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Distinct metabolism of apolipoproteins (a) and B-100 within plasma lipoprotein(a)☆

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ABSTRACT

Objectives. Lipoprotein(a) [Lp(a)] is mainly similar in composition to LDL, but differs in having apolipoprotein (apo) (a) covalently linked to apoB-100. Our purpose was to examine the individual metabolism of apo(a) and apoB-100 within plasma Lp(a).

Materials and Methods. The kinetics of apo(a) and apoB-100 in plasma Lp(a) were assessed in four men with dyslipidemia [Lp(a) concentration: 8.9–124.7 nmol/L]. All subjects received a primed constant infusion of [5,5,5-$^2H_3$] L-leucine while in the constantly fed state. Lp(a) was immunoprecipitated directly from whole plasma; apo(a) and apoB-100 were separated by gel electrophoresis; and isotopic enrichment was determined by gas chromatography/mass spectrometry.

Results. Multicompartmental modeling analysis indicated that the median fractional catabolic rates of apo(a) and apoB-100 within Lp(a) were significantly different at 0.104 and 0.263 pools/day, respectively ($P = 0.04$). The median Lp(a) apo(a) production rate at 0.248 nmol/kg · day^{-1} was significantly lower than that of Lp(a) apoB-100 at 0.514 nmol/kg · day^{-1} ($P = 0.03$).

Conclusion. Our data indicate that apo(a) has a plasma residence time (11 days) that is more than twice as long as that of apoB-100 (4 days) within Lp(a), supporting the concept that apo(a) and apoB-100 within plasma Lp(a) are not catabolized from the bloodstream as a unit in humans in the fed state.

Abbreviations: apo, apolipoprotein; CHD, coronary heart disease; *d*, density; FCR, fractional catabolic rate; GC/MS, gas chromatography/mass spectrometry; IDL, intermediate density lipoprotein (*d* 1.006–1.019 g/mL); KIV_1, kringle IV_1; KIV_2, kringle IV_2; Lp(a), lipoprotein(a); PAS, periodic acid Schiff's base; PCSK9, proprotein convertase subtilisin/kexin type 9; PR, production rate; PS, pool size; TC, total cholesterol; TG, triglycerides; VLDL, very low-density lipoprotein (*d* < 1.006 g/mL).

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1. Introduction

Lipoprotein(a) [Lp(a)] was first described by Käre Berg in 1963 [1]. It is a lipoprotein similar in lipid content and composition to LDL but different in having apolipoprotein (apo) (a) covalently linked to apoB-100 by a disulfide bond [2–4]. Apo(a), a heavily glycosylated protein, shares homology with several regions of plasminogen [5]. It is highly polymorphic in size due to different numbers of the kringle IV type 2 (KIV_2) domain, ranging from a minimum of 3 to more than 40 [6,7].

Elevated plasma concentrations of Lp(a) are associated with increased coronary heart disease (CHD) risk [8–10]. Lp(a) is considered to have a pro-atherogenic effect since the particle preferentially accepts oxidized phospholipids from LDL, leading to lipid deposition in the arterial intima [11] and, thereby, promoting multiple oxidative and inflammatory actions [12]. It is also thought to have a prothrombotic effect due to the similarity that apo(a) has with the fibrinolytic proenzyme plasminogen [13,14]. As a result, elevated Lp(a) concentrations may lead to excess clot formation, which is often the terminal event resulting in coronary artery occlusion. Plasma concentrations of Lp(a) are heritable, vary greatly among ethnic populations, and, in large part, are determined by variations at the apo(a) gene locus, including the number of KIV_2 repeats and specific single nucleotide polymorphisms [15–18].

Lp(a) metabolism is still not fully defined [19]. Initial metabolic studies utilizing radioiodinated Lp(a) showed an inverse association between plasma Lp(a) concentrations and apo(a) isoform size, due mainly to differences in production rates [20]. Apo(a) is synthesized primarily by the liver. Linkage of this glycoprotein with apoB-100-containing lipoproteins to form Lp(a) is thought to occur extracellularly, perhaps in the space of Disse or on the surface of hepatocytes [21], although the concept is debated [22]. Less is known about the sites and mechanisms responsible for the clearance of Lp(a) from plasma. There is evidence that the kidney plays a significant role as Lp(a) plasma concentrations are elevated and the apo(a) catabolic rate is significantly lower in patients with impaired renal function [23]. Direct evidence for renal uptake of Lp(a) in humans is lacking, and recent cell and animal studies point to the liver as the primary site of catabolism, with multiple receptors potentially involved in the process [24,25].

