Atherogenic normolipidemia – a new phenomenon in the lipoprotein profile of clinically healthy subjects

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Abstract

OBJECTIVE: The identification of an atherogenic and a non-atherogenic lipoprotein profile, athero phenotype B vs. non-athero phenotype A, in a group of healthy normolipidemic subjects reveals a new clinical phenomenon in lipoprotein profiles, an atherogenic normolipidemia. Individuals with atherogenic normolipidemia are at increased risk to develop premature atherothrombosis and experience a sudden cardiovascular event. **METHODS:** A quantitative analysis of non-atherogenic and atherogenic lipoproteins in plasma in a group of healthy normolipidemic volunteers who had no clinical signs of cardiovascular system impairment was performed. An innovative electrophoresis method on polyacrylamide gel (PAG) (Lipoprint LDL System, USA) was used for the analysis of plasma lipoproteins. With regard to lipids, total cholesterol and triglycerides in plasma were analyzed with an enzymatic method, CHOD PAP (Roche Diagnostics, FRG). Prostacyclin and thromboxane A2 were analyzed with an ELISA analysis (DRG USA). A new parameter, the score for anti-atherogenic risk (SAAR), was calculated as the ratio between non-atherogenic to atherogenic plasma lipoproteins in examined subjects. **RESULTS:** There was a high concentration of LDL3–7 subfraction ($p<0.0001$) and a slowly increasing triglyceride concentration ($p<0.05$) in the atherogenic subgroup. The non-atherogenic subgroup of healthy subjects was characterized by high SAAR scores, as well as a low concentration of LDL3–7 subfractions ($p<0.0001$). Other statistically significant differences between the atherogenic and non-atherogenic subgroup, including total cholesterol, prostanoid parameters (prostacyklin, thromboxane A2), and lipoproteins values, were not confirmed. **CONCLUSIONS:** The advantages of this new method include:

(i) identification of an atherogenic and a nonatherogenic lipoprotein profile in an individual's plasma
(ii) identification of an atherogenic normolipidemic lipoprotein profile in plasma
(iii) introduction of a new risk measure, the score for anti-atherogenic risk (SAAR), for an estimation of a patient's atherogenic risk of atherothrombosis development.
(iv) the presence of small dense LDL in plasma is decisive for declaration of an atherogenic lipoprotein profile. It is valid for hyperlipidemia and for normolipidemia as well.

ORIGINAL ARTICLE

Abbreviaions and units:

Chol	- cholesterol
HDL	- high density lipoproteins
IDL	- intermediate density lipoproteins
LDL	- low density lipoproteins
LDL	- oxidized LDL (oxid-)
PAG	- polyacrylamide gel
PGI2	- prostacyklin
SAAR	- score for anti-atherogenic risk
sdLDL	- small dense low density lipoproteins
TxA2	- thromboxane A2
TxB2	- thromboxane B2
TAG	- triglycerides, resp. triacylglycerols
VLDL	- very low density lipoperoteins

INTRODUCTION

Generally, normolipidemia is interpreted as an equilibrated state of lipoprotein metabolism, characterized by total cholesterol and triglyceride values within reference ranges. From clinical experience, it is known that patients with normolipidemia are better protected against the development of cardiovascular diseases and degenerative vessel changes.

Conversely, dyslipidemia represents a risk factor for the development of cardiovascular disease, and therefore, dyslipidemia is categorized as an atherogenic risk factor, because of the existence of an atherogenic lipoprotein profile. Atherothrombosis with its consequences, as a critical complication of cardiovascular disease, represents the most frequent cause of premature mortality in economically developed societies all over the world.

An atherogenic lipoprotein profile is characterized by the prominent presence of atherogenic lipoproteins: very low density lipoprotein (VLDL); intermediate density lipoproteins (IDL1, IDL2); and particularly, by the presence of small, dense low density lipoproteins (sdLDL), which form LDL 3–7 subfractions and which are strongly atherogenic (Lamarche *et al.* 1997; Gardner *et al.* 1996; Rajman *et al.* 1996; Halle *et al.* 1998). Small, dense LDL form macromolecular complexes (micels), with an average length under 26.5 nm (265 Angström), which float in the density range d= 1,048–1,065 g/ml. Their strong atherogenic potential is a consequence of their chemical composition and biological characteristics (Table 2) (Berneis & Krauss 2002; Packard 2003).

