Small dense LDL cholesterol and apolipoproteins C-II and C-III in non-diabetic obese subjects with metabolic syndrome

Theodosios D. Filippatos[1], Vasilis Tsimihodimos[1], Michalis Kostapanos[1], Christina Kostara[2], Eleni T. Bairaktari[2], Dimitrios N. Kiortsis[3], Alexandros D. Tselepis[4], Moses S. Elisaf[1]

[1]Department of Internal Medicine, School of Medicine, University of Ioannina, Ioannina, Greece
[2]Laboratory of Clinical Chemistry, School of Medicine, University of Ioannina, Ioannina, Greece
[3]Laboratory of Physiology, Medical School, University of Ioannina, Ioannina, Greece
[4]Laboratory of Biochemistry, Department of Chemistry, University of Ioannina, Ioannina, Greece

Corresponding author:
Prof. Moses S. Elisaf MD, FRSH, FASA, FISA
Department of Internal Medicine
School of Medicine
University of Ioannina
45 110 Ioannina, Greece
Phone: +30 2651 0 97509
Fax: +30 2651 0 97016
E-mail: egepi@cc.uoi.gr

Submitted: 12 June 2008
Accepted: 24 August 2008

Arch Med Sci 2008; 4, 3: 263–269

Abstract

Introduction: Apolipoprotein (apo) C-II is considered as an important activator of lipoprotein lipase (LPL) and is required for efficient lipolysis of triglyceride (TG)-rich lipoproteins. In contrast, excess apo C-II inhibits LPL-mediated hydrolysis of TGs. Apo C-III is an inhibitor of LPL activity. These effects may influence the plasma levels of atherogenic small dense low-density lipoprotein cholesterol (sdLDL-C), since TG concentrations are markers of this subfraction.

Material and methods: We examined the possible influence of apo C-II and C-III plasma levels on sdLDL-C concentration in obese patients with metabolic syndrome (MetS). Plasma apo C-II and C-III were determined by an immunoturbidimetric assay. Obese subjects (n=73) with MetS but without any clinically evident cardiovascular disease were enrolled.

Results: TG, apo C-II and C-III plasma levels progressively increased when study participants were divided according to sdLDL-C tertiles ($P<0.001$ for all 3 trends). The apo C-III/C-II ratio was relatively constant (i.e. ≈2.5) for all tertiles of sdLDL-C. Stepwise multiple linear regression analyses showed that apo C-III levels independently correlated with TG levels, while TG and apo B levels were independently associated with sdLDL-C concentrations. Apo C-II and C-III significantly correlated with sdLDL-C in univariate analysis, but not in multivariate analysis.

Conclusions: Apo C-II and C-III levels are not independent predictors of sdLDL-C levels in obese subjects with MetS.

Key words: metabolic syndrome, apolipoprotein C-II, apolipoprotein C-III, small dense LDL, triglycerides, obesity.

Introduction

The human apolipoprotein (apo) Cs are constituents of chylomicrons, very low-density lipoproteins (VLDL) and high-density lipoproteins (HDL) [1]. Apo Cs influence lipoprotein lipase (LPL)-mediated lipolysis of triglyceride (TG)-rich lipoproteins [1].

Apo C-II is an activator of LPL and is required for efficient lipolysis of TG-rich lipoproteins in the circulation [1, 2]. The total absence of apo C-II or

defects in its structure severely hampers LPL-mediated lipolysis of TG-rich lipoproteins, resulting in markedly elevated levels of plasma TGs [3-5]. In contrast, excess apo C-II inhibits LPL-mediated hydrolysis of TGs [1]. Furthermore, lipid-lowering therapy (e.g. with rosuvastatin) can normalize elevated apo C-II levels [6].

Apo C-III is a powerful inhibitor of LPL activity [1, 7-9]. The amount of apo C-III is a modulator of plasma TG metabolism and may contribute to hypertriglyceridaemia in humans [10]. It has been reported that patients with metabolic syndrome (MetS) have high plasma apo C-III concentrations [11]. Furthermore, plasma apo C-III levels reduction has been reported with weight loss [12] or hypolipidaemic therapy with statins [6, 13-15] or fibrates [16-19].

