes mellitus or pre-diabetic signs, hypercholesterolemia (total cholesterol-level > 6.5 mmol/L) impaired renal function or other concomitant diseases, as well as pregnant patients were excluded. The normotensive groups were composed of nurses, doctors, their relations, and some individuals from sport. Only 1 patient was treated with acetylsalicyl acid. Patients characteristics are shown in Table I.

All patients were advised to fast and abstain from smoking for 12 hours prior to the blood analysis. Total cholesterol, low-density-lipoprotein (LDL) and triglyzerides were determined by standard laboratory techniques. Results are expressed as mean ± SD. Differences between the control group and the other groups were analyzed using the unpaired t-test.

Determination of LDL Oxidation

For the measurement of the susceptibility of LDL to oxidation we used the photometric assessment of LDL oxidation. 10 ml of venous blood was collected and drawn into vacutainer tubes containing EDTA as an anticoagulant. Plasma was recovered by centrifugation for 10 minutes at 1000× g at 4°C and stored at –70°C. 36 hours before the determination of oxidation susceptibility, LDL was isolated by ultracentrifugation and purified by dialysis at 4°C in

Table I: Patient characteristics.

	NT	NT-FH	HT	HT-FH
Patients (n)	14	13	10	11
Females (n)	8	7	2	6
Males (n)	6	6	8	5
Age (years)	39 ± 12	42 ± 7	45 ± 15	51 ± 9*
Height (cm)	169 ± 7	168 ± 8	171 ± 16	166 ± 8
Weight (kg)	77 ± 17	73 ± 15	79 ± 9	75 ± 21
BMI	26 ± 8	26 ± 6	28 ± 5	27 ± 7
Smokers	5	4	4	3

NT = normotensive patients without family histories of hypertension; NT-FH = normotensive patients with family histories of hypertension; HT = hypertensive patients without family histories of hypertension; HT = hypertensive patients with family histories of hypertension; BMI = bodymass index; = $p < 0.05$ versus control-group (NT).

the dark with 3 changes of 1.5 l of 0.15 mol/L NaCl at pH 7.4 within 24 hours to remove EDTA. The LDL-protein content was determined by the method of Lowry [22]. In order to measure LDL oxidation kinetics 175 µg LDL were placed in a quartz cuvette containing 1 ml of phosphate buffered saline (PBS) and 1.67 µmol/L copper [8]. LDL oxidation was monitored by the change in 234 nm absorbance in a spectrophotometer at 22°C [11]. The intra-assay variance of LDL oxidation was 10%. The initial absorbance was taken as baseline and the change in absorbance was recorded every 10 minutes for 30 cycles. The lag-phase was expressed as the intercept given by the tangent of the slope of the absorbance curve in the propagation phase with the baseline. Oxidation of LDL in vitro can be induced with copper [24]. Oxidation kinetics typically show three distinct phases: a lag-phase, a propagation-phase, and a decomposition-phase [10]. The lag-phase is characterized by the consumption of the endogenous LDL antioxidants. Once the antioxidants are consumed the unsaturated fatty acids in LDL can be rapidly oxidized in an autocatalytic process (propagation-phase). In the decomposition-phase lipids and polypeptides break down to different endproducts, e.g. various aldehydes. A longer lag-phase expressed a reduced susceptibility of the LDL particle to oxidation and signified an increased antioxidative resistance of LDL [11].

Determination of LDL-subgroups

Distribution and frequencies of LDL-subgroups were measured by analytical capillary isotachophoresis (ITP) [26, 31]. The analysis was based on the specific prestaining of whole serum lipoproteins with the fluorescent lipophilic dye 7-nitrobenz-2-oxa-1,3-diazole (NBD)-ceramide [27]. Human serum lipoproteins were separated into 10 well-characterisized subgroups according to their electrophoretic mobility.

Aliquots of plasma samples as prepared for measurement of LDL oxidation were stored at –70°C and thawed only once before use for ITP. Separations were performed on a P/ACE 5510 system (Beckman Instruments, Inc., Fullerton, CA, USA) equipped with a 27 cm dimethyl polysiloxane modified fused silica capillary (Restek Rtx-1), inner diameter 180 µm, purchased from Alltech (Unterhaching, Germany). NBD-ceramide was purchased from Molecular Probes (Eugene, OR, USA).

