Inter-relationships between small, dense low-density lipoprotein (LDL), plasma triacylglycerol and LDL apoprotein B in an atherogenic lipoprotein phenotype in free-living subjects

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ABSTRACT

A predominance of small, dense low-density lipoprotein (LDL) is a major component of an atherogenic lipoprotein phenotype, and a common, but modifiable, source of increased risk for coronary heart disease in the free-living population. While much of the atherogenicity of small, dense LDL is known to arise from its structural properties, the extent to which an increase in the number of small, dense LDL particles (hyper-apoprotein B) contributes to this risk of coronary heart disease is currently unknown. This study reports a method for the recruitment of free-living individuals with an atherogenic lipoprotein phenotype for a fish-oil intervention trial, and critically evaluates the relationship between LDL particle number and the predominance of small, dense LDL. In this group, volunteers were selected through local general practices on the basis of a moderately raised plasma triacylglycerol (triglyceride) level (≥1.5 mmol/l) and a low concentration of high-density-lipoprotein cholesterol (<1.1 mmol/l). The screening of LDL subclasses revealed a predominance of small, dense LDL (LDL subclass pattern B) in 62% of the cohort. As expected, subjects with LDL subclass pattern B were characterized by higher plasma triacylglycerol and lower high-density lipoprotein cholesterol (<1.1 mmol/l) levels and, less predictably, by lower LDL cholesterol and apoprotein B levels (P < 0.05; LDL subclass A compared with subclass B). While hyper-apoprotein B was detected in only five subjects, the relative percentage of small, dense LDL-III in subjects with subclass B showed an inverse relationship with LDL apoprotein B (r = −0.57; P < 0.001), identifying a subset of individuals with plasma triacylglycerol above 2.5 mmol/l and a low concentration of LDL almost exclusively in a small and dense form. These findings indicate that a predominance of small, dense LDL and hyper-apoprotein B do not always co-exist in free-living groups. Moreover, if coronary risk increases with increasing LDL particle number, these results imply that the risk arising from a predominance of small, dense LDL may actually be reduced in certain cases when plasma triacylglycerol exceeds 2.5 mmol/l.

Key words: apoprotein B, atherogenic lipoprotein phenotype, coronary heart disease, hyper-apoprotein B, small, dense low-density lipoprotein, triacylglycerol.

Abbreviations: ALP, atherogenic lipoprotein phenotype; apo B, apoprotein B; CHD, coronary heart disease; HDL, high-density lipoprotein; hyperapo-B, plasma concentration of LDL apo B of >1.3 g/l; LDL, low-density lipoprotein; TG, triacylglycerol (triglyceride); TGRL, TG-rich lipoprotein.

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INTRODUCTION

An atherogenic lipoprotein phenotype (ALP) is potentially the most prevalent (but modifiable) lipid-mediated risk factor for coronary heart disease (CHD) in free-living individuals, i.e. people with lifestyles that are unrestricted with respect to diet and physical activity [1,2]. In its most common form, an ALP may be regarded as a sub-clinical risk factor characterized by moderately raised plasma triacylglycerol (TG; triglyceride) levels, low levels of high-density lipoprotein (HDL) and a predominance of small, dense low-density lipoprotein (LDL) (density > 1.040 g/ml) [3]. Since the latter is a relative term which is technically difficult to measure and interpret, it has remained the least well defined component of this dyslipidaemia. The relative risk associated with small, dense LDL is usually expressed phenotypically as LDL subclass patterns ‘A’ (predominantly large LDL), ‘I’ (LDL of intermediate size) and ‘B’ (a predominance of small, dense LDL with a peak particle diameter of less than 25.5 nm), as detected by gradient gel electrophoresis [4]. While this procedure has proved effective in routine applications, the point at which a relative percentage area under a curve constitutes a ‘predominance’ is open to interpretation. This limits the amount of information provided by this technique to a qualitative, phenotypic classification of LDL subclasses. Even so, small LDL particle size has been shown to be predictive of CHD in large prospective studies [5,6].

