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VLDL Cholesterol and VLDL Apolipoprotein B

Preliminary Cross-Sectional Data of the Prospective Epidemiological Study of Company Employees in Westphalia

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Summary: VLDL cholesterol concentrations were determined in 1641 male and 608 female and VLDL apolipoprotein B concentrations in 505 male and 211 female company employees in Westphalia aged 17–70 years.

VLDL cholesterol and VLDL apolipoprotein B values were found to be distributed with positive skew in both sexes but were higher in men than in women (median in men: VLDL cholesterol 0.30 mmol/l, VLDL apolipoprotein B 0.066 g/l, median in women: VLDL cholesterol 0.18 mmol/l, VLDL apolipoprotein B 0.047 g/l).

In males VLDL cholesterol and VLDL apolipoprotein B were closely correlated to each other ($r = 0.757$) as well as to triacylglycerols (VLDL cholesterol: $r = 0.673$, VLDL apolipoprotein B: $r = 0.707$) and HDL cholesterol (VLDL cholesterol: $r = 0.509$, VLDL apolipoprotein B: $r = -0.419$). In females these observed correlations were weaker.

The VLDL cholesterol/VLDL apolipoprotein B ratio was also higher in men (median 4.28 mmol/g) than in women (median 3.15 mmol/g). The ratio correlated to triacylglycerols (men: $r = 0.591$, women: $r = 0.321$). The results suggest that the composition of VLDL may be related to triacylglycerols in serum.

Introduction

Apolipoprotein B represents the functional entity of several lipoproteins in plasma, especially of very low density lipoproteins (VLDL) and low density lipoproteins (LDL). Normally, more than 90% of total apolipoprotein B in plasma is transported in LDL, whereas approximately 4–5% of total apolipoprotein B is found in VLDL (1). While enhanced LDL have been well established as a primary risk factor for coronary heart disease (2, 3) increased VLDL in plasma generally may not be a primary risk factor, but rather a reflection of the existence of other risk factors (4). On the other hand, in several clinical cases increased levels of VLDL and triacylglycerols are obviously associated with premature atherosclerosis (5). The reason for the potential role of VLDL in atherogenesis may be associated with the observation that in several hypertriacylglycerolaemic patients an altered composition of VLDL can be found which is characterised by an enhancement of the cholesterol component of these particles (6). Furthermore, according to studies of *Franceschini* (7) increased concentrations of apolipoprotein B in VLDL are associated with peripheral vascular disease. Therefore, the VLDL composition may be of special clinical and epidemiological significance.

However, the determination of VLDL components has hitherto been a time consuming method, involving the separation of VLDL by ultracentrifugation. Recently a commercial test was developed for the selective precipitation of LDL with polyvinylsulphate (8, 9). Using this procedure VLDL cholesterol as well as VLDL apolipoprotein B (10) can be easily analysed in the superanatant obtained following LDL precipitation.

The present paper reports preliminary results of our epidemiological study in Westphalia (11) regarding the relationship of VLDL apolipoprotein B and VLDL cholesterol to several lipid parameters.

Materials and Methods

Sample material

As test material we used fresh serum from the test series Prospective Epidemiological Study on Company Employees in Westphalia (11).

Methods

Analysis of lipids

Analyses of cholesterol and triacylglycerols were performed with the SMAC Analyser (Technicon GmbH, Bad Vilbel, FRG) as described elsewhere (11).

Analysis of HDL cholesterol

HDL cholesterol was enzymatically analysed using the CHOD-PAP method (Boehringer Mannheim Test Combination No. 187313) as described elsewhere (12).

Analysis of VLDL components

VLDL components were determined in the supernatant obtained following selective precipitation of LDL with polyvinylsulphate as described elsewhere in detail (8, 9). VLDL cholesterol was determined as the difference between cholesterol in the supernatant (which was enzymatically analysed as described for HDL cholesterol) and HDL cholesterol. VLDL apolipoprotein B was determined as described elsewhere in detail (10).

Statistics

Since both parameters were distributed with positive skew, non-parametric statistic methods were used. In correlation analysis *Spearman*'s rank correlation coefficients are given.

Differences in the distribution between subgroups were tested by *Mann-Whitney*'s U-test (two groups) or by the method of *Nemenyi* (multiple comparisons). The level of significance was set at 0.05.

Results

Distribution of VLDL cholesterol and VLDL apolipoprotein B values in normal individuals

In both sexes VLDL cholesterol and VLDL apolipoprotein B values were distributed with positive skew (fig. 1 and fig. 2). Men exhibited obviously higher levels of VLDL cholesterol (median: 0.30 mmol/l, 5th percentile 0.06 mmol/l, 95th percentile 1.19 mmol/l) as well as VLDL apolipoprotein B (median: 0.066 g/l, 5th percentile 0.041 g/l, 95th percentile 0.173 g/l) than women (VLDL cholesterol: median 0.18 mmol/l, 5th percentile 0.033 mmol/l, 95th percentile 0.57 mmol/l, VLDL apolipoprotein B: median 0.047 g/l, 5th percentile 0.030 g/l, 95th percentile 0.075 g/l) ($p < 0.001$ each).