The leading electrolyte consisted of 10 mM HCl, 16 mM 1,3-bis (tris [hydroxymethyl] methylamino) propane (BTP), pH 8.9 and 0.3% w/v hydroxypropylmethylcellulose. The terminating electrolyte contained 20 mM 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS), 16 mM BTP and was adjusted to pH 10.5 with saturated barium-hydroxyde solution. Samples were diluted with leading buffer(1:2 v/v), incubated for 1 min with a half volume of a NBD-ceramide solution (0.5 mg/ml in ethylene glycol/DMSO, 9:1 v/v) and subsequently mixed with the evaluated spacer mixture (3:8, v/v, final concentration of each spacer 0.01 mg/ml). The following compounds in order of decreasing isotachophoretic mobility were used as spacers: N-2-acetamido-2-aminoethanesulfonic acid (ACES), 3-(N-morpholino)-2-hydroxypropanesulfonic acid (MOPSO), octansulfonic acid, 3-(N-morpholino) propanesulfonic acid (MOPS), 3-N-tris (hydroxymethyl) methylamino-2-hydroxypropanesulfonic acid (TAPSO), N-tris (hydroxymethyl) methyl-3-aminopropanesulfonic acid (TAPS), serine, glutamine, methionine, histidine, glycine, valine and norleucine.The spacer mixture was prepared in leading buffer. As internal marker 5-carboxyfluorescein was used. The capillary, samples and buffers were cooled to 20°C during separation. Samples were injected between the leading and terminating buffer by pressure. The separation was performed at a constant of 10 kV and the separated zones were monitored with laser-induced fluorescence detection (exitation 488 nm; emission 520 nm; see Fig. 1).

Results

The results collected from 48 patients are summarized in the Tables II and III. As expected the ambulatory systolic and diastolic blood pressure were, despite antihypertensive therapy, significantly higher in patients with hypertension. The cholesterol-, LDL-, and triglyzerides-level were similar in all patients. Table II shows the kinetics of LDL oxidation as determined by its lag-time. The mean value of the antioxidative resistance of LDL to oxidation in normotensive patients without a family history of hypertension (NT) was 116 ± 36 minutes. However, the mean lag-time for normotensive patients with a family history of hypertension was 92 ± 32 minutes and significantly lower ($p < 0.05$). Furthermore, there was a significant decrease in the lag-phase in hypertensive patients with a family history of hypertension (76 ± 33 minutes, $p < 0.05$). Hyper-

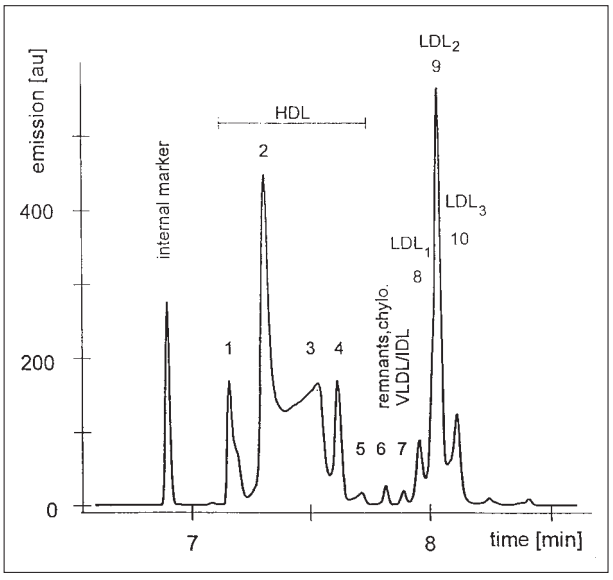

Fig. 1: Lipoprotein profile from a healthy control as determined by analytical ITP. The various lipoprotein subfractions known from gradient ultrazentrifugation are indicated. First peak derives from carboxyfluoresceine as an internal marker.
HDL = high-density lipoprotein; VLDL = very low-density lipoprotein; LDL = low-density lipoprotein; LDL_1 = fast migrating LDL; LDL_2 =intermediate migrating LDL; LDL_3 = slow migration LDL.

tensive patients without a family history of hypertension showed a tendency towards a shorter lag-phase (95 ± 41 minutes).

