HDL cholesterol: atherosclerosis and beyond
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Citation for published version (APA):

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A role for LDL cholesterol in Cortisol Production in Humans

Submitted for publication

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Abstract

Background
Adrenal steroidogenesis is essential for survival in humans. Three-quarters of all cholesterol required for adrenal steroidogenesis is derived from plasma lipoproteins. We previously discovered that both low levels of circulating HDL-c as well as deficiency of the HDL-receptor impaired adrenal steroidogenesis. Here, we set out to investigate whether LDL derived cholesterol contributes to adrenal response to adrenocorticotropic hormone (ACTH) as a sensitive read-out of adrenal function in humans.

Methods
Cortisol response to 250 μg ACTH (cosyntropin) was measured in LDL receptor mutation carriers, apolipoprotein B mutation carriers as well as controls.

Findings
Cortisol response was lower in 123 male LDL receptor mutation carriers compared to 24 male controls (401.63±126.58 vs 793.75±116.91 nmol/L; p<0.001) and also in 64 female LDL receptor mutation carriers versus 25 female controls (429.24±135.15 vs 846.80±152.83 nmol/L; p<0.001). No differences in cortisol response became apparent between LDL receptor mutation carriers and apolipoprotein B mutation carriers.

Interpretation
These data support a role for LDL derived cholesterol in the adrenal response to stress in humans. Importantly, monitoring of adrenal function as a safety outcome measure might be indicated when aggressively lowering LDL cholesterol with novel therapeutics.
A role for LDL in adrenal function

Introduction

Adrenal steroidogenesis is essential for homeostasis and survival in humans and depends on the availability of its precursor cholesterol. Although exact data do not exist, circulating plasma lipoproteins have been suggested to contribute more than 75% of all cholesterol required for adrenal steroidogenesis. Whereas HDL derived cholesterol is indeed important for adrenal steroidogenesis in humans, low plasma levels of HDL-c are not associated with the life threatening disease of adrenal insufficiency in otherwise healthy individuals. In contrast, robust data on the contribution of the other major plasma cholesterol source, low density lipoprotein (LDL), to the adrenal production of hormones, are lacking to date.

Familial hypercholesterolemia (FH) is an autosomal dominant disorder predominantly caused by mutations in the gene encoding the low density lipoprotein receptor (LDLR). FH patients are characterised by extremely high plasma levels of LDL cholesterol due to (partial) loss of function of the hepatic LDLR, resulting in decreased clearance of LDL-c. Next to hepatic cells, the LDLR is abundantly expressed on cells in steroid producing organs such as adrenals, testes and ovaries. Based on the putative role of the LDLR in steroidogenesis it has been postulated that adrenal response to ACTH might be decreased patients with FH, because of a decrease in functional LDL receptors, resulting in decreased LDL-c uptake into the adrenals. However, this hypothesis has only been tested in a handful of FH patients in the 1980’s. LDL metabolism can be perturbed by mutations in the LDLR as well as in apolipoprotein B (APOB), the constituent protein of LDL particles and the protein that binds to the extracellular domain of the receptor. APOB mutations result in impaired binding of the LDL particle to the LDL receptor and thus, a defect in either the receptor (LDLR) or its ligand (apoB) might be hypothesized to have a similar effect on adrenal steroidogenesis. Illingworth and co workers did indeed show that in homozygous LDLR as well as in APOB mutation carriers, adrenal response to ACTH is impaired. An intriguing finding, since a complete deficiency of cholesterol uptake through the LDL pathway by the adrenal gland is likely in this situation. In contrast, the same investigators could not demonstrate any consequences for adrenal hormone production in heterozygous carriers of an LDLR or APOB mutation. The finding that cholesterol uptake via the LDL-receptor pathway is relevant in humans, has consequences for future drug development and the extremely low LDL-c levels attained by that therapy.

We therefore investigate whether LDL derived cholesterol plays a role in adrenal cholesterol uptake in humans. To this end, we assessed cortisol response to adrenocorticotropic hormone (ACTH) in heterozygous LDL receptor mutation carriers and APOB mutation carriers as well as in a control groups.

The clinical relevance of our experiment lies in the fact that novel therapies that lower LDL-c over and above statins, will significantly increase the number of patients reaching very low levels of LDL-c. It is therefore of consequence to the long-term safety of these therapies to understand the relationship between low levels of LDL-c and adrenal function.

We originally hypothesized that heterozygous LDLR and APOB mutation carriers would not have altered adrenal steroidogenesis. Here we present our results.
Methods

Study population
Patients, aged between 40 and 75 years (male) or between 45 and 75 years (female) with a functional LDLR or APOB mutation, were enrolled in the study. ACTH stimulation testing was performed at baseline. As a control group, we recruited healthy normolipidemic individuals matched for age and gender by advertisement. The study was approved by the Institutional Review Board of our hospital, and all participants provided written informed consent.