Others as well as ourselves have reported that patients with MetS exhibit high levels of atherogenic small dense low-density lipoprotein (sdLDL) levels [20-22]. Moreover, we showed that TG concentration is a marker of sdLDL cholesterol (sdLDL-C) levels in human plasma [23]. The effect of apo Cs on TG-rich lipoprotein metabolism may influence the plasma levels of atherogenic sdLDL particles. In the present study we examined for the first time the possible influence of apo C-II and C-III plasma levels on sdLDL-C plasma concentration in obese patients with MetS.

Material and methods

Participants

Consecutive patients attending the Outpatient Obesity and Lipid Clinic of the University Hospital of Ioannina (Ioannina, Greece) were recruited. Eligible patients were those with MetS according to the National Cholesterol Educational Program Adult Treatment Panel III (NCEP ATP III) definition [24]. No participant had either symptomatic ischaemic heart disease or any other clinically evident vascular disease. Patients with impaired hepatic or renal function, type 2 diabetes mellitus (T2DM) and thyroid disorders were excluded from the study. Patients taking antihypertensive drugs on a stabilized dose for at least 8 weeks before entry to the study were considered eligible.

All participants gave their informed consent and the study protocol was approved by the institutional ethics committee.

Laboratory measurements

Lipid and carbohydrate metabolism parameters were determined as previously described [25, 26]. Apo A-I, apo B and apo E were measured with a Behring Nephelometer BN100, and reagents (antibodies and calibrators) from Dade Behring Holding GmbH (Liederbach, Germany) [27]. Apo C-II and C-III were determined by an immunoturbidimetric assay (Kamiya Biomedical Company, Seattle, U.S.A.) [28].

For all measurements in our laboratory, the coefficients of inter- and intra-assay variation were less than 5.0%, and blinded quality-control specimens were included in each assay. Analyses were conducted by the Clinical Chemistry Laboratory of the University Hospital of Ioannina, under regular quality control procedures including the use of reference pools and blinded duplicate samples.

The Clinical Chemistry Laboratory of the University Hospital of Ioannina participates in an External Quality Assurance Services (EQAS) programme provided by Bio-Rad Laboratories, Inc.

LDL subclass analysis

Electrophoresis was performed using a high resolution 3% polyacrylamide gel tube and the Lipoprint LDL System (Quantimetrix, Redondo Beach, CA) according to the manufacturer's instructions. Briefly, 25 μl of sample was mixed with 200 μl of Lipoprint Loading Gel and placed on the upper part of the 3% polyacrylamide gel. After 30 min of photopolymerization at room temperature, electrophoresis was performed for 60 min with 3 mA for each gel tube. Each electrophoresis chamber involved 2 quality controls (sample provided by the manufacturer). For quantification, scanning was performed with a ScanMaker 8700 digital scanner (Mikrotek Co, USA) and iMac personal computer (Apple Computer Inc, USA). After scanning, electrophoretic mobility (Rf) and the area under the curve (AUC) were calculated qualitatively and quantitatively with the Lipoprint LDL System Template and the Lipoware software (Quantimetrix Co, Redondo Beach, CA), respectively. LDL subfractions were estimated by the Rf between the very low-density lipoprotein (VLDL) fraction (Rf 0.0) and the HDL fraction (Rf 1.0). LDL is distributed from Rf 0.32 to Rf 0.64 as 7 bands, whose Rfs are 0.32, 0.38, 0.45, 0.51, 0.56, 0.60 and 0.64 (LDL1 to LDL7, respectively). LDL1 and LDL2 are defined as large, buoyant LDL, while LDL3 up to LDL7 are defined as sdLDL. The cholesterol concentration of each LDL subfraction is determined by multiplying the AUC of each subfraction by the total cholesterol (TC) concentration of the sample.

There is substantial heterogeneity among the methodologies currently used for the analysis of LDL subfractions [29]. So far, there is no standardization programme for methodologies used for the subfractionation of apo B-containing lipoproteins. However, the LDL subfraction analysis obtained by the Lipoprint system is well associated with LDL particle size determined by nuclear magnetic resonance spectroscopy ($P<0.0001$) [30].

Statistical analysis

Values are given as mean ± standard deviation (SD) and median (range) for parametric and non-parametric data, respectively. Continuous variables were tested for lack of normality by the Kolmogorov-Smirnov test and logarithmic transformations were accordingly performed. The Kruskal-Wallis test was used to assess the trend of variables divided according to sdLDL-C tertiles. Spearman's correlation coefficients were used to describe the relationship of TG and sdLDL-C levels with age, waist circumference, body mass index [BMI, calculated by dividing weight (kg) by height squared (m^2)], homeostasis model assessment (HOMA) index, and lipid and apo levels (univariate analysis). Stepwise multivariate linear regression analyses were performed to assess the independent contribution of the variables that significantly associated with TG and sdLDL-C levels in the univariate analysis. Significance was defined as $P<0.05$. All analyses were carried out using SPSS 15.0 (SPSS Inc, Chicago, Ill).

