Relation Between 25-Hydroxyvitamin D₃, Apolipoprotein A-I, and High Density Lipoprotein Cholesterol

Johan Auwerx, Roger Bouillon, and Hugo Kesteloot

In a survey of cardiovascular risk factors in 185 men and 173 women of a Belgian population group, an independent and highly significant positive correlation was found between the serum concentrations of 25-hydroxyvitamin D₃ and apolipoprotein A-I (p<0.001 in both sexes). 25-Hydroxyvitamin D₃ also showed a positive correlation with high density lipoprotein cholesterol levels (p<0.05 in men and p<0.005 in women). This relation was independent of a possible effect of 25-hydroxyvitamin D₃ on serum calcium. Taking the biological variation of 25-hydroxyvitamin D₃ levels (±2 SD=25 ng/ml) into account, these findings could explain variations in the levels of apolipoprotein A-I of 15 mg/100 ml and of high density lipoprotein cholesterol of 4 mg/100 ml. (Arteriosclerosis and Thrombosis 1992;12:671-674)

KEY WORDS • apolipoprotein A-I • apolipoprotein B • atherosclerosis • cholesterol • high density lipoprotein cholesterol • 25-hydroxyvitamin D₃

In industrialized Western countries, coronary heart disease (CHD) accounts for more deaths than any other disease.¹ Hypercholesterolemia and elevated levels of low density lipoprotein cholesterol in particular are linked to this high cardiovascular mortality, whereas high density lipoprotein cholesterol (HDL-C) appears to be inversely related to CHD incidence.² Delineating the factors that influence concentrations of the lipoproteins and their various apolipoprotein constituents is, therefore, of primary importance.

Most³⁴ but not all⁵⁻¹² clinical studies suggest a hypercholesterolemic and hence atherogenic effect of supplements of vitamin D₃. Others report that vitamin D₃ produces a form of atherosclerosis (or vitamin D₃ sclerosis) typified by diffuse fibroelastic thickening of the arterial wall with no evidence of lipid deposition.³ Calcification, smooth muscle cell degeneration, and other forms of arterial damage may also contribute to vitamin D-induced injury to the arterial wall. Moreover, calcium antagonists appear to protect against atherosclerosis, suggesting a role for calcium in the development of atherosclerotic lesions.¹³

The purpose of our study was to examine, as part of a population survey, the interrelation between serum 25-hydroxyvitamin D₃ (25-OHD₃) levels on the one hand and the serum concentration of total cholesterol, HDL-C, and the apolipoproteins A-I (apo A-I) and B (apo B) on the other hand.

Methods

Subjects

The survey was performed in Belgian military personnel and their dependents residing in the former Federal Republic of Germany. Cardiovascular risk factors were evaluated in 185 men (mean age, 37.1±11.1 years) and 173 women (mean age, 36.8±10.2 years). The examination was on a voluntary basis and was conducted in the autumn of 1983. A clinical examination was performed in all participants. The subjects were asked not to smoke for at least 1 hour before examination. After examination a blood sample was drawn for determination of total serum calcium, serum 25-OHD₃, serum gamma glutamyl transpeptidase (γ-GT), total cholesterol, HDL-C, apo A-I, apo B, and total protein.

Methods

Analysis of serum calcium, γ-GT, total cholesterol, HDL-C, apo A-I, and apo B was performed on fresh plasma. Determinations of 25-OHD₃ were performed on a frozen plasma sample.

Total serum calcium was measured by means of complexometric titration with calcein, a fluorescein derivative, with a Corning 940 calcium analyzer. Total protein was measured by the biuret method. Serum γ-GT was determined by the colorimetric method (Boehringer Mannheim). Serum cholesterol was measured with an enzymatic method (Boehringer Mannheim) in an Abbott analyzer, and HDL-C was measured after precipitation with manganese-heparin.*

After precipitation by addition of specific immune sera that were obtained from the Ortho Pharmaceutical

*Conversion factors for SI units are as follows: for calcium, 1 mg/100 ml=0.25 mmol/l; for 25-OHD₃, 1 ng/ml=2.5 nmol/l; and for cholesterol, 1 mg/100 ml=0.026 mmol/l.
TABLE 1. Biological and Anthropometric Variables of the Study Population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Men (n = 185)</th>
<th>Women (n = 173)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>38.7 ± 10.9</td>
<td>37.2 ± 10.4</td>
<td>NS</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>173.8 ± 5.7</td>
<td>161.9 ± 5.9</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>77.8 ± 10.1</td>
<td>65.1 ± 11.7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Serum calcium (mg/100 ml)</td>
<td>9.7 ± 0.4</td>
<td>9.6 ± 0.3</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Log y-GT (log IU/1)</td>
<td>1.2 ± 0.3</td>
<td>0.9 ± 0.3</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Total cholesterol (mg/100 ml)</td>
<td>259.9 ± 54.4</td>
<td>247.9 ± 47.7</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>HDL cholesterol (mg/100 ml)</td>
<td>43.6 ± 10.5</td>
<td>51.9 ± 12.5</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Apo A-I (mg/100 ml)</td>
<td>101.8 ± 23.4</td>
<td>111.3 ± 28.7</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Apo B (mg/100 ml)</td>
<td>133.3 ± 29.1</td>
<td>130.3 ± 27.6</td>
<td>NS</td>
</tr>
<tr>
<td>25-OHD3 (ng/ml)</td>
<td>29.6 ± 12.1</td>
<td>30.4 ± 14.5</td>
<td>NS</td>
</tr>
</tbody>
</table>

y-GT, serum gamma glutamyl transpeptidase; IU, international units; HDL, high density lipoprotein; apo, apolipoprotein; 25-OHD3, 25-hydroxyvitamin D3; NS, not significant.

