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# Plasma apoE is Elevated in Metabolic Syndrome: Importance of Large Very Low Density and Low Density Lipoprotein Particles

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## Abstract

**Background:** Apolipoprotein E (apoE) is carried by all major lipoprotein classes in plasma and is likely to contribute to the development of atherosclerosis. We set out to determine the extent to which plasma apoE is related to various VLDL, LDL and HDL subfractions in subjects with and without metabolic syndrome (MetS).

**Methods:** Plasma lipids, lipoprotein subfractions (nuclear magnetic resonance spectroscopy) and plasma apoE were determined in 60 subjects with and 62 subjects without MetS (APOE ε2/ε2 carriers excluded).

**Results:** Plasma apoE was higher in MetS, coinciding with increased total and large VLDL particles, as well as total LDL particles ($p<0.01$ for each after age, sex and diabetes status adjustment). Age- and sex-adjusted multivariable linear regression analysis revealed that plasma apoE was related positively to the VLDL particle concentration ($p=0.003$), in particular large VLDL ($p<0.001$) and to the LDL particle concentration ($p=0.013$), independent of MetS and diabetes status ($p>0.30$). Plasma apoE was unrelated to HDL particle concentration ($p=0.88$).

**Conclusions:** Plasma apoE is elevated in MetS in conjunction with increased concentrations of (large) VLDL and LDL particles. These novel findings provide a rationale to explore whether preferential association of apoE with (large) VLDL and LDL could modify its influence on atherosclerosis development.

**Keywords:** Apolipoprotein E; Diabetes mellitus; Metabolic syndrome; Lipoprotein subfractions

## Abbreviations:

apoE: apolipoprotein E; apoA-I: apolipoprotein A-I; apoB: apolipoprotein B; CVD: Cardiovascular Disease; LDL: Low Density Lipoprotein; VLDL: Very Low Density Lipoprotein; HDL: High Density Lipoprotein; MetS: Metabolic Syndrome; NMR: Nuclear Magnetic Resonance; T2DM: Type 2 diabetes mellitus; BMI: Body Mass Index; HbA1c: Glycated Haemoglobin

## Introduction

Apolipoprotein E (apoE) is a multifunctional apolipoprotein that is synthesized by a number of tissues and cell types, including liver, adipose tissue and macrophages [1-3]. ApoE is a constituent of all major lipoprotein classes in plasma [4,5]. ApoE is critically involved in lipid homeostasis by facilitating receptor-mediated uptake of apolipoprotein B (apoB)-containing lipoproteins [1-3,6]. It is widely appreciated that apoE has anti-atherogenic properties, as evidenced by accelerated atherosclerosis development in apoE knock-out mice [1-3]. Besides multiple effects on lipoprotein metabolism, apoE also exerts anti-oxidative and anti-inflammatory properties [2,7-9].

Despite the pivotal role of apoE in lipoprotein metabolism, and the importance of genetic variations in APOE on the development of cardiovascular disease (CVD) [1-3,10], remarkably little is known about the association of plasma apoE levels with incident cardiovascular disease. The apoE content in subfractions of low density lipoproteins (LDL) and very low density lipoproteins (VLDL) was reported to be inversely associated with incident coronary heart disease [11]. On the other hand, in elderly subjects, as well as in a subset of women with increased plasma high density lipoprotein (HDL) cholesterol with concurrently high C-reactive protein levels, plasma apoE was found to be associated positively with incident CVD [12-15]. A high apoE content in HDL may also be associated with an increased risk of recurrent CVD [16].

The plasma apoE concentration is known to be elevated in subjects with the metabolic syndrome (MetS) and obesity [9,17,18]. In line, plasma apoE is strongly correlated with plasma triglycerides and triglyceride-rich lipoproteins levels [2,4,5,9,17]. Accordingly, it has been reported that in hypertriglyceridemia the distribution of apoE among lipoproteins is shifted from HDL towards triglyceride-rich remnant lipoproteins [4,5], although apoE enrichment of HDL was recently suggested in obesity [18]. Both VLDL, LDL and HDL particles are highly heterogeneous in size and composition [19,20]. In view of the potential importance of changes in lipoprotein subfraction distribution for cardiometabolic risk [21-24], and the possibility that the association of apoE with CVD could be dependent on its presence

on specific lipoprotein subfractions, it is relevant to discern the relationship of plasma apoE with various lipoprotein subfraction characteristics.

In the absence of data with respect to the relationship of plasma apoE with lipoprotein subfraction distribution, as determined by nuclear magnetic resonance (NMR) spectroscopy, we initiated the present study 1) to determine the extent to which plasma apoE is related to various lipoprotein subfractions, and 2) to assess whether such relationships are altered in subjects with MetS.