Results

We enrolled 73 patients (19 men and 54 women, mean age 51±10 years). The demographic, clinical and laboratory characteristics of the study population are shown in Table I.

TG, apo C-II and apo C-III plasma levels progressively increased when divided according to sdLDL-C tertiles ($P<0.001$ for all trends, Table II). Interestingly, the apo C-III/C-II ratio was relatively constant (i.e. ≈2.5) for every tertile of sdLDL-C (Table II).

In univariate analysis TG levels were significantly associated with TC, apo B, apo E, apo C-II and apo C-III levels, while sdLDL-C levels were significantly correlated with TC, TG, LDL-C, apo B, apo E, apo C-II and apo C-III concentrations (Table III). We next performed stepwise multiple linear regression analyses to assess the independent contributions of parameters which significantly correlated with TG or sdLDL-C levels in univariate analysis. Apo C-III levels were independently correlated with TG levels (Table IV), while TG and apo B levels were independently associated with sdLDL-C concentrations (Table V).

Table I. Baseline demographic, clinical and laboratory characteristics of study participants

N (females/males)	73 (54/19)
Age [years]	51±10
Body weight [kg]	93±18
BMI [kg/m^2]	35±6
Waist circumference [cm]	117±12
Systolic BP [mm Hg]	139±12
Diastolic BP [mm Hg]	89±8
Glucose [mg/dl]	104±13
Insulin [μU/ml]	14 (5-60)
HOMA index	3.5 (1.1-18.2)
TC [mg/dl]	247±51
TG [mg/dl]	188 (86-376)
HDL-C [mg/dl]	51±11
LDL-C [mg/dl]	156±41
Apo A-I [mg/dl]	128±25
Apo B [mg/dl]	108±20
Apo E [mg/dl]	42±13
Apo C-II [mg/dl]	5.3 (2.3-11.1)
Apo C-III [mg/dl]	14.0 (5.6-28.4)
sdLDL-C [mg/dl]	15 (0-61)
Mean LDL particle diameter [nm]	26.4 (24.5-27.7)

Values are expressed as mean ± SD, except for insulin, HOMA index, TG, apo C-II, apo C-III, sdLDL-C and mean particle diameter, which are expressed as median (range).
BMI – body mass index, HOMA – homeostasis model assessment, BP – blood pressure, TC – total cholesterol, TG – triglycerides, HDL-C – high-density lipoprotein cholesterol, LDL-C – low-density lipoprotein cholesterol, Apo – apolipoprotein, sdLDL-C – small dense LDL-C.
To convert values for glucose to mmol/l, multiply by 0.05551. To convert values for insulin to pmol/l, multiply by 6.945. To convert TC, LDL-C, HDL-C and sdLDL-C from mg/dl to mmol/l, multiply by 0.02586.
To convert TG levels to mmol/l, multiply by 0.01129

Discussion

Plasma apo C-III is synthesized by the liver and intestine and its physiological plasma concentration is approximately 12 mg/dl [1]. Apo C-III is a major component of TG-rich lipoproteins and HDL. The majority of apo C-III is associated with TG-rich

Table II. TG, apo C-II and apo C-III plasma levels divided according to sdLDL-C tertiles

sdLDL-C tertiles	TG [mg/dl]	Apo C-II [mg/dl]	Apo C-III [mg/dl]	Apo C-III/C-II ratio
1	160 (86-330)	4.4 (2.3-8.6)	11.1 (6.7-19.6)	2.56 (1.8-3.7)
2	195 (88-279)	5.3 (2.7-8.1)	14.0 (5.6-20.3)	2.50 (1.9-3.6)
3	241 (150-376)	6.7 (3.5-11.1)	15.8 (10.2-28.4)	2.49 (1.9-3.6)
P for trend	<0.001	<0.001	<0.001	0.82

Values are expressed as median (range).
sdLDL-C – small dense low-density lipoprotein cholesterol, TG – triglycerides, Apo – apolipoprotein
To convert TG levels to mmol/l, multiply by 0.01129

