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Cholesterol delivery to the adrenal glands estimated by adrenal venous sampling: An *in vivo* model to determine the contribution of circulating lipoproteins to steroidogenesis in humans

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BACKGROUND: Cholesterol, required for adrenal steroid hormone synthesis, is at least in part derived from circulating lipoproteins. The contribution of high-density lipoproteins (HDL) and low-density lipoproteins (LDL) to adrenal steroidogenesis in humans is unclear.

OBJECTIVE: The aim of the study was to determine the extent to which HDL and LDL are taken up by the adrenal glands using samples obtained during adrenal venous sampling (AVS).

METHODS: AVS was successfully performed in 23 patients with primary aldosteronism. Samples were drawn from both adrenal veins and inferior vena cava (IVC). HDL cholesterol (HDL-C) and lipoprotein particle profiles were determined by nuclear magnetic resonance spectroscopy. Apolipoprotein (apo) A-I and apoB were assayed by immunoturbidimetry.

RESULTS: Plasma HDL-C and HDL and LDL particle concentrations (HDL-P and LDL-P) were not lower in samples obtained from the adrenal veins compared with the IVC (HDL-C, $P = .59$; HDL-P, $P = .06$; LDL-P, $P = .93$). ApoB was lower in adrenal venous plasma than in IVC ($P = .026$; $P < .05$ for right adrenal vein). In 13 patients with an aldosterone producing adenoma (APA), apoB was also lower ($P = .045$) and LDL-P tended to be lower ($P = .065$) in the APA adrenal vein compared with the IVC. ApoA-I was not lower in adrenal venous plasma compared with the IVC, neither in the whole group ($P = .20$) nor in the APA subgroup ($P = .075$).

CONCLUSION: These *in vivo* observations suggest that circulating LDL may contribute to adrenal steroidogenesis in humans as inferred from adrenal venous-IVC apoB concentration differences. AVS is a feasible method to investigate the relationships between lipoproteins and steroidogenesis.

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Introduction

Steroidogenesis by the adrenal glands is a complex enzymatic process by which cholesterol is converted to biologically active steroid hormones.[1] Cholesterol, targeted for the synthesis of adrenal glucocorticoids, mineralocorticoids, and androgens, originates from *de novo* intracellular synthesis, from intracellular catabolism of stored cholesteryl esters, as well as from uptake of cholesterol carried by circulating lipoproteins.[1,2] It has been assumed that cholesterol derived from circulating lipoproteins represents an important source for adrenal steroidogenesis.[3–6]

Studies in mice have indicated that scavenger receptor class B, type I (SRBI) plays a pivotal role in the selective uptake of cholesteryl esters from high-density lipoproteins (HDL) particles, which are subsequently stored intracellular and converted into free cholesterol.[7,8] Accordingly, SRBI deficiency in rodents results in impaired adrenal steroidogenesis.[9–11] Mildly impaired adrenal function has also been documented in human subjects with heterozygous SRBI deficiency.[12] Moreover, it has been suggested that adrenal glucocorticoid production could be impaired in the context of low HDL cholesterol (HDL-C) due to heterozygous deficiency of lecithin–cholesterol acyltransferase (LCAT), which catalyzes the esterification of cholesterol, or to adenosine triphosphate–binding cassette transporter 1 (ABCA1) deficiency, a transmembrane receptor that facilitates cholesterol efflux from cells to (nascent) HDL particles.[5] Apolipoprotein (apo)B-containing lipoproteins, in particular, low-density lipoproteins (LDL), are considered a potentially important source of cholesterol for adrenal steroidogenesis as well.[13–15] These particles are taken up by LDL receptor–mediated endocytosis with subsequent degradation and intracellular release of cholesterol.[1] Adrenal function was, however, found to be uncompromised in subjects with heterozygous LDL receptor deficiency,[16] although modestly impaired adrenal function has been documented in abetalipoproteinemia, which is characterized by the absence of plasma apoB-containing lipoproteins.[17]

