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Apolipoprotein B, oxidized low-density lipoprotein, and LDL particle size in predicting the incidence of metabolic syndrome: the Cardiovascular Risk in Young Finns study

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Abstract

Objective: To test whether serum apolipoprotein B (apoB) and low-density lipoprotein (LDL) particle characteristics (oxidation and mean particle size) predict the incidence of metabolic syndrome (MetS).

Methods: The 6-year follow-up study included 1429 adults (baseline mean age 31.5). Lipids, apoB, and apoA1 were measured at baseline in 2001. LDL oxidation was measured with monoclonal antibody-based enzyme-linked immunosorbent assay (oxLDL-prot) and with a method measuring oxidized lipids in LDL (oxLDL-lipids). Mean LDL particle size was calculated from proton nuclear magnetic resonance spectroscopy data.

Results: Increased concentrations of both oxLDL-measures were associated with increased apoB levels but not with LDL particle size. The odds ratios (95% confidence intervals) for MetS incidence during a 6-year follow up by quartiles of apoB were 2.0 (1.0–3.8) for the second quartile, 3.1 (1.7–5.7) for the third quartile, and 4.2 (2.3–7.6) for the fourth quartile. This association remained after adjusting for age, sex, body mass index, homeostasis model assessment for insulin resistance, C-reactive protein, smoking, LDL cholesterol, oxidized LDL measures ($p \leq 0.01$) in addition to risk factors comprising the MetS ($p = 0.03$). OxLDL-prot and oxLDL-lipids levels were not independently associated with incident MetS after adjusting for apoB. Mean LDL particle size was not associated with the incidence of MetS.

Conclusion: ApoB is associated with increased risk of MetS incidence. We found no clear evidence to suggest that increased LDL oxidation or small mean LDL particle size would facilitate the development of MetS.

Keywords

Apolipoproteins, lipids, lipoproteins, metabolic syndrome, oxidative stress

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Introduction

Dyslipidaemia is a part of the metabolic syndrome (MetS) – a cluster of metabolic components that increase the risk of cardiovascular disease and type 2 diabetes.[1–3] Dyslipidaemia is often associated with obesity[4,5] and is characterized by elevations of triglycerides (TGs), apolipoprotein B (apoB), and small low-density lipoprotein (LDL) particles and by decreased levels of apolipoprotein A1 (apoA1) and high-density lipoprotein (HDL) cholesterol.[4,5] ApoB serves as a structural protein for cholesterol- and TG-containing lipoproteins that are carried from the liver to the site of use, whereas apoA1 containing HDL particles mediate the reverse cholesterol transport from the peripheral tissue to the liver.[4,5] Onat et al. demonstrated that apoB predicts the incidence of MetS.[6] However, data are lacking whether LDL characteristics i.e. oxidation and mean particle size mediate the predictive capacity of apoB on incident MetS.

Higher prevalence of small dense LDL particles (sdLDL) has been shown to associate with MetS.[7] The ratio of antioxidants to cholesterol content in sdLDL is smaller compared to larger buoyant LDL particles and therefore may become more susceptible to oxidation.[8] Sigurdardottir et al. found elevated levels of oxidized LDL (assessed by monoclonal antibody-based enzyme-linked immunosorbent assay) in subjects with MetS.[9] In addition, they found that elevated oxidized LDL levels in metabolic syndrome patients were associated with sdLDL particle size. However, the role of LDL particle size in the development of early metabolic derangement is not clear.[10,11] In the present study, we used proton nuclear magnetic resonance (NMR) spectroscopy to assess the mean LDL particle size.

Oxidized LDL (oxLDL) may also have a role in the development of MetS.[12,13] Previously, Maddux et al.[14] exposed cultured rat muscle cells to oxidative stress and found that the insulin stimulation of glucose transport was abolished, suggesting a link between insulin resistance and oxidative stress. Another functional study by Masella et al. reported that oxidative stress affected the balance between cultured adipose cell proliferation and differentiation.[15] They proposed causative role for oxidative stress in obesity and its clinical complications. Recently, a population-based study by Holvoet et al. found an association between oxLDL (assessed by monoclonal antibody-based enzyme-linked immunosorbent assay) and incident MetS independent of potentially confounding factors during a 5-year follow up.[16]

In the present study we used two different methods to evaluate oxidative lipoprotein modifications: (1) monoclonal antibody-based enzyme-linked immunosorbent assay to detect oxidized apoB in apoB-containing LDL-like particles in serum (oxLDL-prot) and (2) measurements of conjugated dienes in LDL-like particles isolated by precipitation with buffered heparin (oxLDL-lipids).[16,17]

Here we present results on the predictive value of apoB, LDL size, and LDL particle oxidation on the incidence of MetS and its components.

