

# Hyper-betalipoproteinemia LDL 1,2: A newly identified nonatherogenic hypercholesterolemia in a group of hypercholesterolemic subjects

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## Abstract

**OBJECTIVE:** The identification of a non-atherogenic and an atherogenic lipoprotein profile, non-athero phenotype A vs. athero phenotype B, in a group of hypercholesterolemic subjects reveals newly discovered non-atherogenic hypercholesterolemia. Individuals with this type of hypercholesterolemia, or hyper-betalipoproteinemia LDL1,2, are probably not at increased risk to develop a premature atherothrombosis or a sudden cardiovascular event. Examined individuals with hyper-betalipoproteinemia LDL1,2 were divided into two subgroups: individuals under 40 years of age, and older individuals between 46 and 71 years of age. Subjects in the under 40 years of age group did not have any apparent clinical or laboratory-proven impairment of the cardiovascular system. The older subjects with hyper-betalipoproteinemia and a non-atherogenic lipoprotein profile had only mild signs of clinically irrelevant aortic valve sclerosis.

**METHODS:** A quantitative analysis of the lipoprotein spectrum in plasma in a group of hypercholesterolemic subjects was performed. An innovative electrophoresis method on polyacrylamide gel (PAG) was used for the analysis of plasma lipoproteins and for the identification of atherogenic vs. non-atherogenic lipoproteins in plasma. With regard to lipids, total cholesterol and triglycerides in plasma were analyzed with an enzymatic CHOD PAP method (Roche Diagnostics, FRG). A new parameter, the score for anti-atherogenic Risk (SAAR), was calculated as the ratio between non-atherogenic to atherogenic plasma lipoproteins in the examined subjects.

**RESULTS:** There was a high concentration of LDL1, and LDL2 subfractions ( $p < 0.0001$ ), and an extremely low concentration of LDL3–7 ( $p < 0.0001$ ) in the non-atherogenic lipoprotein profile of hyper-betalipoproteinemia LDL1,2 compared to the control group. Higher concentrations ( $p < 0.0001$ ) of lipids and lipoproteins in the non-atherogenic hypercholesterolemia, compared to the control group, were also found. The hyper-betalipoproteinemia LDL1,2 was also characterized by high SAAR values. There was found a higher concentration of HDL large and HDL intermediate subfractions in hypercholesterolemic subjects.

**CONCLUSIONS:** The advantages of this new diagnostic method include:

- identification of the existence of a non-atherogenic hyper-betalipoproteinemia LDL1,2 in examined hypercholesterolemic subjects with untreated hypercholesterolemia
- introduction of a new risk measure, the score for anti-atherogenic risk (SAAR), for the estimation of atherogenic/anti-atherogenic risk.
- the presence of small dense LDL in plasma is decisive for the declaration of an atherogenic lipoprotein profile. It is valid for hyperlipidemia and for normolipidemia as well.

## INTRODUCTION

Hypercholesterolemia represents a risk factor for the development of cardiovascular diseases. In addition to arterial hypertension and nicotine abuse, hypercholesterolemia is considered one of three cardinal risk factors. Cholesterol in plasma is transported by a sophisticated lipoprotein complex system and is also an active part of this lipoprotein system. Different parts of the lipoprotein systems are called lipoprotein families. Every lipoprotein family transports different concentrations of cholesterol in blood plasma, but the major conveyor of cholesterol in plasma is the family of Low Density Lipoproteins, i.e., the LDL family. LDL is considered as an atherogenic part of the lipoprotein system (Kwiterovich 2002a; b). LDL transports a major cholesterol load from the liver to the peripheral cells of the body. Under conditions of impaired LDL catabolism in the periphery, LDL particles persist in the circulation, their physical-chemical characteristics are modified, and the physiological way of LDL degradation – via LDL receptors – fails. The consequences of this sequence of events is the alternative metabolic pathway of LDL degradation through scavenger receptors and formation of cholesterol deposits in the sub-endothelial space of the arterial wall. In this way, the process of atherogenesis and atherothrombosis begins, and LDL particles play a crucial role at the beginning and in the development of this injury process in the vessel walls (Berneis & Krauss 2002; Carmena *et al.* 2004; St-Pierre *et al.* 2005).

