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Correlation of apolipoprotein M with leptin and cholesterol in normal and obese subjects

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Abstract

Apolipoprotein M (apoM) is a recently characterized apolipoprotein that is exclusively expressed in the liver and kidney. In plasma it is present predominantly in high-density lipoprotein (HDL). The physiological function of apoM is not yet known. In the present study we investigated relationships between plasma apoM levels and leptin levels, body mass index (BMI), as well as fasting glucose and other lipid parameters in women with a wide range of BMI (18.9–57.1 kg/m^2, $n = 51$). In univariate analysis, apoM correlated significantly with leptin ($r = 0.54$, $P < 0.001$), BMI ($r = 0{,}70$, $P < 0.001$), fasting insulin ($r = 0.33$, $P = 0.025$), total cholesterol ($r = -0.41$, $P = 0.016$), and LDL-cholesterol ($r = -0.39$, $P = 0.018$). The correlations between apoM and cholesterol and between apoM and leptin remained significant after adjustment for the influence of BMI. Forward stepwise multiple regressions when leptin, BMI, insulin, and cholesterol were entered into a model as independent variables and apoM as the dependent variable, showed that cholesterol and leptin were independent predictors of circulating apoM. These two parameters yielded a value of $r^2 = 0.28$, thereby explaining approximately 30% of the variance in apoM. Hence, we show that apoM is positively related to leptin and negatively related to cholesterol in humans.

Keywords: Apolipoprotein M; Leptin; Cholesterol; Obesity; Lipoproteins

1. Introduction

Apolipoprotein M (apoM) is a recently characterized human apolipoprotein that is mainly associated with high-density lipoprotein (HDL) in human plasma, with a small proportion present in triglyceride-rich lipoproteins and low-density lipoproteins (LDL) [1]. ApoM fulfills the criteria of a HDL-associated apolipoprotein because the majority of apoM in plasma is associated with HDL [2]. In situ hybridization experiments demonstrated that apoM is exclusively expressed in hepatocytes in the liver and in tubular cells in the kidney [3]. Previous studies suggested that apoM might be involved in lipid and/or lipoprotein metabolism in vivo. For example, the proportion of apoM in triglyceride-rich lipoproteins has been shown to be increased in the post-prandial phase [1]. Recently we reported that apoM is also related to the activation of platelet activating factor–receptor (PAF-R) in HepG2 cells, such that PAF enhances apoM secretion and expression in a dose-dependent manner, whereas the PAF-R antagonist lexipafant inhibits apoM expression [4]. However, the physio-pathological function of apoM is still not understood. Because apoM is an HDL-associated apolipoprotein, it is reasonable to hypothesize that the expression of apoM in the liver may be associated with the production of HDL or with hepatic lipid and/or lipoprotein metabolism. If so, apoM might be regulated by hormones regulating lipid and/or lipoprotein metabolism or by plasma lipids.

Leptin is a multifunctional hormone encoded by the *ob* gene [5]; it is synthesized mainly in adipocytes and has been shown to have influences on hepatic lipid and lipoprotein metabolism [6–9]. In most cases it reflects body fat content; that is, serum concentrations of leptin are elevated in individuals who are obese in comparison with those who are lean, and a strong positive correlation has been found between leptin and body fat content as well as between leptin and body mass index (BMI) [10]. Intravenous administration of leptin results in a reduction of food intake and an enhancement of energy expenditure [11]. It was previously reported that there is a positive correlation of leptin with

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systolic and diastolic blood pressure, fasting triacylglycerols, serum uric acid, and fasting glucose and insulin, and a negative correlation with insulin sensitivity [6]. Recently, Liang and Tall reported that leptin treatment in *ob/ob* mice leads to an increase in mRNA levels of apoA-I, apoA-II, apoH, and apoM and to a decrease in apoA-IV in the liver [12]. This would suggest an association between leptin and apoM in mice. However, whether such a relation between apoM and leptin as well as with other lipid parameters exists also in humans is not known. In the present study, therefore, we examined apoM in correlation with plasma leptin, cholesterol, and other lipid parameters in both normal and obese individuals.