Adrenocorticotropic hormone (ACTH) testing
After an overnight fast, participants underwent ACTH testing at 0900 h. A peripheral IV catheter was placed in the antecubital vein. Two baseline blood samples were obtained, 15 minutes and one minute before administration of the ACTH bolus. Subsequent blood samples were drawn 30 minutes and 60 minutes after administration of 250 μg Cortrosyn® (cosyntropin, Amphastar Pharmaceuticals, Rango Cucamonga, CA, USA).

Laboratory analyses
Lipid profiles were routinely measured. For plasma cortisol levels, samples were centrifuged for 15 minutes at 2000 rpm. The SST tubes were refrigerated and send to a central laboratory for analysis (Medical Research Laboratories International, Zaventem, Belgium) or measured by enzyme immunoassay (Siemens Medical Solutions, Los Angeles, CA, USA).

Results

Baseline characteristics
A total of 187 LDLR mutation and 24 APOB mutation carriers as well as 49 healthy controls were enrolled in this study. Normolipidemic individuals were recruited by advertisement and enrolled as controls. Data from males and females were analysed separately. Baseline characteristics are described in tables 1 and 2. Besides lipoproteins, other characteristics were similar between groups.

ACTH testing
Absolute cortisol response was significantly lower in 123 male LDLR mutation carriers compared to 24 matched controls (401.63±126.58 nmol/L vs 793.75±116.91 nmol/L, p<0.001, figure 1) as well as relative cortisol response (120.30±75.54 vs 274.39±112.04, p<0.001, figure 1). This significant difference was also observed in 64 female LDLR mutation carriers compared to 25 female controls (absolute response: 429.24±135.15 nmol/L vs 846.80±152.83 nmol/L, p<0.001; relative cortisol response: 146.35±96.51 vs 225.29±75.25, p=0.01, figure 2). All values in controls were within the reference range.16,17

Furthermore, cortisol response was compared between LDLR mutation and APOB mutation carriers. Cortisol responses did not differ between male carriers (absolute response: 377.97±127.19 nmol/L vs 390.72 vs 164.81 nmol/L, p=0.73; relative response: 109.19±72.72 vs 122.17±115.80, p=0.55, figure 2a) or female carriers (absolute response:
A role for LDL in adrenal function

Figure 1. Cortisol response to cosyntropin testing in male controls vs male LDL receptor mutation carriers (a) and female controls vs female LDL receptor mutation carriers (b). Data are presented as mean ± SD. P for student’s T-test.
### Table 1. Baseline characteristics male and female controls vs LDL receptor mutation carriers

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
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<tbody>
<tr>
<td></td>
<td>controls (n=24)</td>
<td>LDLR mutation carriers (n=123)</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>44·5±15·7</td>
<td>49·5±5·2</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26·5±5·7</td>
<td>27·0±3·5</td>
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<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5·66±1·32</td>
<td>5·35±0·10</td>
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<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>4·03±1·14</td>
<td>3·47±0·84</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1·16±0·27</td>
<td>1·20±0·24</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1·35 (0·92-2·33)</td>
<td>1·22 (0·91-1·54)</td>
</tr>
<tr>
<td>Plasma cortisol (nmol/L)</td>
<td>337·92±149·40</td>
<td>382·51±97·04</td>
</tr>
</tbody>
</table>

Values are means ± SD, p-value for Student’s T-test. Triglyceride concentrations were log-transformed prior to T-test.

### Table 2. Baseline characteristics LDL receptor mutation carriers vs APOB mutation carriers

<table>
<thead>
<tr>
<th></th>
<th>Males</th>
<th>Females</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LDLr mutation carriers (n=186)</td>
<td>APOB mutation carriers (n=13)</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td>53·5±8·2</td>
<td>53·2±10·6</td>
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<tr>
<td>BMI (kg/m²)</td>
<td>27·1±3·4</td>
<td>26·7±3·3</td>
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<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5·41±1·0</td>
<td>5·28±0·73</td>
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<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3·52±0·85</td>
<td>3·39±0·60</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>1·22±0·26</td>
<td>1·29±0·30</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1·22 (0·88-1·57)</td>
<td>0·96 (0·83-1·55)</td>
</tr>
<tr>
<td>Plasma cortisol (nmol/L)</td>
<td>403·78±110·52</td>
<td>415·34±152·02</td>
</tr>
</tbody>
</table>

Values are means ± SD, p-value for Student’s T-test. Triglyceride concentrations were log-transformed prior to T-test.
a. males

b. females

Figure 2b. Cortisol response to cosyntropin testing in male LDLr mutation carriers vs male apoB receptor mutation carriers (a) and female LDLr mutation carriers vs female apoB receptor mutation carriers (b). Data are presented as mean ± SD. P for Student’s T-test.
420.69 nmol/L±147.42 vs 454.73±112.40 nmol/L, p=0.46; relative response 133.62±87.04 vs 158.74 vs 102.95, p=0.38, figure 2b).

