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Parabolic relationship between plasma triacylglycerols and LDL-cholesterol in familial combined hyperlipidaemia: the multiple-type hyperlipidaemia explained?

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ABSTRACT

FCHL (familial combined hyperlipidaemia) is a highly prevalent genetic lipid disorder that accounts for a substantial number of premature cardiovascular events. To date, FCHL has been complicated by the different lipid phenotypes that are present within one family and one individual patient over time. In the present study, we hypothesized that a parabolic relationship between plasma triacylglycerols (triglycerides) and LDL (low-density lipoprotein)-cholesterol can explain this so-called 'multiple-type hyperlipidaemia' in FCHL. Our hypothesis was tested in two well-documented FCHL cohorts [Maastricht ($n = 145$) and Nijmegen ($n = 299$)] that were followed over a 5-year interval. Three groups were constructed depending on plasma triacylglycerols: group A (individuals with both measurements below 1.5 mmol/l), group B (one measurement below and one measurement above 1.5 mmol/l) and group C (both measurement above 1.5 mmol/l). In both male, but not female, cohorts, a significant positive relationship between plasma triacylglycerols and LDL-cholesterol was observed in group A ($P = 0.02$ for Maastricht cohort and $P = 0.001$ for the Nijmegen cohort), a significant negative relationship in group C ($P = 0.01$ for Maastricht cohort and $P = 0.02$ for the Nijmegen cohort), and a relationship intermediate to group A and C in group B. In contrast, both apoB (apolipoprotein B) levels and the prevalence of cardiovascular disease were related with plasma triacylglycerols in a more linear fashion. In conclusion, a parabolic relationship between plasma triacylglycerols and LDL-cholesterol explains the 'multiple-type hyperlipidaemia' in FCHL. In addition, the linear relationship between triacylglycerols and both apoB levels and the prevalence of cardiovascular disease substantiate the use of apoB instead of LDL-cholesterol in the diagnosis of FCHL and the prediction of cardiovascular disease.

Key words: apolipoprotein B (apoB), cardiovascular disease, coronary artery disease, familial combined hyperlipidaemia (FCHL), triacyglycerol, very-low-density lipoprotein (VLDL).

Abbreviations: apoB, apolipoprotein B; BMI, body mass index; BP, blood pressure; CVD, cardiovascular disease; FCHL, familial combined hyperlipidaemia; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-LDL.

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INTRODUCTION

Almost 30 years ago, Goldstein et al. [1] were the first to delineate FCHL (familial combined hyperlipidaemia) as a genetic hyperlipidaemia. Nowadays, it is well-established that in Western societies FCHL is a highly prevalent disease (1 in 100) with an approx. 5-fold increased risk of premature cardiovascular complications [1,2]. It has been estimated that 10 % of patients with premature coronary artery disease are affected by FCHL [1]. Many patients with FCHL have features of the metabolic syndrome, such as insulin resistance [3], visceral obesity [2,4], fatty liver [5,6], low HDL (high-density lipoprotein)-cholesterol and high BP (blood pressure) [7]. With the current epidemic of obesity, it is expected that the prevalence of the metabolic syndrome, and hence FCHL, will increase even further with a subsequent increase in the incidence of cardiovascular complications.

Despite the progressive insights into the clinical syndrome, several important questions around FCHL remain. One of the most challenging is the puzzle of the different plasma lipid phenotypes observed in relatives in families with FCHL, or even more confusing within one patient with FCHL [1,8], i.e. hypercholesterolaemia, hypertriglyceridaemia or the combination of both. This phenomenon is called the 'multiple-type hyperlipidaemia' and it has resulted in the requirement that an elaborate diagnostic procedure in several relatives is needed to robustly establish FCHL in a family and subsequently in one patient with FCHL. Furthermore, once a subject is diagnosed as 'affected', he or she might be non-affected at the next visit due to the same phenomenon. Efforts have therefore been made to circumvent this problem by redefining FCHL with parameters that are more specific and more stable over time, such as apoB (apolipoprotein B) [9,10].

Previous studies by Veerkamp and co-workers [11,12] have demonstrated that the switch in triacylglycerol (triglyceride) phenotype, i.e. from normotriglyceridaemia to hypertriglyceridaemia or vice versa, is associated with changes in BMI (body mass index) and insulin resistance in FCHL. More recently, another metabolic factor, i.e. hepatic fat as reflected by plasma alanine aminotransferase levels, was added to this list in a second Dutch FCHL cohort [13]. As the metabolic pathways contributing to the switch in triacylglycerol phenotype are being elucidated, no factors have yet been linked to the switch in cholesterol phenotype. In the present study, we anticipated that the switch in cholesterol phenotype depends on changes in plasma triacylglycerols.