LDL-cholesterol became a criterion of the degree of atherogenic risk for the development of atherothrombosis. A high LDL-cholesterol concentration in plasma correlates positively with the premature onset of cardiovascular diseases, and is considered a strong cardiovascular risk factor for individuals. From this point of view, the aim of treatment of hypercholesterolemia in secondary as well as in primary prevention, is the reduction of LDL concentration in plasma and a

lowering of the cholesterol level to the "target reference values" (Expert Panel 2001; Backers 2005).

However, in the last decades, the lipoprotein research has focused on the phenomenon of atherogenic and non-atherogenic lipoproteins, atherogenic and non-atherogenic lipoprotein profiles, and phenotype A vs. phenotype B characterization (Austin *et al.* 1990; Van *et al.* 2007). The traditional approach to hypercholesterolemia as an atherogenic risk factor for the development of degenerative diseases of the cardiovascular system became a target of criticism. Castelli published evidence, that more than 75 percent of the patients with an acute coronary syndrome or a myocardial infarction had normal plasma values of cholesterol LDL cholesterol or HDL cholesterol (Castelli 1988; 1992; 1998). Thus, it was necessary to look for other risks factors in plasma, that could cause an acute coronary event. An increased cholesterol level, as a universal explanation for the origin of atherogenesis, was not longer valid.

A reasonable explanation was found in atherogenic lipoprotein subpopulations, the presence of which in plasma, even in very low concentrations, could impair the integrity of the vessel wall and lead to endothelial dysfunction with its fatal consequences: formation of atherothrombotic plaques, acute myocardial infarction, stroke, and sudden death (Nichols & Lundmann 2004; Rizzo & Berneis 2006; Shoji *et al.* 2009; Zhao *et al.* 2009).

Laboratory analysis methods became the essential contribution to the identification of atherogenic lipoprotein entities, which simplified the analysis and quantification of the atherogenic lipoprotein subfractions. Gradient gel electrophoretic separation of LDL and HDL subclasses, or a proton nuclear magnetic resonance spectroscopy were the methods of choice for analysis of these entities (Rainwater *et al.* 1997; Alabakovska *et al.* 2002; Otvos *et al.* 2003).

The electrophoresis of plasma lipoproteins on the polyacrylamide gel (PAG) Lipoprint LDL System represents one of a diagnostic analytical methods for the identification and quantitative evaluation of lipoprotein subfractions, i.e., the atherogenic and non-atherogenic lipoproteins (Hoefner *et al.* 2001). The Lipoprint LDL system can also characterize a non-atherogenic lipoprotein profile, phenotype A, and an atherogenic lipoprotein profile, phenotype B. On the basis of lipoprotein separation by the Lipoprint LDL System, a non-atherogenic normolipidemia, an atherogenic normolipidemia, a non-atherogenic hyper-betalipoproteinemia, and an atherogenic hyper-betalipoproteinemia can be identified (Figures 1–4) (Oravec 2006a; b; 2007a; b).

The aim of this study was to identify, among hypercholesterolemic individuals with untreated hypercholesterolemia, those who had a non-atherogenic hyperlipoproteinemia. These subjects then underwent a medical examination to identify the extent of the arterial vessel damages caused by hypercholesterolemia.

## PATIENTS AND METHODS

In the group of examined hypercholesterolemic subjects, 145 individuals with a non-atherogenic lipoprotein profile were identified. Of the total number, 15 were under 40 years of age without a clinically apparent impairment, and no laboratory signs of cardiovascular diseases. These subjects formed one subgroup of young people (34 years ± 5 years).

The subgroup of older persons consisted of 130 individuals (32 males, 57 ± 11 years of age and 98 females, 62 ± 9 years of age).

The medical examination, which included a physical examination, blood pressure and ECG examination, bicycle stress test, echocardiography, and duplex sonography of the carotid arteries, confirmed that there was no impairment of the cardiovascular system. Only mild

signs of clinically irrelevant aortic valve sclerosis were found in the subgroup of older persons.

