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Normal HDL–apo AI turnover and cholesterol enrichment of HDL subclasses in New Zealand rabbits with partial nephrectomy

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ABSTRACT

Objective. The kidney has been proposed to play a central role in apo AI catabolism, suggesting that HDL structure is determined, at least in part, by this organ. Here, we aimed at determining the effects of a renal mass reduction on HDL size distribution, lipid content, and apo AI turnover.

Methods. We characterized HDL subclasses in rabbits with a 75% reduction of functional renal mass (Nptx group), using enzymatic staining of samples separated on polyacrylamide electrophoresis gels, and also performed kinetic studies using radiolabeled HDL–apo AI in this animal model.

Results. Creatinine clearance was reduced to 35% after nephrectomy as compared to the basal values, but without increased proteinuria. A slight, but significant modification of the relative HDL size distribution was observed after nephrectomy, whereas cholesterol plasma concentrations gradually augmented from large HDL2b (+54%) to small HDL3b particles (+150%, $P<0.05$). Cholesteryl esters were the increased fraction; in contrast, free cholesterol phospholipids and triglycerides of HDL subclasses were not affected by nephrectomy. HDL–apo AI fractional catabolic rates were similar to controls.

Conclusion. Reduction of functional renal mass is associated to enrichment of HDL subclasses with cholesteryl esters. Structural abnormalities were not related to a low apo AI turnover, suggesting renal contribution to HDL remodeling beyond being just a catabolic site for these lipoproteins.

Abbreviations: CETP, cholesteryl ester transfer protein; CHD, coronary heart disease; EDTA, ethylenediaminetetraacetic acid; FCR, fractional catabolic rate; HDL, high-density lipoproteins; HDL-C, HDL-cholesterol; LCAT, lecithin: cholesterol acyltransferase; Nptx, nephrectomized; Ph, phospholipids; PR, production rate.

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1. Introduction

Several clinical and epidemiological studies have demonstrated the negative correlation between HDL-cholesterol (HDL-C) and the incidence of coronary heart disease risk (CHD). The protective effect of HDL particles against CHD has been explained by their ability to transport cholesterol from peripheral tissues to the liver to be eliminated through the bile. In addition, HDLs have antioxidant and anti-inflammatory actions [1,2].

HDLs include a heterogeneous group of lipoproteins that can be classified by size (in decreasing order) into HDL2b, HDL2a, HDL3a, HDL3b, HDL3c [3]. These HDL subclasses differ in their physicochemical properties, and it has been suggested that they have different antiatherogenic characteristics [4,5].

HDLs structure seems to be determinant for their intravascular metabolism and removal from the plasma compartment; small HDLs seem to be catabolized faster than larger ones in mice [6,7]. Indeed, several studies have suggested a relationship between HDLs structure and apo AI turnover [8–11].

The kidney is usually not regarded as an important organ in HDL metabolism because the glomerular filtration barrier prevents large molecules such as lipoprotein particles from being filtered into the pre-urine. Nevertheless, the proximal tubule epithelium expresses receptors involved in the uptake of a broad spectrum of ligands, including apolipoproteins [12]. Furthermore, abnormal renal function is associated with abnormal HDL-cholesterol and apo AI plasma levels [13–15]. These changes could be the result of co-morbidities accompanied by a decreased glomerular excretion rate (i.e., hypertriglyceridemia, insulin resistance, and decreased lipoprotein lipase mass and activity). However, early studies reported that the main catabolic tissue for apo AI–HDL is the kidney, as has been demonstrated in rats using tyramine cellobiose-labeled HDL [16].

Considering the potential role of the kidney in apo AI catabolism [12,14,16] and the relationship between HDL structure and its metabolism [7–11], we hypothesized that the kidney is a determinant of HDL size distribution, chemical composition, and turnover. We performed kinetic studies using labeled HDL–apo AI in rabbits with a 75% reduction of renal mass by nephrectomy, and also characterized HDL subclasses in this animal model.

