Triglyceride-rich lipoprotein metabolism in women: roles of apoC-II and apoC-III

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Abstract (250 words)

Background: Experimental data suggest that apolipoprotein (apo) C-II and C-III regulate triglyceride-rich lipoprotein (TRL) metabolism, but there are limited studies in humans. We investigated the metabolic associations of TRLs with apoC-II and apoC-III concentrations and kinetics in women.

Material and methods: The kinetics of plasma apoC-II, apoC-III and very low-density lipoprotein (VLDL) apoB-100 and triglycerides were measured in the postabsorptive state using stable isotopic techniques and compartmental modeling in 60 women with wide-ranging body mass index (19.5-32.9kg/m²).

Results: Plasma apoC-II and apoC-III concentrations were positively associated with the concentration of plasma triglycerides, VLDL₁- and VLDL₂- apoB-100 and triglyceride (all $P<0.05$). ApoC-II production rate (PR) was positively associated with VLDL₁-apoB-100 concentration, VLDL₁-triglyceride concentration and VLDL₁-triglyceride PR, while apoC-II fractional catabolic rate (FCR) was positively associated with VLDL₁-triglyceride FCR (all $P<0.05$). No significant associations were observed between apoC-II and VLDL₂ apoB-100 or triglyceride kinetics. ApoC-III PR, but not FCR, was positively associated with VLDL₁-triglyceride, and VLDL₂- apoB-100 and triglyceride concentrations (all $P<0.05$). No significant associations were observed between apoC-III and VLDL- apoB-100 and triglyceride kinetic. In multivariable analysis, including homeostasis model assessment score, menopausal status and obesity, apoC-II concentration was significantly associated with plasma triglyceride, VLDL₁ apoB-100 and -triglyceride concentrations and PR. Using the

same multivariable analysis, apoC-III was significantly associated with plasma triglyceride and VLDL₁- and VLDL₂- apoB-100 and triglyceride concentrations and FCR.

Conclusions: In women, plasma apoC-II and apoC-III concentrations are regulated by their respective production rates and are significant, independent determinants of the kinetics and plasma concentrations of TRLs.

Keywords: apolipoprotein C-II, apolipoprotein C-III, lipoprotein metabolism, triglyceride, women

Introduction

Cardiovascular disease (CVD) is a leading cause of morbidity and mortality in women worldwide[1]. In general, women have a higher risk of dying from first myocardial infarction or experience a second cardiovascular event compared with men. In addition, CVD risk factors are more prevalent among women compared with men when standardized for age [1]. Despite this, women remain an under-recognized and understudied population.

Hypertriglyceridaemia is associated with increased CVD risk [2]. It is the most consistent lipid disorder in obesity and type 2 diabetes. Hypertriglyceridaemia is chiefly related to dysregulated triglyceride-rich lipoprotein (TRL) metabolism, including overproduction of very low-density lipoprotein (VLDL) particles and impaired catabolism of TRL and their remnants [3]. These abnormalities are a consequence of insulin resistance, increased lipid substrate availability in the liver and depressed activities of lipoprotein lipase (LPL) and hepatic clearance receptors [4].

Apolipoprotein (apo) C-II and C-III are synthesized and secreted by the liver, and circulate in plasma as components of TRL and high-density lipoprotein (HDL) particles [5, 6]. The prevailing notion is that apoC-II and apoC-III have opposing roles in TRL metabolism. ApoC-II is a required co-activator of LPL activity [5]. By contrast, apoC-III inhibits LPL activity [6]. Despite this, the metabolic regulation of apoC-II and apoC-III, is poorly understood. The exact relationship between apoC-II and TRL metabolism, which could underscore the relationship between hypertriglyceridaemia and CVD risk, is also not well characterized.

We aimed to examine the associations between plasma apoC-II and apoC-III concentrations and kinetics with those of VLDL apoB-100 and triglycerides in women.