Careful study of kinetic data on synthesis and metabolism of lipoprotein particles, as reviewed by Packard and Shepherd [14], has led us to hypothesize that plasma triacylglycerols and LDL (low-density lipoprotein)-cholesterol levels are part of a metabolic continuum that follows a parabolic relationship, i.e. at low triacylglycerol levels (below 1.5 mmol/l), LDL-cholesterol increases in parallel with increasing triacylglycerols levels, whereas a further increase in triacylglycerols above the triacylglycerol cut-off of 1.5 mmol/l is associated with a decline in LDL-cholesterol. This complex relationship would be due to the secretion of different species of VLDL (very-LDL) particles by the liver, i.e.VLDL2 and triacylglycerol-rich VLDL1. Since VLDL2 particles, which are mainly catabolized into LDL particles, are secreted when plasma triacylglycerols are low [14], a positive relationship between plasma triacylglycerols and LDL-cholesterol can be anticipated when plasma triacylglycerols are below 1.5 mmol/l. In contrast, when plasma triacylglycerols increase, VLDL1 particles become abundant, which are not all catabolized into LDL particles [14]. Furthermore, these LDL particles are cholesterol-depleted due to CETP (cholesteryl ester transfer protein)-mediated cholesteryl ester exchange with VLDL1 particles [14]. As a consequence, we hypothesized that, at high triacylglycerols (above 1.5 mmol/l), the relationship between plasma triacylglycerols and LDL-cholesterol is negative (see Figure 1 for a detailed explanation).

A parabolic relationship between plasma triacylglycerols and LDL-cholesterol could theoretically account for the switch in cholesterol phenotype in FCHL. Therefore, in the present study, we investigated the presence of such a parabola in two previously reported well-defined FCHL cohorts that have been followed over time, i.e. the Maastricht and Nijmegen FCHL cohorts [11,13]. In addition, we studied the longitudinal relationship between plasma triacylglycerols and apoB, hallmarks of FCHL, and the prevalence of CVD (cardiovascular disease), the clinically relevant end point of FCHL.

MATERIALS AND METHODS

Study populations

Family members with FCHL were collected and followed prospectively in two cohorts: the Maastricht and Nijmegen cohorts. The Maastricht pedigrees with FCHL ($n = 145$ subjects) were originally recruited based on the cut-off values of 6.5 mmol/l and/or 2.3 mmol/l for plasma cholesterol and triacylglycerols respectively. Their baseline (1999) and follow-up (2004) characteristics have recently been described in detail [13]. The Nijmegen families with FCHL ($n = 299$ subjects) were originally recruited based on the 90th percentile diagnostic criteria for hypercholesterolaemia and hypertriglyceridaemia, which has been described in detail elsewhere, together with their baseline (1994) and follow-up (1999) characteristics [11].

In both cohorts, Type 2 diabetes mellitus was an exclusion criterion for the index patient (= proband) [7,11]. No subjects carried the apoE2/E2 genotype. Furthermore, patients with FCHL were withdrawn from lipid-lowering medication for at least 2 weeks prior to both visits.

The presence of CVD was defined by angina pectoris, myocardial infarction, stroke, peripheral vascular disease or vascular surgery.

The studies were approved by the Ethics Committee of the University Medical Center Nijmegen and the University Hospital Maastricht/Maastricht University. All subjects gave written informed consent.

Measurements

Subjects visited the study centres after an overnight fast. Weight and height were measured with participants wearing light clothing only, and the BMI was calculated as weight divided by height squared (kg/m^2).

Fasting plasma triacylglycerols, total cholesterol, HDL-cholesterol and apoB were determined as described previously [7,11,15]. LDL-cholesterol was calculated with the Friedewald formula [LDL-cholesterol = total cholesterol − HDL-cholesterol − (plasma triacylglycerols × 0.45)] [16]. As this formula is only valid for plasma triacylglycerols < 4.5 mmol/l [16], subjects with triacylglycerol levels above this value were excluded from analyses (six subjects from the Maastricht cohort and seven subjects from the Nijmegen study population). In the Nijmegen FCHL cohort, LDL-cholesterol was also approximated by subtracting VLDL-cholesterol and HDL-cholesterol from total cholesterol levels. VLDL-cholesterol was measured by ultracentrifugation [9].

Statistical analyses

Baseline characteristics of the cohorts are presented as means ± S.D. or medians (interquartile range). A Student's *t* test was used to detect differences in age between FCHL probands (= index patient of one pedigree with FCHL) and their relatives, and between the Maastricht and Nijmegen probands/relatives. The anthropometric and lipid parameters were compared by linear regression with the inclusion of age and sex.

To test the possibility of a parabolic relationship between plasma concentrations of triacylglycerols and LDL-cholesterol as an explanation for the 'multiple-type hyperlipidaemia' in FCHL, three groups were defined (Figure 1): a group with plasma triacylglycerols below 1.5 mmol/l both at baseline and at follow-up (group A), a group with triacylglycerols above 1.5 mmol/l at both time points (group C), and the remaining group in whom plasma triacylglycerols were below 1.5 mmol/l at one time-point and above 1.5 mmol/l at the other moment (group B). The cut-off value of 1.5 mmol/l was used as it is commonly accepted that from this value there is substantial cholesteryl ester exchange between VLDL1 and LDL particles (Figure 1) [14]. As sex hormones are known to influence lipid metabolism [17], all analyses were conducted for male and female subjects separately. A mixed linear model for repeated measurements with a random intercept was used accounting for repeated measurements in one individual. A significant relationship should therefore be interpreted as the intra-individual relationship averaged for all subjects in the particular subgroup. According to our hypothesis, we anticipated a significant positive relationship in group A, a significant, negative relationship in group C and a relationship intermediate to groups A and C in group B (Figure 1). In addition, the regression slopes of the different subgroups were compared with each other by using interaction terms. All analyses were conducted with SPSS 13.0 statistical package (SPSS Inc.).