Alternative procedures for the quantification of the absolute mass or concentration of small, dense LDL based on ultracentrifugation have proved equally, if not more, effective in discriminating coronary risk and in elaborating an intimate relationship between LDL subclasses and plasma TG [7,8].

Efforts to reduce the risk associated with small, dense LDL have until now focused primarily on lowering plasma TG, an approach which has proved efficacious in redistributing LDL towards larger, less dense, and thus less atherogenic, LDL particles [9–11]. However, an elevation in the absolute number of LDL particles. The results demonstrate that a predominance of small, dense LDL and hyperapo-B do not co-exist in the majority of these individuals. They also provide evidence to suggest that the potential of small, dense LDL as a coronary risk factor in an ALP may be diminished through a decrease in the number of these particles as the plasma TG concentration exceeds 2.5 mmol/l.

Part of this work was presented in abstract form at the summer meeting of the British Hyperlipidaemia Association (Bristol; 1997).

MATERIALS AND METHODS

Subject recruitment

Non-smoking male subjects, aged between 30 and 70 years, were selected from a database held at the Royal Berkshire Hospital, Reading, U.K., initially on the basis of raised plasma TG (1.5–4 mmol/l) and low HDL cholesterol (< 1.1 mmol/l). Exclusion criteria at this stage of selection included a body mass index of > 35, a serum cholesterol concentration of > 8.5 mmol/l, a previous history of myocardial infarction or unstable angina, hypertension, endocrine or liver disease, or any medication known to affect lipid metabolism. All screening data taken from medical records remained confidential. The subjects meeting these criteria were invited to volunteer for a fish-oil intervention trial through their local GP. Those interested in taking part were asked to complete a basic ‘health and lifestyle’ questionnaire with help from a qualified interviewer, principally to exclude alcohol abuse (> 2 drinks/day or > 27 units of alcohol/week), the use of dietary restrictions or supplements within the previous 2 months, or dietary fat intakes falling outside 35–45% of total energy. Suitable volunteers were asked to provide a fasting blood sample (screening sample) for the determination of serum lipids, glucose, HDL cholesterol and LDL subclasses in order to verify their ALP status. Subjects meeting the original entry criteria for serum TG and HDL with a relative percentage of small, dense LDL greater than 50% (LDL subclass pattern B) were recruited for the study after tests...
Small, dense low-density lipoprotein

Figure 1 Distribution profiles of plasma LDL subclasses of selected subjects representative of subclass patterns A, I and B, as measured by density-gradient ultracentrifugation

LDL profiles were generated after centrifugation by continuous-flow spectrophotometric detection of separated LDL subclasses using a Beckman fraction recovery system and gel-scan software as described previously [20]. Each spectrophotometric trace (LDL profile) represents a total of 2500 absorbance (optical density) readings at 280 nm, with a read-time average of 0.25 s, giving a total run-through time of 10 min and 25 s for the generation of each LDL profile. Three visually discrete LDL subclasses were recognized by this method of separation: large and least dense LDL-I (density 1.025–1.034 g/ml), LDL-II (density 1.034–1.044 g/ml) and small, dense LDL-III (density 1.044–1.060 g/ml). LDL subclass patterns A, I (intermediate between A and B) and B were determined on the basis of the relative percentage of LDL-III, as shown in Table 2.

for the exclusion of anaemia and liver dysfunction (haemoglobin and liver enzymes).

Ethical consent for the study was obtained from the West Berkshire Area Health Authority and University of Reading Ethics Committees. All subjects gave signed consent prior to participating in the full study.