Individuals with hyperglycemia, diabetics, and those individuals, who were being treated with lipid-lowering drugs were excluded from the study.

The control group consisted of 103 normolipidemic volunteers, all nonsmokers, who had no clinically apparent impairment, or laboratory signs of cardiovascular disease, was recruited from outpatients from the department of internal medicine and practitioners. The average age of the control group was 47.5 ± 8.5 years, with 33 males and 70 females. All subjects gave written, informed consent, and the study was approved by the local ethics committee.

A blood sample from the antecubital vein was collected in the morning after a 12-hour fasting period. EDTA-K<sub>2</sub> plasma was obtained and the concentration

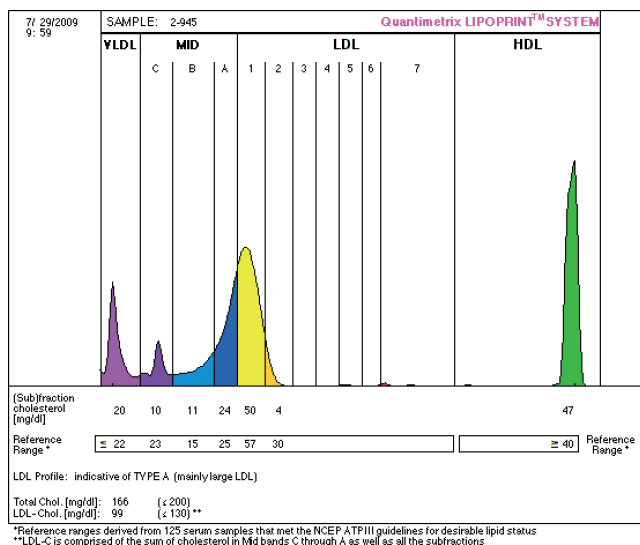


Fig. 1. A non-atherogenic normolipidemia.

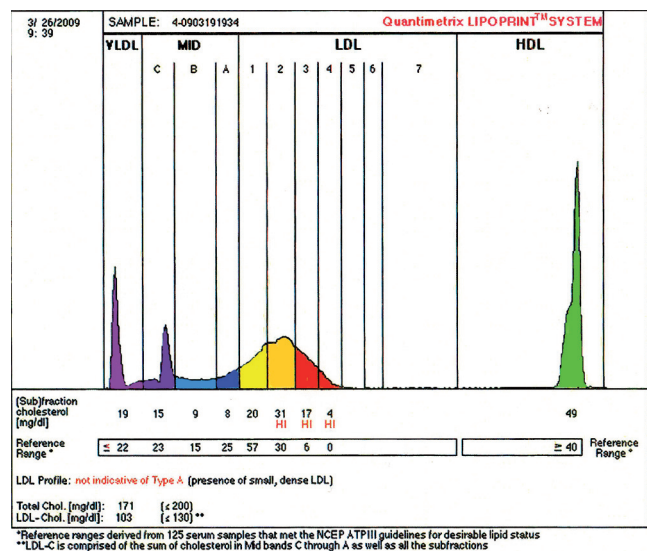


Fig. 2. An atherogenic normolipidemia.

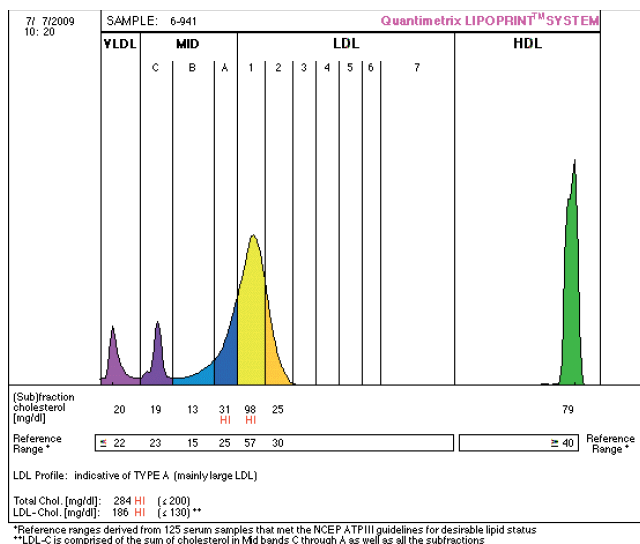


Fig. 3. A non-atherogenic hyper-beta lipoproteinemia LDL1,2.

