The puzzle of high-density lipoprotein in cardiovascular prevention
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Reduced fecal sterol excretion in subjects with familial hypoalphalipoproteinemia

ABSTRACT

**Background** Fecal bile acid and neutral sterol excretion are the obligate endpoints of the reverse cholesterol transport pathway (RCT). In studies in mice, no evidence was found for a relation between HDL cholesterol levels and fecal sterol excretion. In this study, we have evaluated this relationship in patients with isolated low HDL cholesterol versus controls.

**Results** Fecal sterol excretion was studied in 12 subjects with familial hypoalphalipoproteinemia (FHA) and 11 healthy controls. Compared to the controls (8.9 ± 6.3 mg/kg/day), neutral sterol excretion was significantly lower in the FHA group (4.0 ± 2.4 mg/kg/day). Fecal bile acid excretion showed a similar pattern. Across the groups, a strong positive correlation between HDL cholesterol and fecal neutral sterol excretion was found (r=0.53; p=0.01).

**Conclusions** Isolated low HDL cholesterol levels in humans are associated with reduced fecal sterol excretion suggesting that in humans HDL regulates the final step in the RCT pathway at low HDL cholesterol levels.
INTRODUCTION

Reverse cholesterol transport (RCT) is defined as the process by which superfluous cholesterol from peripheral cells is transported to the liver for removal from the body by excretion as neutral sterols and bile acids into the feces (1). High-density lipoprotein (HDL) is considered to be the specific carrier of cholesterol in the RCT pathway (2). Biogenesis of this lipoprotein occurs in the small intestine and liver, where apolipoprotein A-I, its main structural protein, is produced (3). Most of the initial supportive evidence for the RCT pathway was derived from in vitro studies and studies in mice (2). In these studies, no evidence was found for a relation between HDL cholesterol levels and fecal sterol excretion.

In humans, reported data on the relationship between HDL and fecal sterol excretion are scarce and ambiguous. Miettinen and Kesaniemi showed a surprising negative correlation between plasma HDL cholesterol levels and neutral sterol excretion in a large study with 63 male subjects (4). Two very small studies in only 2 patients with low HDL cholesterol due to LCAT deficiency and 2 patients with familial combined hyperlipidemia failed to show a difference in neutral sterol excretion compared to controls (5,6). Intervention studies showed a clear increase in sterol excretion after infusion of pro-apolipoprotein A-I or rHDL in 4 and 16 individuals, respectively (7,8). Yet, recently, doubling HDL cholesterol levels through CETP inhibition had no effect on fecal sterol excretion in 16 individuals (9).

In this study, we addressed the relationship between HDL cholesterol levels and fecal cholesterol excretion in subjects with familial hypoalphalipoproteinemia.

METHODS AND RESULTS

The FHA (familial hypoalphalipoproteinemia) group comprised 12 subjects. Seven individuals were carrier of a mutation in the apoA-I gene (10), and two were carriers of non-synonymous mutations in the LCAT and ABCA1 gene, respectively. In the remaining three subjects, the etiology of their low HDL phenotype is unknown. The control group consisted of 11 healthy volunteers who were matched for age and body mass index. Exclusion criteria for both groups were significant co-morbidity, alcohol abuse, body mass index ≥ 35 kg/m², or hepatic transaminases > 1.5 ULN. The study protocol was approved by the Institutional Review Board of the Academic Medical Center, University Hospital of Amsterdam.

Seven days before and during sample collection all subjects used a standardized diet to control for weight and cholesterol/fat intake. This diet was based on the Dutch adaptation from the American heart Association, National Cholesterol Education Program-‘ Step by step’- eating to lower your high blood cholesterol (No 94-2920, august 1994). This diet contains about 200 mg cholesterol per day.
Fasting total cholesterol, LDL cholesterol, HDL cholesterol and triglycerides were measured by established enzymatic methods (Reagents Boehringer Mannheim and Technicon USA). Apolipoprotein concentrations were assessed using nephelometry. Plant sterols and lathosterol were determined from non-saponifiable plasma material with GC/MS, as described previously (11).