Materials and Methods

Participants

The current study is an extension of a large kinetic trial investigating lipid and metabolism in 60 healthy pre- and postmenopausal women [7]. All women were Caucasian, with a BMI > 18.5 and $< 35 \text{ kg/m}^2$, aged between 35 and 45 years (premenopausal) and 55 and 65 years (postmenopausal) and who reported being weight stable for a period of 2 months before the study. Premenopausal was defined as having regular menses over the past 12 months and blood FSH $< 30 \text{ IU/l}$, whilst postmenopausal status was defined as absence of menses for at least 12 months and blood FSH $> 30 \text{ IU/l}$. Subjects were excluded if they had any condition or treatment that would affect metabolic or hormonal status (including smoking, diabetes, polycystic ovary syndrome or hormone replacement therapy). Smokers or women exceeding alcohol consumption guidelines of $> 30 \text{ g/day}$ were also excluded. The pre- and postmenopausal groups were matched for BMI and waist circumference. The clinical protocol, including administration of D3-leucine and blood sampling, was described previously [7]. All participants provided written consent. The Oxfordshire Clinical Research Ethics Committee approved the study. Reporting of the study conforms to STROBE statement along with references to STROBE statement and the broader EQUATOR guidelines [8].

Measurement of isotopic enrichments and calculation of kinetic parameters

Plasma apoC-II and apoC-III were isolated by ultracentrifugation and isoelectric focusing, delipidated, hydrolyzed and derivatized, as described previously [9]. Isotopic enrichment was determined using gas chromatography-mass spectrometry with selected ion monitoring of derivatized samples. The SAAM II program (The Epsilon Group, VA) was

used to fit the model to the tracer-to-tracee data. The fractional catabolic rates (FCR) of plasma apoC-II and apoC-III were derived from the model parameters giving the best fit. The corresponding production rates (PR) were calculated as the product of FCR and pool size. Laboratory methods for measurement of VLDL triglyceride and apoB-100 kinetics were described previously [7].

Biochemical analyses

Plasma apoC-II concentrations were measured by enzyme-linked immunosorbent assay (Cell Biolab Inc. San Diego, CA). Plasma apoC-III concentrations were measured using Hydragel LP CIII Electroimmunodiffusion (Sebia, France) according to the manufacturer's instructions and detailed previously [7]. Laboratory methods for lipids, lipoproteins and other biochemical analyses were detailed previously [7].

Statistics

Statistical analyses were performed using STATA (Version 12.1; StataCorp, College Station, TX). Associations between apoC-II, apoC-III and VLDL apoB-100 and triglyceride concentrations and kinetics were examined using simple and multiple linear regression methods. Statistical significance was defined at the 5% level using a 2-tailed test.

Results

The 60 women were on average (mean $\pm$ standard deviation) middle-aged (49.7 ± 9.1 years; range: 35-64yrs) with a wide-range of body mass index (BMI 24.9 ± 2.9 kg/m²; range: 19.5-33.0 kg/m²), insulin resistance (homeostasis model assessment [HOMA] score 2.93 ± 1.16 ; range 0.70-8.42), plasma total cholesterol (5.44 ± 0.97 mmol/L; range 3.9-8.9 mmol/L), low-density lipoprotein [LDL] cholesterol (3.32 ± 0.92 mmol/L; range 1.9-6.61 mmol/L), HDL cholesterol (1.65 ± 0.38 mmol/L; range: 0.63-2.62 mmol/L) and apoB (0.85 ± 0.2 g/L; range: 0.51-1.74 g/L). The average plasma apoC-II concentration was 28.4 ± 22.6 mg/L (range 5.80-152.0 mg/L), while plasma apoC-III concentration was 31.2 ± 9.9 mg/L (range 8.3-65.5 mg/L). Fifty-one percent of women were postmenopausal and 46% were overweight-obese (BMI ≥ 25 kg/m²).