Samples and laboratory analysis

Blood samples were taken by venepuncture from fasting subjects (overnight 12 h fast) and collected into EDTA tubes for the determination of lipids, apo B and lipoprotein subclasses, and into fluoride oxalate tubes for the analysis of glucose. Plasma was separated by low-speed centrifugation (1500 g for 10 min) at 4 °C and used for the analysis of serum lipids, glucose and HDL at the University of Reading, and was then transported immediately on wet ice to the University of Surrey for the analysis of LDL subclasses and apo B. Plasma cholesterol, TG and glucose were measured by automated enzymic techniques on a Monarch auto-analyser (Instrumentation Laboratories Ltd., Warrington, U.K.; cholesterol oxidase kit, cat. no. 181618-10; TG kit, cat. no. 181610-60; glucose hexokinase kit, cat. no. 035199-000). LDL apo B was measured in LDL pools (see LDL subclasses below) by immunological assay using a commercially available rabbit antiserum supplied by Roche (kit no. 0736899). Inter-assay coefficients of variation were as follows: plasma cholesterol, 3.2%; TG, 4.2%, apo B and glucose, 4.1%. Plasma HDL cholesterol was isolated by selective anion precipitation using dextran sulphate and MgCl₂·6H₂O for the precipitation of apo B-containing lipoproteins, as previously described [18]. All plasma samples for HDL analysis were diluted (1:1, v/v) with 0.85% (w/v) saline to counteract the adverse effects of large, buoyant TG-rich lipoproteins on precipitation. The inter-assay coefficient of variation for HDL cholesterol was 4%. LDL cholesterol was calculated using the Friedewald formula [19].

LDL subclasses

LDL subclasses were separated directly from plasma by non-equilibrium density-gradient ultracentrifugation using a six-step, curvilinear salt gradient [20]. After centrifugation in a Beckman Optima XL100 ultracentrifuge [(2.02 × 10⁶ g for 23 h) at 23 °C] in a swinging-bucket rotor (Beckman SW40Ti), the separated LDL subclasses were eluted from the tube by upward displacement through a micro-flow cell and detected by continuous spectrophotometric monitoring at 280 nm. The contents of the tube were collected into two pools: an initial pool of 2 ml containing the lighter, TG-rich lipoproteins (TGRL), followed by a 4 ml pool containing the total LDL. The lipoprotein content of these pools was established initially by collecting a large number of fractions across the density gradient and comparing the total protein concentration in these fractions (protein concentration profile) with the continuous-flow lipoprotein profile obtained at 280 nm. LDL subclasses were quantified by dividing proportionately the concentration of apo B in the 4 ml LDL pool on the basis of the relative percentage of each LDL subclass, as calculated from the relative percentage area beneath the profile of individual LDL subclasses. The areas beneath the LDL subclass curves were corrected for variations in the molar absorption coefficient at 280 nm across the lipoprotein profile, as described previously [20]. The within-batch (within-rotor) and between-batch (between-rotor) coefficients of variation for the relative percentages of LDL subclasses were less than 6% and 6.5% respectively, as previously reported [20].

Statistical methods

The normality of the data distribution was assessed by normal-probability plots and the Shapiro–Wilk test for normality. TG values were normalized by square-root
transformation for correlation analysis and plotting. Differences between subject groups, i.e. LDL subclass patterns ‘A’, ‘I’ and ‘B’, were determined by the two-sample t-test for normally distributed data and the Mann–Whitney test for the skewed TG data. Relationships between variables were assessed by calculating Pearson’s correlation coefficients and by simple linear regression. All statistical manipulations were carried out using MINITAB software (Minitab Inc., State College, PA, U.S.A.).