Table 1
Characteristics of the study population ($n = 51$)

Parameter	Mean ± SD
Age (y)	54 ± 12
ApoM (vol)*	2735 ± 661
BMI (kg/m^2)	30.6 ± 10.1
Leptin (ng/mL)	32.3 ± 24.4
Glucose (mmol/L)	5.1 ± 1.1
Insulin (pmol/L)	145 ± 330
Cholesterol (mmol/L)	6.1 ± 1.0
Triglycerides (mmol/L)	1.2 ± 0.6
HDL-cholesterol (mmol/L)	1.5 ± 0.4
LDL-cholesterol (mmol/L)	4.1 ± 1.0

*Vol=volume density (intensity × mm^3)

2. Methods and materials

2.1. Subjects

A total of 51 women (aged 26–61 years, BMI 18.9–57.1 kg/m^2) were included in the study. All subjects were healthy except two obese subjects with diet-regulated type 2 diabetes. No subjects were taking any drugs known to affect liver or kidney function. The protocol of the study was approved by the local ethics committee. All of the subjects included in the study were instructed about the purpose of the study and gave their consent to participate. The blood samples for apoM, leptin, and serum lipid determination were taken in prechilled tubes containing EDTA (final concentration 0.34 mol/L) after an overnight fasting. Samples were immediately centrifuged at 4°C, and plasma was stored at −70°C until analysis.

2.2. Analysis

Plasma apoM concentration was estimated by a dot-blot analysis with a specific rabbit antihuman apoM antibody. Rabbit antiserum against a synthetic peptide corresponding to amino acid residues 103-122 of apoM, or a truncated apoM conjugated to keyhole limpet hemocyanin was raised. Specificity of the antibody was checked by Western blot analysis demonstrating that no cross-reaction had occurred. In brief, 10 μL of plasma was diluted with Tris-HCl buffer (1:20) and 5-μL diluted samples were applied to membrane (Hybond-C, Amersham Biosciences, Piscataway, NJ). All samples were applied to the membrane triplicate. The membrane was quenched in Tris-HCl buffer with 4% Tween and 3% BSA for 2 hours, and sequentially incubated with primary antibody (dilution, 1:2000 in Tris-HCl buffer) overnight at 4°C and then alkaline phosphatase (AP) conjugated secondary antibody for 2 hours at room temperature. The development of AP activity was performed with a commercial visualization system according to the manufacturer's instructions (Dako, Hŏnŏ, Sweden). The relative amount of apoM were analyzed with a Macintosh computer using the software of Quantity One (Version 4.2.1, Bio-Rad Laboratories, Hercules, CA), and presented as arbitrary units, being equivalent to volume density (intensity × mm^2). The same experiments were repeated at least three times. Leptin concentrations were determined with a double-antibody radioimmunoassay using rabbit anti-human leptin antibodies, ^{125}I-labeled human leptin as tracer, and human leptin as standard (Linco Research, St. Charles, MO) [13]. Plasma insulin concentrations were analyzed with a double-antibody radioimmunoassay technique (Linco). Serum lipid parameters and fasting glucose were measured in the Department of Clinical Biochemistry of Lund University Hospital.

2.3. Statistical analysis

Results are reported as mean ± SDM unless stated otherwise. Statistical analysis was performed using the SPSS package (SPSS, Chicago, IL), version 6.1, on a Macintosh computer. The correlation between apoM and other parameters was determined by the stepwise correlation test. Two-tailed tests were used, and a value of $P < 0.05$ was considered to be significant.

3. Results

Table 1 shows the characteristics of the study population. In univariate analysis, apoM correlated significantly with leptin ($r = 0.54$, $P < 0.001$), BMI ($r = 0{,}70$, $P < 0.001$), fasting insulin ($r = 0.33$, $P = 0.025$), total cholesterol ($r = -0.41$, $P = 0.016$), and LDL-cholesterol ($r = -0.39$, $P = 0.018$) but not with glucose ($r = 0.26$, $P = 0.073$ or HDL-cholesterol ($r = -0.078$, $P = 0.65$). Figure 1 shows the univariate correlations with BMI, leptin, and total cholesterol. The correlations between apoM and total cholesterol and between apoM and leptin remained significant after adjustment for the influence of BMI. Forward stepwise multiple regressions, when leptin, BMI, insulin, and total cholesterol were entered into a model as independent variables and apoM as the dependent variable, showed that total cholesterol and leptin were independent predictors of circu-

