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Enhanced apoB48 metabolism in lipoprotein lipase X447 homozygotes

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Abstract

Rationale: Lipoprotein lipase (LPL) X447 homozygotes are characterized by enhanced conversion of TRL apoB100. Here, we set out to investigate whether this LPL variant is also associated with enhanced apoB48 clearance. Therefore, we evaluated apoB48 kinetics in X447 homozygotes in the fed state by infusion of isotope L-[1-^{13}C]-valine and subsequent compartmental modeling.
Methods and results: ApoB48 metabolism was assessed in five X447 homozygotes (X/X genotype) and five S447 homozygotes (S/S genotype). Subjects were continuously fed and received infusion of stable isotope L-[1-^{13}C]-valine. Results were analyzed by SAAM II modeling. Fasting (2.4-fold, $p = 0.02$) as well as non-fasting (1.6-fold, $p = 0.09$) apoB48 concentration was increased in the X447 homozygotes compared to S447 homozygotes. In addition, the X447 homozygotes exhibited a 1.7-fold higher apoB48 poolsize ($p = 0.04$). Interestingly, apoB48 fractional catabolic rate (FCR) was 1.9-fold higher ($p = 0.007$) and apoB48 synthesis was more than two-fold higher ($p = 0.006$) in the X447 homozygotes compared to S447 homozygotes.
Conclusion: In the present study, we show that X447 homozygotes exhibit enhanced apoB48 clearance. Previously, these homozygotes were shown to present with enhanced apoB100 TRL conversion. Combined, this LPLS447X gain of function variant affects apoB48 as well as apoB100 TRL metabolism.

Keywords: Lipoprotein lipase; X447; ApoB48; Chylomicron; Metabolism

1. Introduction

The negative consequences of increased levels of triglyceride (TG)-rich lipoproteins (TRL) on atherogenesis have generally been acknowledged [1,2]. The enzyme lipoprotein lipase (LPL) drives, to a large extend, the processing of these TRL. LPL hydrolyses TG in chylomicrons (CM), as well as in VLDL. These lipoproteins have separate structural apolipoproteins; apoB48 and apoB100, respectively.

The X447 variant in the LPL gene is associated with decreased TG and increased HDL-C as well as a lower incidence of cardiovascular disease (CVD) [3]. Even though the precise mechanism for this apparent cardiovascular protection remains to be determined, X447 homozygotes were recently shown to exhibit enhanced apoB100 TRL conversion [4]. This enhanced conversion was the first in vivo evidence for the gain of function associated with this particular LPL gene variant. In addition, X447 has also been reported to have a direct effect on postprandial apoB48 levels [5]. We hypothesized that the effect of X447 might also extend towards increased apoB48 clearance. To test this hypothesis, we eval-

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uated kinetics of apoB48 in X447 homozygotes in the fed state by infusion of isotope L-[1-^{13}C]-valine and subsequent compartmental modeling.

2. Materials and methods

2.1. Participants

Five male X447 homozygotes (X/X genotype) were selected from the genetic database of the department of Vascular Medicine of the Academic Medical Center in Amsterdam, as reported previously [4]. The control group, i.e. S447 homozygotes (S/S genotype), was described elsewhere [4] and consists of five healthy male individuals that were selected from the same genetic database. The five controls were selected to match the X447 homozygotes with respect to age, body mass index (BMI), smoking habits, lipid levels and use of alcohol. In addition, none of the subjects had signs of cardiovascular disease or used medication. The presence of natural variants in apolipoprotein E (apoE) gene, apoE2 or apoE4, have been shown to affect lipid metabolism [6]. ApoE genotyping was performed in the participants and the subjects did not have E2/E2 or E4/E4 genotype. All participants gave written informed consent. They were asked to refrain from alcohol-containing beverages for at least 3 days before the start of the study. The study protocol was approved by the Institutional Review Board of the AMC. The study conforms to the principles outlined in the Declaration of Helsinki.