Results

The anthropometric data and biological variables of the participants are given in Table 1. As expected, higher values of log y-GT and total serum cholesterol were found in male subjects whereas HDL-C and apo A-I levels were higher in female subjects. Multiple regression analysis was performed on HDL-C and apo A-I levels with the following variables included in the analysis: age, height, weight, total calcium, log y-GT, total protein, and 25-OHD3. In both sexes apo A-I levels showed a significant positive correlation with 25-OHD3 concentrations (r = 0.316 in men and r = 0.274 in women; p < 0.001). HDL-C correlated well with 25-OHD3 (r = 0.264; p < 0.001) in women, whereas in men (r = 0.123) the correlation coefficient did not attain the level of significance. The multiple regression analysis on apo A-I and HDL-C is given in Table 2, and only 25-OHD3 was shown to be consistently and independently correlated with both the apo A-I and the HDL-C concentration. The effect of 25-OHD3 was independent of its relation with the serum calcium level, which was also included in the multiple regression analysis. The biological variation of 25-OHD3 is about 25 ng/ml (±2 SD) and is able to explain a variation in the apo A-I concentration of about 15 mg/100 ml and a variation in the HDL-C concentration of about 4 mg/100 ml, as calculated from the slopes of the multiple regression analysis. Only in men was a significant relation found between body weight (negative) and alcohol consumption expressed as log y-GT (positive) on the one hand and HDL-C and apo A-I values on the other. Total protein concentration appears to be correlated with HDL-C and apo A-I in women. No significant correlation was found between 25-OHD3 and either total serum cholesterol or apo-B levels (Table 3). These two lipid variables were significantly and consistently correlated with age only. Furthermore, in men these two values were significantly correlated with weight, log

TABLE 2. Multiple Regression Analysis on Apolipoprotein A-I and High Density Lipoprotein Cholesterol

<table>
<thead>
<tr>
<th>Apo A-I (mg/100 ml)</th>
<th>Weight (kg)</th>
<th>Total serum calcium (mg/100 ml)</th>
<th>Log y-GT (log IU/1)</th>
<th>25-OHD3 (ng/ml)</th>
<th>Total protein (g/l)</th>
<th>R</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men</td>
<td>b</td>
<td>t</td>
<td>Constant</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>112</td>
<td>-4.29</td>
<td>-0.7</td>
<td>22.4</td>
<td>0.6</td>
<td>0.45</td>
</tr>
<tr>
<td>Women</td>
<td>b</td>
<td>t</td>
<td>95</td>
<td>-13.5</td>
<td>0.5</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-2.6</td>
<td>3.7</td>
<td>4.0</td>
</tr>
<tr>
<td>HDL cholesterol (mg/100 ml)</td>
<td>Men</td>
<td>b</td>
<td>t</td>
<td>68</td>
<td>-0.36</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-5.06</td>
<td>2.7</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>b</td>
<td>t</td>
<td>25</td>
<td>-0.22</td>
<td>0.2</td>
<td>0.47</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-2.94</td>
<td>3.3</td>
<td>2.4</td>
</tr>
</tbody>
</table>

Age and height were found to be not significant.

y-GT, serum gamma glutamyl transpeptidase; IU, international units; 25-OHD3, 25-hydroxyvitamin D3; R, multiple correlation coefficient; apo, apolipoprotein; b, regression coefficient; t, t value for testing the significance of b; HDL, high density lipoprotein.
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genic effect of vitamin D3. In none of these studies, 
emic role for vitamin D3 demonstrated no significant independent correlation with 
total serum cholesterol and apo B levels. 
gated. On the other hand, serum 25-OHD3 levels dem-
ized a hypercholesterolemic and hence an athero-
over, most of the studies in favor of a hyperchoolesterol-
 however, were apolipoprotein levels determined. More-
explain the seasonal variations previously found in 
the present survey only evaluated a part of the year, 
HDL-C concentrations. 20 Because this last study 20 and 
the known seasonal variation of 25-OHD3 
tration and apo A-I (both sexes) and HDL-C (only in 
men) could argue for a role of 25-OHD3 in the 
regulation of apo A-I and apo A-I-containing lipopro-
A-I and HDL-C is causal and that 25-OHD3 exerts a 
cardioprotective role. Carefully controlled interven-
tional and other experimental studies are warranted to 
further clarify this problem.

