Adrenal tumors
Buitenwerf, Edward

DOI:
10.33612/diss.96963155

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2019

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons). The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the “Taverne” license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.
Chapter 4

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

Edward Buitenwerf
Robin P. F. Dullaart
Anneke C. Muller Kobold
Thera P. Links
Wim J. Sluiter
Margery A. Connelly
Michiel N. Kerstens

*Journal of Clinical Lipidology. 2017;11:733–738*
Abstract

**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.
Adrenal lipoprotein uptake

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, 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 mg/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 cortisol$_{\text{side}}$/plasma 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 aldosterone$_{\text{dominant}}$/plasma cortisol$_{\text{dominant}}$ : plasma aldosterone$_{\text{nondominant}}$/plasma cortisol$_{\text{nondominant}}$) was ≥4.0 and the contralateral suppression index (ie, plasma aldosterone$_{\text{nondominant}}$/plasma cortisol$_{\text{nondominant}}$ : plasma aldosterone$_{\text{IVC}}$/plasma 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 HDL-P) were calculated as the sum of the respective lipoprotein subclasses. Weighted-average VLDL, LDL, and HDL sizes were derived from the sum of the diameter of each subclass multiplied by its relative mass percentage based on the amplitude of its methyl NMR signal (21). Quantification of HDL-C was accomplished by converting NMR particle numbers to lipid mass concentration units, assuming that the lipoprotein particles have normal lipid content. HDL cholesterol values correlate well with chemically measured values (21).

ApoB and apoA-I were measured by immunoturbidimetric assay performed on an Olympus AU680 chemistry analyzer using reagents and standards from Beckman Coulter Inc. The intra-assay coefficients of variation of lipoprotein subfractions, HDL-C, and apolipoproteins were all ≤3%.

**Methodological approach and statistical analysis**

To determine the uptake of circulating lipoprotein particles by the adrenal glands, it is necessary that the (apo)lipoprotein concentrations in the adrenal arteries and adrenal veins are comparable. Because catheterization of the adrenal arteries is not possible, we used samples drawn from the IVC instead. We assumed that plasma (apo)lipoprotein concentrations in the IVC and the adrenal arteries are similar as is illustrated by equal (apo)lipoprotein concentrations in the femoral artery and femoral vein (22). Thus, potential lipoprotein uptake by the adrenal gland was estimated by analyzing differences in plasma lipoprotein particle numbers and apolipoproteins in plasma samples between the adrenal veins and the IVC.

Data are expressed as median with interquartile range (IQR) or range. Differences between plasma (apo)lipoprotein-tein concentrations between each of the adrenal veins and the IVC were determined using Friedman’s analysis of variance for paired observations with Duncan’s correction for multiple measurements. In case of APA (unilateral aldosterone production as described previously), differences between the corresponding adrenal vein and the IVC were determined by Wilcoxon’s tests. A 2-sided P value <.05 was considered significant.
Chapter 4

Results

We included 12 men and 11 women between 2008 and 2015. Median age was 57 (IQR, 50–61) years and 24-hour urinary aldosterone excretion was 78 (IQR, 44–125) nmol/24 h (upper limit of normal: 37.6 nmol/24 h) after a 3-day salt loading test. Serum creatinine, electrolytes, plasma total cholesterol, non-HDL cholesterol, HDL cholesterol, and triglyceride levels obtained as part of routine medical care are shown in Table 1. Four patients used a statin at the time of AVS. The selectivity index was >3 in all catheterized adrenal veins (range: 3.12–50.66), indicating that the AVS procedure was technically successful in all cases. Based on the lateralization index and the contralateral suppression index, an APA was diagnosed in 13 subjects. The others were diagnosed with bilateral adrenal hyperplasia.