## Patients and Methods

### Participants

This study was performed in a university hospital setting. Participants (aged > 18 years) were Caucasian, and were recruited by advertisement in local newspapers. Subjects without and MetS, defined according to the revised NCEP-ATP III criteria [25], participated. Three or more of the following criteria were required for categorization of subjects with MetS: waist circumference > 102 cm for men and > 88 cm for women; hypertension (blood pressure ≥ 130⁄85 mmHg or use of anti-hypertensive drugs); fasting plasma triglycerides ≥ 1.70 mmol/L; HDL cholesterol<1.0 mmol/L for men and<1.30 mmol/L for women; fasting glucose ≥ 5.6 mmol/L. Subjects with type 2 diabetes mellitus (T2DM), previously diagnosed by primary care physicians using guidelines from the Dutch College of General Practitioners (fasting plasma glucose ≥ 7.0 mmol/L and/or non-fasting plasma glucose ≥11.1 mmol/L) were allowed to participate. Diabetic subjects who were treated with metformin and/or sulfonylurea were allowed to participate, but subjects using insulin were not eligible. The use of anti-hypertensive medication was allowed. Further exclusion criteria were clinically manifest CVD, renal insufficiency (elevated serum creatinine and⁄or proteinuria), thyroid disorders, liver disease, current smoking, pregnancy and use of lipid lowering drugs. Subjects who were homozygous for the APOE ε2 allele were also excluded. Physical examination did not reveal pulmonary or cardiac abnormalities. All subjects were studied after an overnight fast. Body mass index (BMI) was calculated as weight divided by height squared (in kg/m$^2$). Waist circumference was measured between the 10$^{th}$ rib and the iliac crest.

The medical ethics committee of the University Medical Center Groningen, The Netherlands approved the study. All participants provided written informed consent.

### Laboratory analyses

Venous blood samples were collected into EDTA-containing tubes (1.5 mg⁄mL) for the measurement of plasma lipids and apolipoproteins. Plasma was prepared by centrifugation at 1400 g for 15 min at 4°C. Blood glucose and glycated haemoglobin (HbA1c) levels were measured directly after blood collection. Samples for other assays were stored at -80°C until analysis.

Plasma total cholesterol and triglycerides were assayed by routine enzymatic methods (Roche/Hitachi cat nos 11875540 and 11876023, respectively; Roche Diagnostics GmbH, Mannheim, Germany). HDL cholesterol was measured with a homogeneous enzymatic colorimetric test (Roche/Hitachi, cat no 04713214; Roche Diagnostics GmbH, Mannheim, Germany). Non-HDL cholesterol was calculated as the difference between total cholesterol and HDL cholesterol. LDL cholesterol was calculated by the Friedewald formula in case of a plasma triglyceride concentration ≤ 4.5 mmol/L. ApoA-I and apoB were assayed by immunoturbidimetry (Roche/Cobas Integra Tina-quant catalog no. 03032566 and 033032574, respectively, Roche Diagnostics). ApoE was measured using an immunoturbidimetric assay (cat. no. 417-35906; Wako Inc., Osaka, Japan).

VLDL, LDL and HDL particle profiles were measured by NMR spectroscopy with the LipoProfile-3 algorithm, as described (LipoScience Inc., Raleigh, North Carolina, USA) [19]. Lipoprotein particle subclasses (expressed in concentration units, i.e. μmol/L or nmol/L) were quantified from the amplitudes of their spectroscopically distinct lipid methyl group NMR signals. Diameter range estimates were for VLDL: large VLDL (including chylomicrons if present >60 nm), medium VLDL (35 to 60 nm) and small VLDL (27 to 35 nm), for LDL: IDL (23 to 27 nm), large LDL (21.2 to 23 nm) and small LDL (18 to 21.2 nm), and for HDL: large HDL (9.4 to 14 nm), medium HDL (8.2 to 9.4 nm), small HDL (7.3-8.2 nm). The total VLDL, LDL and HDL particle concentrations were calculated as the sum of the concentrations of the VLDL, LDL and HDL subclasses, respectively. The lipoprotein particle concentrations are regarded to represent an estimate of the particle numbers [24].

Glucose was measured with an APEC glucose analyzer (APEC Inc., Danvers, MA). HbA1c was measured by high-performance liquid chromatography (Bio-Rad, Veenendaal, the Netherlands; normal range: 4.6–6.1%).