The goal of the treatment of hyperlipoproteinemia or dyslipidemia is to reduce the lipid concentration in serum, reaching target values of lipids (total cholesterol and triglycerides), but primarily, reducing the atherogenic potential of serum lipids (Backers 2005; Rizzo & Berneis 2006; Fruchart at al. 2008; Chun *et al.* 2009). From this point of view, the treatment of hyperlipoproteinemia, which does not remove the atherogenic potential of plasma lipids (represented by atherogenic plasma lipoproteins), is less effective.

A new diagnostic electrophoretic method for lipoprotein analysis on polyacrylamide gel (PAG), Lipoprint LDL System, can quantify atherogenic and non-atherogenic lipoprotein populations in patients and identify an atherogenic or, alternatively, a non-atherogenic lipoprotein profile (Hoefner *et al.* 2001). This method contributes to the early diagnosis of cardiovascular degenerative diseases.

The aim of this study was to identify a type of lipoprotein profile in a group of healthy volunteers with no clinical manifested or chemically identified signs of cardiovascular diseases.

PATIENTS AND METHODS

There were 150 healthy normolipidemic volunteers, who did not smoke, and who had no clinical or chemically identified signs of cardiovascular diseases, who were included in the study. Volunteers were recruited among medical students at the medical facility. All subjects gave written, informed consent, and the study was approved by the local ethics commitee of the medical institution. The average age of the subjects was 21.5 ± 2.5 years and the group involved 69 males and 81 females.

A blood sample from the cubital vein was collected in the morning after a 12 hour fasting period. EDTA-K_2

Tab. 1. Plasma concentration of lipids, lipoproteins, prostanoids, and score for anti-atherogenic risk (SAAR) in the group of volunteers.

	Chol	TAG	VLDL	LDL12	LDL37	LDL	HDL	PGI2	TxA2	PG/TxA2	Score
	(mmol/l±SD)							(pg/ml±SEM)			
subjects *non-atherogenic n=140*	4.28 ±0.60	1.15 ±0.39	0.60 ±0.16	1.29 ±0.38	0.03 ±0.003	2.31 ±0.53	1.35 ±0.32	5194 ±604	1277 ±107	4.1 ±2.7	37.8 ±19.7
subjects *atherogenic n=10*	4.25 ±0.54	1.44 ±0.40	0.68 ±0.14	1.16 ±0.24	0.22 ±0.08	2.24 ±0.36	1.32 ±0.31	2467 ±384	868 ± 362	2.8 ±1.6	6.0 ±2.0
subjects *non-athero vs. athero*	**4.27 ±0.60**	**1.17 ±0.39**	**0.61 ±0.16**	**1.28 ±0.37**	**0.04 ±0.004**	**2.30 ±0.52**	**1.34 ±0.32**	**4967 ±589**	**1243 ± 124**	**4.0 ±2.6**	**35.8 ±18.5**
		p<0.05			p<0.0001						p<0.0001

(total number of subjects – n=150)

plasma was obtained and the concentration 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; LDL4; LDL5; LDL6; LDL7; 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).

Prostacyclin (PGI_2), in its chemically stable form, 6-keto-PGF1 alpha, and Thromboxan A_2 (TxA_2), in its chemically stable form, Thromboxan B_2 (TxB_2), were analyzed by the ELISA-method (DRG, USA).

The score for anti-atherogenic risk (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. The cutoff values for a non-atherogenic lipoprotein profile and an atherogenic lipoprotein profile were calculated from the results of 900 Lipoprint LDL analysis.

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

RESULTS

Table 1 shows the lipid, lipoproteins, and prostanoid values and the score for anti-atherogenic risk (SAAR) in the examined group of 150 subjects. In a subgroup of 140 subjects, a non-atherogenic lipoprotein profile, phenotype A, was identified. However, in a group of 10 subjects, an atherogenic lipoprotein profile, phenotype B, using the Lipoprint LDL method, was confirmed. All examined subjects had normal values of cholesterol and triglycerides. A significant difference ($p<0.0001$) between the subgroup with the atherogenic lipoprotein profile, phenotype B, and the subjects with a non-atherogenic lipoprotein profile, phenotype A, was found in subfraction LDL 3–7, i.e., small dense LDL, which represent strong atherogenic lipoproteins. The score for anti-atherogenic risk (SAAR) showed highly statistically significant differences in the values between the two subgroups (atherogenic vs. non-atherogenic subgroup), $p<0.0001$. Differences in plasma concentration of prostacyclin and thromboxan A_2 between both subgroups (athero profile phenotype B vs. non-athero profile phenotype A) were not statistically significant.

