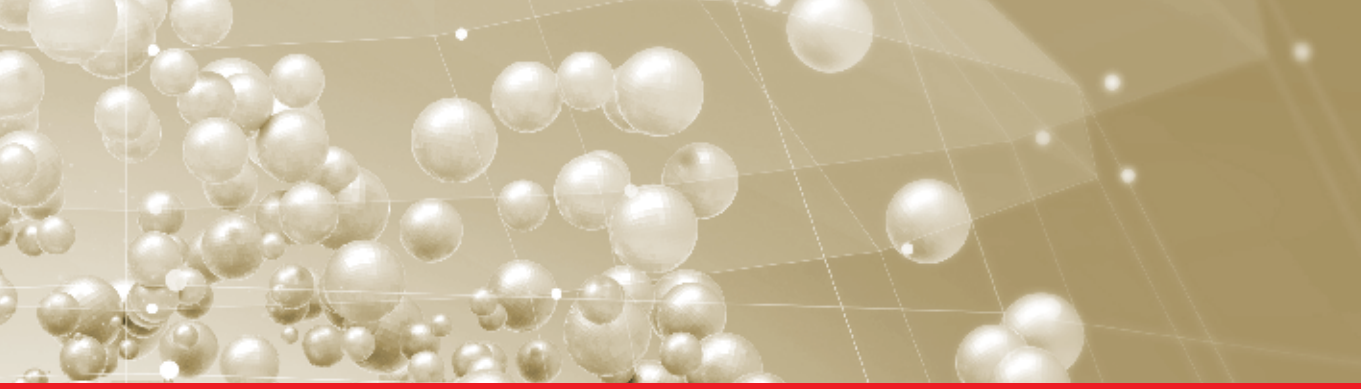


IntechOpen

Lipoproteins
From Bench to Bedside

Edited by Gerhard Kostner and Indumathi Chennamesetty

LIPOPROTEINS - FROM BENCH TO BEDSIDE

Edited by **Gerhard Kostner**
and **Indumathi Chennamesetty**

Lipoproteins - From Bench to Bedside
http://dx.doi.org/10.5772/59331
Edited by Gerhard Kostner and Indumathi Chennamesetty

Contributors

Isaac Karimi, Danial Naseri, Sanja Stankovic, Andriy Zagayko, Anna Kravchenko, Katerina Strelchenko, Anton Shkapo, Tatyana Briuhanova, Mohora Maria, Bogdana Virgolici, Laura Popescu, Horia Virgolici, Elena Daniela Casariu, Olivia Timnea, Stanislav Oravec, Kristina Gruber, Andrej Dukat, Peter Gavornik, Ludovit Gaspar, Elisabeth Dostal, Gerhard Kostner

Notice

First published in Croatia, 2015 by INTECH d.o.o.
eBook (PDF) Published by IN TECH d.o.o.
Place and year of publication of eBook (PDF): Rijeka, 2019.
IntechOpen is the global imprint of IN TECH d.o.o.
Printed in Croatia

Legal deposit, Croatia: National and University Library in Zagreb

Additional hard and PDF copies can be obtained from orders@intechopen.com

Lipoproteins - From Bench to Bedside
Edited by Gerhard Kostner and Indumathi Chennamesetty

p. cm.

ISBN 978-953-51-2178-7

eBook (PDF) ISBN 978-953-51-5407-5

Meet the editors

Prof. Gerhard Kostner was born in 1940 in Vienna, Austria. He studied Chemistry, Biology and Physics at the Karl-Franzens University in Graz and graduated in February, 1966. After spending year and a half as a post-doctor in the laboratory of Pierre Alaupovic in Oklahoma, USA, he returned to Graz and became Assistant Professor in 1970, and full professor for Molecular Biology and Biochemistry in 1991. His current status is Emeritus University Professor. He became Doctor honoris causa in 2013 at the Semmelweiss University in Budapest. Prof. Kostner published more than 350 original articles in peer reviewed journals. His work has more than 5300 citations and he has 1250 impact points.

Dr. Indumathi Chennamsetty is a post doctoral fellow in cardiovascular medicine studying insulin resistance, validating candidate genes identified in a genome-wide association study of insulin resistance. She received her Ph.D. in Molecular Biology studying transcriptional regulation of Lipoprotein(a) from the Institute of Molecular Biology and Biochemistry, Medical University of Graz, Austria. Dr. Chennamsetty research interests are insulin resistance, lipid and lipoprotein metabolism, nuclear receptors and cell signaling.

Contents

Preface

Atherosclerosis is a hallmark in cardiovascular diseases (CVD) and stroke. It is a multifactorial disease triggered by multiple genes. These genes govern the expression of proteins and enzymes involved in lipid and lipoprotein metabolism, inflammation, hemostasis and all events leading to CVD. Looking at the anatomy of an atherosclerotic plaque (Fig.1) it becomes apparent, that the deposition of lipids is a prominent feature in the development and appearance of the arterial stenosis.

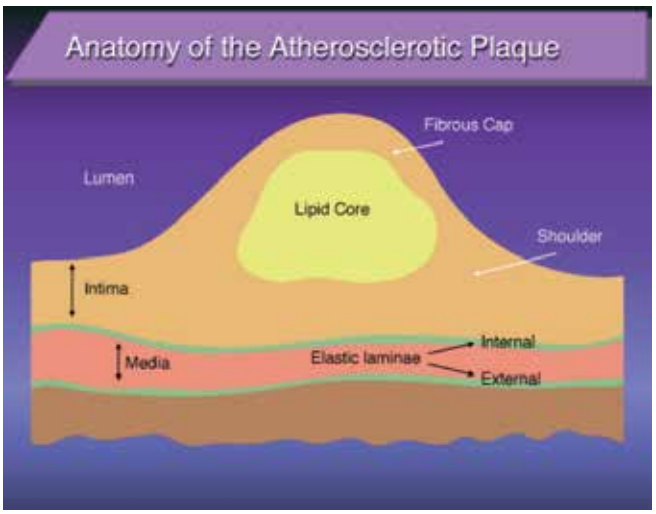


Figure 1. Prominent role of lipids in atherosclerosis.

Since all pathophysiological events are in one way or the other triggered by one or several genes, much efforts are currently directed towards screening of the genome, proteome lipidome or metabolome to uncover abnormalities related to atherogenesis. Genetic epidemiology, however, is expensive, time consuming and in many cases results in associations of specific loci or genes with a disease without giving any clue on the mechanism .

In the present book that is far from giving a complete picture on the pathophysiology of atherosclerosis and CVD we focus on few aspects of the role of lipids and lipoproteins in atherogenesis. In the first part we address the question as to what extent animals like rabbits or hamsters may serve as models for that disease. This is addressed in the chapters of Isaac

Karimi and Andriy Zagayko. Another part of this section focuses on the management of obesity and dyslipoproteinemia in childhood (composed by Maria Mohara).

In the second section of the book two important topics are **addressed**. The article written by Stanislav Oravec and his colleagues highlights the fact, that lipoprotein profiles related to CVD are not necessarily reflected by plasma lipids or lipoprotein-lipid concentrations but rather by specific electrophoretic lipoprotein patterns. In addition, lipoprotein associated phospholipase appears to be a very important analyte related to the cardiovascular risk and inflammatory status of a patient, reviewed by Sanja Stankovic.

Last but not least, an overview is presented by Indumathi Chennamsetty and Gert Kostner on the current status of the most atherogenic lipoprotein, Lp(a) (Fig.2).

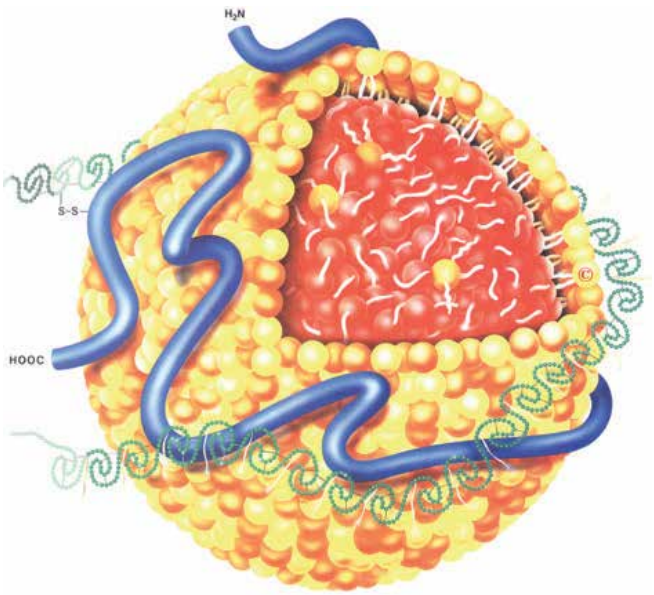


Figure 2. Schematic view of lipoprotein(a)

We are convinced that these snapshots of the role of lipoproteins in the pathophysiology of atherosclerosis adds to the understanding of related diseases not only for scientists but also for practitioners working in clinics and in private practice.

Prof. Gerhard Kostner
Institute of Molecular Biology and Biochemistry,
Medical University of Graz, Austria

Dr. Indumathi Chennamesetty
Stanford University, USA

Section 1

Animal Models and Lipoproteins in Childhood

Chapter 1

Nutritional Modulations Used to Translate a Rabbit Model of Atherosclerosis — A Systematic Review and Meta-analysis

Isaac Karimi, Danial Naseri and Ferdous Karimizand

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/61155

Abstract

Dietary cholesterol has been suggested as a cause of dyslipidemic atherosclerosis with scarce convincing evidence. A systematic review and a meta-analysis were conducted in MEDLINE (2004–2015) to screen randomized controlled trials (RCTs) that used cholesterol-fed rabbits as a model of atherosclerosis. A total of 32 RCTs (n = 1104 New Zealand rabbits; 4.37 ± 2.52 months old) reported lipid and lipoprotein outcomes following cholesterol intake (0.98 ± 0.67%) for a duration of 8.90 ± 7.26 weeks. Cholesterol intakes significantly raised combined lipid and lipoprotein outcomes (standardized mean difference) in a random-effect model by 5.618 (95% CI: 4.592, 6.644; P = 0.0001). The value of I^2, heterogeneity, was 89.387%, indicating real variation. A subgroup analysis based on the duration and amount of cholesterol feeding in a mixed-effects analysis showed combined heterogeneous effects of 2.788 (95% CI: 2.333, 3.244; P = 0.000; Q = 112.206; df = 14) and 5.538 (95% CI: 4.613, 6.463; P = 0.000; Q = 31.622; df = 6), respectively. Random-effect meta-regression conducted using cholesterol moderator did not support causal effects of dietary cholesterol in inducing atherosclerosis, which may be due to significant publication bias. These high levels of heterogeneity among studies may decline fidelity of this animal model for translation of dyslipidemic atherosclerosis.

Keywords: Atherosclerosis, rabbits, cholesterol, lipoproteins, meta-analysis

1. Introduction

Atherosclerosis is a disease that belongs to both antiquity and modern era [1]. This economically important disease is an outcome of both genetic and environmental risk factors and their interactions [2]. Inflammatory and metabolic derangements and their synergy are the main

causes in the etiopathogenesis of atherosclerosis [3]. However, all aspects of atherosclerosis are not known at the present time, and more human and animal studies are requested to decode the black box of atherosclerosis. Since we are not able to do interventional studies in humans, animal models are good simulated and translated tools in this endeavor.

Many seminal reviews (e.g., see [4–6]) have shown that rabbits are good, reliable, and cheap animal models of atherosclerosis, with a high degree of comparability to human's atherosclerosis. In this context, low-density lipoprotein cholesterol (LDL-C) is predominant plasma lipoprotein in rabbits and humans [6].

Despite these data, the amount of dietary cholesterol, the diet formula, and the duration of cholesterol feeding necessary to induce translated atherosclerosis are not conclusive among experimental studies (e.g., see [7,8]). In this line, Prof. Watanabe and colleagues [9] partially solved this problem by producing a genetic rabbit model of hypercholesterolemia many years ago. However, this expensive model is not globally available in all laboratories and is not suitable for translating nutritional interventions that lead to atherosclerosis. Watanabe rabbit is still not a pet animal model for studying atherosclerosis in all laboratories.

In addition, there is not any concert about the methodology used for inducing atherosclerosis in nongenetic animal models like New Zealand rabbits (NZRs) among various studies. In this sense, reliable and loyal translation of atherosclerosis in rabbits were hampered by methodological limitations, including suitable sample size, suboptimal biomarker assay, amount of cholesterol intake, routes of cholesterol intake, and duration of cholesterol intake, among others. The aforementioned limitations result in low statistical power to translate atherosclerosis in animal models like rabbits.

To obtain a more truthful and defined estimation of association between cholesterol intake and atherosclerosis (i.e., the occurrence of dyslipidemia), to do root cause analysis, and to explore the source of heterogeneity among randomized controlled trials (RCTs), we conducted a meta-analysis of RCTs by evaluating the associations of dietary cholesterol with the dyslipidemia component of atherosclerosis in rabbits.

2. Materials and methods

2.1. Study selection

A comprehensive electronic search of the MEDLINE (National Library of Medicine) database was conducted to identify English-written studies published between January 1, 2004, and January 1, 2015, using ("Atherosclerosis"[Mesh]) AND "Rabbits"[Mesh] AND (Animals[Mesh:noexp]) in PubMed as Medical Subject Headings (MeSH). Two investigators (IK and SSM) coincidentally assessed titles and abstracts of articles generated by the literature research after removal of duplicated records. Discrepancies in opinion between the two investigators were adjudicated by a third investigator (GB). We considered the following

http://dx.doi.org/10.5772/61155

criteria in the selected studies for meta-analysis: (1) inclusion of cholesterol in diet was the main part of the dietary intervention; (2) comparison groups could include a control diet or an intervention in which cholesterol intake was quantitatively different from cholesterol intake in the experimental groups; (3) among pharmacological studies, only results of the control group and the cholesterol-fed group were selected, and the rest of the drug-treated groups were excluded; (4) we included studies that reported net quantity of lipid and lipoprotein profiles and studies that reported changes graphically have been excluded; (5) studies that solely considered other end points such as inflammatory end points of atherosclerosis, histopathologic descriptive end points, and vascular imaging of atherosclerosis were excluded; (6) all studies that reported dyslipidemia in genetic engineered models and strains were excluded; and (7) all studies that focused on lipid, and lipid profiles of cholesterol-fed New Zealand rabbits (NZRs) were included to do meta-analysis over their results. In this continuum, 32 articles met the aforementioned eligibility criteria for inclusion in meta-analysis [10–41]. Figure 1 summarizes information on the study selection process.

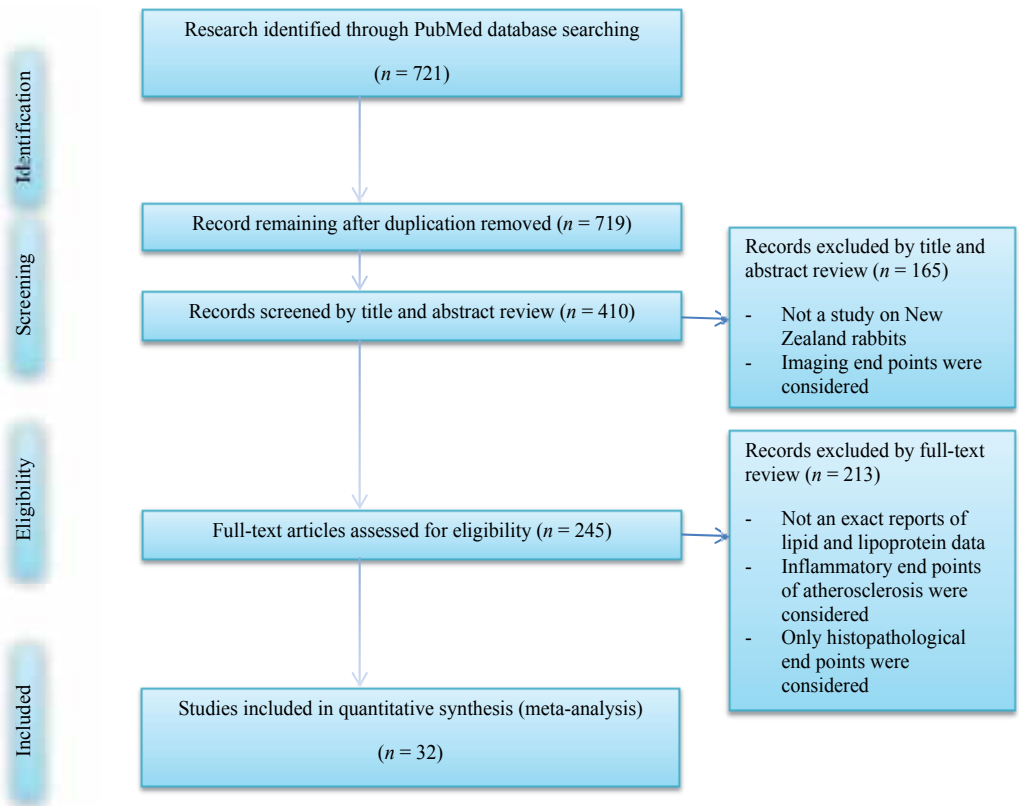


Figure 1. Flow diagram of publication inclusion.

2.2. Statistical analysis

Effect sizes are indices that measure the magnitude of the differences between two groups. For each comparison, individual RCT data for each outcome measure and combined measure were pooled to calculated standardized mean difference (SMD) effect size considering $P < 0.05$ significant level [42] using the Comprehensive Meta-Analysis ver.2.2.064, a software package developed by Biostat (http://www.meta-analysis.com/; Englewood, NJ 08631 USA).

To show substantial heterogeneity among studies, we report fixed-, random-, and mixed-effects meta-analysis [43]. In this continuum, random-effects meta-analysis takes into account the precision of discrete studies and the variation among studies and weights of each study accordingly.

We conducted two subgroup analyses to explore association of dietary cholesterol and duration of cholesterol intake with outcome measures. For each subgroup category, overall net change estimates were calculated using fixed-effects, random-effects, and mixed-effects models, and the heterogeneity of estimates was assessed. We conducted a meta-regression analysis to further examine the effects of cholesterol intake as an explanatory factor on outcome variables using random-effects meta-regression (unrestricted maximum likelihood (UREML)).

The heterogeneity of studies was quantified using chi-square test, Q, and I^2 statistics [44]. Begg's [45] and Egger's tests [46] were employed to identify publication bias.

3. Results and discussion

A total of 721 articles were initially identified after electronic search from January 1, 2004, to January 1, 2015, was restricted to animal studies using usual PubMed filters. Three duplicated articles were subtracted, and 719 unique publications were screened by title and abstract review. In this continuum, articles dealing with non-New Zealand rabbit strains and studying imaging end points like MRI, CT scan, and radiology have been excluded (see Figure 1). The remaining articles were submitted to detail inspection, and articles that focused on inflammatory and histopathologic end points of atherosclerosis without reporting lipid and lipoprotein profiles of studied animals were excluded. In total, 32 eligible studies were included in the systematic review and meta-analysis involving 1104 rabbit subjects [10–41]. We just considered control and cholesterol-fed (treated; model) rabbits for meta-analytic assessment, and other animal groups that received other interventions (drug, nutrients, etc.) have been excluded from the data set. The study identification process is shown in Figure 1.

Of the included RCT, 30 studies reported on outcomes of triacylglycerols (TGs; $n = 638$ animal subjects), 32 studies reported on total cholesterol (TC; $n = 677$ animal subjects), 26 studies reported on LDL-C ($n = 595$ animal subjects), 30 studies reported on high-density lipoprotein cholesterol (HDL-C; $n = 571$ animal subjects), 4 studies reported on LDL-C/HDL-C ($n = 98$ animal subjects), 1 study reported on LDL-C/TC ($n = 8$ animal subjects), 2 studies reported on oxidized LDL-C (ox-LDL-C; $n = 32$ animal subjects), and 3 studies reported on TC/HDL-C ($n = 46$ animal subjects).

http://dx.doi.org/10.5772/61155

Age in the start of studies varied from 1.86 to 9 months old with an average value of 4.37 ± 2.52 months old in 9 studies, and it was not reported in the majority of studies. The duration of nutritional intervention went from 4 to 56 weeks with an average value of 8.90 ± 7.26 weeks in all studies (Figure 2). The majority of studies (56%) reported ≤10 months, while 37.5% of studies reported 10–20 weeks of nutritional intervention to translate a rabbit model of atherosclerosis. The amount of dietary cholesterol surplus that utilized to induce atherosclerosis varied from 0.34% to 4.00% with an average value of 0.98 ± 0.67% in all studies (Figure 3). In this context, 31% of all eligible studies used 0.5% cholesterol in feed, while 46% of studies used 1% cholesterol in their feed.

The approximate analysis and the major ingredients of rations were not usually reported in studies. However, in 40.6% of studies, a range of fat (1% to 10%) with an average value of (5.03 ± 2.80%) from diverse sources like lard has been reported. The 5% fat content has been more frequently (30.76%) utilized among studies that reported the fat content of diets. However, it is not precisely clear from studies that the fat added to the ration is the whole content of dietary fat or surplus fat. Experimental diets must be formulated according to the rabbit requirements of NRC [47] because all macronutrients (carbohydrate, fat, and protein), micronutrients (minerals and vitamins), and other diet-specific bioactive compounds may modulate lipid metabolism and cytokine milieu that lead to atherosclerosis or prevent atherosclerosis. In addition, the cholesterol content of diets needs to be determined since 0.34% cholesterol in diet can trigger dyslipidemia in rabbits, depending on the duration of treatment. The lack of dietary formulations and the approximate analysis are two major shortcomings of studies that report cholesterol-induced atherosclerosis in rabbits.

No consensus for initial weights of rabbits were observed among eligible studies as well as on the changes of weight during experiment and the final weight of the studied animal at the end of dietary intervention. The initial weight of rabbits employed to translate atherosclerosis changed from 1.5 to 3.25 kg with an average value of 2.45 ± 0.59 kg. Final weights (3.29 ± 0.21 kg) were reported only in four studies, in which their rabbits were fed 1% cholesterol for an average duration of 2.17 ± 0.84 months of nutritional intervention. By pooling data from 32 RCTs of 1104 rabbit subjects, the current meta-analysis documented lipid and lipoprotein alterations associated with increased intake of cholesterol. However, dietary formulations, duration of cholesterol feeding, initial and final weights of animals or their weight changes during study, and concise age of animals were not reported in most of these studies and would cause heterogeneity.

The effect of dietary cholesterol inclusion on the combined outcome of lipid and lipoprotein profiles and indices of rabbits in a random-effect model as an analysis model for the meta-analyses was 5.618 (95% confidence interval (CI): 4.592, 6.644; $P = 0.0001$). In this way, SMD effects of dietary cholesterol inclusion were 2.424 mg/dl (95% CI: 1.531, 3.318 mg/dl; $P = 0.000009$) for HDL-C, 7.646 mg/dl (6.266, 9.026; $P = 0.000000000$) for LDL-C, 5.216 (95% CI: 2.155, 8.278; $P = 0.001$) for LDL-C/HDL-C, 4.658 ng/ml (95% CI: 3.321, 5.995 ng/ml; $P = 0.000000000$) for ox-LDL-C, 8.643 mg/dl (95% CI: 7.258, 10.028 mg/dl; $P = 0.00000000$) for TC, 4.392 (95% CI: 2.317, 6.466; $P = 0.00003$) for TC/HDL-C, and 3.173 mg/dl (95% CI: 2.451, 3.896 mg/dl; $P = 0.000000000$) for TGs. The value of I^2 was 89.387%, indicating that nearly all of the

variation is real and sturdily supporting the subgroup analysis and/or the meta-regression. By convention, an I^2 value >75% indicates significant between-study heterogeneity [4]. Subgroup analyses according to whether lipid and lipoprotein changes occurred following the stratification of studies according to the duration of cholesterol feeding in cholesterol-fed rabbits. In this way, based on a mixed-effects analysis model, SMD effects of the duration of cholesterol inclusion was 2.788 (95% CI: 2.333, 3.244; $P = 0.000009$). According to the Q test ($Q = 112.206$, $P < 0.001$), a significant level of heterogeneity has been concluded in the subgroup analysis of studies based on the duration of cholesterol feeding, which reflects the diversity of study design among the 28 comparisons.

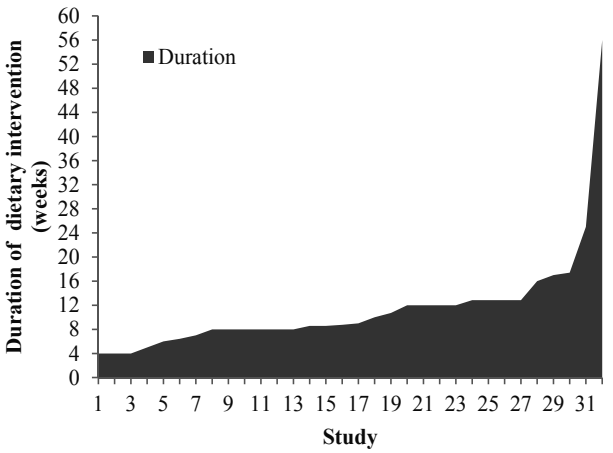


Figure 2. The duration of dietary cholesterol surplus used in a rabbit model of atherosclerosis.

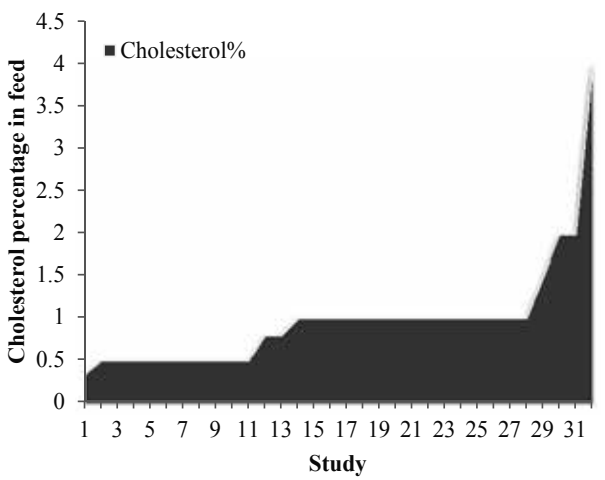


Figure 3. The dietary cholesterol surplus used in a rabbit model of atherosclerosis.

http://dx.doi.org/10.5772/61155

In this continuum, the subgroup analysis based on duration (week) of cholesterol feeding in a mixed-effects analysis showed combined SMD effects, that is, 2.788 (95% CI: 2.333, 3.244; P = 0.000; Q = 112.206; df = 14). Furthermore, SMD effects were 1.414 mg/dl (95% CI: 1.049, 1.780; P = 0.000000000; Q = 166.780; df = 14) for HDL-C, 4.3888 mg/dl (95% CI: 3.779, 4.996; P = 0.000000000; Q = 138.362; df = 13) for LDL-C, 3.367 (95% CI: 2.686, 4.048; P = 0.00000; Q = 35.845; df = 3) for LDL-C/HDL-C, 4.658 ng/ml (95% CI: 3.321, 5.995 ng/ml; P = 0.000000000; Q = 0.163; df = 1) for ox-LDL-C, 3.942 mg/dl (95% CI: 3.408, 4.475 mg/dl; P = 0.00000000; Q = 184.421; df = 17) for TC, 3.725 (95% CI: 2.733, 4.718; P = 0.000; Q = 6.663; df = 2) for TC/HDL-C, and 2.161 mg/dl (95% CI: 1.792, 2.529 mg/dl; P = 0.000000000; Q = 79.221; df = 15) for TGs. The duration of cholesterol intake was an important determinant of the heterogeneity in studies with regard to lipid and lipoprotein outcomes. Based on Q values of outcomes following the subgroup analysis and their comparison with the chi-square table, the high level of heterogeneity has been detected for all outcomes except TC/HDL-C as an atherogenic index. This strange result may be due to the low sample size (n = 3) of the TC/HDL-C index. Therefore, the duration of cholesterol intake may not play an essential role in inducing dyslipidemic atherogenesis in cholesterol-fed rabbit models.

Subgroup analyses according to whether lipid and lipoprotein changes occurred following the stratification of studies according to the amount of cholesterol feeding in cholesterol-fed rabbits. In this continuum, the subgroup analysis based on dietary cholesterol inclusion (%) in of cholesterol-fed rabbits in a mixed-effects analysis showed SMD effects 5.538 (95% CI: 4.613, 6.463; P = 0.000; Q = 31.622;df = 6). Further, the subgroup analysis based on the amount of cholesterol feeding in a mixed-effects analysis showed SMD effects 1.743 mg/dl (95% CI: 1.145, 2.341; P = 0.000000000; Q = 77.709; df = 6) for HDL-C, 7.409 mg/dl (95% CI: 6.173, 8.646; P = 0.000000000; Q = 138.362; df = 4) for LDL-C, 3.208 (95% CI: 1.680, 4.736; P = 0.000000000; Q = 0.967; df = 1) for LDL-C/HDL-C, 4.658 ng/ml (95% CI: 3.321, 5.995 ng/ml; P = 0.000000000; Q = not determined; df = 0) for ox-LDL-C, 8.543 mg/dl (95% CI: 7.309, 9.776 mg/dl; P = 0.00000000; Q = 41.307; df = 6) for TC, 4.255 (95% CI: 2.852, 5.658; P = 0.000; Q = 3.691;df = 1) for TC/HDL-C, and 3.107 mg/dl (95% CI: 2.491, 3.723 mg/dl; P = 0.000000000; Q = 23.675; df = 6) for TGs.

As the amount of dietary cholesterol surplus was considered as a moderator, the P values of Q_R in mixed-model regression were significant for TGs, TC, LDL-C/HDL-C, LDL-C, HDL-C, and combined lipid and lipid profile (P < 0.01; Table 1 and Figure 4), while the P values of Q_R in mixed-model regression were nonsignificant for TC/HDL-C (P = 0.31731; Table 1). Random-effects meta-regression (UREML) was conducted for change in all parameters and indices using the cholesterol moderator (data not shown). The ANOVA table of fixed- and mixed-effect models for combined lipid and lipoprotein profile regression is shown in Table 1. Accordingly, Q_M is significant. Then at least one of the regression coefficients between dietary cholesterol inclusion and lipid and lipoprotein profiles and indices is different from zero. In this sense, the funnel plot (Figure 5) and the results of Egger's tests showed a significant publication bias (data not shown). The subgroup analysis based on the amount of dietary cholesterol surplus showed heterogeneity in all outcomes except LDL-C/HDL-C (n = 2) and TC/HDL-C (n = 2) after comparing their Q values using the chi-square table. Therefore, cholesterol content may not be a pivotal determinant in inducing dyslipidemia in cholesterol-

fed rabbit models, but it raised all lipid and lipoprotein profile and indices in cholesterol-fed rabbits. Moreover, no consensus for atherogenic diets was observed based on their cholesterol and fat contents and the types of their fat, including pure cholesterol, lard, egg yolk powder, coconut oil, and/or safflower oil as well as different nutritional interventions.

ANOVA						
	Fixed-effect regression			Mixed effects regression		
	Q	df	*P*	*Q*	df	*P*
Q_M	44.51969	1	0.00000	5.30338	1	0.02128
Q_R	287.66695	34	0.00000	43.61079	34	0.12506
Q_{TOTAL}	332.18664	35	0.00000	48.91418	35	0.05934

Q_M—model sum of squares compared to chi-square distribution with $p-1$ df (p is number of predictors in the model); Q_R—residual sum of squares compared to chi-square distribution with $k-p-1$ df (k is the number of studies) [48].

Table 1. Fixed- and mixed-effect models—analysis of variance (ANOVA) table for combined lipid and lipoprotein profile regression.

The resulting funnel graphs and the associated statistics based on Egger et al. [46] revealed a significant asymmetry and publication bias among studies. Therefore, publication or other sources of bias could be relevant in evaluating an overall effect of the selected nutritional interventions in lipid and lipoprotein outcomes in the cholesterol-fed rabbit model of (pre)atherosclerosis.

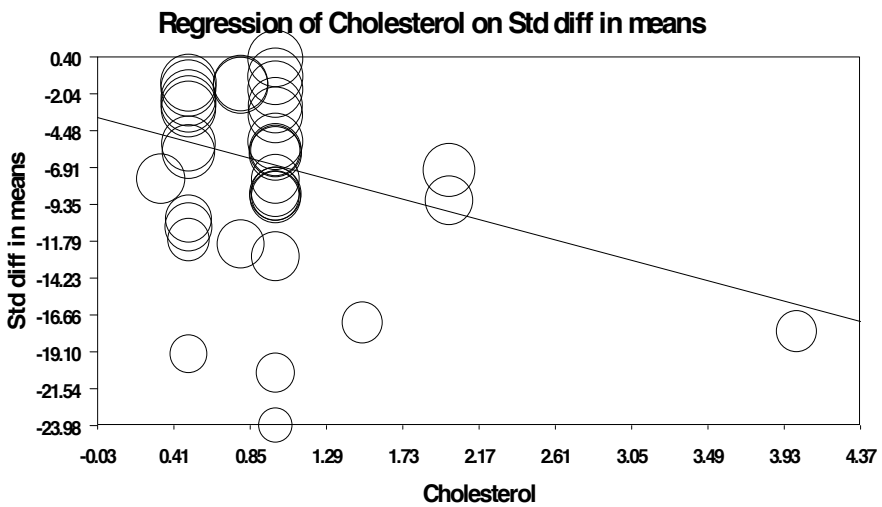


Figure 4. Fixed-effect model—regression of dietary cholesterol surplus on standardized mean difference of lipid and lipid profiles and indices in a rabbit model of atherosclerosis.

http://dx.doi.org/10.5772/61155

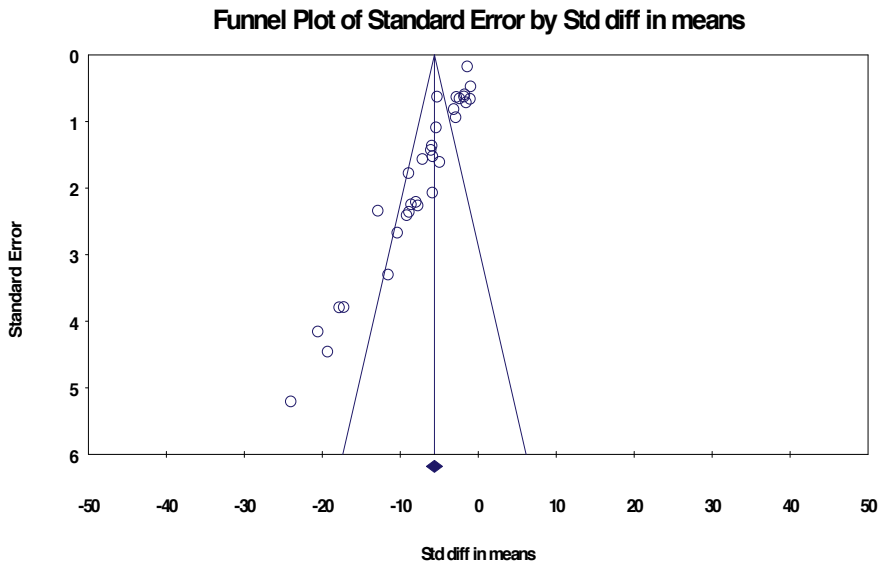


Figure 5. Funnel plot for a meta-analysis of association between dietary cholesterol inclusion and combined effects of lipid and lipoprotein profiles and indices in a rabbit model of atherosclerosis.

4. Conclusions

These systematic review and meta-analyses of RCTs initially assess the robustness of cholesterol-fed rabbit of atherosclerosis since the causal role of cholesterol intake in progression and induction of atherosclerosis is not supported in some clinical trials (e.g., see [49]). These systematic review and meta-analysis showed high levels of heterogeneity among studies that used the cholesterol-fed rabbit model of atherosclerosis, which may be due to the lack of consensus on the dietary ingredients and formulation, the amount of dietary cholesterol surplus, the duration of cholesterol intake, and the analytical methodology in lipid and lipoprotein determination. In sum, for the first time, this meta-analysis showed that dietary cholesterol surplus could not be a reliable determinant of dyslipidemic atherosclerosis in cholesterol-fed rabbits. Rabbits in fact are vegetarians, and plants actually do not contain cholesterol. Thus, by itself, the model for studying atherogenesis by cholesterol feeding is far from being ideal or relevant for the situation in humans.

Acknowledgements

We thank Biostat (http://www.meta-analysis.com/; Englewood, NJ 08631, USA) for permission to use the online Comprehensive Meta-Analysis software despite sanction against Iran. Authors declare no financial support from any association.

Author details

Isaac Karimi[1*], Danial Naseri[2] and Ferdous Karimizand[3]

*Address all correspondence to: isaac_karimi2000@yahoo.com

1 Department of Biology, School of Sciences, Razi University, Daneshgah Street, Tagh-e-Bostan, Kermanshah, Islamic Republic of Iran

2 Department of Poultry, School of Veterinary Medicine, Shiraz University, Shiraz, Islamic Republic of Iran

3 Department of Biology, Faculty of Basic Sciences, Science and Research Branch of the Islamic Azad University, Tehran, Islamic Republic of Iran

References

[1] O'Keefe JH, Cordain L. Cardiovascular disease resulting from a diet and lifestyle at odds with our Paleolithic genome: how to become a 21st-century hunter-gatherer. *Mayo Clin Proc*. 2004;79:101–08.

[2] Zhang BK, Lai X, Jia SJ. Epigenetics in atherosclerosis: a clinical perspective. *Discov Med*. 2015;19(103):73–80.

[3] Rizzo M, Rizvi AA, Rini GB, Berneis K. The therapeutic modulation of atherogenic dyslipidemia and inflammatory markers in the metabolic syndrome: what is the clinical relevance? *Acta Diabetol*. 2009;46(1):1–11.

[4] Yamashita A, Asada Y. A rabbit model of thrombosis on atherosclerotic lesions. *J Biomed Biotechnol*. 2011;2011. doi:10.1155/2011/424929

[5] James C. Russell, Spencer D. Proctor. Small animal models of cardiovascular disease: tools for the study of the roles of metabolic syndrome, dyslipidemia, and atherosclerosis. *Cardiovasc Pathol*. 2006;15:318–330.

[6] Fan J, Kitajima S, Watanabe T, Xu J, Zhang J, Liu E, Chen YE. Rabbit models for the study of human atherosclerosis: from pathophysiological mechanisms to translational medicine. *Pharmacol Ther*. 2015;146C:104–119.

[7] Abdelhalim MA, Moussa SA, Al-Mohy YH. Ultraviolet-visible and fluorescence spectroscopy techniques are important diagnostic tools during the progression of atherosclerosis: diet zinc supplementation retarded or delayed atherosclerosis. *Biomed Res Int*. 2013;2013:652604. doi:10.1155/2013/652604.

[8] Hur SJ, Min B, Nam KC, Lee EJ, Ahn DU. Effect of dietary cholesterol and cholesterol oxides on blood cholesterol, lipids, and the development of atherosclerosis in rabbits. *Int J Mol Sci*. 2013;14(6):12593–12606.

[9] Watanabe Y. Serial inbreeding of rabbits with hereditary hyperlipidemia (WHHL-rabbit). *Atherosclerosis*. 1980;36:261–268.

[10] Zhong J-K, Guo Z-G, Li C, Wang Z-K, Lai W-Y, Tu Y. Probucol alleviates atherosclerosis and improves high density lipoprotein function. *Lipids Health Dis*. 2011;10:210.

[11] Marinou KA, Georgopoulou K, Agrogiannis G, Karatzas T, Iliopoulos D, Papalois A, et al. Differential effect of *Pistaciavera* extracts on experimental atherosclerosis in the rabbit animal model: an experimental study. *Lipids Health Dis*. 2010;9:73.

[12] Lin F-Y, Lin Y-W, Huang C-Y, Chang Y-J, Tsao N-W, et al. A heat shock protein 60 of *Chlamydia pneumoniae*, induces lectin-like oxidized low-density lipoprotein receptor 1 expression in endothelial cells and enhances atherogenesis in hypercholesterolemic rabbits. *J Immunol.* 2011;186:4405–4414.

[13] Shusheng Li, Lei Wan. Experimental study on the preventive mechanism of *Salviaemiltiorrhizae* against atherosclerosis in rabbits models. *J Huazhong Univ Sci Technol*. 2004;24(3):233–235.

[14] Karantonis HC, Antonopoulou S, Perrea DN, Sokolis DP, Theocharis SE, et al. *In vivo* antiatherogenic properties of olive oil and its constituent lipid classes in hyperlipidemic rabbits. *Nutr Metab Cardiovasc Dis*. 2006;16:174–185.

[15] Lorenz M, Fechner M, Kalkowski J, Frölich K, Trautmann A, Böhm V, et al. Effects of lycopene on the initial state of atherosclerosis in New Zealand White (NZW) rabbits. *PLoS One*. 2012;7(1): e30808. doi:10.1371/journal.pone.0030808.

[16] Kabiri1 N, Asgary S, Setorki M. Lipid lowering by hydroalcoholic extracts of *Amaranthuscaudatus* L. induces regression of rabbits atherosclerotic lesions. *Lipids Health Dis*. 2011;10:89.

[17] Valero-Muñoz M, Martin-Fernandez B, Ballesteros S, Martinez-Martinez E, Blanco-Rivero J, et al. Relevance of vascular peroxisome proliferator-activated receptor γcoactivator-1α to molecular alterations in atherosclerosis. *Exp Physiol.* 2013;98.5: 999–1008.

[18] Tsantila N, Karantonis HC, Perrea DN, Theocharis SE, Iliopoulos DG, et al. Atherosclerosis regression study in rabbits upon olive pomace polar lipid extract administration. *Nutr Metab Cardiovasc Dis.* 2010;20:740–747.

[19] Tavridou A, Kaklamanis L, Papalois A, Kourounakis AP, Rekka EA, et al. EP2306 [2-(4-Biphenyl)-4-methyl-octahydro-1,4-benzoxazin-2-ol, hydrobromide], a novel squalene synthase inhibitor, reduces atherosclerosis in the cholesterol-fed rabbit. J Pharmacol *Exp Ther*. 2007;323:794–804.

[20] Charoenwanthanang P, Lawanprasert S, Phivthong-ngam L, Piyachaturawat P, Sanvarinda Y, et al. Effects of *Curcumacomosa* on the expression of atherosclerosis-related cytokine genes in rabbits fed a high-cholesterol diet. *J Ethnopharmacol*. 2011;134:608–613.

[21] Young Um M, Hwang KH, Choi WH, Ahn J, Jung CH, et al. Curcumin attenuates adhesion molecules and matrix metalloproteinase expression in hypercholesterolemic rabbits. *Nutri Res*. 2014;34:886–893.

[22] Yu Y-M, Chang W-C, Wu C-H, Chiang S-Y. Reduction of oxidative stress and apoptosis in hyperlipidemic rabbits by ellagic acid. *J Nutr Biochem*. 2005;16:675–681.

[23] Tovar AMF, Pecly IMD, Rangel EP, Melo-Filho NM, Mourão PAS, et al. Experimentally induced metabolic acidosis in rabbits modulates the interaction of aortic glycosaminoglycan with plasma low-density lipoprotein-an interesting observation about the association of acidosis and atherosclerosis. *Atherosclerosis*. 2007;192:33–39.

[24] Chen W, Tang F, Xie B, Chen S, Huang H, et al. Amelioration of atherosclerosis by tanshinone IIA in hyperlipidemic rabbits through attenuation of oxidative stress. *Eur J Pharmacol*. 2012;674:359–364.

[25] Zulkhairi A, Zaiton Z, Jamaluddin M, Sharida F, Mohd TH, Hasnah B, Nazmi HM, Khairul O, Zanariyah A. Alpha lipoic acid possess dual antioxidant and lipid lowering properties in atherosclerotic-induced New Zealand White rabbit. *Biomed Pharmacother*. 2008;62(10):716–722.

[26] Mohammadia A, Darabi M, Nasry M, Saabet-Jahromi M-J, Malek-Pour-Afshar R, Sheibani H. Effect of opium addiction on lipid profile and atherosclerosis formation in hypercholesterolemic rabbits. *Exp Toxicol Pathol*. 2009;61:145–149.

[27] Tuncer MA, Yaymaci B, Sati L, Cayli S, Acar G, Altug T, Demir R. Influence of *Tribulusterrestris* extract on lipid profile and endothelial structure in developing atherosclerotic lesions in the aorta of rabbits on a high-cholesterol diet. *Acta Histochem*. 2009;111(6):488–500.

[28] Xiong Q, Li J, Jin L, Liu J, Li T. Nasal immunization with heat shock protein 65 attenuates atherosclerosis and reduces serum lipids in cholesterol-fed wild-type rabbits probably through different mechanisms. *Immunol Lett*. 2009;125:40–45.

[29] Yanni AE, Agrogiannis G, Nomikos T, Fragopoulou E, Pantopoulou A, et al. Oral supplementation with L-aspartate and L-glutamate inhibits atherogenesis and fatty liver disease in cholesterol-fed rabbit. *Amino Acids*. 2010;38:1323–1331.

[30] Setorki M, Asgary S, Eidi A, Haerirohani A, Esmaeil N. Effects of apple juice on risk factors of lipid profile, inflammation and coagulation, endothelial markers and atherosclerotic lesions in high cholesterolemic rabbits. *Lipids Health Dis.* 2009;8:39.

[31] Cavallini DCU, Abdalla DSP, Vendramini RC, Bedani R, Bomdespacho LQ, Pauly-Silveira ND, et al. Effects of isoflavone-supplemented soy yogurt on lipid parameters

and atherosclerosis development in hypercholesterolemic rabbits: a randomized double-blind study. *Lipids Health Dis.* 2009;8:40.

[32] Li S-N, Wang X, Zeng Q-T, Feng Y-B, Cheng X, Mao X-B, et al. Metformin inhibits nuclear factor κB activation and decreases serum high sensitivity C-reactive protein level in experimental atherogenesis of rabbits. *Heart Vessels*. 2009;24:446–453.

[33] Nader MA, El-Agamy DS, Suddek GM. Protective effects of propolis and thymoquinone on development of atherosclerosis in cholesterol-fed rabbits. *Arch Pharm Res.* 2010.33(4):637–643.

[34] Houssen ME, Haron MM, Metwally SS, Ibrahim TM. Effects of immunomodulatory drugs on plasma inflammatory markers in a rabbit model of atherosclerosis. *J Physiol Biochem*. 2011;67:115–120.

[35] Chan K-C, Yang M-Y, Lin M-C, Lee Y-J, Chang W-C, Wang C-J. Mulberry leaf extract inhibits the development of atherosclerosis in cholesterol-fed rabbits and in cultured aortic vascular smooth muscle cells. *J Agric Food Chem*. 2013;61:2780–2788.

[36] Ozaki MR, de Almeida EA. Evolution and involution of atherosclerosis and its relationship with vascular reactivity in hypercholesterolemic rabbits. *Exp Toxicol Pathol*. 2013;65:297–304.

[37] Yang P-Y, Almofti MR, Lu L, Kang H, ZhangJ, Li T-J, et al. Reduction of atherosclerosis in cholesterol-fed rabbits and decrease of expressions of intracellular adhesion molecule-1 and vascular endothelial growth factor in foam cells by a water-soluble fraction of *Polygonum multiflorum*. *J Pharmacol Sci*. 2005;99:294–300.

[38] Koniari I, Mavrilas D, Papadaki H, Karanikolas M, Mandellou M, Papalois A, et al. Structural and biomechanical alterations in rabbit thoracic aortas are associated with the progression of atherosclerosis. *Lipids Health Dis*. 2011;10:125.

[39] Ren M, Rajendran R, Ning P, Huat BTK, Nam OC, Watt F, et al. Zinc supplementation decreases the development of atherosclerosis in rabbits. *Free Radical Biol Med*. 2006;41:222–225.

[40] Mosimann AL, Wilhelm-Filho D, da Silva EL. Aqueous extract of *Ilex paraguariensis* attenuates the progression of atherosclerosis in cholesterol-fed rabbits. *BioFactors*. 2006;26:59–70.

[41] Lu Z, Yue-Rong J, Chun-Yu G, Cai-Feng W, Ke-Ji C, Hui-Jun Y. Effects of active components of red Paeonia and *Rhizoma chuanxiong* on angiogenesis in atherosclerosis plaque in rabbits. *Chin J Integr Med*. 2009;15(5):359–364.

[42] Vesterinen HM, Sena ES, Egan KJ, Hirst TC, Churolov L, Currie GL, et al. Meta-analysis of data from animal studies: a practical guide. *J Neurosci Methods*. 2014;221:92–102.

[43] Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *Brit Med J*. 2003;327:557–560.

[44] Deeks J, Higgins J, Altman D. Analysing data and undertaking meta-analyses. In: Higgins J, Green S (eds.). Cochrane Handbook for Systematic Reviews of Interventions. Chichester, UK: John Wiley & Sons, 2008.

[45] Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics*. 1994;50:1088–1101.

[46] Higgins J, Altman D. Assessing risk of bias in included studies. In: Higgins J, Green S (eds.). Cochrane Handbook for Systematic Reviews of Interventions. Chichester, UK: John Wiley & Sons, 2008.

[47] NRC. National Research Council. Nutrient Requirements of Rabbits. Washington, DC: National Academic of Science, 1977.

[48] Lipsey MR, Wilson DB. Practical Meta-analysis. Thousand Oaks, CA: Sage, 2001. pp. 122–124.

[49] Kratz M. Dietary cholesterol, atherosclerosis and coronary heart disease. Handb Exp Pharmacol. 2005;170:195–213.

Chapter 2

Sex and Age Differences in Lipoprotein Metabolism Proatherogenic Changes under the Experimental Metabolic Syndrome in Hamsters

A. Zagayko, G. Kravchenko, K. Strelchenko,
A. Shkapo and T. Briukhanova

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/60759

Abstract

The unbalanced high-calorie diet can be the cause of a number of pathological states, including metabolic syndrome (MS). It is well known that the risk of MS increases with age, but gender differences in age-related lipid metabolism changes under this pathology are not fully understood.

In order to investigate the mechanisms of atherogenic dyslipidemia under the MS, we study the dynamics of some parameters of lipid and lipoprotein metabolism in hamsters of different sex and age. In our experiments, we found some age and gender differences in lipid and lipoprotein metabolism in healthy hamsters and hamsters with MS. In general, the obtained results demonstrate dyslipidemia development in males feeding high-calorie diet, irrespective of age. We suppose that hypertriglyceridemia in males under the high-calorie diet developed due to the accumulation of triacylglycerols (TAGs) in hepatocytes and as a result very low density lipoprotein 1 (VLDL1) over secretion by liver. However, in females feeding high-calorie diet atherogenic dyslipoproteinemia develops only with aging. It can be assumed that the reason why high-calorie diet in females leads to the pathological changes in VLDL morphology and hypertriglyceridemia development is reducing the hepatocytes sensitivity to insulin. Herewith, insulin resistance in females does not cause lipolysis activation in adipose tissue, which is probably associated with the ability of female

sex hormones to suppress lipolysis in adipose tissue regardless of sensitivity to insulin.

Keywords: lipoprotein metabolism, metabolic syndrome, hamsters, age differences

1. Introduction

Metabolic syndrome (MS) is the complex of hormonal and metabolic disorders that increase the risk of type 2 diabetes mellitus and cardiovascular system (CVS) diseases [59, 78]. It was found the close pathogenic link between obesity, hypertension, insulin resistance (IR) and atherogenic dyslipidemia in the 60's of the last century [54].

According to clinical observations, MS was already registered in 20–25% of the adult population of the industrialized countries in 2004 [36, 61]. In epidemiological studies was found that among examined 8814 men and women older than 20 years the incidence of MS according to the US National Cholesterol Education Program (NCEP) criteria was about 24% among men and 25% among women in the USA [5, 6]. In another epidemiological study examining men of all ages, selected by random sampling, MS was diagnosed in 26.2% of the cases [37]. This prevalence of MS in the population increases with age and is highest among the elderly [24, 38]. In the results of other research groups related to the analysis of men and women, conducted in the USA, MS according to NCEP criterion was diagnosed in 6.7% people aged 20–29 years, in 43.5% people aged 60–69 years, and in 42% people aged 70 years [37,38]. It is also known that MS at a younger age is more common in men, but in women the incidence of MS increases gradually with age – especially during menopause [13, 78].

However, gender differences in age-related lipid metabolism changes under MS are not fully understood. In order to investigate the mechanisms of atherogenic dyslipidemia under MS, we study the dynamics of some parameters of lipid and lipoprotein metabolism in hamsters of different sex and age.

2. Material and methods

Experiments were planned to develop a diet-induced MS in Golden Syrian hamsters of different sex and age (4 weeks, 20 weeks, 1 year at the beginning of the experiment), which were kept in a standard vivarium condition. Animals were fed a standard normal diet (intact group), and for 5 weeks a high-calorie diet that contained 29% of fats (predominantly saturated) with fructose addition – 1 g daily per 100 g body weight (MS groups) [27, 63]. Blood and liver samples were taken after decapitation in necessary terms and prepared according to individual procedures.