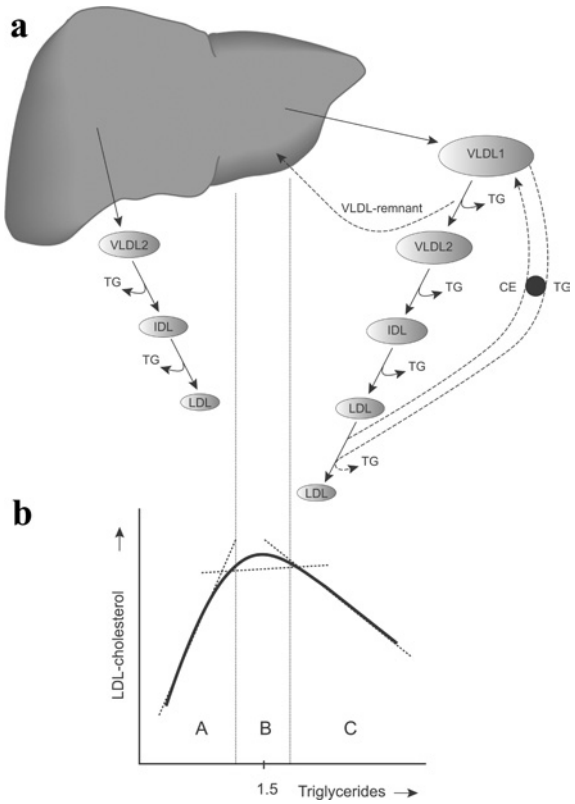

Figure 1 Hypothesized parabolic relationship between plasma triacylglycerols and LDL-cholesterol

In normotriglyceridaemic conditions, triacylglycerols are mainly incorporated in VLDL2 particles, which are mostly degraded into LDL particles. Therefore an increase in VLDL2 particles is expected to lead to an increase in plasma triacylglycerols (TGs) and LDL-cholesterol, explaining part A of the parabolic curve in (b). A further increase in plasma triacylglycerols is accompanied by an increased secretion of triacylglycerol-rich VLDL1 particles. VLDL1 particles exchange cholesteryl ester (CE) and triacylglycerols with LDL (and HDL) particles, mediated by CETP, resulting in cholesterol-depleted LDL particles. Many cholesteryl esters transferred to VLDL1 particles leave the plasma cholesterol pool, since it is estimated that a large proportion of the VLDL1 particles is not catabolized into VLDL2 and eventually to LDL, but is directly taken up as VLDL remnants by, among others, the liver. This would explain the negative correlation between plasma triacylglycerols and LDL-cholesterol (part C of the parabolic curve in b). Part B of the parabolic curve in (b) depicts the transition from VLDL2 secretion to VLDL1 secretion by the liver. This occurs, on average, at plasma triacylglycerols of 1.5 mmol/l. The three dashed lines in (b) indicate the three subgroups that have been constructed to test our hypothesis of a parabolic relationship (see the Materials and methods section). IDL, intermediate-density lipoprotein.

Table 1 Baseline characteristics of probands and relatives with FCHL in the Maastricht and Nijmegen cohorts

Values are means ± S.D., or medians (interquartile range). Values in square brackets indicate males and females respectively. All analyses were Hochberg corrected. *$P < 0.05$ compared with relatives, as determined using a Student's t test; †$P < 0.05$ compared with the Maastricht relatives, age- and sex-corrected; ‡$P < 0.05$ compared with the Maastricht probands, age- and sex-corrected.

	Maastricht FCHL cohort		Nijmegen FCHL cohort	
Characteristic	Relatives	Probands	Relatives	Probands
Male/female (n)	50/68	8/13	115/147	19/11
Age (years)	41.1 ± 15.8	52.3 ± 11.1*	38.2 ± 15.8	48.9 ± 16.4*
BMI (kg/m^2)	25.8 ± 4.2	28.5 ± 4.3	23.9 ± 3.5†	25.3 ± 4.2‡
Total cholesterol (mmol/l)	5.4 ± 1.2	6.4 ± 1.1	5.6 ± 1.3†	6.3 ± 1.3
Triacylglycerols (mmol/l)	1.2 (0.9–1.7)	2.1 (1.3–2.4)*	1.4 (0.8–1.8)	2.1 (1.6–3.0)*
ApoB (g/l)	1.1 ± 0.3	1.4 ± 0.2*	1.1 ± 0.3	1.3 ± 0.3
HDL-cholesterol (mmol/l)	1.0 ± 0.2 [0.8/1.0]	0.9 ± 0.3 [0.9/0.9]	1.2 ± 0.3 [1.1/1.3]†	1.0 ± 0.3 [0.9/1.2]
LDL-cholesterol (mmol/l)	3.9 ± 1.1	4.6 ± 1.2	3.7 ± 1.2	4.3 ± 1.3
CVD (%)	11 [15/8]	33 [42/27]	11 [16/7]	37 [62/5]

RESULTS

Baseline characteristics of Maastricht and Nijmegen FCHL study populations

Baseline characteristics of Maastricht and Nijmegen FCHL probands and their relatives are shown in Table 1. Maastricht and Nijmegen probands with FCHL were significantly older when compared with their relatives. The seemingly marked difference in total cholesterol levels between probands with FCHL and relatives was lost after correction for age and sex ($P = 0.07$ for the Maastricht cohort and $P = 0.32$ for Nijmegen cohort). In contrast, age- and sex-adjusted analyses revealed that probands with FCHL still had significantly higher plasma triacylglycerols (Table 1).