Table 1 shows the plasma concentrations and kinetics of apoC-II, apoC-III and VLDL-apoB-100 and triglycerides. ApoC-II and apoC-III concentrations were significantly associated with their respective PR ($r=0.835$ and $r=0.600$, respectively, both $P<0.01$). In univariate analyses, apoC-II concentration was positively associated with the concentrations of plasma triglyceride ($r=0.391$), apoC-III ($r=0.523$), VLDL₁- ($r=0.370$, $r=0.376$) and VLDL₂- ($r=0.311$ and $r=0.299$) apoB-100 and triglyceride (all $P<0.05$). ApoC-II concentration was also positively associated with the PR of VLDL₁ apoB-100 and triglyceride ($r=0.266$, $r=0.467$, $P<0.05$ for both) but this was not observed for FCR. ApoC-II PR was significantly associated with VLDL₁ apoB-100 and triglyceride concentrations ($r=0.253$ and $r=0.272$, $P<0.05$ for both), and VLDL₁-triglyceride PR ($r=0.272$, $P=0.05$). No significant associations were observed between apoC-II kinetics and VLDL₂ kinetics. In univariate analyses, apoC-III concentration was positively associated with the concentrations of plasma triglyceride ($r=0.501$), VLDL₁- ($r=0.538$, $r=0.554$) and VLDL₂- ($r=0.563$ and $r=0.554$) apoB-100 and triglyceride (all $P<0.05$). Plasma apoC-III was negatively associated

with the FCR, but not PR, of VLDL₁ ($r=-0.290$, $r=-0.340$) and VLDL₂ ($r=-0.355$, $r=-0.390$) apoB-100 and triglyceride (all $P<0.05$). ApoC-III PR, but not FCR, was associated with VLDL₁-triglyceride ($r=0.264$) and VLDL₂- apoB-100 and triglyceride ($r=0.311$ and $r=0.290$) concentrations (all $P<0.05$). No significant associations were observed between apoC-III kinetics and VLDL₂ kinetics. The apoC-III:apoC-II ratio was not significantly associated with VLDL apoB-100 or triglyceride concentrations and kinetics. With the exception of the association between plasma apoC-II concentration and VLDL₁-apoB PR ($r=0.260$, $P=0.06$), the abovementioned significant associations for plasma apoC-II and apoC-III with plasma triglyceride, VLDL-apoB, VLDL-triglyceride concentrations and VLDL kinetic variables remained statistically significance after adjustment for plasma HDL cholesterol concentration (data not shown).

Table 2 shows the relationships between plasma apoC-II and kinetic indices for VLDL₁ and VLDL₂ in multivariable regression models including obesity, HOMA score and menopausal status. Plasma apoC-II concentration was an independent predictor of VLDL₁ apoB-100 and triglyceride concentrations and PR in a multivariable regression model analysis that included HOMA score, menopausal status and obesity (defined as $BMI \geq 25$ kg/m²). None of the abovementioned parameters, including apoC-II, were predictors of VLDL₁ apoB-100 and triglyceride FCR. As seen in Table 3, apoC-III concentration was an independent predictor of VLDL₁- and VLDL₂- apoB-100 and triglyceride concentrations and FCR. No associations with VLDL₁- and VLDL₂- apoB-100 and triglyceride PR were observed. Replacing obesity status with BMI, a continuous variable, in the multivariable regression models in Tables 2 and 3 did not alter the abovementioned results (data not shown). Furthermore, adjustment for age did not significantly alter the relationships between plasma apoC-II or apoC-III.

Discussion

We report on the relationships between plasma apoC-II, apoC-III and VLDL apoB-100 and triglyceride kinetics in women. Plasma apoC-II and apoC-III concentrations are primarily a function of PR: FCR was not significantly associated with plasma concentrations. We showed that high apoC-II concentration is significantly and independently associated with increased production of VLDL₁ apoB-100 and triglyceride leading to elevated VLDL₁ apoB-100 and triglyceride concentrations. We also showed that high apoC-III is significantly and independently associated with impaired catabolism of VLDL₁ and VLDL₂ apoB-100 and triglyceride resulting in elevated VLDL₁ and VLDL₂ apoB-100 and triglycerides. Our observations that plasma apoC-II and apoC-III are positively correlated with plasma triglyceride concentrations concur with earlier reports [5, 6, 10, 11]. We extend previous studies by examining the relationship between plasma apoC-II and apoC-III kinetics with those of VLDL subpopulations, and exclusively in women.