APOE genotyping was performed as follows. DNA was extracted from whole blood using the Qiampmini kit (Qiagen). APOE genotypes (rs429358 and rs7412) were determined by allelic discrimination on a CFX system (Bio Rad), using predesigned primers C-3084793-20 and C-904973-10 and Taqman Universal PCR mastermix (Applied Biosystems, Nieuwerkerka/dIJssel, the Netherlands). The method has been validated against a previously described restriction isotyping procedure [26,27].

### Statistical analysis

SPSS (version 20.0, SPSS Inc. Chicago, IL, USA) was used for data analysis. Results are expressed as mean ± SD or as median (interquartile range). Differences between subjects with and without MetS were determined by unpaired T tests and Chi-square tests where appropriate. Differences in variables between subjects with and without MetS were also determined after adjustment for age, sex and diabetes status. Because of skewed distribution, logarithmically transformed values of triglycerides and lipoprotein subfractions were used for regression analysis. Univariate relationships were determined using Pearson correlation coefficients. Multiple linear regression analyses were carried out to disclose variables which independently contributed to plasma apoE levels. Additionally, multiple linear regression analyses were performed to determine interactions between the presence of MetS or alternatively diabetes status and lipoprotein subfractions impacting on apoE. Interaction terms were calculated as the product terms between the variables of interest. To this end the distributions of the various lipoprotein subfractions were centered to their mean value by subtracting the individual value from their group mean to account for possible outliers. Interaction terms were considered to be statistically significant at two-sided p-values<0.10, as recommended by Selvin [28] and by the Food and Drug Administration authorities [29]. Otherwise, the level of significance was set at two-sided p-values<0.05.

## Results

Sixty subjects with and 62 subjects without MetS were included in the study. Their clinical characteristics are shown in Table 1. Twenty four subjects with MetS and 5 subjects without MetS used anti-hypertensive medication (mostly angiotensin-converting enzyme inhibitors, angiotensin II receptor antagonists and diuretics, either alone or in combination; p<0.001). T2DM was more frequent in subjects with MetS. Oral glucose lowering drugs, i.e. sulfonylurea and metformin, either alone or in combination, were used by 37 diabetic subjects with MetS and by 10 diabetic subjects without MetS (p<0.001). These medications were not used in non-diabetic subjects. Two postmenopausal women with MetS and 1 premenopausal woman used oral contraceptives. Age and sex distribution were not significantly different between subjects with and without MetS. Blood pressure, waist circumference, BMI, glucose and HbA1c levels were higher in MetS subjects; the difference in HbA1c disappeared after adjustment for age, sex and diabetes status (Table 1). The APOE genotype distribution was not significantly different between the groups (Table 1).

| | MetS (n=60) | No MetS (n=62) | p-value | p-value[a] |
|---|---|---|---|---|
| **Age (years)** | 58 ± 9 | 55 ± 10 | 0.07 | |
| **Gender (men/women)** | 32/28 | 32/30 | 0.99 | |
| **Diabetes (yes/no)** | 48/12 | 18/44 | <0.001 | |
| **Systolic blood pressure (mm Hg)** | 146 ± 19 | 131 ± 22 | <0.001 | <0.001 |
| **Diastolic blood pressure (mm Hg)** | 89 ± 9 | 81 ± 11 | <0.001 | <0.001 |
| **BMI (kg/m2)** | 30.1 ± 4.6 | 25.1 ± 3.5 | <0.001 | <0.001 |
| **Waist circumference (cm)** | 104 ± 13 | 86 ± 11 | <0.001 | <0.001 |
| **Glucose (mmol/L)** | 8.7 ± 2.5 | 6.3 ± 1.5 | <0.001 | 0.011 |
| **HbA1c (mmol/mol)** | 48 ± 9 | 39 ± 5 | <0.001 | 0.61 |
| **APOE genotype**<br>**ε2/ε3**<br>**ε3/ε3**<br>**ε3/ε4**<br>**ε4/ε4** | 4<br>38<br>14<br>4 | 2<br>49<br>11<br>0 | 0.091 | |
| **Total cholesterol (mmol/L)** | 5.54 ± 0.95 | 5.56 ± 0.096 | 0.92 | 0.26 |
| **LDL cholesterol (mmol/L)** | 3.37 ± 0.86 | 3.36 ± 0.83 | 0.97 | 0.33 |
| **Non-HDL cholesterol (mmol/L)** | 4.33 ± 0.95 | 3.98 ± 1.04 | 0.057 | 0.006 |
| **HDL cholesterol mmol/L)** | 1.21 ± 0.36 | 1.58 ± 0.39 | <0.001 | <0.001 |
| **Triglycerides (mmol/L)** | 1.95 (1.70-2.52) | 1.15 (0.85-1.59) | <0.001 | <0.001 |
| **ApoA-I (g/L)** | 1.32 ± 0.25 | 1.46 ± 0.20 | 0.001 | <0.001 |
| **ApoB (g/L)** | 0.98 ± 0.22 | 0.90 ± 0.23 | 0.037 | 0.007 |
| **ApoE (g/L)** | 0.042 ± 0.012 | 0.037 ± 0.009 | 0.008 | 0.006 |

