High density lipoprotein as a source of cholesterol for adrenal steroidogenesis: a study in individuals with low plasma HDL-C

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Abstract Few studies have addressed the delivery of lipoprotein-derived cholesterol to the adrenals for steroid production in humans. While there is evidence against a role for low-density lipoprotein (LDL), it is unresolved whether high density lipoprotein (HDL) contributes to adrenal steroidogenesis. To study this, steroid hormone profiles in urine were assessed in male subjects suffering from functional mutations in ATP binding cassette transporter A1 (ABCA1) (n = 24), lecithin:cholesterol acyltransferase (LCAT) (n = 40), as well as in 11 subjects with low HDL cholesterol (HDL-C) without ABCA1/LCAT mutations. HDL-C levels were 39% lower in the ABCA1, LCAT, and low HDL-C groups compared with controls (all P < 0.001). In all groups with low HDL-C levels, urinary excretion of 17-ketogenic steroids was reduced by 33%, 27%, and 32% compared with controls (all P < 0.04). In seven carriers of either type of mutation, adrenocorticotropic hormone (ACTH) stimulation did not reveal differences from normolipidemic controls. In conclusion, this study shows that basal but not stimulated corticosteroid metabolism is attenuated in subjects with low HDL-C, irrespective of its molecular origin. These findings lend support to a role for HDL as a cholesterol donor for basal adrenal steroidogenesis in humans.—Bochem, A. E., A. G. Holleboom, J. A. Romijn, M. Hoekstra, G. M. Dallingga-Thie, M. M. Motazacker, G. K. Hovingh, J. A. Kuivenhoven, and E. S. G. Stroes. High density lipoprotein as a source of cholesterol for adrenal steroidogenesis: a study in individuals with low plasma HDL-C. J. Lipid Res. 2013. 54: 1698–1704.

Supplementary key words steroid hormones • cortisol • dyslipidemia • hyperalphalipoproteinemia • high density lipoprotein cholesterol

The synthesis and secretion of adrenal steroid hormones for regulating stress responses, electrolyte homeostasis, and maintenance of secondary sexual characteristics depends upon the availability of the precursor cholesterol (1). To secure a continuous cholesterol supply, the adrenal glands can synthesize cholesterol, metabolize intracellular esterified cholesterol, or obtain cholesterol from circulating lipoproteins (1). Although exact data are lacking, plasma lipoproteins have been suggested to contribute more than 75% of all cholesterol required for adrenal steroidogenesis (2, 3), but the current literature gives little insight in associations between plasma lipoprotein levels and adrenal function in humans.

With respect to a role for low-density lipoprotein (LDL), it has been shown that LDL receptor-deficient patients suffering from familial hypercholesterolemia display normal urinary excretion of both 17 hydroxycorticosteroids (17-OHCS) and 17 ketogenic steroid metabolites (17-KS) indicative of unaffected adrenal function (4, 5). In addition, low or absent LDL cholesterol (LDL-C) in carriers of one or two defective APOB alleles respectively did not affect basal adrenal function either (6). Combined this suggests that LDL is probably not playing a major role in delivering cholesterol for steroid hormone production in humans. Associations with HDL cholesterol (HDL-C) have thus far only been studied in critically ill patients. In one

Abbreviations: A, androstenedione; ABCA1, ATP binding cassette transporter A1; ACTH, adrenocorticotropic hormone; ALLO, allo-tetrahydrocortisol; CBG, cortisol binding globulin; D, dehydroepiandrosterone; E, etiocholanolon; HA, 11-hydroxyetiocholanolon; KA, 11-ketoandrostenedione; KE, 11-ketoetiocholanolon; 17-KS, 17 ketogenic steroid metabolites; LDL-C, LDL cholesterol; 17-OHCS, 17 hydroxycorticosteroids; P2, pregnaandiol; P3, pregnantriol; SRB1, scavenger receptor type B1; THF, tetrahydrocortisol; THS, 11-deoxytetrahydrocortisol.