Probands and relatives in the Maastricht population were significantly more obese than in the Nijmegen FCHL cohort (Table 1). Furthermore, small, but significant, differences were observed for plasma total cholesterol and HDL-cholesterol levels between the Maastricht and Nijmegen relatives with FCHL.

As reported previously [11,13], the proportion of subjects who switched lipid phenotype during follow-up, i.e. from normotriglyceridaemia to hypertriglyceridaemia, from normocholesterolaemia to hypercholesterolaemia or vice versa, was similar in both cohorts (30% and 28% in the Maastricht and Nijmegen cohorts respectively).

Longitudinal parabolic relationship between plasma triacylglycerols and LDL-cholesterol

A possible parabolic relationship between plasma triacylglycerols and LDL-cholesterol, to explain the 'multiple-type hyperlipidaemia' in FCHL, was first evaluated in males with FCHL from both study populations. As shown in Figure 2(a), mixed linear regression analysis revealed a 5-year positive relationship between plasma triacylglycerols and LDL-cholesterol for Maastricht men with FCHL in group A (plasma triacylglycerols below 1.5 mmol/l at both time points; $P = 0.02$) and group B (plasma triacylglycerols below and above 1.5 mmol/l at different time points; $P = 0.2$). This relationship was significant, but negative, for men in group C (plasma triacylglycerols above 1.5 mmol/l at both time points; $P = 0.01$). According to our hypothesis, the slopes of the regression lines, indicated as β, decreased with increasing plasma triacylglycerols ($\beta_A = 1.8$, $\beta_B = 0.3$ and $\beta_C = -0.7$; Figure 2a). Furthermore, these slopes were all significantly different from each other (β_A compared with β_B, $P = 0.02$; β_B compared with β_C, $P = 0.003$; and β_A compared with β_C, $P < 0.001$; Hochberg corrected). Comparable results were obtained in the male Nijmegen cohort (Figure 2b). Of additional interest, very similar results were obtained when LDL-cholesterol levels, approximated by subtracting VLDL-cholesterol and HDL-cholesterol from total cholesterol levels, were taken for analysis (Figure 2b, inset). Therefore the use of the Friedewald formula does not account for the observed parabola.

The results for the female Maastricht and Nijmegen FCHL cohorts were less consistent: despite a trend towards a parabolic relationship in the Maastricht cohort ($\beta_A = 0.6$, $P = 0.02$; $\beta_B = 0.5$, $P = 0.2$; and $\beta_C = -0.3$, $P = 0.1$), no significant relationship was observed in any subgroup of the Nijmegen cohort ($\beta_A = -0.1$, $P = 0.5$; $\beta_B = -0.2$, $P = 0.3$; and $\beta_C = 0.01$, $P = 0.9$).

Parabolic relationship between plasma triacylglycerols and LDL-cholesterol within individual male patients with FCHL

Further evidence for the longitudinal relationship between plasma triacylglycerols and LDL-cholesterol

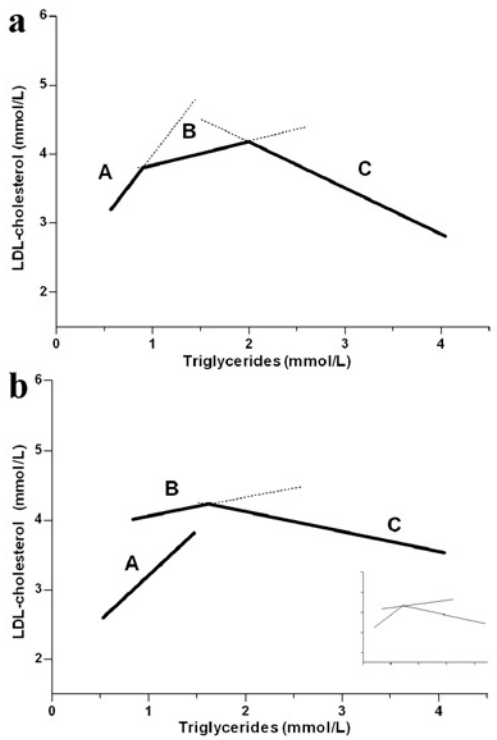

Figure 2 5-Year intra-individual relationship between plasma triacylglycerols and LDL-cholesterol in male Maastricht (a) and Nijmegen (b) FCHL cohorts

(a) Maastricht cohort: group A ($n = 19$), $\beta_A = 1.8$, $P = 0.02$; group B ($n = 19$), $\beta_B = 0.3$, $P = 0.2$; group C ($n = 20$), $\beta_C = -0.7$, $P = 0.01$. (b) Nijmegen cohort: group A ($n = 46$), $\beta_A = 1.0$, $P = 0.001$; group B ($n = 37$), $\beta_B = 0.2$, $P = 0.3$; group C ($n = 51$), $\beta_C = -0.2$, $P = 0.02$. The inset in (b) shows the results for LDL-cholesterol approximated by subtracting VLDL-cholesterol and HDL-cholesterol from total cholesterol in the Nijmegen FCHL population. Regression lines are drawn from the minimum to the maximum triacylglycerols value of each subgroup.

in the male FCHL population described above was assessed by investigating whether one individual can pass completely through the parabolic curve. For this, at least three consecutive measurements in one individual were required with plasma triacylglycerols values below, around and above the parabolic peak value of 1.5 mmol/l. Although such series of data are hard to obtain for each individual, 13 male subjects in the combined study population were measured more than twice and fulfilled each of the following plasma triacylglycerols criteria: one measurement around 1.5 mmol/l, i.e. between the arbitrary values of 1.4 and 1.6 mmol/l, one measurement below 1.4 mmol/l and one measurement above 1.6 mmol/l. Of the 13 subjects who met these criteria, 11 subjects had an increase in LDL-cholesterol levels when plasma triacylglycerol values increased from low to moderate levels, whereas a further increase was accompanied with a decrease in LDL-cholesterol (Figure 3).

