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

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Abbreviated title: Lower adrenal function in individuals with low 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-C without ABCA1/LCAT mutations. HDL-C levels were 39% lower in the ABCA1, LCAT and low HDL-C group, compared to 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 to controls (all p<0.04). In 7 carriers of either type of mutation, 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 cholesterol donor for basal adrenal steroidogenesis in humans.

Keywords
Steroid hormones
Cholesterol
Cortisol
Dyslipidemia
hypoalphalipoproteinemia
Introduction

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 lipoproteins 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 and 17-ketogenic steroid metabolites indicative of unaffected adrenal function (4;5). In addition, low or absent 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 high-density lipoprotein cholesterol (HDL-C) have thus far only been studied in critically ill patients. In one study it has been shown that low HDL-C in such patients was associated with attenuated adrenal responses to synthetic ACTH (7). In support, 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 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 < 5th percentile were screened for mutations in \textit{ABCA1} and \textit{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 \textit{ABCA1} gene. We furthermore enrolled 40 male carriers of mutations in the \textit{LCAT} gene. In addition, subjects with similarly reduced HDL-C levels without mutations in \textit{ABCA1} and \textit{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 mmHg and/or diastolic blood pressure was above 90 mmHg.

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, United Kingdom 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) using a commercial available enzymatic assays (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, USA).

Baseline adrenal steroidogenesis

Urinary excretion of steroid metabolites was analyzed by gas chromatography in 24-hour urine samples as previously described (22;23). Androsteron (A), etiocholanolon (E), dehydroepiandrosteron (D), 11-keto-androsteron (KA), 11-keto-etiocholanolon (KE), 11-hydroxy-androsteron (HA), 11-hydroxy-etiocholanolon (HE), pregnaandiol (P2), Pregnaantriol (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 make up total 17-ketogenic steroids (17-KS), whereas THS, THE, THF, ALLO and P3 are the constituents of total 17-hydroxycorticoids (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, Millford MA) system. Solid Phase extraction was performed on Oasis HLB cartridges (Waters, Millford, MA), chromatographic separation was achieved on a Waters Sunfire C18 column 3.5 μm 2.1 x 50 mm using ammonium acetate mM with 0.1% formic acid as mobile phase and acetonitrile as mobile phase B. Limit of detection 5 nmol/L, intra-assay variation <4%, total assay variation <7%.
Stimulated adrenal steroidogenesis

Random subgroups of 7 ABCA1 and 7 LCAT mutation carriers consented to an ACTH stimulation study (co-syntropin or tetracosactin, 0.25 mg/ml, Novartis Pharma b.v., Arnhem, The Netherlands). After an overnight fast, participants underwent co-syntropin testing at 0900h. Two baseline blood samples were obtained, 15 minutes and 1 minute before administration of the 1μg co-syntropin bolus. Subsequent blood samples were drawn 30 minutes and 60 minutes after co-syntropin 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, Los Angeles, CA). Free cortisol levels were calculated using the method described by Coolens et al (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 T-testing. Categorical data were assessed by \( \chi^2 \)-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 was referred to our clinic for symptoms of adrenal dysfunction. Individuals were matched to male controls for age. In parallel, we also included subjects with equally low HDL-C levels without mutations in ABCA1 or LCAT. Demographic, clinical and biochemical characteristics of all low HDL-C groups as well as controls are listed in table 1. As expected, HDL-C levels were 39% lower in carriers of ABCA1 or LCAT mutations and in the low HDL-C group, compared to normolipidemic controls (p<0.001). 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 group (p=0.34 and 0.39) compared to controls. Detailed analysis of the concentrations of cholesteryl esters and free cholesterol in the total cholesterol fraction and the isolated HDL-C fraction, in a subset of patients, is provided in table 2.

Compared to 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 to the control group. The low HDL-C group had similar baseline characteristics compared to the ABCA1 and LCAT groups.