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study it was shown that low HDL-C in such patients was associated with attenuated adrenal responses to synthetic adrenocorticotropic hormone (ACTH) (7). In support of this, others reported a high incidence of adrenal failure in critically ill individuals with liver disease, with HDL-C being the only variable predictive of adrenal insufficiency (8).

Using adrenal cells, it has been suggested that HDL is the preferred lipoprotein for cholesterol delivery to the adrenal gland (9) and in accordance, scavenger receptor type B1 (SRB1)-mediated cholesterol uptake from HDL has been shown to be the predominant source of cholesterol in mice (10–13). In line, it has been shown that mice lacking SRB1 display an impaired adrenal glucocorticoid stress response (14, 15), lending support to a major role for HDL as a cholesterol donor in mice. In humans, we also showed that adrenal function was compromised in individuals with a functional mutation in SCARB1, the gene encoding SRB1 (16). While this study showed that cholesterol delivery to the adrenals via the HDL-SRB1 pathway is important for adrenal steroidogenesis in humans, it is unclear whether plasma HDL-C levels are associated with adrenal steroidogenesis in humans. To investigate this, we assessed basal and ACTH-stimulated adrenal cortical function in males with low HDL-C due to mutations in either ATP binding cassette transporter 1 (ABCA1) (17) or lecithin:cholesterol acyltransferase (LCAT) and in subjects with low HDL-C without mutations in ABCA1/LCAT, as well as in normolipidemic controls (18). We hypothesized that in subjects with low HDL-C levels, adrenal function would be compromised irrespective of the molecular origin of the low HDL-C.

METHODS

Recruitment of study participants

Male subjects with HDL-C levels below the fifth percentile were screened for mutations in ABCA1 and LCAT (17, 19), of which the functionality was assessed in previously published studies (17, 20, 21). For the current study, we enrolled 24 carriers of mutations in the ABCA1 gene. We furthermore enrolled 40 male carriers of mutations in the LCAT gene. In addition, subjects with similarly reduced HDL-C levels without mutations in ABCA1 and LCAT were included (n = 11). As a control group, normolipidemic age-matched male individuals were recruited by advertisement. None of the included individuals used medication interfering with steroid metabolism. The study was approved by the institutional review board of the Academic Medical Center, Amsterdam, The Netherlands and all participants provided written informed consent.

Questionnaire and biochemical measurements

Medical history, cardiovascular risk factors, use of medication, and family history of cardiovascular disease were assessed using a questionnaire. Brachial artery blood pressures were measured using an oscillometric blood pressure device (Omron 705IT, Hoofddorp, The Netherlands). Hypertension was defined as 1) use of antihypertensive medication, or 2) a systolic blood pressure at visit above 140 mm Hg and/or diastolic blood pressure above 90 mm Hg.

Plasma was obtained after an overnight fast and stored at −80°C. Total cholesterol, LDL-C, HDL-C, and triglyceride levels were analyzed using commercially available enzymatic methods (Randox, Antrim, UK and Wako, Neuss, Germany) on a Cobas Mira autoanalyzer (Roche, Basel, Switzerland). Free cholesterol and total cholesterol were measured before and after precipitation of apoB-containing lipoproteins using phosphotungstic acid (Sigma) and a commercially available enzymatic assay (DiaSys) on a Selectra autoanalyzer (Sopachem). ACTH was determined by an immunoluminometric assay (Nichols Institute, Los Angeles, CA). Aldosterone was measured using a radioimmunoassay (Siemens, Los Angeles, CA).