Longitudinal relationship between plasma triacylglycerols and apoB and the prevalence of CVD

As elevated apoB levels, the equivalent of the amount of atherogenic particles in plasma, are a hallmark of FCHL, we subsequently analysed the longitudinal relationship between plasma triacylglycerols and apoB in the male Maastricht and Nijmegen FCHL cohorts. Of interest, no parabolic relationship was observed. At the lower plasma triacylglycerol range, both cohorts had an increase in apoB with increasing triacylglycerols, which was significant for the Nijmegen cohort ($\beta_A = 0.2$, $P < 0.001$), but not for the Maastricht population ($\beta_A = 0.3$, $P = 0.10$) (Figures 4a and 4b). A further increase in plasma triacylglycerols was not associated with a decrease in plasma apoB levels in either of the cohorts (Figures 4a and 4b), in contrast with what has been observed for LDL-cholesterol. As a consequence, the LDL-cholesterol:apoB ratio, which is indicative of LDL particle size, did decrease with increasing plasma triacylglycerols ($P < 0.001$ for group C in both the Maastricht and Nijmegen cohorts; Figures 4a and 4b, insets). When both male FCHL cohorts were combined to analyse the prevalence of CVD in the three triacylglycerols subgroups, the highest prevalence was observed in group C ($P = 0.04$ for group C compared with group B; $P = 0.006$ for group C compared with group A; as determined using a logistic regression with correction for age; Figure 4c). Of note, the significantly increased prevalence of CVD in group C was similar in both cohorts (40 and 35 % in the Maastricht and Nijmegen cohorts respectively).

DISCUSSION

It has been estimated that approx. 10 % of survivors of a premature myocardial infarction are affected by FCHL [1]. FCHL is currently viewed as a genetically complex disease, implying that multiple genes in combination with environmental factors are responsible for the different metabolic pathways that eventually result in the characteristic hyperlipidaemic phenotype [18]. On top of this complexity, there is the 'multiple-type hyperlipidaemia', i.e. the presence of different lipid phenotypes within one family and one individual patient with FCHL, that complicates its diagnosis, research and treatment. In the present study, we anticipated that a parabolic relationship between plasma triacylglycerols and LDL-cholesterol can account for the switch in plasma cholesterol phenotype in FCHL. This hypothesis was based on the fact that the liver is able to secrete different VLDL particles with different catabolic fates, depending on the actual plasma triacylglycerols concentrations [14].