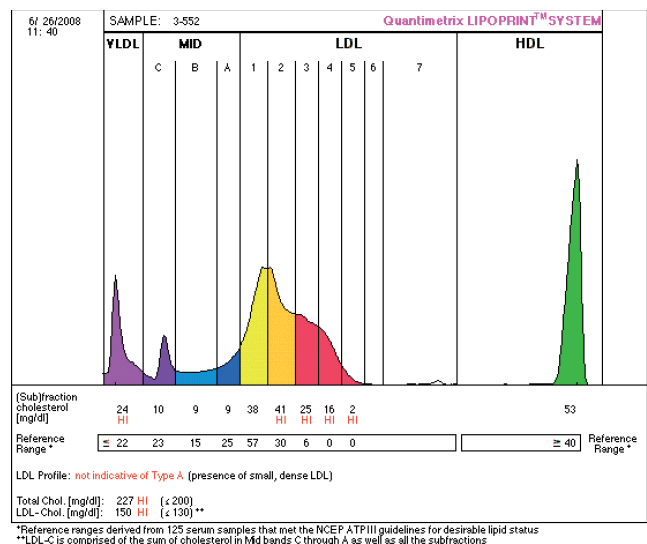


Fig. 4. An atherogenic hyper-beta lipoproteinemia.

of total cholesterol and triglycerides in plasma, using an enzymatic CHOD PAP method (Roche Diagnostics, Germany), was analyzed.

The quantitative analysis of lipoprotein families and lipoprotein subfractions included: VLDL: IDL1: IDL2: IDL3: LDL1: LDL2: LDL3-7: and HDL. A non-atherogenic lipoprotein profile, phenotype A, versus an atherogenic lipoprotein profile, phenotype B, was determined using the Lipoprint LDL System (Quantimetrix Corp., USA) (Hoefner *et al.* 2001). The analysis of HDL subpopulations, including large HDL, intermediate HDL, and small HDL in plasma, was also performed using the Lipoprint HDL System (Morais *et al.* 2003).

The SAAR was calculated as the ratio between non-atherogenic and atherogenic lipoproteins in plasma (Oravec 2007a). SAAR values over 10,8 represented a non-atherogenic lipoprotein profile, whereas values under 9.8 represented an atherogenic lipoprotein profile (Oravec 2007b).

Statistical evaluation of obtained values was performed by an unpaired students' t-test. The level of significance was set at  $p < 0.05$ .

## RESULTS

The subjects with a non-atherogenic hypercholesterolemia had a significantly increased concentration of all lipid and lipoprotein parameters ( $p < 0.0001$ ), except for LDL 3-7 subfractions (small dense LDL), which were significantly lower ( $p < 0.0001$ ), compared to the control group (Table 1). The highest increment of concentrations was found for total cholesterol, LDL cholesterol, HDL cholesterol, IDL3, the LDL1 and LDL2 subfractions. The concentration of LDL1 exceeded the LDL1 concentration in the control group by more than 95 percent. The highest LDL1 concentration in hypercholesterolemic subject exceeded the LDL1 concentration in the control group by approximately three-fold. The increment of LDL2 concentration (33 percent only) did not reach the increment of LDL1 concentrations.

Similar results were obtained, when the group of older hypercholesterolemic subjects was compared to the control group (Table 2).

The differences between a group of younger hypercholesterolemic subjects and older hypercholesterolemic subjects were non significant (Table 3), with the exception of HDL cholesterol in the group of older subjects, where HDL cholesterol was significantly higher ( $p < 0.001$ ).

Table 4 shows the HDL cholesterol concentration and the HDL subfractions, analyzed by the Lipoprint HDL System. The concentration of T-HDL cholesterol (total HDL cholesterol) in the group of hypercholesterolemic subjects was significantly higher ( $p < 0.0001$ ), compared to the control group. An increased concentration was found of both HDL subfractions, the HDL large subfraction ( $p < 0.005$ ) and the HDL intermediate subfraction ( $p < 0.0001$ ) in the hypercholesterolemia

subjects. The difference in the concentration of the HDL small subfraction, between hypercholesterolemic subjects and the control group was not confirmed.