Table III. Spearman's correlation coefficients for TG, sdLDL-C, apo C-II and apo C-III levels

	TG	sdLDL-C	Apo C-II	Apo C-III
Age	0.14	0.12	0.01	0.02
BMI	–0.09	–0.19	–0.19	–0.16
Waist circumference	0.13	0.25	–0.12	–0.14
SBP	0.05	0.05	–0.20	–0.09
HOMA index	0.18	0.06	0.10	0.02
TC	0.37**	0.41**	0.29*	0.25*
TG	–	0.55**	0.70**	0.85**
HDL-C	–0.05	–0.02	0.05	0.07
LDL-C	0.08	0.32**	0.14	0.08
sdLDL-C	0.55**	–	0.45**	0.47**
Apo A-I	0.12	0.13	0.09	0.26*
Apo B	0.35**	0.47**	0.14*	0.24*
Apo E	0.46**	0.46**	0.40**	0.52**
Apo C-II	0.70**	0.45**	–	0.85**
Apo C-III	0.87**	0.52**	0.85**	–

TG – triglycerides, sdLDL-C – small dense low-density lipoprotein cholesterol, BMI – body mass index, SBP – systolic blood pressure, HOMA – homeostasis model assessment, TC – total cholesterol, HDL-C – high-density lipoprotein cholesterol, LDL-C – low-density lipoprotein cholesterol, Apo – apolipoprotein
**P<0.05, **P<0.01*

Table IV. Multivariate regression analysis for the prediction of TG levels*

Parameter	*Beta*	R^2 (%)	P
Apo C-III	0.88	76.8	<0.001

Beta is the standardized regression coefficient, R^2 is the proportion explained by a given independent variable.
**Only significant correlations are shown. Variables included in the model are those which were significantly correlated with TG in univariate analysis.*
TG – triglycerides, Apo C-III – apolipoprotein C-III

Table V. Multivariate regression analysis for the prediction of sdLDL-C levels*

Parameter	*Beta*	R^2 [%]	P
TG	0.40	25.4	<0.001
Apo B	0.35	10.8	0.001

Beta is the standardized regression coefficient, R^2 is the proportion explained by a given independent variable.
**R^2 for the model = 36.2%. Only significant correlations are shown. Variables included in the model are those which were significantly correlated with sdLDL-C in univariate analysis.*
sdLDL-C – small dense low-density lipoprotein cholesterol,
TG – triglycerides, Apo B – apolipoprotein B

lipoproteins in hypertriglyceridaemic subjects [31]. Apo C-III inhibits the hydrolysis of TGs by LPL [32-35]. A positive correlation has been observed between plasma apo C-III levels and plasma TG levels [36, 37]. Moreover, hepatic VLDL apo C-III production is greater in subjects with impaired insulin sensitivity [37, 38], such as our patients. This evidence is in agreement with our multivariate analysis showing that apo C-III is independently correlated with TG levels in our population.

MetS incorporates insulin resistance, which leads to overproduction and hypersecretion of apo B containing VLDL by the liver [39]. Once in the bloodstream, the TG in the core of the VLDL exchanges for cholesteryl esters (CE) in the core of LDL by means of the cholesterol ester transport protein (CETP), producing CE-depleted LDL. As the TG in the core of LDL is hydrolyzed by hepatic lipase, sdLDL is produced. These events, in concert, produce a dyslipidaemia characterized by increased plasma TG levels and an increased number of sdLDL particles [20]. TG levels remain predictive of the risk of vascular events even when LDL-C levels are markedly reduced [40, 41]. The results of multivariate analysis in our study are in accordance with the above findings, since sdLDL-C levels in obese patients with MetS were independently and positively associated with TG levels, as well as with baseline levels of apo B (which correspond to the number of LDL particles).

It has been reported that the levels of apo C-II and C-III were potent predictors of CVD risk in patients with T2DM [42, 43] and patients with coronary heart disease [44-46]. In a prospective, nested case-control study in the Cholesterol and Recurrent Events (CARE) trial (a randomized

placebo-controlled trial of pravastatin in 4159 patients with myocardial infarction and average LDL-C concentrations at baseline), apo C-III in VLDL+LDL particles was one of the independent predictors of coronary events, even when plasma TG levels were entered into the model [47]. Furthermore, in a cohort of type 1 diabetic patients increased apo C-III levels were associated with increases in the circulating levels of small LDL particles, defined by NMR lipoprotein subclass analyses [48]. Additionally, in patients with MetS and T2DM, sdLDL particles are enriched with apo C-III [49]. In the present study apo C-II and C-III concentration, as well as TG levels, progressively increased when divided according to sdLDL-C tertiles. The parallel increase of apo Cs with TG and sdLDL-C levels may imply that there is a genetic factor that influences TG and consequently sdLDL-C concentrations. We were surprised that the apo C-III/C-II ratio remained relatively constant for all tertiles of sdLDL-C in our MetS population. This deserves confirmation in a larger study.