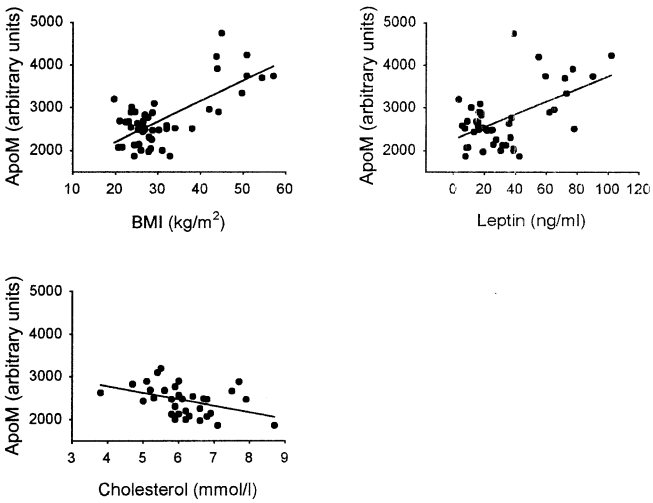

Fig. 1. Relationship between body mass index (BMI), plasma leptin, and plasma cholesterol versus plasma levels of apolipoprotein M (apoM) in 51 women with a wide range of BMI (18.9–57.1 kg/m^2). Regression lines are illustrated for each relationship. ApoM was quantified in arbitrary units and was equivalent to volume density (intensity × mm^2).

lating apoM. These two parameters yielded a value of $r^2 = 0.28$, thereby explaining approximately 30% of apoM.

4. Discussion

In this study, we investigated the plasma apoM levels in relation to plasma leptin and lipid parameters in normal and obese women. We found a significant positive correction between apoM levels and leptin levels and a negative correlation between apoM and total cholesterol in both normal and obese subjects.

Leptin was initially identified in *ob/ob* mice [14]. The main sources of plasma leptin are adipocytes, although other tissues also produce the hormone [15,16]. Plasma leptin concentrations are closely related to body fat content [15,16] and are subject to circadian rhythms [17]. One of the most important hormonal factors affecting plasma leptin levels is insulin, which was reported to increase leptin expression and levels in vivo and in vitro [18,19]. Leptin also shows a pronounced sexual dimorphism, with higher levels in female individuals. This may be dependent on sex hormones, although reports concerning a relationship between leptin and estrogen, progesterone, or testosterone are somewhat contradictory [20–22].

The relationship between leptin and plasma lipid/lipoproteins has been reported in series of studies involving healthy individuals as well as those with various diseases (e.g., growth-hormone deficiency and polycystic ovary syndrome). Some studies have reported no correlation between leptin and serum lipid parameters [23,24]. Other studies have observed a significant positive correlation between leptin and HDL cholesterol [6] or between leptin and triglycerides [25,26]. Mice with genetic defects in leptin exhibit hyperlipidemia, hyperinsulinemia, hyperglycemia, and resistance to insulin [27–29]. Recently, Liang and Tall reported that leptin up-regulated mRNA levels of several HDL apolipoproteins including apoA-I, apoA-II, apoH, and apoM, but not apoC-I and apoE, in *ob/ob* mice [12].

ApoM is a recently characterized, HDL-associated apolipoprotein. The physio-pathological function of apoM is not yet understood. ApoM is mainly associated with HDL with a small proportion present in triglyceride-rich lipoproteins and LDL in human plasma [1]. On human tissue–array blotting analysis, apoM mRNA was expressed exclusively in the liver and kidney, with a minor expression in the fetal liver and fetal kidney [3]. The synthesis of apoM in liver may be associated with the production of HDL, although the physiological importance of the apoM mRNA in kidney is less obvious.

In the present study we demonstrate that the plasma apoM concentration is positively correlated with leptin levels and negatively correlated with total cholesterol in normal and obese subjects. This would suggest that apoM is involved in the metabolism of plasma cholesterol in humans. ApoM, like apoD, is a member of the lipocalin super-family [30]. Most members of the lipocalin superfamily have the ability to carry hydrophobic substance such as cholesterol [31,32]. However, unlike other lipocalin proteins, apoM is hydrophobic and must associate with lipoprotein particles in plasma [1]. It remains to be investigated whether apoM carries cholesterol and influences cholesterol metabolism in vivo.

The relationship between apoM and leptin may be more complex. Liang and Tall reported that leptin up-regulated mRNA levels of apoM in *ob/ob* mice [12], suggesting that leptin could stimulate hepatocytes to produce apoM. However, in another series experiments, it was demonstrated that apoM expression and secretion in HepG2 cells could be inhibited by the administration of recombinant human leptin in a dose-dependent manner (unpublished data). This may indicate that the relation between apoM and leptin is species dependent. The detailed mechanism of positive correlation between apoM and leptin in human needs therefore to be further investigated in different species.

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