Analytical isotachophoresis separated fluorescence stained whole serum lipoproteins according to their electrophoretic mobility within 9 min. The addition of non-fluorescent spacer compounds allowed the discrimination of 10 individual lipoprotein subfractions with laser induced fluorescence detection. A representative isotachopherogram of human serum lipoproteins obtained from a healthy control is shown in Figure 1. Subfractions of HDL are described by peak 1–5, peak 6 represents chylomicon derived particles. Apo B containing lipoproteins are represented by peaks 7–10 including very low density lipoprotein, intermediate density lipoprotein and LDL. LDL-particles were separated into three subfractions with different mobilities. LDL_1 (peak 8) represents the fast migrating subpopulation, LDL_3 (peak 10) the slow migration group and LDL_2 (peak 9) the particles with intermediate mobility. The distribution of 3 different LDL subgroups according to ITP is shown in Table III. The relative proportion of LDL3 in normotensive patients without a family history of hypertension was 19 ± 7%. Compared to this control group a preponderance of LDL_3 20 ± 7% was observed in normotensive patients with a family history of hypertension. Similar trends were noted in the hypertensive groups. The relative occurrence of LDL_3 was with 23.5% significantly higher when compared to that of the control group ($p < 0.05$). LDL_3 was also increased in hypertensive patients without a family history of hypertension.

Table II: Blood pressure, lipid-levels and lag-time.

	NT	NT-FH	HT	HT-FH
BP systolic (mmHg)	121 ± 15	128 ±15	155 ± 15*	152 ± 21**
BP diastolic (mmHg)	78 ± 9	80 ± 8	94 ± 5*	101 ± 11**
Cholesterol (mmol/l)	4.7 ± 0.6	4.8 ± 0.7	5.2 ± 0.7	5.3 ± 0.7
LDL-Cholesterol (mmol/l)	2.7 ± 0.7	2.6 ± 0.6	3.0 ± 0.3	3.0 ± 0.7
Triglyzeride (mmol/l)	1.3 ± 1.5	1.1 ± 0.5	1.6 ± 0.5	1.6 ± 0.
Lag-Time (min)	116 ± 36	92 ± 32*	95 ± 41	76 ± 33*

NT = normotensive patients without family histories of hypertension; NT-FH = normotensive patients with family histories of hypertension; HT = hypertensive patients without family histories of hypertension; HT = hypertensive patients with family histories of hypertension; BP = blood pressure; lag-time = antioxidative capacity to oxidation; = $p < 0.05$ = $p < 0.005$ versus control-group (NT).

Table III: Relative distribution of LDL-subgroups.

LDL-Sub-groups (%)	NT	NT-FH	HT	HT-FH
LDL_1	11.5 ± 9.8	10.7 ± 3.8	14.6 ± 4.2	12.1 ± 4.4
LDL_2	65.9 ± 8.7	66.4 ± 5.9	58.3 ± 11.2	60.2 ± 5.0*
LDL_3	19.3 ± 6.6	20.4 ± 7.4	21.4 ± 4.6	23.5 ± 4.6*

NT = normotensive patients without family histories of hypertension; NT-FH = normotensive patients with family histories of hypertension; HT = hypertensive patients without family histories of hypertension; HT = hypertensive patients with family histories of hypertension; BP = blood pressure; lag-time = antioxidative capacity to oxidation; = $p < 0.05$ = $p < 0.005$ versus control-group (NT).

Discussion

Essential hypertension is caused by a combination of genetic predisposition and enviromental factors/influences [1, 12]. Because hypertension and high plasma LDL, in particular the oxidized LDL, are risk factors for the development atherogenesis and cardiovascular diasease, the aim of the present study was to examine the relationship between LDL oxidizability and genotypic and phenotypic hypertension [15, 29]. Oxidative modification of LDL increases its uptake into macrophages, which in turn contributes to accelerate atherogenesis [10, 39]. Recently, studies have shown that oxidized LDL could attenuate endothelium-dependent vasodilation in vitro [13]. Oxidized LDL could promote the release of free radicals from endothelial cells [34]. In particular superoxide anions, which are in vessels from hypercholesterolemic animals can inactivate NO and peroxynitrite [28]. Furthermore, isoprostanes are formed, which can used as an in-vivo measurement of LDL-Oxidation injury.