Methods

Subjects and study design

The Cardiovascular Risk in Young Finns study included 3596 randomly selected children and adolescents aged 3–18 years at original baseline in 1980. Total of 2283 subjects, aged 24–39 years, participated in the 21-year follow up in 2001 and 2204 subjects in the 27-year follow up in 2007. A total of 1828 subjects participated at both follow-up studies (315 subjects were lost from the follow up between 2001 and 2007). We have previously shown that the characteristics were similar as the baseline study in 1980.[18,19] Subjects who were pregnant or had missing covariates ($n = 127$), and subjects with baseline (2001) MetS ($n = 272$) were excluded. A total of 1429 subjects (621 males and 808 females) were included in the final analyses. Oral contraceptives were used by 229 women in the present study population. OxLDL-lipids data was available for 1367 subjects. Participants gave written informed consent and the study was approved by local ethics committees.

Serum lipid measurements and other biochemical assays

Venous blood samples were drawn after a 12-hour fast. Serum TGs, total cholesterol, HDL cholesterol, apoA1, and apoB were measured as described previously.[20] LDL cholesterol was calculated using the Friedewald formula[21] for subjects with triglycerides <4 mmol/l. Serum glucose was determined enzymically (glucose reagent; Olympus, Ireland) and high-sensitivity serum C-reactive protein (CRP) turbidimetrically (CRP-UL; Wako, USA) on an automated analyser (AU400; Olympus, Japan). Serum insulin concentration was determined by a microparticle enzyme immunoassay (IMx insulin reagent; Abbott Diagnostics, USA) on an IMx instrument (Abbott). Insulin resistance was estimated according to the homeostasis model assessment (HOMA-IR) as the product of fasting glucose and insulin divided by 22.5.[22] OxLDL-prot was quantitatively determined with a commercial enzyme-linked immunoassay (oxidized LDL ELISA kit; Mercodia, Sweden). This competitive ELISA method is based on

the mouse monoclonal antibody 4E6, which is directed against a conformational epitope in oxidized apoB-100 molecule in serum (marked oxLDL-prot in this work).[16] The inter assay coefficient of variation was 12%. Analysis of oxLDL-lipids was based on the determination of the baseline level of conjugated dienes in LDL lipids. The assay procedure consisted of isolation of the LDL fraction by precipitation with buffered heparin, extraction of lipoprotein lipids, and spectrophotometric analysis of conjugated dienes in the lipoprotein lipids at 234 nm. Validation studies for the assay have ruled out interference by nonspecific substances and shown that diene conjugation is a measure of oxidative LDL modification found in all LDL lipid classes. Coefficient of variation was 4.5%. Details of these methods are presented elsewhere.[17]

Mean LDL particle size

Proton NMR data on serum samples were measured at 37.0°C using a Bruker AVANCE III spectrometer operating at 500 MHz (Bruker BioSpin). The data were recorded using a standard pulse sequence with water suppression. The lipoprotein subclasses were calibrated according to high-performance liquid chromatography and the particle concentrations in intermediate-density lipoproteins (IDL; average particle diameter of 28.6 nm) as well as three LDL subclasses (25.5–18.7 nm) were quantified. The mean size for the LDL particles was calculated by weighting the IDL and LDL subclass diameters with their particle concentrations. Details on the NMR spectroscopy and data processing are published elsewhere.[23,24]

Clinical measurements and questionnaires

Height, weight, and waist circumference was measured. Body mass index (BMI) was calculated using the formula weight in kg/ height in metres squared. Blood pressure was measured using a random zero sphygmomanometer with the average of three measurements used in the analyses. Subjects were also asked to fill questionnaires that included questions about oral contraceptives, family history of premature coronary artery disease (positive if present before the age of 55 in first-degree relatives) and smoking habits (yes/no).[20]