RESULTS

Subject characteristics and distribution of LDL subclass phenotypes
Repeat determinations of the pre-selection criteria (plasma TG, 1.5–4.0 mmol/l; HDL cholesterol, < 1.1 mmol/l) in 92 subjects revealed 34% of those selected for screening (31 subjects) to be outside the original exclusion limits for fasting plasma TG (n = 10) and/or HDL cholesterol (n = 21). A ‘predominance’ of small, dense LDL, as found typically in an ALP (subclass pattern B), was defined initially as a relative abundance of small, dense LDL-III in excess of 50%, as measured by the area beneath the ‘paucidisperse’ LDL subclass profile corresponding to LDL-III. This was not an arbitrary decision, but one based on close examination of the LDL subclass profiles and the delineation of profiles into three categories consistent with the classification of LDL subclass patterns A, B and I, as described by Feingold et al. [4] (Figure 1). Subjects with less than 40% LDL-III, and thus in all cases a predominance of LDL-II (density 1.034–1.045 g/ml), were categorized as pattern A, and those with between 40 and 50% LDL-III were categorized as intermediate pattern I. From the reported prevalence of an ALP in North American studies and from the incidence of mild insulin resistance in free-living European populations, it is possible to estimate the frequency of a predominance of small, dense LDL in the free-living population in northern Europe as approx. 25% among middle-aged males. On the basis of this estimate, the detection of 50 subjects with an ALP would normally require the screening of at least 200 individuals. The pre-selection step used in the present study facilitated recruitment, allowing a target figure of 60 volunteers (for dietary intervention with fish oil) to be reached after the screening of only 92 subjects.

Plasma lipids, apo B and LDL subclasses
Of the 92 subjects screened, 62% (56) expressed a predominance of small, dense LDL (> 50%; LDL subclass pattern B). Of the remaining group, 11% were pattern I and 27% were pattern A. As expected, a predominance of small, dense LDL was associated with significantly higher serum TG (P < 0.001; pattern B compared with patterns A and I) and TGRL apo B (P < 0.01; pattern A compared with pattern B) levels, and significantly lower HDL cholesterol (P < 0.05; pattern B compared with pattern A) (Table 1). With one exception, all subjects with > 50% LDL-III had a serum TG concentration of > 1.5 mmol/l. Less predictably, a pre-

Table 1 Plasma lipids, lipoproteins, glucose, TGRL and LDL apo B in the total group and in subjects with LDL subclass patterns A, I and B

<table>
<thead>
<tr>
<th></th>
<th>Total group (92)‡</th>
<th>LDL pattern A (25)‡</th>
<th>LDL pattern I (10)‡</th>
<th>LDL pattern B (57)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma cholesterol (mmol/l)</td>
<td>6.37 ± 0.86 (2.12)</td>
<td>6.49 ± 0.81 (1.72)</td>
<td>5.95 ± 0.57 (1.54)</td>
<td>6.39 ± 0.91 (2.33)</td>
</tr>
<tr>
<td>Plasma TG (mmol/l)</td>
<td>2.49 ± 1.39 (2.12)</td>
<td>1.72 ± 0.52 (1.12)</td>
<td>1.68 ± 0.48 (1.54)</td>
<td>2.96 ± 1.55* (1.15)</td>
</tr>
<tr>
<td>LDL-C (mmol/l)</td>
<td>4.21 ± 1.04</td>
<td>4.55 ± 0.86</td>
<td>4.19 ± 0.48</td>
<td>4.08 ± 1.15#</td>
</tr>
<tr>
<td>HDL-C (mmol/l)</td>
<td>1.00 ± 0.25</td>
<td>1.11 ± 0.28</td>
<td>0.99 ± 0.12</td>
<td>0.96 ± 0.25*</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>5.60 ± 1.05</td>
<td>5.34 ± 0.74</td>
<td>5.59 ± 0.46</td>
<td>5.71 ± 1.22</td>
</tr>
<tr>
<td>TGRL apo B (g/l)</td>
<td>0.27 ± 0.08</td>
<td>0.24 ± 0.06</td>
<td>0.23 ± 0.03</td>
<td>0.28 ± 0.09*</td>
</tr>
<tr>
<td>LDL apo B (g/l)</td>
<td>0.93 ± 0.23</td>
<td>1.01 ± 0.19</td>
<td>0.93 ± 0.25</td>
<td>0.89 ± 0.17†</td>
</tr>
<tr>
<td>Total apo B/ LDL-C ratio</td>
<td>0.29 ‡</td>
<td>0.28 ‡</td>
<td>0.30 ‡</td>
<td>0.29 ‡</td>
</tr>
</tbody>
</table>
Table 2  Distribution of LDL subclasses expressed as relative percentage area (area beneath the curve) and as a function of LDL apo B (measure of LDL particle number) in the total group and in subjects with LDL subclass patterns A, I and B