## DISCUSSION

The findings of hypercholesterolemia in clinically healthy subjects, without clinically apparent signs of cardiovascular disease or laboratory confirmation of cardiovascular disease, and with a negative history for the occurrence of cardiovascular events, stimulated an active search for the hyper-cholesterolemic individuals and undertaking a medical examination of these subjects.

A new, innovative electrophoretic method for the analysis of plasma lipoproteins on polyacrylamide gel (PAG) was used (Hoefner *et al.* 2001). The method can analyze the total lipoprotein spectrum of examined subjects, identify an atherogenic/non-atherogenic lipoprotein profile, and quantify the atherogenic lipoprotein subpopulations in plasma, including strong atherogenic LDL subpopulations – small dense LDL, which form the subfractions LDL 3-7 (Packard 2003).

A medical examination of tested individuals was performed, including physical examination, blood pressure and ECG examination, bicycle stress test, echocardiography, and duplex sonography of the carotid arteries.

The major findings can be summarized as follows:

1. In examined subjects with hypercholesterolemia, a non-atherogenic lipoprotein profile, phenotype A was confirmed, with a high concentration an non-atherogenic lipoprotein profile phenotype A, with a high concentration of LDL1 a LDL2 subfractions. In particular, the LDL1 subfraction exceeded the LDL1 of the control group twice, and in some individual cases, three times.
2. The lipoprotein electrophoresis confirmed only a trace concentration of LDL3-7 subpopulations (1mg LDL3-7 cholesterol/dl, i.e., 0.0256 mmol/l). In the overwhelming majority of subjects (60%) indeed, there was an absence of the atherogenic LDL3-7 in the lipoprotein profile of these subjects. (Plasma lipoprotein profiles for patients with confirmed cardiovascular disease are generally characterized by a high concentration of small dense LDL) (Kwiterovich 2000; Maslowska 2005; Oravec *et al.* 2010a;b; 2011).
3. The concentration of HDL was significantly increased ( $p < 0.0001$ ) compared to the control group, with an overwhelming majority of the non-atherogenic HDL subpopulations: HDL large and HDL intermediate. The concentration of small dense HDL was not increased. Increased concentration of small dense HDL in the lipoprotein spectrum may indicate increased coronary heart disease risk (Muniz & Morais). The structural representation of HDL subpopulations confirms a non-atherogenic type of lipoprotein profiles in examined group of hypercholesterolemic subjects.

**Tab. 1.** Plasma concentration of lipids, lipoproteins, and the SAAR score in the group of hypercholesterolemic subjects and controls.

	T-Chol	TAG	VLDL	IDL1	IDL2	IDL3	LDL1	LDL2	LDL3-7	T-LDL	HDL	SAAR
	(mmol/l ±SD)											
Control n=103	4.16 ±0.59	1.04 ±0.35	0.52 ±0.10	0.36 ±0.15	0.27 ±0.09	0.32 ±0.13	0.86 ±0.26	0.39 ±0.22	0.036 ±0.035	2.23 ±1.11	1.38 ±0.35	39.1 ±21.3
H-βLP n=145	6.71 ±0.90	1.29 ±0.49	0.74 ±0.21	0.55 ±0.16	0.51 ±0.12	0.82 ±0.23	1.68 ±0.36	0.52 ±0.21	0.010 ±0.010	4.09 ±0.69	1.88 ±0.46	76.0 ±17.
Control vs. HLP	<-----p<0.0001----->											

**Tab. 2.** Plasma concentration of lipids, lipoproteins and Score of SAAR in the subgroup of older hypercholesterolemic subjects and controls.