Baseline adrenal steroidogenesis

Urinary excretion of steroid metabolites was analyzed by gas chromatography in 24 h urine samples as previously described (22, 23). Androsteron (A), etiocholanol (E), dehydroepiandrosteron (D), 11-ketoandrosteron (KA), 11-ketoetiocholanol (KE), 11-hydroxy-androsteron (HA), 11-hydroxy-etiocholanol (HE), pregnandiol (P2), pregnantriol (P3), 11-deoxytetrahydrocortisol (THS), tetrahydrocortison (THE), tetrahydrocortisol (THF), and allo-tetrahydrocortisol (ALLO) were measured as readout of adrenal steroidogenesis. A, E, D, KA, KE, HA, and HE made up the total 17-KS, whereas THS, THE, THF, ALLO, and P3 were the constituents of the total 17-OHCS. In addition, urinary free cortisol was determined using solid-phase extraction liquid chromatography-tandem mass spectrometry on a Symbiosis Pharma (Spark Holland, Emmen, The Netherlands) Quattro Premier tandem mass spectrometer (Waters, Milford, MA) system. Solid phase extraction was performed on Oasis HLB cartridges (Waters), chromatographic separation was achieved on a Waters Sunfire C18 column (3.5 μm, 2.1 × 50 mm) using ammonium acetate mM with 0.1% formic acid as mobile phase and acoustic

or

Stimulated adrenal steroidogenesis

Random subgroups of seven ABCA1 and seven LCAT mutation carriers consented to an ACTH stimulation study (cosyntropin or tetracosactin, 0.25 mg/ml; Novartis Pharma B.V., Arnhem, The Netherlands). After an overnight fast, participants underwent cosyntropin testing at 0900 h. Two baseline blood samples were obtained, 15 min and 1 min before administration of the 1 μg cosyntropin bolus. Subsequent blood samples were drawn 30 min and 60 min after cosyntropin administration. Plasma cortisol levels were measured by enzyme immunoassay (Siemens Medical Solutions, Los Angeles, CA), and cortisol binding globulin (CBG) levels were measured with a commercial radioimmunoassay (Siemens Medical Solutions). Free cortisol levels were calculated using the method described by Coolens, van Baelen, and Heyns (24).

Statistical analysis

Unpaired Student’s t-test was performed for analysis of continuous data with a normal distribution. In case of a skewed distribution, data were log-transformed prior to testing. Categorical data were assessed by χ2 testing. A P value of <0.05 was considered statistically significant. A linear regression model was used to correct for differences in LDL-C and statin use.

RESULTS

Population characteristics

We enrolled 24 and 40 carriers of loss-of-function mutations in either ABCA1 or LCAT. Two of the ABCA1 mutation
carriers were compound heterozygotes while three of the
**LCAT** mutation carriers were homozygotes. None of the
participants were referred to our clinic for symptoms of
adrenal dysfunction. Individuals were matched to male
carriers for age. In parallel, we also included subjects with
equally low HDL-C levels without mutations in
**ABCA1** and **LCAT** mutations and in the low
HDL-C group, compared with normolipidemic controls
(\(P = 0.003\)). LDL-C levels were 25% lower in carriers of
**ABCA1** mutations (\(P = 0.003\)), 11% lower in carriers of
**LCAT** mutations (n.s.), and 12% lower in the low HDL-C
fraction, in a subset of patients, is
provided in Table 2.

Detailed analysis of the concentrations of cholesteryl esters
and free cholesterol in the total cholesteryl fraction and the isolated HDL-C fraction, in a subset of patients, is
provided in Table 2.

Compared with controls, hypertension was more prevalent
among **LCAT** mutation carriers (\(P = 0.01\)). Statin use
was more prevalent in the **ABCA1** and **LCAT** mutation
carriers compared with the control group. The low HDL-C
group had similar baseline characteristics compared with
the **ABCA1** and **LCAT** groups.

### Basal adrenal steroidogenesis

Compared with the control group, we identified lower 24 h urinary excretion of 17-KS in carriers of mutations in
**ABCA1** (33%, \(P = 0.003\); Fig. 1A), in **LCAT** (27%, \(P = 0.01\);
Fig. 1. Twenty-four hour urinary steroid excretion in male ABCA1 (A) and LCAT (B) mutation carriers compared with age-matched male controls. Data are presented as mean ± SD. \( p_1 \), the uncorrected \( P \) value for Student’s \( t \)-test; \( p_2 \), the \( P \) value corrected for LDL-C; \( p_3 \), the \( P \) value corrected for statin use.