The relative percentage areas for small, dense LDL-III were used to define the different LDL subclass patterns categories A, I and B illustrated in Figure 1. Pattern A, < 40% LDL-III (consists predominantly of LDL-II); pattern I (intermediate), 40–50% LDL-III; pattern B, > 50% LDL-III. The total LDL apo B concentration was divided proportionately on the basis of the relative percentage areas for LDL subclasses I, II and III to provide an index of LDL particle number within each subclass. All values represent means ± S.D.

<table>
<thead>
<tr>
<th>No. of subjects</th>
<th>Relative percentage area</th>
<th>Apo B (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LDL-I</td>
<td>LDL-II</td>
</tr>
<tr>
<td>Total group</td>
<td></td>
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<tr>
<td>LDL pattern</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>25</td>
<td>13 ± 5</td>
</tr>
<tr>
<td>I</td>
<td>10</td>
<td>11 ± 3</td>
</tr>
<tr>
<td>B</td>
<td>57</td>
<td>5 ± 4</td>
</tr>
</tbody>
</table>

dominance of small, dense LDL-III was frequently associated with lower LDL cholesterol \( P < 0.05 \); pattern A compared with pattern B) and apo B \( P < 0.05 \); pattern A compared with pattern B) levels. It was not possible to discriminate between different LDL subclass patterns (the relative abundance of small, dense LDL-III) on the basis of total plasma or LDL cholesterol, or the ratio of LDL apo B to LDL cholesterol in this group. Only five subjects presented with hyperapo-B (LDL-apo B > 1.3 g/l), three of which had LDL subclass pattern A.

The distribution of LDL subclasses expressed in terms of apo B concentration provided very similar information to the relative percentage areas, with the notable exception for LDL-III in pattern B, where LDL apo B values were proportionally lower (0.59 g/l) than for the relative percentage of LDL-III (69%) (Table 2). The relative percentage of small, dense LDL-III and concentration of LDL apo B (particle number) showed a significant inverse relationship in subjects with LDL subclass pattern B (Figure 2; \( r = -0.57, P < 0.001 \)). The strength of this relationship relied heavily on a subset of individuals with low LDL apo B levels but a significantly higher proportion of small, dense LDL-III (> 70%).

Relationship between plasma TG and LDL subclasses, expressed as relative percentage area and as LDL apo B

The relationships between the relative percentages of LDL subclasses and plasma TG (1.5–4.0 mmol/l) were consistent with that previously reported in patients with CHD and normal healthy groups. For all LDL subclass patterns, the larger and less dense LDL-I and -II subclasses showed a continuous, negative association (results not shown), and small, dense LDL-III showed a statistically positive association, with plasma TG levels (Figure 3a). Despite the reputed existence of a critical threshold value for plasma TG of 1.5 mmol/l, above which LDL becomes small and dense, a significant number of subjects were found with patterns A and I at plasma TG levels above 1.5 mmol/l. Since LDL apo B was inversely related to plasma TG in subjects with pattern B (Figure 3b), when LDL subclasses were expressed in terms of apo B (particle number), the associations with plasma TG were maintained for LDL-I and LDL-II (results not shown), but not for small, dense LDL-III. Hence the concentration of LDL-III increased up to a plasma TG level of 2.5 mmol/l and then diminished above this value. The subjects falling into the latter category represented the group of individuals with a low number of LDL particles but a relatively high percentage of small, dense LDL-III, as described above.