	T-Chol	TAG	VLDL	IDL1	IDL2	IDL3	LDL1	LDL2	LDL3-7	T-LDL	HDL	SAAR
	(mmol/l ±SD)											
Control n=103	4.16 ±0.59	1.04 ±0.35	0.52 ±0.10	0.36 ±0.15	0.27 ±0.09	0.32 ±0.13	0.86 ±0.26	0.39 ±0.22	0.036 ±0.035	2.23 ±1.11	1.38 ±0.35	39.1 ±21.3
H-βLPsen n=130	6.73± 0.91	1.30± 0.48	0.73 ±0.19	0.55 ±0.16	0.52 ±0.13	0.80 ±0.23	1.67 ±0.35	0.52 ±0.22	0.010 ±0.010	4.08 ±0.69	1.93 ±0.45	76.5 ±18.1
	<-----p<0.0001----->											

H-βLPsen - hyperbetalipoproteinemia subgroup of seniors

**Tab. 3.** Plasma concentration of lipids, lipoproteins, and SAAR in the subgroup of younger (n=15) versus older (n=130) hypercholesterolemic subjects.

	T-Chol	TAG	VLDL	IDL1	IDL2	IDL3	LDL1	LDL2	LDL3-7	T-LDL	HDL	SAAR
	(mmol/l ±SD)											
H-βLPjr n=15	6.62 ±0.80	1.20 ±0.60	0.84 ±0.28	0.58 ±0.18	0.44 ±0.08	0.80 ±0.25	1.84 ±0.42	0.54 ±0.18	0.010 ±0.010	4.20 ±0.64	1.46 ±0.24	71.1 ±13.2
H-βLPsen n=130	6.73± 0.91	1.30± 0.48	0.73 ±0.19	0.55 ±0.16	0.52 ±0.13	0.80 ±0.23	1.67 ±0.35	0.52 ±0.22	0.010 ±0.010	4.08 ±0.69	1.93 ±0.45	76.5 ±18.1
	p<0.001											

H-βLPjr - hyperlipoproteinemia subgroup of younger subjects

4. The examined individuals, despite increased total cholesterol and LDL cholesterol values, are healthy, without apparent clinical signs of manifested cardiovascular disease (angina pectoris, cardiac insufficiency, myocardial infarction, or other survived cardiovascular event). There is evidence that an optimal anti-atherogenic LDL profile (see the lipoprotein results) could actually have an vasoprotective effect in tested hypercholesterolemic individuals. Based on the present results, a further, more extensive study will continue to evaluate the Lipoprint electrophoretic method as a standard method for the diagnostics of cardiovascular risk, along with the standard tests now used (ECG examination, bicycle stress test, echocardiography and duplex sonography of the carotid arteries – common normal results).

**Tab. 4.** Plasma concentration of HDL lipoprotein subfractions.

	T-HDL	HDL large	HDL intermediate	HDL small
	(mmol/l ± SD)			
Control (n=103)	1.31 ± 0.29	0.59 ± 0.23	0.56 ± 0.10	0.15 ± 0.09
H-βLP (n=110)	1.51 ± 0.34	0.70 ± 0.46	0.65 ± 0.42	0.15 ± 0.12
	p<0.0001	p<0.005	p<0.0001	n.s.

5. The newly introduced SAAR, a ratio of non atherogenic/atherogenic lipoproteins, also confirms a non-atherogenic lipoprotein constellation in the plasma of hypercholesterolemic individuals.

From the results of examined individuals with hypercholesterolemia these conclusions could be drawn:

1. LDL1 and LDL2 do not fulfil the criteria of atherogenicity for lipoprotein entities, that is usually ascribed to LDL lipoproteins.
2. LDL1 and LDL2 subfractions in hypercholesterolemic individuals, in our study group, created a non-atherogenic hypercholesterolemia – a non-atherogenic hyper-betalipoproteinemia LDL1,2 without the presence of atherogenic small dense LDL (or with traces only), associated with a high concentration of cardiovascular protective HDL subfractions, in plasma lipoprotein spectrum.

We declare the existence of a newly described type of hypercholesterolemia, a **non-atherogenic hyper-betalipoproteinemia LDL 1,2**, characterized by a later-onset beginning of cardiovascular complications, even in those individuals, who are not treated with hypolipidemic therapy.

The hypercholesterolemic subjects of the study group are still undergoing follow-up examinations.

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