Experiments were carried out according to the "European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes" (Strasbourg, 1985).

http://dx.doi.org/10.5772/60759

Lipoprotein fractions (very low density lipoproteins (VLDL); low density lipoproteins (LDL), and high density lipoproteins (HDL)) were determined using electroforesis. Total LDL and apoB-containing lipoproteins (apoB-LP) in blood serum and hepatic cytosol were determined by gradient gel electrophoresis [1]. Using these data (apoB-LP concentration and data of LP fractions percentage), we calculated the content of every LP fraction. Total lipids (TL) were fractionated by thin layer chromatography on the plates with silica layers Silufol U.V.254'' (Sklarny Kavalier, Czech Republic).

Triacylglycerol (TAG) content was determined by enzymatic assay ("KONE," Finland).

Free and esterified cholesterol (CE) was determined by enzymatic assays ("Boehringer Mannheim GmbH diagnostica," Germany). Total lipid concentration was determined by standard test using vanillin reagent (Eagle Diagnostics, USA).

Cholesterol esterification rate and cholesterol ester (CE) transfer was estimated in the HDL fraction received by centrifugation and then incubation of material and measuring of cholesterol and CE before and after incubation (for determination of cholesterol esterification rate – adding 5,5′-dithiobis(2-nitrobenzoic acid) [82].

Lipoprotein lipase (LPL, EC 3.1.1.34) activity and hepatic triglyceride lipase (HL, EC 3.1.1.3) were determined by the method of Lithell and Boberg [52].

Glucose-6-phosphate dehydrogenase (G6PDH, EC 1.1.1.49) activity was measured using assay kit (Cayman Chemical, USA), 6-phosphogluconate dehydrogenase (6PGD, EC 1.1.1.44) using assay kit (Biocompare, USA), and malate dehydrogenase (EC 1.1.1.40) using assay kit (Biovision, USA).

Lysosomal acid lipase (LAL, EC 3.1.1.3) activity was measured in hepatic mitochondrial/lysosomal fraction by the substrate hydrolysis – 4-methylumbelliferone determined fluorometrically (E=449 nm, 410 nm) [86]. Protein content was determined by Lowry in Miller modification.

Statistical analysis was performed using nonparametric van der Waerden criterion [21, 87] with packet Excel and Statistica, and the correlation coefficient was calculated by Spearman.

3. Results

Changes in blood hormone levels observed under the MS led to a shift in the lipolysis/lipogenesis balance and were accompanied by the excessive production of the free fatty acids (FFA).

According to our data, blood FFA levels in animals fed high-calorie diet were significantly increased in all experimental groups except the young females (Table 1).

Indeed, the FFA level was increased by approximately 40% in male experimental animals independently of age, and in young females this index was practically unchanged, but in the adult it increased also by 40% under experimental pathology. However, even such increased FFA level was 17% lower than in intact males of the same age.

Sex	Age	Group	Free fatty acids content, mmol/L
Males	4 weeks	Intact	1.02±0.07
		MS	1.44±0.29*
	20 weeks	Intact	1.64±0.16
		MS	2.29±0.25*
Females	4 weeks	Intact	0.91±0.42
		MS	0.85±0.03
	20 weeks	Intact	0.85±0.04
		MS	1.20±0.14*

Intact groups – animals fed standard normal diet aged 4 weeks and 20 weeks at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 4 weeks and 20 weeks at the beginning of the experiment. Each group was composed of six animals. Mean±S.D. * – p≤0.05 vs the same age intact group.

Table 1. The serum free fatty acids content in male Syrian hamsters with the experimental metabolic syndrome

Our study of lipid and lipoprotein metabolism in the blood serum and liver under modeling MS in Syrian hamsters of different sex and age also indicates the significant changes in lipid metabolism as well as sex and age differences of lipid and lipoprotein metabolism in the health animals and under the experimental MS.

Age	Group	Indices			
		Triacylglycerols, g/L	Total cholesterol, mmol/L	apoB-containing lipoproteins, g/L	High density lipoproteins, g/L
4 weeks	Intact	1.06±0.07	2.93±0.19	4.72±0.23	1.11±0.05
	MS	1.56±0.09*	3.56±0.10*	6.68±0.15*	0.98±0.07
20 weeks	Intact	1.57±0.22	2.84±0.15	5.66±0.34	1.01±0.02
	MS	2.00±0.13*	3.71±0,18*	6.68±0.21*	0.85±0.08
1 year	Intact	1.50±0.10	2.73±0.02	5.21±0.06	1.74±0.13
	MS	2.27±0.13*	3.15±0.08*	7.00±0.22*	2.32±0.13*

Intact groups – animals fed standard normal diet aged 4 weeks, 20 weeks, and 1 year at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 4 weeks, 20 weeks, and 1 year at the beginning of the experiment. Each group was composed of six animals.

Mean±S.D. * – p≤0.05 vs the same age intact group.

Table 2. Some indices of lipid metabolism in blood serum of male Syrian hamsters with the experimental metabolic syndrome

http://dx.doi.org/10.5772/60759

Atherogenic dyslipidemia develops independently of age in males fed high-calorie diet (Table 2). As it can be seen from the data presented, increase of total lipids content in the blood serum of animals is mediated by the increase of apoB-LP level because the HDL content did not change. Herewith, the serum TAG level rose by 47% and 30% relative to intact groups in young and adult animals, respectively (Table 2).

According to our data the accumulation of apoB-LP in the blood and increasing of these class lipoproteins content in the liver undergo simultaneously (Table 3).

Age	Group	Indices				
		Total lipids (TL), mg/g	apoB-containing lipoproteins, mg/g	High density lipoproteins, mg/g	Glucose-6-phosphate dehydrogenase, nmol / mg of protein×min	Lysosomal acid lipase, nmol / mg of protein× min
4 weeks	Intact	104.24±2.52	11.46±0.37	1.25±0.14	3.74±0.33	0.67±0.03
	MS	124.16±2.05*	15.16±0.54*	1.11±0.07	2.80±0.17*	1.09±0.07*
20 weeks	Intact	112.62±2.66	13.03±0.50	0.94±0.10	4.44±0.28	0.54±0.03
	MS	143.59±2.65*	15.69±0.36*	1.10±0.20	3.13±0.28*	1.27±0.09*

Intact groups – animals fed standard normal diet aged 4 weeks and 20 weeks at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 4 weeks and 20 weeks at the beginning of the experiment. Each group was composed of six animals. Mean±S.D. * – p≤0.05 vs the same age intact group.

Table 3. Some indices of lipid metabolism in the liver of male Syrian hamsters with the experimental metabolic syndrome (for the damp tissue)

The significant changes in the lipid and lipoprotein metabolism were observed in the liver of adult male hamsters (1 year old) fed high-calorie diet (Tables 4 and 5). In particular, the changes of apoB-LP composition in the liver (Table 4) led to lipid depletion by lipoproteins.

Indices	Group	
	Intact	MS
Total cholesterol, % of the total content of fraction	9.46±0.81	7.18±0.06*
Triacylglycerols, % of the total content of fraction	45.33±1.39	42.00±1.29*

Intact group – animals fed standard normal diet aged 1 year at the beginning of the experiment. MS group – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 1 year at the beginning of the experiment. Each group was composed of seven animals. Mean±S.D. * – p≤0.05 vs intact group.

Table 4. The composition of apoB-containing lipoproteins post mitochondrial fraction in liver of the 1-year-old male Syrian hamsters with the experimental metabolic syndrome

The low lipid content, predominantly TAGs, in the hepatic apoB-LP composition observed in our experiments (Table 4) indicated the lipolysis activation through the triacylglycerol lipases action (e.g., HL) under experimental MS.

Herewith, the lipoprotein uptake by the liver under experimental MS, obviously, is enhanced, as the content of the apoB-LP in this organ was increased (Table 5).

The liver G6PDH activity in experimental animals of this age group declined by 35% compared to the intact group (Table 5).

As can be seen from the obtained data, the 6PGD that is less than G6PDH is sensitive to damage by free radicals, retained its activity, and the NADP-dependent malate dehydrogenase activity even increased under experimental MS (Table 5).

Indices	Group	
	Intact	MS
Glucose-6-phosphate dehydrogenase, nmol NADPH(H+)/min× mg of protein	4.02±0.17	2.62±0.28*
6-phosphogluconate dehydrogenase, nmol NADPH(H+)/min× mg of protein	1.98±0.15	2.20±0.15
Malate dehydrogenase, nmol NADPH(H+)/min× mg of protein	14.57±0.40	15.09±0.03*
Total lipids, g/100 g for the fresh tissue	11.36±0.69	14.97±0.75*
apoB-containing lipoproteins, mg/g for the fresh tissue	14.71±0.46	18.92±0.84*

Intact group – animals fed standard normal diet aged 1 year at the beginning of the experiment. MS group – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 1 year at the beginning of the experiment. Each group was composed of seven animals. Mean±S.D. * – p≤0.05 vs intact group.

Table 5. Some indices of lipid metabolism in the liver of 1-year-old male Syrian hamsters with the experimental metabolic syndrome

The lowering HDL-cholesterol level is apparently associated with an increased rate of CE transfer from HDL to apoB-LP. According to our data, the CE transfer rate from HDL in animals fed high-calorie diet was increased by 166% and 199% relative to young and adult intact animals, respectively (Table 6).

We recorded the decrease of serum LPL activity and increase in HL activity in young males fed high-calorie diet (Table 7). This may be an additional factor for TAG accumulation in the blood and decrease of the HDL-cholesterol level that we observed in our experiments (see Tables 4 and 6).

Some age-related features in the serum lipid profile were found in the healthy male hamsters with aging (from 4 to 20 weeks). Thus, levels of serum FFA (60%), TAGs (48%), and apoB-LP (20%) increased in 4 weeks old intact males, but the HDL level tended to decrease in 4 weeks

http://dx.doi.org/10.5772/60759

old intact males with the unchanged total lipid an lipoprotein content in the blood serum. All this testifies that lipidosis develops with aging. Also we found out that in adult males, the free cholesterol and cholesterol ester levels were lower than in young animals (20% and 25%, respectively), and the cholesterol ester transfer rate from HDL in adult animals exceeded this index in young animals 191% (Table 6).

Age	Group	Indices			
		HDL cholesterol, mcmole / L	HDL cholesterol esters, in mcmole / L	Cholesterol etherification, mcmole / L × hour	Transfer of cholesterol esters, mcmole / L ×hour
4 weeks	Intact	174.17±18.99	1028.33±12.76	54.92±0.58	20.42±1.76
	MS	80.83±9.17*	810.00±22.78*	49.00±2.50	33.83±1.56*
20 weeks	Intact	138.00±8.00	770.00±32.56	45.50±2.55	59.50±5.39
	MS	164.50±9.97	512.50±0.01*	20.25±2.28*	116.88±9.43*

Intact groups – animals fed standard normal diet aged 4 weeks and 20 weeks at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 4 weeks and 20 weeks at the beginning of the experiment. Each group was composed of six animals. Mean±S.D. * – p≤0.05 vs the same age intact group.

Table 6. The HDL-cholesterol and HDL-cholesterol esters content, cholesterol esterification rate, and cholesterol esters transfer in blood serum of male Syrian hamsters with experimental metabolic syndrome

Age	Group	Indices	
		Lipoprotein lipase (U/ml)	Hepatic triglyceride lipase(U/ ml)
4 weeks	Intact	8±2	51±4
	MS	4±1*	91±3*
20 weeks	Intact	83±2	3±1
	MS	129±3*	2±1

Intact groups – animals fed standard normal diet aged 4 weeks and 20 weeks at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 4 weeks and 20 weeks at the beginning of the experiment. Each group was composed of six animals. Mean±S.D. * – p≤0.05 vs the same age intact groups.

Table 7. The post-heparin plasma lipase activities in male Syrian hamsters with experimental metabolic syndrome

The atherogenic dyslipidemia development significantly depends on the age in females in contrast to males (Table 8).

Age	Group	Indices				
		Glucose-6-phosphate dehydrogenase, nmol/min×mg of protein	Total lipids (TL), mg/g	apoB-containing lipoproteins, mg/g	HDL, mg/g	Lysosomal lipase, nmol/ min×mg of protein
4 weeks	Intact	4.72±0.17*	117.67±4.72	8.87±0.24	1.27±0.08	0.34±0.03
	MS	5.38±0.13*	144.34±5.00*	10.24±0.25*	0.65±0.05*	1.24±0.05*
10 weeks	Intact	5.15±0.22	137.54±3.91	10.65±0.46	0.89±0.07	0.83±0.04
	MS	5.80±0.15	179.22±3.44*	13.44±0.30*	0.46±0.06*	1.33±0.08*

Intact groups – animals fed standard normal diet aged 4 weeks and 20 weeks at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 4 weeks and 20 weeks at the beginning of the experiment. Each group was composed of six animals. Mean±S.D. * – $p \leq 0.05$ vs the same age intact groups.

Table 8. The indices of lipid metabolism in liver homogenate of female Syrian hamsters with experimental metabolic syndrome (for the damp tissue)

In particular, while significant changes in liver apoB-LP content in males was not observed with aging, this index in females increased at growing up in intact animals by 20%, and in animals with experimental MS by 31%. This indicates intensification of liver lipolytic processes in females with aging and may be a manifestation of the lipid metabolism activation that is proved by similar changes in TL content (Table 8).

Moreover, the female liver contains more lipids than male, especially in adulthood – in intact by 22%, while the MS by 24%. This can be explained by the well-known more pronounced effect of estradiol on liver lipid metabolism intensity compared to testosterone.

However, the G6PDH activity in females was significantly higher than in males, especially under MS by 92% in the young ones, and 85% in the adults (Table 8). In addition, this enzyme activity increased with aging. It can also indicate a significant dependence of the liver lipid metabolism rate from hormonal background. As can be seen from the obtained data (Table 9), feeding high-calorie diet did not lead to pronounced atherogenic changes in serum lipid and lipoprotein spectrum in young hamsters-females. The fact that young females had a more favorable serum lipid profile compared to males of the same age group also attracts attention (Tables 9, 10). Thus, the serum total lipid level in young females was 35% lower compared to males, and the total lipoprotein level lower by 32% (in young intact females 4.01±0.31 mg/ml).

Thus, lower serum total lipoprotein level in females may be associated with the decrease of the apoB-LP content (the content of this lipoprotein atherogenic faction was at 39% lower in females compared to males, and the HDL content was similar in animals of both sexes).

As we can see in Tables 9 and 10, in females cholesterol metabolism also was changed in the blood stream, and these changes clearly depended on age: in young females the activity of CE transfer and the cholesterol esterification rate was increased, in the adults was increased only CE transfer, at the same time, the activity of cholesterol esterification rate decreased.

http://dx.doi.org/10.5772/60759

Age	Group	Indices			
		Triacylglycerols, g/L	Total cholesterol, mmol/L	apoB-containing lipoproteins, g/L	HDL, g/L
4 weeks	Intact	0.79±0.04	2.32±0.34	2.92±0.34	1.17±0.07
	MS	0.81±0.04	2.00±0.07	3.24±0.25	1.22±0.06
20 weeks	Intact	0.97±0.03	2.09±0.07	4.40±0.25	0.99±0.01
	MS	2.14±0.06*	1.91±0.17	3.57±0.12*	0.68±0.06*
1 year	Intact	1.48±0.14	2.54±0.08	4.03±0.07	1.85±0.23
	MS	2.20±0.09*	2.40±0.04	3.50±0.10	0.75±0.06*

Intact groups – animals fed standard normal diet aged 4 weeks, 20 weeks, and 1 year at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 4 weeks, 20 weeks, and 1 year at the beginning of the experiment. Each group was composed of six animals. Mean±S.D. * – p≤0.05 vs the same age intact groups.

Table 9. Some indices of lipid metabolism in blood serum of female Syrian hamsters with experimental metabolic syndrome

Serum TAG level and total cholesterol in young females was lower by 26% and 21% as compared to the corresponding values of these parameters in males, and the HDL-cholesterol in females exceeded the value of this index in males by 32% (Tables 9 and 10).

Age	Group	Indices			
		HDL-cholesterol, mcmol/L	HDL-cholesterol esters, mcmol/L	Cholesterol esterification, mcmole / L × hour	Cholesterol esters transfer, mcmole / L ×hour
4 weeks	Intact	230.83±7.46	1004.17±3.75	45.58±4.56	10.75±0.80
	MS	208.83±5.19*	835.50±20.53*	64.33±4.92*	23.83±3.53*
20 weeks	Intact	258.33±13.08	715.83±48.14	80.17±5.02	18.67±1.30
	MS	123.33±7.60*	650.00±22.36	34.25±3.14*	32.08±1.50*

Intact groups – animals fed standard normal diet aged 4 weeks and 20 weeks at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 4 weeks and 20 weeks at the beginning of the experiment. Each group was composed of six animals. Mean±S.D. * – p≤0.05 vs the same age intact groups.

Table 10. The HDL-cholesterol and HDL-cholesterol esters content, the cholesterol etherification activity and cholesterol ester transfer rate in blood serum of female Syrian hamsters with experimental metabolic syndrome

According to our data, the CE transfer rate in young females was 48% less than it was in the serum of males of the same age group (Table 10). The HL activity in intact young females was 28% less in comparison with males (Table 11).

Age	Group	Indices	
		Lipoprotein lipase (U/ml)	Hepatic triglyceride lipase (U/ ml)
4 weeks	Intact	12±1	37±2
	MS	7±1*	66±7*
20 weeks	Intact	9±1	47±2
	MS	5±2*	94±6*

Intact groups – animals fed standard normal diet aged 4 weeks and 20 weeks at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 4 weeks and 20 weeks at the beginning of the experiment. Each group was composed of six animals. Mean±S.D. * – $p \leq 0.05$ vs the same age intact groups.

Table 11. The post-heparin plasma lipase activities in female Syrian hamsters with experimental metabolic syndrome

However, it is interesting that female hamsters have more favorable (antiatherogenic) initial lipid serum profile than males. Thus, the total lipid content in intact young females was lower than in males by 35%, and it was almost 25% in adults. This index was almost two times lower than in males even with the MS in young females. Only adult females and males with experimental pathology hardly differed from each other. The same tendency is typical for the total lipoprotein content and TAG level.

The serum cholesterol content increased by 22% in juvenile age and by 31% in male adults with MS. However, the corresponding index in females remained practically unchanged, which confirms the absence of a direct correlation between the MS development and hypercholesterolemia. At the same time, there were the changes in lipoprotein fractions: in males with the MS the level of apo-B-LP increased (almost by 20% in adulthood), and the HDL content did not change. On the contrary, HDL level with MS decreased by 32% in adult females, and the content of the apoB-LP remained unchanged.

In intact males aged 4–20 weeks under unchanged total serum lipid and lipoprotein content, the levels of following values increased: TAG (by 48%), FFA (by 60%), and apoB-LP (by 19%). However, the HDL level tended to decrease. There were almost the same changes under experimental MS: the TAG level in adult animals with MS was higher by 28% as compared to the young animals with experimental pathology, FFA level – by 59%, HDL level – decreased by 14%, although the level of apoB-LP remained the same (see Tables 1 and 2).

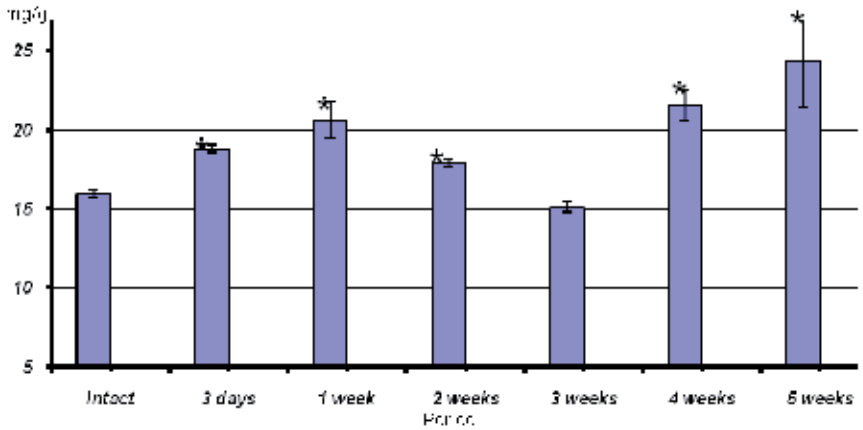
All these data indicate to the hyperlipidemia increase due to age, which is further enhanced by the hyperinsulinemia and IR development. In females these changes were even more pronounced, although not quite so dramatic. In intact females the total lipid content grew with age by 37% (compared with 24% for males), lipoprotein by 32% (although it was lower than 21% for males), TAG by 22% (less than 39% for males), and apoB-LP by 50% (in males it was more than 33%), and the HDL content decreased by 16% (see Tables 1 and 9).

http://dx.doi.org/10.5772/60759

The situation under MS became worse: increase of the TL level in females having MS with aging was 88%, total lipoproteins was 81%, and TAGs was 164%, which was slightly higher than the corresponding indices in males. However, increased apoB-LP content in females with aging under MS was smaller than in the intact animals and 47% less than males in the older age group (see Tables 8 and 9).

For the next series of experiments we used 1-year-old male and female Syrian hamsters that are most likely to MS development and study pathological changes caused by high-calorie diet during 5 weeks in dynamics.

The liver TAG content in the animals fed high-calorie diet increased after 3 days of experiment and remained at a high level in further periods (see Figure 1). The presence of a significant positive correlation between the liver and serum TAG content in the experimental animals (correlation coefficient 0.9) confirms the leading role of the intracellular TAG content increase in the formation of hypertriacylglycerolemia in our experiments.

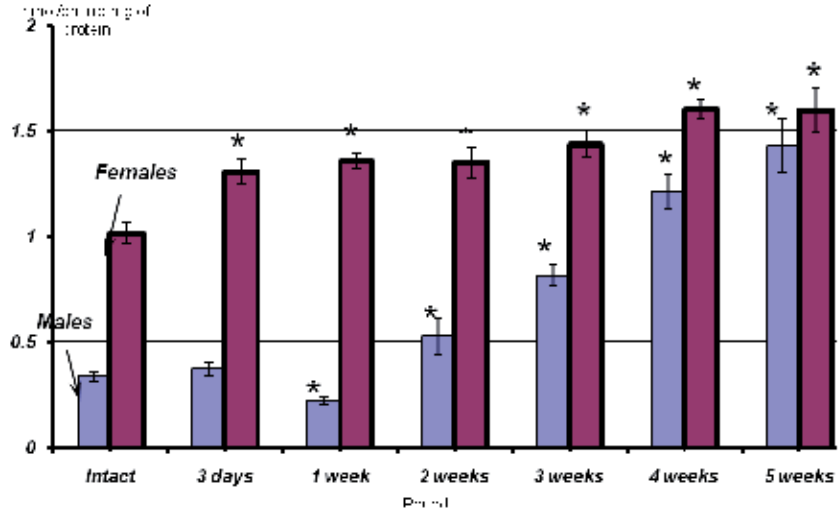


Each group was composed of seven animals. Mean±S.D. * – p≤0.05 vs intact group.
Intact groups – animals fed standard normal diet aged 1 year at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 1 year at the beginning of the experiment.

Figure 1. The liver triglyceride content in male 1-year-old Syrian hamsters under the experimental metabolic syndrome development (mg/g fresh tissue).

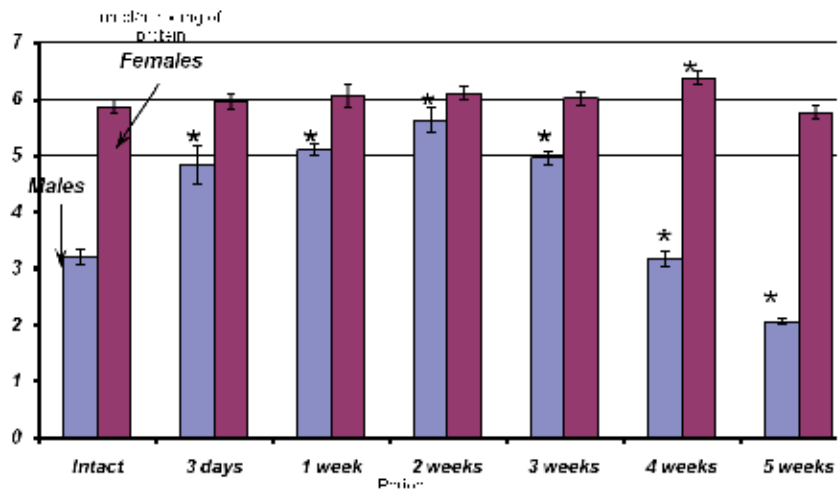
Based on our data, the LAL activity, which reflects the lipoproteins absorption intensity from the blood, decreased in the liver of experimental males at the beginning of our experiments, and it did not change significantly in females, and the enzyme activity increase was observed only after 2 weeks (Figure 2).

As can be seen from the data shown in the Figure 3, the G6PDH activity in males was increasing at the beginning of the experiment, probably because of pentose phosphate pathway activation, and after that it was decreasing, probably due to the lipid peroxidation (LPO) activation. As for females this rate was not changed significantly during the experiment.



Each group was composed of seven animals. Mean±S.D. * – p≤0.05 vs intact group.
Intact groups – animals fed standard normal diet aged 1 year at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 1 year at the beginning of the experiment.

Figure 2. The liver lysosomal acid lipase activity in male and female hamsters under the experimental metabolic syndrome.

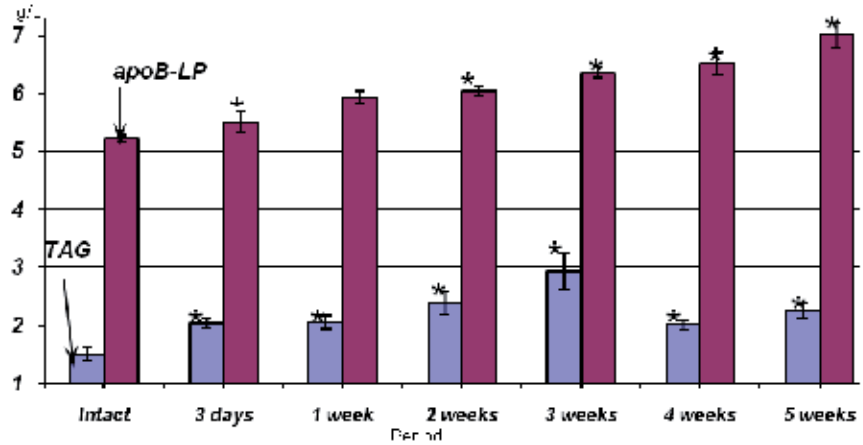


Each group was composed of seven animals. Mean±S.D. * – p≤0.05 vs intact group.
Intact groups – animals fed standard normal diet aged 1 year at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 1 year at the beginning of the experiment.

Figure 3. The liver glucose-6-phosphate dehydrogenase activity in 1-year-old male and female Syrian hamsters under the experimental metabolic syndrome development.

http://dx.doi.org/10.5772/60759

As can be seen from the data obtained (Figure 4), the severe hypertriacylglycerolemia was developing fairly quickly in males fed high-calorie diet during the whole study period. Serum TAG content in the experimental animals increased after 3 days from the beginning of the experiment and reached its maximum value after 3 weeks (197% in regards to intact), and remained at a high level in the subsequent periods (Figure 4).



Each group was composed of seven animals. Mean±S.D. * – p≤0.05 vs intact group.
Intact groups – animals fed standard normal diet aged 1 year at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 1 year at the beginning of the experiment.

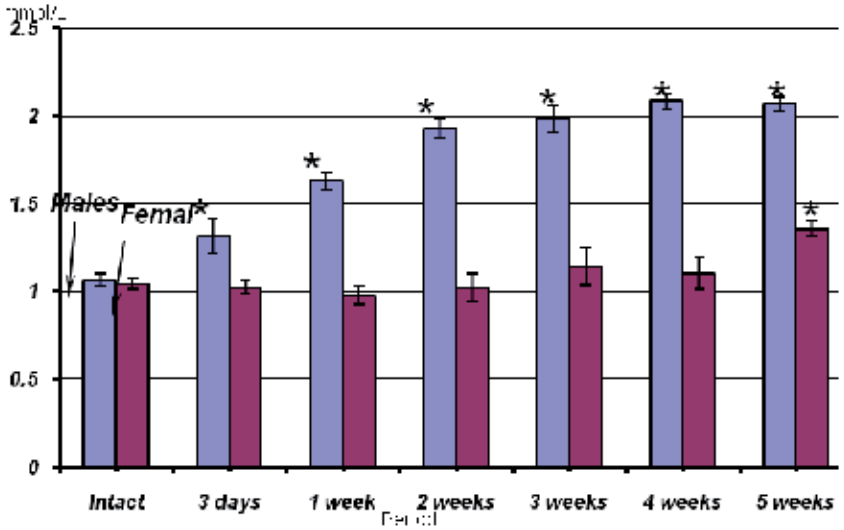
Figure 4. The serum triacylglycerols and apoB-containing lipoproteins content in 1-year-old male Syrian hamsters under the experimental metabolic syndrome development.

At the same time, according to our data, feeding high-calorie diet increased serum apoB-LP content in experimental animals, but the elevated levels of these lipoprotein fractions was observed at later time periods and was relatively less pronounced in comparison with the increased serum TAG levels (Figure 4).

The serum FFA content in animals also was increased after 3 days of the experiment and was increasing in subsequent periods as well (Figure 5).

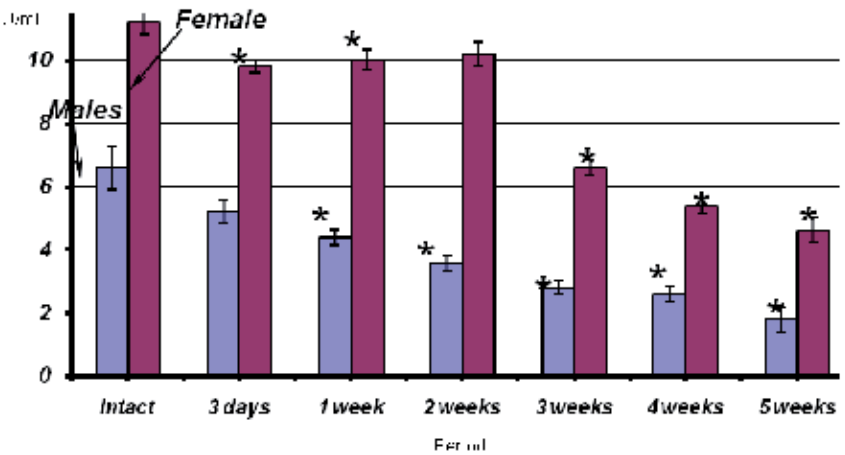
In our experiments, the LPL activity was decreased rapidly since 3 days, during all the study periods (Figure 6), which indicates the stable disorders in VLDL utilization, and may be an additional factor that contributes to the hypertriacylglycerolemia development.

The abnormal cholesterol transport between different subfractions of lipoprotein particles, which leads to the blood atherogenic profile formation, is under discussion. As we have already noted, in our experiments the CE transfer rate was enhanced and this was already observed in the early stages of MS developing (Figure 7). It correlates with the serum TAG content increasing (the correlation coefficient is 0.77) and suggests that changes in apoB-LP morphology is one of the earliest manifestations of MS proatherogenic process.



Each group was composed of seven animals. Mean±S.D. * – p≤0.05 vs intact group.
Intact groups – animals fed standard normal diet aged 1 year at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 1 year at the beginning of the experiment.

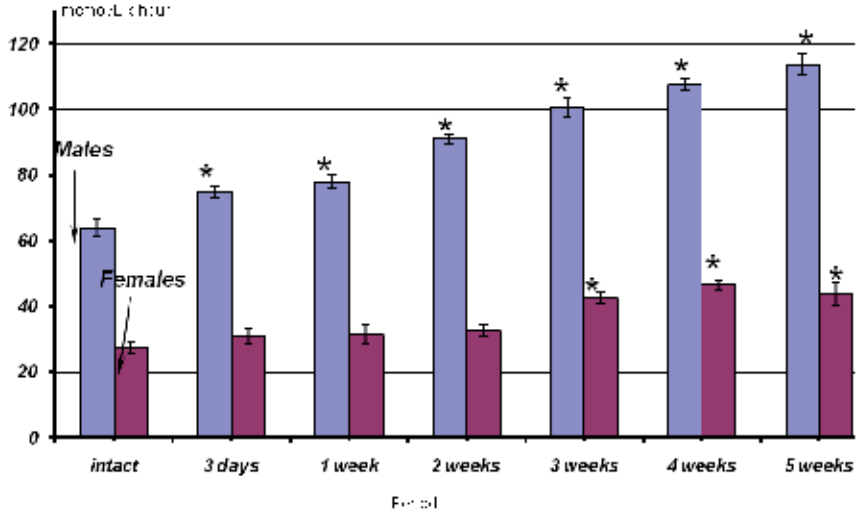
Figure 5. The serum free fatty acids content in 1-year-old male Syrian hamsters under the experimental metabolic syndrome development.



Each group was composed of seven animals. Mean±S.D. * – p≤0.05 vs intact group.
Intact groups – animals fed standard normal diet aged 1 year at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 1 year at the beginning of the experiment.

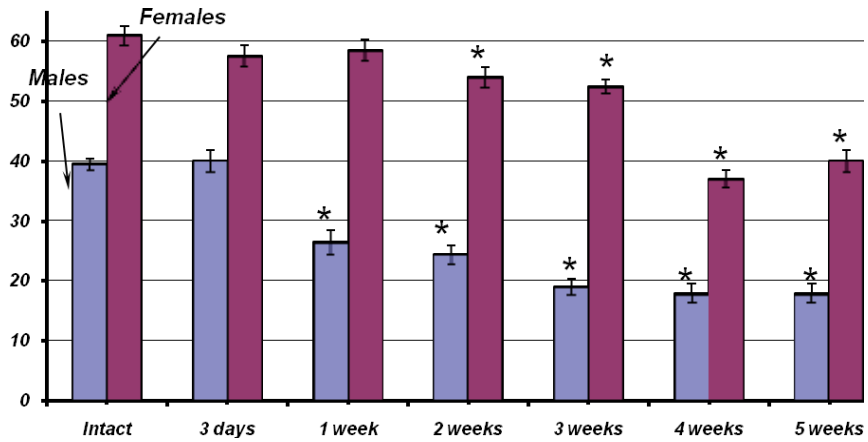
Figure 6. The serum lipoprotein lipase activity in 1-year-old male and female Syrian hamsters under the experimental metabolic syndrome.

http://dx.doi.org/10.5772/60759



Each group was composed of seven animals. Mean±S.D. * – p≤0.05 vs intact group.
Intact groups – animals fed standard normal diet aged 1 year at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 1 year at the beginning of the experiment.

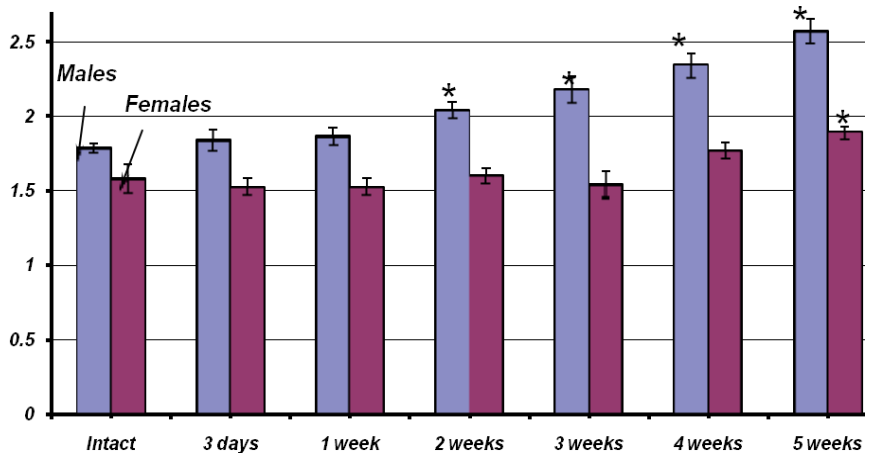
Figure 7. The cholesterol ester transfer rate in serum of 1-year-old male and female Syrian hamsters under the experimental metabolic syndrome development.



Each group was composed of seven animals. Mean±S.D. * – p≤0.05 vs intact group.
Intact groups – animals fed standard normal diet aged 1 year at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 1 year at the beginning of the experiment.

Figure 8. The cholesterol esterification rate in serum of 1-year-old male and female Syrian hamsters under the experimental metabolic syndrome.

The changes in CE transfer activity can be accompanied by cholesterol metabolism changes in the LP composition. In particular, the HDL-cholesterol esterification rate had already decreased to the second week of the experiment in all investigated animals of this age group (Figure 8). However, the overall CE level in these antiatherogenic lipoproteins was decreased (Tables 5, 6, and 10). And this fact again underlines the significant role in CE transfer activation between different classes of lipoproteins in proatherogenic changes of lipid metabolism under the MS.



Each group was composed of seven animals. Mean±S.D. * – p≤0.05 vs intact group.
Intact groups – animals fed standard normal diet aged 1 year at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 1 year at the beginning of the experiment.

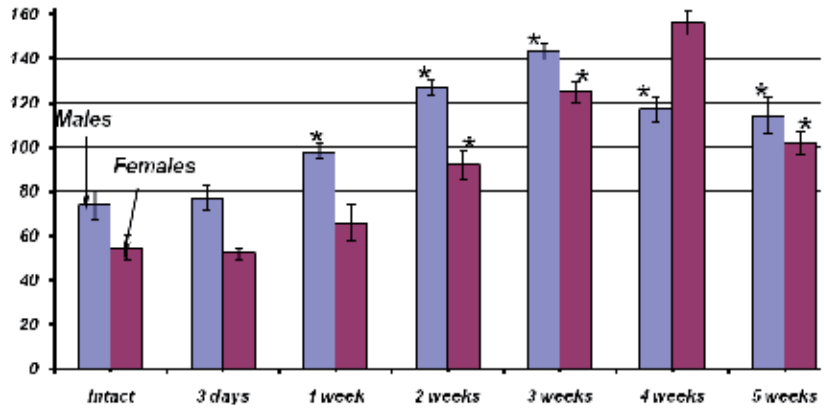
Figure 9. The serum esterified cholesterol content in 1-year-old male and female Syrian hamsters under the experimental metabolic syndrome development.

At the same time, the total CE content in the blood of experimental animals was slightly increased (Figure 9), which is the consequence of the overall lipids accumulation in the blood and liver, and may be associated with more active hepatic cholesterol esterification under the growth of FFA absorption by this organ from the blood. It is known that CE along with the TAG is the transport form of FA; therefore, its number increase should lead to the activation of hepatic CE formation.

Compared with changes in cholesterol metabolism and transfer, HL activity was not increased so quickly with just one week since the beginning of the experiment and remains at a high level in subsequent periods (Figure 10).

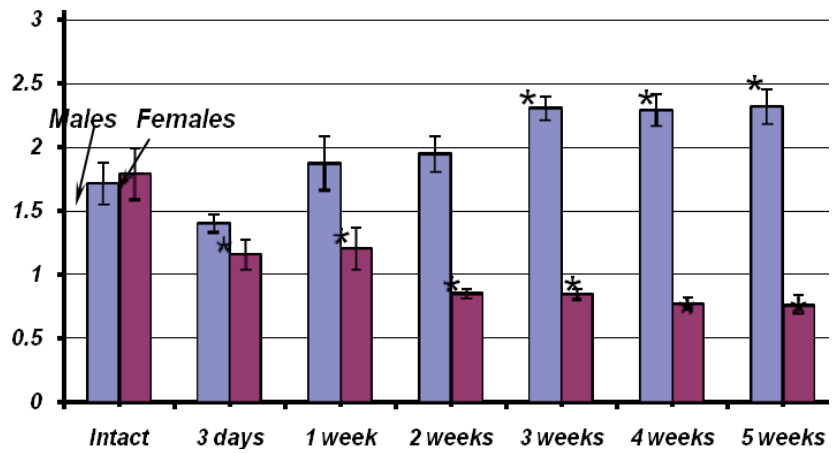
The opposite tendency in the HDL content changes in animals of different sex also attracts attention (Figure 11): we found out that HDL content reduced in females and increased in males in the last stages of MS development, but, as we already pointed out, it was decreased in animals of both sexes in the beginning of the experiment.

http://dx.doi.org/10.5772/60759



Each group was composed of seven animals. Mean±S.D. * – p≤0.05 vs intact group.
Intact groups – animals fed standard normal diet aged 1 year at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 1 year at the beginning of the experiment.

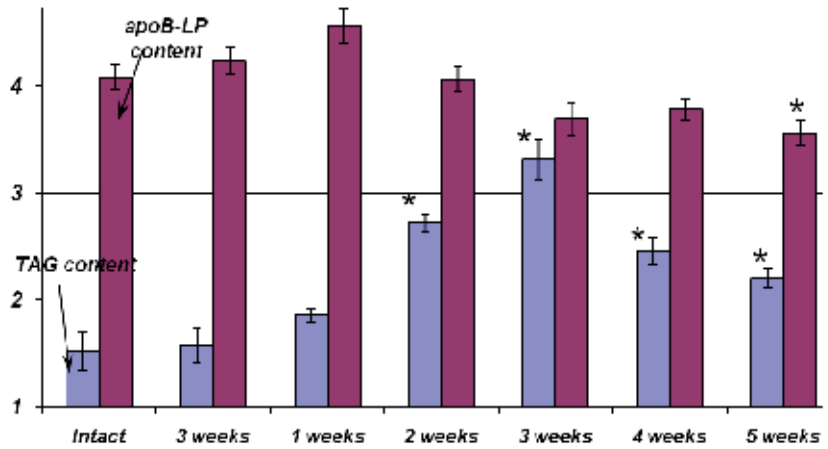
Figure 10. The serum hepatic triglyceride lipase activity in 1-year-old male and female Syrian hamsters under the experimental metabolic syndrome development.



Each group was composed of seven animals. Mean±S.D. * – p≤0.05 vs intact group.
Intact groups – animals fed standard normal diet aged 1 year at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 1 year at the beginning of the experiment.

Figure 11. The serum high-density lipoproteins content in 1-year-old male and female Syrian hamsters under the experimental metabolic syndrome development.

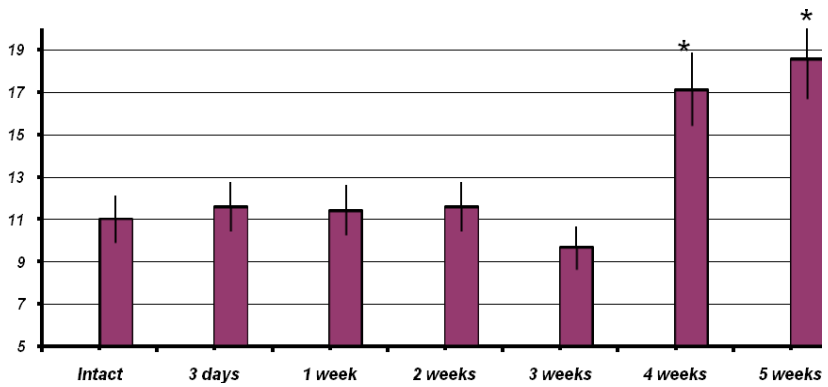
Unlike in males, serum TAG content did not change during the first week in females fed high-calorie diet, and the increase of this index values was observed only after 2 weeks from the beginning of the experiment (Figure 12).



Each group was composed of seven animals. Mean±S.D. * – p≤0.05 vs intact group.
Intact groups – animals fed standard normal diet aged 1 year at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 1 year at the beginning of the experiment.

Figure 12. The serum triacylglycerols and apoB-containing lipoproteins content in 1-year-old female Syrian hamsters under the experimental metabolic syndrome development.

At the same time, in the serum of females fed high-calorie diet the apoB-LP content did not differ from the intact level during the first 4 weeks of the experiment, and after 5 weeks we found out lower values of this index (Figure 12).



Each group was composed of seven animals. Mean±S.D. * – p≤0.05 vs intact group.
Intact groups – animals fed standard normal diet aged 1 year at the beginning of the experiment. MS groups – animals fed during 5 weeks high-calorie diet that contained 29% of fats with fructose addition (1 g daily per 100 g body weight) aged 1 year at the beginning of the experiment.

Figure 13. The liver triacylglycerol content in female Syrian hamsters under the experimental metabolic syndrome development.

http://dx.doi.org/10.5772/60759

The hepatic TAG content did not change during the first three weeks of feeding high-calorie diet and after 4 and 5 weeks since the beginning of the experiment, we found increased values of this index relative to intact 55% and 69%, respectively (Figure 13).

4. Discussion

The numerous experimental and clinical studies suggest that excessive body weight gain is associated with reduction of insulin potency to block lipolysis in adipose tissue [18, 32, 65, 92]. Suppression of lipolysis results in raising blood FFA level and intensifies their intake by insulin-dependent tissues, especially by the liver and muscles [36, 48]. Excessive FFA disrupts insulin binding to hepatocyte receptors and leads to the liver IR development. Such conditions cause gluconeogenesis activation, increase of glucose production by the liver, reduction of the insulin excretion rate, and as the result hyperglycemia and hyperinsulinemia development [48].

The intensive FFA uptake into the muscle cells disturbs the utilization and intracellular glucose metabolism in this tissue [8, 69]. The rates of glycolysis and glycogen synthesis are decreased in muscle cells, and also the uptake of glucose from the blood is considerably reduced. This enhances hyperglycemia and hyperinsulinemia and contributes to IR development.

Thus, raising blood FFA levels due to metabolic activity disorders in the adipose tissue can cause IR under obesity.

Numerous clinical studies proved [40, 72] that the FFA release rate from adipose tissue in women is lower compared with men. This regularity was also observed in obesity. For example, it was found [9] that the rate of FFA release from adipose tissue in men is twice higher compared with women under obesity.

Observed increasing of serum FFA content could be a result of lipid hydrolysis activation in adipose tissue under the body weight gain of animals in our experiment. Thereby (see Table 1), the data of our experiments indicated that the low serum FFA level maintained in young females fed high-calorie diet could be explained by gender differences involving estrogens in the regulation of adipose tissue lipolysis.

The molecular mechanisms that are the basis of these differences should be dependent on the different adipose tissue receptor activity in males and females. It is known [70] that the lipolysis regulation in adipose tissue is carried out mainly at the level of modulation of hormone-dependent lipases activity, particularly, by the insulin and catecholamines action. The activity of lipases is inhibited by insulin action. Catecholamines stimulate the activity of hormone-sensitive lipases indirectly via the β-adrenoreceptors (β-AR), and inhibit enzyme activity via α2-adrenoreceptors (α2-AR). Female sex hormones increase the number of α2-AR in women adipose tissue [58, 70]. It was found out that in women adipose tissue number of α2-AR is greater compared to men, while in men preponderate β-AR [16, 19, 77]. There is evidence that in women adipose tissue hormone-sensitive lipase has low sensitivity to the increased intracellular cAMP that is related to the lower protein kinase A (PKA) activity. It is also known

that estrogens are able to suppress lipolysis even at lower sensitivity of adipocytes to insulin. All of this evidence can determine the lower lipolytic activity in women adipose tissue compared to men.

The sex differences in the regulation of adipose tissue lipolysis become less pronounced with aging [43]. These differences can be associated with significant hormonal changes in women with aging and specifically depend on the decrease in the sex hormone levels [97], increased glucocorticoid hormones secretion, and reduced sensitivity to insulin. These changes increase the risk of MS development in women under obesity with aging.

According to our data, serum FFA level in adult females fed the high-calorie diet increased to the same level as that in same-age males (see Table 1). The latter can be associated with the age-related hormonal changes in the female body (correlation coefficient between age-related changes of FFA content and estradiol is 0.75).

Despite the differences in the release rate of FFA from adipose tissue, the feeding high-calorie diet leads to hyperglycemia and hyperinsulinemia development and aggravates insulin resistance in experimental animals, regardless of gender or age [96, 97]. This indicates that MS development in females is independent to adipose tissue lipolytic activity.

Thus, one of the main features of metabolic proatherogenic changes that we observed under experimental MS was a significant increase in serum FFA level. FFA overabundance could not affect the rest of the lipid metabolism links that leads to general lipid and lipoprotein metabolism disorders and is one of the key components of MS.

Clinical studies [15, 39, 76] show that dyslipidemia developed under MS is characterized by serum TAG level increase, HDL-cholesterol level decrease, and accumulation of LDL that have a high atherogenicity (LDL*B*).

The blood TAG content increase under MS is considered to be the key factor in the atherogenic dyslipidemia formation. A clear correlation between hypertriacylglycerolemia, HDL-cholesterol level decrease, and LDL*B* accumulation in the blood plasma demonstrated in numerous experimental and clinical studies [4, 39, 45, 54, 55, 74].

It is known that hepatic VLDL hyperproduction plays the leading role in the TAG and apoB-LP accumulation in blood under the MS development [62].

So we can suppose that VLDL hepatic production is activated in animals fed a high-calorie diet during our experiments.

Based on these data, we can suppose that lipolysis activation and FFA accumulation in the blood lead to morphological changes of lipoproteins that are secreted by the liver under the MS development.

Mechanisms of VLDL hyperproduction in the liver under FFA intensive uptake by hepatocytes steel remains not fully understood. This activation may occur both using FFA, which intensively enter from the blood, or via the stimulation of *de novo* fatty acids synthesis that is caused by hyperglycemia.

http://dx.doi.org/10.5772/60759

It is known that FFA, which enters into the liver cells from the blood, is mainly used for TAG resynthesis under IR. This leads to increased intracellular TAG content and correlated with increased VLDL secretion rate to the blood [22, 29]. The VLDL composition, which is determined primarily on the second stage of their formation, significantly depends on the intracellular TAG content and hepatocytes sensitivity to insulin [33]. The intense pre-VLDL lipidation involving phospholipase D takes place under the conditions of intracellular TAG content increase and hepatic insulin resistance [7, 64]. Insulin blocks the VLDL formation in the liver [7]. These changes, combined with the TAG intracellular content increase under the IR, determine mainly the VLDL1 formation and secretion by the liver.

In our experiments, the activation of *de novo* fatty acids synthesis in the liver, obviously, did not occur. The G6PDH activity decrease in this organ proved this suggestion (see Table 3). It is known that the activity of G6PDH, which is the main donator of NADP reduced, directly correlates with the lipogenesis activity [38]. A certain contribution to the VLDL formation activation in the liver of animals that was fed high-calorie diet probably makes remnant lipoproteins (RLPs) uptake by the hepatocytes from the blood stream. The increasing LAL activity in the liver of experimental animals (see Table 3) is the evidence of this process. LAL is involved in the RLPs degradation that enters hepatocytes by receptor-mediated transport.

We found the direct correlation between the serum FFA content in animals fed high-calorie diet and apoB-LP content in the liver (correlation coefficient is 0.77). The FFA content is also correlated with the content of the TAG and apoB-LP in the blood serum of the studied animals (coefficient of correlation between the content of FFA and apoB-LP and FFA and TAG is 0.9). Hence, we can suggest that the main cause of TAG-rich lipoproteins hyperproduction by the liver is really the flow of FFA large amounts to this organ from the blood under feeding high-calorie diet.

Therefore, the increase of apoB-LP content in the liver is obviously linked to the activation of TAG synthesis using FFA that undergo to this organ from adipose tissue due to the activation of lipolysis. The high serum FFA content proved the lipolysis activation (see Table 1).

A number of studies have been shown that hypertriacylglycerolemia is always accompanied by the HDL-cholesterol content decrease and LDL*B* accumulation in blood [11]. LDL*B* are highly atherogenic, because of their small size, high sensitivity to oxidative damage, and low affinity to selective LDL receptors.

A clear correlation between blood serum TAG content and LDL*B* was demonstrated in many studies, indicating a prominent role of the TAG content in the blood for the formation of the LDL morphology. It is also known that LDL morphology is determined primarily by the morphology of their precursors – VLDL. VLDL1 has relatively high triacylglycerol content, slowly metabolized and remained for a long time in the blood stream. Increased hepatic VLDL1 secretion leads to the LDL*B* formation, whereas VLDL2 are precursors of LDL*A* that have a low level of atherogenicity and are dominated in the normal state.

Thus, the probable reason for activation of the apoB-LP formation in the liver in males under MS development is the FFA intake from adipose tissue to this organ. However, high serum apoB-LP level registered in our experiments is the evidence for increased hepatic secretion of

these lipoproteins (see Table 2). As mentioned above, increased serum cholesterol level was also observed (see Table 2). Herewith, the HDL level did not change. These changes indicate that the increased cholesterol level rose due to apoB-LP cholesterol content. The determined changes are typical for many people with MS and have specific proatherogenic character.