2.2. Genotyping

We performed LPL gene analyses in more than 2000 DNA samples from healthy male subjects from the genetic database of the department of Vascular Medicine and found only five males to be X447 homozygotes and were willing to participate. The LPL gene analysis was as follows as described by Kuivenhoven et al. [7]. In short, we amplified the target sequence of the *LPL* gene (terminal part of exon 9) by using 5′-TACACTAGCAATGTCTAGGTGA-3′ as upstream primer and 5′-TCAGCTTTAGCCCAGAATGC-3′ as downstream primer. The amplification reactions were performed in 10 mmol/L Tris–HCl (pH 9.0), 50 mmol/L KCl, 0.1% w/v gelatin, 1.5 mmol/L $MgCl_2$, 1% Triton X-100 and 0.2 mg/mL bovine serum albumin containing 0.1–0.5 μg genomic DNA and final concentrations of 200 μmol/L dNTPs and 0.5 μmol/L primers in a total volume of 50 μL. After initial denaturation (10 min, 95 °C), 1.0 U thermostable DNA polymerase (Supertaq; HT Biotechnology Ltd.) was added, followed by 30 amplification cycles of 95 °C (1 min), 60 °C (1 min) and 72 °C (1 min) with a final extension step of 10 min at 72 °C. Twenty percent of the PCR reaction product was used for digestion with 3 U *Mnl* I according to the instructions of the manufacturer (New England Biolabs) in a total volume of 20 μL for 2 h at 37 °C. After electrophoresis of the PCR product in 3% agarose containing ethidium bromide, DNA restriction fragments were visualized and analyzed on a transilluminator.

2.3. Experimental protocol

The protocol for infusion of labeled valine has been described in detail [4]. After baseline blood sampling ($t = 0$), a hospital-made food drink was ingested hourly. This food drink, equivalent to one-twentieth of subjects daily food intake, was ingested every hour and consisted of 14% of calories as protein, 44% as carbohydrates, 42% fat (17% saturated, 17% monounsaturated and 8% polyunsaturated) and 90 mg cholesterol per 1000 kcal as previously published [4]. Five hours after baseline sampling, a priming dose of 17 μmol/kg L-[1-^{13}C]-valine was given intravenously, followed by a continuous infusion of 15 μmol/kg/h for 13 h. Blood samples were obtained from the contralateral arm at baseline and after 5, 5.5, 6, 7, 8, 9, 10, 11, 12, 14, 16 and 18 h.

2.4. Isotope and chemicals

L-[1-^{13}C]-valine (isotope mole fraction > 0.99; Mass-Trace, Woburn, MA, USA) was dissolved in sterile 0.9% saline and sterilized through a 0.22 μm filter [8]. Density solutions were made with KBr in 0.9% NaCl.

2.5. Biochemical measurements

Blood for lipid analysis was drawn in EDTA-coated tubes. Analyses of baseline lipids (TC, HDL-C, LDL-C and TG) and apoB100 are described in detail in Ref. [4]. Plasma apoAI concentration was determined by immunonephelometry (Dade Behring). Plasma apoB48 concentration was measured by a sandwich ELISA using anti-human apoB48 monoclonal antibodies as reported previously [9].

2.6. Isolation of free amino acids, apoB48 from lipoproteins

The isolation of free amino acids from plasma was performed as described in detail elsewhere [10]. Isolation of apoB containing lipoproteins was performed as described below. In short, CM, $VLDL_1$, $VLDL_2$, IDL and LDL were isolated using a discontinuous salt gradient by cumulative ultracentrifugation (UC; Beckman Ultracentrifuge Sw41 Ti rotor; Beckman Instruments). After three subsequent UC spins, chylomicron (CM) fraction (32 min, 40,700 rpm, 4 °C, acceleration 5, brake 5), $VLDL_1$ fraction (3:28 h, 40,700 rpm, 4 °C, acceleration 5, brake 5) and $VLDL_2$, IDL, LDL and bottom fraction (HDL + lipoprotein-deficient plasma) were collected after 17 h. After sample collection of the first and the second UC spin, the UC tubes were refilled using 1 mL, $d = 1.006$ g/mL KBr. All fractions were aliquoted and stored at −20° C. ApoB from all isolated lipoprotein fractions was precipitated and delipidated followed by separation of apoB48 and apoB100 protein by preparative SDS-PAGE