DISCUSSION

Atherosclerosis, with its serious complications such as atherothrombosis, represents the most frequent cause of premature mortality for individuals in economically developed countries all over the world. Premature atherosclerosis development can be found even in young adults and adolescents with high risk factors (Backers 2005; Rizzo & Berneis 2006).

One of the major factors that contributes to the rise and development of atherothrombosis is dyslipidemia, because the overwhelming majority of atherogenic lipoproteins play a key role in the pathomechanism of atherosclerosis (Shoji *et al.* 2009). Dyslipidemia is one of the primary causes of atherosclerosis, with its complications of coronary heart disease, stroke, peripheral obstructive disease, and sudden death. Only early prevention, accurate diagnosis and effective therapy of dyslipidemia can protect against the development of cardiovascular diseases (Backers 2005; Rizzo & Berneis 2006; Fruchart *et al.* 2008; Chun *et al.* 2009; Shoji *et al.* 2009; Haffner 2006).

An innovative laboratory diagnostic method for lipoprotein metabolism disturbances, a lipoprotein electrophoresis on polyacrylamide gel (PAG), the Lipoprint LDL System, can identify and quantify atherogenic lipoproteins in the lipoprotein profile of individuals (Hoefner *et al.* 2001; Oravec 2006 a;b). This method can confirm an atherogenic lipoprotein, phenotype B, vs. a non-atherogenic lipoprotein, phenotype A, based on the predominant presence of a majority of atherogenic, vs. non-atherogenic lipoproteins (Austin at al.1990; Chait *et al.* 1993; Van *et al.* 2007). In our study we used this new diagnostic method to identify lipoprotein profiles in a group of healthy subjects who had no clinical or chemically determined signs of cardiovascular diseases.

An analysis of the lipoprotein profile by the Lipoprint LDL System in healthy subjects revealed a new phenomenon in the composition of lipoproteins and focused our attention on a new clinical-diagnostic reality: an **atherogenic normolipidemia**. Compared to well-known atherogenic dyslipidemia or atherogenic hyperlipoproteinemia, this new phenomenon, called **atherogenic normolipidemia** (Oravec *et al.* 2010;

Tab. 2. Atherogenic potential of small, dense LDL (Berneis & Krauss 2002; Packard 2003).

Small, dense LDL are more highly atherogenic for:
Low recognition by LDL-receptors (configuration alterations Apo B)
Enhanced aptitude for oxidation and acetylation
Oxid-LDL • release of pro-inflammatory cytokines • muscle cell apoptosis
Oxid-LDL • release of metalloproteinase • collagen degradation
Oxid-LDL • enhanced aptitude for trapping by macrophages (scavenger-receptors) • stimulation of foam cell formation • easier penetration into the subendothelial space and formation of cholesterol deposits

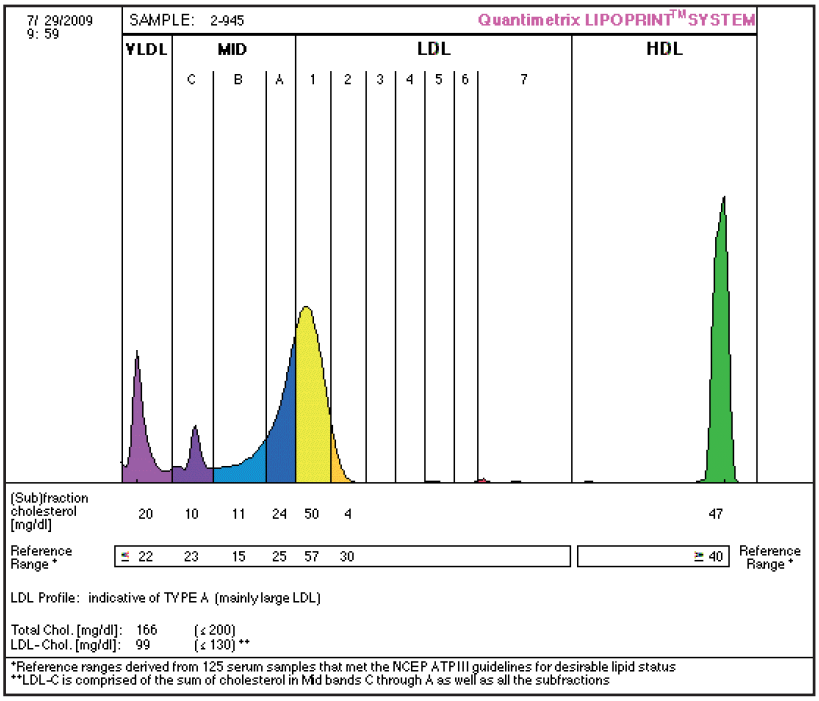

Fig. 1. A non-atherogenic normolipidemia.