Definitions of the metabolic syndrome

To classify MetS, we used the recent definition proposed in a joint statement of the International Diabetes Federation Task Force on Epidemiology and Prevention, the National Heart, Lung and Blood Institute, the American Heart Association, the World Heart Federation, the International Atherosclerosis Society, and the International Association for the Study of Obesity.[25] For waist circumference to define abdominal obesity we used cut-off points of $\geq$102 cm for men and $\geq$88 cm for women. Essentially identical results were seen when using lower cut-off points for waist circumference and other MetS definitions.[3,26]

Incident MetS ($n = 143$) was defined as individuals with prevalent MetS at follow up but not at baseline in 2001. Subjects in control group had no MetS at baseline or at follow up ($n = 1286$).

Statistical methods

The normality assumptions were assessed by examining histograms and normal probability plots. Values for plasma triglycerides, insulin, HOMA-IR, and CRP were $\log_e$ transformed and values for apoB were square-root transformed to correct for skewness. No interaction was observed between sexes in risk factors and incident MetS, indicating that the associations of risk markers and incident MetS were similar between sexes. Therefore, sexes were combined in all models. T-test was used to examine differences in clinical characteristics between control and incident MetS groups. Pearson's correlation coefficient adjusted for age and sex was calculated between oxLDL-prot and oxLDL-lipids.

Next, we stratified subjects into quartiles of apoB, oxLDL measures, and mean LDL particle size. Analysis of variance was used to assess linear trends in continuous oxLDL levels according to quartiles of apoB and oxLDL. Multivariable logistic regression was used to evaluate the association of the quartiles of apoB, oxLDL, and mean LDL particle size with incident MetS. Associations were tested using various models including adjustments for potential confounders: Model 2 was adjusted for age, sex, baseline BMI, HOMA-IR, CRP, smoking and models 3 and 4 further adjusted for oxLDL-prot, oxLDL-lipids, or apoB and finally adjusted for continuous risk factors comprising the MetS. To study the relations of continuous apoB, oxLDL and mean LDL particle size with incidence of MetS components, we performed multivariable logistic regression to assess the odds ratios for incidence of abdominal obesity, hypertension, hypertriglyceridaemia, low HDL cholesterol, and hyperglycaemia. BMI was omitted from the model assessing the incidence of abdominal obesity due to a high multicollinearity between outcome and dependent variable.

In the present study, 20 subjects were using antihypertensive and four subjects using lipid-lowering medication in 2001. We observed essentially similar results when subjects with medications were excluded from the analyses. Statistical analyses were performed with SAS

Table 1. Baseline (2001) characteristics

	Without metabolic syndrome ($n = 1286$)	With incident metabolic syndrome ($n = 143$)	p-value
Age (years)	31.5	33.0	0.0003
Sex (men)	42	52	0.02
Waist circumference (cm)	80 ± 10	90 ± 10	<0.0001
Body mass index (kg/m^2)	23.7 ± 3.4	27.0 ± 3.7	<0.0001
Systolic BP (mmHg)	113 ± 11	119 ± 12	<0.0001
Diastolic BP (mmHg)	69 ± 9	74 ± 10	<0.0001
Total cholesterol (mmol/l)	5.02 ± 0.88	5.29 ± 1.00	0.0008
LDL cholesterol (mmol/l)	3.19 ± 0.80	3.46 ± 0.91	0.0003
HDL cholesterol (mmol/l)	1.34 ± 0.29	1.22 ± 0.30	<0.0001
Apolipoprotein B (g/l)	0.99 (0.83–1.13)	1.11 (0.97–1.25)	<0.0001
oxLDL-prot (U/l)	78.6 ± 22.6	87.3 ± 22.9	<0.0001
oxLDL-lipids (μmol/l)	25.1 ± 7.9	27.4 ± 8.4	0.001
Mean LDL particle size (nm)	23.53 (0.15)	23.51 (0.16)	0.34
Apoprotein A-1 (g/l)	1.50 ± 0.24	1.44 ± 0.26	0.003
Triglycerides (mmol/l)	0.99 (0.80–1.30)	1.23 (1.00–1.70)	<0.0001
Glucose (mmol/l)	4.94 ± 0.50	5.15 ± 0.57	<0.0001
Insulin (mU/l)	5.6 (4.0–8.0)	7.2 (5.0–11.0)	<0.0001
HOMA-IR	1.2 (0.9–1.7)	1.7 (1.2–2.3)	<0.0001
CRP (mg/l)	0.65 (0.28–1.36)	0.91 (0.42–1.91)	0.03
Daily smoking	21	30	0.01
Family history of coronary artery disease	12	11	0.65