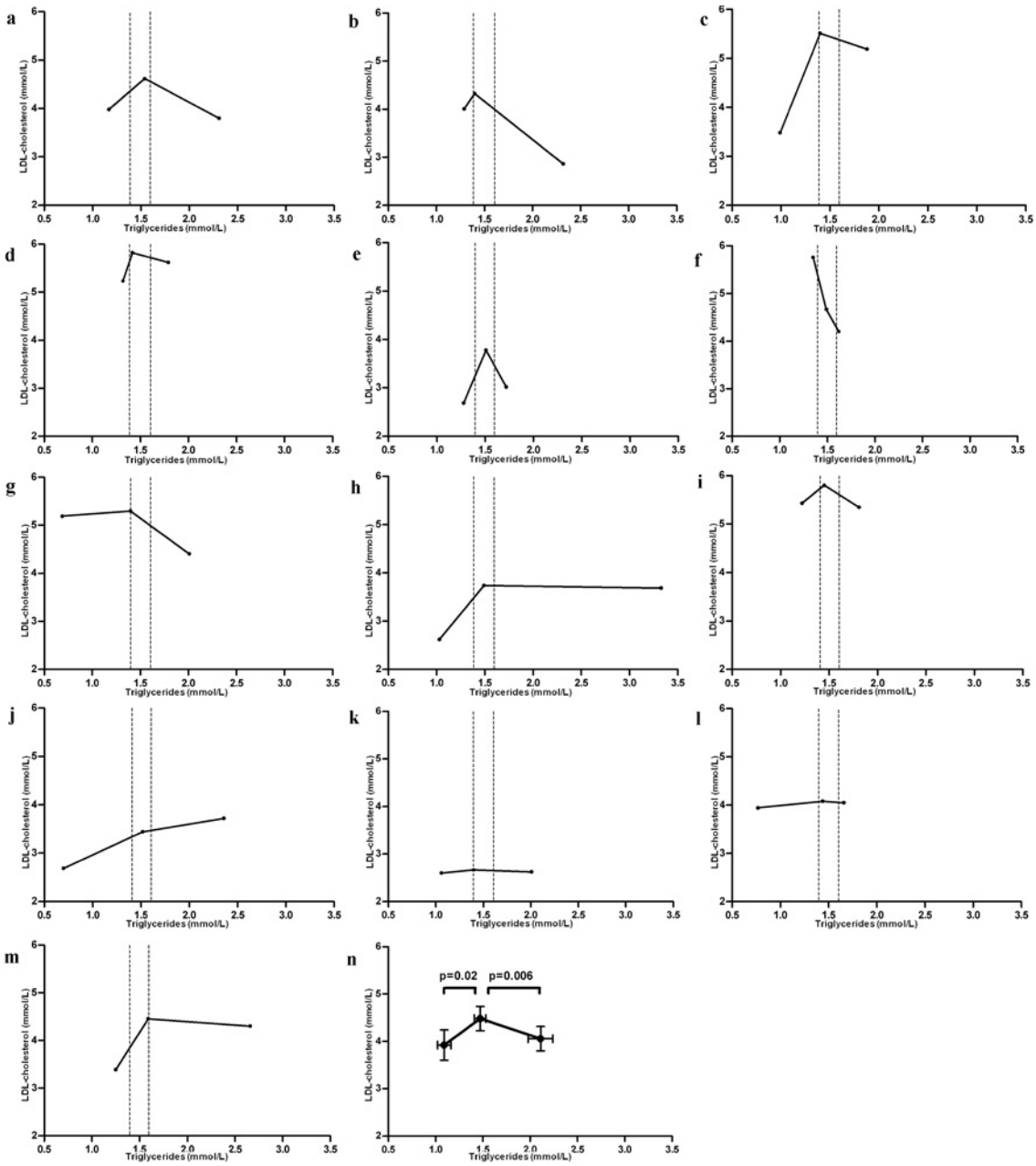

Figure 3 Intra-individual relationship between plasma triacylglycerols and LDL-cholesterol for 13 family members with FCHL with three consecutive triacylglycerol measurements below 1.4 mmol/l, between 1.4 and 1.6 mmol/l and above 1.6 mmol/l

(a–m) Individual traces for the 13 subjects. Dashed vertical lines indicate plasma triacylglycerol levels of 1.4 and 1.6 mmol/l. (n) The mean relationship, with error bars representing S.E.M. is shown (analysed with Wilcoxon's non-parametric test for paired samples).

Our hypothesis was verified in two well-documented FCHL cohorts that have been followed over time [11,13]. We demonstrated recently that the switch in lipid phenotype is very similar in both cohorts [11,13], and we have shown in the present study that baseline characteristics of the Maastricht and Nijmegen FCHL cohorts were comparable with regard to plasma lipid levels and prevalence of CVD. This provided us with the unique opportunity to test our hypothesis in two comparable, but independent, FCHL cohorts. Both male Maastricht and Nijmegen cohorts clearly demonstrated a longitudinal parabolic relationship between plasma triacylglycerols and LDL-cholesterol. This relationship was also present in individual male patients with observations in each

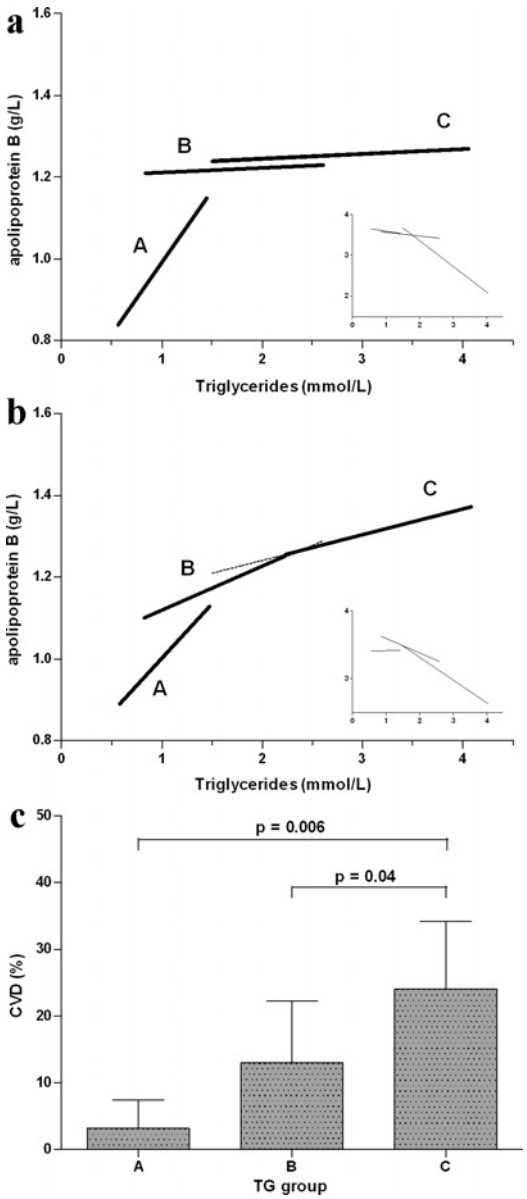

Figure 4 5-Year intra-individual relationship between plasma triacylglycerols and apoB levels in male Maastricht (a) and Nijmegen (b) FCHL cohorts

The insets in (a and b) show the relationship between plasma triacylglycerols and the LDL-cholesterol:apo B ratio, which is indicative of the LDL particle size. (c) Relationship of the three triacylglycerols subgroups with the prevalence of CVD. For this purpose, the male Maastricht and Nijmegen cohorts were combined to increase statistical power (analysed with logistic regression with correction for age).