It is well known that hyperinsulinemia and insulin resistance contribute to the lipolysis activation and lipogenesis suppression under MS. So, the biggest part of the liver post-mitochondrial fraction should be composed from lipoproteins that absorbed from the blood stream, but not those that synthesized in the liver. And presence of lipid-depleted particles also confirmed the intensification of lipoprotein metabolism in the blood stream.

Probably the activation of free radical oxidation plays the key role because of G6PDH strong sensitivity to the reactive oxygen species (ROS) action. As a result, apoB-LP peroxidation is activated and levels of antioxidants (reduced glutathione (GSH), ascorbic acid, and alpha-tocopherol) are decreased in the liver [97].

These changes should probably contribute in maintaining reduced NADP level, which is necessary for glutathione reductase (GR) [97] and effect the cholesterol synthesis. However, the GR activity decline and GSH level decrease indicates the insufficient antioxidant defense systems activity considering lipidosis and domination of oxidative processes under experimental MS. This fact is also confirmed by the alpha-tocopherol content reduction [94, 95].

Hence, based on our results and literature analysis, we can note that VLDL1 formation is activated in the liver of males fed high-calorie diet independently of age. This is probably one of the reasons for the TAG and apoB-LP accumulation in the blood serum of the experimental animals. The activation of the VLDL formation and secretion by the liver under MS is the result of many changes. In particular, increasing intracellular TAG and CE content, which is mediated by high FFA load into hepatocytes, caused probable activation of microsomal triglyceride transfer protein (MTP) and apolipoprotein B100 (apoB100) synthesis and stabilization of apoB100 [99]. Herewith, TAG-enriched VLDL (VLDL1) secretion is increased under hepatocyte insensitivity to insulin and intrahepatic TAG accumulation.

Thus, summarizing the results, we can state that not only hyperinsulinemia and insulin resistance make an important contribution to the MS development, but other factors that are the result of obesity as well: changes in activity of lipogenesis and lipolysis systems, FA accumulation that leads to lipoproteins metabolism disturbances, etc. Naturally this metabolic situation undoubtedly affects the integral state of the body metabolism.

Hence, the increase of the hepatic VLDL1 secretion should cause the significant changes in lipid and lipoprotein metabolism in the blood stream: an increase of TAG content and LDL*B* accumulation in the blood, which have a high aterogenicity. These changes are the characteristics of MS and are considered to be the risk factors for the atherosclerosis development.

Therefore, to study the mechanisms of the dependence between LDL*B* accumulation, serum FFA accumulation, and changes in the VLDL morphology became the next task of our research.

Plasma apoB-LP metabolism is closely linked to HDL metabolism, which carry out the reverse cholesterol transport from peripheral tissues to the liver [9]. The transfer rate of CE from HDL

http://dx.doi.org/10.5772/60759

to apoB-LP involving cholesteryl ester transfer protein (CETP) [91] and hydrolysis of the TAGs in apoB-LP composition involving LPL and HL are important factors of the process of VLDL conversion to LDL in the blood stream [81].

A lot of clinical studies show that increased CETP activity in HDL composition is mostly accompanied by HDL-cholesterol level decrease and blood plasma LDL*B* accumulation and these changes are correlated with the blood TAG content [51, 83].

According to the literature data, the blood TAG content increase is the factor that leads to disorders in the processes of cholesterol reverse transport, which HDL participate in. The transfer of CE from the HDL to apoB-LP with the participation of CETP is the key component of the cholesterol reverse transport. At that the rate and direction of CE transfer depends primarily on the TAG content in VLDL composition. At the normal state, CETP transfers CE from the HDL to LDL that have a high affinity to hepatic LDL receptors containing apolipoproteins B and E (E/B-LDL), and LDL are rapidly removed from the blood stream. Thus, CETP reveals antiatherogenic action by stimulating the reverse transport of cholesterol. VLDL does not accept the CE and turn into LDL involving LPL. The high TAG content in the VLDL composition increases their ability to accept the EC. It was found that VLDL1 became the main acceptors for CE from HDL under hypertriacylglycerolemia when it is caused by the VLDL1 increase in the blood.

Hence, the CETP activation should be atherogenic for two reasons:

- Firstly, CE-enriched VLDL that are formed converted to LDLB with the HL action.
- Secondly, TAG-enriched HDL are formed and hydrolysis of TAGs in their composition involving HL lead to their rapid removal from the blood stream and result in the HDL-cholesterol level decrease.

A recent study has shown the significant changes in cholesterol and HDL metabolism in the blood serum of animals fed high-calorie diet. These proatherogenic changes are suggested as one of the reasons for LDL*B* accumulation in the blood. The determined increasing of serum total cholesterol (see Table 2) in hamsters fed high-calorie diet is obviously related to the high cholesterol in apoB-LP composition because the HDL-cholesterol level decreased (see Table 6).

It was found that levels of HDL-cholesterol and HDL-CE lowered in young males, whereas in adults it decreased only CE content. The CE transfer rate from HDL to apoB-LP is activated under increased blood TAG content that was observed at postprandial period [73], as well as at apoB-LP metabolic disorders [23].

In both cases, the CE transfer activation is a consequence of increased TAG-enriched lipoproteins level in blood. This is also confirmed by the increased neutral lipids content of the apoB-LP composition in hamsters with experimental MS [95-97]. These differences are probably based on different rates of HDL cholesterol esterification in males of different age groups (see Table 6), which is mainly determined by the lecithin: cholesterol acyltransferase (LCAT) activity – the enzyme that is associated with HDL.

Increased CE transfer activity from HDL is suggested to be a consequence of the CETP activation. Increased CETP activity under MS was demonstrated in a large number of studies

[14, 75, 79, 91, 99]. It is known that increased activity of CETP in the blood HDL composition is the result of the CETP synthesis activation in the liver, but the mechanisms of this protein induction remain not fully understood.

Thus, the enhanced CE transfer rate from HDL under hypertriacylglycerolemia that was observed in our experimental animals fed high-calorie diet (see Table 6) caused atherogenic changes because CE transfer primarily to TAG-enriched lipoprotein fractions leads to the CE-enriched VLDL1 accumulation, which are the main LDL*B* precursors. Intensive TAG uptake by HDL in exchange for CE leads to the TAG-enriched HDL accumulation in the blood. TAG-enriched HDL is the preferred substrate for HL and is rapidly removed from the blood stream that leads to the HDL-cholesterol content reduction.

Another factor that can affect the lipoprotein metabolism and atherogenic LDL*B* formation is the transformation of VLDL to LDL. It takes place in the blood stream involving a number of lipases.

Therefore, changes in the activity of enzymes, which catalyze lipid hydrolysis in lipoproteins in the blood stream, particularly LPL and HP, affect significantly lipoprotein metabolism under MS.

The first enzyme in the vascular lipoprotein transformation cascade is the LPL, which is synthesized mainly in adipocytes and myocytes. TAGs in the TAG-enriched lipoproteins composition (chylomicrons (CM) and VLDL) are substrate for the LPL. FFA released during the hydrolysis by LPL are absorbed by adipocytes and muscle cells where they are involved in TAG synthesis or used as an energy source. TAG hydrolysis in the VLDL composition increases the cholesterol availability to be transferred to HDL, so LPL mediates the reverse cholesterol transport. The LPL activity is regulated via transcription activation, translation, and enzyme transport from the cells [49, 55]. Insulin activates LPL in a healthy state that leads to blood TAG content decrease and reverse cholesterol transport stimulation [28].

According to our data, the LPL activity decreased in the blood serum in young male hamsters fed high-calorie diet (see Table 7). Our results corresponded with the literature data about LPL activity decrease under obesity and IR [60]. Mechanisms of LPL inhibition under these conditions remains to be not fully understood, although IR development may contribute.

The other enzyme – HL, necessary for lipoprotein intravascular transformations is synthesized in hepatocytes, secreted and binds to endothelial cell proteoglycans of hepatic vessels. HL hydrolyzes TAGs and phospholipid content of the different lipoprotein fractions and plays a leading role in their metabolism [41]. It was found that HL mediates selective transport of VLDL remnants (rVLDL) to hepatocytes via the LDL-receptors and participates in the reverse cholesterol transport by stimulating the HDL flow to the liver via scavenger receptors (SR-B1). HL hydrolysis TAGs in apoB-LP content hence plays a significant role in their remodeling in the blood stream. It is known that the HL activity makes a great effect on the lipid composition, size, and properties of LDL [15, 22].

HL activity is regulated mainly at the level of transcription involving sex hormones, glucocorticoids, and adipokines [2]. Intension of HL gene transcription also depends on the lipid

intracellular content in hepatocytes, predominantly cholesterol [20]. In our experiments, the HL activity in the blood serum of males fed high-calorie diet rose irrespective of age (see Table 7), which is consistent with literature data. A number of authors reported that the HL activity was increasing under IR, obesity, and high-calorie diet [30]. HL mRNA content increased in the liver of Syrian hamsters fed high-calorie diet that is the evidence of HL synthesis activation. The authors associated this activation with serum adiponectin content decrease because of the ability to suppress the HL synthesis in hepatocytes.

Taking into consideration these data and the data obtained in our studies [95, 96] that proved the reduction of the serum adiponectin content under obesity, we can suggest that one of the reasons of HL activity increase is reduced adiponectin secretion in adipose tissue under high-calorie diet provided in our experiments.

The increased HL activity is seen as one of the key factors of the atherogenic dyslipidemia development under obesity and MS [10]. Some studies demonstrated a clear correlation between the HL activity and the serum LDL*B* content [15]. It is considered that the HL activation leads to increased LDL*B* formation [2]. The latter occurs under the conditions of increasing TAG-enriched VLDL1 content in blood and CETP activation. Moreover, the HL activity increase reduced the HDL-cholesterol level [89]. This happened because the hydrolysis of TAGs in the HDL3 content leads to their transformation into HDL2, which are rapidly removed from the blood stream by the liver. Thus, reducing HDL-cholesterol level observed during our experiments (see Table 6) may be a consequence of HL activity increase.

Hence, we found that serum FFA level increase was accompanied by the activation of the apoB-LP synthesis by the liver in male Syrian hamsters fed high-calorie diet independently of age. The activation of the apoB-LP synthesis causes the increase of TAG and apoB-LP levels in blood. HDL-cholesterol level reduction is obviously a consequence of the cholesterol ester transfer activation from HDL to LDL via the CETP and the HL activation. The development of the atherogenic dyslipidemia, which is the feature of MS, and increased blood atherogenicity are observed as the result of these changes. The received data are agreed with the literature data about the lipid metabolism age-related changes in males that have proatherogenic character [90]. It is known that the level of sex hormones decreases and the level of glucocorticoids secretion increases in men with aging [67]. The blood plasma lipid profile in men is also determined by the level of secretion of sex hormones that have anti-atherogenic properties [93]. A number of studies indicated the direct correlation between blood testosterone and dihydrotestosterone levels and content of HDL-cholesterol [25, 46, 47, 68]. Besides, the high level of sex hormones is correlated with the decrease of TAGs and total cholesterol [12, 47]. Thus, the TAG content increase and HDL-cholesterol content reduction in the male blood serum with aging may be associated with reduced sex hormone secretion [97]. The serum lipid profile changes in males with aging may also be associated with glucocorticoid increased secretion observed in our experiments [95-97].

Thus, in males with aging blood plasma lipid profile undergoes unfavorable changes that are manifested by the FFA and TAG content increase and lowering of HDL-cholesterol level. These changes may be associated with reduced sex hormone levels and increased cortisol secretion. Herewith, atherogenic dyslipidemia develops independently of age under obesity and IR

despite the more favorable blood plasma lipid profile in young males compared with healthy adult animals.

Thus, activation of the *de novo* fatty acid synthesis in the liver, probably, does not occur as was proved by the G6PDH activity decrease (see Tables 3 and 5). It is known that the G6PDH activity, which is one of the main generators of reduced NADPH, directly correlates with the lipogenesis activity.

There is information about glucose-6-phosphate accumulation, which is utilized by pentose-phosphate pathway, occurs under MS. Therefore, the increase of glucose-6-phosphate content can be an important consequence of reduced NADP accumulation in cells. The close correlation was found between NADPH(H+) content and fatty acid synthase activity. Therefore, changes in the activity of dehydrogenases that reduce $NADP^+$ can be an indicator of the lipogenesis intensity. As we have already pointed out, we found out that MS was accompanied by increased NADP-dependent malate dehydrogenase activity and a reduced activity of pentose-phosphate pathway dehydrogenases (see Tables 5 and 8). So *de novo* lipogenesis activation insignificantly contributes to hyperlipidemia development. Herewith, lipid content in the blood serum and liver homogenate increased significantly. These results are consistent with literature data that under MS mitochondrial lipid oxidation inhibition is primary and is no significant influence on the FA and steroids synthesis rate [53, 75].

Despite the physiological serum apoB-LP level, the apoB-LP content increased in the liver of old females in 10 weeks (see Table 8). This is probably connected with the activation of lipid synthesis using FFA, which is released during the hydrolysis of lipoproteins received from the blood stream. The increased activity of liver LAL that catalyzes the hydrolysis of lipids received via receptor-mediated endocytosis is the confirmation of this suggestion. An increase in the apoB-LP content (see Table 8) in the liver of adult females was also found. However, unlike in young animals, the synthesis of apoB-LP TAGs, probably, involved FFA that undergo to the liver from the blood stream due to lipolysis activation in adipose tissue.

The increased serum FFA level in adult females confirms the lipolysis activation (see Table 1). According to the literature data [26] and our study [96] estrogen secretion decreased with aging, and, as a result lipolysis increased in adipose tissue under the reduced insulin sensitivity. This increases the risk of MS in women with aging. Our results suggest that the risk of atherogenic dyslipidemia development, which increases in females with aging, is probably connected with sex hormones metabolism changes with aging, in particular, with increasing extragonadal estrogen production.

We observed the reduction of HDL level in the liver of females independently of age (see Table 8). Considering the literature data [66, 85] regarding the gender features of lipoprotein metabolism, it can be suggested that the decrease of the liver HDL content in females under the experimental MS is connected not only with changes in their formation, but with active uptake by tissues, including adipose tissue, which is less than characteristically for males. It is known that the abdominal fat accumulation in males occurs due to the TAG-enriched lipoproteins because testosterone increases the tissue sensitivity to insulin [31, 80]. In addition, it is known that phospholipid metabolism in females has the higher rate compared to males [71].

http://dx.doi.org/10.5772/60759

As previously mentioned, the CE transfer activation is proatherogenic, because it leads to the apo-B-LP hydrophobic core growth. This fact was confirmed by the decrease of total HDL-cholesterol content in females with the experimental MS and shows the significant dependence the MS development on aging in females compared to males.

As can be seen from the abovementioned information, the changes in lipid and lipoprotein metabolism in the experimental animals differ greatly depending on the age and sex. We have already pointed out the difference in FFA content and its mobilization by the tissues (see Table 1).

According to our data, the serum FFA content in young females is significantly lower compared to the corresponding value in males, and the feeding high-calorie diet along with the developing IR did not lead to the blood FFA level growth in females (see Table 1). This data is consistent with the literature about the lower FFA release rate from adipose tissue in women is mediated by the antilipolytic estrogen activity [56, 84, 88]. Furthermore, estrogens can suppress lipolysis in adipose tissue in women even with reduced insulin sensitivity in the adipose tissue. This may explain the absence of serum FFA content growth in 4-week-old females fed high-calorie diet.

The absence of lipolysis activation in the adipose tissue in young females fed high-calorie diet is probably the reason for the absence of atherogenic changes in the blood serum in animals of this experimental group even under obesity and insulin resistance.

At the same time, sex differences are revealed in other indices (see Tables 2 and 9). Thus, the total serum lipid level in females with MS was elevated more significantly than in males and especially in adult females (by 58% as compared to the intact animals, while it was only 16% for males of this group).

The obtained data are consistent with literature data about sex differences in the lipid and lipoprotein metabolism [25, 71]. There is sufficient evidence that the serum lipids in women are less favorable for atherosclerosis development as compared to men, which is mainly associated with low serum TAG content in women and high HDL-cholesterol level [42, 56, 84, 88].

These differences are considered to explain the higher risk of CVS disease in men at young age compared with women [46].

Therefore, lipid and lipoprotein metabolism sex differences revealed on a number of key stages, mainly:

- The different lipolytic activity in adipose tissue of men and women
- The differences in the liver lipid and lipoprotein metabolism
- The different CE transfer rate associated with lower CETP activity in women
- The different levels of basal HL activity

It is well known that the HL activity is regulated by hormones via the transcription activation, and estrogens inhibit this enzyme synthesis by binding with estrogen-sensitive areas in the promoter of its gene [44].

According to the literature data, the HL activity in women is approximately 2 times lower compared with men [22], which correlates with a LDL lower level in women in health and obesity and even under MS.

With a feeding high-calorie diet, the CE transfer rate from HDL and HL activity in the serum of young females increased (see Tables 10 and 11). But as it is known, these changes have atherogenic character under the serum TAG level increase, which we did not record during our experiments (see Table 9).

Let us pay our attention to the fact that LPL activity in females was significantly higher than in males, as in intact and under MS, and with aging, the activity of this enzyme was decreased. Furthermore, the HL activity in post-heparin serum was increased in all animals with experimental pathology, and this increasing should reflect the growth of hormone-sensitive lipases activity in response to excessive cortisol production [96, 97] and is primarily adaptive. This increase under MS, on the contrary, leads to the FFA accumulation, dyslipidemia, and dyslipoproteinemia that, finally, can lead to the atherosclerosis development. Moreover, the activity of this enzyme was higher in males (see Tables 7 and 11) and increases with aging indicate the serum lipolysis activation in the animal ontogenesis.

The absence of changes in LPL activity in adult males with MS should be noted (see Table 7). It is well known [22] that males have a higher propensity to atherosclerosis, which also increases with aging, and the given results seem to be paradoxical. However, with a very high (almost 40% higher than in females in the same group) triacylglycerol lipase (TGL) activity and low (60% lower than in females in the same group) LPL activity, even in the absence of changes in this enzyme activity, the ratio between TGL activity and LPL activity in males of this group was 3.5 times higher than in females in the same group. Hence, it is obvious that the high risk of atherogenesis remains. In addition, LPL activity in intact males is 30–35% lower than in females.

The comparison of the obtained data about sex and age features under MS development with the literature evidence indicates that young females have more favorable blood serum lipid profile compared with males due to the lipolysis low rate in adipose tissue in females, low CETP activity, which determines the CE transfer rate from HDL, and low basal HL activity. The expressed atherogenic dyslipidemia was not observed in young females fed high-calorie diet, even under obesity and IR. One of the reasons may be the lack of lipolysis activation in adipose tissue, which is associated with the powerful antilipolytic estrogen activity.

The TAG content increasing was observed in the serum of 20-week-old females fed high-calorie diet. The apoB-LP content did not change (see Tables 5 and 9), which indicates the TAG-enriched VLDL1 accumulation in serum in the animals of this experimental group. According to data about the elevated serum FFA level in adult females fed a high-calorie diet, it can be assumed that the cause of hepatic VLDL1 formation is hepatic absorption of a large number of FFA from the blood, which was released as a result of the lipolysis activation in adipose tissue.

These results are the confirmation of the suggestion that sex differences in the lipolysis regulation in adipose tissue become less pronounced with aging. The latter can be associated

http://dx.doi.org/10.5772/60759

with significant hormonal changes in the body of females with aging. There are changes in the sex hormones secretion – to be more specific, the increase of cortisol secretion (according to our data, the content of cortisol in the serum of adult animals is 54% higher than the value of this indicator in the 4 weeks animals [95, 96]) and decrease of insulin sensitivity. Moreover, we found out that the serum estradiol level in young females was increasing while feeding high-calorie diet, while the serum of adult animals had not changed, which may be an additional factor of age-related differences in lipolytic activity in adipose tissue of females under MS.

We also found a decrease in the serum HDL and HDL-cholesterol content in the adult females fed high-calorie diets (see Table 9). All this gives us the opportunity to state the atherogenic dyslipidemia development in 20-week-old females fed high-calorie diet. However, unlike males, changes in serum lipid profile of adult females in the proatherogenic side may be associated with the TAG-enriched lipoprotein accumulation in the blood and a HDL and HDL-cholesterol content decrease. The latter, obviously, is the result of CE transfer growth rate from HDL (see Table 10) and increased HL activity (see Table 11), which is accompanied by a HDL-cholesterol level decrease under hypertriacylglycerolemia.

Therefore, our data show that by feeding high-calorie diet the expressed atherogenic dyslipidemia in females developed only in adulthood. Probably, this is mediated by different lipolytic activity in adipose tissue in young and adult females and is associated with their hormonal status changes with aging. The favorable cardiovascular risk serum lipid profile that was observed in adult animals fed high-calorie diet is associated with TAG blood content increase and with the HDL decrease. Probably it occurs due to the liver VLDL1 secretion activation and disorders in intravascular lipoprotein remodeling.

To establish mechanisms of atherogenic dyslipidemia development under high-calorie diet, we investigated some lipid and lipoprotein indices dynamics of metabolism in the serum and liver in hamsters during experimental MS development. Also the correlation analysis of the obtained data was conducted. In a series of experiments, we used male and female Syrian hamsters that were 1 year old at the beginning of the experiment (group of animals with predisposition to MS).

As mentioned above, it is known that the liver cells are able to secrete two different VLDL fractions: VLDL1 and VLDL2, which differ in size and density. TAG-enriched VLDL1 have large size and low density. VLDL2 are smaller, denser, and contain smaller amounts of TAG in comparison with VLDL1. It is known that in various pathological conditions, including obesity and hepatic IR, the liver secretes mainly VLDL1, which is the main reason of hypertriacylglycerolemia under these conditions. VLDL morphology essentially depends on the intracellular TAG content and the sensitivity of hepatocytes to insulin.

In our experiment, IR is probably not the main reason of serum TAG increase in the male hamsters in the early stages, as was evidenced by the lack of correlation between these indices, also a later IR development compared with the blood TAG increase (see Figure 1) [96].

Thus, we can suggest that hypertriacylglycerolemia in males developed by feeding a high-calorie diet because of liver preferential VLDL1 secretion due to the TAG accumulation in hepatocytes.

The reasons of the liver TAG content increasing can be as follows:

- The intensive uptake of lipoprotein particles from the blood by hepatocytes
- The enhanced TAG formation in hepatocytes due to the FA *de novo* synthesis or FA uptake from the blood stream

This data suggests that although liver lipolysis activation does occur, it does not play a key role in the MS development.

Moreover, the lack of positive correlation between the TAG content and the liver LAL activity shows that the lipoprotein uptake from the blood stream does not play a key role in the hepatic TAG accumulation in our experiments.

The TAG content increasing in the male hamsters' liver fed high-calorie diet probably occurs because of esterification of FFA coming from the blood. And the presence of a positive correlation between the liver TAG and FFA content (the correlation coefficient at early stages – 0.97) and the liver TAG content and serum FFA level (the correlation coefficient at the early stages – 0.98) demonstrates this fact.

Another evidence of the key role of blood FFA in the formation of MS pathogenic complex is the change in NADPH-generating enzyme G6PDH activity in the liver of the experimental animals. These data indicate the absence of the significant lipogenesis activation within the models used and highlight the key role of extra-hepatic lipolysis activation.

It is well-known that the excessive triacylglycerolemia between the food uptake develops due to the growth of TAG-enriched lipoproteins in blood [50], which may be a result of increased hepatic VLDL secretion and/or changes of its morphology.

A significant serum TAG content increase in the studied animals, with the later and less visible apoB-LP growth, confirms the idea that the main reason of hypertriacylglycerolemia in males under the MS development initial period is the VLDL morphology change toward the TAG enriching.

The main FFA source in serum is the TAG release from adipose tissue due to its hydrolysis. Therefore, the FFA content increase is usually associated with the activation of lipolysis in adipose tissue.

The data that was previously discussed along with the positive correlation between the serum TAG and FFA content (correlation coefficient – 0.85) proved the fact that the activation of lipolysis in adipose tissue is the reason for hypertriacylglycerolemia in the male hamsters fed a high-calorie diet. This leads to an intensive FFA uptake by the liver, growth of intracellular TAG in hepatocytes, and causes the preferential VLDL1 secretion.

Although the lipolysis regulation in adipose tissue is carried out involving many factors including pancreatic hormones, glucocorticoids, and adipokines, its activation may take place

under different conditions including the MS. It is a well-known fact that insulin inhibits lipolysis in adipose tissue under healthy conditions, whereas lipolysis is activated under IR.

The adipose tissue factor adiponectin also has an antilipolytic action, whereas cortisol can increase the lipolytic activity of adipose tissue [98]. We found out the significant positive correlation (coefficient of correlation – 0.87) between the serum FFA and cortisol content in males fed high-calorie diet, and the negative correlation between the FFA and adiponectin content (correlation coefficient – 0.90) [96]. The correlation between the FFA content and IR was not observed at the very beginning of our experiments, which indicates the predominance of hypercortisolemia and hypoadiponectinemia over IR as the reason of lipolysis activation in adipose tissue of males fed high-calorie diet.

Thus, based on the results of our analysis and literature data, we can suggest that the lipolysis activation in adipose tissue is the base of lipid and lipoprotein metabolic disorders in males fed high-calorie diet. And the lipolysis activation is a consequence of the hormonal status disorders, namely, the cortisol increased secretion and adiponectin decreased secretion. The adiponectin decreased secretion is probably a consequence of the adipose tissue rapid growth because of overeating. Lipolysis activation in adipose tissue ultimately leads to impaired lipid metabolism in the liver, in particular the vast VLDL1 secretion that leads to the hypertriacylglycerolemia development.

As we noted above, all these processes led to the excessive formation of the most atherogenic LDL*B*. It is generally considered that the reasons of LDL*B* accumulation in blood under hypertriacylglycerolemia are the following:

- The disorders in lipoproteins remodeling in the blood stream [50]
- The disorders in lipoprotein's particle utilization in the hepatocytes and peripheral organs [3, 17, 57]

As already mentioned, LPL plays a significant role in the utilization of TAGs in lipoprotein composition, which is localized predominantly in adipose and muscle tissue. Hydrolysis of TAGs in the VLDL composition catalyzed by this enzyme leads to VLDL transformation into intermediate-density lipoproteins (IDL), which later turns into LDL, and FFA, revealed as a result of hydrolysis, absorbed by adipocytes and muscle cells.

The abnormal cholesterol transport between different lipoprotein subfraction particles that leads to the blood atherogenic profile formation is under discussion.

As we have already noted, in our experiments the CE transfer rate was enhanced and this was already observed in the early stages of MS developing (see Figure 7). It correlates with the serum TAG content increasing (the correlation coefficient is 0.77) and suggests that changes in apoB-LP morphology is one of the earliest manifestations of MS proatherogenic process.

These results are corresponded to the well-known fact from above that the key factor determining the CE transfer rate is the total serum TAG content. Thus, increased cholesterol esterification and CE transfer between lipoprotein particles make a significant contribution to the atherogenic LDL*B* formation.

Another factor that significantly affects this process is HL activation. As we have already mentioned, HL is associated with proteoglycans of liver blood vessels endothelial cells and hydrolyzes TAGs and phospholipids (PL) in the composition of the various lipoprotein fractions and plays a leading role in their metabolism.

It is known that activation of increased HL activity under CE transfer and growth of blood TAG content determined in our experiments is one of the main reasons of HDL-cholesterol content decrease. This is linked with the fact that TAG hydrolysis in the HDL_3 composition leads to their transformation to HDL2, which are rapidly removed from the blood stream by the liver.

According to our data, the HL activity increase is accompanied by LAL activity increase in the liver (compared with Figures 2 and 10), which shows the intense lipoprotein uptake (probably HDL) from the blood stream.

Hence, our results suggest that changes in VLDL secretion are associated with the MS development FFA accumulation in the blood and elevated hepatic FFA uptake then followed changes in the CE transfer activity and after all was HL activation. This leads to the LDL*B* accumulation and cholesterol reverse transport disorder.

At the same time, it is apparent that changes in lipoprotein enzymatic transformations are led to their abnormal composition. This fact is confirmed by earlier enzymatic changes compared with changes in the blood lipid fractions content. Furthermore, the lipoprotein content changes earlier than their composition, which should reflect the balance disorder of their secretion and absorption. Probably, the latter is related with HDL metabolism in the blood and liver uptake under the condition of EC enrichment.

Thus, the TAG-enriched apoB-LP accumulation, which was accompanied by an increased CE transfer rate and increased HL activity, was found out in blood serum of male Syrian hamsters fed high-calorie die. It is known that such changes have a pronounced proatherogenic character, because they lead to the formation of atherogenic LDL fractions – LDL*B* and lower of HDL cholesterol.

The reason for hypertriacylglycerolemia development in the experimental animals in our investigation, probably, is the lipolysis activation in adipose tissue due to cortisol secretion elevation and decreased adiponectin secretion, which was observed under body weight gain.

The absence of a positive correlation between the serum TAG content and apoB-LP, as well as the serum and liver TAG content in experimental animals, suggests that the serum TAG content increase in females fed high calorie diet is not associated with increased hepatic VLDL secretion.

Based on these statements we can suggest that the cause of hypertriacylglycerolemia in females in our experiments, probably, is the predominant only liver VLDL1 fraction secretion by liver and/or diminished VLDL utilization because of the LDL activity decrease.

As is mentioned above, the intensive formation of TAG-enriched VLDL1 in liver and their secretion to the blood may occur due to the growth of the intracellular TAG content, including the intensive FFA inflow from the blood, and reducing of the hepatocytes sensitivity to insulin.

http://dx.doi.org/10.5772/60759

In our experiments, the liver TAG content increase in females occurs in later periods as compared to the hypertriacylglycerolemia development in animals fed high-calorie diet (see Figure 13) and these indices are not correlated. In addition, feeding a high-calorie diet did not lead to serum FFA increase in females during the first 4 weeks (see Figure 5), which indicates that there were no significant lipolysis activation in adipose tissue of the experimental animals in the first period of our experiments.

Our current data suggest that lipolysis activation in adipose tissue and intensive FFA flow from the blood and liver cells are not the main reasons of the TAG accumulation in the serum of females when consuming a high-calorie diet.

There is sufficient evidence that hepatic VLDL1 formation may occur under hepatic IR [7, 15, 76]. It is known that the hepatocyte sensitivity to insulin determines the VLDL morphology. Insulin suppresses the pre-VLDL lipidation, and as a result VLDL2 characterized by low TAG content are formed in the liver. The activation of enzymes that transfer TAGs to pre-VLDL leading to the formation of TAG-enriched VLDL1 was recorded under reducing the cell sensitivity to insulin.

Thus, the obtained results allow to make the assumption that the main reason for VLDL morphology defects that lead to hypertriglyceridemia in females fed high-calorie diet is the reduced sensitivity of liver cells to insulin. This IR in females does not cause lipolysis activation in adipose tissue, which probably relates to the ability of female sex hormones to suppress the lipolysis in adipose tissue without dependence on insulin sensitivity.

Another possible reason of hypertriacylglycerolemia in females is diminished TAG-enriched lipoproteins utilization because of the reduced LPL activity.

According to our data, this enzyme activity in the serum of females fed high-calorie diet decreased after 3 days from the beginning of the experiment and it was even lower than in later periods (see Figure 6).

We suppose that in our experiments the reason for serum LPL activity reducing was also the insulin sensitivity decrease in females, which is evidenced by the presence of the significant negative correlation between this enzyme activity and IR index (coefficient of correlation between them makes –0.87). Inhibition of LPL activity under IR and obesity diminished the TAG-enriched lipoproteins utilization and can be considered as one of the hypertriacylglycerolemia causes. In the context of our experiments, the LPL activity reduction in the early terms of MS development correlated with the growth of serum TAG content (correlation coefficient – 0.80).

5. Conclusion

The obtained results suggest that the MS begins to develop differently in individuals of different sex. In males, the starting point for MS development is the increase in adipose tissue mass, changes of its endocrine activity, and as a result the hypercortisolemia development,

decreased adiponectin secretion, which is caused by the lipolysis activation in adipose tissue and with time, provokes metabolic and hormonal shifts and the IR development. In females, the MS development begins with the IR appearance, which activates the other pathogenetic factors, although they are delayed by estrogens in the first stages.

Our data are consistent with literature data and demonstrate that feeding high-calorie diet causes the atherogenic dyslipidemia development in experimental animals, which is the consequence of metabolic disorders in adipose tissue and liver as well as lipid and lipoprotein metabolic disorders in the bloodstream.

Our current studies revealed some age and gender features of lipid metabolism disorders mediated by body weight gain. In particular, it was demonstrated that the tendency to atherogenic dyslipidemia in males does not significantly depend on age, but it increases with age in females.

The hormonal disturbances that cause lipolysis activation in adipose tissue in males are the bases of hypertriacylglycerolemia development, which in turn provokes the further blood lipid profile deterioration. The hypertriacylglycerolemia in females is associated with lipid metabolism disorders in the liver due to hepatic IR. The body weight gain of the experimental animals is of great importance as to the formation of these disorders.

Author details

A. Zagayko*, G. Kravchenko, K. Strelchenko, A. Shkapo and T. Briukhanova

*Address all correspondence to: andrey.zagayko@gmail.com

National University of Pharmacy, Kharkiv, Ukraine

References

[1] Adler L. Chemical precipitation of apolipoprotein B-containing lipoproteins facilitates determination of LDL particle size / L. Adler, J.S. Hill, J. Frohlich // Clinical Biochemistry. – 2000. – Vol. 33, N 3. – P. 187-190.

[2] Annema W. Role of hepatic lipase and endothelial lipase in high-density lipoprotein–mediated reverse cholesterol transport / W. Annema, U.J.F. Tietge // Current Atherosclerosis Reports. – 2011. – Vol. 13, N 3. – P. 257-265.

[3] Bartels E.D. Hepatic expression of microsomal triglyceride transfer protein and in vivo secretion of triglyceride-rich lipoproteins are increased in obese diabetic mice / E.D. Bartels, M. Lauritsen, L.B. Nielsen // Diabetes. – 2002. – Vol. 51. – P. 1233 –1239.

http://dx.doi.org/10.5772/60759

[4] Barter Philip J. The causes and consequences of low levels of high density lipoproteins in patients with diabetes / J. Barter Philip // Diabetes & Metabolism - Journal. – 2011. – Vol. 35, N 2. – P. 101-106.

[5] Beltrán-Sánchez H. Prevalence and trends of metabolic syndrome in the adult U.S. population, 1999–2010 / H. Beltrán-Sánchez, M.O. Harhay, M.M. Harhay et al. // Journal of the American College of Cardiology. – 2013. – Vol. 62, N 8. – P. 697-703.

[6] Bethene R.E. Prevalence of metabolic syndrome among adults 20 years of age and over, by sex, age, race and ethnicity, and body mass index: United States, 2003–2006 / R.E. Bethene // National Health Scientific Reports. – 2009. – N 13. – P. 1-7.

[7] Bochem A.E. The promise of cholesteryl ester transfer protein (CETP) inhibition in the treatment of cardiovascular disease / A.E. Bochem, J.A. Kuivenhoven, E.S.G. Stroes // Current Pharmaceutical Design. – 2013. – Vol. 19, N 17. – P. 3143-3149.

[8] Boden G. Obesity, insulin resistance and free fatty acids / G. Boden // Current Opinion in Endocrinology, Diabetes, and Obesity. – 2011. – Vol. 18, N 2. – P. 139-143.

[9] Browning J.D. The effect of short-term fasting on liver and skeletal muscle lipid, glucose, and energy metabolism in healthy women and men / J.D. Browning, J. Baxter, S. Satapati et al. // The Journal of Lipid Research. – 2012. – Vol. 53. – P. 577-586.

[10] Brunzell J.D. The effect of hepatic lipase on coronary artery disease in humans is influenced by the underlying lipoprotein phenotype / J.D. Brunzell, A. Zambon, S.S. Deeb // Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids. – 2012. – Vol. 1821, N 3. – P. 365-372.

[11] Camhi S.M. The relationship of waist circumference and BMI to visceral, subcutaneous, and total body fat: sex and race differences / S.M. Camhi, G.A. Bray // Obesity. – 2012. – Vol. 19, N 2. – P. 402-408.

[12] Cattabiani C. Relationship between testosterone deficiency and cardiovascular risk and mortality in adult men / C. Cattabiani, S. Basaria, C.P. Ceda et al. // Journal of Endocrinological Investigation. – 2012. – Vol. 35, N 1. – P. 104-120.

[13] Carr M.C. The emergence of the metabolic syndrome with menopause / M.C. Carr // The Journal of Clinical Endocrinology & Metabolism. – 2003. – Vol. 88. – P. 2404-2411.

[14] Chapman M.J. Triglyceride-rich lipoproteins and high-density lipoprotein cholesterol in patients at high risk of cardiovascular disease: evidence and guidance for management / M.J. Chapman, H.N. Ginsberg, P. Amarenco et al. // European Heart Journal. – 2011. doi:10.1093/eurheartj/ehr112.

[15] Chatterjee C. Hepatic lipase, high density lipoproteins, and hypertriglyceridemia / C. Chatterjee, D.L. Sparks // The American Journal of Pathology. – 2011. – Vol. 178, N 4. – P. 1429-1433.

[16] Chen Y. Endogenous hormones and coronary heart disease in postmenopausal women / Y. Chen, A. Zeleniuch-Jacquotte, A.A. Arslan et al. // Atherosclerosis. – 2011. – Vol. 216, N 2. – P. 414-419.

[17] Choi S.H. Increased very low density lipoprotein (VLDL) secretion, hepatic steatosis, and insulin resistance / S.H. Choi, H.N. Ginsberg // Trends in Endocrinology & Metabolism. – 2011. – Vol. 22, N 9. – P. 353-363.

[18] Choi K. Molecular mechanism of insulin resistance in obesity and type 2 diabetes / K. Choi, Y.-B. Kim // The Korean Journal of Internal Medicine. – 2010. – N 25. – P. 119-129.

[19] Crandall C.J. Endogenous sex steroid levels and cardiovascular disease in relation to the menopause / C.J. Crandall, E. Barrett-Connor // Endocrinology and Metabolism Clinics of North America. – 2013. – Vol. 42, N 2. – P. 227-253.

[20] Deeb S.S. Hepatic lipase and dyslipidemia: interaction among genetic variants, obesity, gender and diet / S.S. Deeb, A. Zambon, M.C. Carr et al. // The Journal of Lipid Research. – 2003. – Vol. 44. – P. 1279-1286.

[21] Dobs A.S. Interrelationships among lipoprotein levels, sex hormones, anthropometric parameters, and age in hypogonadal men treated for 1 year with a permeation-enhanced testosterone transdermal system / A.S. Dobs, P.S. Bachorik, S. Arver et al. // The Journal of Clinical Endocrinology & Metabolism. – 2001. – Vol. 86. – P. 1026-1033.

[22] Ebbert J.O. Fat depots, free fatty acids, and dyslipidemia / J.O. Ebbert, M.D. Jensen // Nutrients. – 2013. – Vol. 5, N 2. – P. 498-508.

[23] Evans G.F. Inhibition of cholesteryl ester transfer protein in normocholesterolemic and hypercholesterolemic hamsters: effects on HDL subspecies, quantity and lipoprotein distribution / G.F. Evans, W.R. Bensch, L.D. Apelgren et al. // The Journal of Lipid Research. – 1994. – Vol. 35. – P. 1634-1645.

[24] Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) Executive summary of the third report of The National Cholesterol Education Program (NCEP) // Journal of the American Medical Association. – 2001. – Vol. 285. – P. 2486-2497.

[25] Fernandez-Balsells M.M. Adverse effects of testosterone therapy in adult men: a systematic review and meta-analysis / M.M. Fernandez-Balsells, M.H. Murad, M. Lane et al. // The Journal of Clinical Endocrinology & Metabolism. – 2010. – Vol. 95, N 6. – P. 2560-2575.

[26] Finan B. Targeted estrogen delivery reverses the metabolic syndrome / B. Finan, B. Yang, N. Ottaway et al. // Nature Medicine. – 2012. – Vol. 18, N 12. – P. 1847-1856.

[27] Foster M.T. Social defeat increases food intake, body mass, and adiposity in Syrian hamsters / M.T. Foster, M.B. Solomon, K.L. Huhman et al. // American Journal of

http://dx.doi.org/10.5772/60759

Physiology - Regulatory, Integrative and Comparative Physiology. – 2006. – Vol. 290. – P. R1284-R1293.

[28] Foufelle F. Mechanism of storage and synthesis of fatty acids and triglycerides in white adipocytes / F. Foufelle, P. Ferré // Physiology and Physiopathology of Adipose Tissue. – New York: Springer. – 2013. – P. 101-121.

[29] Frohnert B.I. Relation between serum free fatty acids and adiposity, insulin resistance, and cardiovascular risk factors from adolescence to adulthood / B.I. Frohnert, Jr. R. Jacobs, J. Steinberger // Diabetes. – 2013. – Vol. 62, N 9. – P. 3163-3169.

[30] Frühbeck G. Regulation of adipocyte lipolysis / G. Frühbeck, L. Méndez-Giménez, J.A. Fernández-Formoso et al. // Nutrition Research Reviews. – 2014. – P. 1-31.

[31] Fujimoto S. Impaired metabolism–secretion coupling in pancreatic β-cells: role of determinants of mitochondrial ATP production / S. Fujimoto, K. Nabe, M. Takehiro et al. // Diabetes Research and Clinical Practice. – 2007. – Vol. 77, N 3. – P. 2-10.

[32] Girousse A. Partial inhibition of adipose tissue lipolysis improves glucose metabolism and insulin sensitivity without alteration of fat mass / A. Girousse, G. Tavernier, C. Valle et al. // A Peer-Reviewed Open-Access Journal. – 2013. – Vol. 11, N 2. doi: 10.1371/journal.pbio.1001485.

[33] Goldberg I.J. Triglycerides and heart disease: still a hypothesis? / I.J. Goldberg, R.H. Eckel, R. McPherson // Arteriosclerosis, Thrombosis, and Vascular Biology. – 2011. – Vol. 31, N 8. – P. 1716-1725.

[34] Grefhorst A. Acute hepatic steatosis in mice by blocking β-oxidation does not reduce insulin sensitivity of very-low-density lipoprotein production / A. Grefhorst, J.H oekstra, T.G.J. Derks et al. // The American Journal of Physiology-Gastrointestinal and Liver Physiology. – 2005. – Vol. 289. – P. G592-G598.

[35] Grundy S.M. Metabolic syndrome pandemic // Arteriosclerosis, Thrombosis, and Vascular Biology. – 2008. – N 28. – P. 629-636.

[36] Grundy S.M. Clinical management of metabolic syndrome / S.M. Grundy, B. Hansen, S.C. Smith et al. // Circulation. – 2004. – Vol. 109. – P. 551-556.

[37] Grundy S.M. Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition / S.M. Grundy, H. Brewer, J.L. Cleeman et al. // Circulation. – 2004. – Vol. 109. – P. 433-438.

[38] Grundy S.M. Clinical management of metabolic syndrome: report of the American Heart Association/National Heart, Lung, and Blood Institute/American Diabetes Association conference on scientific issues related to management / S.M. Grundy, B. Hansen, S.C. Smith et al. // Circulation. – 2004. – Vol. 109. – P. 551-556.

[39] Guay V. Effect of short-term low- and high-fat diets on low-density lipoprotein particle size in normolipidemic subjects // V. Guay, B. Lamarche, A. Charest et al. // Metabolism. – 2012. – Vol. 61, N 1. – P. 76-83.

[40] Homko C.J. Effects of free fatty acids on glucose uptake and utilization in healthy women / C.J. Homko, P. Cheung, G. Boden // Diabetes. – 2003. – Vol. 52. – P. 487-491.

[41] Huang J.P. The diverse mechanisms of cardiomyopathy and heart failure in obese and non-obese insulin-resistant rat models / J.P. Huang, L.M. Hung // The FASEB Journal. – 2014. – Vol. 28, N 1 Supplement. – C. 1086.2.

[42] Jankowska E.A. Circulating estradiol and mortality in men with systolic chronic heart failure / E.A. Jankowska, P. Rozentryt, B. Ponikowska et al. // Journal of the American Medical Association. – 2009. – Vol. 301, N 18. – P. 1892-1901.

[43] Jaworski K. Hormonal regulation of lipolysis in adipose tissue / K. Jaworski, E. Sarkadi-Nagy, R. Duncan et al. // The American Journal of Physiology-Gastrointestinal and Liver Physiology. – 2007. – Vol. 256. – P. 2901-2907.

[44] Jensen E.V. Estrogen action: a historic perspective on the implications of considering alternative approaches / E.V. Jensen, H.I. Jacobson, A.A.Walf et al. // Physiology & Behavior. – 2010. – Vol. 99, N 2. – P. 151-162.

[45] Jocken J.W.E. Insulin-mediated suppression of lipolysis in adipose tissue and skeletal muscle of obese type 2 diabetic men and men with normal glucose tolerance / J.W.E. Jocken, G.H. Goossens, H. Boon et al. // Diabetologia. – 2013. – Vol. 56, N 10. – P. 2255-2265.

[46] Jones T.H. Testosterone deficiency: a risk factor for cardiovascular disease? / T.H. Jones // Trends in Endocrinology & Metabolism. – 2010. – Vol. 21, N 8. – P. 496-503.

[47] Jones T. H. The effects of testosterone on risk factors for, and the mediators of, the atherosclerotic process / T.H. Jones, F. Saad // Atherosclerosis. – 2009. – Vol. 207, N 2. – P. 318-327.

[48] Karpe F. Fatty acids, obesity, and insulin resistance: time for a reevaluation / F. Karpe, J.R. Dickmann, K.N. Frayn // Diabetes. – 2011. – Vol. 60. – P. 2441-2449.

[49] Kersten S. Physiological regulation of lipoprotein lipase / K. Sander. // Biochimica et Biophysica Acta. – 2014. – Vol. 1841, N 7. – P. 919-933.

[50] Krauss R.M. Lipoprotein subfractions and cardiovascular disease risk / R.M. Krauss // Current Opinion in Lipidology. – 2010. – Vol. 21, N 4. – P. 305-311.

[51] Li Y. Lipoprotein lipase: from gene to atherosclerosis / Y. Li, P.-P. He, D.-W. Zhang et al. // Atherosclerosis. – 2014. – Vol. 237, N 2. – P. 597–608.

[52] Lithell H. Determination of lipoprotein-lipase activity in human skeletal muscle tissue / H. Lithell, J. Boberg // Biochimica et Biophisica Acta. – 1978. – Vol. 528. – P. 58-68.

[53] Ma K. Increased beta-oxidation but no insulin resistance or glucose intolerance in mice lacking adiponectin / K. Ma, A. Cabrero, P.K. Saha et al. // Journal of Biological Chemistry. – 2002. – Vol. 277. – P. 34658 – 34662.

[54] Marcello A. Mechanisms of diabetic dyslipidemia: relevance for atherogenesis / A. Marcello, P. Giovanni, F. Carla et al. // Current Vascular Pharmacology. – 2012. – Vol. 10, N 6. – P. 684-686.

[55] Martins A.R. Mechanisms underlying skeletal muscle insulin resistance induced by fatty acids: importance of the mitochondrial function / A.R. Martins, R.T. Nachbar, R. Gorjao // Lipids in Health and Disease. – 2012. – Vol. 11-30. doi: 10.1186/1476-511X-11-30.

[56] McCullough A.J. Epidemiology of the metabolic syndrome in the USA / A.J. McCullough //Journal of Digestive Diseases. – 2011. – Vol. 5, N 12. – P. 333-340.

[57] Mead J.R. Lipoprotein lipase: structure, function, regulation, and role in disease/ J.R. Mead, S.A. Irvine, D.P. Ramji // Journal of Molecular Medicine. – 2002. – Vol. 80. – P. 753–769.

[58] Mendelsohn M.E. Estrogen actions in the cardiovascular system / M.E. Mendelsohn // Climacteric. – 2009. – Vol. 12, N 1. – P. 18-21.

[59] Meshkani R. Hepatic insulin resistance, metabolic syndrome and cardiovascular disease / R. Meshkani, K. Adeli // Clinical Biochemistry. – 2009. – Vol. 42, N 13. – P. 1331-1346.

[60] Meyer M.R. Obesity, insulin resistance and diabetes: sex differences and role of oestrogen receptors / M.R. Meyer, D.J. Clegg, E.R. Prossnitz et al. // Acta Physiologica. – 2011. – Vol. 203. – P. 259-269.

[61] Misra A. The metabolic syndrome in South Asians: epidemiology, determinants, and prevention / A. Misra, L. Khurana // Metabolic Syndrome Relative Disorders. – 2009. – Vol. 6, N 7. – P. 497-514.

[62] Nakajima K. Postprandial lipoprotein metabolism: VLDL vs chylomicrons / K. Nakajima, T. Nakano, Y. Tokita et al. // Clinica Chimica Acta. – 2011. – Vol. 15-16, N 412. – P. 1306-1318.

[63] Nistor A. The hyperlipidemic hamster as a model of experimental atherosclerosis / A. Nistor, A. Bulla, D.A. Filip et al. // Atherosclerosis. – 1987. – Vol. 68. – P. 159-173.

[64] Parks J.S. Hepatic ABC transporters and triglyceride metabolism / J.S. Parks, S. Chung, G.S. Shelness et al. // Current Opinion in Lipidology. – 2012. – Vol. 23, N 3. – P. 196-200.

[65] Qatanani M. Mechanisms of obesity-associated insulin resistance: many choices on the menu / M. Qatanani, M.A. Lazar // Genes & Development. – 2007. – P. 1443-1455.

[66] Razzouk L. Ethnic, gender, and age-related differences in patients with the metabolic syndrome / L. Razzouk, P. Muntner // Current Hypertension Reports. – 2009. – Vol. 11, N 2. – P. 127-132.

[67] Rohrmann S. Body fatness and sex steroid hormone concentrations in US men: results from NHANES III / S. Rohrmann, M.S. Shiels, D.S. Lopez et al. // Cancer Causes & Control. – 2011. – Vol. 22, N 8. – P. 1141-1151.

[68] Saad F. The role of testosterone in the metabolic syndrome: a review / F. Saad, L. Gooren // The Journal of Steroid Biochemistry and Molecular Biology. – 2009. – Vol. 114, N 1. – P. 40-43.

[69] Samuel V.T. Mechanisms for insulin resistance: Ccommon threads and missing links / V.T. Samuel, G.I. Shulman // Cell. – 2012. – Vol. 148, N 5. – P. 852-871.

[70] Schmidt S.L. Adrenergic control of lipolysis in women compared with men / S.L. Schmidt, D.H. Bessesen, S. Stotz et al. // Journal of Applied Physiology. – 2014. – Vol. 117, N 9. – P. 1008-1019.

[71] Schwertz D.W. Sex differences in the response of rat heart ventricle to calcium / D.W. Schwertz, J.M. Beck, K.J. Mowalski et al. // Biological Research For Nursing. – 2004. – Vol. 5. – P. 286-298.

[72] Shah P. Elevated free fatty acids impair glucose metabolism in women: decreased stimulation of muscle glucose uptake and suppression of splanchnic glucose production during combined hyperinsulinemia and hyperglycemia / P. Shah, A. Vela, A. Basu et al. // Diabetes. – 2003. – N 52. – P. 38-42.

[73] Shinkai H. Cholesteryl ester transfer-protein modulator and inhibitors and their potential for the treatment of cardiovascular diseases / H. Shinkai // Journal of Vascular Health and Risk Management. – 2012. – Vol. 8. – P. 323-331.

[74] Stephane S. Fatty acids - induced lipotoxicity and inflammation / S. Savary, D. Trompier, P. Andreoletti et al. // Current Drug Metabolism. – 2012. – Vol. 13, N 10. – P. 1358-1370.

[75] Taghibiglou C. Mechanisms of hepatic very low density lipoprotein overproduction in insulin resistance. Evidence for enhanced lipoprotein assembly, reduced intracellular ApoB degradation, and increased microsomal triglyceride transfer protein in a fructose-fed hamster model / C. Taghibiglou, A. Carpentier, S.C. Van Iderstine et al. // The Journal of Biological Chemistry. – 2000. – Vol. 275. – P. 8416–8425.

[76] Tan C.E. Relations between plasma lipids and postheparin plasma lipases and VLDL and LDL subfraction patterns in normolipemic men and women / C.E. Tan, L. Foster, M.J. Caslake et. al. // Arteriosclerosis, Thrombosis, and Vascular Biology. – 1995. – Vol. 15. – P. 1839-1848.

[77] Tan Y.Y. Gender differences in risk factors for coronary heart disease / Y.Y. Tan, G.-C. Gast, Y.T. van der Schouw // Maturitas. – 2010. – Vol. 65, N 2. – P. 149-160.

[78] The Metabolic Syndrome / edited by H. Beck-Nielsen. – Verlag Wien: Springer, 2013. – 228 p.

[79] Tiwari S. Intracellular trafficking and secretion of VLDL / S. Tiwari, S.A. Siddiqi // Arteriosclerosis, Thrombosis, and Vascular Biology. – 2012. – Vol. 39. – P. 1079-1086.

