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# The effect of ageing and obesity on the expression of dyslipidemia in children from FCH families.

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**Running headline:** Ageing and the FCH phenotype

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## Abstract

**Objective:** To delineate the mechanism(s) responsible for the increased secretion of very low density lipoprotein particles (VLDL) in patients with familial combined hyperlipidemia (FCH).

**Methods:** In 194 young adults (<25 years) recruited from FCH-families, we investigated how plasma lipids, (apo)lipoproteins, and body mass index (BMI) varied with age. Furthermore, we performed a 5-year follow-up study of clinical and biochemical characteristics of a subset of this population (n=85), stratified by apolipoprotein B levels (below or above the 75<sup>th</sup> percentile adjusted for age and gender).

**Results:** Plasma apoB concentration ( $r=0.45$ ,  $P<0.0001$ ), triglyceride concentration ( $r=0.45$ ,  $p<0.0001$ ), LDL-subfraction profile ( $r=-0.46$ ,  $p<0.0001$ ) and BMI ( $r=0.51$ ,  $p<0.0001$ ) were significantly associated with age. Plasma apoB concentration in the hyperapoB group was already elevated at young age, whereas other characteristics of FCH, as observed in adults, including triglyceride-levels  $>1.5 \text{ mmol.l}^{-1}$  and/or small dense LDL were observed only sporadically. After 5-year follow-up, BMI increased in both groups and this increase was associated with changes in apoB ( $r=0.27$ ,  $p<0.05$ ), TG ( $r=0.30$ ,  $p<0.01$ ), VLDL-c ( $r=0.22$ ,  $p<0.05$ ), VLDL-TG ( $r=0.25$ ,  $p<0.05$ ) and HDL-c ( $r= -0.27$ ,  $p<0.05$ )

**Conclusions:** We found indirect evidence of a primary, presumably genetically-determined, increase in plasma apoB concentration occurring early in life of FCH-family offspring. However, ageing-related post maturation increases in adipose tissue mass also appear to contribute to an aggravation and/or modulation of this genetically determined apoB-overproduction

## Introduction

Familial combined hyperlipidemia (FCH) is the most common of the familial atherogenic dyslipoproteinemias with a prevalence of up to 5.7 % in the general population [1]. The lipid profile of FCH is complex and affected subjects may present with hypercholesterolemia, hypertriglyceridemia or mixed hyperlipidemia; worse yet, the lipid profile may vary over time in the same subject. Thus, based on lipids, the hallmarks of FCH are variability amongst subjects and within subjects. Amongst all this variability, increased concentrations of apolipoprotein B (apoB) and small dense low-density lipoprotein (sdLDL) stand out as the two most consistent abnormalities amongst FCH subjects [2-6]. These abnormalities might result from a decreased fractional catabolic rate of apoB-containing particles [7], but the majority of studies show that the increased concentrations of apoB and the presence of sdLDL are rather a consequence of increased secretion of very low density lipoprotein (VLDL) particles [8-13]. However, the specific abnormalities responsible for an increased secretion of VLDL remain unknown. One hypothesis is that increased hepatic free fatty acid (FFA) flux due to increased transmembrane adipose tissue FFA flux is responsible [2]. As upper body adipose tissue mass increases, so does transmembrane adipocyte FFA flux and therefore this model predicts age-related expression of the disorder. A second model posits that a primary disorder in regulation of hepatic sterol balance could be responsible for increased VLDL secretion in which case expression should not be age-dependent [14,15]. The first model is more consistent with a phenotype of hyperTG-hyperapoB with increased secretion of relatively triglyceride-rich VLDL, the second with a phenotype of normoTG-hyperapoB and increased secretion of relatively cholesterol-rich VLDL. However, neither model alone would explain the heterogeneity of the lipid phenotypes in FCH.

The objective of the current study was to determine whether we could find evidence for one or the other of these models, one reflecting the fundamental genetically determined metabolic abnormality, the other reflecting the impact of age-related factors such as expansion of adipose tissue mass. Therefore, we explored the phenotype expression of FCH in young adults recruited from families affected with FCH. Moreover, the effect of time and subsequent increases in weight on the phenotype expression in these subjects was determined in a 5-year follow-up study.