This study shows that LDL derived from normotensive and hypertensive offsprings of hypertensive parents differ in their susceptibility to lipid peroxidation in comparison to normotensive subjects without genetic predisposition. The lag-time was significantly shorter in patients with a family history of hypertension independent of blood pressure. In comparison to the control group, we found that the mean lag-time in hypertensive patients without a family history of hypertension had a tendency to be lower. It is not known by which direct mechanism established hypertension could influence LDL oxidation, nor do we know whether hypertension is even the reason for higher LDL oxidation.

As an explanation for the differences in susceptibility to LDL oxidation genetic origins may be suggested. There is evidence for genetic influences on the LDL subfractions patterns varying in chemical composition, density, size, metabolic properties and possibly atherogenic potential [3, 20, 32]. The LDL subgroup profile is mostly defined according to its density. This does not take into account the fact that, particles have the same density but different biological function. ITP is simply and reproducible technique for the determination of LDL subgroups according to their electrophoretic mobility [26, 31]. The migration behavior of the particles depends primarily on ionic charge. The test duration lasts only minutes. This method is a helpful tool to analyze the distribution of most atherogenic lipoproteins, which assists to relate it to the risk of vascular diseases.

We detected three subfractions: the fast migrating subpopulation LDL_1, the intermediate migrating LDL_2, and the slow migration LDL_3. There are indications that specifically LDL_3 consists of small, heavy dense particles [26]. A preponderance of small, dense LDL particles has been associated with an atherogenic lipoprotein profile and a

threefold increased risk of cardiovascular disease [2]. The three LDL subfractions isolated by density ultracentrifugation differ in their susceptibility to lipid peroxidation in vitro. Thus indicating that the dense LDL and the light LDL were not as well protected from oxidative modification and were more extensively oxidized in time in comparison to the very light LDL [16, 36, 37]. This implies that the heavy, small, dense LDL has a higher atherogenic potential than the other subfractions [38]. One explanation may be the significantly higher concentration of polyunsaturated fatty acids in dense LDL, which might result in increased oxidative modification and in an excessive uptake by the scavenger receptor of macrophages, an important step in the pathogenesis of atherosclerosis. It is also possible that a change in protein content may lead to conformational changes in the core of the subfractions, which could result in a more obvious exposure of unsaturated fatty acids in the heavy LDL particles to free radicals, therefore, explaining the higher rate of oxidation of dense LDL [18, 19].

To study the basis for the relationship between susceptibility to LDL-oxidation and genotypic hypertension we investigated the distribution of subgroups in our normotensive and hypertensive patients with and without a family history of hypertension. In addition to the increase of the susceptibilty to LDL oxidation in normotensive patients with a family history of hypertension and hypertensive patients we found a relative predominance of slow migrating LDL_3. It is possible that this fraction consisted of dense particles. Our finding provides a hypothetical explanation for the higher susceptibility to oxidation and atherogenicity of LDL in patients with a family history of hypertension. The genetic influences in determing the distribution of subgroups may contribute to the explanation of familial clustering of hypertension and vascular disease [9].

A limitation of the present study could be that hypertensive patients were treated accordingly to clinical practice with antihypertensive medication. Calcium antagonists, beta-blockers and ace-inhibitors are known to increase the resistance of LDL to oxidation [7, 23]. However, this objection can be rejected, as antihypertensive therapy would in fact, prevent the oxidation of LDL rather than decrease the lag-times of LDL oxidation.

In summary, our results indicate that both normotensive patients with a family history of hypertension and hypertensive patients may have an increased risk of vascular disease caused by an enhanced atherogenic potential of LDL. Thus, genetic predisposition for hypertension already renders low-density lipoprotein more susceptible to oxidation without phenotypic manifestation of hypertension. It is shown that in humans the LDL subgroup pattern is partly determined by hereditary. This could be an explanation for the higher susceptibility to LDL oxidation in normotensive patients with a family history of hypertension.

Acknowledgement: This work was supported by research Grant BR 1423/2-1 (C Bracht) from the National Research Foundation of Germany.

