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Metabolism of apolipoprotein A-II containing triglyceride rich ApoB lipoproteins in humans

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Objective: To characterize human triglyceride-rich lipoproteins (TRL) with and without apoA-II and to study their metabolism *in vivo*.
Methods: Plasma from 11 participants on a controlled diet given a bolus infusion of [D_5]L-phenylalanine to label apoB was combined into four pools and applied to anti-apoA-II immunoaffinity columns. Fractions with and without apoA-II were separated into VLDL and IDL by ultracentrifugation; lipids and apolipoproteins were measured. For kinetic measurements, apoB was isolated and hydrolyzed to the constituent amino acids. Tracer enrichment was measured by GCMS. Metabolic rates were determined by SAAM-II.
Results: VLDL and IDL with apoA-II comprised 7% and 9% of total VLDL and IDL apoB respectively. VLDL with apoA-II was enriched in apoC-III, apoE, and cholesterol compared to VLDL without apoA-II. Mean apoB FCR of VLDL with apoA-II was significantly lower than for VLDL without apoA-II (2.80 ± 0.96 pools/day v.s. 5.09 ± 1.69 pools/day, $P = 0.009$). A higher percentage of VLDL with apoA-II was converted to IDL than was cleared from circulation, compared to VLDL without apoA-II (96 ± 8% vs. 45 ± 22%; $P = 0.007$). The rate constants for conversion of VLDL to IDL were similar for VLDL with and without apoA-II. Thus, a very low rate constant for clearance accounted for the lower FCR of VLDL with apoA-II.
Conclusion: VLDL with apoA-II represents a small pool of VLDL particles that has a slow FCR and is predominantly converted to IDL rather than cleared from the circulation.
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1. Introduction

Apolipoprotein A-II (apoA-II) is the second most abundant protein constituent of high-density lipoprotein (HDL), accounting for about 20% of HDL protein [1]. The mean plasma total apoA-II concentration is about 30–35 mg/dL [2]. Although apoA-II is mainly found associated with HDL, a small proportion is associated with the apoB lipoproteins, especially chylomicrons and very-low-density lipoprotein (VLDL) [3].

Animal models have shown that apoA-II can directly affect VLDL metabolism [4–6]. Transgenic mice expressing human apoA-II at two to three times higher than the normal concentration of mouse apoA-II displayed hypertriglyceridemia [4]. The lipoprotein lipase mediated lipolysis of VLDL particles containing apoA-II was impaired in these mice [4]. Secretion of VLDL was not affected [4]. A subsequent study confirmed that human apoA-II transgenic mice had reduced hydrolysis of triglycerides from chylomicrons and VLDL, but also found a 25% increase in triglyceride secretion [5]. These findings suggest that the hypertriglyceridemia in human apoA-II overexpressing mice is associated with increased secretion and defective catabolism by lipoprotein lipase of triglyceride-rich lipoproteins.

The role of apoA-II in human VLDL metabolism is unclear. Patients who have severe hypertriglyceridemia (Type V hyperlipoproteinemia) have apoB lipoproteins that are enriched in apoA-

Abbreviations: TRL, triglyceride rich lipoproteins; VLDL, very low density lipoproteins; apoB, apolipoprotein B; apoA-II, apolipoprotein A-II; LPL, lipoprotein lipase; FCR, fractional catabolic rate.

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II that are poor substrates for lipoprotein lipase [3]. Patients with Tangier disease have VLDL that contain a large amount of apoA-II that is also a poor substrate for lipoprotein lipase, and they have mild hypertriglyceridemia [3,7]. Alaupovic et al. separated apoB lipoproteins from patients with severe hypertriglyceridemia or Tangier disease into lipoproteins with or without apoA-II [3]. Importantly, the apoB lipoproteins from both groups that contained apoA-II had impaired reactivity to lipoprotein lipase compared to lipoproteins that did not contain apoA-II [3]. In other respects, the lipid and apolipoprotein composition of the apoB lipoproteins that had apoA-II was similar to VLDL that did not have apoA-II. These studies in mice and humans raise the possibility that apoA-II contributes to hypertriglyceridemia by reducing lipolysis in plasma of triglyceride-rich lipoproteins. It is also possible that apoA-II, as a surface protein, interferes with clearance of VLDL from the circulation by apoE or apoB interacting with hepatic receptors, similar to the action of another VLDL surface protein, apoC-III [8].

It is unknown whether apoA-II affects VLDL metabolism in normolipidemia or in common phenotypes of mild hypertriglyceridemia. No studies have directly examined the relationship of apoA-II to VLDL metabolism *in vivo* in humans. To do this, we studied the metabolism of VLDL and IDL that contain apoA-II compared to those that do not have apoA-II in individuals who have normal or mildly increased fasting triglyceride levels.

2. Materials and methods

2.1. Subjects

11 participants evaluated were recruited as part of a previous study. [8,9] Exclusion criteria included secondary hyperlipidemia; Apo E2/E2, E4/E4, and E2/E4 genotypes and use of medications that affect lipid metabolism. The study was approved by the Human Subjects Committees at Harvard School of Public Health and Brigham and Women's Hospital. All participants gave informed consent.