We showed that high apoC-II is associated with increased VLDL₁ particle production. The underlying mechanism for this association is unclear. It is possible that elevated apoC-II directly enhances VLDL production. Recent studies reported a paradoxical, slower rate of TRL synthesis in apoC-II deficient mice [12]. Alternatively, the association may reflect secretion of apoC-II as a component of VLDL. **In contrast, we found no association between plasma apoC-II and the FCRs of VLDL-apoB and triglycerides. Given the role of apoC-II as activator of LPL, the result was unexpected. However, the lack of association might reflect that low concentrations of apoC-II are sufficient to fully activate LPL. At higher concentrations, apoC-II is present in excess and the expected associations between concentration and kinetics might not be observed. In hyperlipidaemic subjects, however, an**

association between apoC-II and FCR might be evident because of a lower apoC-II/VLDL particle ratio, although this remains to be demonstrated. Future studies that examine the metabolism of apoC-II in TRL and other lipoprotein fractions, particularly HDL, may better explain this relationship [13].

Of interest, we observed that overweight-obese women had higher plasma apoC-II concentrations compared with normal weight women (21.7 ± 1.86 vs. 36.3 ± 5.72 mg/L, $P=0.01$), which was chiefly related to higher apoC-II production (1.01 ± 0.14 vs. 1.72 ± 0.26 mg/kg/day, $P=0.01$). We also found that the difference in apoC-II production remained significance between the two groups after adjusting for VLDL₁ and VLDL₂ apoB-100 and triglyceride (all $P<0.05$), with implication that the regulation of apoC-II production is likely to be independent of VLDL production. This result also suggests that weight reduction may be useful to lower apoC-II concentrations as it is secreted by white adipose tissue [14].

Our study found that high apoC-III concentration, as opposed to apoC-II, was associated impaired VLDL₁ and VLDL₂ catabolism. This is consistent with the notion that apoC-III inhibits LPL activity and/or diminishes apoB- and apoE-mediated TRL clearance [6, 15-17]. It is noteworthy that high apoC-III was primarily associated with impaired conversion of VLDL₁ to VLDL₂ apoB-100 and triglyceride (data not shown). Furthermore, high apoC-III was chiefly associated with impaired direct catabolism of VLDL₂ apoB-100 and triglyceride. Our findings support the inhibition of apoC-III as a therapeutic target for hypertriglyceridaemia and CVD risk reduction [6]. Consistent with this notion, the selective inhibition of apoC-III with antisense drugs in hypertriglyceridaemic patients significantly lowered triglyceride concentrations [18]. Although the precise mechanism of action on VLDL metabolism remains to be elucidated, we would speculate that the FCRs of VLDL apoB-100 and VLDL-triglycerides would be increased with apoC-III inhibition.

Our study has limitations. First, the study is limited by its cross-sectional design and correlations are not proof of causality. Second, we studied a relatively homogeneous group of healthy white women. Future studies in different ethnicity and disease states are warranted. Because of the study design, we did not study women in the age range 46-54 yrs. Including women in this age bracket may have impacted our conclusions. Despite this, we noted that age was not significantly associated with any kinetic indices of VLDL₁ and VLDL₂ apoB and triglycerides across the study group or within the premenopausal or postmenopausal groups (data not shown). Third, we examined the kinetics of plasma apoC-II and apoC-III only. Future studies on the metabolism of these apolipoproteins within the TRL and HDL fractions would be of interest. The metabolism of apoC-I also warrants study, given its emerging role in regulating TRL metabolism [14]. Finally, we did not measure LPL mass or activity. Measurement of LPL mass or activity, and its inter-relationship with apoC-II and apoC-III may further corroborate our findings.

In conclusion, we propose that in women, plasma apoC-II and apoC-III are key regulators of TRL metabolism, independent of obesity, menopausal status and insulin resistance. While high concentrations of apoC-II and apoC-III are associated with elevated VLDL particle concentrations, increased production of VLDL particle underscores the relationship between high apoC-II and elevated VLDL concentration. By contrast, impaired catabolism of VLDL particles underscores the relationship between high apoC-III and elevated VLDL concentrations.

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