## Methods

### Study population

The study population consisted of kindred of FCH families with known FCH recruited in 1994, 1999 or 2004 and followed up in 1999 and/or 2004 [6, 16, 17]. All FCH kindreds were recruited by family studies of probands attending the out-patient clinic of the University Medical Centre Nijmegen. Families were included when, besides a proband presenting with combined hyperlipidemia, at least 1 first-degree relative presented with hypertriglyceridemia (HTG), hypercholesterolemia (HC), or a combined hyperlipidemia. Furthermore, at least the proband or one of the first-degree relatives had to have cardiovascular disease (CVD) before the age of 60 years. Families were excluded when a secondary cause of the hyperlipidemia was diagnosed in the proband (ie, diabetes mellitus, hypothyroidism, and hepatic or renal impairment). None of the probands was homozygous for the apo E2 allele, and none of the probands or first-degree relatives had tendon xanthomas. All subjects in this study were Caucasian.

On every visit, all subjects filled in a questionnaire about their previous medical history, medication use, smoking and drinking habits. Cardiovascular disease was defined by stroke, transient ischaemic attack, myocardial infarction, angina pectoris and/or peripheral vascular disease. On all occasions, weight and height were measured and body-mass index (BMI) was calculated as body weight (in kilograms) divided by the square of height (in meters). Furthermore, venous blood was drawn by venipuncture after an overnight fast and after withdrawal of lipid lowering medication (if used) for 4 weeks. A total of 194 subjects younger than 25 years were recruited from 29 different families (6.6 subjects/family) with an average family size of 30 subjects/family. These patients were seen on one or more visits, and for this group, associations between age, apoB, TG, LDL-subfraction profile and BMI were determined. Associations between BMI, apoB and age were also determined in a control group of 177 healthy, unaffected siblings from children from families with a diagnosis of heterozygous familial hypercholesterolemia (FH). In all these subjects FH diagnosis was excluded by molecular diagnosis: none of the subjects tested positive for LDL-receptor gene mutations or apolipoprotein B mutations.

FCH-family subjects were subdivided into two different phenotypes according to their apoB level at their recruitment visit. They were classified as “normoapoB” when their apoB was < 75<sup>th</sup> percentile, adjusted for age and gender, or they were classified as “hyperapoB” when their apoB level exceeded the 75<sup>th</sup> percentile (reference data obtained from the NHANES III) [18]. The 75<sup>th</sup> percentile of apoB was chosen since this represents a level of 1200 mg/l in adults, which is the level at which subjects were separated and analyzed with regard to their cardiovascular risk in the Framingham Study [19] and the Quebec Cardiovascular Study [20]. Furthermore, it is the level which Veerkamp et al.

proposed to use as a cut-off value to diagnose FCH in the absence of percentile scores for TC and TG [6]. For 85 subjects recruited from 23 different FCH families, 5-year follow-up data were available and they were selected for further data analysis. By using the 75<sup>th</sup> percentile, 23 subjects of these subjects (27%) were classified as “hyperapoB” at their recruitment visit, and the other 62 subjects were classified as “normoapoB”. The effect of the 5-year follow-up on clinical- and biochemical parameters was compared across these groups. The study was carried out in accordance with the declaration of Helsinki of the World Medical Association, and approved by the Medical Ethics Committee of the Radboud University Nijmegen Medical Centre. All participants provided written informed consent.