Table 1: Baseline characteristics of 23 subjects with primary aldosteronism

<table>
<thead>
<tr>
<th>Measure</th>
<th>Value (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>11/12</td>
</tr>
<tr>
<td>Age (y)</td>
<td>57 (22-73)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29 (18-44)</td>
</tr>
<tr>
<td>Creatinine (mmol/L)</td>
<td>72 (55-204)</td>
</tr>
<tr>
<td>Sodium (mmol/L)</td>
<td>144 (137-148)</td>
</tr>
<tr>
<td>Potassium (mmol/L)</td>
<td>3.6 (2.3-4.5)</td>
</tr>
<tr>
<td>Serum aldosterone (pmol/L)</td>
<td>540 (220-3220)</td>
</tr>
<tr>
<td>PRC (ng/L)</td>
<td>2.5 (0.7-16.8)</td>
</tr>
<tr>
<td>Urinary aldosterone after SLT (nmol/24 h)</td>
<td>78 (37-522)</td>
</tr>
<tr>
<td>PA subtype (APA/BAH)</td>
<td>13/10</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>4.70 (3.10-7.90)</td>
</tr>
<tr>
<td>Non-HDL cholesterol (mmol/L)</td>
<td>3.10 (1.50-6.50)</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1.30 (0.80-3.00)</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.44 (0.53-5.16)</td>
</tr>
</tbody>
</table>

APA, aldosterone producing adenoma; BAH, bilateral adrenal hyperplasia; BMI, body mass index; HDL, high-density lipoproteins; PA, primary aldosteronism; PRC, plasma renin concentration, SLT, sodium loading test. Data are expressed as numbers or median (range).

In the whole group, no significant differences were found in the VLDL-P, LDL-P, HDL-P, or HDL-C concentrations between samples from the adrenal veins and the IVC (Table 2). In addition, VLDL, LDL, and HDL lipoprotein subfractions (data not shown) and sizes (Table 2) were not different between adrenal veins and IVC samples. Of note, the apoB concentration was lower in the right adrenal vein compared with the IVC. Plasma apoA-I concentrations were not different between samples from the adrenal veins and the IVC. Results were similar after excluding 4 statin using patients (data not shown).
Adrenal lipoprotein uptake

Table 2: Lipoprotein particle concentrations, sizes, HDL-C, and apolipoproteins in the IVC and median differences in each adrenal vein compared with the IVC in 23 patients undergoing adrenal venous sampling

<table>
<thead>
<tr>
<th>Apolipoprotein</th>
<th>IVC</th>
<th>Left adrenal vein, IVC</th>
<th>Right adrenal vein, IVC</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL-P (nmol/L)</td>
<td>44.0 (29.8 - 55.2)</td>
<td>1.7 (–5.5 - 4.6)</td>
<td>0.1 (–8.8 - 7.6)</td>
<td>.84</td>
</tr>
<tr>
<td>LDL-P (nmol/L)</td>
<td>878 (602 - 1100)</td>
<td>4 (–53 - 58)</td>
<td>9 (–89 - 74)</td>
<td>.93</td>
</tr>
<tr>
<td>HDL-P (nmol/L)</td>
<td>27.2 (24.1 - 29.6)</td>
<td>0.3 (–0.1 - 1.3)</td>
<td>0.9 (–0.3 - 1.9)</td>
<td>.06</td>
</tr>
<tr>
<td>VLDL size (nm)</td>
<td>49.7 (45.9 - 54.2)</td>
<td>–0.4 (–2.6 - 1.7)</td>
<td>–0.3 (–2.1 - 2.8)</td>
<td>.57</td>
</tr>
<tr>
<td>LDL size (nm)</td>
<td>21.1 (20.5 - 21.5)</td>
<td>–0.3 (–0.6 - 0.1)</td>
<td>–0.15 (–4.23 - 0.2)</td>
<td>.13</td>
</tr>
<tr>
<td>HDL size (nm)</td>
<td>9.0 (8.5 - 9.5)</td>
<td>0.0 (–0.1 - 0.1)</td>
<td>0.0 (–0.1 - 0.1)</td>
<td>.93</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.01 (0.85 - 1.32)</td>
<td>0.0 (0.0 - 0.5)</td>
<td>0.0 (–0.26 - 0.08)</td>
<td>.59</td>
</tr>
<tr>
<td>ApoA-I (g/L)</td>
<td>1.57 (1.40 - 1.74)</td>
<td>–0.00 (–0.08 - 0.10)</td>
<td>–0.03 (–0.09 - 0.05)</td>
<td>.20</td>
</tr>
<tr>
<td>ApoB (g/L)</td>
<td>0.81 (0.64 - 0.94)</td>
<td>–0.01 (–0.03 - 0.02)</td>
<td>–0.02 (–0.04 - 0.01)</td>
<td>.026</td>
</tr>
</tbody>
</table>