[80] Tsai E.C. Association of bioavailable, free, and total testosterone with insulin resistance: influence of sex hormone-binding globulin and body fat / E.C. Tsai, A.M. Matsumoto, W.Y. Fujimoto et al. // Diabetes Care. – 2004. – Vol. 27. – P. 861-868.

[81] Turner S. Measurement of reverse cholesterol transport pathways in humans: in vivo rates of free cholesterol efflux, esterification, and excretion / S. Turner, J. Voogt, M. Davidson et al. // Journal of the American Heart Association. – 2012. – Vol. 1. doi: 10.1161/JAHA.112.001826.

[82] Tvorogova M.G. Correlations of lipoprotein metabolism indicators in persons with low and high cholesterol ester transport activity / M.G. Tvorogova, T.A. Rozhkova, V.V. Kukharchuk et al. [English Abstract, Journal Article] // Voprosu Medicinskoiy Khimii. – 1999. – Vol. 45, N 4. – P. 332-338.

[83] Vasan R.S. Association of circulating cholesteryl ester transfer protein activity with incidence of cardiovascular disease in the Ccommunity / R.S. Vasan, M.J. Pencina, S.J. Robins et al. // Circulation. – 2009. – Vol. 120. – P. 2414-2420.

[84] Vitale C. Gender differences in the cardiovascular effect of sex hormones / C. Vitale, M.E. Mendelsohn, G.M. Rosano // Nature Reviews Cardiology. – 2009. – Vol. 6, N 8. – P. 532-542.

[85] Wang H. Skeletal muscle–specific deletion of lipoprotein lipase enhances insulin signaling in skeletal muscle but causes insulin resistance in liver and other tissues / H. Wang, L.A. Knaub, D.R. Jensen et al. // Diabetes. – 2009. – Vol. 58, N 1. – P. 116-124.

[86] Wilson P.W.F. Triglycerides, HDL-cholesterol and coronary artery disease: a Framingham update on their interrelations / P.W.F. Wilson, M.G. Larson, W.P. Castelli // Canadian Journal of Cardiology. – 1994. – Vol. 10. – P. 5B-9B.

[87] Wu G. The distribution of lipoprotein lipase in rat adipose tissue. Changes with nutritional state engage the extracellular enzyme/ G. Wu, G. Olivecrona, T. Olivecrona // The Journal of Biological Chemistry. – 2003. – Vol. 278. – P. 11925-11930.

[88] Xing D. Estrogen and mechanisms of vascular protection / D. Xing, S. Nozell, Y.F. Chen et al. // Arteriosclerosis, Thrombosis, and Vascular Biology. – 2009. – Vol. 29, N 3. – P. 289-295.

[89] Yasuda T. Update on the role of endothelial lipase in high-density lipoprotein metabolism, reverse cholesterol transport, and atherosclerosis / T. Yasuda, T. Ishida, D.J. Rader // Circulation Journal: Official Journal of the Japanese Circulation Society. – 2010. – Vol. 74, N 11. – P. 2263-2270.

[90] Yatsuya H. Race-and sex-specific associations of obesity measures with ischemic stroke incidence in the Atherosclerosis Risk in Communities (ARIC) study / H. Yatsuya, A.R. Folsom, K. Yamagishi et al. // Stroke. – 2010. – Vol. 41, N 3. – P. 417-425.

[91] Yazdanyar A. Role of phospholipid transfer protein in high-density lipoprotein–mediated reverse cholesterol transport / A. Yazdanyar, C. Yeang, X.-C. Jiang // Current Atherosclerosis Reports. – 2011. – Vol. 13, N 3. – P. 242-248.

[92] Ye J. Mechanisms of insulin resistance in obesity / J. Ye // Frontiers in Medicine. – 2013. – Vol. 1, N 7. – P. 14-24.

[93] Yeap B.B. Androgens and cardiovascular disease / B.B. Yeap // Current Opinion in Endocrinology, Diabetes and Obesity. – 2010. – Vol. 17, N 3. – P. 269-276.

[94] Zagayko A.L. Antioxidant complexes and lipoprotein metabolism – experience of grape extracts application under metabolic syndrome and neurogenic stress / A. L. Zagayko, G.B. Kravchenko, M.V. Voloshchenko et al. // Lipoproteins - Role in Health and Diseases. – Rijeka, Croatia: InTech, 2012. – P. 445-488.

[95] Zagayko A.L. Antioxidant status changes in golden Syrian hamsters with experimental metabolic syndrome / A.L. Zagayko, L.N. Voronina, P.A. Kaliman et al. // Ukrainian Biochemistry Journal. – 2008. – Vol. 80, N 3. – P. 103-109.

[96] Zagayko A.L. Grape polyphenols increase the activity of HDL enzymes in old and obese rats / A.L. Zagayko, G.B. Kravchenko, O.A. Krasilnikova et al. // Oxidative Medicine and Cellular Longevity. – 2013, art. 593761, 7 pages (http://www.hindawi.com/journals/omcl/2013/593761/).

[97] Zagayko A.L. The sexual differences of changes in some lipid metabolism parameter in syrian hamster with experimental metabolic syndrome under hypercaloric diet / A.L. Zagayko, L.N. Voronina, K.V. Strelchenko // Ukrainian Biopharmaceutical Journal. – 2008. – Vol. 1, N 1. – P. 31-33.

[98] Zerradi M. Androgens, body fat distribution and adipogenesis / M. Zerradi et al. // Current Obesity Reports. – 2014. – Vol. 3, N 4. – P. 396-403.

[99] Zhang Z. Role of cholesterol ester mass in regulation of secretion of ApoB100 lipoprotein particles by hamster hepatocytes and effects of statins on that relationship / Z. Zhang, K. Cianflone, A.D. Sniderman // Arteriosclerosis, Thrombosis, and Vascular Biology. – 1999. – Vol. 19. – P. 743-752.

Chapter 3

Obese Childhood Dyslipidemia Management Beyond Statins — MUFA, PUFA, and Sea-buckthorn Supplements

Bogdana Virgolici, Laura Anca Popescu,
Horia Virgolici, Daniela Elena Casariu,
Olivia Timnea and Maria Mohora

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/61001

Abstract

The dyslipidemia pattern usually associated with childhood obesity consists of a combination of elevated triglyceridemia, decreased plasma high density lipoprotein cholesterol concentration and LDL-c concentration at the upper limit of the normal range. This type of dyslipidemia is associated with dense and small LDL, which are proatherogenic. High circulating levels of oxidized LDL were described in extreme pediatric obesity, in children with high fructose intake and are associated with insulin resistance. The worst effect on blood lipids have trans and saturated fatty acids. But the amount of total energy intake plays more important role in lipid profiles. In childhood obesity it seems that insulin resistance precedes the development of the metabolic syndrome feature and insulin resistance is correlated with dyslipidemia. Insulin resistance increases free fatty acid flux to the liver by decreased inhibition of lipolysis and also by increased de novo lipogenesis. Fish oil is rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and has hypotriglyceridemic effect in comparison to monounsaturated fatty acids. Passive in utero exposure to a hyperlipidemic environment may have programmed these children for accelerated atherosclerosis. The infant formula should be enriched with long chain fatty acids because this supplementation is associated with lower blood pressure during later childhood. In obese children, supplements with Omega-3 polyunsaturated fatty acids improve lipid profile, blood pressure values and inflammatory markers. Omega-3 fatty acids prevent metabolic syndrome, by reducing hepatic steatosis, visceral fat, by reducing serum triglycerides and improving insulin sensitivity. Potentially all compounds of

the Sea buckthorn berry, including flavonols, carotenoids, fatty acids, tocopherols and phytosterols can affect the metabolic profile. Special features of the berry oils are high proportions of palmitoleic acid as well as vitamin E, carotenoids, and sterols. The palmitoleic acid stimulates muscle insulin action, suppresses hepatosteatosis and prevent the deleterious effects of saturated fatty acids and high glucose on human pancreatic beta-cell turnover and function. Phenolic compounds and flavonoids from sea buckthorn ameliorate bodyweight, blood glucose, and serum lipid profile. By reducing triglyceridemia and by improving the blood pressure levels, sea buckthorn pulp oil may prevent metabolic syndrome in obese children. The treatment is recommended in hypertriglyceridemic waist phenotype obese children. Omega-3 supplements and sea buckthorn pulp oil supplements reversed the carotid intima media thickness values in obese children and they have beneficial effects in childhood obesity

Keywords: MIFA, PUFA, childhood obesity

1. Introduction

The World Health Organization (WHO) regards childhood obesity as one of the most serious global public health challenges for the 21st century. Childhood obesity is associated with a higher chance of obesity, premature death, and disability in adulthood. But in addition to increased future risks, overweight and obese children are at an increased risk of developing various health problems. Visceral obesity leads to insulin resistance, mediated by free fatty acids and adipokines [1, 2].

The present review deals with the management of obesity and dyslipoproteinemia in childhood and emphasis the beneficial effects of supplements with Omega-3 fatty acids and Seabuckthorn (Hippophae rhamnoides) pulp oil obtained by cold pressing.

2. Dyslipidemia in childhood obesity

The dyslipidemia pattern usually associated with childhood obesity consists of a combination of elevated triglyceridemia, decreased plasma level high-density lipoprotein cholesterol (HDL-c) concentration, and low-density lipoprotein cholesterol (LDL-c) concentration at the upper limit of the normal range. According to the nuclear magnetic resonance spectroscopy results, this type of dyslipidemia is associated with dense and small LDL, less stable HDL, a reduction in total HDL-c, and in large HDL particles [3]. Small and dense LDL particles are associated also with visceral fat and insulin resistance. In school-age children, greater total and central adiposity are associated with smaller LDL particle size and lower HDL-c plasma levels [4].

http://dx.doi.org/10.5772/61001

The LDL can be easily oxidized; they have low affinity for LDL receptors and they penetrate the intima [5]. The macrophages from the vessel wall take the oxidized LDL and they are transformed into foam cells, and so the process of atherosclerosis starts. GGT (Gamma Glutamyl Transpeptidase), an enzyme known to modulate the redox status of the thiol proteins, can also catalyze the oxidation of LDL from the circulation, augmenting the development of atherosclerosis [6]. In the plasma, GGT constitutes complexes with albumin and lipoproteins [7]; while in the atheromatous plaques, GGT is colocalized with oxidized LDL and foam cells [8].

HDL inhibits LDL oxidation. The antioxidant activity of HDL can be explained by its proteins, which link transitional metals, and by two intrinsic enzymatic systems: acetylhydrolase and paraoxonase [9].

High circulating levels of oxidized LDL were described in extreme pediatric obesity [10] in children with high fructose intake [11] and are associated with insulin resistance [12].

More than half of the obese children have dyslipidemia. Improper dietary habits, such as fast foods and snacks, rich in saturated and trans-fatty acids have an important contribution for dyslipidemia. Decreased physical activity and unhealthy eating habits are noticed to have higher incidence in adolescents. These lifestyle modifications, increased susceptibility to insulin resistance, and hormonal changes make pubertal subjects prone to metabolic syndrome. Dyslipidemia present in metabolic syndrome (hypertriglyceridemia and low HDL-c) is associated with insulin resistance, with inflammatory markers (C reactive protein) and with a protrombotic status [13, 14, 15].

In a recent study [16] done on 139 children, the prevalence of dyslipidemia among overweight and obese children was 50.4%. Dyslipidemia patterns were: hypertriglyceridemia 31.9%, low HDL-c 29.7%, high non-HDL-c 15.8%, hypercholesterolemia 11.9%, and elevated LDL-c 10.7%. The dyslipidemia was often (> 50%) present among those with increased waist circumference and family history of dyslipidemia.

The consumption of fructose has recently increased and it seems that in adolescents, fructose represents 12% of the total daily intake [11]. In overweight children, higher fructose intake from sweets and sweetened drinks predicts smaller LDL particle size [4].

Although the caloric intake from fat and different types of fatty acids influence the plasma lipid profile, the amount of total energy intake plays a more important role in lipid profiles [17].

The worst effect on blood lipids have trans and saturated fatty acids [18]. Partially hydrogenated vegetable oil or fish oil are very rich in trans fatty acids that increase LDL-c and decrease HDL-c, so they must be avoided in the diet. C12-16 saturated fatty acids increase both LDL-c and HDL-c levels, but stearic acid has a neutral effect on the plasma lipid profile comparable to that of oleic acid [19].

Replacement of saturated fatty acids by polyunsaturated fatty acids (PUFA) or monounsaturated fatty acids (MUFA) lowers both LDL-c and HDL-c [20].

Supplementation with MUFA is supported by authoritative bodies. When MUFA replaces saturated fatty acids it reduces total cholesterol, and when MUFA replaces carbohydrates it decreases triglycerides and increases HDL [21].

The atherogenic lipid profile is more severe in obese children, especially in boys, who are insulinoresistent. The severity of obesity estimated by the value of body mass index (BMI) is of a lesser importance in comparison with insulin resistance on the atherogenic lipid profile [22].

The atherogenic index can be calculated in many ways, but the most used procedures are: as the ratio between total cholesterol to HDL cholesterol or as a ratio apoB/apoA1. By assessing atherogenic indexes, it has been demonstrated that overweight and obese children have twice higher risk of atherosclerosis [23] and that advancing puberty and advancing age are atherogenic risk factors for obese boys [24].

The relation between plasma lipids and prediabetes in obese prepubertal children is proved by the association of saturated fatty acids in triglycerides with the HOMA-IR (homeostatic model assessment of insulin resistance) [25].

In children, the process of atherosclerosis starts at an early age and is linked to visceral obesity [26]. The common carotid artery intima-media thickness (C-IMT) measured by ultrasound imaging is a marker of preclinical atherosclerosis. C-IMT relates to the severity and extent of coronary artery disease and predicts the likelihood of cardiovascular events in adults.

True primary prevention of atherosclerosis, as contrasted with primary prevention of clinically manifest atherosclerotic disease, must begin in childhood or adolescence [27].

Results from the Young Finns Study have shown that conventional childhood risk factors, such as dyslipidemia, obesity, elevated blood pressure, and smoking, are predictive of subclinical atherosclerosis in young adults [28]. The same authors of the study underline the good news that the adverse cardiometabolic effects of childhood overweight/obesity are reversed among those who become nonobese adults.

The relation cause-effect between dyslipidemia and insulin resistance is not well established but they are interdependent. Most of the researchers consider that insulin resistance precedes the development of the metabolic syndrome feature, including dyslipidemia [29].

Insulin resistance increases free fatty acid flux to the liver by decreased inhibition of lipolysis and also by increased de novo lipogenesis [30].

Supplements with docosahexaenoic acid (DHA) reduce plasma concentrations of free fatty acids of LDL-c and the ratio triacylglycerols/HDL-c, improving insulin resistance [31].

Eicosapentaenoic acid (EPA) increases the anti-inflammatory monocyte cytokine IL-10 expression and reduces arterial stiffness, which may contribute to the antiatherogenic effect of EPA in obese dyslipidemic patients [32].

http://dx.doi.org/10.5772/61001

2.1. Effects of MUFA and PUFA supplements on dyslipidemia associated to childhood obesity

The MUFA from n-7 and n-9 classes can be synthesized in our body from acetyl-coA, but the essential PUFA from n-3 and n-6 classes are required in the diet. A balance between n-6 and n-3 PUFA must be maintained in the diet. There is no consensus about the value of this ratio, but the most used value is around 5. Linoleic acid (C18 delta 9,12) represents the parental fatty acid for class n-6 and linolenic acids (C18 delta 9,12,15) for class n-3, respectively. Naturally, the structure of unsaturated fatty acids is cis fatty acids. PUFA are incorporated in the structure of membrane phosphatides involved in cell fluidity, permeability, and signal transduction. n-3 PUFA are important in the brain development in the fetus and also in early postnatal life [19].

Differences in the composition of dietary fat may also contribute to adipose tissue development by altering rates of adipocyte differentiation and proliferation. Relatively low intake of n-3 PUFA and excessive dietary linoleate may contribute to excessive adipose tissue [33].

Postmortem examination of fetuses delivered from hypercholesterolemic mothers demonstrated that passivity in utero exposure to a hyperlipidemic environment may have programmed these children for accelerated atherosclerosis [34].

It was demonstrated that an enhanced maternal-fetal n-3 PUFA status was associated with lower childhood adiposity [35]. But, in another study, supplementation with n-3 fatty acids during pregnancy and lactation didn't influence significantly the fat mass in the offspring during the first year of life [36]. Good news is that fish oil supplementation during lactation affects blood pressure and body composition of children [37] and that nutritional interventions may improve plasma long-chain PUFA profile and metabolic outcomes of normolipidaemic obese children [38].

According to the European directive, the infant formula should be enriched with long-chain PUFA because this supplementation is associated with lower blood pressure during later childhood. In clinical studies done in adults, fish oil supplementation gave varying results on blood pressure values but most of them showed a lowering in the blood values [19].

A decrease in serum n-3 PUFA, especially DHA, and an increase in saturated FA was noticed in obese children versus lean controls. The subcutaneous adipose tissue and not the visceral adipose tissue was correlated to the changes in PUFA and saturated FA, suggesting an abnormal essential FA metabolism in obese adolescents [39].

Fish oil is rich in EPA and DHA and has a hypotriglyceridemic effect in comparison to MUFA, but supplementation only with DHA does not share the hypotriglyceridemic effect. Fish may be more beneficial than fish oil supplementation. The favorable effect of cis-MUFA on cardiovascular diseases is unlikely, but of those n-3 PUFA is suggestive [19].

Omega-3 PUFA supplementation was associated with a reduced level for triglycerides and an up-regulated expression of the gene encoding peroxisome proliferator activated receptor-α (PPARα), a transcription factor that increases fatty acid oxidation and down-regulates pro-inflammatory genes [40].

Omega-3 fatty acids can prevent prediabetes and diabetes mellitus development because these PUFA are ligands for peroxisome proliferator receptor activator gamma (PPARγ) involved in insulin sensitivity and also, by constituting of the novel biologically active lipid mediators (resolvins and protectins), which also increase insulin sensitivity. The obesity-induced hepatic steatosis can be prevented by Omega-3 PUFA because they decrease endogenous lipid production by inhibiting the expression of the transcription factor, sterol response element binding protein-1c, SREBP-1c [41].

In accordance with the above molecular effects, beneficial effects of Omega-3 PUFA in obesity were demonstrated in clinical and experimental studies. It was shown that Omega-3 fatty acids prevent metabolic syndrome by reducing hepatic steatosis and visceral fat, by reducing serum triglycerides, and improving insulin sensitivity [42, 43].

Omega-3 fatty acids, by inhibiting hepatic lipogenesis, reduce a jéun and postprandial triglyceridemia [44] and improve the quality of platelets membrane phospholipids. In extrahepatic tissues, the activation of lipoprotein lipase has also a great contribution to the hypotriglyceridemic effect of Omega-3 fatty acids. They have also an antiatherogenic effect by reducing the quantity of small and dense LDL [45].

Many clinical and experimental studies have demonstrated that omega-3 PUFA have lipid-lowering effects. The improvement in lipid profile is due mainly to enhanced fatty acid beta-oxidation and suppression of fatty acid synthesis in the liver [46, 47, 48, 49].

During PUFA treatment, the gene expression of the lipogenesis enzyme sterol regulator element binding protein-1c (SREBP-1c) is decreased while the fatty acid oxidation in the liver is increased via the activation of peroxisome proliferator activated receptor-α (PPAR-α) [50, 51].

Dyslipidemia and insulin resistance are associated with non-alcoholic fatty liver disease (NAFLD). Diet rich in lipids and augmented lipolysis in adipose tissues increase the hepatic pool of fatty acids for the liver. Hyperglycaemia and hyperinsulinemia increase lipogenesis in the liver and contribute to hepatic steatosis [52, 53].

Omega-3 PUFA have beneficial effects in liver steatosis by decreasing triglyceridemia and by reducing the muscle intramyofibrillar triglycerides [51].

By upregulating the genes involved in insulin sensitivity, namely glucose transporters (GLUT-2/GLUT-4), peroxisome proliferator activated receptor-γ (PPAR-γ), and insulin receptor signaling (IRS-1/IRS-2) [41], Omega-3 PUFA increase insulin sensitivity.

Lipid intake should represent 25–35% of total energy intake. In USA, saturated FA represents 11% of total energy intake, PUFA 7%, and MUFA 12%. Excess consumption of fatty acids, above the intake recommended, lead to weight gain that is detrimental for body health [21].

When in the diet, saturated fatty acids and trans fatty acids are replaced by cis-MUFA and PUFA, plasma levels for total cholesterol and LDL-c are reduced, while HDL-c concentration remains unchanged. According to the value of the calculated atherogenic index (total cholesterol/HDL-c), this should mean a beneficial effect. Diets rich in cis-MUFA and PUFA may

http://dx.doi.org/10.5772/61001

improve insulin sensitivity; and cis-MUFA compared with carbohydrate and saturated fatty acids reduced HOMA-IR values [19].

When replacing carbohydrate and saturated fat, MUFA consumption can be beneficial. MUFA has positive impact on surrogate markers, but the potential impact on disease outcome remains unclear. When MUFA replaces saturated fatty acids it reduces total cholesterol and when MUFA replaces carbohydrates it decreases triglycerides and increases HDL [21].

Nowadays, the recommendation is to replace solid fats with oils rich in MUFA and PUFA and the most recommended diet is the Mediterranean diet. A randomized controlled trial done on 7,000 patients demonstrated that Mediterranean diet beats low-fat diet. In the study, the two groups of subjects with Mediterranean diet that were supplemented with either olive oil (approximately 4 tablespoons/day) or nuts (average of 3 servings/day) versus the group with low-fat diet reduced the risk for major cardiovascular events [54].

2.2. Effects of supplements with Sea-buckthorn fractions on dyslipidemia associated to childhood obesity

Sea-buckthorn (Hippophae rhamnoides) is a plant of seashores and cliffs; it can be found in Central Asia and Europe, all the way from the shores of the Black Sea to the northwestern shores of the continent. In addition, Sea-buckthorn has spread to Canada and the United States and nowadays the plant has been currently domesticated. Both the berries and the leaves of the plant can be used as dietary supplements.

According to Greek legend, the sick and wounded horses of warriors would be allowed to roam and feed on the shrub. The horses would return more strong and healthy. Sea-buckthorn is a special food that is used to help astronauts maintain their health in space [55].

Since ancient times, Sea-buckthorn berries have been used for their beneficial effects on blood circulation, skin regeneration, and anti-inflammatory effects. The berry contains oil, both in the seed and in the soft parts (pulp oil from the flesh and peel). Special features of the oils are high proportions of palmitic (16:0), oleic (18:1n-9), palmitoleic (16:1n-7), linoleic (18:2n-6), and α-linolenic (18:3n-3) acids as well as vitamin E, carotenoids, and sterols [56].

While the seed oil is rich in linoleic and α-linolenic acids, the pulp oil is a very rich source of palmitoleic acid. This acid is an essential fatty acid that is rare to find in the natural form. Macadamia nuts are the only other source of Omega-7 fatty acids and only in trace amounts [57].

The pulp oil provides a 1:1 ratio of Omega-3/Omega-6. The pulp can be consumed either as a juice or a puree. All the components of the berry contain flavonoides, but the seed residues and the leaves are the most important sources of flavonoides (particularly quercetin, isorhamnetin, kaempherol) [58]. Flavonoides and vitamin C have anti-inflammatory effects and act through a synergic mechanism [59].

The Sea-buckthorn fruit is a very rich source of vitamin C (695 mg per 100 grams), about 12 times greater than oranges, placing Sea-buckthorn fruit among the most enriched plant sources of vitamin C [60].

The origin (subspecies of more than seven) and harvesting time of the berries, as well as oil isolation technology, influence the oil composition [61]. The amounts of bioactive compounds in sea buckthorn berry vary depending on the subspecies, area and year of cultivation, and the maturity of the berries [62].

The method used for extracting Sea-buckthorn influences the potency and the quality of the oil. Cold pressing, hot pressing, solvent extraction, and maceration in other carrier oils are the most used ways for Sea-buckthorn oil extraction, but each method have its own disadvantages. For example, cold-pressing may be ideal, but the extraction rate is quite low. Hot-pressing destroys healthy nutrients and solvent extraction may contaminate the oil with hazardous solvents. The best way to obtain Sea-buckthorn oil is through supercritical CO_2-extraction.

Potentially all compounds of the berry, including flavonols, carotenoids, fatty acids, tocopherols, tocotrienos, and phytosterols of the pulp oil, can affect the metabolic profile. Clinical and experimental studies have demonstrated a wide range of positive effects of the sea buckthorn oils and flavonoides on the lipid profile. Animal and in vitro studies have suggested that sea buckthorn oils [63, 64, 65] and alcohol extracts and flavonoid preparations [66, 67, 68, 69] have antioxidant and anti-inflammatory activities and may beneficially affect serum glucose and triglyceride concentrations. The effect was observed in participants who had a metabolic profile that reflected higher cardiometabolic risk.

In two clinical studies [70, 71] involving healthy men, the intake for 8 weeks of Sea-buckthorn juice versus placebo or supplementation with 5 g of Sea-buckthorn berry oil for 4 weeks versus coconut oil control had no significant influence on the lipid profile (total cholesterol, LDL-c, HDL-c, and triglycerides), but decreased moderately the susceptibility of LDL to oxidation [71].

In healthy, normolipidemic adults having healthy diets, consumption of Sea-buckthorn berry did not affect the circulating concentration of lipid markers, but increased the fasting plasma concentration of quercetin and isorhamnetin indicating that Sea-buckthorn berry is a good dietary source of flavonols [72].

There is a paucity of clinical and animal experiments focused on Sea-buckthorn oil effects on dyslipidemia associated to obesity and the studies are lacking in childhood obesity. Also, in some published data, the tested oils are not defined and some studies should have a better design. The influence of the oils on the plasma lipid levels needs further investigation.

In the experimental study done on white albino rabbits fed with high cholesterol, CO_2 extracted Sea-buckthorn seed oil treatment (1 ml for 30 days) had significant anti-atherogenic and cardioprotective activity. The treatment increased the HDL-c plasma levels and restored the acetylcholine-induced vasorelaxant effect to that of normal values [73].

A randomized, double-blind, crossover study including two 4-week periods with either 3 g/day of black currant seed oil or 2.8 g/day of fish oil separated by a 4-week washout period was done on 15 healthy females. The results showed that black currant seed oil had minor changes on serum n3 fatty acids. Serum levels of LDL cholesterol were lower ($p < 0.05$) after black currant seed oil compared to fish oil. Plasma glucose concentration decreased during the

http://dx.doi.org/10.5772/61001

fish oil supplementation ($p < 0.05$). The results underline the beneficial effects of berries and berry seed oils on serum lipid profile [74].

In a study group including 49 atopic dermatitis patients who took 5 g (10 capsules) of seed oil, pulp oil, or paraffin oil daily for 4 months, a significant ($p < 0.05$) increase in the level of high-density lipoprotein cholesterol from 1.38 to 1.53 mmol/L was observed in the pulp oil group [75].

It was demonstrated that a high-MUFA, cholesterol-lowering diet may be superior to a low-fat diet because it lowers triglyceridemia and does not decrease the plasma level of HDL cholesterol [76].

C16:1n7-palmitoleate is known as an adipose tissue-derived lipid hormone that works at the crosstalk between lipid and sugar metabolism. The acid stimulates muscle insulin action and suppresses hepatosteatosis [77]. The following lines will present the effects of palmitoleic acid on insulinodependent tissues.

In vitro studies done on rat L6 skeletal muscle cells, it was demonstrated that palmitoleic might facilitate uptake and utilization of glucose by upregulation of the activities of the glucose transporters GLUT1 and GLUT4 [78].

On a spontaneous model of obese type 2 diabetes rats, researchers demonstrated that repeated administration of palmitoleic acid increased insulin sensitivity by down-regulating mRNA expressions of proinflammatory adipocytokine genes (TNFα and resistin) in white adipose tissue and by decreasing hepatic lipid accumulation. Palmitoleic acid down-regulates the mRNA of lipogenic genes (as an example, SREBP-1) in the liver and reduces both the plasma triglyceride and hepatic triglyceride levels [79].

It is worth mentioning the effects of C16, n-7 on pancreatic tissue. It was demonstrated that palmitoleic and oleic acids prevent the deleterious effects of saturated fatty acids and high glucose on human pancreatic beta-cell turnover and function via Bcl-2 [80].

In a recent randomized crossover study, 80 overweight women were divided into four groups with different supplements intake for 30 days: dried Sea-buckthorn berries, Sea-buckthorn oil, Sea-buckthorn phenolics ethanol extract mixed with maltodextrin (1:1), or frozen bilberries. Sea-buckthorn-induced effects were mainly on serum triglycerides and very-low-density lipoprotein (VLDL) and its subclasses in the groups with higher metabolic risk. From the supplements, Sea-buckthorn oil induced a decreasing trend in serum total, IDL, and LDL cholesterol and apolipoprotein B in participants with the baseline metabolome of higher cardiometabolic risk [81].

In the Sea-buckthorn, most antioxidants appear to accumulate in the seeds relative to the pulp, leaves, or stem despite most flavonoids being in the leaves. The total phenolic content of the leaves is high and is between 47.06–66.03 mg/g rutin equivalents (RE) [82].

Phenolic compounds and flavonoids ameliorate bodyweight, blood glucose, and serum lipid profile. Also flavonoids from seeds and leaves have anti-obesity and hypoglycemic effect.

Clinical studies have demonstrated that the berry and the ethanol fraction from Sea-buckthorn pulp has beneficial effects on postprandial glucose and insulin levels [83].

Experimental studies done in diabetic rats demonstrated that flavonoids present in water extracts of Sea-buckthorn seeds have hypoglycemic and triglyceride-lowering effect [84].

The fibers and polyphenols in Sea-buckthorn (Hippophaë rhamnoides) extraction residues delay also the postprandial lipemia [85].

Quercetin, as an important flavonoid in the Sea-buckthorn, has also hypolipidemic effect [86].

Quercetin inhibits fatty acid and triacylglycerol synthesis in rat liver cells [87].

In vitro studies demonstrated that quercetin and isorhamnetin have protective effects against oxidized LDL-induced endothelial cell injuries. The flavonoids' beneficial effects might derive from their antioxidant activity and from their capability in modulating the expression of eNOS (endothelial nitric oxide synthase) and LOX-1(lipooxygenase-1) [66].

There are some molecules that are (currently known to be) unique to Sea-buckthorn named flavonoid glycosides [88] and they have antioxidant activity.

High-fat diet obese C57BL/6J mice treated with 70% ethanolic extract of Sea-buckthorn at 500–1,000 mg/kg bodyweight over 13 weeks had lower hepatic and serum total cholesterol, lower hepatic triglycerides and serum leptin level versus non-treated mice. Triglyceridemia and insulinemia were similar in the studied group. The study demonstrated that the Sea-buckthorn intervention was effective in preventing body weight gain and fat accumulation in the liver. The molecular mechanism of this effect is the increase of the hepatic mRNA expression of peroxisome proliferator-activated receptor (PPAR) α and PPAR-γ while the level of the hepatic key enzyme in the fatty acid synthase was decreased [89].

Many compounds from Sea-buckthorn help lose extra weight. Omega-7 in Sea-buckthorn sends messages to the brain, telling it to stop storing calories as excess fat. Sea-buckthorn oil stimulates healthy bowel moves, thereby protecting cell membranes from oxidative and physical stress.

In overweight or obese women, the intake of different berries and berry fractions for 33 days with washout periods of 30–39 days have resulted in positive effects such as a significantly decrease in the waist circumference and in the level of vascular cell adhesion and intercellular adhesion molecules [90].

2.3. Association of the atherogenic indexes with antropometric, inflammatory, oxidative stress and endothelial dysfunctional markers in childhood obesity

It was demonstrated that in obese children with metabolic syndrome, there is a positive association between the high waist circumference and the atherogenic index (total cholesterol/HDL-c) [91].

Endothelial dysfunction, inflammation, and oxidative stress are present in childhood obesity. Especially during puberty, there are some pro-inflammatory and pro-oxidative changes

http://dx.doi.org/10.5772/61001

associated with a relative insulin resistance. The association of the inflammatory and oxidative stress markers with the high value of the apo B/apo A1 ratio in obese children underlies the action in the cluster of different pathogenic mechanisms, augmenting the atherosclerosis development [26, 92].

Apolipoproteins B and A-1 are proposed as markers with value in pediatric lipid risk assessment. High apo B and low apo A-1 levels, usually present in obese children and adolescents reflect a lipoprotein profile predisposing to the development of subclinical atherosclerosis in adulthood [93], [94].

The concentrations of plasma apolipoprotein (apo) B are often increased in childhood obesity, partly due to the hepatic overproduction of apo B containing lipoproteins [95,96].

Many researchers consider apo B as a better predictor of vascular risk than LDL because apo B, in comparison with LDL, is more strongly associated with other cardiovascular risk factors [97].

3. Diet and weight loss

A comparative study between severely obese children versus moderately obese children demonstrated a markedly more unfavorable cardio-metabolic risk profile in the first group. The study highlights that severely obese children need to receive particular attention regarding obesity treatment [98].

A recent study demonstrated that the strongest negative predictor of weight loss is waist circumference. The study underscores that obese children with abdominal fat distribution need more intensive interventions [99].

In a review about the impact of dietary and physical activity in obese children, including the studies that have been done in the last 35 years, demonstrated that diet-only and diet-plus-exercise interventions resulted in weight loss and metabolic profile improvement. Diet-only intervention reduced triglycerides and LDL levels, while diet-plus-exercise interventions reduced fasting glycaemia and insulinemia and increased HDL-c concentration [100].

In obese children and adolescents, weight loss improved the values of the parameters included in the metabolic syndrome criteria with the exception of HDL-c concentration. The reduction in fasting triglycerides concentration (but not waist circumference) was the only significant predictor of metabolic syndrome change [101].

Diet modifications and physical activity have been included in obesity management, but have shown relatively limited success among severely obese children and adolescents. However, the parents of obese children should know that a healthy lifestyle is important for better physical and mental health no matter how much or how little weight is lost. The physician should help patients to cope obesity for psychosocial functioning and must motivate them to make use of the available healthcare resources.

Pharmacotherapy for obesity has side effects (growth problems, lessened self-esteem, unhealthy weight-control mechanisms) and frequently fails to be efficacious because obesity is a multifactorial, polygenic disease [102,103].

4. Own results

We did some studies in obese children and adolescents (n = 41 and n = 48) and analytical evaluations were performed before and immediately after supplements were administrated. In one study we gave Sea-buckthorn pulp oil (800 mg/day for 60 days) and placebo, and in another study we gave Omega-3 fatty acids (DHA 130 mg and EPA 25 mg) associated with vitamins (A 200 μg, D 1.25 μg, E 2.5 mg, and C 30 mg). As a control, we enrolled 30 lean children. All the children under medications or those with chronic disease (endocrine, inflammatory, or hereditary diseases) or smokers were excluded. The participants were instructed not to change their lifestyle (dietary and drinking habits, physical activity) during the whole studies and not to take any additional medication including vitamin supplements.

The atherogenic indexes (calculated either as total cholesterol/HDL-c or apoB/apo-A1) were calculated in obese children. Higher values for atherogenic indexes have predictive values for atherosclerosis development. The atherogenic indexes were correlated with other cardiovascular risk factors such as: waist circumference (Figure 1), C reactive protein (CRP), GGT activity (Figure 2), diastolic blood pressure, and malondialdehyde (MDA)-marker of lipid peroxidation (Figure 3). The multiple associations of atherogenic index with dyslipidemia, antropometric, inflammatory, and oxidative stress markers underline that in obesity there is a cluster of patogenic mechanisms that contribute to atherosclerosis. Also, the atherogenic indexes were associated with subclinical atherosclerotic disease markers as carotid intima media thickness-CIMT (positive correlation) (Figure 4) and with brachial artery flow-mediated dilation-FMD (Flow Mediated Dilatation (negative correlation) (Figure 5) [104, 105].

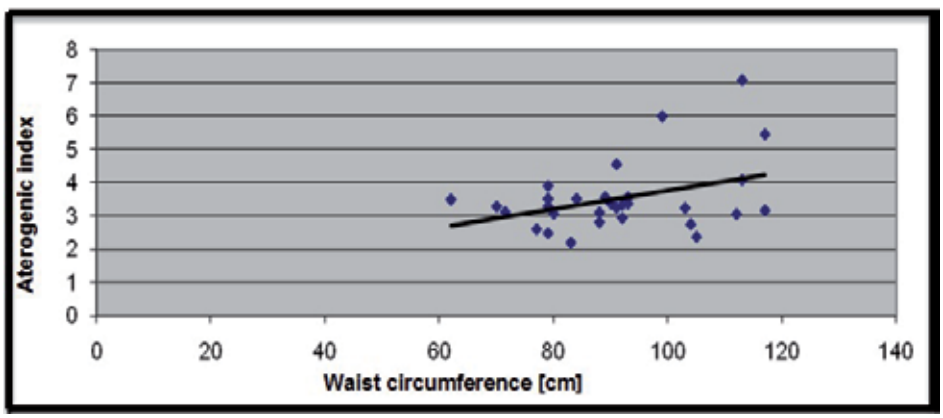


Figure 1. Correlation between atherogenic index and waist circumference (r = 0.33). The atherogenic index was calculated as the ratio between total cholesterol/HDL-c.

http://dx.doi.org/10.5772/61001

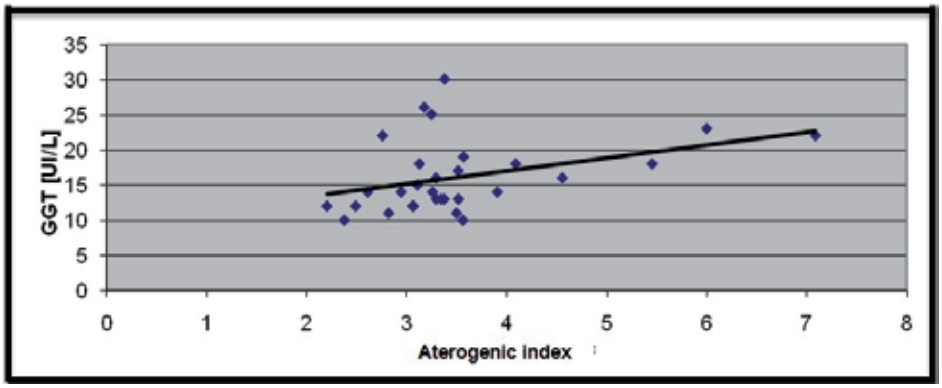


Figure 2. Correlation between GGT activity with aterogenic index (r = 0.42). The atherogenic index was calculated as the ratio between total cholesterol/HDL-c.

By hydrolyzing the glutathione, the GGT has a pro-oxidant effect. Also, the products of the GGT reaction may themselves lead to increased free radical production, particularly in the presence of iron. High serum GGT activity was positively associated with the risk of coronary heart disease, type 2 diabetes mellitus, and stroke. We have demonstrated a positive correlation between GGT activity and the cholesterol/HDL-c ratio value [104] (Figure 2).

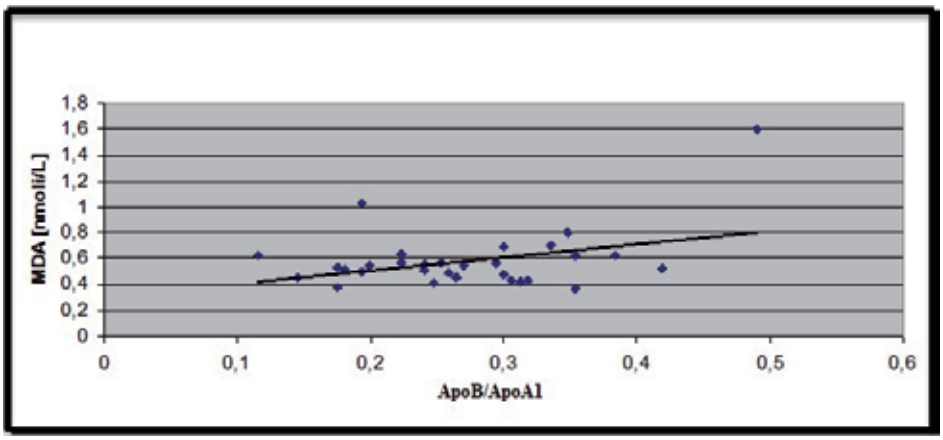


Figure 3. Correlation between MDA and apoB/apo A-1 (r = 0.39).

There is a paucity of studies on systemic oxidative stress in childhood obesity but almost all of them have demonstrated higher levels of serum malonyldialdehyde, a marker of lipid peroxidation. There is a direct relation between dyslipidemia and lipid peroxidation and both contribute to a pro-inflammatory phenotype in childhood obesity [105, 106, 107] (Figure 5). NADPH oxidase activity (respiratory burst) in monocytes is increased in childhood obesity and together with serum lipid peroxidation contributes to an increased systemic oxidative stress [105].

Our research team demonstrated that in obese children versus lean ones, plasma total cholesterol and triglycerides were higher, while HDL-c level was lower. LDL-c concentrations were similar in the studied groups. The treatment (800 mg/day, for 60 days) with Sea-buckthorn pulp oil (obtained by cold pressing) reduced significantly the total plasma cholesterol, the apo B/apo A-I ratio and the plasma triglycerides. A weak improvement of HDL-c and LDL-c levels was noticed [105]. We consider that the composition of the Sea-buckthorn pulp oil rich in MUFA and phytosterols is responsible for this effect [108].

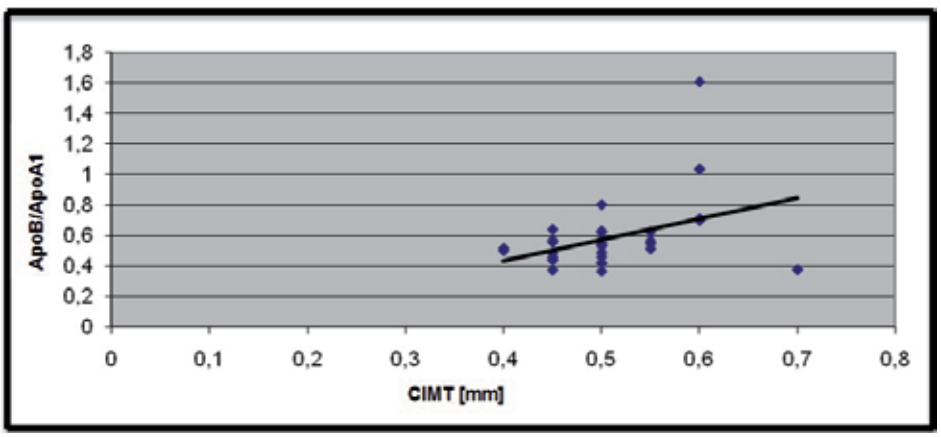


Figure 4. Correlation between apo B/apo A-1 with CIMT (r = 0.38).

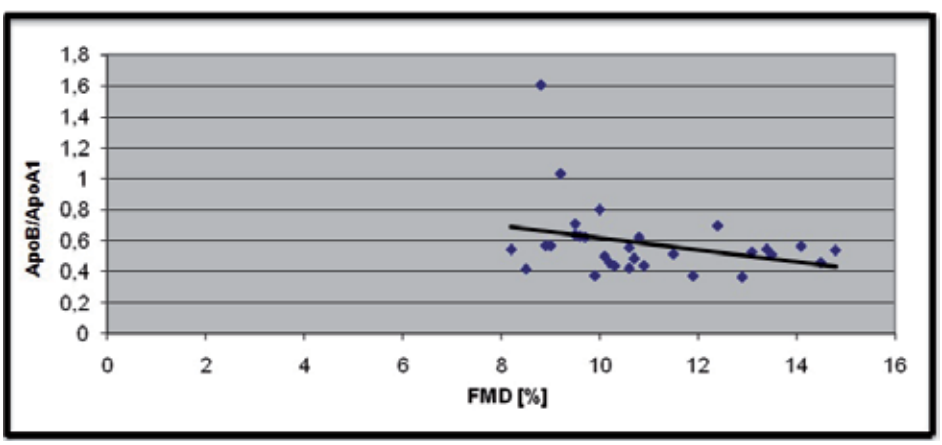


Figure 5. Correlation between apo B/apo A-1 with FMD (r = -0.36).

Dyslipidemia, inflammation, insulin resistance, and oxidative stress are important culprits for atherosclerosis in obesity. Hyperleptinemia and hypoadiponectinemia represent links between inflammation and vascular dysfunction in obese children. Atherosclerosis begins with endothelium dysfunction and insulin resistance is an important trigger. Puberty alerts

http://dx.doi.org/10.5772/61001

some of the inflammatory markers associated with endothelial dysfunction in obese children and leptin concentration rises. Leptin is a biomarker of vascular dysfunction, while adiponectin improves endothelial cells function. In our study done on 48 obese children, the intake of Omega-3 fatty acids (DHA 130 mg and EPA 25 mg) associated with vitamins (A 200 μg, D 1.25 μg, E 2.5 mg, and C 30 mg) for 3 months has resulted in improved lipid profile. The values for total cholesterol, LDL-c, and triglycerides were decreased, while the HDL-c level was increased [109]. We showed that in the obese children, supplements with Omega-3 fatty acids improved not only the lipid profile, but also the blood pressure values and inflammatory markers. By lowering leptin, increasing adiponectin, and by decreasing the HOMA-IR values, Omega-3 PUFA reduces the risk of cardiovascular disease development in adulthood [109].

According to the ATP III (adult treatment panel) modified criteria [110] in our studies, more than 55% of the obese children had triglyceridemia higher than 100 mg/dl and triglyceridemia was correlated with ALT (Alanine aminotransferase) activity. Our results are in accordance with others. In a study done on 700 overweight and obese children aged 7–18 years, ALT activity was correlated with BMI, waist circumference, blood pressure values, triglyceridemia, and insulin resistance [111]. Calcaterra et al. proposed the high value of ALT as a screening marker for metabolic syndrome in obese children [112].

In one of our studies, we divided the obese children in two groups: hypertriglyceridemic waist fenotype (n = 17) and obese nonhypertriglyceridemic (n = 24). Modified ATP III cut points for serum triglycerides (≥110 mg/dL) and waist circumference (≥90th percentile for age and sex) were used to divide obese children. Metabolic and inflammatory parameters were measured before and after Sea-buckthorn pulp oil intake (800 mg/day, 60 days) and the best improvement of the measured plasma variables was observed in hypertriglyceridemic waist phenotype obese children. We demonstrated that high serum values for triglycerides were associated with low values for HDL-C and the treatment reduced significantly the levels of triglycerides and improved the HDL-C concentration [113].

Hepatic de novo lipogenesis is augmented in hypertriglyceridemic waist phenotype obese children and this can be demonstrated by the high values of ALT. In our study, treatment with Sea-buckthorn pulp oil reduced ALT activity and triglyceridemia and improved blood pressure levels. By reducing triglyceridemia and by improving the blood pressure levels, Sea-buckthorn pulp oil may prevent metabolic syndrome in obese children [105, 113]. The study underlines that treatment with Sea-buckthorn pulp oil should be recommended in hypertriglyceridemic waist phenotype obese children. Also, in obese children, the intake of Omega-3 fatty acids (DHA 130 mg and EPA 25 mg per day) associated with vitamins (A 200 μg, D 1.25 μg, E 2.5 mg, and C 30 mg) for 3 months reduced the blood pressure values (systolic and diastolic) significantly [109].

Some of the most important effects for Omega-3 supplements and Sea-buckthorn pulp oil supplements are to prevent atherosclerosis and to reverse the CIMT values [105,109].

In a high-fat diet mouse model, we induced non-alcoholic fatty liver disease to NMRI mice and the treatment with normocaloric/normolipidic diet and Omega-3 fatty acids (DHA 130 mg

and EPA 25 mg per day) had reversed liver histopatological results from steatohepatitis to normal aspect and improved the plasma lipid profile [114].

5. Treatment of dyslipidemia associated to childhood obesity

Both in adults and in children, the extent of atherosclerotic lesions is significantly correlated with a modified serum lipid profile (increased total and LDL cholesterol, triglycerides, low HDL cholesterol) together with elevated blood pressure and waist circumference.

Reversal of vascular functional abnormalities with early therapy with statins and supplementation with antioxidant vitamins and Omega-3 fatty acids has been observed in children with familial hypercholesterolemia.

According to an AHA Scientific Statement, "LDL cholesterol lowering drug therapy is recommended only in those children ≥10 years of age whose LDL cholesterol remains extremely elevated after an adequate 6- to 12-month trial of diet therapy. Drug therapy was to be considered for children with LDL cholesterol levels ≥190 mg/dL and in those with LDL cholesterol ≥160 mg/dL together with either the presence of ≥2 other cardiovascular disease risk factors or a positive family history of premature cardiovascular disease" [34].

Omega-3 PUFA represents the first choice in treating hypertrigliceridemia in childhood obesity because they do not have adverse reactions, they are safe, and they have good tolerance. Also, different Sea-buckthorn fractions do not have side effects [110–86] and they have great future as food supplements in preventing obesity complications. Future research work will show if the beneficial changes of the supplements revert back if the treatment is stopped.

Cook and Kavey recommend that "any medication except Omega-3 fish oil in youths with combined dyslipidemia should be undertaken only with the assistance of a lipid specialist" [3].

Author details

Bogdana Virgolici[1*], Laura Anca Popescu[1], Horia Virgolici[1], Daniela Elena Casariu[2], Olivia Timnea[3] and Maria Mohora[1]

*Address all correspondence to: hvirgolici@yahoo.com

1 University of Medicine and Pharmacy "Carol Davila", Bucharest, Romania

2 General Practitioner, Medicine Office, Bucharest, Romania

3 Ecological University, Bucharest, Romania

References

[1] Knight JA. Diseases and disorders associated with excess body weight. Ann. Clin. Lab Sci. 2011;41:107–121.

[2] Boden G. Obesity, insulin resistance and free fatty acids. Curr. Opin. Endocrinol. Diabetes Obes. 2011;18:139–143.

[3] Cook and Kavey. Pediatr Clin North Am. Author manuscript, p.3; (accessed in PMC 1 December 2012).

[4] Aeberli I, Zimmermann MB, Molinari L, Lehmann, l'Allemand D, Spinas GA, Berneis K. Fructose intake is a predictor of LDL particle size in overweight schoolchildren. Am J Clin Nutr 2007;86(4):1174–1178.

[5] Beauloye V, Zech F, Thi Mong HT, Clapuyt P, Maes M, Brichard SM. Determinants of early atherosclerosis in obese children and adolescents. J Clin Endocrinol Metab. 2002;92(8):3025–3032.

[6] Paolicchi A, Emdin M, Passino C, Lorenzini E, Titta F, Marchi S, Malvaldi G, Pompella A. Beta-Lipoprotein and LDL associated serum gamma-glutamyltransferase in patients with coronary atherosclerosis. Atherosclerosis. 2006;186:80–85.

[7] Huseby NE. Multiple forms of serum gamma-glutamyltransferase. Association of the enzyme with lipoproteins. Clin Chim Acta. 1982;124:103–112.

[8] Paolicchi A, Emdin M, Ghliozeni E, Ciancia E, Passino C, Popoff G, Pompella A. Images in cardiovascular medicine. Human atherosclerotic plaques contain gamma-glutamyl transpeptidase enzyme activity. Circulation. 2004;109:1440.

[9] Kota SK, Meher LK, Kota SK, Jammula S, Krishna SVS, Modi KD. Implications of serum paraoxonase activity in obesity, diabetes mellitus, and dyslipidemia. Indian J of Endocrinology and metabolism. 2013;17(3):402–412.

[10] Norris AL, Steinberger J, Steffen LM, Metzig AM, Schwarzenberg SJ, Kelly AS. Circulating oxidized LDL and inflammation in extreme pediatric obesity. Obesity (Silver Spring). 2011;19(7):1415–1419.

[11] Vos MB, Weber MB, Welsh J, et al. Fructose and oxidized low-density lipoprotein in pediatric nonalcoholic fatty liver disease: A pilot study. Arch Pediatr Adolesc Med. 2009;163(7):674–675.

[12] Chalasani N, Deeg MA, Crabb DW. Systemic levels of lipid peroxidation and its metabolic and dietary correlates in patients with nonalcoholic steatohepatitis. Am J Gastroenterol. 2004;99(8):1497–1502.

[13] Jessup A, Harrell JS. The metabolic syndrome: Look for it in children and adolescents, too! Clinical diabetes. 2005;1(23):26–32.

[14] Vos M, Barlow SE. Update in childhood and adolescent obesity. Pediatric clinics of North America. 2011;58(6):xv–xvii.

[15] Steinberger J, Daniels SR, Eckel RH, Hayman L, Lustig RH, McCrindle B, Mietus-Snyder ML. Progress and Challenges in Metabolic Syndrome in Children and Adolescents. Circulation. 2009;119:628–647.