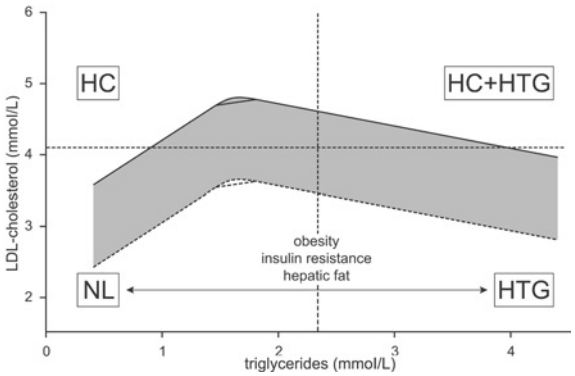

Figure 5 Conceptual framework of the parabolic relationship between plasma triacylglycerols and LDL-cholesterol as an explanation for the 'multiple-type hyperlipidemia' in FCHL

The grey-shaded area represents the 95 % confidence interval for the combined male Maastricht and Nijmegen cohorts. The horizontal and vertical lines indicate the cut-off values for hypercholesterolaemia (HC) and hypertriglyceridaemia (HTG) respectively, as defined by the NCEP ATPIII (National Cholesterol Education Program Adult Treatment Panel III) criteria. NL, normolipidaemia.

of the different triacylglycerols segments, substantiating the evidence that one individual can indeed pass completely through the parabolic curvature. In addition, this relationship was not simply due to the use of the Friedewald formula, since similar results were obtained with an alternative approximation of LDL-cholesterol. The results in the female cohorts were less consistent, which is probably due to the well-known inter- and intra-individual scattering effects of oestrogens on lipid levels in a population with different oestrogen levels [17].

We believe that the present results, combined with our findings obtained previously, account for the 'multiple-type hyperlipidaemia' in FCHL, as shown in Figure 5. In previous studies, we have shown that the switch in plasma triacylglycerols is accompanied by changes in obesity, insulin resistance and the amount of hepatic fat [11–13]. As these metabolic factors cause a switch in plasma triacylglycerols, they automatically induce changes in plasma LDL-cholesterol and, when substantial, cause a switch in cholesterol phenotype.

A striking observation in the present study is the absence of a parabolic, and the presence of an almost linear, relationship between plasma triacylglycerols and the amount of atherogenic particles in plasma, as reflected by apoB levels. This observation is pivotal in several respects. First, given the fact that the majority of the plasma apoB is accounted for by LDL particles [19], the discrepancy between the curves for LDL-cholesterol and apoB is most probably explained by a change in LDL particle composition, i.e. the development of small-dense LDL, as shown by a decrease in the LDL-cholesterol:apoB ratio. This finding is in concordance with our hypothesis. Secondly, although the parabolic relationship for LDL-cholesterol offers an explanation for the multiple-type hyperlipidaemia in FCHL, the linear relationship for apoB may serve as a solution for the accompanying diagnostic difficulties, since it is expected that apoB levels switch less frequently from normal to

abnormal over time. Indeed, many reports have shown a lower temporal variability for apoB levels and have therefore advocated the use of apoB instead of cholesterol levels in diagnosing FCHL [9,10,20,21]. Lastly, and most importantly, the present study suggests that apoB levels are superior to LDL-cholesterol in predicting cardiovascular events, at least in hypertriglyceridaemic patients with FCHL, since the relationship between plasma triacylglycerols and the prevalence of CVD was also linear. Again, it is anticipated that the presence of small-dense LDL particles, which are more atherogenic than buoyant LDL particles [22,23], is responsible for this discrepancy. These findings corroborate the use of apoB in the ongoing debate of LDL-cholesterol compared with apoB levels in predicting cardiovascular events [24].

In summary, the present 5-year follow-up study in two well-documented FCHL cohorts has demonstrated that plasma triacylglycerols and LDL-cholesterol are related in a parabolic fashion in male family members with FCHL. These findings account for the 'multiple-type hyperlipidaemia' in FCHL and, therefore, improve our understanding of the natural history of this highly prevalent disease. Furthermore, the more linear relationship between plasma triacylglycerols and both apoB levels and the prevalence of CVD may provide a solution to this complex phenomenon, and advocates further the use of apoB instead of LDL-cholesterol levels in the diagnosis of FCHL and the prediction of cardiovascular complications.