Apo, apolipoprotein; HDL-C, high-density lipoprotein cholesterol; HDL-P, high-density lipoprotein particle concentration; IVC, inferior vena cava; LDL-P, low-density lipoprotein particle concentration; VLDL-P, very low-density particle concentration. Data are expressed as median (interquartile range).

*P value: overall P value by Friedman’s 2-way analysis of variance for paired observations with subsequent Duncan correction for multiple comparisons. †P < .05 from IVC.

We next tested whether lipoprotein uptake would be higher in adrenal glands harboring an APA. In 13 subjects diagnosed with APA, apoB was significantly lower in the adrenal vein at the side of the APA compared with the IVC (Table 3; Figure 1). Accordingly, LDL-P tended to be lower in the adrenal vein at the APA side compared with IVC. HDL-P, HDL-C, and apoA-I concentrations were not significantly different in the APA draining adrenal vein (Table 3).

Figure 1: Apolipoprotein B (apoB) concentrations in the inferior vena cava (IVC) and adrenal vein at the side were the aldosterone producing adenoma (APA) was located in 13 APA patients.
Table 3: Lipoprotein particle concentrations, sizes, HDL-C, and apolipoproteins in 13 patients diagnosed with an APA

<table>
<thead>
<tr>
<th>Apolipoprotein</th>
<th>IVC</th>
<th>APA side, IVC</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL-P (nmol/L)</td>
<td>35.9 (27.2 – 47.2)</td>
<td>3.0 (–8.8 – 6.7)</td>
<td>.650</td>
</tr>
<tr>
<td>LDL-P (nmol/L)</td>
<td>716 (574 – 1050)</td>
<td>–17 (–101 – 4.5)</td>
<td>.065</td>
</tr>
<tr>
<td>HDL-P (nmol/L)</td>
<td>26.8 (22.7 – 28.1)</td>
<td>0.3 (–0.2 – 1.2)</td>
<td>.077</td>
</tr>
<tr>
<td>VLDL size (nm)</td>
<td>48.5 (46.5 – 52.0)</td>
<td>–0.9 (–3.5 – 1.9)</td>
<td>.294</td>
</tr>
<tr>
<td>LDL size (nm)</td>
<td>21.2 (20.5 – 51.5)</td>
<td>–0.3 (–0.7 – 0.1)</td>
<td>.132</td>
</tr>
<tr>
<td>HDL size (nm)</td>
<td>9.1 (8.9 – 10.0)</td>
<td>0.0 (–0.1 – 0.1)</td>
<td>.348</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.06 (0.87 – 1.31)</td>
<td>0.0 (0.0 – 0.1)</td>
<td>.672</td>
</tr>
<tr>
<td>ApoA-I (g/L)</td>
<td>1.65 (1.51 – 1.75)</td>
<td>–0.03 (–0.11 – 0.01)</td>
<td>.075</td>
</tr>
<tr>
<td>ApoB (g/L)</td>
<td>0.72 (0.51 – 0.94)</td>
<td>–0.01 (–0.06 – 0.00)</td>
<td>.045</td>
</tr>
</tbody>
</table>

APA, aldosterone producing adenoma; Apo, apolipoprotein; LDL-P, low-density lipoprotein particle concentration; HDL-C, high-density lipoprotein cholesterol; HDL-P, high-density lipoprotein particle concentration; VLDL-P, very low-density particle concentration.