In the group in which VLDL cholesterol was determined, 28% of the women took oral contraceptives at the time of the study; in the group for VLDL apolipoprotein B determination, this figure was 22.8%. Women who took the "pill" showed marginally but not significantly raised levels of VLDL

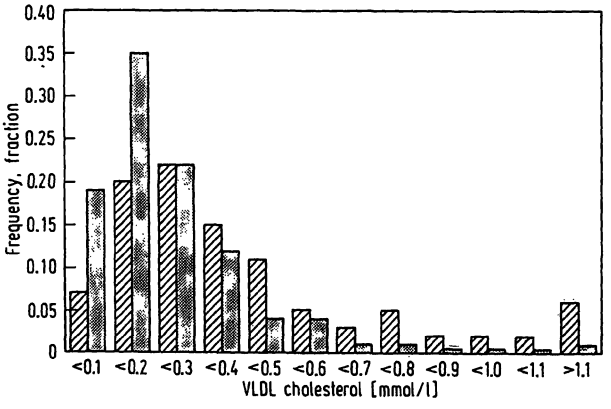

Fig. 1. Distribution of VLDL cholesterol (mmol/l) in normal individuals. On the horizontal axis the upper bounds of the intervals are given.
▨ men, n = 1641; ▩ women, n = 608

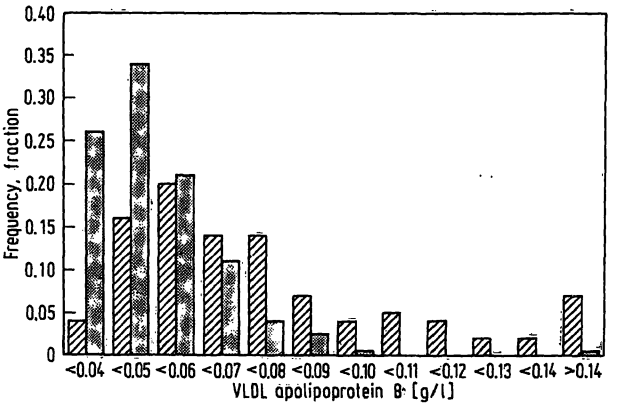

Fig. 2. Distribution of VLDL apolipoprotein B (g/l) in normal individuals. On the horizontal axis the upper bounds of the intervals are given.
▨ men, n = 505; ▩ women, n = 211

Tab. 1. Correlation coefficients between VLDL cholesterol, VLDL apolipoprotein B and lipid parameters.

	VLDL cholesterol		VLDL apolipoprotein B	
	Men (n = 1641)	Women (n = 608)	Men (n = 505)	Women (n = 211)
Cholesterol	0.089***	−0.015	0.176***	−0.142*
Triacylglycerols	0.673***	0.365***	0.707***	0.250***
HDL cholesterol	−0.509***	−0.344***	−0.419***	−0.245***

* $p < 0.05$ *** $p < 0.001$

cholesterol (median: 0.192 mmol/l, 0.175 mmol/l respectively), whereas the difference in VLDL apolipoprotein B values was more pronounced (median: 0.057 g/l and 0.046 g/l, $p < 0.01$). This difference was age-independent. There were no statistically relevant differences for the two parameters for women who had never or had in the past been on the pill.

Correlation of VLDL cholesterol and VLDL apolipoprotein B with lipid parameters

In men there was a strong positive correlation between VLDL cholesterol and VLDL apolipoprotein B values ($r = 0.757$, $n = 505$). In women the positive correlation between these parameters was weaker ($r = 0.362$, $n = 211$).

In men both VLDL components were significantly correlated with all the lipid parameters that were considered (tab. 1), but the correlations with cholesterol were weaker than those with triacylglycerols and HDL cholesterol. In women the correlations between both VLDL components and lipid parameters were much weaker than in men (tab. 1). In both sexes and both VLDL components the correlation coefficients of triacylglycerols were the highest. Obviously, the VLDL cholesterol values increased more than VLDL apolipoprotein B values with increasing triacylglycerols.

VLDL cholesterol/VLDL apolipoprotein B ratio

To test whether the composition of VLDL may be related to lipid parameters, the VLDL cholesterol/VLDL apolipoprotein B ratio was computed. Like the single parameters the ratio was higher in males (median 4.28 mmol/g, 5th percentile 0.94 mmol/g, 95th percentile 8.94 mmol/g) than in females (median 3.15 mmol/g, 5th percentile 0.77 mmol/g, 95th percentile 8.50 mmol/g) (fig. 3, $p < 0.001$). The correlation analysis also showed comparable results to those of the single parameters (tab. 2).

Tab. 2. Correlation coefficients between VLDL cholesterol/VLDL apolipoprotein B ratio and lipid parameters.