References

1 Allemann Y, Weidmann P. Cardiovascular, metabolic and hormonal dysregulation in normotensive offspring of essential hypertensive parents. J Hypert 1995; 13: 163–73.
2 Austin MA, Breslow JL, Hennekens CH, Buring JE, Willett WC, Krauss RM. Low-density lipoprotein subclass patterns and risk of myocardial infarction. JAMA 1988; 260: 1917–21.
3 Austin MA, Brunzell JD, Fitch WL, Krauss RM. Inheritance of low density lipoprotein subclass patterns in familial combined hyperlipdemia. Arteriosclerosis 1990; 10: 520–30.
4 Bracht C, Locher R, Suter P, Vetter W. LDL-Oxidation bei essentieller Hypertonie. Kardiovaskuläre Medizin 1997; 1: 54–8.
5 Campos H, Genest JJ, Blijlevens E, McNamara J, Jenner JL, Ordovas JM. Low density lipoprotein size and coronary artery disease. Arterioscler Thromb 1992; 12: 187–95.
6 Chait A, Brasg RL, Tribble DL, Krauss R. Susceptibility of small, dense low density lipoproteins to oxidative modification in subjects with the atherogenic lipoprotein phenotype pattern B. Am J Med 1994; 94: 350–6.
7 Croft KD, Dimmitt SB, Moulton C, Beilin LJ. Low density lipoprotein composition and oxidizability in coronary artery disease apparent favourable effect of beta-blockers. Atherosclerosis 1992; 97: 123–30.
8 de Graaf J, Hak-Lemmers HL, Hectors MP, Demacker PN, Hendriks JC, Stalenhoef AF. Enhanced susceptibility to in vitro oxidation of the dense low density lipoprotein subfraction in healthy subjects. Arteriosclerosis and Thrombosis 1991; 11: 298–306.
9 de Graaf J, Swinkels DW, de Haan AF, Demacker PN, Stalenhoef AF. Both inherited susceptibility and enviromental exposure determine the low-density lioprotein-subfraction pattern distribution in healthy Dutch families. Am J Hum Gene 1992; 51: 1256–1310.
10 Esterbauer H, Jürgens G, Quehenberger O, Koller E. Autoxidation of human low density lipoprotein: a loss of polyunsaturated fatty acids and vitamin E and generation of aldehydes. J Lipid Res 1987; 28: 495–509.
11 Esterbauer H, Striegl G, Puhl H, Rotheneder M. Contiuous monitoring of in vitro oxidation of human low density lipoprotein. Free Rad Res Commun 1989; 67–75.
12 Folkow B. Physiological aspects of primary hypertension. Physiol Rev 1982; 62: 347–61.
13 Galle J, Ochslen M, Schollmeyer P, Wanner C. Oxidized lipoproteins inhibit endothelium-dependent vasodilations effects of pressure and high density lipoprotein. Hypertens 1994; 23: 556–64.
14 Grundy SM. Oxidized LDL and atherogenesis: relation to risk factors for coronary heart disease. Clin Cardiol 1993; 16 (4 Suppl 1): 13–5.
15 Hypertension Detection and Follow-up Program Cooperative Group. The effect of treatment on mortality in "mild" hypertension: Results of the Hypertension Detection and Follow-up Program. New Engl J Med 1982; 307: 976.
16 Jun-Jun W, Xiao-Zhuan L, Yi-Yi Z, Lu-Yan L. Correlation between susceptibility of LDL subfractions to in vitro oxidation and in vivo oxidized LDL. Clin Biochem 2000; 3: 71–3.
17 Kannel WB, Sorlie P. Hypertension in Framingham. In: Paul O (editor): Epidemiology and control of hypertension. Stratton International Medical Book Cooperation, New York and Georg Thieme Verlag, Stuttgart 1975; 553–92.
18 Karpe F, Tornvall P, Olivecrona T, Steiner G, Carlson LA, Hamsten A. Composition of human low density lipoprotein: effects of postprandial triglyceride-rich lipoproteins, lipoprotein lipase, hepatic lipase and cholesteryl ester transfer protein. Atherosclerosis 1993; 98: 33–49.
19 Kinoshita M, Krul ES, Schönfeld G. Modification of the core lipids of low density lipoproteins produces selevtive alterations in the expression of apoB-100 epitopes. J Lipid Res 1990; 31: 701–8.
20 Krauss RM, Burke DJ. Identification of multiple subclasses of plasma low density lipoproteins in normal humans. J Lipid Res 1982; 23: 97–104.