Data are expressed as median (interquartile range). Median concentrations in the inferior vena cava (IVC) and median differences in the adrenal vein corresponding to the side of the APA are shown.

Discussion

Based on measurements of plasma (apo)lipoproteins and subfraction concentrations in samples from the adrenal veins and the IVC, we show here for the first time that the apoB concentration in adrenal veins is lower compared with the IVC. The apoB concentration was particularly lower in the adrenal vein at the side of the APA, conceivably as a result of the higher rate of steroid production by the adenoma. Accordingly, the decrease in the venous plasma LDL-P concentration at the APA side was close to statistical significance. Our findings, therefore, agree with the concept that steroidogenesis in humans may to some extent be driven by circulating LDL particles as supplier of cholesterol to the adrenal glands.

Neither HDL-C nor HDL-P and apoA-I were lower in adrenal venous plasma compared with the IVC. Moreover, HDL size was not decreased as would be expected if the adrenal glands were to a considerable extent using cholesteryl esters from circulating HDL via SRBI-mediated uptake (9,10,23). However, these findings should be interpreted with caution because we cannot entirely exclude that the lack of difference in HDL variables is attributable to a type II error. Thus, the extent to which circulating HDL may contribute to adrenal steroidogenesis as determined during tetracosactide-stimulated adrenal glucocorticoid output remains uncertain.
The AVS procedure was performed during stimulation with tetracosactide as recommended (19). This allowed us to delineate adrenal uptake of (apo)lipoproteins during maximally stimulated cortisol output. In comparison, SRBI mutation carriers showed mildly diminished cortisol stimulation in response to tetracosactide in particular when variation in cortisol binding globulin levels was taken into account, whereas the cortisol response to tetracosactide was not attenuated in men with low HDL-C due to heterozygous lecithin–cholesterol acyltransferase or adenosine triphosphate–binding cassette transporter 1 deficiency (5). Of further relevance, no positive relationship between urinary cortisol metabolite secretion as an estimate of cortisol production, and plasma HDL-C has been documented in several population-based studies (24–26). Altogether, the importance of circulating HDL for adrenal steroidogenesis in subjects without rare genetic deficiencies affecting HDL metabolism is still uncertain.

It could be argued that our findings do not represent normal physiology as we examined subjects with primary aldosteronism under stimulating conditions with tetracosactide. However, it should be noted that, because of its invasive character and inherent risks, studies describing AVS in healthy subjects are not available. The continuous intravenous administration of tetracosactide stimulates the adrenal steroid biosynthesis, dampens the pulsatility of adrenal steroid secretion, and is, therefore, expected to enhance the uptake of circulating cholesterol, thereby improving the sensitivity to demonstrate relevant changes in the lipoprotein profile. Furthermore, the mechanism of cholesterol uptake by the adrenal gland is not likely to be different between patients with primary aldosteronism and healthy subjects.

Obviously, the interpretation of the present findings depends on the validity of the assumption that the plasma concentrations of LDL and HDL in the adrenal arteries and IVC are similar (22). It is likely that adrenal venous blood is to some extent mixed with accessory vein blood flow during the AVS procedure (19). This might diminish apparent adrenal venous-IVC differences in the plasma concentrations of the various lipoproteins, and hence result in underestimation of the magnitude of adrenal lipoprotein uptake. However, in the setting of highest expected difference (ie, at the APA side), we did find a significant gradient in apoB concentrations, which we regard as a proof of the concept that this novel approach is feasible to assess lipoprotein uptake in vivo.

In conclusion, the in vivo observations described here support the possibility that circulating LDL may contribute to adrenal steroidogenesis in humans.
Chapter 4

Acknowledgments

The authors would like to thank Labcorp for determining lipoprotein particle profiles with nuclear magnetic resonance spectroscopy free of charge. This current work was presented in abstract form at the Endocrine Society's annual meeting 2016.
References


PART II

Adrenal medulla: optimization of current diagnostic strategies for PPGL