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REFERENCES

1 Goldstein, J. L., Schrott, H. G., Hazzard, W. R., Bierman, E. L. and Motulsky, A. G. (1973) Hyperlipidemia in coronary heart disease. II. Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidemia. J. Clin. Invest. **52**, 1544–1568
2 Voors-Pette, C. and de Bruin, T. W. A. (2001) Excess coronary heart disease in familial combined hyperlipidemia, in relation to genetic factors and central obesity. Atherosclerosis **157**, 481–489
3 van der Kallen, C. J., Voors-Pette, C., Bouwman, F. G. et al. (2002) Evidence of insulin resistant lipid metabolism in adipose tissue in familial combined hyperlipidemia, but not type 2 diabetes mellitus. Atherosclerosis **164**, 337–346
4 Purnell, J. Q., Kahn, S. E., Schwartz, R. S. and Brunzell, J. D. (2001) Relationship of insulin sensitivity and ApoB levels to intra-abdominal fat in subjects with familial combined hyperlipidemia. Arterioscler. Thromb. Vasc. Biol. **21**, 567–572
5 de Bruin, T. W. A., Georgieva, A. M., Brouwers, M. C. G. J., Heitink, M. V., van der Kallen, C. J. and van Greevenbroek, M. M. (2004) Radiological evidence of nonalcoholic fatty liver disease in familial combined hyperlipidemia. Am. J. Med. **116**, 847–849
6 Brouwers, M. C. G. J., Bilderbeek-Beckers, M. A. L., Georgieva, A. M., van der Kallen, C. J. H., van Greevenbroek, M. M. J. and de Bruin, T. W. A. (2007) Fatty liver is an integral feature of familial combined hyperlipidaemia: relationship with fat distribution and plasma lipids. Clin. Sci. **112**, 123–130
7 Keulen, E. T., Voors-Pette, C. and de Bruin, T. W. A. (2001) Familial dyslipidemic hypertension syndrome: familial combined hyperlipidemia, and the role of abdominal fat mass. Am. J. Hypertens. **14**, 357–363
8 Brunzell, J. D., Albers, J. J., Chait, A., Grundy, S. M., Groszek, E. and McDonald, G. B. (1983) Plasma lipoproteins in familial combined hyperlipidemia and monogenic familial hypertriglyceridemia. J. Lipid Res. **24**, 147–155
9 Veerkamp, M. J., de Graaf, J., Hendriks, J. C., Demacker, P. N. and Stalenhoef, A. F. (2004) Nomogram to diagnose familial combined hyperlipidemia on the basis of results of a 5-year follow-up study. Circulation **109**, 2980–2985
10 Sniderman, A. D., Castro Cabezas, M., Ribalta, J. et al. (2002) A proposal to redefine familial combined hyperlipidaemia – third workshop on FCHL held in Barcelona from 3 to 5 May 2001, during the scientific sessions of the European Society for Clinical Investigation. Eur J. Clin. Invest. **32**, 71–73
11 Veerkamp, M. J., de Graaf, J., Bredie, S. J., Hendriks, J. C., Demacker, P. N. and Stalenhoef, A. F. (2002) Diagnosis of familial combined hyperlipidemia based on lipid phenotype expression in 32 families: results of a 5-year follow-up study. Arterioscler. Thromb. Vasc. Biol. **22**, 274–282
12 Veerkamp, M. J., de Graaf, J. and Stalenhoef, A. F. (2005) Role of insulin resistance in familial combined hyperlipidemia. Arterioscler. Thromb. Vasc. Biol. **25**, 1026–1031
13 Brouwers, M. C. G. J., van Greevenbroek, M. M. J., Vermeulen, V. M. M.-J., van Lin, J. M. J. P., van der Kallen, C. J. H. and de Bruin, T. W. A. (2007) Five-year follow-up of waist circumference, insulin and ALT levels in familial combined hyperlipidemia. Clin. Sci. **113**, 375–381
14 Packard, C. J. and Shepherd, J. (1997) Lipoprotein heterogeneity and apolipoprotein B metabolism. Arterioscler. Thromb. Vasc. Biol. **17**, 3542–3556
15 Bos, G., Dekker, J. M., Nijpels, G., de Vegt, F., Diamant, M., Stehouwer, C. D., Bouter, L. M. and Heine, R. J. (2003) A combination of high concentrations of serum triglyceride and non-high-density-lipoprotein-cholesterol is a risk factor for cardiovascular disease in subjects with abnormal glucose metabolism: The Hoorn Study. Diabetologia **46**, 910–916
16 Friedewald, W. T., Levy, R. I. and Fredrickson, D. S. (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin. Chem. **18**, 499–502
17 Campos, H., Walsh, B. W., Judge, H. and Sacks, F. M. (1997) Effect of estrogen on very low density lipoprotein and low density lipoprotein subclass metabolism in postmenopausal women. J. Clin. Endocrinol. Metab. **82**, 3955–3963
18 Shoulders, C. C., Jones, E. L. and Naoumova, R. P. (2004) Genetics of familial combined hyperlipidemia and risk of coronary heart disease. Hum. Mol. Genet. **13**, R149–R160
19 Sniderman, A., Vu, H. and Cianflone, K. (1991) Effect of moderate hypertriglyceridemia on the relation of plasma total and LDL apoB levels. Atherosclerosis **89**, 109–116
20 Brouwers, M. C. G. J., Kono, N., van Greevenbroek, M. M. J., van der Kallen, C. J. H., Lusis, A. J., de Bruin, T. W. A. and Cantor, R. M. (2006) Longitudinal differences in familial combined hyperlipidemia quantitative trait loci. Arterioscler. Thromb. Vasc. Biol. **26**, e118–e119

21 Demacker, P. N., Veerkamp, M. J., Bredie, S. J., Marcovina, S. M., de Graaf, J. and Stalenhoef, A. F. (2000) Comparison of the measurement of lipids and lipoproteins versus assay of apolipoprotein B for estimation of coronary heart disease risk: a study in familial combined hyperlipidemia. Atherosclerosis **153**, 483–490
22 Krauss, R. M. (1995) Dense low density lipoproteins and coronary artery disease. Am. J. Cardiol. **75**, 53B–57B
23 Austin, M. A., Breslow, J. L., Hennekens, C. H., Buring, J. E., Willett, W. C. and Krauss, R. M. (1988) Low-density lipoprotein subclass patterns and risk of myocardial infarction. JAMA, J. Am. Med. Assoc. **260**, 1917–1921
24 Barter, P. J., Ballantyne, C. M., Carmena, R. et al. (2006) Apo B versus cholesterol in estimating cardiovascular risk and in guiding therapy: report of the thirty-person/ten-country panel. J. Intern. Med. **259**, 247–258

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