Basal adrenal steroidogenesis

Compared to the control group, we identified lower 24 hour urinary excretion of 17-ketogenic steroids (17-KS) in carriers of mutations in ABCA1 (33%; p=0.003; figure 1a), in LCAT (27%; p=0.01, figure 1b) and in the low HDL-C group (30%; p=0.04 and 34%; p=0.02; figure 1). These differences remained statistically significant after correcting for differences in plasma
LDL-C and statin use using a linear regression model (p=0.01 and p=0.02 for carriers of 

\textit{ABCA1} mutations or \textit{LCAT} mutations vs. controls, respectively). The mean 17-KS steroid excretion of 14.1 µmol/24hr in the \textit{ABCA1} mutation carriers was below the reference values for 17-KS in the appropriate age group (23).

The lower levels of urinary 17-hydroxy corticosteroids (17-OHCS) in both \textit{ABCA1} and \textit{LCAT} mutation carriers as well as the low HDL-C group did not reach statistical significance (p=0.11 and 0.15, respectively, figure 1a-b). The full panel of steroid metabolites is presented in figure 2a-b. No gene-dose effect was observed when comparing the 2 compound heterozygous \textit{ABCA1} mutation carriers or the 3 homozygous \textit{LCAT} mutation carriers to heterozygous carriers.

Free urinary cortisol was not significantly different between \textit{ABCA1} mutation carriers and controls (60.10±31.66 vs 59.27±39.96 nmol/24h, p=0.62) or \textit{LCAT} mutation carriers and controls (50.33±36.34 vs 57.81±35.19, p=0.31).

Plasma levels of ACTH did not differ significantly between \textit{ABCA1} mutation carriers and controls (23.11±12.62 vs 21.64±15.10, p=0.25) or \textit{LCAT} mutation carriers and controls (22.00±14.25 vs 24.57±23.08, p=0.81).

\textit{Adrenal response to cosyntropin}

In a cosyntropin stimulation test, the cortisol response to physiological levels of ACTH is measured as an approach to assess adrenal cortical function (25;26). The peak serum cortisol response to ACTH was comparable in \textit{ABCA1} and \textit{LCAT} mutation carriers and did not differ from controls (p=0.10 and 0.87 respectively, figure 3a-b). Also peak plasma level of free cortisol, taking into account possible differences in cortisol binding globulin (CBG) levels (16), were not different. Plasma lipid levels did not differ significantly before and after cosyntropin testing (data not shown).
Discussion

The present study demonstrates that under normal conditions male individuals with low levels of HDL-C irrespective of molecular origin have significantly lower urinary excretion of total 17-KS compared to 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, however, not 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. Since 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 to 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
to the quantification of a full urinary steroid metabolites in diagnosing the presence of hypocortisolism (30;31).

In accordance with LCAT’s mechanism of action, cholesteryl ester concentration is lower in LCAT mutation carriers, with the most pronounced effect in homozygous carriers. Since adrenal steroidogenesis was not further decreased in homozygous carriers compared to heterozygous carriers, there is no indication that the adrenal gland has a preference for cholesteryl 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 most likely reflects 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.

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 intra-adrenal signalling and steroidogenesis due to cholesterol toxicity as proposed for pancreatic beta-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 non-carrier 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 LDL-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 17KS were particularly decreased in carriers compared to controls. Since 17KS are metabolites derived largely from the androgenic pathway, this finding hints towards 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 adrenals 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.

Acknowledgements
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Reference List


Figure 1. **24-hour urinary steroid excretion in male *ABCA1* and *LCAT* mutation carriers compared to age-matched male controls.**

Data are presented as mean ± SD. $P^1$ is the uncorrected p-value for Student’s T-test; $P^2$ is the p-value corrected for LDL-C and $P^3$ is the p-value corrected for statin use. 17-KS is 17-ketogenic steroids; 17-OHCS is 17-hydroxycorticosteroids.

Figure 2. **Urinary steroid metabolites in male *ABCA1* and *LCAT* mutation carriers compared to age-matched male controls.**

Data are presented as mean ± SD.