### **Laboratory measurements**

TC and TG concentrations were determined using commercially available enzymatic reagents (Boehringer-Mannheim, Germany, catalogue. No. 237574 and Sera Pak, Miles, Belgium, catalogue. No. 6639, respectively). High density lipoprotein-cholesterol (HDL-c) was determined by the polyethylene glycol 6000 method [21]. VLDL was isolated from whole plasma by sequential ultracentrifugation at density 1.006 g/ml for 16 hours in a fixed angle rotor in a Beckman L7-55 ultracentrifuge (Beckman, Palo Alto, USA). LDL cholesterol was calculated by subtraction of VLDL cholesterol and HDL cholesterol from total plasma cholesterol. A continuous variable K representing the LDL subfraction profile of each individual was determined in 1994 and 1999. A negative K value ( $K < -0.1$ ) reflects a more dense LDL subfraction profile, and a positive K value ( $K > -0.1$ ) reflects a more buoyant profile[22]. Total plasma apoB concentration was determined by immunonephelometry, as recently described elsewhere [23]. Plasma glucose was determined by a commercially available glucose oxidation method (GOD-PAP, Hitachi 747; Roche Molecular Biochemicals, Indianapolis, IN). Plasma insulin-concentrations was assessed by means of radioimmunoassay (in-house RIA with an inter-assay coefficient of variation of 10.3% [24]. Insulin resistance was assessed by the homeostasis model assessment (HOMA) index and calculated using the formula  $HOMA\text{-index} = \text{fasting serum insulin (mU/l)} \times \text{fasting plasma glucose (mmol/l)} / 22.5$ , [25].

### **Statistical analysis**

All values are expressed as mean  $\pm$  SD or as absolute numbers with percentages, unless stated otherwise. HOMA-index, BMI, TG, VLDL-C and VLDL-TG were logarithmically transformed to obtain normal distributions before statistical analysis. Differences between subjects with a hyperapoB phenotype and subjects with a normoapoB phenotype were tested for statistical significance by using the 2-tailed Fisher exact test for dichotomous variables and a two-way ANOVA with family-number as a random factor for continuous variables. A linear mixed model with repeated measurements was used to test differences in lipid and (apo)lipoprotein concentrations, BMI, and HOMA for statistical significance between both groups

(normoapoB and hyperapoB) and between the points of measurement (before and after 5-year follow up), for each dependent variable separately. The interaction between time and group was also included in the model. The dependent variables were TC, TG, LDL-C, HDL-C, VLDL-C, VLDL-TG, apoB, BMI, and HOMA. The independent variables were time (T=0 and T=5) and group (normoapoB and hyperapoB) and their interactions. Age was added as a covariate and family number as a random factor in the linear mixed model when the effect of the independent variables was examined. Binary logistic regression was used to test for changes in incidence of dichotomous variables during follow-up with the appropriate likelihood ratio for each dependent variable assessed separately. The dependent variables were lipid-lowering medication use [yes/no], smoking [yes/no], consumption of >1 alcoholic beverage per day [yes/no], and CVD [yes/no]. The independent variables were identical to those described in the linear mixed model. Univariate correlations between age, apoB and BMI as well as between intra-individual changes in plasma apoB and BMI and changes in the various apolipoproteins during follow-up were quantified by calculating Spearman's rho. For all tests, statistical significance was accepted at the 95% confidence level ( $p < 0.05$ ). Statistical analyses were performed using SPSS 12.0.1 for Windows (SPSS Inc., Chicago Illinois, USA).

## Results

### Characteristics of young adults from FCH-families stratified by apoB concentration

The subjects with a normoapoB phenotype ( $n=135$ ) were significantly younger than the subjects with a hyperapoB phenotype ( $n=59$ ) ( $18.9\pm 4.0$  years compared to  $20.9\pm 3.4$  years,  $p<0.001$ ). Gender distribution was equal across both groups, with 53% women in the normoapoB group vs 55% in the hyperapoB group. Clinical and biochemical characteristics for both groups are shown in table 1. The subjects in the hyperapoB group had a significantly higher BMI than the normoapoB group. In addition, subjects with a hyperapoB phenotype had a significantly more atherogenic lipid profile. TC, TG, LDL-C, VLDL-C and VLDL-TG concentrations as well as the VLDL-C/TG ratio were all significantly increased, while HDL-c concentrations were decreased. A mean K-value of  $-0.18$  reflects the presence of small dense LDL within the hyperapoB group. Although HOMA-index was higher in the hyperapoB-group, this difference reached no statistical significance.