Data in mean ± SD or in median (interquartile range). Apo: apolipoproteins; BMI: body mass index; HbA1c, glycated hemoglobin; HDL: high density lipoproteins; LDL: low density lipoproteins; non-HDL: non-high density lipoproteins. LDL cholesterol was calculated in 57 subjects with and in 60 subjects without MetS. [a]p-value: p-value after adjustment for age, sex and diabetes status.

**Table 1:** Clinical characteristics, plasma glucose, glycated hemoglobin (HbA1c), apolipoproten E (apoE) genotype, plasma lipids, apolipoproteins (apos) and lipoprotein subfractions in 60 subjects with and in 62 subjects without metabolic syndrome (MetS).

Plasma total cholesterol and LDL cholesterol were not different between the groups (Table 1). Taking account of age, sex and diabetes status, non-HDL cholesterol, triglycerides and apoB levels were higher, whereas HDL cholesterol and apoA-I were lower in MetS subjects (Table 1). Plasma apoE was higher in MetS (Table 1), but was not significantly different between subjects with and without T2DM (0.042 ± 0.012 g/L vs. 0.039 ± 0.009 g/L, p=0.68).

The VLDL particle concentration was higher in MetS, which was due to higher concentrations of large and medium VLDL particles (Table 2); the difference in small VLDL particles was significant after

adjustment for age, sex and T2DM). The LDL particle concentration was also higher in MetS, which was due to higher concentration of small LDL particles. The HDL particle concentration was not different between subjects with and without MetS, but the HDL subfraction distribution was shifted towards less large HDL particles and more small HDL particles in MetS (Table 2).

| | MetS (n=60) | No MetS (n=62) | p-value | p-value[a] |
|---|---|---|---|---|
| VLDL particle concentration (nmol/L) | 74.7 (58.7-96.9) | 58.5 (39.8-86.4) | <0.001 | 0.001 |
| Large VLDL (nmol/L) | 9.7 (5.3-14.3) | 2.7 (1.0-5.3) | <0.001 | <0.001 |
| Medium VLDL (nmol/L) | 37.8 (22.4-44.3) | 20.9 (11.0-40.0) | 0.001 | <0.001 |
| Small VLDL (nmol/L) | 27.7 (16.8-43.4) | 34.0 (21.0-44.7) | 0.32 | <0.001 |
| LDL particle concentration (nmol/L) | 1342 (1095-1530) | 1077 (922-1333) | <0.001 | <0.001 |
| IDL (nmol/L) | 210 (135-258) | 191 (141-241) | 0.66 | 0.73 |
| Large LDL (nmol/L) | 404 (288-597) | 503 (435-639) | 0.047 | 0.11 |
| Small LDL (nmol/L) | 715 (481-923) | 358 (246-532) | <0.001 | <0.001 |
| HDL particle concentration (μmol/L) | 32.5 (28.7-37.1) | 34.3 (31.7-36.4) | 0.12 | 0.071 |
| Large HDL (μmol/L) | 3.1 (1.93-5.4) | 6.1 (3.4-8.5) | <0.001 | <0.001 |
| Medium HDL (μmol/L) | 10.6 (6.6-14.0) | 12.4 (9.5-15.6) | 0.006 | 0.030 |
| Small HDL (μmol/L) | 18.3 (15.6-21.4) | 15.3 (12.5-18.5) | 0.001 | 0.016 |

Data in median (interquartile range). IDL: intermediate density lipoproteins. [a]p-value: p-value after adjustment for age, sex and diabetes status.

**Table 2:** Very low density lipoprotein (VLDL), low density lipoprotein (LDL) and high density lipoprotein (HDL) subfraction characteristics in 60 subjects with and in 62 subjects without metabolic syndrome (MetS).