Discussion

The data in our study point to a definite role for LDL derived cholesterol in adrenal steroidogenesis since both LDL receptor as well as APOB mutation carriers were characterised by a decreased response to synthetic ACTH when compared to controls. To our knowledge, this is the first time that adrenal response to ACTH has been assessed in a large cohort of individuals with impaired LDL-c uptake in tissues.

Our finding that cholesterol uptake via the LDLR plays a role in adrenal steroidogenesis supports the observations by Illingworth who showed impaired response to ACTH in homozygous carriers of LDLR mutations. However, the authors could not confirm these findings in heterozygous LDLR mutation carriers, which is likely due to inclusion of only four subjects. However, we find cortisol responses to be decreased in both heterozygous LDLR as well as APOB mutation carriers. These data in individuals with impaired cholesterol uptake in adrenal cells, as a consequence of two different defects, validate each other and point to a crucial role for the LDL derived cholesterol in adrenal steroidogenesis.

Plasma lipoproteins have been shown to supply 75% of the cholesterol necessary for adrenal steroidogenesis. However, the relative contribution of LDL and HDL derived cholesterol is unknown. HDL derived cholesterol has been shown to influence basal adrenal function, as reflected by urinary steroid metabolite excretion. However, adrenal response to ACTH was not decreased in low HDL-c subjects, but was lower in individuals with a deficiency of the HDL-receptor SRB1, as we showed recently. This indicates that other cholesterol sources than HDL can supply the adrenal gland with substrate for steroidogenesis in situations of stress. Our current finding that response to ACTH is indeed impaired in LDL receptor mutation carriers indicates that LDL derived cholesterol may constitute that cholesterol source and is, at least in part, responsible for adrenal steroidogenesis in situations of stress. Adrenal steroidogenesis has been extensively assessed in intervention trials aiming to reduce LDL-c in children with FH. While there is no effect of long-term statin therapy on maturation and growth, subtle differences were noted in the hypothalamic-adrenocortical axes, indicating a mild influence of circulating LDL-c on hormone production. These differences did not translate into clinical consequences. Nevertheless, it does seem prudent not to decrease LDL-c in children to extremely low levels, in line with US guidelines.

Clinical implications

The contribution of LDL derived cholesterol to acute adrenal steroidogenesis does not translate into clinical signs of adrenal insufficiency across a wide range of circulating LDL-c levels. Therefore, LDL-lowering according to the current guidelines for cardiovascular risk reduction is unlikely to have consequences for adrenal steroidogenesis. However, recent recommendations to decrease LDL-c as low as possible might have an effect on adrenal steroidogenesis in situations where adrenal hormones are of acute importance for
the individual. In heterozygous LDL receptor mutation carriers, with only half the adrenal uptake capacity for LDL-c, further lowering of available substrate might have untoward consequences. Our data also suggest that the assessment of adrenal function might be indicated in ongoing trials with novel and potent LDL-c lowering compounds that lower LDL-c to below physiological levels.

**Limitations**
All included patients were on statin therapy, but none of the controls. However, statins have been consistently shown not to influence adrenal function in adults. Due to a limited number of healthy volunteers who were willing to undergo ACTH testing, we enrolled a smaller number of controls relative to patients. However, the groups were well-matched and group size was addressed by our statistical analysis.

**Interpretation**
Our study is the first to address the role of LDL derived cholesterol in adrenal steroidogenesis in a large cohort with enough power to yield a statistically significant difference. The findings that adrenal steroidogenesis is impaired in heterozygous LDLR and APOB mutation carriers does not only increase our understanding of cholesterol metabolism in man, but it has also implications for current developments in cardiovascular prevention.

**Conclusion**
Our data support a role for LDL derived cholesterol in adrenal steroidogenesis in the acute setting. It is the first time that adrenal steroidogenesis was assessed in a large cohort of patients with a molecularly defined deficiency in cholesterol uptake in the adrenal gland. Our findings suggest that aggressive LDL lowering to very low LDL-c values, as recently advocated, might result in safety issues in terms of adrenal function. LDL-c lowering with novel and potent strategies should be accompanied by adequate safety testing in future clinical trials.
Reference List