Little is known about the contribution of circulating lipoproteins to adrenal glucocorticoid synthesis in humans without genetic abnormalities affecting plasma levels or binding capacities of HDL and LDL. Adrenal venous sampling (AVS) is currently recommended as the preferred diagnostic procedure in patients with primary aldosteronism to differentiate between a unilateral aldosterone producing adenoma (APA) and bilateral adrenal hyperplasia (BAH).[18] This procedure provides a unique opportunity to compare HDL and LDL particle concentrations and characteristics in plasma obtained from the adrenal veins with the infra-adrenal inferior vena cava (IVC). We tested whether such lipoprotein measurements could provide an *in vivo* estimate of lipoprotein cholesterol uptake by the adrenal glands.

Subjects and methods

The studies were performed in a university hospital setting and have been exempted for approval according to the Dutch Medical Research Involving Human Subjects Act. This report is based on patient data and material acquired during routine care. Study subjects were hypertensive patients with biochemically confirmed primary aldosteronism (ie, elevated plasma aldosterone-renin ratio and nonsuppressible 24-hour urinary aldosterone excretion after a 3-day salt loading test) who underwent a successful AVS procedure for subtype classification. AVS was performed according to international recommendations.[19] In brief, tetracosactide (Synacthen) was administered intravenously during the procedure at an infusion rate of 50 μg/h starting 30 minutes before the procedure. The sequential blood sampling technique was performed, starting with catheterization of the right adrenal vein. Peripheral blood samples were drawn from the infra-adrenal part of the IVC. Catheter positioning was checked fluoroscopically using an iodine-containing X-ray contrast agent (Iomeron300) and confirmed with a rapid intraprocedural plasma cortisol measurement. A selectivity index (ie, plasma $\text{cortisol}_{\text{side}}$/plasma $\text{cortisol}_{\text{IVC}}$) $>$ 3.0 confirmed that the blood sample was taken from the adrenal vein.[18] Lateralization of aldosterone secretion was considered to be compatible with the presence of an APA when the lateralization index (ie, plasma $\text{aldosterone}_{\text{dominant}}$/plasma $\text{cortisol}_{\text{dominant}}$: plasma $\text{aldosterone}_{\text{nondominant}}$/plasma $\text{cortisol}_{\text{nondominant}}$) was $\geq$4.0 and the contralateral suppression index (ie, plasma $\text{aldosterone}_{\text{nondominant}}$/plasma $\text{cortisol}_{\text{nondominant}}$: plasma $\text{aldosterone}_{\text{IVC}}$/plasma $\text{cortisol}_{\text{IVC}}$) was $<$1.0.[18] The patients were studied after an overnight fast.

Laboratory methods

Plasma aldosterone was assayed with a competitive fixed-time solid-phase radioimmunoassay as described (Coat-a-Count; Siemens Medical Solutions Diagnostics).[20] Cortisol was measured by electrochemiluminescence immunoassay (Roche Modular Systems, Mannheim, Germany). Nonfasting plasma levels of total cholesterol, HDL-C, and triglycerides obtained during outpatient clinic visits were measured by routine biochemical methods. Non-HDL cholesterol was calculated as the difference between total cholesterol and HDL cholesterol. Very low–density lipoprotein (VLDL), LDL, and HDL particle profiles were measured by nuclear magnetic resonance (NMR) spectroscopy with the LipoProfile-3 algorithm, as described (LipoScience Inc; Laboratory Corporation of America Holdings Raleigh, NC).[21] Lipoprotein subclasses were quantified from the amplitudes of their spectroscopically distinct lipid methyl group NMR signals. Diameter range estimates were for VLDL (including chylomicrons if present): $>$60 nm to 27 nm, for LDL: 18 nm to 27 nm, and for HDL: 14 nm to 8.2 nm. The VLDL, LDL, and HDL particle concentrations (VLDL-P, LDL-P, and