In univariate regression analysis including all subjects, plasma apoE levels were correlated positively with plasma total cholesterol, non-HDL cholesterol, LDL cholesterol, triglycerides and apoB (Table 3). Comparable relationships were observed in subjects with and without MetS separately, although apoE was not significantly correlated with LDL cholesterol and apoB in MetS subjects. In all subjects combined, plasma apoE was inversely correlated with HDL cholesterol, but not in the subjects with and without MetS separately. Plasma apoE was unrelated to apoA-I. In addition, apoE was correlated positively with the VLDL particle concentration in the combined subjects (Table 3). The strongest univariate relationship of apoE with individual VLDL subfractions was observed for large VLDL particles. There was also a positive relationship of apoE with the LDL particle concentration. In the combined subjects, plasma apoE was correlated positively with IDL and small LDL. Plasma apoE was not related to the HDL particle concentration, although apoE was correlated inversely with large HDL particles in the combined subjects, and positively with medium HDL particles in subjects without MetS. Figure 1 shows the univariate correlations of plasma apoE with the VLDL particle concentration, large VLDL and the LDL particle concentration.

Multivariable linear regression analyses were first performed to disclose the extent to which plasma apoE was independently related with various lipoprotein subfractions (Table 4). In a model, which included age, sex, diabetes status, the presence of MetS and the VLDL, LDL and HDL particle concentrations, plasma apoE was related positively to the VLDL and the LDL particle concentration, but not to the HDL particle concentration (Table 4, model A). Subsequent analysis, now including the individual VLDL subfractions, showed that apoE was independently related to large VLDL (Table 4, model B). After additional adjustment for the use of glucose lowering drugs and antihypertensive medication, the relationship of apoE with the VLDL and the LDL particle concentrations remained statistically significant (cf. Table 4, model A: VLDL particle concentration: $\beta$=0.272, p=0.006; LDL particle concentration: $\beta$=0.246, p=0.013; data not shown). Likewise, the relationship of apoE with large VLDL remained present

after additional adjustment for the use of these medications (cf. Table 4, model B: large VLDL: β=0.478, p<0.001; data not shown).

| | Total population (n=122 ) | MetS (n=60) | No MetS (n=62) |
|---|---|---|---|
| Total cholesterol | 0.429[c] | 0.336[b] | 0.577[c] |
| Non-HDL cholesterol | 0.507[c] | 0.414[c] | 0.578[c] |
| LDL cholesterol | 0.298[c] | 0.220 | 0.401[b] |
| HDL cholesterol | -0.246[b] | -0.207 | -0.114 |
| Triglycerides | 0.648[c] | 0.599[c] | 0.661[c] |
| ApoA-I | -0.144 | -0.137 | 0.01 |
| ApoB | 0.404[c] | 0.249 | 0.524[c] |
| VLDL particle concentration | 0.397[c] | 0.277 [a] | 0.454[c] |
| Large VLDL | 0.509[c] | 0.462[c] | 0.490[c] |
| Medium VLDL | 0.322[c] | 0.118 | 0.434[c] |
| Small VLDL | -0.006 | -0.133 | 0.197 |
| LDL particleconcentration | 0.369[c] | 0.209 | 0.430[c] |
| IDL | 0.219[a] | 0.075 | 0.482[c] |
| Large LDL | -0.134 | -0.052 | -0.189 |
| Small LDL | 0.340[c] | 0.252 | 0.301[a] |
| HDL particle concentration | -0.001 | -0.061 | 0.192 |
| Large HDL | -0.180[a] | -0.018 | -0.209 |
| Medium HDL | 0.042 | 0.023 | 0.265[a] |
| Small HDL | 0.113 | -0.006 | 0.083 |

Pearson correlation coefficients are shown. Apo: apolipoproteins; HDL: high density lipoproteins; IDL: intermediate density lipoproteins; LDL: low density lipoproteins; VLDL: very low density lipoproteins. Triglycerides and lipoprotein subfractions are logarithmically transformed. LDL cholesterol was calculated in 57 subjects with and in 60 subjects without MetS. [a]p<0.05; [b]p≤0.01; [c]p≤0.001.

**Table 3:** Relationships of plasma apolipoprotein E with apoA-I, apoB, lipoproteins and lipoprotein subfraction characteristics in 60 subjects with and 62 without metabolic syndrome (MetS)

Although plasma apoE was related positively to the LDL particle concentration, there was no significant independent contribution of the individual LDL subfractions to apoE (Table 4, model C). In addition, there was also no independent relationship of apoE with the individual HDL subfractions (Table 4, model D). Of note, all these analyses showed that the association of plasma apoE levels with MetS was no longer significant when taking lipoprotein subfractions into account.

We next examined the extent to which the relationship of apoE with the VLDL particle concentration, large VLDL particles and the LDL particle concentration, i.e. those lipoprotein subfraction characteristics to which apoE was independently related, were modified in the context of MetS or T2DM. Except for a marginally significant positive interaction of the presence of MetS with the large VLDL on apoE (cf. Table 4, model B: β=0.200, p=0.086), there were no interactions of the presence of MetS or T2DM with either the VLDL particle concentration, large VLDL particles or the LDL particle concentration on apoE (β=-0.095 to 0.127, p=0.29 to p=0.80; data not shown).