While the majority of Lp(a) particles resemble LDL in density and composition, apo(a) immunoreactivity has been reported across the entire density distribution of lipoproteins, with up to 2% of immunoreactive apo(a) being in the floatation density (d) <1.006 g/mL fraction of fasting normolipidemic plasma [26–28]. In the fed state, as well as in hypertriglyceridemic individuals, the amount of apo(a) associated with triglyceride-rich lipoproteins can be increased markedly [28–31]. The origin of Lp(a) particles associated with the $d < 1.006$ g/mL fraction is not clear. They could be derived from the liver in response to a fatty meal and/or produced by remodeling events in the circulation.

Historically the metabolism of apo(a) has been assessed in Lp(a) isolated from plasma by ultracentrifugation at d 1.05–1.15 g/mL (see Discussion). In this density range, apo(a) and apoB-100 in Lp(a) have been found to have similar rates of catabolism [23,32,33]. We found, however, that when Lp(a) was isolated from non-fasting, whole plasma using lectin-mediated affinity chromatography and no ultracentrifugation, apo(a) in Lp(a) was cleared from plasma at half the rate of apoB-100 in Lp(a) [34], a finding which contradicts the concept of Lp(a) being cleared from circulation as an integral particle. In the present study, we have used a more specific isolation procedure, namely, immunoprecipitation with a monoclonal antibody against human apo(a), to assess the individual metabolism of the apo(a) and apoB-100 moieties within plasma Lp(a). Kinetic parameters were determined in subjects with dyslipidemia while in the constantly fed state.

2. Methods

2.1. Study Design and Subjects

This study aimed to compare the kinetic parameters of apo(a) and apoB-100 in plasma Lp(a) in untreated healthy volunteers. The study constituted part of a larger randomized, double-blind, placebo-controlled, crossover study examining the effects of extended-release niacin 2 g/day (Niaspan, Abbott Laboratories, Abbott Park, IL) and extended-release niacin 2 g/day in combination with lovastatin 40 mg/day (Advicor, Abbott Laboratories, Abbott Park, IL), relative to placebo, on the metabolism of apoB-100, apoB-48, and apoA-I [35]. Each phase lasted 12 weeks and was separated by a 4-week washout phase. While the present study was not designed to examine the effects of niacin on Lp(a) metabolism, the availability of plasma samples from the niacin phase did allow us to assess the kinetics of Lp(a) apo(a) and Lp(a) apoB-100 separately in a metabolic condition known to lower Lp(a) levels [36]. These data are included as Supplementary Material.

Five men (age: 52.8 ± 4.9 y; BMI: 30.1 ± 1.7 kg/m^2) with dyslipidemia were enrolled in the study [35]. They were eligible to participate based on the following plasma lipid criteria: triglyceride (TG) concentration ≥150 mg/dL, LDL cholesterol concentration ≥130 mg/dL, and HDL cholesterol concentration <40 mg/dL. Exclusion criteria included age <40 years, myocardial infarction in the past 6 months, smoking, thyroid dysfunction, liver or kidney disease, liver cancer, diabetes mellitus, stroke, and current use of medications known to affect lipid metabolism. The study protocol was approved by the Institutional Review Board of Tufts Medical Center and Tufts University Health Sciences; and written informed consent was obtained from each study subject.

At the end of each phase, the subjects underwent a 15 h primed-constant infusion of deuterated leucine ([5,5,5-$^2H_3$]-L-leucine, C/D/N Isotopes, Pointe-Claire, Quebec), 10 μmol/kg body weight per hour, under constantly fed conditions, as previously described [35,37]. Briefly, the subjects were fed hourly for 20 h with small identical meals, the composition of which complied with the Therapeutic Lifestyle Changes diet (<30% of calories as total fat, <7% saturated fat, <200 mg/day cholesterol) [38]; the meals started 5 h before and continued throughout the infusion. Blood samples were collected into tubes containing EDTA (0.15%) just before the infusion (0 h) and at 30, 35, 45 min and 1, 1.5, 2, 3, 4, 6, 9, 12, 14, and 15 h during the infusion. Plasma was separated by centrifugation at 2500 rpm at 4 ° C for 30 min, divided into aliquots for Lp(a) measurement or isolation, and stored at −80 °C until analysis. Freshly separated plasma from each infusion time point was also subjected to sequential density ultracentrifugation to