Contrary to the above findings, in the present study we report that apo C-II and C-III are not independently associated with sdLDL-C levels. It seems that the effect of apo Cs is abolished when TG levels are entered into a model explaining sdLDL-C levels. Furthermore, evidence exists that apo C-II and C-III are atherogenic via non-sdLDL-C pathways. Excessive apo C-II and C-III may contribute to the atherogenicity of TG-rich particles, since increased apo C-II inhibits LPL-mediated hydrolysis of TGs [1], while apo C-III delays the lipolysis of these lipoproteins [50] and impairs their hepatic uptake, possibly by interfering with the interaction of apo E in lipoproteins with LDL receptors [7, 51]. Inhibition of rapid clearance of TG-rich lipoproteins by the liver may be atherogenic if it results in their enhanced uptake by low-affinity, high-capacity pathways in cells involved in atherosclerosis. Moreover, disturbances in TG-rich lipoprotein metabolism alter HDL metabolism. The formation of TG-enriched HDL, generated by increased neutral lipid exchange with TG-rich VLDL, accelerates the catabolism of HDL particles [52]. The accumulation of apo C-II and C-III in plasma by inhibiting the catabolism of TG-rich particles may favour the formation of unstable TG-rich HDL, thereby increasing the catabolic rate of HDL and CVD risk. Recent studies also showed an effect of apo C-III on vascular and inflammatory parameters. Kawakami et al. [53, 54] demonstrated that apo C-III increases the expression of vascular cell adhesion molecule-1 protein and intercellular cell adhesion molecule-1 protein in vascular endothelial cells, leading to subsequent recruitment of circulating monocytes. Apo C-III also activates nuclear factor-κB, a regulator for inflammation in atherogenesis [55], suggesting that apo C-III may stimulate diverse inflammatory responses through monocyte activation.

In conclusion, we report that apo C-III is the best marker of TG levels in obese subjects with MetS, while TG and apo B levels independently contribute to sdLDL-C concentrations. Apo C-II and C-III concentration, as well as TG levels, progressively increased when divided according to sdLDL-C tertiles. The parallel increase of apo Cs with TG and sdLDL-C levels may imply that there is a genetic factor that influences TG and consequently sdLDL-C concentrations. In multivariate analysis, apo C-II and C-III levels were not independent predictors of sdLDL-C levels in obese subjects with MetS. The effect of apo Cs is abolished when TG levels are entered into a model explaining sdLDL-C levels. The possible atherogenic role of apo Cs may represent a potential therapeutic target, but larger studies are needed to clarify the participation of these apos in the atherosclerotic process.