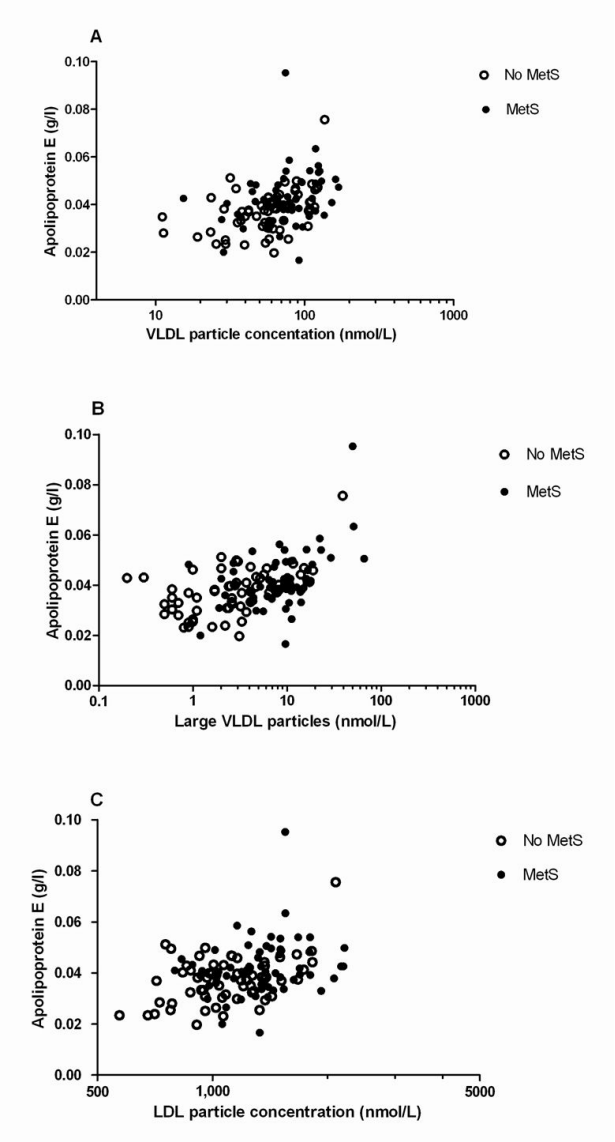


**Figure 1:** Relationship of plasma apolipoprotein E (apoE) with the very low density lipoprotein (VLDL) particle concentration (A), with large VLDL (B) and with the low density lipoprotein (LDL) particle concentration (C) in 60 subjects with metabolic syndrome (Mets) and 62 subjects without MetS. Pearson correlation coefficients are shown in Table 3.

| | Model A | | Model B | | Model C | | Model D | |
|---|---|---|---|---|---|---|---|---|
| | β | p-value | β | p-value | β | p-value | β | p-value |
| **Age** | -0.045 | 0.61 | -0.028 | 0.74 | -0.032 | 0.73 | -0.050 | 0.58 |
| **Sex (men/women)** | -0.122 | 0.20 | -0.152 | 0.095 | -0.218 | 0.033 | -0.029 | 0.79 |
| **Diabetes (yes/no)** | -0.016 | 0.87 | -0.044 | 0.66 | 0.034 | 0.76 | 0.00 | 0.99 |
| **MetS (yes/no)** | 0.107 | 0.310 | -0.052 | 0.63 | 0.126 | 0.260 | 0.142 | 0.19 |
| **VLDL particle concentration** | 0.280 | 0.003 | | | 0.257 | 0.014 | 0.324 | 0.001 |
| **LDL particle concentration** | 0.239 | 0.013 | 0.205 | 0.029 | | | 0.293 | 0.006 |
| **HDL particle concentration** | 0.013 | 0.88 | 0.000 | 1.00 | -0.014 | 0.89 | | |
| **Large VLDL** | | | 0.503 | <0.001 | | | | |
| **Medium VLDL** | | | -0.051 | 0.67 | | | | |
| **Small VLDL** | | | -0.010 | 0.91 | | | | |
| **IDL** | | | | | 0.125 | 0.169 | | |
| **Large LDL** | | | | | -0.040 | 0.68 | | |
| **Small LDL** | | | | | 0.084 | 0.47 | | |
| **Large HDL** | | | | | | | 0.096 | 0.45 |
| **Medium HDL** | | | | | | | 0.072 | 0.50 |
| **Small HDL** | | | | | | | -0.080 | 0.48 |

VLDL: very low density lipoproteins; LDL: low density lipoproteins; HDL: high density lipoproteins. β: standardized regression coefficient.