Excretion of total 17-KS compared with normolipidemic controls, indicative of disturbed basal corticosteroid synthesis. A parallel reduction in hydroxycorticosteroid excretion in both groups did not reach statistical significance which may suggest a preferential adrenal pathway for HDL driven steroidogenesis. Mutations in either ABCA1 or LCAT did not, however, affect the response to ACTH, while physical examination and a standardized questionnaire for signs of adrenal insufficiency (16) did not reveal symptoms of clinically relevant adrenal dysfunction. These findings are in line with those in mice, and tissue culture showing that the supply of plasma lipoprotein-derived cholesterol is used for basal adrenal steroidogenesis, but that this pathway is not able to respond to acute stress (27–29). For a proper quick response, endogenous adrenal cortisol reserves are secreted upon stimulation. Because steroidogenic tissues are rapidly depleted of cortisol following stimulation, replenishing is thought to occur via uptake of cholesterol from lipoproteins.

We previously reported that individuals with reduced SRB1 function displayed mild adrenal insufficiency in addition to a reduced urinary excretion of steroid hormones (16). Thus reduced SRB1 function on adrenal cells has a larger impact on adrenal steroidogenesis when compared with low levels of plasma HDL-C caused by reduced ABCA1 or LCAT function.

There were no differences in urinary excretion of free cortisol. However, particularly in the lower ranges the quantification of free cortisol has been shown to be less reliable as compared with the quantification of full urinary steroid metabolites in diagnosing the presence of hypocortisolism (30, 31).

In accordance with LCAT’s mechanism of action, cholesterol ester concentration is lower in LCAT mutation carriers, with the most pronounced effect in homozygous carriers. Because adrenal steroidogenesis was not further decreased in homozygous carriers compared with heterozygous carriers, there is no indication that the adrenal gland has a preference for cholesterol esters as a substrate for steroidogenesis. This is supported by the fact that a similar reduction in steroid metabolites was observed in subjects with low HDL-C not related to defects in LCAT.

Plasma levels of ACTH did not differ between the low HDL-C group and controls. The latter more likely reflects
We would like to discuss three limitations of our study. First, adrenal cells express ABCA1 (32). Thus reduced adrenal ABCA1 expression could conceptually lead to accumulation of cholesterol in adrenal cells, compromising the fact that the decrease in urinary steroid metabolites is only mild, thereby precluding a compensatory ACTH increase. In line, none of the study participants reported signs of hypocortisolism.

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![Fig. 2. Urinary steroid metabolites in male ABCA1 (A) and LCAT (B) mutation carriers compared with age-matched male controls. Data are presented as mean ± SD.](image)

![Fig. 3. Peak serum cortisol increase after cosyntropin administration in male ABCA1 (A) and LCAT (B) mutation carriers versus male controls. Data are presented as mean ± SD. *P* values for Student’s *t*-test.](image)
intra-adrenal signaling and steroidogenesis due to cholesterol toxicity as proposed for pancreatic β-cell dysfunction in individuals carrying ABCA1 mutations (33, 34). Although we cannot exclude this possibility, mutations in LCAT as well as low HDL-C in noncarrier individuals are associated with similar reductions in HDL-C and urinary steroid metabolite excretion, making an ABCA1-specific effect implausible.

A second limitation is that carriers of ABCA1 mutations showed significantly lower levels of plasma LDL-C levels in addition to low levels of HDL-C. This could mean that combined low levels of HDL-C and LDL-C account for the effects observed, but as already discussed, a role for LDI-C in human adrenal corticoid production is unlikely (4–6). In line, statistical corrections for the observed reductions in LDL-C did not affect outcome.

Third, in the urinary steroid metabolites, the 17-KS were particularly decreased in carriers compared with controls. Because 17-KS are metabolites derived largely from the androgenic pathway, this finding hints toward the preferential use of HDL-derived cholesterol in adrenal steroidogenesis. Further studies are needed to dissect whether, and to what extent, HDL-derived cholesterol contributes to steroidogenesis in either adrenal or testicular steroid production.