2.2. Dietary period

All study subjects were provided complete diets rich in either monounsaturated fat or carbohydrate for 3 weeks before the kinetic study. The participants as outpatients were required to eat one meal each weekday on-site, either lunch or dinner. The monounsaturated fat diet consisted of 37% fat (8% saturated fat, 24% monounsaturated fat, and 5% polyunsaturated fat), 48% carbohydrate, and 15% protein. The carbohydrate diet consisted of 20% fat (7% saturated fat, 8% monounsaturated fat, and 5% polyunsaturated fat), 65% carbohydrate, and 15% protein. Participants were instructed not to consume alcohol or any other source of calories. Energy intake was adjusted to keep body weight constant during the study period.

2.3. Kinetic study

Participants received a bolus injection of 1.2 μmol/kg [D5] L-phenylalanine (Tracer Technologies, Cambridge, MA). Blood samples were collected every 30 min for the first two hours and hourly thereafter for a total of 14 h. To maintain a constant postprandial state, small hourly meals were consumed. The daily intake was divided into 12 portions to obtain the hourly intake level. Hourly food intake started 3 h before the tracer was administered. This technique has been used previously by Zheng et al. in the same kinetic protocol with these participants [9].

2.4. Separation of lipoproteins

Because the plasma from these participants in this kinetics protocol was obtained and used in a previous study, quantities were limited. A pooling scheme was therefore devised to create enough sample at baseline and at each time point during the tracer infusion so that the lipoprotein fractionation could yield sufficient quantity for measurements of tracer enrichment and composition of apoA-II containing VLDL which we determined had a very low plasma concentration. The 11 participants were divided into four groups each with 2–3 individuals and their plasma was combined to create 4 pools for analysis of kinetics and composition. One pool came from normolipidemic participants (N = 3), and three came from mildly hypertriglyceridemic participants (N = 2, 3, 3). Two pools each came from participants on the low-fat diet and the high-monounsaturated fat diets (Table 1). The availability of sufficient plasma governed the selection of the specific participants that were pooled. We emphasize that the aim of this study was to compare the kinetics of TG-rich lipoproteins that contained apoA-II with those that did not apoA-II, and not to explore modulation of the kinetics by diet type for which this protocol is not suited. Another reason for pooling is to reduce the between-subjects variability in kinetic parameters, and to reduce the chance of outliers.

An equal amount of plasma from each individual in a group was pooled for each time point. Pooled plasma from each time point was loaded into 20 mL Econo-Pac columns (Bio-Rad Laboratories, Hercules, CA) packed with 2.5 mL of anti–apoA-II resin prepared from polyclonal sheep anti–human apoA-II antibody bound to Sepharose 4B Resin at a minimum concentration of 10 mg antibody/mL resin (Academy Biomedical Company Inc, Houston, TX). Samples and resin were incubated for 16 h at 4 °C with mixing. The unbound fraction was eluted from the column by gravity followed by washes with phosphate buffered saline. The bound fraction was then eluted from the columns with 3 mol/L sodium thiocyanate in phosphate-buffered saline. The efficiency of the immunoaffinity separation (percent of apoA-II removed from plasma by the resin) was 93%.The two immunofractions were then fractionated by density ultracentrifugation [9]. The fractions were centrifuged in an L8-70M instrument (Beckman, Brea, CA) equipped with a Ti25 rotor for 1 h at 25,000 rpm to float light VLDLs [Svedberg units of flotation (Sf) 60-400]. After light VLDL was collected by aspiration, the initial volume was restored with d = 1.006 g/mL phosphate buffered saline and dense VLDL (Sf: 20–60) was isolated by spinning for 16 h at 25,000 rpm. Following collection of dense VLDL, the initial volume was restored with potassium bromide (KBr) solution to simultaneously adjust the density to 1.025 g/mL. The samples were then spun at 25,000 rpm for 16 h to isolate IDL (1.006–1.025 g/mL). Finally, after IDL was collected, the sample density was again adjusted with KBr to 1.050 g/mL, the samples were spun for 24 h at 25,000 rpm, and LDL (1.025–1.050) was collected.

We combined light and dense VLDL data because the individual concentrations and tracer enrichments were too low for reliable measurement. Also, in this population, apoA-II concentrations in LDL were near or below the detection limit (0.001 mg/dL), not exceeding 0.01 mg/dL or 0.5% of total for any individual. Furthermore, the percentage of LDL apoB that contained apoA-II was less than 1% and was not detectable in most samples by GC–MS. Therefore, this study focused on apoB lipoproteins in the VLDL and IDL fractions. In this study, VLDL and IDL refer to the lipoproteins present in the respective density fractions, both apoB48 and apoB100.

2.5. Measurement of apoB tracer enrichment and pool size

ApoB was precipitated from the lipoproteins with isopropanol, a norleucine internal standard was added, and the mixture was

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