### Effect of age on plasma lipids, (apo)lipoproteins and body mass index

As would be expected, in the total group of subjects recruited from FCH families, there was a significant positive correlation between age and BMI ( $r=0.51$ ,  $p<0.0001$ ). When subjects were analyzed after stratification for age ( $<20$  years), a significant association was found between age and BMI for subjects aged  $<20$  years ( $r=0.49$ ,  $p<0.0001$ ), but not for subjects aged  $>20$  years ( $r=0.09$ ,  $p=NS$ ). The significant positive association in subjects aged  $<20$  years however, was not specific for subjects recruited from FCH-families. In the control group of unaffected FH-family offspring (aged 12-19 years, 46% women, mean plasma apoB concentration  $820\pm 181$  mg.l<sup>-1</sup>, mean plasma TG concentration  $0.84\pm 0.41$  mmol.l<sup>-1</sup>, mean BMI  $20.1\pm 3.4$  kg.m<sup>-2</sup>) we found a similar significant association between BMI and age ( $r= 0.37$ ,  $p<0.0001$ , Figure 1). When the FCH subjects were stratified according to their plasma apoB level, there was a significant association between age and BMI in both groups (normoapoB:  $r = 0.45$ ,  $p<0.001$ , hyperapoB:  $r = 0.47$ ,  $p<0.001$ , Figure 1).

In the FCH population, a significant positive correlation was also present between age and apoB ( $r=0.45$ ,  $p<0.001$ ) and this association remained significant when subjects were analyzed after stratification according to their plasma apoB level (normoapoB phenotype:  $r = 0.45$ ,  $p<0.001$ , hyperapoB phenotype:  $r = 0.47$ ,  $p<0.001$ , Figure 2). The association between age and apoB was strongest in subjects aged  $<20$  years ( $r=0.31$ ,  $p=0.003$ ) whereas there was no significant association between age and apoB in subjects aged  $>20$  years ( $r=0.18$ ,  $p=NS$ ). Again, the relation between age and apoB in subjects aged  $<20$  years was not specific for subjects from FCH-families: a significant



association was also found in the control group of unaffected FH-family offspring ( $r=0.21$ ,  $p=0.01$ , Figure 2). In both the normoapoB-group and the hyperapoB group, plasma TG concentrations were associated positively with BMI (respectively  $r=0.29$ ,  $p<0.001$  and  $r=0.44$ ,  $p<0.001$ ), and this was also the case for our control group unaffected siblings of children with FH ( $r=0.32$ ,  $p<0.001$ ).

When the subgroup with a hyperapoB-phenotype ( $n=59$ ) was analyzed separately, it appeared that 53% of the subjects had plasma triglycerides  $<1.5 \text{ mmol.l}^{-1}$ , which is roughly equivalent to TG-values  $<75^{\text{th}}$  percentile adjusted for age and gender according to the population based PROCAM-study cohort [26]. The vast majority (73%) of these subjects had large buoyant LDL, reflected by a K-value  $>-0.10$  (Figure 3). On the other hand, 80% of the subjects with TG-levels  $>1.5 \text{ mmol.l}^{-1}$  had sdLDL, as reflected by a K-value  $<-0.10$ . However, under the age of 20 years, the presence of TG-levels  $> 1.5 \text{ mmol.l}^{-1}$  and/or the presence of sdLDL was observed only sporadically. The presence of sdLDL in subjects with a hyperapoB phenotype correlated negatively with age ( $r = -0.46$ ,  $p<0.0001$ ). Thus, plasma apoB levels were increased before plasma triglycerides increased or sdLDL was present.

The early rise in plasma apoB levels in subjects under 20 years comes with an early rise in VLDL-c concentrations ( $0.38\pm0.16$  vs  $0.23\pm0.13 \text{ mmol/l}$ ,  $p<0.05$  hyperapoB vs normoapoB) and an elevated VLDL-c/TG ratio ( $0.34\pm0.07$  vs  $0.28\pm0.08$ ,  $p<0.05$  hyperapoB vs normoapoB) as well. Older hyperapoB subjects ( $>20$  years) exhibit even higher VLDL-c concentrations than younger hyperapoB subjects ( $<20$  years) ( $0.74\pm0.47$  vs  $0.38\pm0.16 \text{ mmol/l}$ ). This is a reflection of the presence of an increased number of more VLDL-particles rather than being a reflection of a change in composition of these particles, since they have higher apoB levels than younger hyperapoB subjects ( $1175\pm146$  vs  $1018\pm109 \text{ mg/l}$ ,  $p<0.01$ ) but not a higher VLDL-c/TG ratio ( $0.36\pm0.08$  vs  $0.34\pm0.07$ ,  $p=\text{NS}$ ).