Figure 3. **Peak serum cortisol increase after cosyntropin administration in male *ABCA1* and *LCAT* mutation carriers versus male controls.**

Data are presented as mean ± SD. P values for Student’s T-test.
Table 1. Characteristics of male ATP-binding cassette transporter 1 (ABCA1) and Lecithin-cholesterol acyltransferase (LCAT) mutation carriers and matched male controls
<table>
<thead>
<tr>
<th>Characteristics</th>
<th>ABCA1 Carriers (n=24)</th>
<th>ABCA1 Controls (n=24)</th>
<th>p value</th>
<th>Low HDL-C (n=11)</th>
<th>p value</th>
<th>LCAT Carriers (n=40)</th>
<th>LCAT Controls (n=40)</th>
<th>p value</th>
<th>Low HDL-C (n=11)</th>
<th>p value</th>
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<td>Age -yr</td>
<td>45.2±20.6</td>
<td>45.5±18.5</td>
<td>0.96</td>
<td>44.6±12.0</td>
<td>0.91</td>
<td>46.2±12.8</td>
<td>45.2±14.9</td>
<td>0.84</td>
<td>44.6±12.0</td>
<td>0.71</td>
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<td>BMI (kg/m²)</td>
<td>25.3±3.3</td>
<td>25.1±5.2</td>
<td>0.92</td>
<td>26.3±8.8</td>
<td>0.81</td>
<td>26.3±2.9</td>
<td>24.9±4.5</td>
<td>0.17</td>
<td>26.3±8.8</td>
<td>0.99</td>
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<td>Current smoker (%)</td>
<td>8 (33.3)</td>
<td>4 (17.4)</td>
<td>0.21*</td>
<td>3 (27)</td>
<td>0.72*</td>
<td>7 (18.4)</td>
<td>4 (10.8)</td>
<td>0.35*</td>
<td>3 (27)</td>
<td>0.52*</td>
</tr>
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<td>Alcohol users - no (%)</td>
<td>17 (71)</td>
<td>19 (79)</td>
<td>0.51*</td>
<td>9 (82)</td>
<td>0.49*</td>
<td>36 (90)</td>
<td>33 (83)</td>
<td>0.33*</td>
<td>9 (82)</td>
<td>0.46*</td>
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<td>Coronary artery disease-no (%)</td>
<td>7 (29.2)</td>
<td>1 (4.8)</td>
<td>~</td>
<td>4 (46)</td>
<td>~</td>
<td>9 (23.7)</td>
<td>1 (2.9)</td>
<td>~</td>
<td>4 (36)</td>
<td>~</td>
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<td>Diabetes mellitus - no (%)</td>
<td>3 (13.6)</td>
<td>0 (0)</td>
<td>0.07*</td>
<td>1 (9)</td>
<td>0.71*</td>
<td>1 (2.6)</td>
<td>0 (0)</td>
<td>0.32*</td>
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<td>0.34*</td>
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<td>Hypertension - no (%)</td>
<td>7 (29.2)</td>
<td>3 (12.5)</td>
<td>0.16*</td>
<td>1 (9)</td>
<td>0.19*</td>
<td>13 (32.5)</td>
<td>4 (10)</td>
<td>0.01*</td>
<td>19 (8)</td>
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<td>Statin use - no (%)</td>
<td>8 (33)</td>
<td>2 (8)</td>
<td>0.02*</td>
<td>4 (33)</td>
<td>0.84*</td>
<td>15 (38)</td>
<td>2 (5)</td>
<td>&lt;0.001*</td>
<td>4 (36)</td>
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<td><strong>Blood pressure (mmHg)</strong></td>
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<td>Systolic</td>
<td>136±16</td>
<td>132±13</td>
<td>0.34</td>
<td>127±11</td>
<td>0.29</td>
<td>139±17</td>
<td>132±13</td>
<td>0.14</td>
<td>127±11</td>
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<td>Diastolic</td>
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<td>80±10</td>
<td>0.75</td>
<td>78±11</td>
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<tr>
<td>Aldosterone (nmol/L)</td>
<td>0.20±0.14</td>
<td>0.23±0.12</td>
<td>0.55</td>
<td>0.22±0.19</td>
<td>0.87</td>
<td>0.19±0.11</td>
<td>0.24±0.14</td>
<td>0.12</td>
<td>0.22±0.19</td>
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<td>ACTH (ng/L)</td>
<td>23.11±12.62</td>
<td>21.64±15.10</td>
<td>0.25*</td>
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<td></td>
<td>22.00±14.25</td>
<td>24.57±23.08</td>
<td>0.81*</td>
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<td>Total cholesterol (mmol/L)</td>
<td>3.9±1.2</td>
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<td>0.001</td>
<td>4.5±2.1</td>
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<td>LDL cholesterol (mmol/L)</td>
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<td>HDL cholesterol (mmol/L)</td>
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<td>1.3±0.3</td>
<td>&lt;0.001</td>
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<td>Triglycerides (mmol/L)</td>
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<td>Median</td>
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<td>0.65*</td>
<td>1.5</td>
<td>0.02*</td>
<td>1.25</td>
<td>1.25</td>
<td>0.22*</td>
<td>1.5</td>
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<td>Urinary free cortisol (nmol/24h)</td>
<td>60.10±31.66</td>
<td>59.27±39.96</td>
<td>0.62*</td>
<td>n.a.</td>
<td></td>
<td>50.33±36.34</td>
<td>57.81±35.19</td>
<td>0.31*</td>
<td>n.a.</td>
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</table>
Blood pressure measurements were available in 21 \textit{ABCA1} mutation carriers and 17 controls and in 34 \textit{LCAT} mutation carriers and 26 controls. Plasma ACTH levels were available in 18 \textit{ABCA1} mutation carriers and 18 controls and in 20 \textit{LCAT} mutation carriers and 31 controls. Urinary cortisol levels were available in 22 \textit{ABCA1} mutation carriers and 22 controls and in 35 \textit{LCAT} mutation carriers and 36 controls. Values are means ± SD unless otherwise indicated. \( P \) for Student’s T-test. *Triglycerides, plasma ACTH and urinary cortisol were logtransformed prior to T-test. \( \# \) \( P \) for \( \chi^2 \) test. No t-test was performed for history of coronary artery disease since referral bias was present. Partial overlap exists between the two control cohorts. N.a. stands for not available.
Table 2. Free cholesterol and cholesteryl ester values in a subset lecithin-cholesterol acyltransferase (LCAT) mutation carriers and controls