Model A: model including age, sex, diabetes status, presence of MetS, VLDL, LDL and HDL particle concentrations

Model B: model including age, sex, diabetes status, presence of MetS, VLDL subfractions, LDL and HDL particle concentrations

Model C: model including age, sex, diabetes status, presence of MetS, LDL subfractions, and VLDL and HDL particle concentrations

Model D: model including age, sex, diabetes status, presence of MetS, HDL subfractions, and VLDL and LDL particle concentrations

**Table 4:** Multivariable linear regression analysis demonstrating relationships of plasma apolipoprotein E with lipoprotein subfraction characteristics in all subjects combined (60 subjects with metabolic syndrome (MetS) and 62 subjects without MetS)

Finally, multivariable linear regression analyses were repeated now only including APOE ε3/ε3 and ε3/ε4 carriers, comprising 52 subjects with and 60 subjects without MetS. In this analysis, apoE was still independently related to the VLDL and the LDL particle concentration (Table 5, model A), and – in a subsequent analysis – to large VLDL (Table 5, model B). Again, no independent relationship of apoE with individual LDL and HDL subfractions was observed (Table 5, model C and D).

## Discussion

While the present report has confirmed that plasma apoE is elevated in MetS, a novel finding is that plasma E elevations in MetS coincide with increases in the concentration of both VLDL and LDL particles, as determined by NMR spectroscopy. In univariate regression analysis, apoE was related positively to the concentrations of (large) VLDL and LDL particles. Multivariable linear regression analyses demonstrated that the relationships of apoE with these lipoprotein fractions remained present taking account of the presence of MetS and T2DM. Of note, these analyses also demonstrated that the relationship of plasma apoE with MetS was no longer present when the higher VLDL and LDL particle concentrations in MetS were taken into account. These findings substantiate the notion that both VLDL and LDL particles are major carriers of apoE in plasma. Moreover, there was no important modification of the presence of MetS or T2DM on the relationship of plasma apoE with VLDL and LDL subfraction characteristics. This suggests that these relationships were not substantially altered in the context of MetS and chronic hyperglycemia.

Our observation that the VLDL particle concentration is elevated in MetS, along with increased large (approximately three-fold) and medium (approximately two-fold)VLDL particles, and that the LDL concentration is increased as well, along with increased small LDL particles, extends previous findings showing comparable changes in lipoprotein subfractions, determined by NMR in (non-diabetic) subjects with rather severe insulin resistance [23], as well by non-denaturing polyacrylamide gel gradient electrophoresis in subjects

with MetS [30]. Another report emphasized predominance of large VLDL in MetS, determined by size exclusion high performance liquid chromatography, even independent of insulin resistance and enlarged waist circumference [31]. The elevated plasma apoE in MetS agrees with previous reports [9,17,18]. We found no difference in plasma apoE between subjects with and without T2DM. This remarkable observation suggests that there is no strong relationship of plasma apoE with chronic hyperglycemia. This finding should be interpreted with caution, since T2DM patients participating in the present study were in general adequately controlled.

| | Model A | | Model B | | Model C | | Model D | |
|---|---|---|---|---|---|---|---|---|
| | β | p-value | β | p-value | β | p-value | β | p-value |
| **Age** | -0.016 | 0.86 | 0.002 | 0.98 | 0.007 | 0.95 | -0.027 | 0.78 |
| **Sex (men/women)** | -0.164 | 0.10 | -0.196 | 0.042 | -0.280 | 0.010 | -0.056 | 0.63 |
| **Diabetes (yes/no)** | 0.231 | 0.76 | -0.070 | 0.50 | 0.026 | 0.83 | -0.009 | 0.94 |
| **MetS (yes/no)** | 0.108 | 0.32 | -0.057 | 0.61 | 0.131 | 0.26 | 0.147 | 0.19 |
| **VLDL particle concentration** | 0.243 | 0.013 | | | 0.213 | 0.049 | 0.304 | 0.004 |
| **LDL particle concentration** | 0.402 | 0.006 | 0.249 | 0.011 | | | 0.360 | 0.002 |
| **HDL particle concentration** | -0.057 | 0.96 | -0.018 | 0.85 | -0.063 | 0.56 | | |
| **Large VLDL** | | | 0.493 | <0.001 | | | | |
| **Medium VLDL** | | | -0.059 | 0.63 | | | | |
| **Small VLDL** | | | -0.045 | 0.63 | | | | |
| **IDL** | | | | | 0.161 | 0.091 | | |
| **Large LDL** | | | | | -0.033 | 0.75 | | |
| **Small LDL** | | | | | 0.084 | 0.49 | | |
| **Large HDL** | | | | | | | 0.130 | 0.33 |
| **Medium HDL** | | | | | | | 0.049 | 0.67 |
| **Small HDL** | | | | | | | -0.115 | 0.34 |

VLDL: very low density lipoproteins; LDL: low density lipoproteins; HDL: high density lipoproteins. β: standardized regression coefficient.