### Effect of 5-year follow-up on lifestyle variables

At the time of their recruitment, mean age in the normoapoB group was  $19.4\pm 3.7$  years, and in the hyperapoBgroup  $20.9\pm3.8$  years ( $p=0.09$ ). None of the subjects used lipid-lowering medication. Seven out of 23 subjects (30%) smoked cigarettes in the hyperapoB group compared to 23 out of 62 (37%) in the normoapoB group ( $p=0.48$ ). The number of subjects using  $>1$  alcoholic beverage/day (two in the normoapoB-group, and one in the hyperapoB-group) was not significantly different ( $p= 0.29$ ). None of the participants had any past or present history of cardiovascular disease at the time of their recruitment.

After a 5 year follow-up, only one subject in both groups used lipid-lowering medication. The percentage subjects consuming  $>1$  alcoholic beverage/day was significantly higher than at the recruitment visit ( $p<0.001$ ), but no significant differences across the groups were observed. The percentage subjects

smoking cigarettes did not change significantly compared to recruitment ( $p=0.87$ ). After 5-year follow-up still none of the subjects had any past or present CVD.

### **Effect of 5-year follow-up on plasma lipid and (apo)lipoprotein concentrations and body mass index**

Table 2 shows the baseline BMI, lipid and (apo)lipoprotein concentrations and HOMA-indices for the 23 subjects with a plasma level of apoB > 75<sup>th</sup> percentile adjusted for age and gender, and for the 62 subjects with a plasma level of apoB < 75<sup>th</sup> percentile. In these groups, the same (significant) differences in baseline BMI, HOMA, K-value and lipid and (apo)lipoprotein concentrations between the two groups were observed as presented for the whole group of subjects ( $n=194$ ) above. A significant effect of 5-year follow-up on BMI was found in the linear mixed model. However, this effect was not group-dependent (i.e. there was no time-to group interaction in the model). In both groups, BMI increased significantly after 5 year follow-up ( $p<0.001$  and  $p=0.002$  respectively). This increase in BMI could be attributed to a mean weight-gain of 5.1 kg (from 64.8 to 70.9 kg) in the normoapoB group and to a weight gain of 4.5 kg (from 79.5 to 84 kg) in the hyperapoB group. The difference in weight between the two groups was substantial both at the beginning and at the end of the 5 year follow-up period.

In both groups, apoB, TC and LDL-C concentrations increased after 5 years of follow-up, but these increases reached statistical significance only in the normoapoB-group ( $p=0.02$ ,  $p<0.001$  and  $p=0.002$  respectively). For both ApoB, LDL-C and TC, however, time-to-group interaction was not significant (i.e. the increase in ApoB, TC and LDL-C concentration in the normoapoB-group was not significantly different from the increase in the hyperapoB group). The higher plasma concentrations of TG, VLDL-C, and VLDL-TG as well as the higher VLDL-C/TG-ratio, and the higher HOMA-index in the hyperapoB-group at the recruitment visit remained higher after 5-year follow-up. Both in the normoapoB and in the hyperapoB-group, only small changes in mean-values were recorded, that reached significance only in the larger normoapoB-group. As a result, there was no significant effect of 5-year follow-up on these parameters using the linear mixed model, nor was there a significant time-to-group interaction for any of these parameters. Since the K-value was determined in 1994 and 1999 only and not in 2004, the number of hyperapoB-subjects with 5-year follow-up data on the K-value ( $n=10$ ) was too small to draw solid conclusions regarding the effect of 5-year follow-up on the occurrence of sdLDL in this group. In the normoapoB-subjects ( $n=26$ ) however, we observed a significant decrease in K-value with increasing age ( $p<0.001$ ).

### **Factors affecting intra-individual changes in BMI and apoB after 5-year follow-up**

Univariate regression analysis (n=85) revealed that intra-individual changes in apoB-concentration after follow-up were associated not only with changes in TC, LDL-C and VLDL-C, but also with changes in BMI, VLDL-TG and TG-concentration (Table 3). Furthermore, intra-individual changes in BMI were associated with changes in ApoB, VLDL-c, VLDL-TG, HDL-c and TG-concentrations (Table 3).