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=29)</th>
<th>Heterozygous carriers (n=29)</th>
<th>p value</th>
<th>Homozygous carriers (n=3)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cholesterol mmol/L</td>
<td>5.3±1.2</td>
<td>4.5±1.2</td>
<td>0.002</td>
<td>4.1±0.6</td>
<td>0.09</td>
</tr>
<tr>
<td>FC</td>
<td>1.56±0.35</td>
<td>1.4±0.4</td>
<td>0.04</td>
<td>2.1±0.3</td>
<td>0.02</td>
</tr>
<tr>
<td>CE</td>
<td>3.65±1.04</td>
<td>3.0±0.9</td>
<td>0.01</td>
<td>2.0±0.6</td>
<td>0.01</td>
</tr>
<tr>
<td>FC/CE ratio</td>
<td>0.48±0.30</td>
<td>0.5±0.1</td>
<td>0.73</td>
<td>1.1±0.4</td>
<td>0.001</td>
</tr>
<tr>
<td>Cholesterol after apoB precipitation</td>
<td>1.3±0.4</td>
<td>0.7±0.3</td>
<td>0.03</td>
<td>0.04±0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FC</td>
<td>1.78±0.71</td>
<td>0.1±0.1</td>
<td>0.13</td>
<td>0.0±0.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CE</td>
<td>0.7±0.2</td>
<td>0.6±0.2</td>
<td>0.02</td>
<td>0.04±0.01</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>FC/CE ratio</td>
<td>0.24±0.06</td>
<td>0.1±0.12</td>
<td>0.50</td>
<td>1.1±0.4</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

P for Student’s T-test, compared to controls.
Figure 1.

a. *ABCA1*

b. *LCAT*
Figure 2a. ABCA1

controls (N=24)
ABCA1 mutation carriers (N=24)
low HDL-C controls (N=11)
Figure 2b. *LCAT*

- LCAT mutation carriers (N=40)
- Controls (N=40)
- Low HDL-C controls (N=11)
Figure 3.

a. *ABCA1*

- Controls (N=7)
- *ABCA1* mutation carriers (N=7)

b. *LCAT*

- Controls (N=7)
- *LCAT* mutation carriers (N=7)

Peak serum cortisol response (nmol/l)