References

1. Jong MC, Hofker MH, Havekes LM. Role of ApoCs in lipoprotein metabolism: functional differences between ApoC1, ApoC2, and ApoC3. Arterioscler Thromb Vasc Biol 1999; 19: 472-84.
2. Wang CS. Structure and functional properties of apolipoprotein C-II. Prog Lipid Res 1991; 30: 253-8.
3. Cox DW, Breckenridge WC, Little JA. Inheritance of apolipoprotein C-II deficiency with hypertriglyceridemia and pancreatitis. N Engl J Med 1978; 299: 1421-4.
4. Breckenridge WC, Little JA, Steiner G, Chow A, Poapst M. Hypertriglyceridemia associated with deficiency of apolipoprotein C-II. N Engl J Med 1978; 298: 1265-73.
5. Fojo SS, Brewer HB. Hypertriglyceridaemia due to genetic defects in lipoprotein lipase and apolipoprotein C-II. J Intern Med 1992; 231: 669-77.
6. Kostapanos MS, Milionis HJ, Filippatos TD, et al. A 12-week, prospective, open-label analysis of the effect of rosuvastatin on triglyceride-rich lipoprotein metabolism in patients with primary dyslipidemia. Clin Ther 2007; 29: 1403-14.
7. Chan DC, Chen MM, Ooi EM, Watts GF. An ABC of apolipoprotein C-III: a clinically useful new cardiovascular risk factor? Int J Clin Pract 2008; 62: 799-809.
8. Schonfeld G, George PK, Miller J, Reilly P, Witztum J. Apolipoprotein C-II and C-III levels in hyperlipoproteinemia. Metabolism 1979; 28: 1001-10.
9. McConathy WJ, Gesquiere JC, Bass H, Tartar A, Fruchart JC, Wang CS. Inhibition of lipoprotein lipase activity by synthetic peptides of apolipoprotein C-III. J Lipid Res 1992; 33: 995-1003.
10. Ginsberg HN, Le NA, Goldberg IJ, et al. Apolipoprotein B metabolism in subjects with deficiency of apolipoproteins CIII and AI. Evidence that apolipoprotein CIII inhibits catabolism of triglyceride-rich lipoproteins by lipoprotein lipase in vivo. J Clin Invest 1986; 78: 1287-95.
11. Olivieri O, Bassi A, Stranieri C, et al. Apolipoprotein C-III, metabolic syndrome, and risk of coronary artery disease. J Lipid Res 2003; 44: 2374-81.
12. Fernandez ML, Metghalchi S, Vega-López S, Conde-Knape K, Lohman TG, Cordero-Macintyre ZR. Beneficial effects of weight loss on plasma apolipoproteins in postmenopausal women. J Nutr Biochem 2004; 15: 717-21.
13. Schoonjans K, Peinado-Onsurbe J, Fruchart JC, Tailleux A, Fievet C, Auwerx J. 3-Hydroxy-3-methylglutaryl CoA