Values are mean ± SD, median (interquartile range) or %. BP, blood pressure; CRP, C-reactive protein; HOMA-IR, homeostasis model assessment; HDL, high-density lipoprotein; LDL, low-density lipoprotein; oxLDL-lipids, oxidized lipids in LDL; oxLDL-prot, oxidized apoB in LDL.

9.1 with statistical significance inferred as a two-tailed p-value ≤ 0.05.

Results

Clinical characteristics

In Table 1, characteristics at the baseline 2001 were compared between the subjects with incident MetS and controls (no MetS at baseline 2001 or at follow up at 2007). Subjects with incident MetS were older and had higher risk factor levels compared to controls at baseline except for mean LDL size and family history of coronary artery disease. The correlation coefficient between oxLDL-prot and oxLDL-lipids was $r = 0.46$ ($p < 0.0001$).

OxLDL concentrations with respect to apoB and mean LDL particle size

To assess whether oxLDL levels are dependent on apoB or LDL particle size, we plotted oxLDL concentrations according to apoB quartiles and LDL particle quartiles simultaneously. The results are shown in Figure 1 in the online appendix. The oxLDL and apoB levels had a positive correlation but the oxLDL concentrations were relatively stable for all LDL particle sizes.

ApoB, oxLDL, and mean LDL particle size in predicting incident MetS and its components

Next, we assessed the role of apoB, oxLDL, and mean LDL particle size in predicting MetS incidence in more detail. As shown in Tables 2 and 3, the incidence of MetS was associated with higher quartiles of apoB, oxLDL-prot, and oxLDL-lipids in models adjusted for age and sex (Model 1). ApoB remained significantly associated with incident MetS after further adjusting for baseline BMI, HOMA-IR, CRP, smoking, and oxLDL (Table 2, Models 2, 3, and 4). Similar results were obtained when BMI was replaced by waist circumference. In addition, the results for apoB and oxLDL measures remained similar when LDL cholesterol or mean LDL particle size was included into the models

Table 2. Apolipoprotein B predicting incidence of metabolic syndrome

apoB	*n*	Model 1	Model 2	Model 3	Model 4	Model 5
Quartile 1	352	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)
Quartile 2	350	2.0 (1.0–3.8)	1.7 (0.9–3.6)	1.6 (0.8–3.2)	1.7 (0.8–3.8)	1.5 (0.7–2.9)
Quartile 3	350	3.1 (1.7–5.7)	2.4 (1.3–4.6)	2.8 (1.3–4.6)	2.8 (1.4–5.9)	2.4 (1.2–4.8)
Quartile 4	377	4.2 (2.3–7.6)	2.8 (1.5–5.1)	2.0 (0.9–4.2)	3.0 (1.4–6.6)	2.5 (1.3–4.9)
p for trend		<0.0001	0.001	0.01	0.005	0.03

Values are odds ratio (95% confidence interval). Model 1: adjusted for age and sex. Model 2: model 1 + baseline body mass index, homeostasis model assessment, C-reactive protein, and smoking. Model 3: model 2 + oxidized low-density lipoprotein (oxLDL) protein. Model 4: model 2 + oxLDL lipids. Model 5: adjusted for waist circumference, systolic blood pressure, triglycerides, HDL cholesterol, and glucose.