2. Material and methods

2.1. Animals

New Zealand White male rabbits (3.0 to 3.5 kg) were anesthetized with xylazine and inhaled isoflurane. In a first intervention, two branches of the left renal artery were acutely infarcted by ligation. Two weeks later, a second surgery was performed to completely remove the right kidney. In order to preserve adrenal function in nephrectomized rabbits, the perirenal fat was carefully separated from the kidney and the glands were not manipulated. Sham surgical experiments were performed in 10 rabbits to be used as comparison group for kinetic studies. Following the second operation, animals were given a 4-week convalescence period, after which all animals showed a full recovery and returned to normal dietary and behavioral habits. Animals had free access to a standard chow diet and water. Assessments were made 4 to 5 weeks after the second surgery. Twenty-four-hour urine collections were performed in metabolic cages for determination of proteinuria and creatinine clearance. All procedures were performed in accordance with the Scientific and Ethics Committees of the Instituto Nacional de Cardiología "Ignacio Chávez".

2.2. Isolation of HDL particles and analysis of their structure

Twelve-hour fasting blood samples were drawn from the marginal ear vein for the HDL structure analysis as previously described [8]. Briefly, HDLs were separated by ultracentrifugation in a Beckman optima TLX table centrifuge and dialyzed against 0.09 M Tris/0.08 M boric acid/3 mM EDTA buffer, pH 8.4.

The homogeneity and hydrodynamic diameter of HDL particles were estimated as previously described [17–19] with slight modifications. Briefly, HDL samples were separated according to their size by non-denaturing 3%–30% polyacrylamide gradient gel electrophoresis. Gels were stained for total cholesterol, phosphatidylcholine, and triglycerides using non-commercially available enzymatic mixtures recently developed by our group [17,18]. Cholesterol esterase, cholesterol oxidase, and peroxidase at a final concentration of 0.075 U/mL, 0.05 U/mL, and 0.25 U/mL, respectively, were dissolved in a 150 mM NaCl, 8.6 mM Na_2HPO_4, 1.4 mM NaH_2PO_4 buffer (PBS), pH 7.4. For free-cholesterol staining, cholesterol esterase was omitted in the enzymatic mixture [19]. The reactive mixture for phosphatidylcholine staining contained phospholipase D, choline oxidase, and peroxidase at a final concentration of 0.15 U/mL, 60 U/mL, and 0.25 U/mL, respectively. For triglycerides, the enzymatic mixture contained 40 U/mL lipase, 0.1 U/mL glycerol kinase, 0.4 U/mL glycerol 3-phosphate oxidase, and 0.25 U/mL peroxidase in PBS. The mixture also contained 1 mmol/L $MgCl_2$, and 0.25 mmol/L ATP. All reaction mixtures also included 3 mM sodium cholate, 0.1% Triton 100X, 0.4 mM thiazolyl blue tetrazolium bromide, 0.6 mM phenazine methosulphate, and carboxymethylcellulose at 1.4% as viscosant agent. Incubation times ranged between 60 and 75 min at 37 °C in the dark for any lipid stained on electrophoresis gels [17–19].

Electrophoresis gels were gently washed in PBS and scanned in a GS-670 BioRad densitometer (scan 1), destained and further re-stained for proteins with Coomassie R-250 and scanned (scan 2). The relative proportions of each HDL subclass determined per protein were estimated by optical densitometry analysis of scan 2, using as reference proteins (thyroglobulin, 17 nm; ferritin, 12.2 nm; catalase, 10.4 nm; lactate dehydrogenase, 8.2 nm; and albumin, 7.1 nm; high-molecular weight calibration kit, Amersham Pharmacia Biotech, Buckinghamshire, UK) [3]. Relative proportion of each HDL subclass is expressed as the percentage of the total HDL area under the curve. For the classification of the HDL subclasses, we considered the following size intervals: HDL3c, 7.94–8.45 nm; HDL3b, 8.45–8.98 nm; HDL3a, 8.98–9.94 nm; HDL2a, 9.94–10.58 nm; and HDL2b, 10.58–13.59 nm [3,17].