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

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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

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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 concentrations 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 Lipoprint 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 [m²]), 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).

**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
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

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

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

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Beta</th>
<th>R² (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apo C-III</td>
<td>0.88</td>
<td>76.8</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Beta is the standardized regression coefficient, R² 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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Beta</th>
<th>R² (%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>TG</td>
<td>0.40</td>
<td>25.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Apo B</td>
<td>0.35</td>
<td>10.8</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Beta is the standardized regression coefficient, R² is the proportion explained by a given independent variable. *R² 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
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 apolipoproteins in the atherosclerotic process.

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Plasma apoC-II and apoC-III and small dense LDL