reductase inhibitors reduce serum triglyceride levels through modulation of apolipoprotein C-III and lipoprotein lipase. FEBS Lett 1999; 452: 160-4.
14. Dallinga-Thie GM, Berk-Planken II, Bootsma AH, Jansen H; Diabetes Atorvastatin Lipid intervention (DALI) Study Group. Atorvastatin decreases apolipoprotein C-III in apolipoprotein B-containing lipoprotein and HDL in type 2 diabetes: a potential mechanism to lower plasma triglycerides. Diabetes Care 2004; 27: 1358-64.
15. Hunninghake DB, Stein EA, Bays HE, et al. Rosuvastatin improves the atherogenic and atheroprotective lipid profiles in patients with hypertriglyceridemia. Coron Artery Dis 2004; 15: 115-23.
16. Turay J, Grniakova V, Valka J. Changes in paraoxonase and apolipoprotein A-I, B, C-III and E in subjects with combined familiar hyperlipoproteinemia treated with ciprofibrate. Drugs Exp Clin Res 2000; 26: 83-8.
17. Saku K, Sasaki J, Arakawa K. Effects of slow-release bezafibrate on serum lipids, lipoproteins, apolipoproteins, and postheparin lipolytic activities in patients with type IV and type V hypertriglyceridemia. Clin Ther 1989; 11: 331-40.
18. Davidson MH, Bays HE, Stein E, Maki KC, Shalwitz RA, Doyle R; TRIMS Investigators. Effects of fenofibrate on atherogenic dyslipidemia in hypertriglyceridemic subjects. Clin Cardiol 2006; 29: 268-73.
19. Lemieux I, Salomon H, Després JP. Contribution of apo CIII reduction to the greater effect of 12-week micronized fenofibrate than atorvastatin therapy on triglyceride levels and LDL size in dyslipidemic patients. Ann Med 2003; 35: 442-8.
20. Gazi I, Tsimihodimos V, Filippatos T, Bairaktari E, Tselepis AD, Elisaf M. Concentration and relative distribution of low-density lipoprotein subfractions in patients with metabolic syndrome defined according to the National Cholesterol Education Program criteria. Metabolism 2006; 55: 885-91.
21. 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 Ther 2007; 7: 53-72.
22. Otvos JD, Collins D, Freedman DS, et al. Low-density lipoprotein and high-density lipoprotein particle subclasses predict coronary events and are favorably changed by gemfibrozil therapy in the Veterans Affairs High-Density Lipoprotein Intervention Trial. Circulation 2006; 113: 1556-63.
23. Gazi I, Lourida ES, Filippatos T, Tsimihodimos V, Elisaf M, Tselepis AD. Lipoprotein-associated phospholipase A2 activity is a marker of small, dense LDL particles in human plasma. Clin Chem 2005; 51: 2264-73.
24. 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: 3143-421.
25. Filippatos TD, Kiortsis DN, Liberopoulos EN, Georgoula M, Mikhailidis DP, Elisaf MS. Effect of orlistat, micronised fenofibrate and their combination on metabolic parameters in overweight and obese patients with the metabolic syndrome: the FenOrli study. Curr Med Res Opin 2005; 21: 1997-2006.
26. Filippatos TD, Gazi IF, Liberopoulos EN, et al. The effect of orlistat and fenofibrate, alone or in combination, on small dense LDL and lipoprotein-associated phospholipase A2 in obese patients with metabolic syndrome. Atherosclerosis 2007; 193: 428-37.
27. Steinmetz J, Tarallo P, Fournier B, Caces E, Siest G. Reference limits of apolipoprotein A-I and apolipoprotein B using an IFCC standardized immunonephelometric method. Eur J Clin Chem Clin Biochem 1995; 33: 337-42.
28. Sakurabayashi I, Saito Y, Kita T, Matsuzawa Y, Goto Y. Reference intervals for serum apolipoproteins A-I, A-II, B, C-II, C-III, and E in healthy Japanese determined with a commercial immunoturbidimetric assay and effects of sex, age, smoking, drinking, and Lp (a) level. Clin Chim Acta 2001; 312: 87-95.
29. Ensign W, Hill N, Heward CB. Disparate LDL phenotypic classification among 4 different methods assessing LDL particle characteristics. Clin Chem 2006; 52: 1722-7.
30. Hoefner DM, Hodel SD, O'Brien JF, et al. Development of a rapid, quantitative method for LDL subfractionation with use of the Quantimetrix Lipoprint LDL System. Clin Chem 2001; 47: 266-74.
31. Fredenrich A, Giroux LM, Tremblay M, Krimbou L, Davignon J, Cohn JS. Plasma lipoprotein distribution of apoC-III in normolipidemic and hypertriglyceridemic subjects: comparison of the apoC-III to apoE ratio in different lipoprotein fractions. J Lipid Res 1997; 38: 1421-32.
32. Jong MC, Rensen PC, Dahlmans VE, van der Boom H, van Berkel TJ, Havekes LM. Apolipoprotein C-III deficiency accelerates triglyceride hydrolysis by lipoprotein lipase in wild-type and apoE knockout mice. J Lipid Res 2001; 42: 1578-85.
33. Schaap FG, Nierman MC, Berbee JF, et al. Evidence for a complex relationship between apoA-V and apoC-III in patients with severe hypertriglyceridemia. J Lipid Res 2006; 47: 2333-9.
34. Ito Y, Azrolan N, O'Connell A, Walsh A, Breslow JL. Hypertriglyceridemia as a result of human apo CIII gene expression in transgenic mice. Science 1990; 249: 790-3.
35. Maeda N, Li H, Lee D, Oliver P, Quarfordt SH, Osada J. Targeted disruption of the apolipoprotein C-III gene in mice results in hypotriglyceridemia and protection from postprandial hypertriglyceridemia. J Biol Chem 1994; 269: 23610-6.
36. Chan DC, Watts GF, Nguyen MN, Barrett PH. Apolipoproteins C-III and A-V as predictors of very-low-density lipoprotein triglyceride and apolipoprotein B-100 kinetics. Arterioscler Thromb Vasc Biol 2006; 26: 590-6.
37. Cohn JS, Tremblay M, Batal R, et al. Increased apoC-III production is a characteristic feature of patients with hypertriglyceridemia. Atherosclerosis 2004; 177: 137-45.
38. Cohn JS, Patterson BW, Uffelman KD, Davignon J, Steiner G. Rate of production of plasma and very-low-density lipoprotein (VLDL) apolipoprotein C-III is strongly related to the concentration and level of production of VLDL triglyceride in male subjects with different body weights and levels of insulin sensitivity. J Clin Endocrinol Metab 2004; 89: 3949-55.
39. Adeli K, Taghibiglou C, Van Iderstine SC, Lewis GF. Mechanisms of hepatic very low-density lipoprotein overproduction in insulin resistance. Trends Cardiovasc Med 2001; 11: 170-6.
40. Athyros VG, Kakafika AI, Papageorgiou AA, et al.; GREACE Study Collaborative Group. Atorvastatin decreases triacylglycerol-associated risk of vascular events in coronary heart disease patients. Lipids 2007; 42: 999-1009.
41. Miller M, Cannon CP, Murphy SA, Qin J, Ray KK, Braunwald E; PROVE IT-TIMI 22 Investigators. Impact of triglyceride levels beyond low-density lipoprotein cholesterol after acute coronary syndrome in the PROVE IT-TIMI 22 trial. J Am Coll Cardiol 2008; 51: 724-30.
42. Gervaise N, Garrigue MA, Lasfargues G, Lecomte P. Triglycerides, apo C3 and Lp B: C3 and cardiovascular risk in type II diabetes. Diabetologia 2000; 43: 703-8.