In conclusion, we demonstrate that basal adrenal steroidogenesis is compromised in males with low levels of plasma HDL-C, establishing a role for HDL-derived cholesterol in adrenal steroidogenesis in humans.

The authors would like to thank C. A. M. Koch and J. F. Los for their assistance in expanding the pedigrees and J. Peter for his help in the identification of ABCA1 and LCAT mutations.

REFERENCES

1. Hu, J., Z. Zhang, W. J. Shen, and S. Azhar. 2010. Cellular cholesterol delivery, intracellular processing and utilization for biosynthesis of steroid hormones. Nutr. Metab. (Lond.). 7: 47.

2. Borkowski, A., C. Delcroix, and S. Levin. 1972. Metabolism of adrenal cholesterol in man, 1. In vivo studies. J. Clin. Invest. 51: 1646–1678.

3. Borkowski, A. J., S. Levin, C. Delcroix, A. Mahler, and V. Verhas. 1967. Blood cholesterol and hydrocortisone production in man: quantitative aspects of the utilization of circulating cholesterol by the adrenals at rest and under adrenocorticotropin stimulation. J. Clin. Invest. 46: 797–811.

4. Illingworth, D. R., A. M. Lees, and R. S. Lees. 1983. Adrenal cortical function in homozygous familial hypercholesterolemia. Metabolism. 32: 1045–1052.

5. Illingworth, D. R., N. A. Alam, and S. Lindsey. 1984. Adrenocortical response to adrenocorticotropic in homozygous familial hypercholesterolemia. J. Clin. Endocrinol. Metab. 58: 206–211.

6. Illingworth, D. R., T. A. Kenny, and E. S. Orwoll. 1982. Adrenal function in homozygous and homozygous hypobetalipoproteinemia. J. Clin. Endocrinol. Metab. 54: 27–35.

7. van der Voort, P. H., R. T. Gerritsen, A. J. Bakker, E. C. Boerma, M. A. Kuiper, and L. de Heide. 2003. HDL-cholesterol level and cortisol response to synacthen in critically ill patients. Intensive Care Med. 29: 2199–2203.

8. Marik, P. E., T. Gayowski, and T. E. Starzl. 2005. The hepatoadrenal syndrome: a common yet unrecognized clinical condition. Crit. Care Med. 33: 1254–1259.

9. Yaguchi, H., K. Tsutsui, K. Shimono, M. Omura, H. Sasano, and T. Nishikawa. 1998. Involvement of high density lipoprotein as substrate cholesterol for steroidogenesis by bovine adrenal fasciculotubular cells. Life Sci. 62: 1587–1595.

10. Azhar, S., and E. Reaven. 2002. Scavenger receptor class BI and selective cholesterol ester uptake: partners in the regulation of steroidogenesis. Mol. Cell. Endocrinol. 195: 1–26.

11. Azhar, S., S. Leers-Sucheta, and E. Reaven. 2003. Cholesterol uptake in adrenal and gonadal tissues: the SR-BI and ‘selective’ pathway connection. Front. Biosci. 8: v899–v1029.

12. Reaven, E., Y. D. Chen, M. Spicher, and S. Azhar. 1984. Morphological evidence that high density lipoproteins are not internalized by steroid-producing cells during in situ organ perfusion. J. Clin. Invest. 74: 1384–1397.

13. Azhar, S., D. Stewart, and E. Reaven. 1989. Utilization of cholesterol-rich lipoproteins by perfused rat adrenals. J. Lipid Res. 30: 1799–1810.

14. Hoekstra, M., I. Meurs, M. Koenders, R. Outt, R. B. Hildebrand, J. K. Kruitj, M. van Eck, and T. J. Van Berkel. 2008. Absence of HDL cholesterol ester uptake in mice via SR-BI impairs an adequate adrenal glucocorticoid-mediated stress response to fasting. J. Lipid Res. 49: 738–745.