[16] Casavalle PL, Lifshitz F, Romano LS, Pandolfo M, Caamaño A, Boyer PM, Rodríguez PN, Friedman SM. Prevalence of dyslipidemia and metabolic syndrome risk factor in overweight and obese children. Pediatr Endocrinol Rev. 2014;12(2):213–23.

[17] Mohseni-Takalloo S, Mirmiran P, Hosseini-Esfahani F, Azizi F. Dietary fat intake and its relationship with serum lipid profiles in tehranian adolescents. Journal of Food and Nutrition Research. 2014;2(6):330–334. Available online at http://pubs.sciepub.com/jfnr/2/6/10 © Science and Education Publishing. DOI:10.12691/jfnr-2-6-10.

[18] Brouwer IA, Wanders AJ, Katan MB. Effect of animal and industrial trans fatty acids on HDL and LDL cholesterol levels in humans–A quantitative review. PLoS One. 2010;5(3):e9434.

[19] Nordic Nutrition Recommendations 2012, Integrating nutrition and physical activity, 978-92-89-2670-4, http://dx.doi.org/10.6027/Nord2014-002 Nord 2014:002, ISSN 0903-7004© Nordic Council of Ministers 2014, Layout and ebook production: Narayana Press, p.217-249.

[20] Siri-Tarino PW, Sun Q, Hu FB, Krauss RM. Saturated fat, carbohydrate, and cardiovascular disease. The American journal of clinical nutrition. 2010;91(3):502–509.

[21] Position of the Academy of Nutrition and Dietetics: Dietary fatty acids for healthy adults 2212-2672. Journal of the academy of nutrition and dietetics. 2014;14(1):136–153, http://dx.doi.org/10.1016/j.jand.2013.11.001.

[22] Wójcik M, Januś D, Maślanka A, Olchawa-Czech A, Radwańska M, Dylag K, Dolezal-Ołtarzewska K, Roztoczyńska D, Tyrawa K, Zygmunt-Górska A, Kumorowicz-Czoch M, Kalicka-Kasperczyk A, Ciechanowska M, Stelmach M, Nazim J, Starzyk J. Disorders of lipid metabolism in adolescents with simple obesity. Przegl Lek. 2010;67(11):1168–71.

[23] Rizk NM, Yousef M. Association of lipid profile and waist circumference as cardiovascular risk factors for overweight and obesity among school children in Qatar. Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy 2012;5:425–432.

[24] Güven A, Sanisoglu SY. Pubertal progression and serum lipid profile in obese children. Journal of Pediatric Endocrinology & Metabolism. 2008;21:135–146.

[25] Gil-Campos M, del Carmen Ramírez-Tortosa M, Larqué E, Linde J, Aguilera CM, Cañete R, Gil A. Metabolic syndrome affects fatty acid composition of plasma lipids in obese prepubertal children Saturated FA in TG were associated with HOMA-IR Lipids. 2008;43(8):723–32. DOI: 10.1007/s11745-008-3203-4. Epub 2008 Jul 1.

[26] Berenson GS, Srinivasan SR, Bao W, Newman III WP, Tracy RE, Wattigney WA. Association between multiple cardiovascular risk factors and atherosclerosis in children and young adults. The Bogalusa Heart Study. N Engl J Med. 1998;338:1650–1656.

[27] Strong JP, Malcom GT, McMahan CA, Tracy RE, Newman III WP, Herderick EE, et al. Prevalence and extent of atherosclerosis in adolescents and young adults: Implications for prevention from the Pathobiological Determinants of Atherosclerosis in Youth Study. Jama. 1999;281(8):727–735.

[28] Juonala M, Viikari JS, Raitakari OT. Main findings from the prospective cardiovascular risk in young Finns study. Curr Opin Lipidol. 2013;24(1):57–64. DOI: 10.1097/MOL.0b013e32835a7ed4.

[29] Petersen KF, Dufour S, Savage DB, Bilz S, Solomon G, Yonemitsu S, Cline GW, Befroy D, Zemany L, Kahn BB, Papademetris X, Rothman DL, Shulman GI. The role of skeletal muscle insulin resistance in the pathogenesis of the metabolic syndrome. Proc. Natl. Acad. Sci. USA. 2007;104(31):12587–12594.

[30] Chin HK, Zobair MY. Nonalcoholic fatty liver disease: A manifestation of the metabolic syndrome. Cleveland Clinic Journal of Medicin. 2008;75(10):721–728.

[31] Kelley DS, Adkins Y, Woodhouse LR, Swislocki A, Mackey BE, Siegel D. Docosahexaenoic acid supplementation improved lipocentric but not glucocentric markers of insulin sensitivity in hypertriglyceridemic men. Metab Syndr Relat Disord. 2012 Feb; 10(1):32–8.

[32] Satoh-Asahara N, Shimatsu A, Sasaki Y, Nakaoka H, Himeno A, Tochiya M, Kono S, Takaya T, Ono K, Wada H, Suganami T, Hasegawa K, Ogawa Y. Highly purified eicosapentaenoic acid increases interleukin-10 levels of peripheral blood monocytes in obese patients with dyslipidemia. Diabetes Care. 2012;35(12):2631–9. DOI: 10.2337/dc12-0269. Epub 2012 Aug 21.

[33] Ailhaud G, Guesnet P, Cunnane SC. An emerging risk factor for obesity: Does disequilibrium of polyunsaturated fatty acid metabolism contribute to excessive adipose tissue development? Br J Nutr. 2008;100(3):461-70. DOI: 10.1017/S0007114508911569. Epub 2008 Feb 28.

[34] McCrindle BW, Urbina EM, Dennison BA, Jacobson MS, Steinberger J, Rocchini AP, Hayman LL, Daniels SR. Drug therapy of high-risk lipid abnormalities in children and adolescents. Circulation. 2007;115:1948–1967.

[35] Donahue SM, Rifas-Shiman SL, Gold DR, Jouni ZE, Gillman MW, Oken E. Prenatal fatty acid status and child adiposity at age 3 y: Results from a US pregnancy cohort. Prenatal fatty acid status and child adiposity at age 3 y: Results from a US pregnancy cohort. Am J Clin Nutr. 2011;93(4):780–8. DOI: 10.3945/ajcn.110.005801. Epub 2011 Feb 10.

[36] Hauner H, Much D, Vollhardt C, Brunner S, Schmid D, Sedlmeier EM, Heimberg E, Schuster T, Zimmermann A, Schneider KT, Bader BL, Amann Gassner U. Effect of re-

ducing the n-6:n-3 long-chain PUFA ratio during pregnancy and lactation on infant adipose tissue growth within the first year of life: an open-label randomized controlled trial. Am J Clin Nutr. 2012;95(2):383-94. DOI: 10.3945/ajcn.111.022590. Epub 2011 Dec 28.

[37] Asserhøj M, Nehammer S, Matthiessen J, Michaelsen KF, Lauritzen L. Maternal fish oil supplementation during lactation may adversely affect long-term blood pressure, energy intake, and physical activity of 7-year-old boys. J Nutr. 2009;139(2):298–304. DOI: 10.3945/jn.108.095745. Epub 2008 Dec 17.

[38] Verduci E, Radaelli G, Salvioni M, Riva E, Giovannini M. Plasma long-chain fatty acids profile and metabolic outcomes in normolipidaemic obese children after one-year nutritional intervention. Acta Paediatr. 2011;100(4):585–9. DOI: 10.1111/j.1651-2227.2010.02120.x. Epub 2011 Jan 12.

[39] Karlsson M, Mårild S, Brandberg J, Lönn L, Friberg P, Strandvik B. Serum phospholipid fatty acids, adipose tissue, and metabolic markers in obese adolescents. Obesity (Silver Spring). 2006;14(11):1931–9.

[40] Mejía-Barradas CM, Del-Río-Navarro BE, Domínguez-López A, Campos-Rodríguez R, Martínez-Godínez Md, Rojas-Hernández S, Lara-Padilla E, Abarca-Rojano E, Miliar-García Á. The consumption of n-3 polyunsaturated fatty acids differentially modulates gene expression of peroxisome proliferator-activated receptor alpha and gamma and hypoxia-inducible factor 1 alpha in subcutaneous adipose tissue of obese adolescents. Endocrine. 2014;45(1):98–105. DOI: 10.1007/s12020-013-9941-y. Epub 2013 Apr.

[41] González-Périz A, Horrillo R, Ferré N, Gronert K, Dong B, Morán-Salvador E, Titos E, Martínez-Clemente M, López-Parra M, Arroyo V, Clària J. Obesity-induced insulin resistance and hepatic steatosis are alleviated by omega-3 fatty acids: A role for resolvins and protectins. FASEB J. 2009;23(6):1946–57. DOI: 10.1096/fj.08-125674. Epub 2009 Feb 11.

[42] Sato A, Kawano H, Notsu T, Ohta M, Nakakuki M, Mizuguchi K, Itoh M, Suganami T, Ogawa Y.Antiobesity effect of eicosapentaenoic acid in high-fat/high-sucrose diet-induced obesity: importance of hepatic lipogenesis. Diabetes 2010;59(10):2495–504.

[43] Barre DE. The role of consumption of alpha-linolenic, eicosapentaenoic and docosahexaenoic acids in human metabolic syndrome and type 2 diabetes—A mini-review. J Oleo Sci. 2007;56(7):319–25.

[44] Weintraub MS, Zechner R, Brown A, et al. Dietary polyunsaturated fat of the w-6 and w-3 series reduce postprandial lipoprotein concentrations: acute and chronic effects of fat saturation on postprandial lipoprotein metabolism. J Clin Invest. 1988;83:1884–93.

[45] Clarke SD. The multi-dimensional regulation of gene expression by fatty acids: Polyunsaturated fats as nutrient sensors. Curr Opin Lipidol. 2004;15:13–8.

[46] Mozaffarian D, Wu JH. (n-3) fatty acids and cardiovascular health: are effects of EPA and DHA shared or complementary? J Nutr. 2012;142(3):614S–625S.

[47] Yanagita T, Nagao K. Functional lipids and the prevention of the metabolic syndrome. Asia Pac J Clin Nutr. 2008;17(Suppl 1):189–191.

[48] Shapiro H, Tehilla M, Attal-Singer J, Bruck R, Luzzatti R, Singer P. The therapeutic potential of long-chain omega-3 fatty acids in nonalcoholic fatty liver disease. Clin Nutr 2011;30(1):6–19.

[49] Shirouchi B, Nagao K, Inoue N, Ohkubo T, Hibino H, Yanagita T. Effect of dietary omega 3 phosphatidylcholine on obesity-related disorders in obese Otsuka Long-Evans Tokushima fatty rats. J Agric Food Chem. 2007;55(17):7170–7176.

[50] Di Minno MN, Russolillo A, Lupoli R, Ambrosino P, Di Minno A, Tarantino G. Omega-3 fatty acids for the treatment of non-alcoholic fatty liver disease. World J Gastroenterol. 2012;18(41):5839–5847.

[51] Delarue J, LeFoll C, Corporeau C, Lucas D. N-3 long chain polyunsaturated fatty acids: a nutritional tool to prevent insulin resistance associated to type 2 diabetes and obesity? Reprod Nutr Dev. 2004;44(3):289–299.

[52] Quiroga AD, Li L, Trötzmüller M, Nelson R, Proctor SD, Köfeler H, Lehner R. Deficiency of carboxylesterase esterase-x results in obesity, hepatic steatosis, and hyperlipidemia. Hepatology 2012;56(6):2188–2198.

[53] Assy N. Nutritional recommendations for patients with non-alcoholic fatty liver diseases. World J Gastroenterol. 2011;17(29):3375–3376.

[54] Estruch R, Ros E, Salas-Salvadó J, Martínez-González MA, et al. for the PREDIMED Study Investigators. Primary prevention of cardiovascular disease with a Mediterranean diet. On line publication NEJM. February 25, 2013DOI: 10.1056/NEJMoa1200303.

[55] Cao QH, Qu WJ, Niu W, Deng YX, Wang YW, Xie JJ. The antihyperglycemic effect of flavonoids from hippophae rhamnoides l.on diabetic rats induced by streptozotocin. Acta Nutrimenta Sinica. 1956;0(02).

[56] Yang B, Kallio H. Effects of Sea buckthorn oil on skin. Asia Pacific Personal Care. 2003;4(5):46–49.

[57] Maguire LS, O'Sullivan SM, Galvin K, O'Connor TP, O'Brien NM. Fatty acid profile, tocopherol, squalene and phytosterol content of walnuts, almonds, peanuts, hazelnuts and the macadamia nut. Int J Food Sci Nutr. 2004;5:171–178. [PubMed]

[58] Sharma UK, Sharma K, Sharma N,. Sharma A, Singh HP, Sinha AK. Microwave-assisted efficient extraction of different parts of Hippophae rhamnoides for the comparative evaluation of antioxidant activity and quantification of its phenolic

constituents by reverse-phase high-performance liquid chromatography (RP-HPLC). J. Agric. Food Chem. 2008;56 (2):374–379. DOI: 10.1021/jf072510j.

[59] Elliott Middleton JR, Kandaswami C, Theoharis C. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacol Rev. 2000;52:673–751. http://pharmrev.aspetjournals.org/content/52/4/673.full.

[60] Bal LM, Meda V, Naik SN, Satya S. Sea buckthorn berries: A potential source of valuable nutrients for nutraceuticals and cosmoceuticals. Food Research International. 2011;44:1718–1727.

[61] Yang B, Kallio H. Composition and physiological effects of Sea buckthorn (*Hippophaë*) lipids. Trends Food Sci Technol. 2002;13(5):160–167.

[62] Gao X, Ohlander M, Jeppsson N, Bjork L, Trajkovski V. Changes in antioxidant effects and their relationship to phytonutrients in fruits of Sea buckthorn (*Hippophae rhamnoides* L.) during maturation. J Agric Food Chem. 2000;48:1485–1490. http://www.nature.com/ejcn/journal/v62/n9/full/1602831a.html#bib11.

[63] Ruan A, Min H, Meng Z, Lu Z. Protective effects of sea buckthorn seed oil on mouse injury induced by sulfur dioxide inhalation. Inhal Toxicol. 2003;15:1053–8 [PubMed].

[64] Upadhyay NK, Kumar R, Mandotra SK, Meena RN, Siddiqui MS, Sawhney RC, Gupta A. Safety and healing efficacy of Sea buckthorn (*Hippophae rhamnoides* L.) seed oil on burn wounds in rats. Food Chem Toxicol. 2009;47:1146–53 [PubMed].

[65] Wu D, Meng Z. Effect of sulfur dioxide inhalation on the glutathione redox system in mice and protective role of Sea buckthorn seed oil. Arch Environ Contam Toxicol. 2003;45:423–8 [PubMed].

[66] Bao M, Lou Y. Flavonoids from seabuckthorn protect endothelial cells (EA.hy926) from oxidized low-density lipoprotein induced injuries via regulation of LOX-1 and eNOS expression. J Cardiovasc Pharmacol. 2006;48:834–41 [PubMed].

[67] Gupta A, Kumar R, Pal K, Singh V, Banerjee PK, Sawhney RC. Influence of Sea buckthorn (*Hippophae rhamnoides* L.) flavone on dermal wound healing in rats. Mol Cell Biochem. 2006;290:193–8 [PubMed].

[68] Geetha S, Ram MS, Singh V, Ilavazhagan G, Sawhney RC. Effect of seabuckthorn on sodium nitroprusside-induced cytotoxicity in murine macrophages. Biomed Pharmacother. 2002;56:463–7 [PubMed].

[69] Goel HC, Prasad J, Singh S, Sagar RK, Kumar IP, Sinha AK. Radioprotection by a herbal preparation of *Hippophae rhamnoides*, RH-3, against whole body lethal irradiation in mice. Phytomedicine. 2002;9:15–25 [PubMed].

[70] Eccleston C, Baoru Y, Tahvonen R, Kallio H, Rimbach GH, Minihane AM. Effects of an antioxidant-rich juice (Sea buckthorn) on risk factors for coronary heart disease in humans. J Nutr Biochem. 2002;13(6):346-354.

[71] Johansson AK, Korte H, Yang B, Stanley JC, Kallio HP. Sea buckthorn berry oil inhibits platelet aggregation. J Nutr Biochem. 2000;11(10):491–5.

[72] Larmo PS, Yang B, Hurme SA, Alin JA, Kallio HP, Salminen EK, Tahvonen RL. Effect of a low dose of Sea buckthorn berries on circulating concentrations of cholesterol, triacylglycerols, and flavonols in healthy adults. Eur J Nutr. 2009;48:277–82 [PubMed].

[73] Basu M, Prasad R, Jayamurthy P, Pal K, Arumughan C, Sawhney RC. Anti-atherogenic effects of Sea buckthorn (Hippophaea rhamnoides) seed oil. Phytomedicine. 2007;14(11):770–7.

[74] Tahvonen R, Schwab U, Linderborg K, Mykkänen HM, Kallio HP, et al. Black currant seed oil and fish oil supplements differ in their effects on fatty acid profiles of plasma lipids, and concentrations of serum total and lipoprotein lipids, plasma glucose and insulin. J Nutr Biochem. 2005;16:353–359.

[75] Yang B, Kalimo K., Mattila L, Kallio S, Katajisto J, Peltola O, Kallio H. Effect of dietary supplementation with Sea buckthorn (*Hippophaë rhamnoides*) seed and pulp oils on atopic dermatitis. J. Nutr. Biochem. 1999;10:622–630.

[76] Kris-Etherton PM, Pearson TA, Wan Y, Hargrove RL, Moriarty K, Fishell V, Etherton TD. High–monounsaturated fatty acid diets lower both plasma cholesterol and triacylglycerol concentrations. Am J Clin Nutr. 1999;6:1009–1015.

[77] Cao H, Gerhold K, Mayers JR, Wiest MM, Watkins SM, Hotamisligil GS. Identification of a lipokine, a lipid hormone linking adipose tissue to systemic metabolism. Cell. 2008 Sep 19;134(6):933–44. DOI: 10.1016/j.cell.2008.07.048.

[78] Dimopoulos N, Watson M, Sakamoto K, Hundal HS. Differential effects of palmitate and palmitoleate on insulin action and glucose utilization in rat L6 skeletal muscle cells. Biochem J. 2006;399:473–481. DOI: 10.1042/BJ20060244.

[79] Yang ZH, Miyahara H, Hatanaka A.. Chronic administration of palmitoleic acid reduces insulin resistance and hepatic lipid accumulation in KK-Ay Mice with genetic type 2 diabetes. Lipids in Health and Disease. 10(2011):120.

[80] Maedler K, Oberholzer J, Bucher P, Spinas GA, Donath MY. Monounsaturated fatty acids prevent the deleterious effects of palmitate and high glucose on human pancreatic beta-cell turnover and function. Diabetes. 2003;52:726–733. DOI: 10.2337/diabetes.52.3.726.

[81] Larmo PS, Kangas AJ, Soininen P, Lehtonen HM, Suomela JP, Yang B, Viikari J, Ala-Korpela M, Kallio H. Effects Sea buckthorn and bilberry on serum metabolites differ according to the baseline metabolic profiles in overweight women: A randomized cross-over trial. Am. J. Clin. Nutr. 2013;98: 945–951.

[82] Kumar Y, Dutta R, Prasad D, Misra K. Subcritical water extraction of antioxidant compounds from Seabuckthorn (Hippophae rhamnoides) leaves for the comparative evaluation of antioxidant activity. Food Chemistry. 1 August 2011;127(3):1309–1316.

[83] Lehtonen HM, Järvinen R, Linderborg K, Viitanen M, Venojärvi M, Alanko H, Kallio H. Postprandial hyperglycemia and insulin response are affected by Sea buckthorn (Hippophaë rhamnoides ssp. turkestanica) berry and its ethanol-soluble metabolites. Eur J Clin Nutr. 2010 Dec;64(12):1465–71. DOI: 10.1038/ejcn.2010.173. Epub 2010 Sep 8. PMID: 20823898.

[84] Zhang W, Zhao J, Wang J, Pang X, Zhuang X, Zhu X, Qu W. Hypoglycemic effect of aqueous extract of seabuckthorn (Hippophae rhamnoides L.) seed residues in streptozotocin-induced diabetic rats. Phytother Res. 2010 Feb;24(2):228–32. DOI: 10.1002/ptr.2917.

[85] Linderborg KM, Lehtonen HM, Järvinen R, Viitanen M, Kallio H. The fibres and polyphenols in Sea buckthorn (Hippophaë rhamnoides) extraction residues delay postprandial lipemia. Int J Food Sci Nutr. 2012 Jun;63(4):483–90. DOI: 10.3109/09637486.2011.636346. Epub 2011 Nov 18.

[86] Juźwiak S, Wójcicki J, Mokrzycki K, Marchlewicz M, Białecka M, Wenda-Rózewicka L, Gawrońska-Szklarz B, Droździk M. Effect of quercetin on experimental hyperlipidemia and atherosclerosis in rabbits. Pharmacol Rep. 2005;57(5):604–9.

[87] Gnoni GV, Paglialonga G, Siculella L. Quercetin inhibits fatty acid and triacylglycerol synthesis in rat-liver cells. Eur J Clin Invest. 2009;39(9):761-8. DOI: 10.1111/j.1365-2362.2009.02167.x. Epub 2009 Jun 8.

[88] Gao W, Chen C, Kong DY. Hippophins C-F, four new flavonoids, acylated with one monoterpenic acid from the seed residue of Hippophae rhamnoides subsp. sinensis. J Asian Nat Prod Res. 2013;15(5):507–14. DOI: 10.1080/10286020.2013.787989. Epub 2013 May 3.

[89] Pichiah PB, Moon HJ, Park JE, Moon YJ, Cha YS. Ethanolic extract of seabuckthorn (Hippophae rhamnoides L) prevents high-fat diet-induced obesity in mice through down-regulation of adipogenic and lipogenic gene expression. Nutr Res. 2012;32(11):856-64. DOI: 10.1016/j.nutres.2012.09.015.

[90] Lehtonen, HM, Suomela JP, Tahvonen R, Yang B, Venojärvi M, Viikari J, Kallio H. Different berries and berry fractions have various but slightly positive effects on the associated variables of metabolic diseases on overweight and obese women. European Journal of Clinical Nutrition. 2011; 65(3):394–401. DOI: 10.1038/ejcn.2010.268.

[91] Kwiterovich PO. The Johns Hopkins Textbook of Dyslipidemia: Baltimore, Maryland; 2010.

[92] Montero D, Walther G, Perez-Martin A, Roche E, Vinet A. Endothelial dysfunction, inflammation, and oxidative stress in obese children and adolescents: Markers and effect of lifestyle intervention. Obesity Reviews. 2012;13(5):441–55.

[93] Juonala M, Viikari JS, Kähönen M et al. Childhood levels of serum apolipoproteins B and A-1 predict carotid intima-media thickness and brachial endothelial function in adulthood: The cardiovascular risk in young Finns study. J. Am. Coll. Cardiol. 2008;52:293–299.

[94] Whitfield JB. Gamma glutamyl transferase. Crit. Rev. Clin. Lab. Sci. 2001;38(4):263–355.

[95] Klop B, Elte JWF, Cabezas MC. Dyslipidemia in Obesity: Mechanisms and Potential Targets. Nutrients. 2013;5:1218–1240.

[96] Franssen R, Monajemi H, Stroes ES, Kastelein JJ. Obesity and dyslipidemia. Med. Clin. North. Am. 2011;95:893–902.

[97] Sattar N, Williams K, Sniderman AD, D'Agostino Jr. R, Haffner SM. Comparison of the associations of apolipoprotein B and low-density lipoprotein cholesterol with other cardiovascular risk factors in the Insulin Resistance Atherosclerosis Study (IRAS). Circulation. 2003;108:2312–2316.

[98] Rank M, Siegrist M, Wilks DC, Langhof H, Wolfarth B, Haller B, Koenig W, Halle M. The cardio-metabolic risk of moderate and severe obesity in children and adolescents. J Pediatr. 2013 Jul;163(1):137–42. DOI: 10.1016/j.jpeds.2013.01.020. Epub 2013 Feb 20.

[99] Uysal Y, Wolters B, Knop C, Reinehr T. Components of the metabolic syndrome are negative predictors of weight loss in obese children with lifestyle intervention. Clin Nutr. 2014 Aug;33(4):620–5. DOI: 10.1016/j.clnu.2013.09.007. Epub 2013 Sep 18.

[100] Ho M, Garnett SP, Baur LA, Burrows T, Stewart L, Neve M, Collins C. Impact of dietary and exercise interventions on weight change and metabolic outcomes in obese children and adolescents: A systematic review and meta-analysis of randomized trials. JAMA Pediatr. 2013 Aug 1;167(8):759–68. DOI: 10.1001/jamapediatrics.2013.1453.

[101] Hobkirk JP, King RF, Gately P, Pemberton P, Smith A, Barth JH, Harman N, Davies I, Carroll S. The predictive ability of triglycerides and waist (hypertriglyceridemic waist) in assessing metabolic triad change in obese children and adolescents. Metab Syndr Relat Disord. 2013 Oct;11(5):336–42. DOI: 10.1089/met.2012.0152. Epub 2013 Jun 12.

[102] Sherafat-Kazemzadeh R, Yanovski SZ, Yanovski JA, Pharmacotherapy for childhood obesity: Present and future prospects, Int J Obes (Lond) 2013 Jan;37(1):1–15. Published online 2012 Aug 28. DOI: 10.1038/ijo.2012.144.

[103] Mühlig Y, Wabitsch M, Moss A, Hebebrand D. Weight Loss in Children and Adolescents. Dtsch Arztebl Int. 2014 Nov;111(48):818–824. Published online 2014 Nov 28. DOI: 10.3238/arztebl.2014.0818.

[104] Casariu ED, Vîrgolici B, Lixandru D, Stancu M, Greabu M, Totan A, Miricescu D, Mohora M. Relations for APO B/APO A-I ratio with different cardiovascular risk factors, in obese children. Romanian J. Biophys. 2012;22(2):83–94.

[105] Virgolici B, Lixandru D, Casariu ED, Stancu M, Greabu M, Totan A, Miricescu D, Mohora M. Sea buckthorn pulp oil treatment prevents atherosclerosis in obese children. ISRN, Hindawi, Volume 2013 (2013), Article ID 164941, 9 pages.

[106] Codoñer-Franch P, Boix-García L, Simó-Jordá R, Del Castillo-Villaescusa C, Maset-Maldonado J, Valls-Bellés V. Is obesity associated with oxidative stress in children? International Journal of Pediatric Obesity. 2010;5(1):56-63.

[107] Desideri G, De Simone M, Iughetti L, Rosato T, Iezzi ML, Marinucci MC, Cofini V, Croce G, Passacquale G, Necozione S, Ferri C. Early activation of vascular endothelial cells and platelets in obese children. J. Clin. Endocrinol. Metab. 2005;90:3145–3152.

[108] Larmo P. The health effects of Sea buckthorn berries and oils, Department of Biochemistry and Food Chemistry, Turku, Sweden, ISBN 978-951-29-4459-0, 2011.

[109] Popescu LA, Vîrgolici B, Păcurar D, Timnea O, Ranetti AE, Orăşeanu D, Zăgrean L. Beneficial effects of Omega-3 fatty acids in nonalcoholic fatty liver disease, in childhood obesity. Farmacia. 2013;3(61):598–608.

[110] Daniels SR, Arnett DK, Eckel RH, et al. Overweight in children and adolescents: pathophysiology, consequences, prevention and treatment. Circulation. 2005;111:1999–2012.

[111] Fan X, Chen SK, Tang Q, Luo JS, Feng Y. Relationship between alanine aminotransferase and overweight or obesity in children. Zhongguo Dang Dai Er Ke Za Zhi. 2011;13(12):951–4.

[112] Calcaterra V, Muratori T, Klersy C, Albertini R, Caramagna C, Brizzi V, Larizza D. Early-onset metabolic syndrome in prepubertal obese children and the possible role of alanine aminotransferase as marker of metabolic syndrome. Ann Nutr Metab. 2011;58(4):307–14.

[113] Casariu ED, Virgolici B, Lixandru D, Miricescu D, Totan A, Popescu LA, Mohora M. Sea buckthorn pulp oil treatment can prevent metabolic syndrome in hypertriglyceridemic waist fenotype obese children. Farmacia. 2013;61(6):1043–1053.

[114] Popescu LA, Virgolici B, Lixandru D, Miricescu D, Condrut E, Timnea O, Ranetti AE, Militaru M, Mohora M, Zăgrean L. Effect of diet and Omega-3 fatty acids in NAFLD. RJME. 2013;54(3 Suppl):785–790.

Section 2

Role of Lipoproteins in Atherogenesis

Chapter 4

The Assessment of the Atherogenic Lipoprotein Profile in Cardiovascular Diseases by Lipoprint System Analysis

Stanislav Oravec, Kristina Gruber, Andrej Dukat,
Peter Gavornik, Ludovit Gaspar and Elisabeth Dostal

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/60989

Abstract

Research focus: Identification of incidence of an atherogenic lipoprotein phenotype B in four representative diagnoses of cardiovascular diseases: a) arterial hypertension, b) coronary heart disease, c) lower extremity arterial disease, d) ischemic stroke Research methods: A clinical study included 366 patients with a diagnosis of arterial hypertension (n=107), coronary heart disease (n= 104), lower extremity arterial disease (n= 100) and ischemic stroke (n= 55). The control group consisted of 150 healthy normotensive and normolipemic volunteers, all non-smokers, without signs of cardiovascular disease. In all tested individuals (or subjects) lipid parameters in serum: cholesterol and triglycerides were analyzed, using the enzymatic CHOD-PAP method, Roche Diagnostics Germany. Lipoproteins in serum lipoprotein spectrum by Lipoprint LDL system were analyzed and an atherogenic and a non-atherogenic lipoprotein profile idetified. The Score of the Anti-Atherogenic Risk (SAAR) was calculated as the ratio between non-atherogenic and atherogenic lipoproteins. Results: More than 80 percent of tested patients with cardovascular diseases have an atherogenic lipoprotein profile, with a high level of strongly atherogenic small dense LDL. The aterogenic profile was found in arterial hypertension 78.5%, in coronary heart disease in 81.7%, in lower extremity arterial disease in 80 %, and in patients who survived an ischemic stroke in 85%. Main conclusion: The atherogenic lipoprotein profile was found to be the overwhelming lipoprotein profile in tested cardiovascular diseases A new phenomenon- atherogenic normolipidemia - as a risk factor for the development

of cardiovascular disease, would be established as a new term used in the diagnostics of dyslipoproteinemias

Keywords: atherogenic lipoproteins, atherogenic lipoprotein profile, small dense LDL, cardiovascular diseases

1. Introduction

In the last few decades, lipoprotein research has focused on the phenomenon of atherogenic and non-atherogenic lipoproteins, specifically, atherogenic and non-atherogenic lipoprotein profiles phenotype A and phenotype B [7, 18, 60] after it was reported that more than 75% of patients with an acute coronary syndrome or myocardial infarction had normal plasma values of cholesterol, Low Density Lipoprotein cholesterol (LDL cholesterol) and High Density Lipoprotein cholesterol (HDL cholesterol) [15 - 17].

Thus, it was necessary to look for other risks factors in plasma, the presence of which in relevant quantities could cause damage to endothelial cells and resultant endothelial dysfunction [59]. This called into question whether an increased total cholesterol level, or increased LDL-cholesterol, as a criterion for the degree of atherogenic risk, provided a universal explanation for the origin of atherogenesis. 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 [Table 1]: formation of atherothrombotic plaques, acute myocardial infarction, ischemic stroke, or sudden death [39, 59, 64].

The predominance of atherogenic lipoproteins in plasma is characteristic for the atherogenic lipoprotein spectrum, phenotype B. When present in plasma in high concentrations, these lipoproteins contribute to ischemic vascular impairment [6, 8, 57, 60]. The process of degenerative changes in vessels results in the formation of atheromatous vascular plaques. These later play an important role in the formation of stable or unstable angina (pectoris), and critical ischemia of peripheral and/or cerebral arteries as well [39,56]. When atherogenic lipoproteins in plasma are present in small quantities, we obtain a picture of a non-atherogenic lipoprotein profile, phenotype A.

Various methods have been developed (gradient gel electrophoresis, ultracentrifugation, magnetic resonance spectroscopy, endothelial models for testing lipoprotein cytotoxicity) to identify atherogenic lipoproteins [2, 26, 45, 48], but because of technical and financial issues, long-term analyses and high operating costs, the previously mentioned methods were used primarily in basic research. Simple analytical procedures for routine distribution were lacking and the possibility of their implementation in every day laboratory practice remained limited.

An electrophoretic method by which to separate lipoproteins on polyacrylamide gel (PAG) with the use of Lipoprint LDL System [29, 41] has become a milestone in routine laboratory

analysis and in diagnosing metabolism disorders of lipoproteins. It enables the analysis of 12 lipoprotein subfractions: VLDL, IDL 1-3, LDL 1-7, and HDL.

1. The Lipoprint LDL system identifies and quantifies

   a. Atherogenic lipoproteins (VLDL, IDL1, IDL2, and LDL3-7, so-called small dense LDL)

   b. Non-atherogenic lipoprotein entities (IDL3, HDL)

   c. Lipoproteins with uncertain atherogenicity (LDL1, LDL2)

2. And determines

   a. The atherogenic vs. non-atherogenic lipoprotein spectrum, phenotype B vs. phenotype A

Atherogenic lipoprotein spectrums are characterized according to the predominance of atherogenic lipoproteins: very low density (VLDL); intermediate density IDL1 and IDL2; and by the presence of small dense-low density lipoproteins (sd-LDL). The last represented small dense LDL are highly atherogenic LDL subfractions that form fractions LDL3-7. As the name implies, they are smaller than the other types of LDL with a diameter < 26.5 nm (265 Angströms) and they float within the density range of 1.048–1.065 g/ml, that is, higher than LDL1 and LDL2. On the separating polyacrylamide gel (PAG) sd-LDL are detected as subtle bands on the anodic end of the gel, right behind HDL, that migrate to the head of separated lipoproteins.

Small dense LDL are highly atherogenic for [11, 14, 46]:	
* Low recognition by LDL-receptors (configuration alterations Apo B)	→
* Enhanced aptitude for oxidation and acetylation	→
* Oxide-LDL	→ release of pro-inflammatory cytokines → muscle cell apoptosis
* Oxide-LDL	→ release of metalloproteinase → collagen degradation
* Oxide-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	

Table 1. Atherogenicity of small dense LDL

In our studies were analyzed serum lipoprotein spectrums in patients with newly recognized a) arterial hypertension, b) coronary heart disease, c) lower extremity arterial disease, and d) in patients who survived a stroke. As mentioned earlier, an analytical method for a quantitative evaluation of lipoprotein fractions was used, and the incidence of an atherogenic lipoprotein

spectrum phenotype B (vs. phenotype A) in these four representatives of cardiovascular diseases was identified. At the same time, a lipoprotein spectrum of a control group of healthy individuals was examined and tested for the incidence of phenotype B.

2. Arterial hypertension

Arterial hypertension (AH) (Fig. 3) is one of the most serious cardiovascular diseases. More than 20% of the adult population suffers from this disease. AH is one of the risk factors for atherosclerosis development of coronary, brain, and peripheral arteries, together with the main cardinal risk factors, that is, dyslipoproteinemia and tobacco smoking [14,20,38]. Atherogenic LDL subfractions also play a role in the development of the arterial hypertension [32, 43].

AH is a permanent, long-lasting increase in blood pressure of more than 140/90 mmHg in people of middle age. In people older than 70 years of age, values higher than 160/95 mmHg are considered increased. For more extensive guidelines see the Statement of WHO/ISH (International Society of Hypertension) on the management of hypertension [61].

Dyslipoproteinemia, which frequently accompanies AH and multiplies the risk of atherosclerosis development, can also be considered one of the multiple sources that give rise to AH [35, 64].

Atherogenic lipoproteins in plasma cause endothelial dysfunction, increase vessel tone, and support the development of AH, which terminates in organ ischemia [8,50,51,55,57,59].

2.1. Patients

In our study 107 patients with newly diagnosed arterial hypertension were examined. Repeated blood pressure (BP) examination confirmed an increased blood pressure more than 150 mmHg for systolic and more than 90 mmHg for diastolic blood pressure in all hypertensive patients. Average systolic blood pressure was 172 ±19 mmHg and average diastolic blood pressure was 102 ±10 mmHg. The group of hypertensive patients comprised 66 men and 41 women. The average age of the men was 50 ± 17.6 years and the average age of the women was 51.0 ± 13.4 years.

The control group consisted of 150 healthy normotensive and normolipemic volunteers, all non-smokers, without signs of cardiovascular disease and without biochemical signs of lipid metabolism disorders. The average age of the subjects was 21 years, and the control group involved 50 males and 100 females. Volunteers were recruited from medical students at the Medical Faculty, who gave written, informed consent, and the study was approved by the local ethics committee.

2.2. Methods

A blood sample from an antecubital vein was obtained in the morning after a 12-hour fasting period. Total cholesterol and triglycerides in serum were analyzed from lipid parameters,

http://dx.doi.org/10.5772/60989

using the enzymatic CHOD-PAP method, Roche Diagnostics Germany. To determine the non-atherogenic lipoprotein phenotype A and the atherogenic lipoprotein phenotype B, the Lipoprint LDL System Quantimetrix CA, USA, was used.

The Score of the Anti-Atherogenic Risk (SAAR) was calculated as the ratio between non-atherogenic and atherogenic lipoproteins in serum [42]. SAAR values over 10.8 represented a non-atherogenic lipoprotein profile, whereas values under 9.8 represented an atherogenic lipoprotein profile. The cut off values for a non-atherogenic lipoprotein profile and an atherogenic lipoprotein profile were calculated from the results of 940 Lipoprint LDL analyses. Using the Quantimetrix Lipoprint LDL system interpretation, all 940 individuals were examined (general group of subjects) and tested for the occurrence of atherogenic vs. non-atherogenic lipoprotein profile and were divided into the two subgroups of subjects with an LDL profile:

- Indicative of Type A, that is, a non-atherogenic lipoprotein profile phenotype A
- Not indicative of Type A, that is, an atherogenic lipoprotein profile, phenotype B [29]

Statistical evaluation of obtained values was performed with an unpaired student's t-test. The level of significance was accepted at $p < 0.05$.

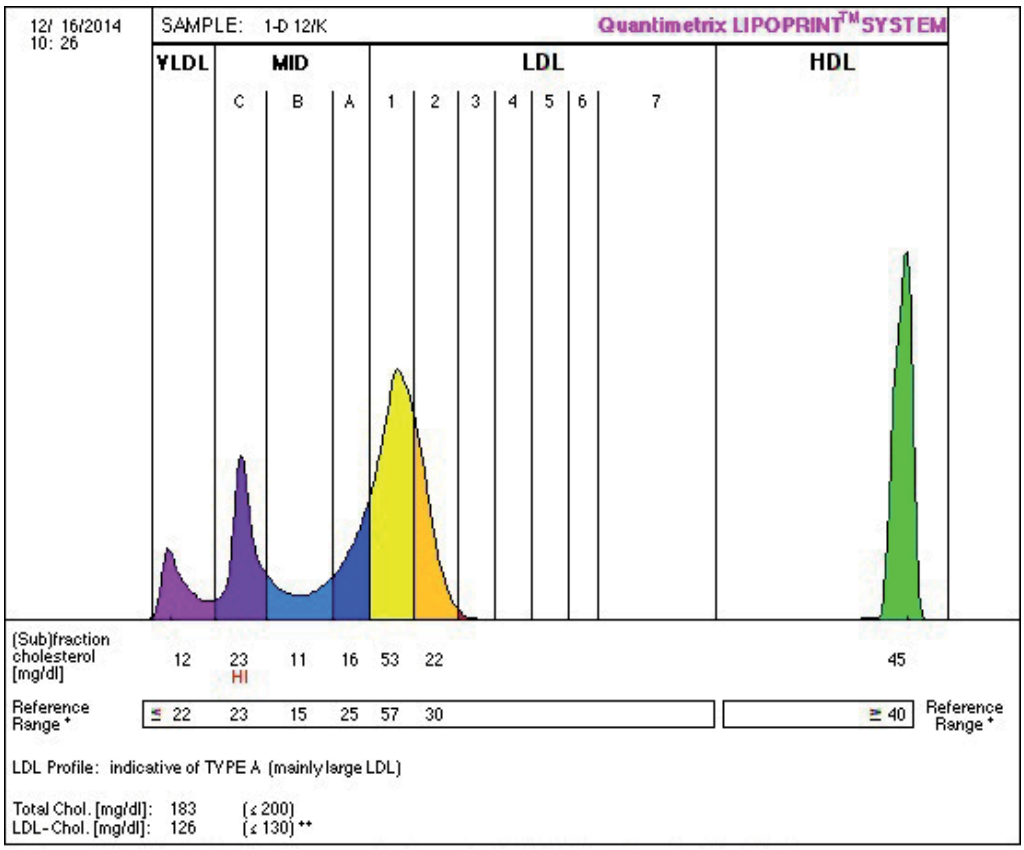
2.3. Results

In the control group shown in Table 2, along with the individuals with non-atherogenic normolipidemia, that is, an ideal lipoprotein profile (Fig. 1), a subgroup of normolipidemic individuals with an atherogenic lipoprotein profile was also identified. This group represented people with an atherogenic normolipidemia (Fig. 2). These people are clinically healthy, without clinical or laboratory signs of cardiovascular diseases, but with a positive familial history for cardiovascular diseases (myocardial infarction) in the parents' or grandparents' generation. The triglycerides and LDL3-7 concentrations in the control group with the atherogenic profile, compared to the individuals with a non-atherogenic lipoprotein profile, were increased ($p < 0.05$, respectively, $p < 0.0001$). The Score of the Anti-Atherogenic Risk (SAAR) for a non-atherogenic lipoprotein profile is a sensitive indicator by which to differentiate between an atherogenic and non-atherogenic plasma lipoprotein constellation (non-atherogenic vs. atherogenic: $p < 0.0001$).

	Chol	**TAG**	**VLDL**	**LDL1, 2**	**LDL3-7**	**LDL**	**HDL**	**Score**
	(mmol/l±SD)							
Control	4.28	1.15	0.60	1.29	0.03	2.31	1.35	37.8
	±0.60	±0.39	±0.16	±0.38	±0.003	±0.53	±0.32	±19.7
(non atherogenic profile n =140)								
Control	4.25	1.44	0.68	1.16	0.22	2.24	1.32	6.0
	±0.54	±0.40	±0.14	±0.24	±0.08	±0.36	±0.31	±2.0

	Chol	TAG	VLDL	LDL1, 2	LDL3-7	LDL	HDL	Score
	(mmol/l±SD)							
(atherogenic profile n = 10)								
Control	**4.27**	**1.17**	**0.61**	**1.28**	**0.04**	**2.30**	**1.34**	**35.8**
	±0.60	±0.39	±0.16	±0.37	±0.004	±0.52	±0.32	±18.5
(total number n=150)								
Non-atherogenic vs. atherogenic								
		p<0.05			**p< 0.0001**			**p< 0.0001**
Non-atherogenic profile, 93.4 % vs. atherogenic profile, 6.6 %, in control group								

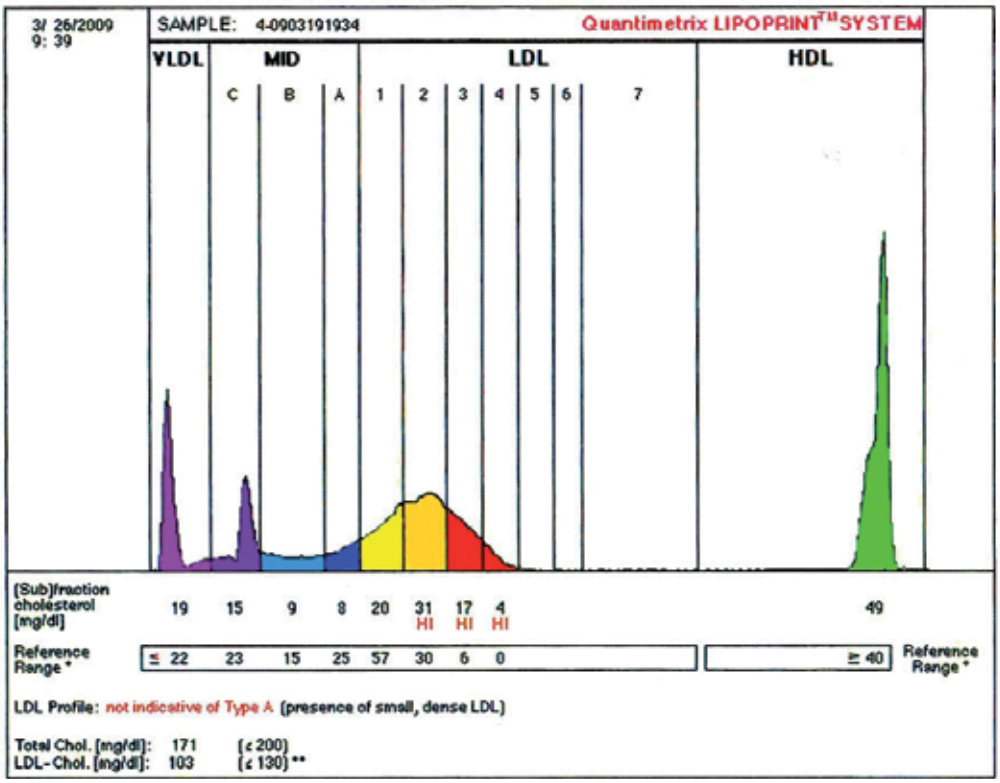
Table 2. Serum concentration of lipids, lipoproteins, and SAAR-score in the control group



*Reference ranges derived from 125 serum samples that met the NCEP ATPIII guidelines for desirable lipid status
**LDL-C comprised of the sum of cholesterol in Md bands C through A as well as all the subfractions

Figure 1. Non-atherogenic normolipidemia – Control group, SAAR score: 62.5

http://dx.doi.org/10.5772/60989



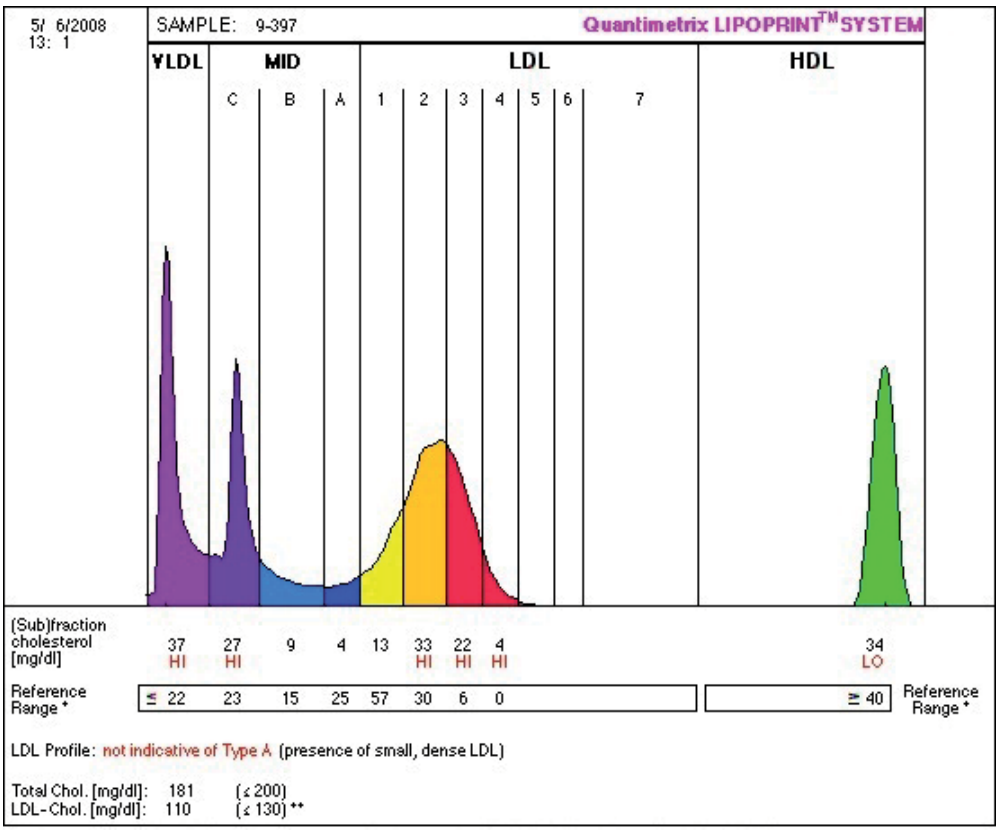
*Reference ranges derived from 125 serum samples that met the NCEP ATPIII guidelines for desirable lipid status
**LDL-C comprised of the sum of cholesterol in Md bands C through A as well as all the subfractions

Figure 2. Atherogenic normolipidemia – atherogenic subgroup of control group atherogenic small dense LDL are present in LDL 3,4 subfractions SAAR score: 2.7

A non-atherogenic lipoprotein profile in the control group was confirmed in 93.4% healthy normolipidemic individuals, and an atherogenic lipoprotein profile was found in 6.6%.

Table 3 shows high statistical significance for the analyzed lipid and lipoprotein parameters between the control group and the group of subjects with arterial hypertension ($p < 0.0001$, and for HDL, $p < 0.03$).

In Table 4, 78.5% of patients with arterial hypertension have an atherogenic lipoprotein profile. There is a highly significantly increased concentration of small dense LDL (subfractions LDL3-7) in a subgroup of AH-patients, who have an atherogenic profile, compared to the concentration of small dense LDL in the subgroup of AH-patients with a non-atherogenic profile, which confirms the predominance of atherogenic lipoproteins in AH-patients and the creation of atherogenic lipoprotein profile, phenotype B, as well. SAAR in patients with AH is low, that is, 9.2 (cut off is 10.8), and confirms also the predominance of atherogenic lipoproteins in serum.



*Reference ranges derived from 125 serum samples that met the NCEP ATPIII guidelines for desirable lipid status
**LDL-C comprised of the sum of cholesterol in Md bands C through A as well as all the subfractions

Figure 3. Arterial hypertension with a borderline hypertriglyceridemia, small dense LDL are present in LDL3, 4 subfractions, SAAR score: 0.9

	Chol	TAG	VLDL	LDL1,2	LDL3-7	LDL	HDL	Score
	(mmol/l SD)							
Control	**4.27**	**1.17**	**0.61**	**1.28**	**0.04**	**2.30**	**1.34**	**35.8**
	±0.60	±0.39	±0.16	±0.37	±0.004	±0.52	±0.32	±18.5
(total number n=150)								
AH	**5.19**	**2.28**	**0.97**	**1.54**	**0.35**	**3.00**	**1.25**	**9.2**
	±1.10	±1.07	±0.34	±0.55	±0.25	±0.91	±0.34	± 4.5
(total number n= 107)								
Control vs. AH								
	p<0.0001						**p< 0.03**	**p< 0.0001**

Table 3. Serum concentration of lipids, lipoproteins, and SAAR-score in AH patients vs.