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\[ \gamma-GT, \text{ total protein whereas in women, height} \]
\[ \text{seemed to exert a borderline significant negative influence on total cholesterol and apo B levels.} \]

\textbf{Discussion}

The average levels of and the differences in total 
cholesterol, HDL-C, and apo A-I between men and 
women are typical of a Western population in the age 
range considered.17 The most important finding in our 
survey is the strong and consistent correlation between 
serum 25-OHD3 levels and serum apo A-I and HDL-C 
levels, independent of other factors that were included in 
the multiple regression analysis and that influence 
apo A-I and HDL-C. Indeed, by means of the multiple 
regression analysis we were able to demonstrate an 
independent relation between the serum levels of apo 
A-I or HDL-C and 25-OHD3 in men and women. The 
significant linear correlation between 25-OHD3 concentra-
tion and apo A-I (both sexes) and HDL-C (only in 
women) could argue for a role of 25-OHD3 in the 
regulation of apo A-I and apo A-I-containing lipopro-
tein particles. Furthermore, the results of this study are 
in accordance with a recent study by the Russian group of 
Antonenko et al,18 who showed that vitamin D-defi-
cient rickets is associated with a decrease in HDL-C 
content. Because of the significant relation between 
25-OHD3 and apo A-I and HDL-C, we hypothesize that 
the known seasonal variation of 25-OHD3 could also 
explain the seasonal variations previously found in 
HDL-C concentrations.20 Because this last study20 and 
the present survey only evaluated a part of the year, 
extrapolation to the whole year remains to be investi-
gated. On the other hand, serum 25-OHD3 levels dem-
onstrated no significant independent correlation with 
total serum cholesterol and apo B levels.

Several\textsuperscript{3-8} but not all\textsuperscript{12,18} previous studies have 
described a hypercholesterolemic and hence an athero-
egenic effect of vitamin D3. In none of these studies, 
however, were apolipoprotein levels determined. Moreover, 
most of the studies in favor of a hypercholesterole-
mic role for vitamin D3\textsuperscript{6,7} were intervention studies 
that were performed by adding large amounts of vitamin 
D to the diet, thus creating an unphysiological situation 
with a vitamin D excess. The Tromsø Heart Study 
argued that patients with a myocardial infarction had a 
higher vitamin D intake.\textsuperscript{6} This finding, however, can be 
criticized because of the difficulties in obtaining precise 
dietary histories and the problems encountered when 
assessing the vitamin D content of food. Later on, direct 
measurement of 25-OHD3 revealed no differences in 
25-OHD3 levels between survivors of a myocardial 
infarction and control subjects.\textsuperscript{11,12}

In conclusion, our study showed the existence of a 
significant positive relation between serum 25-OHD3 
and both HDL-C and apo A-I. This is potentially very 
important in view of the difficulty of influencing the 
HDL-C at the population level.21 On the other hand, 
serum 25-OHD3 was not significantly related to total 
cholesterol and apo B levels. It is, however, premature 
to conclude that the relation between 25-OHD3 and apo 
A-I and HDL-C is causal and that 25-OHD3 exerts a 
cardioprotective role. Carefully controlled interven-
tional and other experimental studies are warranted to 
further clarify this problem.

\begin{table}
\centering
\caption{Multiple Regression Analysis on Apolipoprotein B and Total Cholesterol}
\begin{tabular}{llcccccc}
\hline
Variable & Constant & Age (years) & Height (cm) & Weight (kg) & Log \(\gamma\)-GT (log IU/l) & Total serum calcium (mg/100 mg) & 25-OHD3 (ng/ml) & Total protein (g/l) & \(R\) \\
\hline
Apo B (mg/100 ml) & & & & & & & & & \\
\hline
Men & & & & & & & & & \\
b & -108 & 1.05 & \ldots & 0.56 & 19.01 & \ldots & \ldots & 1.75 & 0.58 \\
t & 5.95 & \ldots & 2.94 & 2.48 & \ldots & \ldots & \ldots & 3.39 & \\
Women & & & & & & & & & \\
b & 101 & 0.82 & \ldots & -0.68 & \ldots & 11.35 & \ldots & \ldots & 0.39 \\
t & 4.32 & \ldots & 2.04 & \ldots & 2.29 & \ldots & \ldots & \\
Total cholesterol (mg/100 ml) & & & & & & & & & \\
Men & & & & & & & & & \\
b & -248 & 2.05 & \ldots & 1.00 & 39.79 & \ldots & \ldots & 3.95 & 0.63 \\
t & 6.52 & \ldots & 2.95 & 2.89 & \ldots & \ldots & \ldots & 4.24 & \\
Women & & & & & & & & & \\
b & 261 & 1.44 & \ldots & -1.19 & \ldots & \ldots & \ldots & 1.67 & 0.38 \\
t & 4.35 & \ldots & -2.01 & \ldots & \ldots & \ldots & \ldots & 2.24 & \\
\hline
\end{tabular}
\textsuperscript{\gamma-GT, serum gamma glutamyl transpeptidase; IU, international units; 25-OHD3, 25-hydroxyvitamin D3; R, multiple correlation coefficient; apo, apolipoprotein; b, regression coefficient; t, t value for testing the significance of b.}
\end{table}


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