Table 3. Oxidized apoB in low-density lipoprotein (LDL) and oxidized lipids in LDL predicting incidence of metabolic syndrome

	n	Model 1	Model 2	Model 3
Oxidized apoB in LDL				
Quartile 1	338	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)
Quartile 2	365	1.7 (1.0–3.2)	1.7 (0.9–3.2)	1.4 (0.7–2.8)
Quartile 3	355	2.1 (1.2–3.8)	1.8 (1.0–3.4)	1.3 (0.7–2.8)
Quartile 4	371	2.7 (1.5–4.6)	1.9 (1.0–3.4)	1.6 (0.7–3.7)
p for trend		<0.0001	0.03	0.46
Oxidized lipids in LDL				
Quartile 1	336	1.0 (Ref)	1.0 (Ref)	1.0 (Ref)
Quartile 2	342	1.2 (1.0–3.2)	1.1 (0.6–1.9)	0.9 (0.5–1.6)
Quartile 3	342	1.1 (1.2–3.8)	0.8 (0.4–1.5)	0.6 (0.3–1.1)
Quartile 4	347	2.2 (1.5–4.6)	1.6 (0.9–2.7)	1.5 (0.8–3.0)
p for trend		0.0004	0.10	0.70

Values are odds ratio (95% confidence interval). Model 1: adjusted for age and sex. Model 2: model 1 + baseline body mass index, homeostasis model assessment, C-reactive protein, and smoking. Model 3: Model 2 + apoB.

(data not shown). When the model was adjusted for the components of MetS as continuous variables (waist circumference, systolic blood pressure, TGs, HDL cholesterol, glucose), the association remained significant for apoB as shown in Table 2, Model 5. In contrast, the association between oxLDL-prot and incident MetS as well as between oxLDL-lipids and incident MetS was attenuated when apoB (Table 3, Model 3) or TG concentration (data not shown) was included into the model as a covariate. Mean LDL particle size was not associated with incident MetS (p always >0.68) in any of the models (data not shown). Results were similar when adjusting for oral contraceptives in women.

The results of continuous apoB and oxLDL-prot in predicting incidence of each dichotomous MetS component are shown in Table I (available in online appendix). The model included apoB, oxLDL-prot, age, sex, BMI, LDL cholesterol, HOMA-IR, CRP, and smoking as explanatory variables. Essentially similar results were observed for apoB when oxLDL-prot was replaced with oxLDL-lipids or mean LDL particle size (Tables II and III, available in online appendix). ApoB was significantly associated with 6-year incidence of central obesity, hypertension, triglyceridaemia, and low HDL. No significant independent associations for oxLDL-prot, oxLDL-lipids, or mean LDL particle size with incident MetS components during 6-year follow up were observed (Tables I–III, available in online appendix).

Discussion

The present study sought to give insight to dyslipidaemia in the development of MetS. OxLDL-prot and oxLDL-lipids were depending on the apoB concentration but not on the mean LDL particle size. ApoB

predicted incident MetS and was associated with the incidence of obesity, hypertension, triglyceridaemia, and low HDL cholesterol. No association between the oxLDL measures and incident MetS or the MetS components were observed after adjusting for apoB.

In the present study we demonstrated that subjects with incident MetS had elevated levels of total cholesterol, LDL cholesterol, TGs, apoB, and oxidized LDL as well as reduced levels of HDL cholesterol and apoA1 and smaller mean LDL particle size compared to controls. To our knowledge, this is the first population-based study demonstrating association between apoB and the incidence of MetS in young adults. This association remained independent of age, sex, BMI, HOMA-IR, CRP, smoking, LDL cholesterol, and oxLDL measures in addition to risk factors included in MetS. In line with these data, Onat et al. recently demonstrated that high levels of apoB predicts the incidence of MetS in 2348 middle-aged to elderly men and women.[6] However, their potential confounders were limited to markers of obesity and inflammation. We also found that apoB predicted the incidence of central obesity, hypertension, hypertriglyceridaemia, and low HDL. These data suggest that the prospective association of apoB with incident MetS and its components is likely related to increased levels of TG-rich, large apoB-containing particles, a well-known characteristic of MetS. In addition, apoB accounts for the number of oxidized IDL and LDL as well as TG-rich lipoproteins and therefore appears to give a better reflection of risk for MetS.[4,5] It is also possible that part of the risk associated with increased apoB levels is mediated by the heterogeneity of LDL particles. Previously, the cross-sectional Multi-Ethnic Study of Atherosclerosis reported substantial heterogeneity of LDL particle number with low or very low levels of LDL cholesterol in patients with type 2 diabetes.[27] In addition they showed that measuring LDL particle number was superior in predicting subclinical atherosclerosis compared to LDL cholesterol.[28] In the Framingham Heart Study, small LDL particle number was elevated in the presence of MetS and increased with the number of MetS components.[29] However, in the present longitudinal study, we found no evidence to suggest that mean LDL particle size would predict the development of MetS. Further, no clear evidence was found that higher LDL oxidation would relate to smaller LDL size. Instead, both oxLDL measures indicated increased LDL oxidation for higher concentrations of apoB. These data suggest that higher oxLDL levels may be due to increased production of apoB containing lipoprotein particles rather than a tendency of smaller LDL particles in the circulation.[16,30]