## Discussion

To the best of our knowledge, this is the first study that has examined the effect of age and body weight on phenotype expression of FCH in a large cohort of children and young adults recruited from FCH families. We show that an elevated apoB concentration is the first expression of FCH in children and young adults. Furthermore, our data support the hypothesis that age-related factors, such as expansion of adipose tissue mass, contribute to the phenotype expression in patients with FCH.

We found that in FCH plasma apoB, plasma triglycerides, BMI, and LDL-particle size were all associated with age. These associations were independently of the apoB phenotype (normoapoB or hyperapoB). Even more, the associations between age, BMI and apoB were not specific for subjects recruited from FCH-families: similar association, were also present in a control group of unaffected siblings of children with FH.

The majority of subjects with an elevated apoB but younger than 20 years had no elevated plasma triglyceride levels or sdLDL, although these traits are frequently observed in adult FCH patients. This suggests that increased secretion of apoB may be the primary abnormality in FCH. On the other hand, the positive trends between plasma TG concentration and age, between age and BMI and between BMI and plasma triglyceride concentration, suggest that increasing adipose tissue mass is a major driver of plasma triglycerides and that this is a secondary trend affecting the expression of FCH. This is in concordance with our finding that the 59 subjects with a hyperapoB phenotype in our study not only had a higher plasma apoB concentration, but they also had a higher BMI as well as a more atherogenic lipid profile than their 135 normoapoB relatives. The combination of a higher BMI and higher plasma TG levels points to a role of adipose tissue in the pathogenesis of their elevated apoB levels by virtue of an increased hepatic FFA flux.

The results of the 5-year-follow-up were in line with these findings. BMI and apoB increased in both the normoapoB and the hyperapoB groups (although the latter not significantly). Furthermore, the intra-individual increase in BMI after 5-year follow-up appeared to be significantly associated with intra-individual changes in plasma apoB concentration. Of importance, the influence of changes in BMI on levels of apoB was not confined to a specific phenotype. Taken together, these findings favour the hypothesis that increased hepatic apoB secretion in subjects with FCH might be augmented by post-maturational changes in adipose tissue mass.

The observed lack of change in mean plasma concentrations of VLDL-C, VLDL-TG, HOMA-index, TG and apoB after 5-year follow-up might be related to the relatively small increases in mean value of BMI. Datillo et al. [27] showed in a meta-analysis that every kilogram weight-reduction is associated

with a change in TG of 0.015 mmol/l. Since mean weight change in our study was only 4.8 kg, we could not have expected more than 0.072 mmol/l increase in plasma levels of TG. Our actual data are concordant with this relationship. The same may well be true for VLDL-C, VLDL-TG and HOMA-values. Alternatively, in a recent publication, Noakes et al. [28] identified dietary composition and physical exercise as significant mediators of the relation between weight gain and plasma lipid and lipoprotein concentrations. Since these parameters were not assessed in the current study, they could have acted as confounders. Beyond these factors, it is worth emphasizing that no simple relationship between BMI and apoB expression should be anticipated. To the extent adipose tissue mass influences the level of plasma apoB, it does so based on its effect on hepatic FFA flux. Lower body adipose tissue is associated with low adipocyte transmembrane FFA fluxes and therefore low hepatic FFA fluxes. By contrast, upper body adipose tissue is associated with higher adipocyte transmembrane FFA fluxes and higher hepatic FFA fluxes. Upper body adipose tissue mass does not expand until the lower body adipose tissue reservoirs are filled [29]. This explains why there is a tighter relation between waist-to-hip ratio and plasma apoB, insulin resistance, and the risk of vascular disease than between BMI and these indices [30].