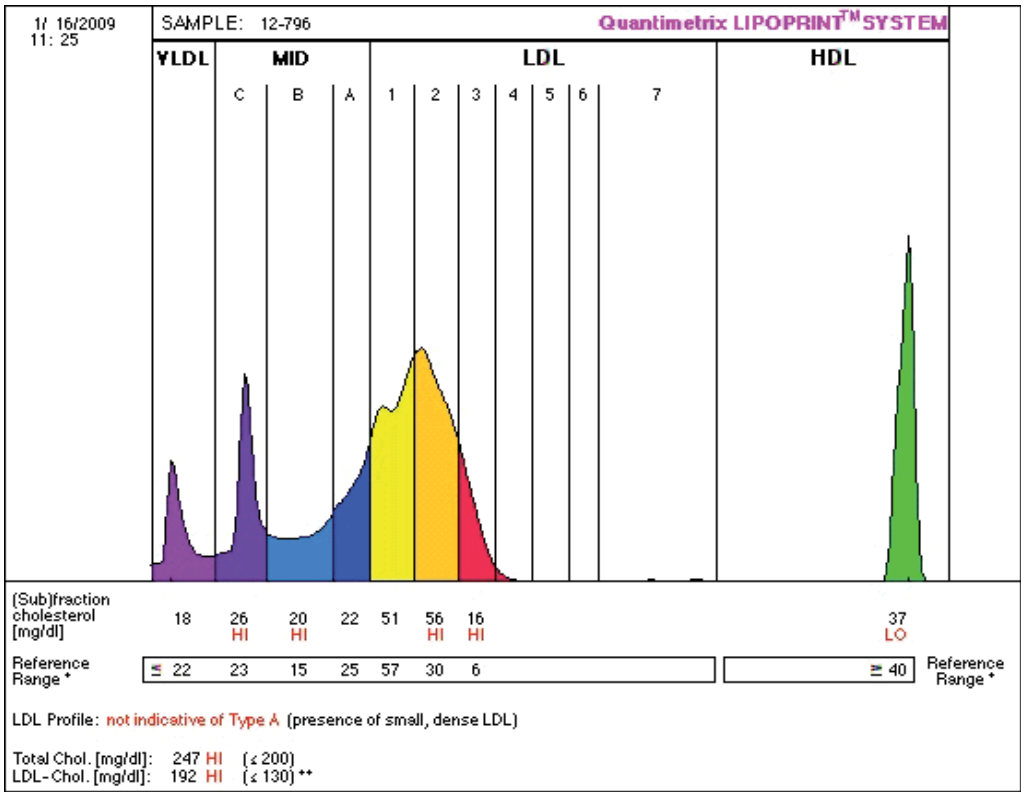
	Chol	TAG	VLDL	LDL1,2	LDL3-7	LDL	HDL	Score
	(mmol/l SD)							
AH	5.32	1.56	0.84	1.78	0.08	3.02	1.49	24.2
	±0.98	±0.55	±0.31	±0.44	±0.04	±0.71	±0.34	±13.6
(non-atherogenic profile n= 23)								
AH	5.15	2.48	1.01	1.47	0.42	2.99	1.18	5.1
	±1.14	±1.34	±0.35	±0.58	±0.31	±0.96	±0.34	± 2.0
(atherogenic profile n= 84)								
AH	**5.19**	**2.28**	**0.97**	**1.54**	**0.35**	**3.00**	**1.25**	**9.2**
	±1.10	±1.07	±0.34	±0.55	±0.25	±0.91	±0.34	± 4.5
(total number n=107)								
Non-atherogenic vs. atherogenic								
	n.s.	**p<0.002**	**p<0.05**	**p<0.02**	**p< 0.0001**	**n.s.**	**p< 0.001**	**p< 0.0001**
Atherogenic 78.5% vs. non-atherogenic 21.5% – arterial hypertension								

Table 4. Serum concentration of lipids, lipoproteins, and SAAR-score in patients with arterial hypertension

3. Coronary heart disease

Coronary heart disease (CHD) (Fig. 4) is a common manifestation of cardiovascular diseases and is frequently associated with lipid and lipoprotein metabolism disorders. Hypercholesterolemia and hypertriglyceridemia, as well as combined hyperlipoproteinemia are regular features that accompany CHD [22,50,51]. Pathophysiologically, the cause of myocardial ischemia is a disproportion, or imbalance, between myocardial oxygen supply and oxygen demand. Ischemia in stable angina is generally due to fixed atheromatous stenosis of one or more coronary arteries as a consequence of impaired lipoprotein metabolism and the formation of lipid atheromas in the coronary arteries [5, 33, 34,49].

However, clinically, stable angina is not the only form of manifestation of coronary heart disease. Stable angina, as an ischemia due to fixed atheromatous stenosis, can turn into a myocardial ischemia due to plaque rupture with thrombosis and spasm of the artery (instable angina). In addition, myocardial necrosis (myocardial infarction), caused by acute occlusion of a coronary artery (due to plaque rupture and thrombosis), can have fatal consequences for disabled persons. It can be supposed that the modified forms of lipoproteins can play an important role in any form of clinical manifestation of coronary heart disease. Recently, clinical studies reported that the atherogenic lipoprotein populations (lipoprotein subfractions), presented in the plasma lipoprotein spectrum in high concentrations, play an important role in the development of atherosclerotic changes in the arterial wall [14, 38, 39].



*Reference ranges derived from 125 serum samples that met the NCEP ATPIII guidelines for desirable lipid status
**LDL-C comprised of the sum of cholesterol in Md bands C through A as well as all the subfractions

Figure 4. Coronary heart disease combined with an atherogenic hypercholesterolemia. High concentration of atherogenic small dense LDL in LDL 3,4 subfractions SAAR score: 5.1

We distinguish facultative atherogenic very low density lipoproteins, VLDL, and their remnants, intermediate density lipoproteins, IDL, low density lipoproteins, LDL (considered a lipoprotein family with high atherogenicity), and high density lipoproteins, HDL. Modified lipoprotein entities in all these lipoprotein families can play a role in the formation of atherogenic lipoproteins, which accelerate the atherogenesis in the artery walls, including in the coronary arteries.

In our study, we focused on the determination of the incidence of an atherogenic lipoprotein phenotype in patients with coronary heart disease – in stable angina patients.

3.1. Patients

In our study, 104 patients with newly diagnosed coronary heart disease were examined. The diagnosis of CHD (stable angina pectoris grade I or II) was confirmed by medical examination, laboratory results, resting ECG, results of echocardiography, and duplex ultrasound of the carotid arteries.

http://dx.doi.org/10.5772/60989

3.2. Methods

See methods published in the section "Arterial hypertension (AH)."

3.3. Results

The results of lipid parameters presented in Table 5 confirm a highly significant increased concentration of analyzed lipid and lipoprotein parameters ($p<0.0001$) in CHD-patients, compared to control values and a low value of the SAAR. These low values (< 10.8) are regularly found in atherogenic lipoprotein phenotype B.

	Chol	**TAG**	**VLDL**	**LDL1,2**	**LDL3-7**	**LDL**	**HDL**	**Score**
	(mmol/l SD)							
Control	**4.27**	**1.17**	**0.61**	**1.28**	**0.04**	**2.30**	**1.34**	**35.8**
	±0.60	±0.39	±0.16	±0.37	±0.004	±0.52	±0.32	±18.5
(total number n=150)								
CHD	**5.25**	**2.41**	**0.99**	**1.52**	**0.41**	**3.06**	**1.18**	**5.6**
	±1.15	**±1.72**	**±0.43**	**±0.47**	**±0.29**	**±0.85**	**±0.29**	**± 3.8**
(total number n= 104)								
Control vs. CHD								
				p<0.0001				

Table 5. Serum concentration of lipids, lipoproteins, and SAAR-score in CHD patients vs. control group

	Chol	**TAG**	**VLDL**	**LDL1,2**	**LDL3-7**	**LDL**	**HDL**	**Score**
	(mmol/l±SD)							
CHD	5.26	1.44	0.82	1.73	0.13	3.11	1.29	12.7
	±0.99	±0.50	±0.27	±0.49	±0.06	±0.80	±0.31	±5.1
(non-atherogenic profile = 19)								
CHD	5.25	2.63	1.02	1.47	0.48	3.05	1.16	4.0
	±1.19	±2.03	±0.47	±0.46	±0.35	±0.86	±0.28	±3.5
(atherogenic profile n= 85)								
CHD	**5.25**	**2.41**	**0.99**	**1.52**	**0.41**	**3.06**	**1.18**	**5.6**
	±1.15	±1.72	±0.43	±0.47	±0.29	±0.85	±0.29	±3.8
(total number n=104)								
Non-atherogenic vs. atherogenic profile								
		p<0.01		**p<0.05**	**p< 0.0001**	**n.s.**		**p< 0.0001**

Note: In the column without published p values, the differences in the evaluated parameter were not significant (n.s.)

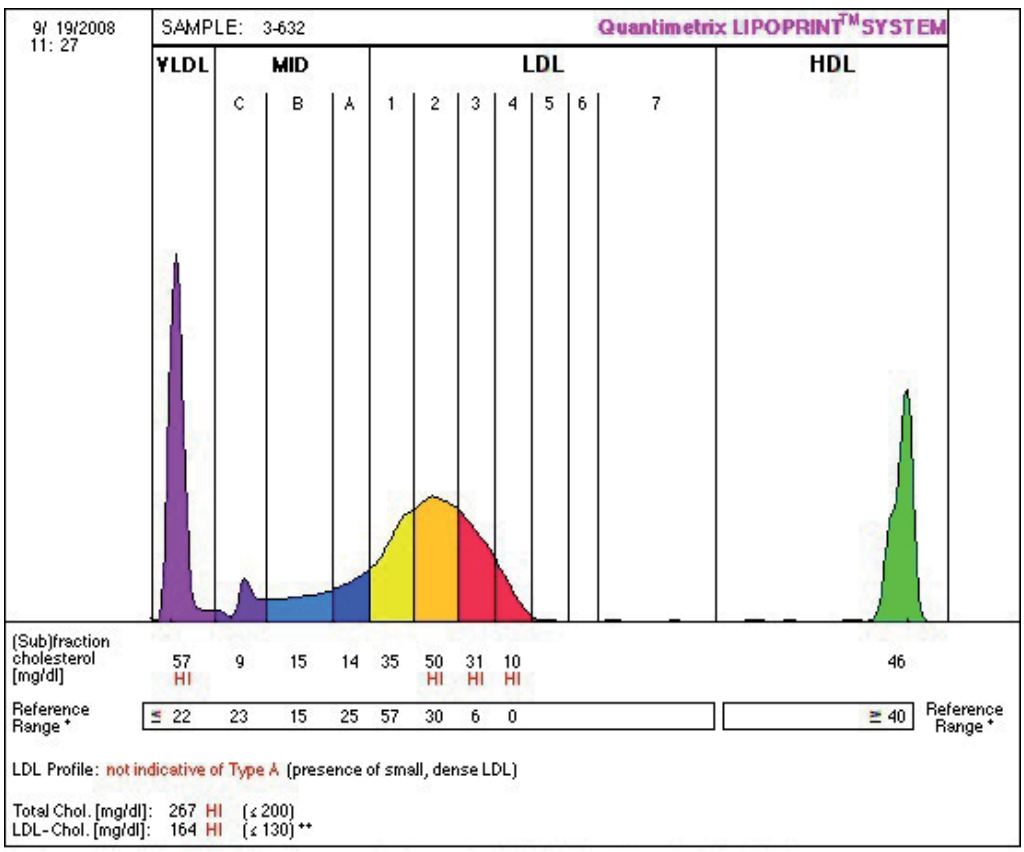
Atherogenic 81.7% vs. non-atherogenic 18.3%, in coronary heart disease

Table 6. Serum concentration of lipids, lipoproteins, and SAAR-score in coronary heart disease

In Table 6, an atherogenic lipoprotein phenotype B is present in 81.7% of patients with CHD. An increased concentration of small dense LDL (LDL3-7) in the CHD-patient subgroup with an atherogenic lipoprotein profile, compared to the results of the CHD-patient subgroup with a non-atherogenic lipoprotein profile ($p<0.0001$), confirms a predominance of atherogenic lipoproteins in the serum of patients with CHD.

4. Lower extremity arterial disease

Lower extremity arterial disease (LEAD) (Fig. 5) is a common atherogenic disease of the cardiovascular system. Patients with LEAD exhibit normal to high atherogenic dyslipoproteinemia [8, 31, 50 -52, 62].



*Reference ranges derived from 125 serum samples that met the NCEP ATPIII guidelines for desirable lipid status
**LDL-C comprised of the sum of cholesterol in Md bands C through A as well as all the subfractions

Figure 5. Lower extremity arterial disease with combined atherogenic hyperlipoproteinemia with high concentration of atherogenic small dense LDL (LDL3,4 subfractions) SAAR score: 1.5

http://dx.doi.org/10.5772/60989

Almost all lower extremity arterial disease is due to atherosclerotic changes in artery vessels, and the pathology of LEAD is also similar to coronary heart disease. The most important risk factor for the development and progression of atherosclerotic LEAD are tobacco smoking, arterial hypertension, and hyperlipidemia. Other risk factors include diabetes mellitus, low physical activity, and a diet rich in lipids and carbohydrates. However, dyslipidemia plays an important role. Increased lipid levels of cholesterol and triglycerides are generally accepted as important risk factors for the development of atherosclerosis [14,25, 47].

In the last few decades, there has been much discussion about which atherogenic lipoproteins participate in the formation of the atherogenic lipoprotein profile, phenotype B. Atherogenic lipoproteins in relevant concentration in the blood serum are responsible for the acceleration of the development of atherogenic cardiovascular diseases, including the development of LEAD. The LDL subpopulations of small dense LDL are considered to be strongly atherogenic lipoprotein entities in the plasma/serum lipoprotein spectrum [38,59] with consequent acceleration of endothelial dysfunction and formation of the atheromatous subendothelial plaques in the arteries [21]. In the present study, we have focused on determining the incidence of an atherogenic lipoprotein phenotype, along with determining the role of atherogenic serum lipoproteins, in patients with lower extremity arterial disease.

4.1. Patients

In the clinical study, 100 patients with newly diagnosed lower extremity arterial disease were examined. The study included 55 males and 45 females: the average age of males was 56.0 years ±11 years and the average age of females 52.5 years ± 14 years. The patients had C2a degree, according to the Claudication classification: [proximal type (AP), the first degree (P1) with dyslipidemia]. Patients were ex-smokers.

LEAD was diagnosed according to the history of disease, intermittent claudication, the medical examination, including physical examination (Ratschow's test in the modification according to Linhart, see the Angiological Section of Slovak Medical Chamber) [23, 24, 27, 28] and examination of the ankle-brachial (pressure) index (ABPI) [40, 55, 57].

4.2. Methods

See methods published earlier in the section "Arterial hypertension (AH)."

4.3. Results

Results of lipid parameters presented in Table 7 confirm the highly significant increased concentration of analyzed lipid and lipoprotein parameters in LEAD-patients ($p<0.0001$), compared to control values. The low values of the SAAR, which is generally low (< 10.8) in the atherogenic lipoprotein phenotype, also confirms the atherogenic lipoprotein constellation in the serum of LEAD-patients.

	Chol	TAG	VLDL	LDL1,2	LDL3-7	LDL	HDL	Score
	(mmol/l SD)							
Control	**4.27**	**1.17**	**0.61**	**1.28**	**0.04**	**2.30**	**1.34**	**35.8**
	±0.60	±0.39	±0.16	±0.37	±0.004	±0.52	±0.32	±18.5
(total number n=150)								
LEAD	**5.29**	**2.21**	**0.96**	**1.58**	**0.39**	**3.11**	**1.21**	**7.2**
	±1.21	**±1.05**	**±0.37**	**±0.51**	**±0.28**	**±0.96**	**±0.31**	**± 4.5**
(total number n= 100)								
Control vs. LEAD								
	«.....p<0.0001.....»							

Table 7. Serum concentration of lipids, lipoproteins, and SAAR-score in LEAD-patients vs. control group

	Chol	TAG	VLDL	LDL1,2	LDL3-7	LDL	HDL	Score
	(mmol/l±SD)							
LEAD	5.37	1.81	0.86	1.82	0.10	3.18	1.33	17.4
	±0.95	±0.51	±0.26	±0.54	±0.03	±0.82	±0.29	± 6.5
(non-atherogenic profile n= 20)								
LEAD	5.28	2.31	0.98	1.52	0.46	3.09	1.18	4.6
	±1.28	±1.18	±0.39	±0.50	±0.34	±0.99	±0.32	± 4.0
(atherogenic profile n= 80)								
LEAD	**5.29**	**2.21**	**0.96**	**1.58**	**0.39**	**3.11**	**1.21**	**7.2**
	±1.21	±1.05	±0.37	±0.51	±0.28	±0.96	±0.31	± 4.5
(total number n=100)								
Non-atherogenic vs. atherogenic								
	p<0.01	p<0.001		p<0.05	p< 0.0001			p< 0.0001

Atherogenic 80% vs. non-atherogenic 20% in lower extremity arterial disease

Table 8. Serum concentration of lipids, lipoproteins, and SAAR-score in lower extremity arterial disease

In 80% of patients (Table 8), LEAD was associated with an atherogenic lipoprotein phenotype. An increased concentration of small dense LDL (LDL3-7 subgroups) in the LEAD-patients with an atherogenic lipoprotein profile, compared to the results from the LEAD-patients with a non-atherogenic lipoprotein profile ($p<0.0001$), confirms the predominance of atherogenic lipoproteins in serum in the subgroup of patients with an atherogenic lipoprotein profile.

http://dx.doi.org/10.5772/60989

5. Stroke

Stroke (Fig. 6) is the leading cause of mortality and of long-term morbidity in the populations of developed industrialized countries in the world. The atherogenic serum lipoproteins in high concentrations create an atherogenic lipoprotein profile, which plays a key role in the acute onset of cardiovascular and cerebrovascular events, that is, stroke [54,55]. Cerebral stroke attack remains a frequent medical problem and is the third most frequent cause of mortality all over the world. It represents a heterogeneous group of diseases with more than 150 known causes. In 25–39% of strokes, the cause leading to the acute cerebrovascular event cannot usually be definitively explained [4].

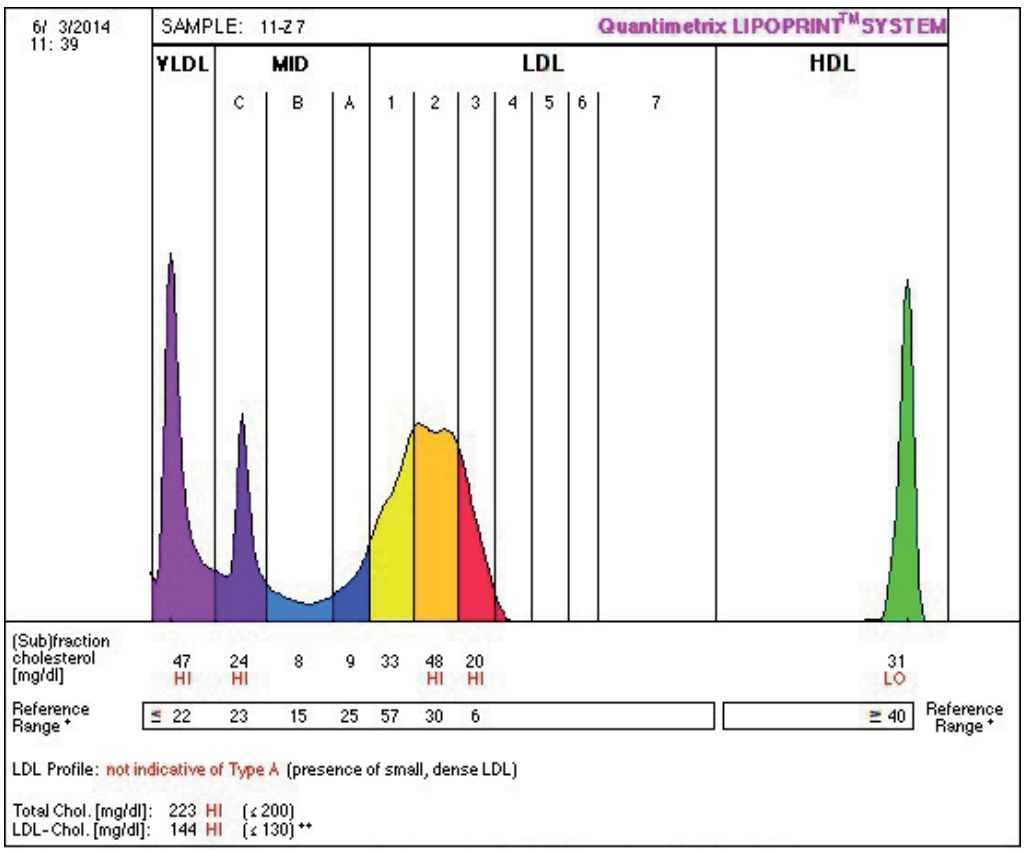


Figure 6. Patient survived an ischemic stroke with combined atherogenic hyperlipoproteinemia high concentration of VLDL, VLDL remnants and atherogenic small dense LDL, i.e. LDL 3, 4 subfractions. SAAR score: 2.4*Reference ranges derived from 125 serum samples that met the NCEP ATPIII guidelines for desirable lipid status**LDL-C comprised of the sum of cholesterol in Md bands C through A as well as all the subfractions

Dyslipidemia represents a risk factor for the development of cardiovascular disease, and thus dyslipidemia has been classified as an atherogenic phenomenon. The goal of the treatment of

hyperlipoproteinemia, that is, of dyslipidemia, is to reduce the lipid concentration in serum to established target values of lipids (cholesterol and triglycerides), but the primary goal is to reduce the atherogenic potential of serum lipids [9, 21, 53]. Dyslipoproteinemia is also the key phenomenon in the pathogenesis of the onset of atherosclerotic alterations in brain vessels [64]. Accompanied by high cholesterol levels – a classic risk factor for the development of cardiovascular diseases – an increased concentration of triglycerides in the blood serum can also play an important role in atherogenesis [3,58].

There are several studies that have provided evidence for the relation between carotid artery stenosis and an ischemic cerebral event [55]. However, the causal inter-relation between dyslipidemia and stroke has not been explained sufficiently [3, 4, 63]. Relapsing ischemic strokes account for one-fourth of all strokes in a year and are a strong evidence for a failure of secondary prevention [10]. This hard reality leads rightly so to the idea of optimal stroke prevention through the selection of individuals, who are at risk of stroke [13]. The aim of this pilot study was to identify the atherogenic lipoproteins and determine the lipoprotein profile in subjects who had suffered an ischemic cerebrovascular event, that is, stroke.

5.1. Patients

The study included 55 patients, 23 men, with an average age of 64 years ± 13 years, and 32 women, average age 74 years ± 13 years, who survived an ischemic cerebrovascular event, that is, a large-artery atherosclerosis subtype of stroke. To determine the subtype of ischemic stroke, the original TOAST (Trial of ORG 10172 in Acute Stroke Treatment) [1] criteria were used. The diagnosis of subtype was based on the risk factor profiles, clinical features, and results of diagnostic tests, including CT scan/MRI, vascular imaging (carotid duplex, transcranial Doppler), EEG – electroencephalography, echocardiography (transesophageal/ transthoracic), assessment of prothrombotic syndromes [1,30], activated partial thromboplastin time (aPTT), and international normalized ratio (INR).

5.2. Methods

See methods published in the section "Arterial hypertension (AH)."

A blood sample from the antecubital vein was obtained throughout the 24 hours after the onset of cerebrovascular event.

5.3. Results

The results of lipid parameters presented in Table 9 confirm a highly significantly increased concentration of analyzed lipid and lipoprotein parameters ($p<0.0001$) in people who survive a stroke, compared to control values, and also a low value on the SAAR, which is generally low (< 10.8) in an atherogenic lipoprotein phenotype.

In Table 10, an atherogenic lipoprotein phenotype was identified in 85.5 % of the patients who survive a cerebral ischemic stroke. The increased concentration of small dense LDL (LDL3-7

	Chol	TAG	VLDL	LDL1,2	LDL3-7	LDL	HDL	Score
	(mmol/l SD)							
Control	**4.27**	**1.17**	**0.61**	**1.28**	**0.04**	**2.30**	**1.34**	**35.8**
	±0.60	±0.39	±0.16	±0.37	±0.004	±0.52	±0.32	±18.5
(total number n=150)								
Stroke	**5.19**	**2.21**	**1.08**	**1.56**	**0.29**	**2.91**	**1.09**	**6.40**
	±1.10	±0.91	±0.34	±0.57	±0.24	±0.82	±0.32	±4.22
(total number n= 55								
Control vs. stroke								
	«.....p<0.0001.....»							

Table 9. Serum concentration of lipids, lipoproteins, and SAAR-score in stroke patients vs. control group.

	Chol	TAG	VLDL	LDL1,2	LDL3-7	LDL	HDL	Score	LDL1	LDL2
	(mmol/l±SD)									
Stroke	5.54	1.70	0.93	2.19	0.14	3.30	1.31	13.74	1.22	0.92
	±1.30	±0.44	±0.14	±0.86	±0.07	±1.01	±0.35	±1.36	±0.45	±0.44
(non-atherogenic profile n= 8)										
Stroke	5.14	2.29	1.11	1.48	0.31	2.86	1.06	5.33	0.72	0.76
	±1.11	±0.94	±0.37	±0.41	±0.23	±0.72	±0.29	±3.32	±0.26	±0.26
(atherogenic profile n = 47)										
Stroke	**5.19**	**2.21**	**1.08**	**1.56**	**0.29**	**2.91**	**1.09**	**6.40**	**0.78**	**0.78**
	±1.06	±0.91	±0.35	±0.53	±0.23	±0.77	±0.31	± 4.22	±0.33	±0.28
(total number n= 55)										
Non-atherogenic vs. atherogenic										
	n.s.	**n.s.**	**n.s.**	**p<0.002**	**p<0.05**	**n.s.**	**n.s.**	**p< 0.0001**	**n.s.**	**p< 0.0001**

Atherogenic 85.5 % vs. non-atherogenic 14.5 % in stroke patients.

Table 10. Serum concentration of lipids, lipoproteins, and SAAR-score in patients with stroke.

subgroups) in the atherogenic lipoprotein profile of patients with stroke, compared to the results in a non-atherogenic lipoprotein profile, is mild, but significant ($p<0.05$). The difference in the SAAR between the two subgroups was highly significant ($p<0.0001$), which also confirmed the overwhelming atherogenic lipoprotein constellation in patients who survived a stroke. The concentration of LDL1 was significantly higher in the subgroup of stroke-patients with a non-atherogenic lipoprotein profile ($p<0.0001$), however, the difference in the LDL2 lipoprotein subfraction was not significant.

6. Discussion

In the last few decades, lipoprotein research has focused on the phenomenon of atherogenic and non-atherogenic lipoproteins, and on the phenotype A vs. phenotype B characterization, as a consequence of the published evidence that the majority of the patients with an acute coronary syndrome or patients who survive a myocardial infarction had normal plasma values of cholesterol, LDL-cholesterol, and HDL-cholesterol [15-17]. A reasonable explanation for this fact was to posit a new, active atherogenic substance in plasma, an atherogenic lipoprotein subfraction, 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. Several clinical studies reported observations that in the plasma of patients with coronary heart disease there are subfractions of lipoproteins, which could play a crucial role in atherodegenerative processes and form the atherothrombotic plaques [5, 33, 34, 37, 39,49]. The Quebec Cardiovascular Study, a prospective study of 2,103 men [33,34] concluded that " a significant proportion of the risk for heart disease associated with small, dense LDL particles may be independent of variations in plasma lipid concentrations. Small LDL particles and elevated apo B levels were found to be the most predictive indications for ischemic heart disease ".

For this reason, patients who were suffering from cardiovascular diseases were examined in order to quantify the atherogenic lipoproteins in serum and to determine the incidence of an atherogenic lipoprotein profile in patients who had a diagnosis of cardiovascular diseases.

The clinical studies included 366 patients with a diagnosis of arterial hypertension (n=107), coronary heart disease (n= 104), lower extremity arterial disease (n= 100), and ischemic stroke (n= 55). Patients were tested with the diagnostic method Lipoprint LDL System, which quantifies atherogenic lipoproteins and identifies an atherogenic and a non-atherogenic lipoprotein profile [29]. This was a fundamental methodological contribution of this new analytical and diagnostic method.

Our study confirmed that more than 80% of tested patents with cardiovascular diseases have an atherogenic lipoprotein profile, with a high level of strongly atherogenic small dense LDL. The atherogenic lipoprotein profile was found to be the overwhelming lipoprotein profile in tested cardiovascular diseases. Such a profile was found in arterial hypertension in 78.5%, in coronary heart disease in 81.7%, in lower extremity arterial disease in 80%, and in patients who survived an ischemic stroke in 85%. The average atherogenic lipoprotein profile in all these tested diagnoses in the study was found to be of 81.3%.

This study also highlights the observation that, in the atherogenic lipoprotein profiles, in all diagnoses, compared to the non-atherogenic profiles, the concentration of total cholesterol is lower (n.s.) and the concentration of triglycerides is higher (even statistically significant; in AH, CHD, LEAD, as well as in the control group, up to $p<0.002$). Hypertriglyceridemia accompanied the hypercholesterolemia in all tested diagnoses, that is, in AH, CHD, LEAD, and stroke). The concentration of triglycerides, compared to the control group, was significantly increased ($p<0.0001$) and proportionally even higher than cholesterol. From this result,

it can be assumed that triglycerides/hypertriglyceridemia can play a much more important role than was generally accepted, as until now the most important role in the pathogenesis of vascular degenerative atherosclerotic injury was attributed to cholesterol and hypercholesterolemia. Our present results are in agreement with other authors, who have called attention to hypertriglyceridemia as a risk factor for cardiovascular diseases [8,12,19, 36,58], as triglyceride-rich lipoproteins can generate small dense LDL in high quantities [46].

The strong atherogenic lipoproteins – small dense LDL – have been found in the lipoprotein profile of all diagnostic groups [25,52,55,56]. Their presence is decisive for an atherogenic profile declaration. This is a rule that is valid not only for a hyperlipidemia, but also for a normolipidemia.

In the case of normolipidemia (see the atherogenic lipoprotein profile in the control group), a new phenomenon could be established – atherogenic normolipidemia [44] – as a risk factor for the development of cardiovascular disease. A special form of normolipidemia can also be atherogenic. This is new knowledge, and this new knowledge could help in the prevention and treatment of cardiovascular disease.

Acknowledgements

This study was supported by an EU structural research fund Interreg III AT-SR, project code: 1414-02-000-28 in years 2006-2008.

We would like to acknowledge the excellent technical assistance of MTA Barbara Reif, MTA Judith Trettler, and MTA Karin Waitz, Krankenanstalten Dr. Dostal, Vienna, Austria, and also to acknowledge the excellent technical assistance of MTA Olga Reinoldova, 2nd Department of Internal Medicine, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic.

Author details

Stanislav Oravec[1*], Kristina Gruber[2], Andrej Dukat[1], Peter Gavornik[1], Ludovit Gaspar[1] and Elisabeth Dostal[3]

*Address all correspondence to: stanislavoravec@yahoo.com

1 2nd Department of Internal Medicine, Faculty of Medicine, Comenius University, Bratislava, Slovak Republic

2 Department of Internal Medicine, Landesklinikum, Thermenregion Baden, Austria

3 Krankenanstalten Dr. Dostal, Vienna, Austria

References

[1] Adams HP, Benedixen BH, Kapelle LJ, Biller J, Love BB, Gordon DL, Marsh EE. Classification of subtype of acute ischemic stroke. Definition for use in a multicenter clinical trial:TOAST Trial of Org 10172 in Acute Stroke Treatment. Stroke 1993; 24(1): 35–41

[2] Alabakovska SB, Todorova BB, Labudovic DD, Tosheska KN. Gradient gel electrophoretic separation of LDL and HDL subclasses on BioRad Mini Protean II and size phenotyping in healthy Macedonians. Clin Chim Acta 2002; 317: 119–123

[3] Amarenco P, Labreuche J, Touboul PJ. High-density lipoprotein cholesterol and risk of stroke and carotid atherosclerosis. A systemic review. Atherosclerosis 2008; 196: 489–496

[4] Amarenco P, Bogousslavsky J, Caplan LR, Donnan GA, Hennerici MG. Classification of stroke subtypes. Cerebrovasc Dis 2009; 27: 493–501

[5] Arsenault BJ, Lemieux I, Despres J-P, Wareham NJ, Luben R, Kastelein JP, Khaw K-T, Boekholdt SM. Cholesterol levels in small LDL particles predict the risk of coronary heart disease in the EPIC-Norfolk prospective population study. Eur Heart J 2007; 28(22): 2770–2777

[6] Austin MA, Breslow JL, Henekens CH, Buring JE, Willett WC, Krauss RM. Low-density lipoprotein subclass patterns and risk of myocardial infarction. JAMA 1988; 260:1917–1921

[7] Austin MA, King MC, Vranizan KM, Krauss RM. Atherogenic lipoprotein phenotype. A proposed genetic marker for coronary heart disease risk. Circulation 1990; 82: 495–506

[8] Austin MA, Hokanson JE, Edwards KL. Hypertriglyceridemia as a cardiovascular risk factor. Am J Cardio 1994; 81: 7B–12B

[9] Backers J. Effect of lipid lowering drug therapy on small-dense low density lipoprotein. Ann Pharmacol 2005; 39(3): 523–526

[10] Bejot Y, Benatru I, Roaud O, Fromont A, Besancenot JP, Moreau T, Giroud M. Epidemiology of stroke in Europe: Geographic and environmental differences. J Neurol Sci 2007; 262(1–2): 85–88

[11] Berneis KK, Krauss RM. Metabolic origins and clinical significance of LDL heterogeneity. J Lipid Res 2002; 43: 1363–1379

[12] Brunzell JD. Hypertriglyceridemia. N Engl J Med 2007; 357(10): 1009–1017

[13] Bulas J. Interdisciplinárny prístup k liečbe náhlej cievnej mozgovej príhody zo strany Internistu.(A complex approach to the treatment of an acute cerebral-vascular event from the position of internist). Interna Medicina 2006; 6(9): 1363–1379

[14] Carmena R, Duriez P, Fruchart JC. Atherogenic lipoprotein particles in atherosclerosis. Circulation 2004; 109: III2–III7

[15] Castelli WP. Cholesterol and lipids in the risk of coronary artery disease – The Framingham Heart Study. Can J Cardiol 1988; (Suppl A): 5A–10A

[16] Castelli WP. Epidemiology of triglycerides; a view from Framingham. Am J Cardiol 1992; 70: 43–49

[17] Castelli WP. The new pathophysiology of coronary artery disease. Am J Cardiol 1998; 82(Suppl 2): 60–85

[18] Chait A, Brazo RL, Tribble DL, Krauss RM. Susceptibility of small, low-density lipoproteins to oxidative modification in subjects with the atherogenic lipoprotein phenotype, pattern B. Amer J Med 1993; 94: 350–356

[19] Cullen P. Evidence that triglycerides are an independent coronary heart disease risk factor. Am J Cardiol 2000; 86: 943–949

[20] Felmeden DC, Spencer CGC, Blann AD, Beewers DG, Lip YH. Low-density lipoprotein subfractions and cardiovascular risk in hypertension. Relationship to endothelial dysfunction and effects of treatment. Hypertension 2003; 41: 528–533

[21] Fruchart JC, Sacks FM, Hermans MP et al. The residual risk reduction initiative: A call to action to reduce residual vascular risk in dyslipidemic patients. Diab Vasc Res 2008; 5(4): 319–335

[22] Gardner CD, Fortman SP, Krauss, RM. Association of small low-density lipoprotein particles with the incidence of coronary artery disease in men and women. JAMA 1996; 276: 875–881

[23] Gavorník P. Všeobecná angiológia. Angiologická propedeutika. Cievne choroby-2. vydanie (General angiology, propedeutics of angiology, Vessel diseases, 2nd ed). Comenius University, Bratislava, 2001, 268 p.

[24] Gavorník P. Etiopatogenéza a nová klinicko-etiologicko-anatomicko-patologická (CEAP) klasifikácia končatinovej choroby (Ethiopathogenesis and a new clinical ethio-anatomico-pathological (CEAP) classification of the low extremity arterial disease). Interna Medicina 2010; 10: 115–123

[25] Gazi IF, Tsimihodimos V, Tselepis AD, Elisaf M, Mikhailidis DP. Clinical importance and therapeutic modulation of small dense low-density lipoprotein particles. Expert Opin Biol Therapy 2007; 7(1): 53–72

[26] Hirany SV, Othman Y, Kutscher P, Rainwater D, Jialal I, Devaraj S. Comparison of low-density lipoprotein size by polyacrylamide tube gel electrophoresis and polyacrylamide gradient gel electrophoresis. Am J Clin Pathol 2003; 119: 439–445

[27] Hirsch AT, Criqui MH, Treat-Jacobson D, Regensteiner JG, Creager MA, Olin JN, Krook SH, Hunninghake DB, Comerota AJ, Walsh ME, McDermott MM, Hiatt WR.

Peripheral arterial disease detection, awareness and treatment in primary care. JAMA 2001; 286(11): 1317–1322

[28] Hirsch AT, Haskal ZJ, Hertzer NR et al. ACC/AHA 2005 Guidelines for the management of patients with peripheral arterial disease. J Am Coll Cardiol 2006; 47: 1239–1312

[29] Hoefner DM, Hodel SD, O'Brien JF, Branum EL, Sun D, Meissner I, McConnell JP. Development of a rapid quantitative method for LDL subfraction with use of the Quantimetrix Lipoprint LDL system. Clin Chem 2001; 472: 266–274

[30] Kalita Z. Akutní cévní mozkové príhody. Diagnostika, patofyziologie, management (The acute cerebral-vascular events. Diagnostics, pathophysiology, management). Maxdorf, Praha, 2006, 623 p

[31] Kasko M, Gaspar L, Dukat A, Gavornik P, Oravec S. High-density lipoprotein profile in newly-diagnosed lower extremity artery disease in Slovak population without diabetes mellitus. Neuroendocrinol Lett 2014; 35(6): 531–535

[32] Kim YK, Seo HS, Lee EM, Na JO, Choi CU, Lim HE, Kim EJ, Rha SW, Park CG, Oh DJ. Association of hypertension with small, dense low-density lipoprotein in patients without metabolic syndrome. J Hum Hypertension 2012; 26: 670–676

[33] Lamarche B, Tchernof A, Moorjani S, Cantin B, Dagenais RG, Lupien PJ, Despres J-P. Small dense low-density lipoprotein particles as a predictor of the risk of ischemic heart disease in men. Prospective results from the Quebec Cardiovascular Study. Circulation 1997; 95: 69–75

[34] Lamarche B, Lemieux I, Despres J-P. The small, dense LDL phenotype and the risk of coronary heart disease: Epidemiology, pathophysiology and therapeutic aspects. Diabetes Metab 1999; 25: 199–211

[35] Landray MJ, Edmunds E, Li Saw Hee FL, Hughes BA, Beewers DG, Lip Gyh. Abnormal low density lipoprotein subfraction profile in patients with untreated hypertension. Q J Med 2002; 95: 165–171

[36] McBride PE. Triglycerides and risk for coronary artery disease. JAMA 2007; 298(3): 336–338

[37] Mudd JO, Borlaug BA, Johnston PV, Kral BG, Rouf R, Blumenthal RS, Kwiterovich PO, Jr. Beyond low-density lipoprotein cholesterol: Defining the role of low-density lipoprotein heterogeneity in coronary artery disease. J Am Coll Cardiol 2007; 50(18): 1735–1741

[38] National Cholesterol Education Program (NCEP), Expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III) final report. Circulation 2002; 106: 3143–3421

[39] Nicholls S, Lundmann P. The emerging role of lipoproteins in atherogenesis: Beyond LDL cholesterol. Semin Vasc Med 2004; 4: 187–195

[40] Norgren L, Hiatt WR, Dormandy JA, Nehler MR, Harris KA, Fowkes FG. TASC II Working Group. Inter-society consensus for the management of peripheral arterial disease. J Vascul Surg 2007; 45(1 Suppl): S5–S67

[41] Oravec S. Identifikácia subpopulácií LDL triedy – Aktuálny prínos v diagnostike porúch metabolizmu lipoproteínov a ochorení kardiovaskulárneho systému (Identification of LDL subfractions – A contribution in the diagnostics of lipoprotein metabolism diseases and cardiovascular diseases). Med Milit Slov 2006; 8: 32–34

[42] Oravec S. Nové perspektívy v diagnostike porúch metabolizmu lipoproteínov-prínos v interpretácii výsledkov (New perspectives in the diagnostics of lipoprotein metabolism diseases – a contribution in the interpretation of results). Med Milit Slov 2007; 9: 42–45

[43] Oravec S, Dukat A, Gavornik P, Caprnda M, Kucera M, Ocadlik I. Contribution of the atherogenic lipoprotein profile to the development of arterial hypertension. Bratisl Lek Listy 2011; 12(1): 4–7

[44] Oravec S, Dukat A, Gavornik P, Lovasova Z, Gruber K. Atherogenic normolipidemia – A new phenomenon in the lipoprotein profile of clinically healthy subjects. Neuroendocrinol Endocrinol Lett 2011; 32(3): 317-321

[45] Otvos JD, Jeyarajah EJ, Bennet SW, Krauss RM. Development of a proton nuclear magnetic resonance spectroscopic method for determining plasma protein concentrations and subspecies distribution from a single, rapid measurement. Clin Chem 1992; 38: 1632–1638

[46] Packard CJ. Triacylglycerol-rich lipoproteins and the generation of small dense low-density lipoprotein. Biochem Soc Trans 2003; 31: 1066–1069

[47] Pollak AW, Kramer CM. LDL lowering in peripheral arterial disease. Are there benefits beyond reducing cardiovascular morbidity and mortality ? Clin Lipidol 2012; 7(2): 141–149

[48] Rainwater DL, Moore PH Jr, Shelledy WR, Dyer TD, Slifer SH. Characterization of a composite gradient gel for the electrophoretic separation of lipoproteins. J Lipid Res 1997; 38: 1261–1266

[49] Rajman I, Kendall MJ, Cramb R, Holder RL, Salih M, Gammage MD. Investigation of low density lipoprotein subfractions as a coronary risk factor in normotriglyceridemic men. Atherosclerosis 1996; 125: 231–242

[50] Rizzo M, Berneis K. Low density lipoprotein size and cardiovascular prevention Europe J Int Med 2006; 17: 77–80

[51] Rizzo M, Berneis K. Low-density-lipoprotein size and cardiovascular risk assessment QJM – Int J Med 2006; 99: 1–14

[52] Rizzo M, Pernice V, Frasheri A, Berneis K. Atherogenic lipoprotein phenotype and LDL size and subclasses in patients with peripheral arterial disease. Atherosclerosis 2008; 197: 237–241

[53] Rubins HB, Davenport J, Babikian V et al. Reduction in stroke with gemfibrozil in men with coronary heart disease and low HDL cholesterol: The veterans affairs HDL intervention trial (VA-HIT). Circulation 2001; 103(23): 2828–2833

[54] Sarkar PD, Rautaray SS. Oxidized LDL and paraoxonase status in ischemic stroke patients. Indian J Physiol Pharmacol 2008; 52(4): 403–407

[55] Shoji T, Hatsuda S, Tsuchikura S, Shinohara K, Komoto E, Koyama H, Emoto M, Nishizawa Y. Small dense low-density lipoprotein cholesterol concentration and carotid atherosclerosis. Atherosclerosis 2009; 202: 582–588

[56] St-Pierre AC, Cantin B, Daganais GR, Mauriege P, Bernard PM, Despres JP, Lamarche B. Low density lipoprotein subfractions and the long-term risk of ischemic heart disease in men: 13-year follow-up data from the Quebec Cardiovascular Study. Arterioscler Thromb Vasc Biol 2005; 25: 553–559

[57] Superko HR. Lipoprotein subclasses and atherosclerosis. Front Biosci 2001; 6: D355–D365

[58] Tanne D, Koren-Morgan N, Graff E, Goldbourt U. Blood lipids and first ever ischemic stroke, transient ischemic attack in the bezafibrate infarction prevention (BIP) registry: High triglyceride constitute an independent risk factor. Circulation 2001; 104: 2892–2897

[59] Vakkilainen J, Mäkimattila S, Seppälä-Lindroos A, Vehkavaara S, Lahdenperä S, Groop PH, Taskinen MR, Järvinen HY. Endothelial dysfunction in men with small LDL particles. Circulation 2000; 102: 716–721

[60] Van J, Pan J, Charles MA, Krauss R, Wong N, Wu X. Atherogenic lipid phenotype in a general group of subjects. Arch Pathol Lab Med 2007; 131: 1679–1685

[61] Whitworth JA. 2003 World Health Organisation (WHO), International Society of hypertension (ISH) Statement on management of hypertension. J Hypertension 2003; 21: 1983–1992

[62] Wierzbicki AS. Quality as well as quantity? Beyond low-density lipoprotein-cholesterol – The role of particle size. Int J Clin Pract 2007; 61: 1780–1782

[63] Vrablik M. Prevence cévních mozkových príhod hypolipidemickou léčbou. Remedia. Interetové stránky ceského farmakoterapeutického dvoumesícniku, rubrika PND, 2. duben 2009 (The prevention of the stroke by hypolipidemic treatment. Remedia). In-

http://dx.doi.org/10.5772/60989

ternet Website of Czech Pharmaco therapeutic two-monthly Journal, column PND, April 2, 2009

[64] Chun Xia Zhao, Ying Hua Cui, Qiao Fan, Pei Hua Wang, Ruitai Hui, Cianflone K, Dao Wen Wang. Small dense low-density lipoproteins and associated risk factors in patients with stroke. Cerebrovasc Dis 2009; 27: 99–104

Chapter 5

Lipoprotein-Associated Phospholipase A_2 – Pathophysiological Role and Clinical Significance as a Cardiovascular Biomarker

Sanja Stankovic and Milika Asanin

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/60608

Abstract

Within the last decade, a broad range of biomarkers associated with an increased risk for death and cardiovascular/cerebrovascular endpoints have been identified. Epidemiological studies clearly indicate that lipoprotein-associated phospholipase A_2 (Lp-PLA_2) has the potential to become clinically useful emerging biomarker in the true sense, linking plaque biology with cardiovascular/cerebrovascular event rate. Lipoprotein-associated phospholipase A_2 is a specific vascular inflammatory marker, a risk factor, a prognostic biomarker, and also a therapeutic target. This chapter will summarize our current knowledge on Lp-PLA_2 with emphasis on its potential pathophysiological mechanisms of action and on clinical relevance as cardiovascular/cerebrovascular biomarker. This chapter gives comprehensive, systematic review of studies assessing the significance of Lp-PLA_2 in cardiovascular/cerebrovascular diseases with emphasis on clinical benefit of pharmacologic inhibition of Lp-PLA_2.

Keywords: biomarker, cardiovascular disease, lipoprotein-associated phospholipase A_2

1. Introduction

Although an atherogenic lipoprotein phenotype was reported as a predictor of cardiovascular/cerebrovascular disease, numerous recent studies have recognized additional lipid-related markers as emerging biomarkers to identify patients with cardiovascular/cerebrovascular disease risk. Among them the most promising biomarkers for cardiovascular/cerebrovascular risk assessment is lipoprotein-associated phospholipase A_2 (Lp-PLA_2).

2. Lipoprotein-associated phospholipase A_2: structure and biology

Lipoprotein-associated phospholipase A_2 (Lp-PLA_2), also known as platelet-activating factor acetylhydrolase (PAF-AH), belongs to the phospholipase A_2 superfamily [1,2]. This Ca^{2+}-independent phospholipase is encoded by *PLA2G7* gene that consists of 12 exons and 11 introns located on chromosome 6p21.2 to 12 [3,4]. Lp-PLA_2 is protein of 45,4 kDa that consists of 441 amino acid residues [5]. The major sources of Lp-PLA_2 in plasma are T lymphocytes, monocytes/macrophages, activated bone marrow-derived mast cells, and liver cells [6-8]. The secreted Lp-PLA_2, circulates in plasma in active form. It predominantly binds to LDLs, and in a much smaller extent to HDLs, Lp(a), lipoprotein remnants, and platelet-borne microparticles [6,9-12]. Indeed, Lp-PLA_2 is highly associated with the smallest LDL and HDL subclasses [13] and with electronegative LDL, which overlaps with small dense LDL [14]. Lp-PLA_2 bound to HDL has a much lower specific activity compared to when bound to LDL [10]. Different distribution of Lp-PLA_2 in various plasma lipoproteins affects its functions.

Lp-PLA_2 catalyzes hydrolysis of the acetyl group at sn-2 position of PAF to generate lyso-PAF and acetate [15]. On the other side, Lp-PLA_2 cleaves oxidatively modified lipoproteins from the sn-2 position of the apoB100-containing lipoproteins into oxidized nonesterified fatty acids (oxFFAs) and lysophosphatidylcholine.

3. Actions of lipoprotein-associated phospholipase A_2

We can think about Lp-PLA_2 as a friend and foe at the same time. On the one hand, it functions as PAF-AH, which hydrolyzes inflammatory mediator PAF, inhibits foam cell formation, enhances cholesterol efflux in macrophages, and exerts its atheroprotective role [16].

On the other hand, Lp-PLA_2 has a proatherogenic role. Lp-PLA_2 relates to a number of different proatherogenic biological processes. Lp-PLA_2 hydrolyzes oxidized phospholipids on modified LDL particles within the arterial intima, and thus contributes to the initiation and progression of atheroma. Lp-PLA_2 is produced by macrophages and foam cells within atherosclerotic plaque. Its expression is mainly confined to plaque areas with massive lipid accumulation and leukocyte infiltration, cellular necrosis, and calcification, suggesting that Lp-PLA_2 is a marker for rupture-prone plaque [16]. The amount of Lp-PLA_2 and its by-product, lysophosphatidylcholine in the coronary circulation is proportional to the extent of the atheroma and indirectly affects local endothelial function [17]. Proatherogenic activities of lysophosphatidylcholine are: expression of adhesion molecules; upregulation of cytokines and CD40 ligand by T-cells, cytotoxic at concentrations higher than 30–50 μM; stimulation of macrophage proliferation; release of arachidonic acid from endothelial cells; induction of MCP-1 and genes for growth factors; release of myeloperoxidase; migration of vascular smooth muscle cells; chemoattractant for monocytes, macrophages, and T-cells; induction of apoptosis in smooth muscle cells and macrophages; involvement in the antigenicity of oxidized LDL; inhibition of endothelium-derived nitric oxide [18-20]. Furthermore, oxFFAs promote atherosclerosis by direct and

http://dx.doi.org/10.5772/60608

indirect increasing of oxidative stress and the presence of oxidized LDL and other lipoproteins in the plasma and arterial walls, thereby initiating fatty streak formation [21].

4. Assays for Lp-PLA_2 mass and activity determination

It is well known that circulating Lp-PLA_2 can be measured by different assays ascertaining either its mass or activity. There is no definite decision about the potential superiority of the tests.

According to the literature data, Lp-PLA_2 levels were determined preferentially using PLAC® test. Lp-PLA_2 mass was determined by diaDexus PLAC Test ELISA, sandwich enzyme immunoassay, followed by diaDexus PLAC® test based for Lp-PLA_2 mass measurement on turbidimetric immunoassay technology. More recently, enzyme assay PLAC® Test for measurement of Lp-PLA_2 activity has been developed and commercially available. The preferred sample type is EDTA plasma, and serum is also acceptable. It should be noted that methodological issues associated with Lp-PLA_2 measurement make comparisons between studies difficult.

Recently published meta-analysis of 32 prospective studies in persons with stable vascular disease or recent acute ischemic event revealed a moderate correlation between mass and activity of Lp-PLA_2 [24]. The PROVEIT-TIMI 22 study [25] has also found a moderate correlation between Lp-PLA_2 activity and mass measured at baseline and at 30-day after ACS. There is still controversy about the method of estimating Lp-PLA_2 level. While Koenig et al. [26] reported that Lp-PLA_2 mass was the better risk predictor of future cardiovascular events than Lp-PLA_2 activity, Persson et al. [27] has reached the opposite conclusion. Jenny et al. [28] showed no difference in Lp-PLA_2 activity and mass with respect to risk prediction.

Currently, there is no consensus on the best method to estimate Lp-PLA_2 level. A consensus panel recommendation for incorporating Lp-PLA_2 testing into the cardiovascular disease risk assessment guidelines used Lp-PLA_2 mass for stratifying patients [23].

Blood samples should be refrigerated after processing and should be kept frozen for long-term storage. There are no restrictions to the time of day that the sample should be drawn and no dietary restrictions. In contrast to other emerging risk markers, a very minimal biological variation in Lp-PLA_2 concentrations has been demonstrated among individuals monitored serially over several weeks [29].

In addition, Lp-PLA_2 levels are typically unaffected by conditions of systemic inflammation, such as osteoarthritis and chronic obstructive pulmonary disease, whereas markers of inflammatory response are often elevated by these conditions. The normal population medians for men and women are in the range of 230–250 ng/mL, and a value of >300 ng/mL may be considered elevated [30].

Gender differences in Lp-PLA_2 levels were found; men had significantly higher levels than women. Also, a significant association between Lp-PLA_2 levels and smoking was noticed.

These finding has been observed in previous studies [31,32,33,34]. Lower Lp-PLA_2 levels in women could be explained by estrogen-mediated down-regulation of Lp-PLA_2 expression, due to lower concentrations of LDL cholesterol in women or estrogen-related decrease in platelet activating factor acetyl hydrolase activity [35,36]. Estrogen-replacement therapy can significantly reduce Lp-PLA_2 activity in healthy postmenopausal women [37], while administration of steroids with progesterone-like activity increases Lp-PLA_2 activity [38]. Smoking may increase the carrier (LDL) and the substrate (oxidized LDL) for Lp-PLA_2 [39].

5. Association of Lp-PLA_2 with cardiovascular disease risk

Lp-PLA_2 is an emerging inflammatory biomarker, characterized by high vascular specificity and low biovariability. High Lp-PLA_2 levels are indicative of rupture-prone plaque [22]. Numerous epidemiological and clinical studies examined association between Lp-PLA_2 concentration/enzyme activity and cardiovascular disease risk in apparently healthy individuals, in subjects with stable cardiovascular disease, acute coronary syndrome (ACS), heart failure (HF), stroke, transient ischemic attack (TIA) support, trying to answer in which patients would determination of Lp-PLA_2 be the most valuable. There are novel data about the prognostic significance of Lp-PLA_2 as a predictor of short-term or long-term outcome in patients with cardiovascular disease. To evaluate this, systematic literature review concerning the association of Lp-PLA_2 with cardiovascular disease risk and prognostic implications was done. The studies on this issue were extracted from relevant electronic databases (Medline (http://www.ncbi.nlm.nih. gov/pubmed/), Embase (http://www. embase.com/), Google Scholar (http://scholar. google.com/), Yahoo (http:// www. yahoo. com/), Kobson (http:// www.kobson.nb.rs/), ClinicalTrails.gov.) and the obtained results were included in the text.