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Figure 3  Relationships between plasma TG and (a) the relative percentage area of small, dense LDL-III, (b) LDL apo B and (c) small, dense LDL-III apo B (LDL particle number)

(a) The distribution of small, dense LDL-III as measured by relative percentage area showed a significant positive correlation with plasma TG (triglyceride) in the total group (regression line shown: \( r = 0.63, P < 0.001 \)). This correlation was upheld for subjects with subclass pattern B ( ) but not for those with patterns A ( ) or I ( ). (b) LDL apo B showed a significant negative correlation with plasma TG in the total group (regression line shown: \( r = -0.42, P < 0.001 \)). The significance of this correlation was greater in subjects with LDL subclass pattern B ( ) but not significant in subjects with either pattern A ( ) or pattern I ( ). (c) The concentration of small, dense LDL-III in terms of apo B (particle number) showed a significant correlation with plasma TG up to a TG value of 2.5 mmol/l in the total group (solid regression line shown: \( r = 0.39, P < 0.01 \)) which was diminished above this value, chiefly in subjects with subclass pattern B ( ) (indicated by the broken line). This correlation was not upheld for any individual LDL subclass pattern. Plasma TG was shown to be non-parametrically distributed, and was square-root transformed for the scatter plots (a–c) and correlation analysis. The maximum value on the abscissa was set at 3 mmol/l to enhance the graphic illustration of the inter-relationships in these scatter plots. This excluded three subjects with plasma TG levels in excess of this value.

DISCUSSION

This study reports a procedure for the recruitment of free-living subjects with an ALP for a fish-oil intervention trial. Subjects were initially pre-selected from an existing hospital database on the basis of the two principal features of an ALP that can be measured routinely, namely a moderately raised plasma TG level and low HDL. Those meeting the entry criteria were accessed through their GP, and screened at the Universities of Reading and Surrey for confirmation of their plasma lipid levels and to verify their ALP status, by the measurement of LDL subclasses. While this process produced a recruitment rate for an ALP of 62%, it was clearly designed to bias the selection towards subjects with a predominance of small, dense LDL. As such, the results convey no information with respect to the prevalence of an ALP in the free-living population, which would require the random selection of subjects from a substantially larger free-living population. Nevertheless, this procedure was successful in targeting a group of volunteers predisposed to increased CHD risk, who represent an appropriate and predictably responsive group for a dietary intervention trial with fish oil. It is noteworthy that a major proportion of these individuals were unremarkable with respect to their total plasma and LDL cholesterol levels and, although at potentially increased risk, would otherwise escape any form of clinical management directed at blood lipids.

Serum cholesterol is now recognized to be only a weak predictor of coronary risk within populations [21]. Furthermore, variations in total and LDL cholesterol levels fail to provide an adequate scientific basis on which to explain the relationship between diet and CHD. One plausible explanation for this lies in the nature of atherogenicity in LDL. This is now known to reside in subtle modifications in the structural properties of LDL that accompany a decrease in its particle size [22,23] and an increase in the number of LDL particles (hyperapo-B) [21], and not in its cholesterol content per se. While hyperapo-B is invariably characterized by an abundance of small, dense LDL, the extent to which increased particle number contributes to the risk associated with small, dense LDL in an ALP in free-living individuals is unknown. The present study shows that a predominance of small, dense LDL may not always be found in association with a raised number of LDL particles (LDL apo B). Although hyperapo-B and small, dense LDL commonly co-exist in familial combined hyperlipidaemia, it is interesting to note that a significant number of individuals with this condition and with LDL subclass pattern B have been found with total apo B values well below 1.3 g/l [24]. In the present study, LDL apo B levels showed an inverse relationship with the relative abundance of small, dense LDL-III in subjects with LDL subclass pattern B (Figure 2), most notably in subjects...
with a serum TG level above approx. 2.5 mmol/l. This finding provides support for expressing the level of small, dense LDL-III in terms of the concentration of plasma apo B. It also might suggest that the mechanisms that regulate the concentration of apo B and thus LDL particle number may be distinct from those regulating the distribution of LDL particle size.