43. Lee SJ, Campos H, Moye LA, Sacks FM. LDL containing apolipoprotein CIII is an independent risk factor for coronary events in diabetic patients. Arterioscler Thromb Vasc Biol 2003; 23: 853-8.
44. Gerber Y, Goldbourt U, Segev S, Harats D. Indices related to apo CII and CIII serum concentrations and coronary heart disease: a case-control study. Prev Med 2003; 37: 18-22.
45. Alaupovic P, Fesmire JD, Hunnighake D, et al. The effect of aggressive and moderate lowering of LDL-cholesterol and low dose anticoagulation on plasma lipids, apolipoproteins and lipoprotein families in post coronary artery bypass graft trial. Atherosclerosis 1999; 146: 369-79.
46. Luc G, Fievet C, Arveiler D, et al. Apolipoproteins C-III and E in apoB- and non-apoB-containing lipoproteins in two populations at contrasting risk for myocardial infarction: the ECTIM study. Etude Cas Temoins sur 'Infarctus du Myocarde. J Lipid Res 1996; 37: 508-17.
47. Sacks FM, Alaupovic P, Moye LA, et al. VLDL, apolipoproteins B, CIII, and E, and risk of recurrent coronary events in the Cholesterol and Recurrent Events (CARE) trial. Circulation 2000; 102: 1886-92.
48. Klein RL, McHenry MB, Lok KH, et al.; DCCT/EDIC Research Group. Apolipoprotein C-III protein concentrations and gene polymorphisms in type 1 diabetes: associations with lipoprotein subclasses. Metabolism 2004; 53: 1296-304.
49. Davidsson P, Hulthe J, Fagerberg B, et al. A proteomic study of the apolipoproteins in LDL subclasses in patients with the metabolic syndrome and type 2 diabetes. J Lipid Res 2005; 46: 1999-2006.
50. Ebara T, Ramakrishnan R, Steiner G, Shachter NS. Chylomicronemia due to apolipoprotein CIII overexpression in apolipoprotein E-null mice. Apolipoprotein CIII-induced hypertriglyceridemia is not mediated by effects on apolipoprotein E. J Clin Invest 1997; 99: 2672-81.
51. Aalto-Setälä K, Fisher EA, Chen X, et al. Mechanism of hypertriglyceridemia in human apolipoprotein (apo) CIII transgenic mice. Diminished very low density lipoprotein fractional catabolic rate associated with increased apo CIII and reduced apo E on the particles. J Clin Invest 1992; 90: 1889-900.
52. Ascaso J, Gonzalez Santos P, Hernandez Mijares A, et al. Management of dyslipidemia in the metabolic syndrome: recommendations of the Spanish HDL-Forum. Am J Cardiovasc Drugs 2007; 7: 39-58.
53. Kawakami A, Aikawa M, Libby P, Alcaide P, Luscinskas FW, Sacks FM. Apolipoprotein CIII in apolipoprotein B lipoproteins enhances the adhesion of human monocytic cells to endothelial cells. Circulation 2006; 113: 691-700.
54. Kawakami A, Aikawa M, Alcaide P, Luscinskas FW, Libby P, Sacks FM. Apolipoprotein CIII induces expression of vascular cell adhesion molecule-1 in vascular endothelial cells and increases adhesion of monocytic cells. Circulation 2006; 114: 681-7.
55. Kawakami A, Aikawa M, Nitta N, Yoshida M, Libby P, Sacks FM. Apolipoprotein CIII-induced THP-1 cell adhesion to endothelial cells involves pertussis toxin-sensitive G protein- and protein kinase C alpha-mediated nuclear factor-kappaB activation. Arterioscler Thromb Vasc Biol 2007; 27: 219-25.