21 Lee DM, Alaupovic P. Studies of the composition and structure of plasma lipoproteins: isolation, composition and immunochemical characterization of low density lipoprotein subfractions of human plasma. Biochemistry 1970; 9: 2244–52.
22 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. J Biol Chem 1951; 193: 265–75.
23 Lupo E, Locher R, de Graaf J, Vetter W. In vitro antioxidant activity of calcium antagonists against LDL oxdition compared with alpha-tocopherol. Biochem Biophys Res Commun 1994; 203: 1803–8.
24 McNamara J, Campos H, Ordovas JM, Peterson J, Wilson PW, Schaefer EJ. Effect of gender, age and lipid status on low-density lipoprotein subfraction distribution. Arteriosclerosis 1987; 7: 483–90.
25 National Institutes of Health. The fifth report of the Joint National Committee on Detection, Evaluation, and Treatment of High Blood Pressure. NIH publication no 93–1088, 1993.
26 Nowicka G, Brüning T, Grothaus B, Kahl G, Schmitz G. Characterization of apolipoprotein B-containing lipoproteins separated by preparative free flow isotachophoresis. J Lipid Res 1990; 31: 1173–86.
27 Nowicka G, Brüning T, Böttcher A, Kahl G, Schmitz G. Macrophage interaction of HDL subclasses separated by free flow isotachophoresis. J Lipid Res 1990; 31: 1947–63.
28 Ohara Y, Peterson TE, Harrison DG. Hypercholesterolemia increases endothelial superoxide anion production. J Clin Invest 1993; 91: 2546–51.
29 Pyörälä K. Interpopulation correlations between serum cholesterol level and the occurence of coronary heart disease. Eur Heart J 1987; 8: 23–30.
30 Schaffner T, Taylor K, Bartucci EJ, Fischer-Dzoga K, Beeson JH, Glagov S, et al. Arterial foam cells with distinctive immunomorphologic and histochemical features of macrophages. Am J Pathol 1980; 100: 57–80.
31 Schmitz G, Möllers C, Richter V. Analytical capillary isotachophoresis of human serum lipoproteins. Electrophoresis 1997; 18: 1807–13.
32 Shimano H, Yamada N, Ishibashi S, Mokuno H, Mori N, Gotoda T. Oxidation-labile subfraction of human plasma low density lipoprotein isolated by ion-exchange chromatography. J Lipid Res 1991; 32: 763–73.
33 Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol: modifications of low density liproproteins that increases its atherogenicity. N Engl J Med 1989; 320: 1196–7.
34 Stewart DJ, Monge JC. Hyperlipidemia and endothelial dysfunction. Curr Opin Lipid 1993; 4: 319–24.
35 Swinkels DW, Hak-Lemmers HL, Demacker PN: Single spin density gradient ultracentrifugation method for the detection and isolation of light and heavy low density lipoprotein subfraction. J Lipid Res 1987; 28: 1233–9.
36 Tan CE, Chew LS, Chio LF, Tai ES, Lim HS, Lim SC. Cardiovascular risk factors and LDL subfraction profile in type 2 diabetes mellitus subjects with good glycaemic controll. Diabetes Res Clin Pract 2001; 51: 107–14.
37 Vasankari T, Ahotupa M, Toikka J, Mikkola J, Irjala K, Pasanen P, et al. Oxidized LDL and thickness of carotid intima-media are associated with coronary atherosclerosis in middle-aged men lower levels ofo oxidized LDL with statin therapy. Atherosclerosis 2001; 155: 403–12.
38 Wallace AJ, Humphries SE, Fisher RM, Mann JI, Chisholm A, Sutherland WH. Genetic factors associated with response of LDL subfraction to change in the nature of dietary fat. Atherosclerosis 2000; 149: 387–94.
39 Witztum JL. The oxidation hypothesis of atherosclerosis. The Lancet 1994; 344: 793–5.
40 Witztum JL, Steinberg D. Role of oxidized low density lipoprotein in atherogenesis. J Clin Invest 1991; 88: 1785–92.

Christiane Brockes, Universitätsspital, Abteilung für Hypertonie, Rämistraße 100, CH-8091 Zürich
E-mail: christiane.brockes@epost.de