Stool was collected in plastic buckets in pools of 3 consecutive days. Stool collection was started at day 7 (12). All stool was collected during a 3 day period. After weighing and homogenization, three portions of 50 cc were freeze-dried and the water content of the stool samples was calculated. The freeze dried stool samples were crushed to a homogenous mass and fecal neutral sterols and bile acid were determined by chromatography as previously described (13). The CV’s are 4.9% for bile salt determinations and 5.4% for the neutral sterol determination.

Data are presented as mean ± standard deviation (SD). Differences between the two groups were tested with student’s t-test for normally distributed variables and the non-parametric Mann-Whitney rank test was used for skewed variables. Spearman correlation coefficients were calculated to assess correlations between variables. A p-value < 0.05 was considered to indicate statistical significance.

Baseline characteristics of the two study groups are listed in Table 1. Controls were matched to the FHA group for age and body mass index. HDL cholesterol levels were significantly lower in FHA subjects compared to controls (0.8 vs. 1.6 mmol/L in controls; p<0.001). LDL cholesterol tended to be higher in the FHA group (3.7 vs. 3.0 mmol/L in controls respectively, p=0.05) which was in line with the finding of higher apolipoprotein B levels in these individuals (p=0.001). The mean campesterol concentration of 11.2 ± 4.8 µmol/l was higher in the FHA patients compared with the control subjects (6.3 ± 2.8 µmol/l; p = 0.007). The mean lathosterol concentration in the FHA group was equal to that in controls (6.4 ± 3.5 vs 6.1 ± 4.9; P = 0.8). The campesterol to cholesterol ratio was significantly higher in the FHA patients compared to controls (2.2 ± 1.1 vs 1.3 ± 0.7 µmol/mmol; p=0.03). Baseline lathosterol to cholesterol ratio were similar in both groups.

Panel A of Figure 1 shows that FHA subjects presented with a significant reduction in neutral sterol excretion compared to controls i.e., 4.0 ± 2.4 mg/kg/day vs. 8.9 ± 6.3 mg/kg/day (p=0.01). Data on fecal bile acid excretion provided a similar pattern: panel B shows that the FHA subjects tended to have a lower fecal bile acid excretion compared to controls (2.2 ± 2.0 mg/kg/day vs. 3.1 ± 1.2; p=0.06).

HDL cholesterol levels were strongly inversely related with triglycerides (r=−0.64, p<0.001) and positively correlated with neutral sterol excretion (r=0.53, p=0.01). A positive correlation was also observed for HDL cholesterol and fecal bile acid excretion (r=0.55 , p=0.007). The lathosterol/cholesterol ratio correlated positively with LDL cholesterol, triglycerides and apolipoprotein B but not with HDL cholesterol and apolipoprotein B. On the other hand, the campesterol/cholesterol ratio was inversely correlated with HDL cholesterol and apoA-I.
anticipated, the excretion rates of neutral sterols and bile acids were strongly positively related 
(r=0.55, p=0.007).

**Table 1.** Demographic, lipid and apolipoprotein parameters of individuals with familial 
hyperalphalipoproteinemia (FHA) and normolipidemic controls.