In 2008, the panel's recommendation [23] incorporated Lp-PLA_2 testing as an adjunct to traditional risk factors in assessing future cardiovascular risks. It endorses Lp-PLA_2 testing in moderate-risk persons determined as having simply two risk factors. An Lp-PLA_2 >200 ng/mL warrants reclassification of the moderate-risk patient as high cardiovascular risk and should prompt reduction of the LDL-C target from 130 mg/dL to 100 mg/dL. The panel also recommends Lp-PLA_2 testing for patients with coronary artery disease (CAD) or CAD risk equivalents (diabetes, ischemic stroke, etc.) be considered at very high risk when Lp-PLA_2 is elevated, warranting reduction in the LDL-C target from 100 mg/dL to 70 mg/dL. Today, Lp-PLA_2 measurement for cardiovascular disease risk stratification of patients is included in four international clinical guidelines: 2012 European Guidelines on CVD Prevention in Clinical Practice European Society of Cardiology – Lp-PLA_2 may be measured as a part of a refined risk assessment in patients at high risk of a recurrent acute atherothrombotic event. Class IIb; 2010 ACCF/AHA Guideline for Assessment of Cardiovascular Risk in Asymptomatic Adults – Lp-PLA_2 testing may be considered in intermediate-risk asymptomatic adults; 2011 AHA/ASA Guidelines for the Primary Prevention of Stroke Measurement of inflammatory markers such as high sensitive C-reactive protein (hs-CRP) or Lp-PLA_2 in patients without CVD may be considered to identify patients who may be at increased risk of stroke; 2012 AACE Guidelines for Management of Dyslipidemia and Prevention of Atherosclerosis Test for Lp-

http://dx.doi.org/10.5772/60608

PLA_2, which in some studies has demonstrated more specificity than highly sensitive CRP, when it is necessary to further stratify a patient's CVD risk [40-43].

Some studies suggested that Lp-PLA_2 predicts risk complementary to hs-CRP, although when compared with hs-CRP, Lp-PLA_2 in some studies is suggested to be a more promising marker of risk prediction. In multiple previous studies, correlation analysis revealed no association of Lp-PLA_2 with CRP [28,31,44-47]. It can be expected because CRP is an acute-phase reactant and its elevation can be caused by a wide range of inflammatory conditions. CRP shows intraindividual variability of ~40%. On the other hand, Lp-PLA_2 is not affected by systemic inflammation; it is a specific marker of vascular inflammation. Also, it shows significantly lower biologic variability than CRP, and higher stability in states of myocardial ischemia [48].

Lp-PLA_2 is suggested to be a more promising marker of risk prediction than CRP [49]. Winkler et al. [50] showed that increased Lp-PLA_2 levels in moderate-risk patients with hs-CRP<3 mg/L doubled the risk for cardiac death. The WOSCOPS study [44] found that Lp-PLA_2 was significantly associated with cardiovascular risk, compared with hs-CRP. Stankovic et al.'s [32] results confirmed that Lp-PLA_2 have better risk prediction than CRP.

Although, the majority of published studies showed a significant relationship between Lp-PLA_2 levels and cardiovascular events, there are several important differences across ethnic groups; for example, African-Americans and Caucasians with respect to Lp-PLA_2. Activity of Lp-PLA_2 was higher among African-Americans with CAD. The difference in Lp-PLA_2 activity levels between CAD and non-CAD patients was higher among African-Americans. Also, the Lp-PLA_2 index was independently associated with the extent of CAD among African-Americans [51-53].

From the WOSCOPS publication (West of Scotland Coronary Prevention Study, WOSCOPS) [44], which revealed a positive association between elevated circulating concentrations of Lp-PLA_2 and the risk of coronary heart disease (CHD), the interest in Lp-PLA_2 as a biomarker for cardiovascular disease rapidly increased. The vast body of evidence derived from prospective epidemiologic studies, two meta-analyses (79,036 participants in 32 prospective studies, and 52,995 subjects participated in 33 studies) and review revealed the positive association of elevated Lp-PLA_2 with cardiovascular risk [24,54-56]. Furthermore, Lp-PLA_2 has been confirmed to predict the presence of CAD, even among patients undergoing coronary angiography. Uniquely, Lp-PLA_2 predicted the risk of CAD death, but not all cause death [57].

LpPLA_2 has been considered as a prognostic marker in patients with CAD. Li et al. [58] identified prognostic value of Lp-PLA_2 baseline measurement for major adverse cardiac event (MACE) (cardiovascular death, nonfatal myocardial infarction, and target vessel revascularization) in ACS patients during follow-up for a median of 6 months. Mockel et al. [59] demonstrated that Lp-PLA_2 levels in the first 7 hours after onset of symptoms may be an independent predictor of MACE within 42 days in ACS patients. PROVEIT-TIMI 22 study [25] has shown that Lp-PLA_2 in patients randomized to atorvastatin or pravastatin measured 30 days after ACS are associated with an increased risk of cardiovascular events (death, myocardial infarction, unstable angina, revascularization, or stroke) over 24 months of follow-up. Gerber et al. MACE [48] found that Lp-PLA_2 level measured early after myocardial infarction is

strongly and independently associated with 1-year mortality. Stankovic et al. [32] demonstrated that the Lp-PLA_2 may have short-term predictive value in pure STEMI patients treated by primary percutaneous coronary intervention (PCI). They concluded that pre-interventional plasma Lp-PLA_2 level is an independent predictor of 30-day MACE in patients with first anterior STEMI treated by primary PCI, and suggested that Lp-PLA_2 level could help in very early risk stratification of STEMI patients treated by PCI.

Previously published studies examined and suggested the association between Lp-PLA_2 and heart failure (HF) incidence in a population-based cohort of healthy individuals, and in people older than 65 years [60]. Baseline Lp-PLA_2 levels are associated with a high risk of developing heart failure in 3,991 adults older than 65 years, independent of coronary risk factors [61]. Lp-PLA_2 activity is significantly associated with congestive heart failure in 5,531 persons older than 65 years [62]. Gerber et al. [63] evaluated the association of Lp-PLA_2 with mortality in subjects with diagnosed HF. Lp-PLA_2 was strongly and independently associated with mortality in patients under 80 years of age. Moldoveanu et al.'s study [64] in 208 patients with HF found significantly increased Lp-PLA_2 activity in HF patients with preserved ejection fraction (EF) than in HF with reduced EF. The literature data about the association of Lp-PLA_2 and heart failure on admission in patients with acute myocardial infarction are missing. Stankovic et al. [32] suggested that patients with the first anterior STEMI who had higher levels of Lp-PLA_2 had a worse prognosis, but not with a greater probability of developing HF. In Raichlin et al.'s [65] study in heart transplant patients, Lp-PLA_2 correlated with the progression of cardiac allograft vasculopathy and increased risk of cardiovascular events/death suggesting that it could be therapeutic target in heart transplant patients.

In 2005, the US Food and Drug Administration (FDA) approved Lp-PLA_2 blood test for assessing patients at risk for ischemic stroke. The Rotterdam Study was the first population-based study that determined the impact of elevated Lp-PLA_2 on stroke. It identified that Lp-PLA_2 activity was an independent predictor of ischemic stroke in the middle-aged healthy men and women population [31]**.** In the ARIC study, healthy middle-aged adults with increased levels of both Lp-PLA_2 and hs-CRP had an 11-fold higher incidence of stroke than individuals with low Lp-PLA_2 and hs-CRP levels [45]. This association between Lp-PLA_2 mass/activity and first ischemic stroke was confirmed in the Malmo Diet and Cancer Study [66], Bruneck Study [67], and Cardiovascular Health Study [28]. The Tsekepis et al. study [68] showed that Lp-PLA_2 correlated with the intima-media thickness in patients with beta-thalassemia, suggesting that Lp-PLA_2 may be implicated in premature carotid atherosclerosis.

Although some reports inconclusively found this positive association of Lp-PLA_2 and first-ever and recurrent stroke, little is known about its influence on stroke outcome. Elkind et al. [69] measured Lp-PLA_2 mass and activity in relation to outcome in first ischemic stroke patients, determined as recurrent stroke, recurrence of vascular events, and mortality. Lp-PLA_2 was a good predictor of recurrent stroke risk. Delgado et al. [70] investigated the temporal profile of Lp-PLA_2 mass and activity within the first 24 hours after stroke and found significant changes in Lp-PLA_2 concentrations early after stroke onset. Patients with higher Lp-PLA_2 mass were more likely to be resistant to intravenous t-PA administration with very low early recanalization rates.

http://dx.doi.org/10.5772/60608

Lp-PLA_2, an inflammatory biomarker, has been described as able to predict risk of first-ever or recurrent stroke and myocardial infarction [71]. Moreover, Lp-PLA_2 may also have a role in the pathophysiology of cerebrovascular disease, particularly in strokes of atherosclerotic etiology, since its expression is enhanced in atherosclerotic carotid lesions together with markers of oxidative damage, inflammation, and instability [72].

Sarlon-Bartoli et al. [73] reported that Lp-PLA_2 mass is increased in patients with high-grade carotid stenosis and unstable plaque and suggest that Lp-PLA_2 could be an important biomarker for classifying carotid plaque as vulnerable and predict neurological risk of a carotid stenosis in asymptomatic subjects. Although these findings have the potential to improve cerebrovascular disease stratification, correlation with ultrasonic or MRI markers of plaque instability or the presence of infarction on brain imaging must be performed [74].

Shoamanesh et al. [75] investigated the association between circulating biomarkers of inflammation including Lp-PLA_2 and MRI markers of cerebral small vessel disease in 1,763 stroke-free Framingham offspring. They observed higher levels of lipoprotein-associated phospholipase A_2 mass in patients with greater white matter hyperintensity volumes and silent cerebral infarcts. These results could improve stroke risk prognosis.

The accumulating results of numerous studies demonstrate a significant positive association between Lp-PLA_2 levels and incident cardiovascular disease and heart failure. A dissociation could be noted between mass and activity in terms of risk prediction. Several of the epidemiology studies have measured only Lp-PLA_2 mass (26,30,33,44,47,57,63,65,69), whereas others measured only enzyme activity (31,50,62,64,67) and a few have measured both in the same study population (25,27,28, 76). Recently published meta-analysis assessed the 32 prospective studies of Lp-PLA_2 and cardiovascular outcomes. For each standard deviation Lp-PLA_2 increase, the relative risks for the primary endpoint of coronary heart disease were 1.10 (1.05–1.16) and 1.11 (1.07–1.16) for Lp-PLA_2 activity and mass, respectively. The relative adjusted risks for ischemic stroke were 1.08 (0.97–1.20) and 1.14 (1.02–1.27); vascular mortality 1.16 (1.09–1.24) and 1.13 (1.05–1.22); and nonvascular mortality 1.10 (1.04–1.17); and 1.10 (1.03–1.18) for Lp-PLA_2 activity and mass, respectively. The final decision whether to measure the Lp-PLA_2 mass or its enzyme activity may help transition of this biomarker from research to routine clinical practice.

6. Lp-PLA_2 and genetic influences

Several Lp-PLA_2 gene polymorphisms and their role in affecting the regulation or production of LpPLA_2 assessed as activity and mass were described; many in small studies and some in recently published genome-wide association studies. It is known that genetic factors account for 62% of the variation in Lp-PLA_2 activity [76,77].

Although familial factors explain about one-half and one-quarter of the variance in Lp-PLA_2 activity and mass, respectively [78,79], few genetic determinants of Lp-PLA_2 have been identified. The first genome-wide association study using data from 6,668 Caucasian subjects

in population-based Framingham Heart Study identified one locus associated with Lp-PLA_2 mass, and four loci associated with Lp-PLA_2 activity [80]. Twelve SNPs in the region of chromosome 6p12.3 near the gene for PLA2G7 were associated with Lp-PLA_2 mass at a genome-wide level of significance. The top hit SNP rs1805017, is a nonsynonymous change (H92R) within the PLA2G7 gene. It was found that T allele that corresponds to the amino acid histidine was associated with higher Lp-PLA_2 mass. On the other side, four loci achieving genome-wide significance for association with Lp-PLA_2 activity was identified: a) first within the APOE/APOC1 gene cluster on chromosome 19q13.32 (rs41377151); b) second locus on chromosome 1p13.3, which includes the genes PSRC1 (rs599839), CELSR2 (rs4970834); c) third locus within an intron of SCARB1 on chromosome 12q24.31 (rs10846744); d) fourth locus in ZNF259 gene and BUD13 gene on chromosome 11q23.3 near the apolipoprotein gene cluster APOA5/APOA4/APOC3/APOA1 (rs12286037, rs11820589). Investigation from Suchindran's study was extended by Grallert et al. who made meta-analysis with additional four cohorts [81]. They performed genome-wide association study as part of the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium in five population-based studies with the aim to identify genetic loci related to Lp-PLA_2 mass/activity [82]. This study, in the popoulation of 13,664 subjects, revealed the association of PLA2G7 loci variants with Lp-PLA_2 mass/activity, and genetic variants (APOC1, CELSR2, LDL, ZNF259, SCARB1) related to low-density lipoprotein cholesterol levels with Lp-PLA_2 activity.

Also, Lp-PLA_2 mass was associated with SNP rs247616 on chromosome 16 within the cholesteryl ester transfer protein gene. The T allele of rs247616 was associated with higher HDL-C concentration and higher mean Lp-PLA_2 mass [83]. The additional value of this study was the revealed significant association of four polymorphisms (in APOC1, CELSR2, SCARB1, ZNF259), but not PLA2G7 with coronary heart disease. Large-scale analysis focusing on variants in PLA2G7 did not identify any SNP that achieved experiment-wide statistical significance [79].

The most frequently studied SNPs within PLA2G7 are: variants in exon 9 (Val279Phe; rs45619133), exon 11 (Val379Ala; rs1051931), exon 7 (Ile198Thr, Iso195Thr; rs1805018), and exon 4 (Arg92His; rs1805017) [84-86].

The V279F SNP was found in subjects of Asian ancestry. The Val279Phe substitution is located within the catalytical domain of Lp-PLA_2 and leads to reduction of enzyme activity in V/F heterozygous individuals or complete loss of enzymatic activity in homozygous F/F individuals. The 279F null allele is relatively frequent in Japan, with approximately 25% and 2% of the population carrying one or two copies. Its prevalence declines toward the West, has intermediate frequencies in China and Korea, is rare in the Middle East, and almost completely absent in European populations. The results from the association studies on this V279F variant and coronary artery disease have thus far been inconclusive. Li et al. [87] in Chinese Han population, found significant association of V279F and coronary artery disease, indicating that carrier of F allele increases the risk of coronary artery disease. This result was consistent with the Yamada et al. [88] study (850 cases/1,684 controls), which found that subjects carrying the mutant allele are at higher risk of arterial events (MI or stroke), and the Shimokata study (3,085 subjects with coronary artery disease/2,163 controls) [89]. The Jang et al. [90] study in two

http://dx.doi.org/10.5772/60608

different patient sets (2,890 men diagnosed with CAD before age 60/3,128 male controls and 877 CAD cases/1,230 controls) confirmed that deficiency in Lp-PLA_2 activity due to carriage of PLA2G7 279F allele protects from CAD in Korean men. Nevertheless, in the South Korean population (532 cases/670 controls) and Chinese study (827 cases/947 controls), V279F variant results in an unexpectedly opposite outcome [91,92]. Meta-analysis of 14 association studies focusing on R92H polymorphism in PLA2G7 gene and risk of CHD in 8,280 cases/5,656 controls indicate 92H allele had probably increased the risk of CHD [92]. The missense polymorphisms I198T and A379V are identified mainly in Caucasians. A379V variant in which alanine is substituted by valine is functional. Some studies explored the contribution of A379V to Lp-PLA_2 activity with contradictory results; some found the association of A379V variant with increased [86,93] and some with decreased Lp-PLA_2 activity [94].

There are also different reports about the association between the A379V variant and cardiovascular disease [94-97]. A recent Taiwanese population study reported that subjects carrying the 379 V allele had increased severity of coronary atherosclerosis [94]; two studies in Caucasian subjects reported that the 379 V allele was associated with decreased atherosclerosis risk [93,96]; a large meta-analysis showed that the 379 V allele was associated with Lp-PLA_2 activity, but not with cardiovascular risk markers [97]; and another study showed no association [95].

Intriguingly, in the study conducted by Liu and colleagues [94], the outcome was quite contradictory. They found that in the Chinese Taiwan Han population, A379V variant is significantly associated with Lp-PLA_2 activity and the severity of coronary atherosclerosis. Recently, a meta-analysis including a total of 12 studies shows that in the populations from European ancestry, among the 7 SNPs, A379V variant shows the strongest association with Lp-PLA_2 activity; however, no significant correlation is found between PLA2G7 variants and cardiovascular risk markers, coronary atheroma, or CHD [97].

7. Lp-PLA_2 as a therapeutic target

It is well known that lipid-altering medications, including statins, fenofibrate, prescription of omega-3 fatty acids, for weight loss, have been shown to reduce Lp-PLA_2 levels. The degree of its reduction correlates with the extent of lipid lowering.

Lp-PLA_2 was identified as a potential novel target of therapy. The most used therapy targeting Lp-PLA_2 in plasma in advanced stages of clinical investigation is darapladib. Darapladib is a selective, potent, reversibile, oral inhibitor of lipoprotein-associated phospholipase A_2. The basic idea of applying darapladib is to improve patient outcomes in addition to evidence-based treatments and potentially reduce cardiovascular and cerebrovascular events by decreasing cytokines concentrations, stabilizing atherosclerotic plaque, inhibiting macrophage infiltration, and thickening of the connective tissue cap.

The first study that showed as a secondary endpoint a change in coronary artery plaque necrotic core with darapladib after 12 months of treatment was Integrated Biomarkers and

Imaging Study (IBIS)-2 [98]. It was a multicenter, randomized, double-blind placebo-controlled study that included 330 patients with angiographically confirmed coronary artery disease. Inhibition of Lp-PLA_2 with darapladib also prevented necrotic core expansion of coronary plaque as measured on intravascular ultrasound.

One multicenter, randomized, double-blind placebo-controlled study examined the effects of darapladib on biomarkers of cardiovascular risk in 959 CAD and CAD-risk equivalent patients who were previously randomized to atorvastatin 20 mg or 80 mg and then randomized to oral darapladib 40, 80, 160 mg, or placebo for 12 weeks. Overall dose-dependent inhibition of Lp-PLA_2 activity was sustained over the study period and was present in both atorvastatin dose groups, at different baseline LDL cholesterol < or ≥70 mg/dl, and high-density lipoprotein cholesterol HDL-C < or ≥40 mg/dl [99].

The first study that examined the effects of darapladib on Lp-PLA_2 activity in Japanese dyslipidemic patients with/without the Val279Phe single-nucleotide polymorphism (SNP) of the *PLA2G7* gene showed that darapladib produced sustained inhibition of Lp-PLA_2 activity [100].

Two large-scale studies with hard clinical endpoints were completed: the Stabilization of Atherosclerotic Plaque by Initiation of Darapladib Therapy (STABILITY) trial, with 15,828 randomized patients, and the Stabilization of Plaques using Darapladib-Thrombolysis In Myocardial Infarction 52 Trial (SOLID-TIMI 52), with an estimated recruitment of 13,026 patients [101-103].

The SOLID-TIMI 52 was a randomized, double-blind, placebo-controlled, multicenter, and event-driven trial that determined the clinical benefit of direct inhibition of Lp-PLA_2 activity with darapladib in patients after an acute coronary syndrome (non-ST-elevation or ST-elevation myocardial infarction). Subjects were randomized to darapladib (160 mg enteric-coated tablet daily) or matching placebo within 30 days after acute coronary syndrome. The primary endpoint was the composite of coronary heart disease death, nonfatal myocardial infarction, or nonfatal stroke, and secondary endpoints were major and total coronary events, individual components of the primary endpoint, and all-cause mortality. Patients were followed up for a median of 2.5 years. In patients who experienced an ACS event, direct inhibition of Lp-PLA_2 with darapladib added to optimal medical therapy and initiated within 30 days of hospitalization did not reduce the risk of major coronary events.

The STabilization of Atherosclerotic plaque By Initiation of darapLadIb TherapY (STABILITY) trial was a double-blind trial in patients randomized more than one month after a myocardial infarction to double-blind darapladib (160 mg daily) or placebo (daily). The primary endpoint was a composite of cardiovascular death, myocardial infarction, or stroke. Secondary endpoints included the components of the primary endpoint as well as major coronary events (death from coronary heart disease, myocardial infarction, or urgent coronary revascularization for myocardial ischemia) and total coronary events (death from coronary heart disease, myocardial infarction, hospitalization for unstable angina, or any coronary revascularization). Patients were followed up for a median of 3.7 years. In patients with stable coronary heart disease, darapladib did not significantly reduce the risk of the primary composite endpoint of cardiovascular death, myocardial infarction, or stroke.

http://dx.doi.org/10.5772/60608

Darapladib is a selective Lp-PLA_2 inhibitor that is under investigation for its potential to stabilize high-risk atherosclerotic plaques and potentially reduce cardiovascular events [104,105]. A phase 2 clinical trial in 959 patients with CHD or CHD-risk equivalents demonstrated that darapladib was effective at producing sustained inhibition of plasma Lp-PLA_2 activation in patients on atorvastatin therapy. This study was a post hoc analysis of this phase 2 trial studying high-risk patients with a diagnosis of peripheral arterial disease (PAD). Despite a more aggressive baseline risk factor profile, darapladib was equally effective at reducing Lp-PLA_2 in patients with and without PAD [106]. Johnson et al. [107] assessed the effects of darapladib on both plasma and plaque Lp-PLA_2 activity in patients undergoing elective carotid endarterectomy randomized to darapladib 40 mg (n = 34), 80 mg (n = 34), or placebo (n = 34) for 14 days. Patients were followed by carotid endarterectomy 24 hours after the last dose of study medication. Darapladib reduced plasma and plaque Lp-PLA_2 activity compared with placebo.

8. Conclusion

Within the last decade, a broad range of biomarkers associated with an increased risk for death and cardiovascular/cerebrovascular endpoints have been identified. Epidemiological studies clearly indicated that Lp-PLA_2 has the potential to become a clinically useful biomarker because it promotes independent information in the diagnosis, and especially cardiovascular/cerebrovascular risk stratification. In the future, we can expect new drugs (new Lp-PLA_2 inhibitors) that will affect patient's management, and assessing the effect of Lp-PLA_2 inhibition on cardiovascular endpoints can provide definitive answers.

However, further clinical validation in well-designed observational and interventional studies is needed before these recommendations can be properly evaluated in order to include them in the clinical diagnostic algorithms.

Currently, Lp-PLA_2 measurement has only been reserved to patients with moderate and high cardiovascular risk, rather than healthy population or low-risk patients, since the values of Lp-PLA_2 in these population groups are insignificant. Also, the evaluation of Lp-PLA_2 in combination with noninvasive imaging could be expected. The formation of best-case model from Lp-PLA_2 and other biomarkers can yield the best patient stratification algorithm. The next step could be cost-effectiveness analysis of more accurate risk stratification with biomarker testing. Future studies need to focus on exploring the potential of this biomarker and evaluating the effects of Lp-PLA_2 inhibition on human populations.

The final decision on which test to use for Lp-PLA_2 determination, test based on mass or activity of Lp-PLA_2, together with the development of commercially available automated, robust, valid, high throughput, cost-effective test capable of increasing agility and reducing the analytical imprecision can be adopted routinely in clinical practice for better risk stratification and therapeutic choice in patients with cardiovascular/cerebrovascular disease.

Author details

Sanja Stankovic* and Milika Asanin

*Address all correspondence to:

Clinical Center of Serbia, Belgrade, Serbia

References

[1] Schaloske RH, Dennis EA. The phospholipase A2 superfamily and its group numbering system. *Biochim Biophys Acta* 2006;1761(11):1246-1259.

[2] Burke JE, Dennis EA. Phospholipase A2 biochemistry. *Cardiovasc Drugs Ther* 2009; 23:49-59.

[3] Tew DG, Southan C, Rice SQ, Lawrence MP, Li H, Boyd HF, Moores K, Gloger IS, Macphee CH. Purification, properties, sequencing, and cloning of a lipoprotein-associated, serine-dependent phospholipase involved in the oxidative modification of low-density lipoproteins. *Arterioscler Thromb Vasc Biol* 1996;16(4):591-599.

[4] Zalewski A, Macphee C. Role of lipoprotein-associated phospholipase A2 in atherosclerosis: biology, epidemiology, and possible therapeutic target. *Arterioscler Thromb Vasc Biol* 2005;25(5):923-931.

[5] Tjoelker LW, Wilder C, Eberhardt C, Stafforini DM, Dietsch G, Schimpf B, Hooper S, Le Trong H, Cousens LS, Zimmerman GA, Yamada Y, McIntyre TM, Prescott SM, Gray PW. Anti-inflammatory properties of a platelet-activating factor acetylhydrolase. *Nature* 1995;374(6522):549-553.

[6] Stafforini DM, Elstad MR, McIntyre TM, Zimmerman GA, Prescott SM. Human macrophages secret platelet-activating factor acetylhydrolase. *J Biol Chem* 1990;265(17): 9682-9687.

[7] Asano K, Okamoto S, Fukunaga K, Shiomi T, Mori T, Iwata M, Ikeda Y, Yamaguchi K. Cellular source(s) of platelet-activating-factor acetylhydrolase activity in plasma. *Biochem Biophys Res Commun* 1999;261(2):511-514.

[8] Tarbet EB, Stafforini DM, Elstad MR, Zimmerman GA, McIntyre TM, Prescott SM. Liver cells secrete the plasma form of platelet-activating factor acetylhydrolase. *J Biol Chem* 1991;266(25):16667-16673.

[9] Mitsios JV, Vini MP, Stengel D, Ninio E, Tselepis AD. Human platelets secrete the plasma type of platelet-activating factor acetylhydrolase primarily associated with microparticles. *Arterioscler Thromb Vasc Biol* 2006;26(8):1907-1913.

[10] Tellis CC, Tselepis AD. The role of lipoprotein-associated phospholipase A2 in atherosclerosis may depend on its lipoprotein carrier in plasma. *Biochim Biophys Acta* 2009;1791(5):327-338.

[11] Ostermann G, Kostner GM, Gries A, Malle E, Till U. The contribution of individual lipoproteins to the degradation of platelet-activating factor in human serum. *Haemostasis* 1989;19(3):160-168.

[12] Blencowe C, Hermetter A, Kostner GM, Deigner HP. Enhanced association of platelet-activating factor acetylhydrolase with lipoprotein (a) in comparison with low density lipoprotein. *J Biol Chem* 1995;270(52): 31151-31157.

[13] Tselepis AD, Dentan C, Karabina SA, Chapman MJ, Ninio E. PAF degrading acetylhydrolase is preferentially associated with dense LDL and VHDL-1 in human plasma. Catalytic characteristics and relation to the monocyte-derived enzyme. *Arterioscler Thromb Vasc Biol* 1995;15:1764-1773.

[14] Benítez S, Sánchez-Quesada JL, Ribas V, Jorba O, Blanco-Vaca F, González-Sastre F, Ordóñez-Llanos J. Platelet-activating factor acetylhydrolase is mainly associated with electronegative low-density lipoprotein subfraction. *Circulation* 2003;108(1):92-96.

[15] Stafforini DM, McIntyre TM, Carter ME, Prescott SM. Human plasma platelet-activating factor acetylhydrolase. Association with lipoprotein particles and role in the degradation of platelet activating factor. *J Biol Chem* 1987;262:4215-4222.

[16] Bhatti S, Hakeem A, Cilingiroglu M. Lp-PLA(2) as a marker of cardiovascular diseases. *Curr Atheroscler Rep* 2010; 12(2):140-144.

[17] Lavi S, McConnell JP, Rihal CS, Prasad A, Mathew V, Lerman LO, Lerman A. Local production of lipoprotein-associated phospholipase A2 and lysophosphatidylcholine in the coronary circulation: association with early coronary atherosclerosis and endothelial dysfunction in humans. *Circulation* 2007;115:2715-2721.

[18] Macphee CH. Lipoprotein-associated phospholipase A2: a potential new risk factor for coronary artery disease and a therapeutic target. *Curr Opin Pharmacol* 2001;1(2): 121-125.

[19] Kume N, Cybulsky MI, Gimbrone MA Jr. Lysophosphatidylcholine, a component of atherogenic lipoproteins, induces mononuclear leukocyte adhesion molecules in cultured human and rabbit arterial endothelial cells. *J Clin Invest* 1992;90:1138-1144.

[20] Quinn MT, Parthasarathy S, Steinberg D. Lysophosphatidylcholine: a chemotactic factor for human monocytes and its potential role in atherogenesis. *Proc Natl Acad Sci USA* 1988;85:2805-2809.

[21] Vittos Oana, Toana B, Vittos A, Moldoveanu E. Lipoprotein-associated phospholipase A2 (Lp-PLA2): a review of its role and significance as a cardiovascular biomarker. *Biomarkers* 2012;17(4): 289-302.

[22] Zalewski A, Nelson JJ, Hegg L, Macphee C. Lp-PLA2: a new kid on the block. *Clin Chem* 2006;52(9):1645-1650.

[23] Davidson MH, Corson MA, Alberts MJ, Anderson JL, Gorelick PB, Jones PH, Lerman A, McConnell JP, Weintraub HS. Consensus panel recommendation for incorporating lipoprotein-associated phospholipase A2 testing into cardiovascular disease risk assessment guidelines. *Am J Cardiol* 2008;101:51F-57F.

[24] Thompson A, Gao P, Orfei L, Watson S, Di Angelantonio E, Kaptoge S, Ballantyne C, Cannon CP, Criqui M, Cushman M, Hofman A, Packard C, Thompson SG, Collins R, Danesh J, for the Lp-PLA(2) Studies Collaboration. Lipoprotein-associated phospholipase A(2) and risk of coronary disease, stroke, and mortality: collaborative analysis of 32 prospective studies. *Lancet* 2010;375:1536-1544.

[25] O'Donoghue M, Morrow DA, Sabatine MS, Murphy SA, McCabe CH, Cannon CP, Braunwald E. Lipoprotein-associated phospholipase A2 and its association with cardiovascular outcomes in patients with acute coronary syndromes in the PROVE IT-TIMI 22 (PRavastatin Or atorVastatin Evaluation and Infection Therapy – Thrombolysis In Myocardial Infarction) trial. *Circulation* 2006;113(14):1745-1752.

[26] Koenig W, Khuseyinova N, Lowel H, Trischler G, Meisinger C. Lipoprotein-associated phospholipase A2 adds to risk prediction of incident coronary events by C-reactive protein in apparently healthy middle-aged men from the general population: results from the 14-year follow-up of a large cohort from southern Germany. *Circulation* 2004;110:1903-1908.

[27] Persson M, Hedblad B, Nelson JJ, Berglund G. Elevated Lp-PLA2 levels add prognostic information to the metabolic syndrome on incidence of cardiovascular events among middle-aged nondiabetic subjects. *Arterioscler Thromb Vasc Biol* 2007; 27(6): 1411-1416.

[28] Jenny NS, Solomon C, Cushman M, Tracy RP, Nelson JJ, Psaty BM, Furberg CD. Lipoprotein-associated phospholipase A(2) (Lp-PLA(2)) and risk of cardiovascular disease in older adults: results from the Cardiovascular Health Study. *Atherosclerosis* 2010; 209(2):528-532.

[29] Wolfert RL, Kim NW, Selby RG. Biological variability and specificity of lipoprotein associated phospholipase A2, a novel marker of cardiovascular risk. *Circulation* 2004;110(Suppl III):III-309. (Abstract).

[30] Ballantyne CM, Hoogeveen RC, Bang H, Coresh J, Folsom AR, Heiss G, Sharrett AR. Lipoprotein-associated phospholipase A2, high-sensitivity C-reactive protein, and risk for incident coronary heart disease in middle-aged men and women in the Atherosclerosis Risk in Communities (ARIC) study. *Circulation* 2004;109:837-842.

[31] Oei HH, van der Meer IM, Hofman A, Koudstaal PJ, Stijnen T, Breteler MM, Witteman JC. Lipoprotein-associated phospholipase A2 activity is associated with risk of

coronary heart disease and ischemic stroke: the Rotterdam Study. *Circulation* 2005;111(5):570-575.

[32] Stankovic S, Asanin M, Trifunovic D, Majkic-Singh N, Miljković A, Ignjatovic S, Mrdovic I, Matic D, Savic L, Ostojic M, Vasiljevic Z. Utility of lipoprotein-associated phospholipase A2 for prediction of 30-day major adverse coronary event in patients with the first anterior ST-segment elevation myocardial infarction treated by primary percutaneous coronary intervention. *Clin Lab* 2012;58(11-12):1135-1144.

[33] Sabatine MS, Morrow DA, O'Donoghue M, Jablonksi KA, Rice MM, Solomon S, Rosenberg Y, Domanski MJ, Hsia J; PEACE Investigators. Prognostic utility of lipoprotein-associated phospholipase A2 for cardiovascular outcomes in patients with stable coronary artery disease. *Arterioscler Thromb Vasc Biol* 2007;27(11):2463-2469.

[34] Hatoum IJ, Nelson JJ, Cook NR, Hu FB, Rimm EB. Dietary, lifestyle, and clinical predictors of lipoprotein-associated phospholipase A2 activity in individuals without coronary artery disease. *Am J Clin Nutr* 2010;91(3):786-793.

[35] Miyaura S, Maki N, Byrd W, Johnston JM. The hormonal regulation of platelet-activating factor acetylhydrolase activity in plasma. *Lipids* 1991;26:1015-1020.

[36] Mendelsohn ME, Karas RH. The protective effects of estrogen on the cardiovascular system. *N Engl J Med* 1999; 340:1801-1811.

[37] Yoshimura T, Ohshige A, Maeda T, Ito M, Okamura H. Estrogen replacement therapy decreases platelet-activating factor acetylhydrolase activity in post-menopausal women. *Maturitas* 1999;31:249-253.

[38] Ohshige A, Ito M, Koyama H, Maeda T, Yoshimura T, Okamura H. Effects of estrogen and progesterone on platelet activating factor acetylhydrolase activity in ovariectomised rats. *Artery* 1994;21:234-242.

[39] Yanbaeva DG, Dentener MA, Creutzberg EC, Wesseling G, Wouters EF. Systemic effects of smoking. *Chest* 2007;131:1557-1566.

[40] Perk J, De Backer G, Gohlke H, Graham I, Reiner Z, Verschuren M, Albus C, Benlian P, Boysen G, Cifkova R, Deaton C, Ebrahim S, Fisher M, Germano G, Hobbs R, Hoes A, Karadeniz S, Mezzani A, Prescott E, Ryden L, Scherer M, Syvänne M, Scholte op Reimer WJ, Vrints C, Wood D, Zamorano JL, Zannad F; European Association for Cardiovascular Prevention & Rehabilitation (EACPR); ESC Committee for Practice Guidelines (CPG). European Guidelines on cardiovascular disease prevention in clinical practice (version 2012). The Fifth Joint Task Force of the European Society of Cardiology and Other Societies on Cardiovascular Disease Prevention in Clinical Practice (constituted by representatives of nine societies and by invited experts). *Eur Heart J* 2012;33(13):1635-1701.

[41] Greenland P, Alpert JS, Beller GA, Benjamin EJ, Budoff MJ, Fayad ZA, Foster E, Hlatky MA, Hodgson JM, Kushner FG, Lauer MS, Shaw LJ, Smith SC Jr, Taylor AJ, Weintraub WS, Wenger NK, Jacobs AK, Smith SC Jr, Anderson JL, Albert N, Buller CE,

Creager MA, Ettinger SM, Guyton RA, Halperin JL, Hochman JS, Kushner FG, Nishimura R, Ohman EM, Page RL, Stevenson WG, Tarkington LG, Yancy CW; American College of Cardiology Foundation; American Heart Association. 2010 ACCF/AHA guideline for assessment of cardiovascular risk in asymptomatic adults: a report of the American College of Cardiology Foundation/American Heart Association Task Force on Practice Guidelines. *J Am Coll Cardiol* 2010;56(25):e50-e103.

[42] Goldstein LB, Bushnell CD, Adams RJ, Appel LJ, Braun LT, Chaturvedi S, Creager MA, Culebras A, Eckel RH, Hart RG, Hinchey JA, Howard VJ, Jauch EC, Levine SR, Meschia JF, Moore WS, Nixon JV, Pearson TA; American Heart Association Stroke Council; Council on Cardiovascular Nursing; Council on Epidemiology and Prevention; Council for High Blood Pressure Research; Council on Peripheral Vascular Disease; and Interdisciplinary Council on Quality of Care and Outcomes Research. Guidelines for the primary prevention of stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke* 2011;42(2):517-584.

[43] Jellinger PS, Smith DA, Mehta AE, Ganda O, Handelsman Y, Rodbard HW, Shepherd MD, Seibel JA; AACE Task Force for Management of Dyslipidemia and Prevention of Atherosclerosis. American Association of Clinical Endocrinologists' Guidelines for Management of Dyslipidemia and Prevention of Atherosclerosis. *Endocr Pract* 2012;18 (Suppl 1):1-78.

[44] Packard CJ, O'Reilly DS, Caslake MJ, McMahon AD, Ford I, Cooney J, Macphee CH, Suckling KE, Krishna M, Wilkinson FE, Rumley A, Lowe GD. Lipoprotein-associated phospholipase A2 as an independent predictor of coronary heart disease. West of Scotland Coronary Prevention Study Group. *N Engl J Med* 2000;343(16):1148-1155.

[45] Ballantyne CM, Hoogeveen RC, Bang H, Coresh J, Folsom AR, Chambless LE, Myerson M, Wu KK, Sharrett AR, Boerwinkle E. Lipoprotein-associated phospholipase A2, high-sensitivity C-reactive protein, and risk for incident ischemic stroke in middle-aged men and women in the Atherosclerosis Risk in Communities (ARIC) study. *Arch Intern Med* 2005;165(21):2479-2484.

[46] Corsetti JP, Rainwater DL, Moss AJ, Zareba W, Sparks CE. High lipoprotein-associated phospholipase A2 is a risk factor for recurrent coronary events in postinfarction patients. *Clin Chem* 2006;52:1331-1338.

[47] Brilakis ES, McConnell JP, Lennon RJ, Elesber AA, Meyer JG, Berger PB. Association of lipoprotein-associated phospholipase A2 levels with coronary artery disease risk factors, angiographic coronary artery disease, and major adverse events at follow-up. *Eur Heart J* 2005;26:137-144.

[48] Gerber Y, McConnell JP, Jaffe AS, Weston SA, Killian JM, Roger VL. Lipoprotein-associated phospholipase A2 and prognosis after myocardial infarction in the community. *Arterioscler Thromb Vasc Biol* 2006;26:2517-2522.

[49] Anderson JL. Lipoprotein-associated phospholipase A2: an independent predictor of coronary artery disease events in primary and secondary prevention. *Am J Cardiol* 2008;101:23F-33F.

[50] Winkler K, Hoffmann MM, Winkelmann BR, Friedrich I, Schäfer G, Seelhorst U, Wellnitz B, Wieland H, Boehm BO, März W. Lipoprotein-associated phospholipase A2 predicts 5-year cardiac mortality independently of established risk factors and adds prognostic information in patients with low and medium high-sensitivity C-reactive protein (the Ludwigshafen risk and cardiovascular health study). *Clin Chem* 2007;53(8):1440-1447.

[51] Yusuf S, Hawken S, Ounpuu S, Dans T, Avezum A, Lanas F, McQueen M, Budaj A, Pais P, Varigos J, Lisheng L. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet* 2004; 364:937-952.

[52] 2002 Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation* 2002; 106:3143-3421.

[53] Anuurad E, Ozturk Z, Enkhmaa B, Pearson TA, Berglund L. Association of lipoprotein-associated phospholipase A2 with coronary artery disease in African-Americans and Caucasians. *J Clin Endocrinol Metab* 2010;95(5):2376-2383.

[54] Corson MA, Jones PH, Davidson MH. Review of the evidence for the clinical utility of lipoprotein-associated phospholipase a2 as a cardiovascular risk marker. *Am J Cardiol* 2008;101[suppl]:41F-50F.

[55] Madjid M, Ali M, Willerson JT. Lipoprotein-associated phospholipase A2 as a novel risk marker for cardiovascular disease: a systematic review of the literature. *Tex Heart Inst J* 2010;37:25-39.

[56] Mallat Z, Lambeau G, Tedgui A. Lipoprotein-associated and secreted phospholipases A2 in cardiovascular disease: roles as biological effectors and biomarkers. *Circulation* 2010;122:2183-2200.

[57] May HT, Horne BD, Anderson JL, Wolfert RL, Muhlestein JB, Renlund DG, Clarke JL, Kolek MJ, Bair TL, Pearson RR, Sudhir K, Carlquist JF. Lipoprotein-associated phospholipase A2 independently predicts the angiographic diagnosis of coronary artery disease and coronary death. *Am Heart J* 2006;152 (5):997-1003.

[58] Li N, LiS, Yu C, Gu S. Plasma Lp-PLA2 in acute coronary syndrome: association with major adverse cardiac events in a community-based cohort. *Postgrad Med* 2010;122(4): 200-205.

[59] Möckel M, Müller R, Vollert J, Müller C, Danne O, Gareis R, Störk T, Dietz R, Koenig W. Lipoprotein-associated phospholipase A2 for early risk stratification in patients with suspected acute coronary syndrome: a multi-marker approach: the North

Wuerttemberg and Berlin Infarction Study-II (NOBIS-II). *Clin Res Cardiol* 2007;96(9): 604-612.

[60] van Vark LC, Kardys I, Bleumink GS, Knetsch AM, Deckers JW, Hofman A, Stricker BH, Witteman JC. Lipoprotein-associated phospholipase A2 activity and risk of heart failure: the Rotterdam Study. *Eur Heart J* 2006;27:2346-2352.

[61] Suzuki T, Solomon C, Jenny NS, Tracy R, Nelson JJ, Psaty BM, Furberg C, Cushman M. Lipoprotein-associated phospholipase A(2) and risk of congestive heart failure in older adults: the Cardiovascular Health Study. *Circ Heart Fail* 2009;2:429-436.

[62] Furberg CD, Nelson JJ, Solomon C, Cushman M, Jenny NS, Psaty BM. Distribution and correlates of lipoprotein-associated phospholipase A2 in an elderly cohort: the Cardiovascular Health Study. *J Am Geriatr Soc* 2008;56:792–799.

[63] Gerber Y, Dunlay SM, Jaffe AS, McConnell JP, Weston SA, Killian JM, Roger VL. Plasma lipoprotein-associated phospholipase A2 levels in heart failure: association with mortality in the community. *Atherosclerosis* 2009;203(2):593-598.

[64] Moldoveanu E, Serban M, Marta D, Serban I, Huica R. Lipoprotein-associated phospholipase A2 in patients with preserved left ventricular ejection fraction. *Biomarkers* 2011;16(7):587-589.

[65] Raichlin E, McConnell JP, Bae JH, Kremers WK, Lerman A, Frantz RP. Lipoprotein-associated phospholipase A2 predicts progression of cardiac allograft vasculopathy and increased risk of cardiovascular events in heart transplant patients. *Transplantation* 2008;85:963-968.

[66] Persson M, Berglund G, Nelson JJ, Hedblad B. Lp-PLA2 activity and mass are associated with increased incidence of ischemic stroke: a population-based cohort study from Malmö, Sweden. *Atherosclerosis* 2008;200(1):191-198.

[67] Tsimikas S, Willeit J, Knoflach M, Mayr M, Egger G, Notdurfter M, Witztum JL, Wiedermann CJ, Xu Q, Kiechl S. Lipoprotein-associated phospholipase A2 activity, ferritin levels, metabolic syndrome, and 10-year cardiovascular and non-cardiovascular mortality: results from the Bruneck study. *Eur Heart J* 2009;30:107-115.

[68] Tselepis AD, Hahalis G, Tellis CC, Papavasiliou EC, Mylona PT, Kourakli A, Alexopoulos DC. Plasma levels of lipoprotein-associated phospholipase A(2) are increased in patients with β-thalassemia. *J Lipid Res* 2010;51(11):3331-3341.

[69] Elkind MS, Tai W, Coates K, Paik MC, Sacco RL. High-sensitivity C-reactive protein, lipoprotein-associated phospholipase A2, and outcome after ischemic stroke. *Arch Intern Med* 2006;166:2073-2080.

[70] Delgado P, Chacón P, Penalba A, Pelegrí D, Merino C, Ribó M, Rubiera M, Álvarez-Sabin J, Montaner J. Temporal profile and prognostic value of Lp-PLA2 mass and activity in the acute stroke setting. *Atherosclerosis* 2012;220(2):532-536.

[71] Gorelick PB. Lipoprotein-associated phospholipase A2 and risk of stroke. *Am J Cardiol* 2008;101:34F-40F.

[72] Mannheim D, Herrmann J, Versari D, Gössl M, Meyer FB, McConnell JP, Lerman LO, Lerman A. Enhanced expression of Lp-PLA2 and lysophosphatidylcholine in symptomatic carotid atherosclerotic plaques. *Stroke* 2008;39:1448-1455.

[73] Sarlon-Bartoli G, Boudes A, Buffat C, Bartoli MA, Piercecchi-Marti MD, Sarlon E, Arnaud L, Bennis Y, Thevenin B, Squarcioni C, Nicoli F, Dignat-George F, Sabatier F, Magnan PE; RISC Study Group. Circulating lipoprotein-associated phospholipase A2 in high-grade carotid stenosis: a new biomarker for predicting unstable plaque. *Eur J Vasc Endovasc Surg* 2012;43(2):154-159.

[74] Kakkos SK, Tsolakis IA. The emerging role of lipoprotein-associated phospholipase A2 in cerebrovascular disease. *Eur J Vasc Endovasc Surg* 2012; 43:160.

[75] Shoamanesh A, Preis SR, Beiser AS, Vasan RS, Benjamin EJ, Kase CS, Wolf PA, DeCarli C, Romero JR, Seshadri S. Inflammatory biomarkers, cerebral microbleeds, and small vessel disease: Framingham Heart Study Neurology 2015;84(8):825-32.

[76] Caslake MJ, Packard CJ, Suckling KE, Holmes SD, Chamberlain P, Macphee CH. Lipoprotein-associated phospholipaseA(2), platelet-activating factor acetylhydrolase: a potential new risk factor for coronary artery disease. *Atherosclerosi*s 2000;150:413-419.

[77] Ishihara M, Iwasaki T, Nagano M, Ishii J, Takano M, Kujiraoka T, Tsuji M, Hattori H, Emi M. Functional impairment of two novel mutations detected in lipoprotein-associated phospholipase A2 (Lp-PLA2) deficiency patients. *J Hum Genet* 2004;49:302-307.

[78] Guerra R, Zhao B, Mooser V, Stafforini D, Johnston JM, Cohen JC. Determinants of plasma platelet-activating factor acetylhydrolase: heritability and relationship to plasma lipoproteins. *J Lipid Res* 1997;38: 2281-2288.

[79] Schnabel R, Dupuis J, Larson MG, Lunetta KL, Robins SJ, Zhu Y, Rong J, Yin X, Stirnadel HA, Nelson JJ, Wilson PW, Keaney JF, Vasan RS, Benjamin EJ. Clinical and genetic factors associated with lipoprotein-associated phospholipase A2 in the Framingham Heart Study. *Atherosclerosis* 2009;204:601-607.

[80] Suchindran S, Rivedal D, Guyton JR, Milledge T, Gao X, Benjamin A, Rowell J, Ginsburg GS, McCarthy JJ. Genome-wide association study of Lp-PLA2 activity and mass in the Framingham Heart Study. *PLoS Genet* 2010;6:e1000928.

[81] Grallert H, Dupuis J, Bis JC, Dehghan A, Barbalic M, Baumert J, Lu C, Smith NL, Uitterlinden AG, Roberts R, Khuseyinova N, Schnabel RB, Rice KM, Rivadeneira F, Hoogeveen RC, Fontes JD, Meisinger C, Keaney JF Jr, Lemaitre R, Aulchenko YS, Vasan RS, Ellis S, Hazen SL, van Duijn CM, Nelson JJ, März W, Schunkert H, McPherson RM, Stirnadel-Farrant HA, Psaty BM, Gieger C, Siscovick D, Hofman A, Illig T, Cushman M, Yamamoto JF, Rotter JI, Larson MG, Stewart AF, Boerwinkle E, Witteman JC, Tracy RP, Koenig W, Benjamin EJ, Ballantyne CM. Eight genetic loci associated with variation in lipoprotein-associated phospholipase A2 mass and activity

and coronary heart disease: meta-analysis of genome-wide association studies from five community-based studies. *Eur Heart J* 2012; 33(2):238-251.

[82] Psaty BM, O'Donnell CJ, Gudnason V, Lunetta KL, Folsom AR, Rotter JI, Uitterlinden AG, Harris TB, Witteman JC, Boerwinkle E, on behalf of the CHARGE Consortium. Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium: design of prospective meta-analyses of genomewide association studies from 5 cohorts. *Circ Cardiovasc Genet* 2009;2:73-80.

[83] Willer CJ, Sanna S, Jackson AU, Scuteri A, Bonnycastle LL, Clarke R, Heath SC, Timpson NJ, Najjar SS, Stringham HM, Strait J, Duren WL, Maschio A, Busonero F, Mulas A, Albai G, Swift AJ, Morken MA, Narisu N, Bennett D, Parish S, Shen H, Galan P, Meneton P, Hercberg S, Zelenika D, Chen WM, Li Y, Scott LJ, Scheet PA, Sundvall J, Watanabe RM, Nagaraja R, Ebrahim S, Lawlor DA, Ben-Shlomo Y, Davey-Smith G, Shuldiner AR, Collins R, Bergman RN, Uda M, Tuomilehto J, Cao A, Collins FS, Lakatta E, Lathrop GM, Boehnke M, Schlessinger D, Mohlke KL, Abecasis GR. Newly identified loci that influence lipid concentrations and risk of coronary artery disease. *Nat Genet* 2008;40:161-169.

[84] Bell R, Collier DA, Rice SQ, Roberts GW, MacPhee CH, Kerwin RW, Price J, Gloger IS. Systematic screening of the LDL-PLA2 gene for polymorphic variants and case control analysis in schizophrenia. *Biochem Biophys Res Commun* 1997;241:630-635.

[85] Kruse S, Mao XQ, Heinzmann A, Blattmann S, Roberts MH, Braun S, Gao PS, Forster J, Kuehr J, Hopkin JM, Shirakawa T, Deichmann KA. The Ile198Thr and Ala379Val variants of plasmatic PAF-acetylhydrolase impair catalytical activities and are associated with atopy and asthma. *Am J Hum Genet* 2000;66:1522-1530.

[86] Hoffmann MM, Winkler K, Renner W, Winkelmann BR, Seelhorst U, Wellnitz B, Boehm BO, Marz W. Genetic variants and haplotypes of lipoprotein associated phospholipase A2 and their influence on cardiovascular disease (The Ludwigshafen Risk and Cardiovascular Health Study). *J Thromb Haemost* 2009;7:41-48.

[87] Li L, Qi L, Lv N, Gao Q, Cheng Y, Wei Y, Ye J, Yan X, Dang A. Association between lipoprotein associated phospholipase A2 gene polymorphism and coronary artery disease in the Chinese Han population. *Ann Hum Genet* 2011;75(5):605-611.

[88] Yamada Y, Ichihara S, Fujimura T, Yokota M. Identification of the G994→ T missense in exon 9 of the plasma platelet-activating factor acetylhydrolase gene as an independent risk factor for coronary artery disease in Japanese men. *Metabolism* 1998;47(2):177-181.

[89] Shimokata K, Yamada Y, Kondo T, Ichihara S, Izawa H, Nagata K, Murohara T, Ohno M, Yokota M. Association of gene polymorphisms with coronary artery disease in individuals with or without nonfamilial hypercholesterolemia. *Atherosclerosis* 2004;172(1):167-173.

[90] Jang Y, Waterworth D, Lee JE, Song K, Kim S, Kim HS, Park KW, Cho HJ, Oh IY, Park JE, Lee BS, Ku HJ, Shin DJ, Lee JH, Jee SH, Han BG, Jang HY, Cho EY, Vallance

P, Whittaker J, Cardon L, Mooser V. Carriage of the V279F null allele within the gene encoding Lp-PLA2 is protective from coronary artery disease in South Korean males. *PLoS One* 2011;6(4): e18208.

