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The positive relationship of serum paraoxonase-1 activity with apolipoprotein E is abrogated in metabolic syndrome

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ABSTRACT

Background: High density lipoproteins (HDL) contain paraoxonase-1 (PON-1), which has strong anti-oxidative properties. Apolipoprotein E (apoE) may enhance PON-1 activity *in vitro*, but the extent to which PON-1 activity is determined by circulating apoE levels is unknown. Here we determined relationships of serum PON-1 activity with apoE in subjects without and with metabolic syndrome (MetS).
Methods: We measured PON-1 activity (arylesterase activity), plasma apoE and serum amyloid A (SAA) in 93 subjects without and in 75 subjects with MetS (25 and 54 subjects with Type 2 diabetes mellitus (T2DM), respectively; $p < 0.001$).
Results: PON-1 activity was lower in MetS ($p < 0.005$) coinciding lower HDL cholesterol, apoA-I ($p < 0.001$)) and SAA levels ($p < 0.01$), whereas apoE was increased in relation to higher triglycerides ($p < 0.01$). In subjects without MetS, PON-1 activity was correlated positively with apoE ($r = 0.376$, $p < 0.001$), but this relationship was absent in MetS subjects ($r = 0.085$, $p = 0.47$). Multiple linear regression analysis showed that the relationship of PON-1 activity with apoE was different in subjects with MetS compared to subjects without MetS ($\beta = -0.270$, $p = 0.014$ for the interaction between apoE and MetS), independently from age, sex, T2DM, use of glucose lowering drugs, anti-hypertensives and the inverse relation with SAA levels ($p = 0.008$). Of the individual MetS components, apoE only interacted with low HDL-C on PON-1 activity ($\beta = -0.175$, $p = 0.074$). The relationship of apoE with PON-1 activity was neither modified by T2DM ($p = 0.49$), nor by SAA ($p = 0.79$).
Conclusion: Higher apoE levels may confer higher PON-1 activity. The relationship of PON-I activity with total plasma apoE is apparently abrogated in MetS.

1. Introduction

The inverse relationship of incident atherosclerotic cardiovascular disease (CVD) with plasma levels of high density lipoprotein (HDL) cholesterol is unequivocally established [1,2]. However, genetic association studies, as well as the recently reported lack of clinical efficacy of pharmacological interventions that are aimed at raising HDL cholesterol have resulted in a shift of concept concerning the mechanisms by which HDL confers atheroprotection [3–8]. As a result, it is increasingly appreciated that intrinsic functional properties of HDL are physiologically important in explaining the beneficial effects of HDL on atherosclerosis development [9–14]. Among other anti-oxidative protein factors, HDL particles contain paraoxonase-1 (PON-1), which is thought to play an essential role in the ability of HDL to confer cardioprotection in rodent models and in humans [6,9–11,13,15–18].

Serum PON-1 activity is likely to be decreased in clinical conditions associated with low HDL cholesterol including the metabolic syndrome (MetS) and Type 2 diabetes mellitus (T2DM) [15,19–22]. Nonetheless, our current knowledge with respect to the mechanisms responsible for regulating PON-1 activity is incomplete. Apolipoprotein (apo) A-I, the most abundant apolipoprotein constituent of HDL, stabilizes PON-1 and is required for optimal PON-1 function [9,23]. Of further relevance, the pro-inflammatory protein, serum amyloid A (SAA), which was found to be elevated in MetS [21,24], may displace both apoA-I and PON-1 from HDL particles, thereby impairing PON-1 activity [23,25]. In agreement with these *in vitro* data, we have shown recently that PON-I activity is inversely related to SAA levels [21], and that higher SAA is a determinant of impaired HDL anti-oxidative functionality [24].

During the past few years, the concept is evolving that apoE may have anti-oxidative properties [26,27], besides well known roles in

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the metabolism of both triglyceride-rich apoB-containing lipoproteins and of HDL [28–31]. Interestingly, *in vitro* experiments have shown that reconstituted apoE-containing HDL particles stabilize PON-1 in a manner similar to that of apoA-I [32]. This stabilization process increases PON-1 activity and improves its ability to inhibit LDL oxidation [32]. In keeping with such a proposed positive contribution of apoE to PON-1 action, it was also documented that administration of an apoE-mimetic compound stimulates PON-1 activity in apoE null mice, although this study did not report effects of apoE deficiency as such on PON-1 activity [33]. Of further interest, hepatic PON-1 expression may be dependent on variation in apoE genotype in mouse models humanized for the apoE gene [34]. Given the proposed role of apoE on PON-1 regulation, it is plausible to postulate that circulating apoE may be a determinant of PON-1 activity in humans. In view of decreased PON-1 activity [19–21] and higher total plasma apoE levels but lower HDL-associated apoE in MetS [35], it is also relevant to test whether the possible relationship of PON-1 activity with apoE levels is modified in MetS subjects.