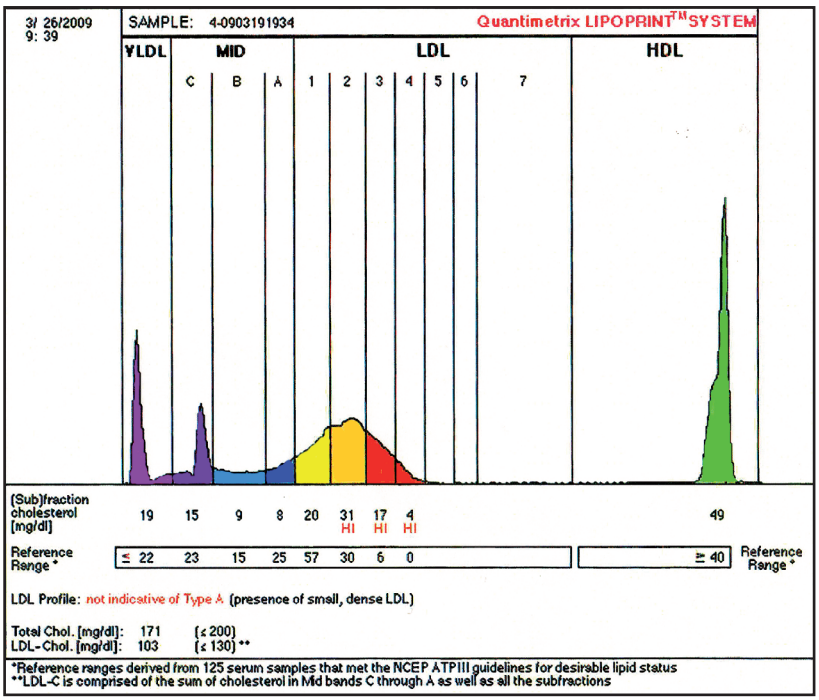

Fig. 2. An atherogenic normolipidemia.

2011), is not identifiable by common biochemical diagnostic analyses. It represents a serious cardiovascular risk for the individuals with this type of normolipidemia, as these individuals, who are at great cardiovascular risk, are till now not identified, diagnosed, or treated. The **atherogenic normolipidemia** enlarges the population at risk for a cardiovascular event, as these individuals at risk are not included in the protective measures of primary cardiovascular prevention.

The identification of the type of lipoprotein profile (atherogenic vs. non-atherogenic) with this innovative electrophoretic method for lipoprotein analysis represents a beneficial contribution to lipid diagnostics, and provides a new interpretation of lipoprotein profiles, which exceeds the current knowledge and clinical practice (Figures 1 and 2).

The score for anti-atherogenic risk (SAAR) is a newly introduced parameter. It can determine an individual's

degree of anti-atherogenic risk, and is calculated as the ratio of non-atherogenic to atherogenic serum lipoproteins (Oravec 2007a;b; 2010). This method reflects the degree of risk for individuals who are in danger of experiencing a premature cardiovascular event. High SAAR values were found in the non-atherogenic subgroup. Individuals with an atherogenic lipoprotein profile have low SAAR values and demonstrate atherogenic normolipidemia, but are at risk for the premature development of cardiovascular diseases. This score correlates well with the phenotype of the lipoprotein profile, created by the Lipoprint LDL System.

Values of prostacyclin and thromboxane A_2 in the examined subjects also provided information about endothelial function. Suppression of endothelial prostanoid secretion (prostacyclin and thromboxane A_2) found in the subpopulation with an atherogenic phenotype B, was indicative of impaired endothelial secretion of prostanoids and endothelial dysfunction in this subgroup. However, the suppression of prostanoid secretion in the atherogenic lipoprotein profile group was discrete and the differences in prostanoid values between both subgroup were not statistically significant.

A great benefit of an innovative electrophoretic lipoprotein analysis is an identification of a new phenomenon in the diagnostics of lipoprotein disturbances, called atherogenic normolipidemia. Individuals with this type of normolipidemia are at high risk for cardiovascular event. However, till now, they are not identified, not selected and not included in the protective measures of primary cardiovascular prevention.

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