A considerable amount of the variation in the relative abundance of small, dense LDL is known to be determined by plasma TG levels or, more specifically, the concentration and composition of TGRL, particularly large TG-rich very-low-density lipoprotein species [25]. These TGRLs promote the cholesteryl-ester-transfer-protein-mediated net transfer of TG into LDL, which then undergoes remodelling into smaller particles via the action of hepatic TG lipase. On the other hand, hyperapo-B results from the overproduction and secretion of apo B in very-low-density lipoprotein, and may be linked to the formation of small, dense LDL through mechanisms of insulin resistance [26]. Failure of insulin action has been linked to the postprandial elevation of TGRL and non-esterified fatty acids; the latter are known to stimulate the secretion of apo B in the liver [27]. Thus, at least in theory, it is reasonable to expect hyperapo-B and small, dense LDL to co-exist as common features of insulin-resistant dyslipidaemia. The finding of a predominance of small, dense LDL without hyperapo-B in free-living individuals, but the frequent co-existence of these lipid abnormalities in hyperlipidaemic (insulin-resistant) states, implicates the involvement of other predisposing factors in the aetiology of these conditions. Whatever the underlying cause, genetic predisposition and specific diet–gene interactions are likely to contribute to the development of these two phenomena.

The lower limit for the plasma TG concentration for inclusion in the intervention study was set at 1.5 mmol/l, since this value was previously shown to represent a critical threshold in terms of a raised concentration of small, dense LDL [7,28]. In one of these previous studies [7], when plasma TG exceeded this value the relative CHD risk associated with a raised concentration of small, dense LDL-III (> 100 mg of total lipoprotein mass/100 ml of plasma), which presumably reflected an increase in both the relative abundance (‘predominance’) and number of small, dense LDL particles in diseased and post-myocardial infarction survivors, became highly significant. It is not exactly clear how the value of 100 mg/dl corresponds to the risk associated with hyperapo-B, i.e. LDL apo B > 1.3 g/l. In clinically proven CHD, the increased risk may well originate from an increase in the number of small, dense LDL particles. This information will not be conveyed through the classification of LDL subclass pattern B.

The relationship between plasma TG and small, dense LDL-III in the present study is consistent with earlier findings, and re-affirms the positive and continuous relationship between these two parameters up to a fasting TG level of approx. 4 mmol/l in male subjects [24]. However, while this trend applies to both the relative percentage and total lipoprotein mass of LDL-III, when small, dense LDL is expressed as a function of LDL apo B, the positive relationship with plasma TG disappears once the latter exceeds 2.5 mmol/l (Figure 3c), largely because plasma TG and LDL apo B are inversely related (Figure 3b). The implications of this finding are two-fold. First, in subjects with TG > 2.5 mmol/l, the detection of LDL subclass pattern B might, in the absence of hyperapo-B, result in an overestimation of the CHD risk associated with this condition, as indicated above. Secondly, it might suggest that the risk mediated through small, dense LDL is not continuous, and that individuals with the greatest number of small, dense LDL-III particles present with only moderately raised plasma TG (1.5–2.5 mmol/l). Evidence from kinetic studies in severely hypertriglyceridaemic patients supports the possibility that, as plasma TG rises above 2.5 mmol/l, LDL is reduced even further in size to smaller particles that are cleared very rapidly or ‘hypercatabolized’ from the circulation [29].

The interpretation of CHD risk associated with small, dense LDL in an ALP is now of increasing importance, in view of the high frequency of this phenotype among free-living groups and the role of diet as a means of reducing this potential risk. The present study raises an important question: are free-living and otherwise healthy individuals with a predominance of small, dense LDL but without hyperapo-B still at increased CHD risk? Further case-control and prospective studies with firm clinical end-points will be required to resolve this issue.

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