<table>
<thead>
<tr>
<th></th>
<th>FHA</th>
<th>Controls</th>
<th>p†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>45 ± 12</td>
<td>50 ± 12</td>
<td>0.3</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25 ± 4.5</td>
<td>25 ± 3.9</td>
<td>0.9</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>3 (25)</td>
<td>1 (0)</td>
<td></td>
</tr>
<tr>
<td>male, n</td>
<td>6</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
<td>0.8 ± 0.2</td>
<td>1.6 ± 0.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.2 ± 1.0</td>
<td>5.0 ± 1.1</td>
<td>0.6</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
<td>3.7 ± 0.7</td>
<td>3.0 ± 0.9</td>
<td>0.05</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.3 [1.0-2.1]</td>
<td>0.8 [0.5-1.2]</td>
<td>0.05</td>
</tr>
<tr>
<td>Apolipoprotein A-I (mg/L)</td>
<td>102 ± 32</td>
<td>165 ± 25</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Apolipoprotein B (mg/L)</td>
<td>141 ± 33</td>
<td>89 ± 30</td>
<td>0.001</td>
</tr>
<tr>
<td>Non-cholesterol sterols:</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>- campesterol µmol/L</td>
<td>11.2 ± 4.8</td>
<td>6.3 ± 2.8</td>
<td>0.007</td>
</tr>
<tr>
<td>- lathosterol µmol/L</td>
<td>6.4 ± 3.5</td>
<td>6.1 ± 4.9</td>
<td>0.8</td>
</tr>
<tr>
<td>- campesterol/cholesterol ratio, µmol/mmol</td>
<td>2.2 ± 1.1</td>
<td>1.3 ± 0.7</td>
<td>0.03</td>
</tr>
<tr>
<td>- lathosterol/cholesterol ratio, µmol/mmol</td>
<td>1.2 ± 0.5</td>
<td>1.1 ± 0.8</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Values are mean ± SD or median [interquartile range]. p† for comparison FHA versus controls

**DISCUSSION**

The current study shows for the first time that isolated low HDL cholesterol in humans is associated with a reduction of fecal neutral and acidic sterol output.

To our knowledge, Mietinnen and Kesaniemi (4) published the only large study in which the relation between plasma HDL levels and neutral sterol excretion was directly investigated. In 63 normolipidemic middle-aged male volunteers, these investigators reported a significant negative correlation between serum HDL cholesterol and fecal neutral sterol excretion (4) whilst no correlation was found between LDL or VLDL and neutral sterols. In the current analysis, we observed a significant positive correlation between serum HDL cholesterol and neutral sterol excretion when all 23 participants (subjects with low or normal HDL cholesterol levels) were included. It is tempting to speculate that at (high) normal HDL cholesterol levels, transport through the liver is saturated and hepatic cholesterol uptake systems have become
rate-limiting. This may also explain why a 100% increase of HDL cholesterol levels after the use of CETP inhibitors did not cause any change in fecal sterol excretion (9).

Total sterol output in the FHA patients was approximately 460 mg/day, suggesting very low \textit{de novo} cholesterol synthesis. However the lathosterol/cholesterol ratio was not in concert with this finding. We have no explanation for this discrepancy but it is possible that an altered lipoprotein turnover in the FHA patients underlies this phenomenon. It should be noted that the lathosterol/cholesterol ratio has never been validated as an indicator of cholesterol synthesis in subjects with FHA. The campesterol/cholesterol ratio was higher in FHA subjects, suggesting increased cholesterol absorption compared to control subjects which is in line with the low \textit{de novo} synthesis rate.

**CONCLUSIONS**

Glomset (1) proposed the RCT concept almost 4 decades ago. Since then, multiple steps in this pathway have been studied in detail but mainly in animals. Interestingly, combined data from recent animal studies indicate that the plasma HDL cholesterol levels are not related to the amount of cholesterol removed from the body via bile and feces. We here show that in subjects with isolated low HDL cholesterol, fecal sterol excretion rate was markedly decreased compared to age and gender matched controls. The correlation between HDL cholesterol levels and excretion parameters suggests that HDL cholesterol levels are a marker for the rate of cholesterol excretion from the body at least in individuals with isolated low HDL cholesterol.

**ACKNOWLEDGMENTS**

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Figure 1. Fecal neutral sterol (panel A) and bile acids (panel B) excretion in subjects with familial hyperalphalipoproteinemia (FHA, n=12) and normolipidemic controls (n=11). Data are presented as box plots with presentation of median values.
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