Model A: model including age, sex, diabetes status, presence of MetS, VLDL, LDL and HDL particle concentrations

Model B: model including age, sex, diabetes status, presence of MetS, VLDL subfractions, and LDL and HDL particle concentrations

Model C: model including age, sex, diabetes status, presence of MetS, LDL subfractions, and VLDL and HDL particle concentrations

Model D: model including age, sex, diabetes status, presence of MetS, HDL subfractions, and VLDL and LDL particle concentrations

**Table 5:** Multivariable linear regression analysis demonstrating relationships of plasma apolipoprotein E with lipoprotein subfraction characteristics in APOE ε3/ε3 and ε3/ε4 carriers (52 subjects with metabolic syndrome (MetS) and 60 subjects without MetS).

It is well appreciated that triglyceride-rich lipoproteins are major carriers of apoE in human plasma [2,4,5]. Accordingly, plasma apoE was strongly related to triglycerides in the present report. In addition, apoE was independently related to the VLDL concentration. Of the individual VLDL subfractions the closest relationship with apoE was observed for large VLDL. Importantly, increased hepatic production of large VLDL particles is considered to be a primary liporegulatory abnormality in MetS. In view of the precursor-product relationship between (large) VLDL and (small) LDL [32,33], the currently documented relationships of apoE with (large) VLDL and LDL appear to be consistent with the possibility that the apoE content in (large) VLDL may represent a determinant of LDL metabolism [34]. On the other hand, overexpression of human apoE in mice results in hypertriglyceridemia which is due to both hepatic VLDL overproduction and impaired VLDL catabolism [35].

In the light of higher levels of plasma apoE in MetS, the close relationship of apoE with large VLDL particles, and the increased VLDL apoE production rate observed in hypertriglyceridemia [36], it seems paradoxical that apoE most probably exerts anti-atherogenic effects [1-3,7-9]. Our novel finding regarding the independent relationships of plasma apoE with the concentrations of both VLDL and LDL particles complements recent findings showing that specific VLDL and LDL subfractions that carry apoE may confer protection against CVD development [11]. Although these relationships suggest that VLDL and LDL particle characteristics should be taken into account when evaluating the association of apoE with incident CVD, it is obvious that the extent to which apoE could modify CVD risk that is

attributable the VLDL and LDL subfraction concentrations remains to be more precisely established. In this regard it is important to emphasize that the NMR spectroscopy analysis that we used does not allow to determine the apoE content in various lipoprotein subfractions. Finally, it should be noted that there were no positive relationships of apoE with HDL cholesterol and apoA-I in univariate analysis. There was also no independent relationship of apoE with HDL subfraction characteristics in multivariable linear regression analyses.

Several other methodological aspects and limitations of our study need to be discussed. First, in view of the cross-sectional design of our study, cause-effect relationships of apoE with lipoprotein subfraction characteristics cannot be assessed with certainty. Second, we excluded subjects using lipid lowering drugs in order to avoid treatment-induced effects on lipoproteins and lipoprotein subfractions. Nonetheless, close relationships of plasma apoE with (large) VLDL and LDL were already demonstrated under modestly dyslipidemic circumstances. Third, we excluded subjects with APOE ε2/ε2 genotype in order to circumvent effects of dysbetalipoproteinemia on the relationships of apoE with lipoprotein subfractions. Furthermore, additional analyses that were restricted to APOE ε3/ε3 and ε3/ε4 carriers yielded similar results, underscoring that the relationships of apoE with lipoprotein subfraction characteristics were not to a considerable extent confounded by pathophysiologically important structural variations in apoE. Fourth, we employed a high-throughput NMR spectroscopy analysis to determine lipoprotein subfraction characteristics. There is considerable agreement between this method and more conventional lipoprotein subfraction analyses, but some discrepancies between assay methods cannot be excluded [37].

In conclusion, this study suggests to our knowledge for the first time that elevated plasma apoE is closely related to increased concentrations of (large) VLDL and LDL particles in MetS. These findings provide a rationale to explore whether the preferential association of apoE with (large) VLDL and LDL could modify its influence on atherosclerosis development.

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