[91] Hou L, Chen S, Yu H, Lu X, Chen J, Wang L, Huang J, Fan Z, Gu D. Associations of PLA2G7 gene polymorphisms with plasma lipoprotein-associated phospholipase A2 activity and coronary heart disease in a Chinese Han population: the Beijing Atherosclerosis Study. *Hum Genet* 2009;125:11-20.

[92] Wang Q, Hao Y, Mo X, Wang L, Lu X, Huang J, Cao J, Li H, Gu D. PLA2G7 gene polymorphisms and coronary heart disease risk: a meta-analysis. *Thromb Res* 2010;126(6):498-503.

[93] Ninio E, Tregouet D, Carrier JL, Stengel D, Bickel C, Perret C, Rupprecht HJ, Cambien F, Blankenberg S, Tiret L. Platelet-activating factor-acetylhydrolase and PAF-receptor gene haplotypes in relation to future cardiovascular event in patients with coronary artery disease. *Hum Mol Genet* 2004;13:1341-1351.

[94] Liu PY, Li YH, Wu HL, Chao TH, Tsai LM, Lin LJ, Shi GY, Chen JH. Platelet-activating factoracetylhydrolase A379V (exon11) gene polymorphism is an independent and functional risk factor for premature myocardial infarction. *J Thromb Haemost* 2006;4:1023-1028.

[95] Wootton PT, Stephens JW, Hurel SJ, Durand H, Cooper J, Ninio E, Humphries SE, Talmud PJ. Lp-PLA2 activity and PLA2G7 A379V genotype in patients with diabetes mellitus. *Atherosclerosis* 2006:189:149-156.

[96] Abuzeid AM, Hawe E, Humphries SE, Talmud PJ. Association between the Ala379Val variant of the lipoprotein associated phospholipase A2 and risk of myocardial infarction in the north and south of Europe. *Atherosclerosis* 2003;168:283-288.

[97] Casas JP, Ninio E, Panayiotou A, Palmen J, Cooper JA, Ricketts SL, Sofat R, Nicolaides AN, Corsetti JP, Fowkes FG, Tzoulaki I, Kumari M, Brunner EJ, Kivimaki M, Marmot MG, Hoffmann MM, Winkler K, März W, Ye S, Stirnadel HA, Boekholdt SM, Khaw KT, Humphries SE, Sandhu MS, Hingorani AD, Talmud PJ. PLA2G7 genotype, lipoprotein-associated phospholipase A2 activity, and coronary heart disease risk in 10 494 cases and 15 624 controls of European Ancestry. *Circulation* 2010;121(21):2284-2293.

[98] Serruys PW, García-García HM, Buszman P, Erne P, Verheye S, Aschermann M, Duckers H, Bleie O, Dudek D, Bøtker HE, von Birgelen C, D'Amico D, Hutchinson T, Zambanini A, Mastik F, van Es GA, van der Steen AF, Vince DG, Ganz P, Hamm CW, Wijns W, Zalewski A; Integrated Biomarker and Imaging Study-2 Investigators. Effects of the direct lipoprotein-associated phospholipase A(2) inhibitor darapladib on human coronary atherosclerotic plaque. *Circulation* 2008; 118:1172-1182.

[99] Mohler ER 3rd, Ballantyne CM, Davidson MH, Hanefeld M, Ruilope LM, Johnson JL, Zalewski A; Darapladib Investigators. The effect of darapladib on plasma lipopro-

tein- associated phospholipase A2 activity and cardiovascular biomarkers in patients with stable coronary heart disease or coronary heart disease risk equivalent: The results of a multicenter, randomized, double-blind, placebo-controlled study. *J Am Coll Cardiol* 2008; 51:1632-1641.

[100] Daida H, Iwase T, Yagi S, Ando H, Nakajima H. Effect of darapladib on plasma lipoprotein-associated phospholipase A2 activity in Japanese dyslipidemic patients, with exploratory analysis of a PLA2G7 gene polymorphism of Val279Phe. *Circ J* 2013;77(6):1518-1525.

[101] O'Donoghue ML, Braunwald E, White HD, Serruys P, Steg PG, Hochman J, Maggioni AP, Bode C, Weaver D, Johnson JL, Cicconetti G, Lukas MA, Tarka E, Cannon CP. Study design and rationale for the Stabilization of pLaques usIng Darapladib-Thrombolysis in Myocardial Infarction (SOLID-TIMI 52) trial in patients after an acute coronary syndrome. *Am Heart J* 2011;162(4):613-619.

[102] O'Donoghue ML, Braunwald E, White HD, Lukas MA, Tarka E, Steg PG, Hochman JS, Bode C, Maggioni AP, Im K, Shannon JB, Davies RY, Murphy SA, Crugnale SE, Wiviott SD, Bonaca MP, Watson DF, Weaver WD, Serruys PW, Cannon CP; SOLID-TIMI 52 Investigators, Steen DL. Effect of darapladib on major coronary events after an acute coronary syndrome: the SOLID-TIMI 52 randomized clinical trial. *JAMA* 2014;312(10):1006-1015.

[103] STABILITY Investigators, White HD, Held C, Stewart R, Tarka E, Brown R, Davies RY, Budaj A, Harrington RA, Steg PG, Ardissino D, Armstrong PW, Avezum A, Aylward PE, Bryce A, Chen H, Chen MF, Corbalan R, Dalby AJ, Danchin N, De Winter RJ, Denchev S, Diaz R, Elisaf M, Flather MD, Goudev AR, Granger CB, Grinfeld L, Hochman JS, Husted S, Kim HS, Koenig W, Linhart A, Lonn E, López-Sendón J, Manolis AJ, Mohler ER 3rd, Nicolau JC, Pais P, Parkhomenko A, Pedersen TR, Pella D, Ramos-Corrales MA, Ruda M, Sereg M, Siddique S, Sinnaeve P, Smith P, Sritara P, Swart HP, Sy RG, Teramoto T, Tse HF, Watson D, Weaver WD, Weiss R, Viigimaa M, Vinereanu D, Zhu J, Cannon CP, Wallentin L. Darapladib for preventing ischemic events in stable coronary heart disease. *N Engl J Med* 2014;370(18):1702-1711.

[104] White H. Editorial: why inhibition of lipoprotein-associated phospholipase A2 has the potential to improve patient outcomes. *Curr Opin Cardiol* 2010;25:299-301.

[105] Wilensky RL, Shi Y, Mohler ER 3rd, Hamamdzic D, Burgert ME, Li J, Postle A, Fenning RS, Bollinger JG, Hoffman BE, Pelchovitz DJ, Yang J, Mirabile RC, Webb CL, Zhang L, Zhang P, Gelb MH, Walker MC, Zalewski A, Macphee CH. Inhibition of lipoprotein-associated phospholipase A2 reduces complex coronary atherosclerotic plaque development. *Nat Med* 2008;14:1059-1066.

[106] Berger JS, Ballantyne CM, Davidson MH, Johnson JL, Tarka EA, Lawrence D, Trivedi T, Zalewski A, Mohler ER 3rd. Peripheral artery disease, biomarkers, and darapladib. *Am Heart J* 2011;161(5):972-978.

[107] Johnson JL, Shi Y, Snipes R, Janmohamed S, Rolfe TE, Davis B, Postle A, Macphee CH. Effect of darapladib treatment on endarterectomy carotid plaque lipoprotein-associated phospholipase A2 activity: a randomized, controlled trial. PLoS One 2014;9(2):e89034.

Chapter 6

Lipoprotein(a) — A Hallmark in Atherosclerosis and Cardiovascular Diseases

Indumathi Chennamsetty and Gert M. Kostner

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/61078

Abstract

Lipoprotein(a) [Lp(a)] consists of a low-density lipoprotein (LDL)-like core and apo(a), a large molecular weight glycoprotein. Apo(a) is highly homologous to plasminogen, yet in contrast exhibits a unique size polymorphism that is characterized by an increasing number of kringle-IV (K-IV) repeats. The number of K-IV repeats ranges from n = 2 to n = 40 or even higher. Apo(a) is synthesized almost exclusively in the liver and there is still some debate whether the assembly of Lp(a) from LDL and apo(a) occurs inside the liver cells or in the circulating blood. The plasma Lp(a) concentration is markedly skewed reaching from <1 mg/dl up to >200 mg/dl. The plasma concentration is >90% genetically determined and correlates negatively with the number of K-IV repeats. In the apo(a) promoter, there are numerous response elements for transcription factors and nuclear receptors that regulate apo(a) expression. The HNF4α binding sequence appears to be the most important one in that respect, yet further work needs to be done to unravel the key features of apo(a) biosynthesis under different conditions. Importantly, activation of FXR causes the dissociation of HNF4α α from its response element and in turn a significant downregulation of apo(a) transcription.

Undoubtedly, Lp(a) is one of the most atherogenic lipoproteins and recent large epidemiological studies document quite impressively that Lp(a) is an independent causal risk factor for coronary heart disease (CHD) and myocardial infarction. This fact led to the development of specific medications to reduce Lp(a) in patients with high plasma concentrations. Among the registered lipid-lowering drugs, only nicotinic acid has a consistently significant Lp(a)-lowering effect, and we recently succeeded in elucidating the mode of action of this drug. There are numerous medications in the pipeline for the treatment of hyper-Lp(a). Among those that are currently in clinical trials, CETP inhibitors, PCSK9 antibodies, MTP inhibitors as well as antisense oligonucleotides (ASO), such as the specific $APO(a)_{Rx}$® from ISIS, which is directed against apo(a)-mRNA and appears to be the most promising drug as it lowers Lp(a) levels by more than 90%.

Lp(a) emerged as an important screening parameter to assess coronary atherosclerosis risk. Its quantitation in the clinical laboratory was, for a long time, quite problematic

since commonly accepted reference materials and standardized analytical methods were lacking. However, newer commercial assays based on nephelometry or turbidimetry, or ELISA using monoclonal antibodies that recognize single epitopes in apo(a), warrant comparable interlaboratory results.

Keywords: Metabolism, Fibrinolysis, Reference values, Medication

1. Introduction

Lipoprotein(a) [Lp(a)] was uncovered in 1963, and its role in atherogenesis has been a matter of debate for many years. This was caused to a certain extent by the fact that the function of Lp(a) was—and still is—unknown. Also, there exists no specific therapy for reducing elevated blood levels of Lp(a). Lp(a) consists of an LDL-like core and a specific antigen, apo(a). Apo(a) exhibits a great homology to plasminogen. For this reason, it was long believed that Lp(a) may play a role in hemostasis and fibrinolysis. There are numerous publications dealing with the role of Lp(a) in hemostasis (reviewed in ref. [1]) providing evidence that the atherogenicity of Lp(a) in fact might be due to a certain extent to pathophysiological effects in fibrinolysis. These findings, however, appear to be of little relevance for practical considerations. Of much greater importance is the causal relationship of elevated plasma Lp(a) with the incidence of atherosclerosis, coronary heart diseases and stroke [2–4]. Of note, on the other hand, are the findings that plasma Lp(a) levels rise with age, i.e. that nonagenarians exhibit significantly higher Lp(a) plasma levels than younger generations [5].

2. Lp(a) metabolism

The protein part of Lp(a) consists of two main components, apoB-100 and apo(a) [6]. ApoB-100, the main component of LDL, is biosynthesized in the liver and LDL is the end-product of VLDL catabolism. Yet, LDL also appears to be synthesized directly and secreted from the liver. Liver LDL, however, displays a different composition from VLDL-derived LDL. Apo(a) consists of 11 unique "kringle-IV's" (K-IV) that are highly homologous to kringle-4 of plasminogen. In addition, apo(a) has a variable number of so-called repetitive K-IVs, which is one of the main puzzles in the immunochemical quantification of Lp(a) (see below). In addition to the presence of K-IVs, apo(a) possesses one kringle-V and a nonactive protease domain; further details on the structure of apo(a) may be found in ref. [7]. The exact mode of the assembly of Lp(a) from LDL and apo(a) might be irrelevant for Lp(a) quantifications, yet it has important implications for the development of Lp(a)-lowering drugs and the interpretation of their mode of action. Mixing recombinant apo(a) with LDL in the test tube and incubation for a few minutes leads to the formation of an intact Lp(a) particle that is indistinguishable from native Lp(a). This led to the assumption that the assembly of Lp(a) takes part outside the liver in circulating blood. Turnover studies carried out in the laboratory of H. Dieplinger (Innsbruck), on the other hand, revealed that the synthesis rate of protein components of Lp(a), i.e. apoB-100 and apo(a), are

http://dx.doi.org/10.5772/61078

identical but distinct from the synthesis rate of apoB-100 in LDL [8]. This appears to be a strong argument for the intracellular assembly of Lp(a).

It is well established that the black population has strikingly different plasma Lp(a) concentrations compared with the white population (black individuals have the highest and Asians have the lowest plasma Lp(a) levels [9]). In addition to these ethnic differences, there are great differences of plasma Lp(a) levels in any ethnic group ranging from <1 mg/dl to >200 mg/dl. This heterogeneity is caused, on one hand, by numerous polymorphisms in the apo(a) promoter and on the other hand by the variable number of K-IV repeats: individuals with a high copy number of K-IV have lower plasma Lp(a) levels and those with a low number copy number have higher plasma Lp(a) levels.

3. The biosynthesis of apo(a)

The locus for the apo(a) gene is situated at chromosome 6 (6q26–q276). The biosynthesis of apo(a) is characteristic of that for any glycoprotein, and the negative correlation of the number of K-IV repeats with the plasma concentration has been explained by longer cellular residence times causing a more efficient intracellular degradation of large molecular weight apo(a) isoforms.

The rate of apo(a) biosynthesis, on the other hand, is significantly influenced by the promoter activity and its activation by transcription factors and nuclear receptors. We provided evidence that the apo(a) promoter contains response elements for >70 transcription factors including HNFs, FXR, PPARs, RXR, SREBPs, CCAAT-Enhancer, IL-6 in addition to numerous others that play important roles in the lipid and lipoprotein metabolism [10]. The presence of these multiple transcription factor binding sites led to the assumption that the regulation of apo(a) transcription might be complex and influenced by numerous metabolic features. Our research group was in fact the first to characterize important response elements in the apo(a) promoter that are key for apo(a) transcription and the abundance of apo(a) in blood plasma [10]. The most significant response element is that of HNF4α at position –826 to –814 in the apo(a) promoter. HNF4α $\beta\nu\delta\nu\gamma$ is competed by farnesoid-X receptor (FXR), a nuclear receptor that plays an important role in bile acid metabolism. Thus, elevated plasma bile acid levels that activate FXR lead to a profound reduction of Lp(a) biosynthesis. These metabolic relationships are schematically displayed in Fig. 1.

4. The Lp(a) catabolism

There exist numerous gaps not only in the understanding of Lp(a) biosynthesis but even more so in Lp(a) catabolism. Very little is known on the site of uptake, the mode of cell binding, internalization and intracellular degradation. *In vivo* studies have been performed mostly on animals that by themselves do not produce Lp(a). These latter studies revealed that >50% of Lp(a) is taken up by the liver and that protein degradation products are secreted into bile [11].

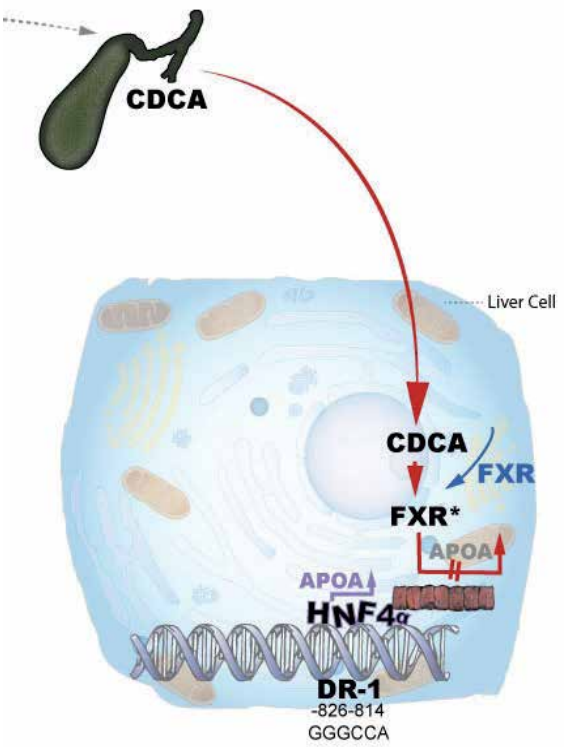


Figure 1. Inhibition of apo(a) transcription by bile acids. Chenodeoxycholic acid (CDCA), the FXR agonist with the highest affinity in humans, binds and activates FXR leading to a displacement of that complex from the cytoplasm to the nucleus. The complex interferes with HNF4α binding to DR-1, a key response element in the apo(a) promoter and in turn silences apo(a) transcription. With permission of the Medical University of Graz (copyrights held by the MUG Graz, Austria).

Significant amounts of radioactivity from labeled Lp(a), however, were also found in kidney, spleen, lung and pancreas, yet it is unknown whether these organs are of relevance for Lp(a) catabolism in humans. Since the liver is the principal organ for the LDL-receptor-mediated catabolism of apoB-containing lipoproteins, it was of interest to study this particular pathway for Lp(a) catabolism. *In vivo* studies carried out in our laboratory as well as by other groups, however, revealed that Lp(a) only has a low affinity to the LDL-R. The main argument for this allegation is the fact that Lp(a) is catabolized in homozygous FH patients with the same rate as compared to healthy control individuals [12]. Since pathways involved in Lp(a) catabolism —and in particular the role of specific receptors—is of eminent importance for strategies to develop Lp(a)-lowering drugs, many attempts have been made to identify binding proteins (receptors) that might be specific for Lp(a). Actually there is hardly any lipoprotein receptor that had not been found to bind Lp(a), including LRP, VLDL-R, asialo-glycoprotein receptor, different scavenger receptors and others. Unfortunately, all these results are based on *in vitro* studies that may have little relevance for the *in vivo* situation. One pathway that appears to be a hot candidate for Lp(a) catabolism is the binding of apo(a) kringle to lysine (Lys)-rich cell surface proteins. Along these lines, we actually demonstrated in previous experiments that

http://dx.doi.org/10.5772/61078

feeding of Lys analogues such as tranexamic acid or δ-amino valeric acid to transgenic apo(a) or Lp(a) mice increased the Lp(a) plasma levels by a factor of 1.5–2, and this correlated with a lower cell uptake and a higher Lp(a) degradation [13].

5. Lp(a): A causal risk factor for atherosclerosis, CHD and stroke

In MedLine and other databanks, there are more than 1500 papers listed dealing with this topic. Thus, it is almost impossible to consider all these publications in this report. Therefore, in this article, we concentrate mainly on the most recent findings; this does not imply that older references might be of lesser relevance. Semiquantitative measurements of Lp(a) in a Scandinavian collective by the "father" of Lp(a), Kare Berg, revealed that individuals with a more pronounced "sinking pre-β band" [= Lp(a)] is found in lipoprotein electrophoresis correlated with the appearance of angina pectoris and CHD [14]. The first quantitative measurements of Lp(a) were in fact carried out by rocket electrophoresis in our laboratory in cooperation with P. Avogaro from Venice. In that case-control study, where 183 probands were included, it was found that the relative risk (RR) of suffering from myocardial infarction (MI)—depending on the applied cutoff value—was approximately 2-fold higher than in healthy controls [15]. This led to the adoption of an upper cutoff value of 30 mg/dl in most subsequent studies. In our first publication, we also could show that patients with type-IIa hyperlipoproteinemia (familial hypercholesterolemia, FH) exhibited a 6-fold higher risk of myocardial infarction (MI). Most of the subsequent studies that were published from various laboratories confirmed a positive correlation of Lp(a) plasma levels with CHD and MI. Some of the studies in fact were also negative, i.e. no relation of Lp(a) with atherosclerotic diseases could be established (for a review, see ref. [16]). A stab in the back to Lp(a) research in fact was given in 1993 by the article from Ridker et al. [17], who could not find any significant relation between Lp(a) and the risk for CHD in a nested case-control evaluation in the Physician's Health Study with almost 15,000 probands "In this prospective study of predominantly middle-aged white men, we found no evidence of association between Lp(a) level and risk of future MI. These data do not support the use of Lp(a) level as a screening tool to define cardiovascular risk among this population." These findings of Ridker et al [17] might have been based on the fact that the methodology used for Lp(a) quantification was subject to criticism.

Some years later, Lp(a) research encountered a revival after the publication of new data from several research groups in 2009–2011. These studies comprised >100.000 patients or probands and, for the first time, revealed beyond any doubt a significant causal relationship between elevated plasma Lp(a) and CHD ([2–4, 18,19]). Of note are studies from the last 3 years which underline the significance of Lp(a) as a risk factor for atherosclerotic cardiovascular diseases:

The PROCARDIS Consortium asked the question that had been discussed for a long time, whether different apo(a) isoforms with different number of K-IV repeats would exert differences in their atherogenicity [20]. There were actually indications in the literature that not only the actual plasma concentration of Lp(a) but also the size polymorphism may reflect the risk of atherosclerosis. Thus, in the PROCARDIS study, including some 1000 patients and a similar

number of control individuals, plasma Lp(a) concentrations were measured by latex-enhanced immune-turbidimetry (see below) and apo(a) isoforms were assayed by SDS-polyacrylamide gel electrophoresis followed by immune blotting, using the isoform-standard from Immuno A.G., Vienna. Unfortunately, Immuno A.G. does not exist anymore and isoform standards are nowadays hard to obtain. The authors of PROCARDIS calculated the odds ratio (OR) of patients and controls between the first and last quintile before and after adjusting for the number of K-IV repeats. In both calculations, an OR of 2.05 ($p < 0.001$) was found, i.e. no difference could be observed whether the apo(a) size polymorphism was taken into consideration or not. This report appears to quite definitely conclude this debate and is proof that Lp(a) exerts its atherogenicity through its plasma concentration and not through possible structural differences in K-IV repeats. In an editorial to this report, F. Kronenberg (Innsbruck) pointed out that the analysis of SNPs—in particular rs41272114, rs10455872 and rs3798220, which exhibit the strongest association to plasma Lp(a) concentrations can neither be taken as surrogates nor as substitutions for the number of K-IV repeats. He further pointed out that more than half the number of individuals with isoforms containing less than 22 K-IV repeats are not recorded by this SNP analysis mentioned above.

In a further publication by the PROCARDIS Consortium published in ATVB [21], the question was asked as to what extent the LPA "null allele" (rs41272114) might influence the plasma concentration of Lp(a) in heterozygous individuals and if it might be a determinant for atherogenic risk. In this study comprising some 8000 CAD patients, an allele frequency for rs41272114 of approximately 3% was found. Patients containing the null allele exhibited significantly lower plasma Lp(a) levels as compared to control individuals without the rs41272114 allele (OR 0.79; $p = 0.023$). According to findings from the group of G. Utermann [22], the rs41272114 SNP represents a donor-splice site mutation leading to the biosynthesis of a truncated apo(a) with only 7 K-IVs (K-IV 1–7) in total and no K-V or protease domain. As a consequence of the absence of K-IV type 9, which contains the only free –SH group in apo(a) and is responsible for the covalent binding to apoB-100, the truncated apo(a) fragments are well secreted from the liver into the blood but do not assemble with LDL and thus are rapidly degraded and removed from the circulation. The PROCARDIS study also proved that individuals with only one apo(a) isoform exhibit a large variation in their plasma Lp(a) concentrations and that there exists a sigmoid correlation between the number of K-IV repeats and plasma Lp(a) levels. The question of the mechanism that causes this variation, however, could not be answered by this study. The authors of the PROCARDIS Consortium claimed, on the basis of their results, that in future epidemiological studies by SNP analysis for the assessment of the CAD risk, the rs41272114 polymorphism must be taken into consideration as a matter of state-of-the-art experiments.

Further support of the hypothesis published in 1981 by our group [15] indicating that Lp(a) might be a significant risk factor for MI comes from the "Bruneck Study" comprising 826 male and female probands [23]. In a recall survey after 15 years, it was found that the inclusion of Lp(a) in the Framingham algorithm for the risk assessment of CHD, an improvement of 0.016 in the C-index was reached. Consideration of Lp(a) plasma levels improved the hit rate in the prediction of CHD by 40%.

http://dx.doi.org/10.5772/61078

6. Lp(a) and stroke

The question as to what extent Lp(a) might also be causally related to stroke was addressed in numerous publications (reviewed in ref. [24]). Sultan et al. [25] recently published the results of his meta-analysis, where he included 10 published papers dealing with ischemic stroke in children. Setting the cutoff level for Lp(a) at 30 mg/dl, a positive association between Lp(a) and stroke was found with a Mantel-Haenszel OR of 4.24 ($p < 0.00001$).

As mentioned above, the physiological function of Lp(a) is in the dark. Concerning the pathophysiology, the work of Tsimikas et al. from San Diego is noteworthy because they believe that the high affinity of Lp(a) for oxidized phospholipids might be responsible for its atherogenicity [26]. Oxidized phospholipids are known to promote the synthesis of inflammatory cytokines that recruit monocytes and T-lymphocytes. Monocytes differentiate to macrophages that phagocytose oxLDL and are transformed to foam cells, hallmarks in atherogenesis. Negatively charged phospholipids such as Ox-Phos are key components in oxLDL and also bind a specific protein, β-2-glykoprotein-I (β2-GPI). The latter also forms a complex with Lp(a). In a recently published paper, it was reported that the plasma levels of Lp(a), Ox-Lp(a) and β2GP-I-Lp(a) in stroke patients were significantly higher than in controls (124 patients vs. 64 controls). In addition, a positive correlation of these plasma parameters with the severity of stroke was established [27]. These findings point towards the assumption that Lp(a) might not neutralize ox-PL but in contrast boosts its atherogenic properties.

7. Medication of patients with elevated plasma Lp(a)

Although recent publications might be taken as a clear hint for the causal relationship between elevated Lp(a) and CHD, a final proof might be only obtained from intervention studies with Lp(a)-lowering drugs (see ref. [28]). Unfortunately, there hardly exists any drug that may specifically lower plasma Lp(a). As a consequence of the work cited above, however, intensive efforts have been taken to develop such therapeutic agents. In addition, any new lipid-lowering or HDL-raising drug that is in clinical trials is checked for its potential action on Lp(a). There exist several serious recommendations for the treatment of relevant patients (reviewed in ref. [16]), but many of them are based on anecdotal observations, or are of little practical value or low efficacy. As mentioned above, Lp(a) is hardly bound by the LDL-R and thus specific drugs acting solely by increasing LDL-R activity are mostly inactive in the treatment of hyper-Lp(a) patients. Statins that belong to this sort of drugs are therefore not the drugs of choice—and actually there are reports that statins even may lead to an elevation of Lp(a) [29]. Unfortunately, the pathomechanism of the LDL-raising effect of this observation is unknown.

The only current method that reduces plasma Lp(a) levels to a satisfactory extent is apheresis, and it has been shown that lowering Lp(a) by this method reduced the CHD risk significantly [30]. Apheresis is therefore strongly advised in the secondary prevention of patients with very high plasma Lp(a) (>80 mg/dl).

Another current possibility is medication with nicotinic acid or derivatives thereof. Nicotinic acid (niacin), its amide or different retard compounds were, for a long time, in the markets of numerous countries because of their HDL-raising properties. In addition, these compounds reportedly reduce plasma Lp(a) by some 30% [31]. In recent studies, we succeeded to uncover the mode of action of this drug at a molecular level: the *APOA* promoter contains several cAMP response elements that impact the apo(a) transcription [32]. Nicotinic acid interferes in liver with the binding activity of cAMP to these elements and reduces the biosynthesis of apo(a) (see cartoon in Fig. 2).

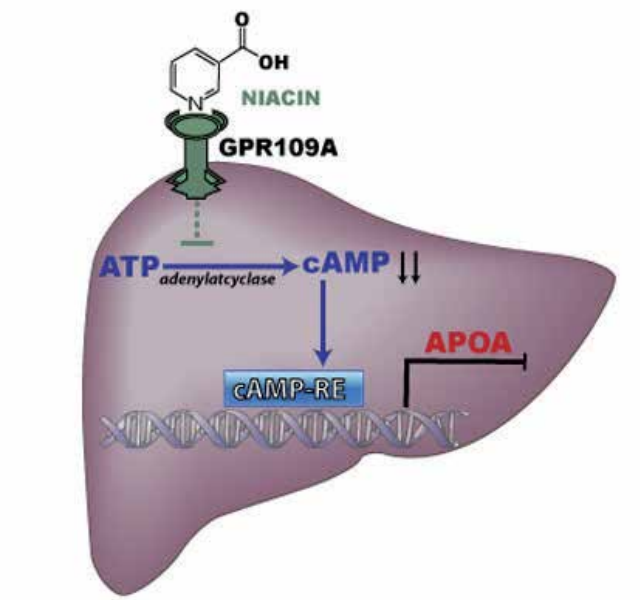


Figure 2. Influence of nicotinic acid on apo(a) biosynthesis: Proposed mode of action. cAMP regulates apo(a) biosynthesis by binding to specific cAMP response elements in the *APOA* promoter. Nicotinic acid inhibits adenylate cyclase, the key enzyme of cAMP biosynthesis in the liver and in turn lowers its intracellular concentration, leading to a lower expression of the *APOA* gene. With permission of the Medical University of Graz (copyrights held by the MUG Graz, Austria).

A major side effect of nicotinic acid is the activation of prostaglandin D_2, particularly in the skin, which causes the dilatation of blood vessels by binding to DP1 (PGD2 receptors) and in turn causing skin flushing (red face). Thus, nicotinic acid is not very well appreciated by most patients and, as a consequence, it was removed from the market in most countries. Another drawback for nicotinic acid was the outcome of the HPS2-THRIVE study (http://www.nejm.org/doi/full/10.1056/NEJMoa1300955). In this trial, 25,673 patients were treated with a standard statin background therapy plus a nicotinic acid supplement consisting of 2 g extended-release niacin + 400 mg of the DP1 antagonist laropiprant or a matching placebo. As it turned out, the supplement nicotinic acid-laropiprant therapy did not reduce CHD risk but increased the incidence of serious adverse events.

http://dx.doi.org/10.5772/61078

8. Selective medication for hyper-Lp(a)

In our study cited in ref. [10], we actually found that patients suffering from extrahepatic cholestasis exhibited very low Lp(a) plasma concentrations. After treating these patients and curing them from cholestasis, Lp(a) levels vent up significantly. In this study, we succeeded in pinpointing FXR as the most important repressor for apo(a) biosynthesis (see also Fig. 2). Unfortunately, FXR is a pluripotent nuclear receptor that plays eminent roles not only in bile acid and glucose metabolism but also influences the activity of LXR, the master regulator of cellular cholesterol metabolism. There exist negative feedback loops not only between FXR and LXR but also between FXR and other transcription factors, cytokines and interleukins. Thus, the application of FXR activation must be done with great caution and may be not feasible at all for prolonged applications. Nevertheless, such FXR agonists are in development and are currently being tested for their action on the plasma levels of Lp(a). Phenex, a SME that specializes on the development of antagonists and agonists of nuclear receptors, has the FXR agonist Px-102® in clinical trials (http://www.phenex-pharma.com/pdf/PR-Phenex-Phase%20I%20finished_5%20M%20Euros_engl.pdf). Px-102® significantly affects plasma cholesterol levels in laboratory animals and is also tested for its potential effect on liver tumors. No data has been released so far on its potential effect on Lp(a).

Other selective Lp(a)-lowering agents are currently under investigation. They comprise mostly drugs that were originally drafted to lower LDL cholesterol or increase HDL cholesterol. Among them, inhibitors of PCSK-9, CETP, MTP and thyromimetica are worth noting.

8.1. PCSK-9 inhibitors

Recently, it has been published in *Circulation* that AMG145, the monoclonal antibody against PCSK-9 from Amgen®, at a dose of 105 mg Q2W reduced plasma Lp(a) levels on average by 32% [33]. At this dose, the authors observed a reduction of plasma LDL-C and of apoB by 60% and 50%, respectively. It must be stressed, however, that among the 626 male and female patients, approximately half of them had Lp(a) levels below the median concentration of 43 nmol/L. Although the Lp(a)-lowering effect of AMG-145 correlated significantly with the reduction of LDL-C, patients with low Lp(a) showed a much bigger relative reduction of Lp(a) than patients of the 3rd or 4th Lp(a) quartile. At a dosage of 420 mg, Q4W patients of the 4th quartile did not respond at all with a reduction of Lp(a). According to unconfirmed communications AMG-145 might be registered in the 2nd half of 2015, yet the treatment costs will certainly be significantly higher as compared to that of statin therapy.

In a similar study with SAR236553, the PCSK-9 antibody from Sanofi®, an average reduction of Lp(a) of up to 28.6% was observed [34].We actually consider these trials as pilot studies as they do not address all the questions of the mode of action of PCSK-9 inhibitors on Lp(a). It is well known that these drugs increase the activity of LDL-R, particularly in the liver, and this receptor has a relatively low affinity to Lp(a).

8.2. CETP inhibitors

CETP stands for "cholesterol ester exchange/transfer protein." It catalyzes the exchange of CE and triglycerides between VLDL or LDL and HDL. In fact, many years ago, we published that Lp(a) also serves as a substrate for CETP [35]. On theoretical grounds, CETP inhibitors should be ideal for the treatment of stroke patients that exhibit significantly elevated Lp(a) and reduced HDL levels [36]. The development of drugs containing the CETP inhibitors Torcetrapib and Dalcetrapib has been stopped because of unwanted side effects. Anacetrapib and Evacetrapib, on the other hand, are currently in phase II clinical trials. Concerning Anacetrapib, it was published in several reports that it reduces Lp(a) by up to 25%, yet details of this study are still lacking.

Further medications such as Eprotirome, a thyromimetikum, Lomitapide, an MTP inhibitor from Aegerion, and Mipomersen, an antisense oligonucleotide targeting apoB, all reportedly reduce Lp(a); however, it is rather uncertain that these drugs will ever be admitted for the indication, high Lp(a).

The most promising medication, at the time being, appears to be $APO(a)_{rx}$, a specific antisense drug from ISIS®. In that respect, it is noteworthy that we published in 2001 that by RNA interference, a 100% inhibition of the expression of ap(a) may be accomplished in transgene apo(a) mice [39]. ISIS®, in fact, claims from a phase I study that in patients with Lp(a) of 10–100 mg/dl, a reduction of up to 90% was reached (http://www.isispharm.com/Pipeline/Therapeutic-Areas/Cardiovascular.htm#ISIS-APOARx). If ISIS ® succeeds in admitting their antisense drug $APO(a)_{rx}$, we consider this strategy as the most specific and effective in treating hyper-Lp(a).

9. How and when should Lp(a) be measured

Actually, Lp(a) is not measured routinely in clinical laboratories because of the following reasons: (i) there exists no effective treatment regime to lower plasma Lp(a); (ii) the currently available Lp(a) assays are not standardized and results from different laboratories vary considerably (see ref. [16]).

i. As mentioned above, very effective and partially specific Lp(a) medications are in clinical trials and it is hoped that some of them might soon be on the market. Even if it should take several years before such drugs are admitted, knowledge of the plasma Lp(a) value gives additional important information to judge CHD risk. It has been reported previously that plasma Lp(a) levels stay pretty constant over months and years and may hardly be influenced by diet and living conditions. Systematic studies within single individuals, however, revealed quite large fluctuations. Patients with elevated or borderline Lp(a) values therefore should be assayed for Lp(a) at several occasions.

ii. It is true that most commercial Lp(a) assays are not standardized, and the accuracy and precision of these assays needs to be seriously revised. Since there is a strong

http://dx.doi.org/10.5772/61078

genetic component in Lp(a) plasma levels, Lp(a) should also be measured in all relatives of such index patients mentioned above. In addition, we advise that Lp(a) should be measured in the plasma of all premature myocardial infarction and stroke patients in addition to patients with borderline CHD risk because elevated Lp(a) puts them into a higher risk group. Since a specific practicable Lp(a) therapy is currently lacking, patients at increased CHD risk that exhibit elevated Lp(a) must be treated quite more rigorously with conventional lipid-lowering therapy than similar patients with low Lp(a). The monitoring of Lp(a), in addition, is advised in patients that undergo state-of-the-art therapy but still show a progression of atherosclerosis or vascular diseases, in all FH patients and in patients with genetic lipoprotein disorders, in patients with low HDL or high homocysteine, or in patients with disorders of hemostasis or fibrinolysis. Finally, we recommend assaying Lp(a) in patients with diabetes mellitus and autoimmune diseases.

In a consensus report of the European Atherosclerosis Society, monitoring of plasma Lp(a) is recommended in patients at a 10-year atherosclerosis risk of >3%. Particular attention should be paid to hemodialysis patients and patients with any form of kidney disease. In kidney disease, it is important before targeting hyperlipoproteinemias by medication to treat the primary disease as well as possible and to concentrate on modifiable CHD risk factors such as LDL-C, high blood pressure, smoking, blood glucose and obesity. Apheresis in addition to nicotinic acid therapy must be considered in these patients if feasible, although evidence-based results are still lacking.

10. What needs to be kept in mind when measuring Lp(a)

The first laboratory methods for measuring plasma Lp(a) were radial immune diffusion, rocket electrophoresis and later nephelometry. Today, high-throughput methods are mostly requested comprising ELISE, DELFIA nephelometry and turbidimetry. In all these methods, one must consider the fact that the molecular mass of Lp(a) and apo(a) varies quite strikingly within large limits, that Lp(a) contains apoB-100 in addition to apo(a), that Lp(a) exhibits great affinities to other proteins, e.g. β2-GPI, and in particular that apo(a) contains repetitive structures: the number of repetitive K-IV repeats varies from 2 to approximately 40 or more. This causes, in many assays, an overestimation of Lp(a) concentrations in patients with large apo(a) isoforms and an underestimation in patients with small apo(a) isoforms. Finally, one must consider the presence of small apo(a) fragments in plasma that are not bound to LDL. Yet the concentration of these fragments correlates positively with Lp(a) levels. In order to circumvent some problems in the quantitative analysis of Lp(a), ELISA and DELFIA methods have been suggested where the capture antibody binds relatively unspecific all apo(a) isoforms yet the detection antibody is monoclonal and recognizes only one epitope in apo(a). Other assays use anti-apoB as a detection antibody. This, however, is biased by the fact that in hyperlipidemic samples, one Lp(a) particle may bind additional apoB-containing lipoproteins leading to an overestimation of plasma Lp(a) levels.

As a consequence of these challenges, a group of experts in the field vent together that tackled these problems by propagating various reference standards and methods. We also participated in this survey using our in-house laboratory methods and antibodies [40]. A major problem that came out from this study was the different reference materials used in the assays. Even the use of the WHO/IFCC Reference Material as a common calibrator did not result in satisfactory harmonization of Lp(a) values [41].

We consider most of the considerations, based on theoretical grounds, of little importance in commercial routine clinical assays. There are three important questions that need to be solved: (1) What methods are apo(a)-isoform insensitive? (2) How can be units in mg/dl be transformed into nmol/L? (3) What are the cutoff levels to be adopted for risk stratification?

11. What needs to be considered in measuring Lp(a)?

Considering all these puzzles, we propagate the following: Our preferred commercial assays are based on latex-enhanced immune-nephelometry or -turbidimetry. This is based on the consideration that the size of latex particles in comparison to the size of Lp(a) is very high and the size polymorphism of apo(a) becomes negligible. In addition, the latter methods are highly precise and may be applied in high-throughput. ELISA and DELFIA methods may be isoform-insensitive if monoclonal antibodies are used that recognize only one epitope on apo(a).

Another possibility that still needs confirmation is to assay apo(a) fragments in urine. In urine, apo(a) fragments are secreted by a mechanism that has not been fully explored so far. These fragments consist mostly of repetitive K-IV structures of different lengths and thus what is measured accurately even by the use of polyclonal antibodies is the concentration of K-IVs, mostly of type 2, that have been shown to correlate significantly with the plasma Lp(a) concentration [42]. In the work cited in ref. [42], we could actually show that the discriminatory power of urinary apo(a) fragments is at least as good—if not even better—than that of plasma Lp(a).

12. Should Lp(a) levels be expressed as mass units or molar units?

This question is partly academic since there is, at the moment, no validated commercial assay on the market that gives accurate and reliable molar values. One must also consider that most of the individuals are heterozygous, i.e. they have 2 kinds of Lp(a) particles in their blood with quite large differences in the molecular mass. In our laboratory, we use mass values since the majority of the published epidemiological studies publish their values in mg/dl or mg/L. In addition, the cutoff values propagated in the Consensus Report of the European Atherosclerosis Society are given in mg/dl.

Keeping in mind that not only the molecular mass but also the composition of Lp(a) varies quite remarkably, we must think practicable for the time being. Assuming a molecular mass

http://dx.doi.org/10.5772/61078

for Lp(a) of 3,150.000 Daltons, a value that sounds quite realistic on the basis of quasielastic light-scattering data, a conversion factor of 3.17 for converting mass into molar units has been proposed: 1 mg/dl apo(a) corresponds roughly to 3.17 nmol/L. It should be pointed out, however, that in the US, a conversion factor of 2.5 has been proposed. This factor may be calculated on the grounds of a molecular mass of 4 million.

What are the most realistic cutoff values? Most of the results from recent studies assumed that Lp(a) is not a continuous risk factor but rather that a significant risk starts at a certain border value. This in fact is not supported by any evidence-based study, yet on practical considerations, cutoff levels have been propagated. In the original study where Lp(a) was quantitatively measured in our laboratory, we published that at a cutoff point of 30 mg/dl, the relative risk for myocardial infarction in that particular collective was 1.75 and at a cutoff value of 50 mg/dl, the relative risk was 2.5 [15]. These values are very close to those that have been obtained in numerous large epidemiological studies including meta-analyses of prospective trials published by many laboratories. The European Atherosclerosis Society propagates, in a consensus report that is mostly based on data from the Copenhagen Heart Study, a cutoff value of 50 mg/dl, corresponding to approximately 150 nmol/L.

Author details

Indumathi Chennamsetty[1*] and Gert M. Kostner[2]

*Address all correspondence to: induc@stanford.edu

1 Stanford Cardiovascular Medicine, Stanford, CA, USA

2 Institute of Molecular Biology and Biochemistry, Medical University of Graz, Austria

A German version of this manuscript has been published in: J Lab Med 201539:71-80.

References

[1] Voetsch B, Loscalzo J (2004). Genetics of thrombophilia: impact on atherogenesis. Current Opinion in Lipidology 15: 129–143.

[2] Clarke R, Peden JF, Hopewell JC, et al. (2009). Genetic variants associated with Lp(a) lipoprotein level and coronary disease. The New England Journal of Medicine 361: 2518–2528.

[3] Emerging Risk Factors Collaboration (2009). Lipoprotein(a) concentration and the risk of coronary heart disease, stroke, and nonvascular mortality. JAMA 302: 412–423.

[4] Kamstrup PR, Tybjaerg-Hansen A, Steffensen R, Nordestgaard BG (2009). Genetically elevated lipoprotein(a) and increased risk of myocardial infarction. JAMA 301: 2331–2339.

[5] Zuliani G, Bader G, Imbastaro T, et al. (1995). Lipoprotein(a) plasma levels and apo(a) isoforms are not associated with longevity or disability in a sample of Italian octo-nonagenarians. Associazione Medica Sabin. Aging 7: 385–391.

[6] Kostner KM, Kostner GM (2002). Lipoprotein(a): still an enigma? Current Opinion in Lipidology 13: 391–396.

[7] Kostner KM, Kostner GM (2002). Lipoprotein(a): still an enigma? Current opinion in lipidology 13: 391–396.

[8] Frischmann ME, Ikewaki K, Trenkwalder E, et al. (2012). In vivo stable-isotope kinetic study suggests intracellular assembly of lipoprotein(a). Atherosclerosis 225: 322–327.

[9] Kronenberg F, Utermann G (2013). Lipoprotein(a): resurrected by genetics. Journal of Internal Medicine 273: 6–30.

[10] Chennamsetty I, Claudel T, Kostner KM, et al. (2011). Farnesoid X receptor represses hepatic human APOA gene expression. The Journal of Clinical Investigation 121: 3724–3734.

[11] Kostner GM, Wo X, Frank S, Kostner K, Zimmermann R, Steyrer E (1997). Metabolism of Lp(a): assembly and excretion. Clinical Genetics 52: 347–354.

[12] Krempler F, Kostner GM, Roscher A, Haslauer F, Bolzano K, Sandhofer F (1983). Studies on the role of specific cell surface receptors in the removal of lipoprotein(a) in man. The Journal of Clinical Investigation 71: 1431–1441.

[13] Frank S, Hrzenjak A, Kostner K, Sattler W, Kostner GM (1999). Effect of tranexamic acid and delta-aminovaleric acid on lipoprotein(a) metabolism in transgenic mice. Biochimica et Biophysica Acta 1438: 99–110.

[14] Berg K (1963). A new serum type system in man—the Lp system. Acta Pathologica et Microbiologica Scandinavica 59: 369–382.

[15] Kostner GM, Avogaro P, Cazzolato G, Marth E, Bittolo-Bon G, Qunici GB (1981). Lipoprotein Lp(a) and the risk for myocardial infarction. Atherosclerosis 38: 51–61.

[16] Kostner KM, Marz W, Kostner GM (2013). When should we measure lipoprotein (a)? European Heart Journal 34: 3268–3276.

[17] Ridker PM, Hennekens CH, Stampfer MJ (1993). A prospective study of lipoprotein(a) and the risk of myocardial infarction. Jama 270: 2195–2199.

http://dx.doi.org/10.5772/61078

[18] Li Y, Luke MM, Shiffman D, Devlin JJ (2011). Genetic variants in the apolipoprotein(a) gene and coronary heart disease. Circulation Cardiovascular Genetics 4: 565–573.

[19] Erqou S, Thompson A, Di Angelantonio E, et al. (2010). Apolipoprotein(a) isoforms and the risk of vascular disease: systematic review of 40 studies involving 58,000 participants. Journal of the American College of Cardiology 55: 2160–2167.

[20] Hopewell JC, Seedorf U, Farrall M, et al. (2014). Impact of lipoprotein(a) levels and apolipoprotein(a) isoform size on risk of coronary heart disease. Journal of Internal Medicine 276: 260–268.

[21] Kyriakou T, Seedorf U, Goel A, et al. (2014). A common LPA null allele associates with lower lipoprotein(a) levels and coronary artery disease risk. Arteriosclerosis, Thrombosis, and Vascular Biology 34: 2095–2099.

[22] Ogorelkova M, Gruber A, Utermann G (1999). Molecular basis of congenital lp(a) deficiency: a frequent apo(a) 'null' mutation in caucasians. Human Molecular Genetics 8: 2087–2096.

[23] Willeit P, Kiechl S, Kronenberg F, et al. (2014). Discrimination and net reclassification of cardiovascular risk with lipoprotein(a): prospective 15-year outcomes in the Bruneck Study. Journal of the American College of Cardiology 64: 851–860.

[24] Ferraro M, Spagnuolo V, Sprovieri M, Mauro GF (2008). [Lipoprotein (a) and stroke]. Minerva Medica 99: 399–409.

[25] Sultan SM, Schupf N, Dowling MM, Deveber GA, Kirton A, Elkind MS (2014). Review of lipid and lipoprotein(a) abnormalities in childhood arterial ischemic stroke. International Journal of Stroke: Official Journal of the International Stroke Society 9: 79–87.

[26] Hung MY, Witztum JL, Tsimikas S (2014). New therapeutic targets for calcific aortic valve stenosis: the lipoprotein(a)-lipoprotein-associated phospholipase A2-oxidized phospholipid axis. Journal of the American College of Cardiology 63: 478–480.

[27] Ma LJ, Wu J, Niu DM, et al. (2014). Serum lipoprotein(a) complexes with beta2-glycoprotein I levels in patients with ischemic stroke. Clinica Chimica Acta 429: 163–167.

[28] Norata GD, Ballantyne CM, Catapano AL (2013). New therapeutic principles in dyslipidaemia: focus on LDL and Lp(a) lowering drugs. European Heart Journal 34: 1783–+.

[29] Kostner GM, Gavish D, Leopold B, Bolzano K, Weintraub MS, Breslow JL (1989). HMG CoA reductase inhibitors lower LDL cholesterol without reducing Lp(a) levels. Circulation 80: 1313–1319.

[30] Jaeger BR, Richter Y, Nagel D, et al. (2009). Longitudinal cohort study on the effectiveness of lipid apheresis treatment to reduce high lipoprotein(a) levels and prevent

major adverse coronary events. Nature Clinical Practice Cardiovascular Medicine 6: 229–239.

[31] Gurakar A, Hoeg JM, Kostner G, Papadopoulos NM, Brewer HB (1985). Levels of lipoprotein Lp(a) decline with neomycin and niacin treatment. Atherosclerosis 57: 293–301.

[32] Chennamsetty I, Kostner KM, Claudel T, et al. (2012). Nicotinic acid inhibits hepatic APOA gene expression: studies in humans and in transgenic mice. Journal of Lipid Research 53: 2405–2412.

[33] Desai NR, Kohli P, Giugliano RP, et al. (2013). AMG145, a monoclonal antibody against proprotein convertase subtilisin kexin type 9, significantly reduces lipoprotein(a) in hypercholesterolemic patients receiving statin therapy: an analysis from the LDL-C Assessment with Proprotein Convertase Subtilisin Kexin Type 9 Monoclonal Antibody Inhibition Combined with Statin Therapy (LAPLACE)-Thrombolysis in Myocardial Infarction (TIMI) 57 Trial. Circulation 128: 962–969.

[34] McKenney JM, Koren MJ, Kereiakes DJ, Hanotin C, Ferrand AC, Stein EA (2012). Safety and efficacy of a monoclonal antibody to proprotein convertase subtilisin/kexin type 9 serine protease, SAR236553/REGN727, in patients with primary hypercholesterolemia receiving ongoing stable atorvastatin therapy. Journal of the American College of Cardiology 59: 2344–2353.

[35] Groener JEM, Kostner GM (1987). Lipid transfer protein-catalyzed exchange of cholesteryl ester between high-density-lipoproteins and Apo-B-containing lipoproteins. Journal of Lipid Research 28: 1053–1056.

[36] Kostner GM, Marth E, Pfeiffer KP, Wege H (1986). Apolipoprotein-a-I, apolipoprotein-Aii and HDL phospholipids but not Apo-B are risk indicators for occlusive cerebrovascular-disease. European Neurology 25: 346–354.

[37] Cuchel M, Meagher EA, Theron HD, et al. (2013). Efficacy and safety of a microsomal triglyceride transfer protein inhibitor in patients with homozygous familial hypercholesterolaemia: a single-arm, open-label, phase 3 study. Lancet 381: 40–46.

[38] Thomas GS, Cromwell WC, Ali S, Chin W, Flaim JD, Davidson M (2013). Mipomersen, an apolipoprotein B synthesis inhibitor, reduces atherogenic lipoproteins in patients with severe hypercholesterolemia at high cardiovascular risk. Journal of the American College of Cardiology 62: 2178–2184.

[39] Frank S, Gauster M, Strauss J, Hrzenjak A, Kostner GM (2001). Adenovirus-mediated apo(a)-antisense-RNA expression efficiently inhibits apo(a) synthesis in vitro and in vivo. Gene Therapy 8: 425–430.

[40] Tate JR, Berg K, Couderc R, et al. (1999). International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) standardization project for the measurement of

http://dx.doi.org/10.5772/61078

lipoprotein(a). Phase 2: Selection and properties of a proposed secondary reference material for lipoprotein(a). Clinical Chemistry and Laboratory Medicine 37: 949–958.

[41] [41] Marcovina SM, Albers JJ, Scanu AM, Kennedy H, Giaculli F, Berg K, Couderc R, Dati F, Rifai N, Sakurabayashi I, Tate JR, Steinmetz A (2009). Use of a reference material proposed by the International Federation of Clinical Chemistry and Laboratory Medicine to evaluate analytical methods for the determination of plasma lipoprotein(a) Clinical Chemistry 46: 1956–1967.

[42] Kostner KM, Maurer G, Huber K, et al. (1996). Urinary excretion of Apo(a) fragments —Role in Apo(a) catabolism. Arteriosclerosis Thrombosis and Vascular Biology 16: 905–911.

Edited by Gerhard Kostner and Indumathi Chennamesetty

In mammalian blood plasma there exist some 6 major lipoprotein classes. Under physiological conditions lipoproteins are certainly beneficial as they transport nutrients and steroids to numerous organs for further metabolism. On the other hand, under pathophysiological conditions most lipoprotein classes promote atherogenesis except of HDL that are considered to be anti-atherogenic. Lipoprotein research is a wide field comprising basic science, analytical methods and clinical investigations. Thus this issue does not raise the claim to give a comprehensive picture of the current knowledge, but rather focuses on specific questions related to animal models in lipoprotein research as well as features of the most atherogenic lipoprotein, Lp(a).

ISBN 978-953-51-5407-5

Photo by monsitj / DollarPhoto

IntechOpen