	Men (n = 505)	Women (n = 211)
Cholesterol	0.069	0.036
Triacylglycerols	0.591***	0.321***
HDL cholesterol	−0.483***	−0.293***

*** $p < 0.001$

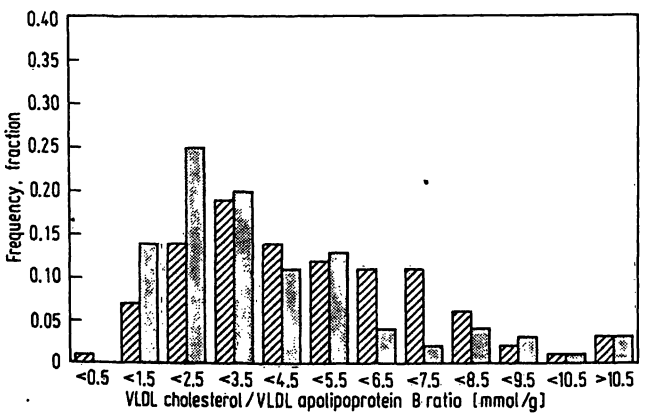

Fig. 3. Distribution of VLDL cholesterol/VLDL apolipoprotein B ratio in normal individuals.
▨ men, n = 505; ▩ women, n = 211

The highest correlation coefficients were found between triacylglycerols and the ratio of VLDL components in males ($r = 0.591$) as well as in females ($r = 0.321$). Furthermore, VLDL cholesterol as well as VLDL apolipoprotein B values in the three subgroups of men with normal (<1.71 mmol/l) and high (>2.28 mmol/l) triacylglycerols differed from each other (tab. 3, $p < 0.01$). Since the differences in VLDL cholesterol were greater than in VLDL apolipoprotein B the ratio showed significantly different values ($p < 0.05$, tab. 3). The number of hyperlipidaemic women was too small to test this observation in females.

Tab. 3. Medians of VLDL cholesterol, VLDL apolipoprotein B and VLDL cholesterol/VLDL apolipoprotein B ratio in subgroups with different triacylglycerol concentrations (men only).

Triacylglycerol concentration	VLDL cholesterol (mmol/l)	VLDL apolipoprotein B (g/l)	VLDL cholesterol/VLDL apolipoprotein B ratio (mmol/g)
<1.71 mmol/l	0.25 (n = 1044)	0.058 (n = 316)	3.33 (n = 316)
1.71–2.28 mmol/l	0.40 (n = 261)	0.080 (n = 82)	4.55 (n = 82)
>2.28 mmol/l	0.78 (n = 336)	1.18 (n = 107)	7.69 (n = 107)

Discussion

In contrast to the determination of apolipoprotein B in total plasma, the determination of apolipoprotein B as a component of lipoproteins, especially of VLDL, is not yet a routine method, due to the time consuming separation of lipoproteins by ultracentrifugation. Using the recently developed selective precipitation procedure for LDL (8), VLDL apolipoprotein B can be determined correctly and easily in the supernatant (10). Our VLDL apolipoprotein B values as well as VLDL cholesterol values for males agree well with the results recently described by *Vega & Grundy* (15). To our knowledge, VLDL apolipoprotein B has not yet been extensively investigated in females. As described for VLDL cholesterol (16) the apolipoprotein B values obtained in females are obviously lower than in males.

The present study showed strikingly close correlations between triacylglycerols and VLDL apolipoprotein B as well as between triacylglycerols and VLDL cholesterol. However, in both sexes the correlation between triacylglycerols and VLDL apolipoprotein B was weaker than the corresponding correlation between triacylglycerols and VLDL cholesterol. Hypertriacylglycerolaemia may therefore be reflected rather by increased VLDL cholesterol than by increased VLDL apolipoprotein B in plasma. Since in hypertriacylglycerolaemic individuals (>2.28 mmol/l) VLDL cholesterol was more enhanced than VLDL apolipoprotein B in hypertriacylglycerolaemic individuals the ratio VLDL cholesterol/VLDL apolipoprotein B is clearly higher than in normotriacylglycerolaemic subjects. Our results agree well with recent findings of *Eisenberg* et al. (6, 17). These authors have shown that hypertriacylglycerolaemia resulted in an altered composition of VLDL particles which are enriched in cholesterol at the cost of apolipoproteins. The reason for this observation has been suggested to be a reflection of an enhanced activity of lipid transfer reactions between lipoproteins in hypertriacylglycerolaemic plasma (6, 17, 18). According to this view, the presence of a high concentration of triacylglycerol-rich lipoproteins in plasma induces accelerated transfer of cholesteryl esters from LDL and HDL to VLDL and triacylglycerols in the opposite direction (19).

This transfer may result in VLDL particles relatively enriched in cholesteryl esters (and LDL and HDL particles relatively enriched in triacylglycerols). Since the triacylglycerol level in plasma in females is lower than in males (16), and since prevalence of hypertriacylglycerolaemia in females is relatively low (11), it can be suggested that in females VLDL are relatively poorer in cholesterol and relatively richer in apolipoproteins than in males. This supposition was supported by our observation that in females the ratio VLDL cholesterol/VLDL apolipoprotein B is lower than in males. Another indicator for differences in VLDL composition between males and females is our observation that in females the correlation between VLDL cholesterol and VLDL apolipoprotein B was relatively weak, while in males the corresponding correlation was relatively close.

The significance of alterations in VLDL composition in relation to the risk of atherosclerosis is an open question and deserves further investigation.

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