15. Hoekstra, M., D. Ye, R. B. Hildebrand, Y. Zhao, B. Lammers, M. Süttinger, J. Kuiper, T. J. Van Berkel, and M. van Eck. 2009. Scavenger receptor class B type I-mediated uptake of serum cholesterol is essential for optimal adrenal glucocorticoid production. J. Lipid Res. 50: 1039–1046.

16. Vergeer, M., S. J. Corporaal, R. Franssen, I. Meurs, R. Outt, G. K. Hovingh, M. Hoekstra, H. Sierts, G. M. Dallinga-Thie, M. M. Motzacker, et al. 2011. Genetic variant of the scavenger receptor BI in humans. N. Engl. J. Med. 364: 136–145.

17. Gandini, C., A. W. Schimmel, J. Peter, A. E. Bochem, A. G. Holleboom, M. Vergeer, R. D. Pillaert, G. M. Dallinga-Thie, G. K. Hovingh, K. L. Khoo, et al. 2010. Identification and characterization of novel loss of function mutations in ATP-binding cassette transporter AI in patients with low plasma high-density lipoprotein cholesterol. Atherosclerosis. 213: 492–498.

18. Duivenvoorden, R., E. de Groot, B. M. Olsen, J. S. Lameris, R. J. van der Geest, E. S. Stroes, J. J. Kastelein, and A. J. Nedernev. 2009. In vivo quantification of carotid artery wall dimensions: 3.0-Tesla MRI versus B-mode ultrasound imaging. Circ. Cardiovasc. Imaging. 2: 293–298.

19. Duivenvoorden, R., A. G. Holleboom, B. van den Bogaard, A. J. Nedernev, E. de Groot, B. A. Atten, A. W. Schimmel, G. K. Hovingh, J. J. Kastelein, J. A. Kuivenhoven, et al. 2011. Cholesterol acyltransferase gene mutations have accelerated atherogenesis as assessed by carotid 3.0-T magnetic resonance imaging. J. Am. Coll. Cardiol. 58: 2481–2487.
cholesterol during gonadotropin-induced desensitization of steroidogenic response in luteinized rat ovary. *J. Biol. Chem.* **258**: 3735–3740.

29. Schumacher, M., M. Schwarz, and F. Leidenberger. 1985. Desensitization of mouse Leydig cells in vivo: evidence for the depletion of cellular cholesterol. *Biol. Reprod.* **33**: 335–345.

30. Snow, K., N. S. Jiang, P. C. Kao, and B. W. Scheithauer. 1992. Biochemical evaluation of adrenal dysfunction: the laboratory perspective. *Mayo Clin. Proc.* **67**: 1055–1065.

31. Streeten, D. H., G. H. Anderson, Jr., T. G. Dalakos, D. Seeley, J. S. Mallov, R. Eusebio, F. S. Sunderlin, S. Z. Badawy, and R. B. King. 1984. Normal and abnormal function of the hypothalamic-pituitary-adrenocortical system in man. *Endocr. Rev.* **5**: 371–394.

32. Wellington, C. L., E. K. Walker, A. Suarez, A. Kwok, N. Bissada, R. Singaraja, Y. Z. Yang, L. H. Zhang, E. James, J. E. Wilson, et al. 2002. ABCA1 mRNA and protein distribution patterns predict multiple different roles and levels of regulation. *Lab. Invest.* **82**: 273–283.

33. Brunham, L. R., J. K. Kruit, T. D. Pape, J. M. Timmins, A. Q. Reuwer, Z. Vasanji, B. J. Marsh, B. Rodrigues, J. D. Johnson, J. S. Parks, et al. 2007. Beta-cell ABCA1 influences insulin secretion, glucose homeostasis and response to thiazolidinedione treatment. *Nat. Med.* **13**: 340–347.

34. Vergeer, M., L. R. Brunham, J. Koetsveld, J. K. Kruit, C. B. Verchere, J. J. Kastelein, M. R. Hayden, and E. S. Stroes. 2010. Carriers of loss-of-function mutations in ABCA1 display pancreatic beta-cell dysfunction. *Diabetes Care.* **33**: 869–874.