Previous studies have shown that high LDL oxidation is related to MetS.[13,16,31] Data from the Young Adult Longitudinal Trends in Antioxidants study have shown that mean HOMA-IR increased with increasing levels of oxLDL-prot independent of adiposity.[31] Further, Holvoet et al. recently found a significant association between oxLDL-prot and incident MetS during their 5-year follow up among 1889 men and women (age 33–45 years) independent of age, sex, LDL cholesterol, and BMI and thus suggested a causal role for LDL oxidation in the development of MetS.[16] In the present study, in addition to the enzyme-linked immunosorbent assay for oxLDL-prot, a diene conjugation-based assay for oxidized LDL lipids was also applied. We found that the associations between the oxLDL measures and incident MetS were diluted after adjusting for the apoB. The results were similar for oxLDL-prot and oxLDL-lipids. Similar results were obtained using all MetS definitions.[3,26] The discrepancy between our results and those of Holvoet et al. may be due to differences in study populations, measurement protocols, or statistical methods.[17] Most importantly, however, apoB was not included as potential confounder in the study of Holvoet et al.[16] Although *in vitro* studies and animal models have suggested that LDL oxidation may have an important role in the pathogenesis of obesity and insulin resistance,[14,15] the evidence in clinical studies have not been consistent.[32]

Due to the heterogeneous nature of the chemistry of LDL composition and oxidation, proper determination of oxLDL is problematic.[17] In the present study we used two methods to evaluate oxidative modification of LDL particles.[16,17] Our oxLDL-prot measure is based on the enzyme-linked immunosorbent assay with antibodies (mAb-4E6) directed against a conformational epitope in oxidized apoB-100 molecules in serum.[16] However, the specificity of the monoclonal antibodies against apoB epitopes in various apoB-containing lipoprotein particles is unclear. The apoB-lipoprotein metabolism is a continuum with no definite borderline between small very-low-density lipoprotein and IDL (and LDL) particles. Thus, antibodies against conformational epitopes in apoB are likely to recognize modified apoB in all similar apoB-containing lipoprotein particles. In fact, these TG-enriched apoB-containing lipoprotein particles have also been demonstrated to possess atherogenic effects similar to LDL particles.[33] Nevertheless, TG-rich large apoB-containing particles contribute generally only around 15% of total apoB in serum, the LDL and IDL particles containing the rest of circulating apoB.[24,34] Due to these inherent complexities in lipoprotein physiology and molecular detection, the results on LDL oxidation should be interpreted with caution and further studies on the specific role of apoB in various lipoprotein particles and oxidation are needed. A potential limitation to mention in this study is the possible loss of original

participants during the long-term follow up that may have caused bias due to differential loss to follow up. However, we have previously shown that the present study cohort is well representative of the original study population.[18,19] Because our study cohort had normal lipid characteristics, the generalizability of our results is limited to ambulatory white European subjects. Therefore the results may not be applicable to all populations with elevated apoB levels.[35] These include patients with familiar hypercholesterolaemia or with type II Frederickson hyperlipidaemia who may have high LDL cholesterol and LDL particle numbers in the absence of developing hypertriglyceridaemia and low HDL cholesterol.

Accumulating evidence is increasingly coming to light that apoB measurements are likely to be better than conventional lipid measures in assessing metabolic problems and risk for atherosclerosis. Our findings emphasize the role of apoB as an early metabolic risk marker and its importance in the prevention and treatment of dyslipidaemia characteristic for MetS in the population of apparently healthy young adults. We found no clear evidence to suggest that either LDL oxidation or mean LDL particle size would predict the development of MetS independently of apoB concentrations.

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Conflicts of interest

None declared.

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