The present study was initiated to determine the extent to which serum PON-1 activity is related to apoE levels in subjects without and with metabolic syndrome (MetS), and to establish whether such a possible relationship remains present when taking SAA levels into account.

2. Patients and methods

2.1. Participants

The medical ethics committee of the University Medical Center Groningen, The Netherlands approved the study. All participants provided written informed consent. This study was performed in a university hospital setting. The subjects (aged > 18 years) were Caucasian, and were recruited by advertisement in local newspapers. Subjects without and with metabolic syndrome (MetS), defined according to the revised NCEP-ATP III criteria [36], 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 <1.3 mmol/l for women; fasting glucose ≥5.6 mmol/l. Subjects with Type 2 diabetes mellitus, 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 with treated by primary care physicians with diet alone or diet in combination with metformin and/or sulfonylurea. The use of anti-hypertensive medication was allowed, but subjects using insulin not eligible. Further exclusion criteria were clinically manifest cardiovascular disease, renal insufficiency (elevated serum creatinine and/or proteinuria), thyroid disorders, liver disease, current smoking, pregnancy and use of lipid lowering drugs. Physical examination did not reveal pulmonary or cardiac abnormalities. All subjects were studied after an overnight fast. BMI was calculated as weight divided by height squared (in kg/m^2). Waist circumference was measured between the 10th rib and the iliac crest.

2.2. Laboratory analyses

Venous blood samples were collected into EDTA-containing tubes (1.5 mg/ml) for the measurement of plasma lipids, apolipoproteins and SAA. Serum was obtained for the measurement of PON-1 activity. Plasma and serum samples were prepared by centrifugation at 1400 g for 15 min at 4 °C. Blood glucose and glycated hemoglobin (HbA1c) levels were measured directly after blood collection. Samples for other assays were stored at −80 °C until analysis.

Serum PON-1 enzymatic activity was measured as its arylesterase activity, i.e. as the rate of hydrolysis of phenyl acetate into phenol as described previously [22,37]. Arylesterase activity is expressed in kilo units (kU) per liter of serum; 1 U is equivalent to 1 μmol of phenyl acetate hydrolyzed per min. The inter-assay CV amounts to 8%. Arylesterase activity, measured with this assay is correlated positively with PON-1 enzymatic activity toward paraoxon, assayed as described earlier ($n = 1527$; $r = 0.42$, $p < 0.001$, derived from Ref. [37]).

Plasma total cholesterol (TC) and triglycerides were assayed by routine enzymatic methods (Roche/Hitachi cat nos 11875540 and 11876023, respectively; Roche Diagnostics GmbH, Mannheim, Germany). HDL cholesterol (HDL-C) was measured with a homogeneous enzymatic colorimetric test (Roche/Hitachi, cat no 04713214; Roche Diagnostics GmbH, Mannheim, Germany). Non-HDL cholesterol (non-HDL-C) was calculated as the difference between TC and HDL-C. 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).

SAA protein was measured by a monoclonal antibody-based sandwich SAA1 enzyme-linked immunosorbent assay [21,38]. Human apo-SAA was purified from the HDL_3 fraction of acute phase serum, linked to helix pomatia hemocyanin, and injected into Balb/c mice to produce monoclonal antihuman-SAA antibodies. The antibodies used are the capture antibody Reu.86.5, which reacts to all acute phase SAA subtypes and the coupled to Horse radish peroxidase detection antibody Reu.86.1, which reacts to the major SAA1 subtype. The assay is standardized against the international standard for SAA protein (WHO code 92/680). The inter-assay CV is 7.0%.

Glucose was analyzed 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%).

2.3. Statistical analysis

SPSS 20 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, Mann–Whitney *U* and Chi-square tests where appropriate. Differences in variables between subjects with and without MetS were also determined after adjustment for age and sex. Because of skewed distribution, logarithmically transformed values of triglycerides and SAA were used for regression analysis. Univariate relationships were calculated using Pearson correlation coefficients. Multiple linear regression analyses were carried out to disclose variables which independently contributed to PON-1 activity in subjects without and with MetS. Additionally, multiple linear regression analyses were performed to determine interactions between variables impacting on PON-I activity. Interaction terms were calculated as the product terms between apo E and the presence of MetS, or alternatively between apoE and individual MetS components or diabetes status. To this end the distribution of apo E levels was centered to its 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 [39] and by the Food and Drug Administration authorities [40].