Our study has limitations. Almost all our subjects were post adolescent and follow-up data are available for only a subset and in these only over a relatively short period of time. Nevertheless, we think these data are of interest. In the first place, very limited data are available on the phenotypic expression of FCH in young subjects from affected families, and the current study represents the largest data set available. Second, they confirm our previous studies in adults that plasma apoB is the most reliable marker of affected individuals. Third, they indicate that FCH is expressed relatively early in life. At an average age of 21.9 years, the absolute apoB concentration of the 59 subjects in the hyperapoB group was 1119 mg.l<sup>-1</sup>, a value for apoB that is only slightly less than the level (1200 mg.l<sup>-1</sup>) used in adults to diagnose FCH [6]. Although TG, VLDL-C and VLDL-TG concentrations as well as the HOMA-index were also elevated in the hyperapoB-subjects, the mean values for these parameters were much lower than the corresponding values found in adult FCH patients [16]. These findings are in line with an earlier (albeit cross-sectional) study demonstrating a high incidence (86%) of elevated plasma apoB in children and young adults from families with FCH, whereas the incidence of the hyperapoB-hyperTG phenotype was only 35% [31].

Taken together, the available data suggests there may be two processes augmenting the elevated plasma apoB levels in FCH. The first would be a primary defect, responsible for the high apoB levels early in life, possibly related to sterol balance within the liver [32]. Multiple lines of evidence have established that regulation of hepatic sterol content is a fundamental metabolic role for apoB secretion and that the rate of hepatic apoB secretion is directly related to the mass of cholesterol ester on the endoplasmic reticulum. Hepatic FFA flux is only one of several determinants of cholesterol ester

mass. It seems possible therefore that the final phenotypic expression of FCH is the interplay between a primary genetically determined abnormality in hepatic sterol balance and the superimposed secondary effects of increased FFA flux resulting from the expansion of upper body adipose tissue mass.

**In conclusion:** In the present study we found indirect evidence of a presumably genetically determined increased apoB production rate occurring early in life of FCH-family offspring. Furthermore, we showed that ageing-related factors such as post maturation increases in adipose tissue mass contributed to an aggravation and/or modulation of this genetically determined apoB overproduction.

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## Reference List

- 1 Hopkins PN, Heiss G, Ellison RC, et al. (2003) Coronary artery disease risk in familial combined hyperlipidemia and familial hypertriglyceridemia: a case-control comparison from the National Heart, Lung, and Blood Institute Family Heart Study. *Circulation* **108**, 519-523.
- 2 Bredie SJ, Tack CJ, Smits P, Stalenhoef AF. (1997) Nonobese patients with familial combined hyperlipidemia are insulin resistant compared with their non affected relatives. *Arterioscler Thromb Vasc Biol* **17**, 1465-1471.
- 3 Bredie SJ, Demacker PN, Stalenhoef AF. (1997) Metabolic and genetic aspects of familial combined hyperlipidaemia with emphasis on low-density lipoprotein heterogeneity. *Eur J Clin Invest* **27**, 802-811.
- 4 de Graaf J, Stalenhoef AF. (1998) Defects of lipoprotein metabolism in familial combined hyperlipidaemia. *Curr Opin Lipidol* **9**, 189-196.
- 5 de Graaf J, Veerkamp MJ, Stalenhoef AF. (2002) Metabolic pathogenesis of familial combined hyperlipidaemia with emphasis on insulin resistance, adipose tissue metabolism and free fatty acids. *J R Soc Med Suppl*, 46-53.
- 6 Veerkamp MJ, de Graaf J, Hendriks JC, Demacker PN, Stalenhoef AF. (2004) Nomogram to diagnose familial combined hyperlipidemia on the basis of results of a 5-year follow-up study. *Circulation* **109**, 2980-2985.
- 7 Aguilar- Salinas CA, Hugh P, Barrett R, Pulai J, Zu XL, Schonfeld G. (1997) A familial combined hyperlipidemic kindred with impaired apolipoprotein B catabolism. Kinetics of apolipoprotein B during placebo and pravastatin therapy. *Arterioscler Thromb Vasc Biol.* **17**:72-82.
- 8 Kissebah AH, Alfarsi S, Adams PW. (1981) Integrated regulation of very low density lipoprotein triglycerides and apolipoprotein B kinetics in man: normolipidemic subjects, familial hypertriglyceridemia and familial combined hyperlipidemia. *Metabolism* **30**:856-868.
- 9 Kissebah AH, Alfarsi S, Evans DJ. (1984) Low density lipoprotein metabolism in familial combined hyperlipidemia. *Arteriosclerosis* **4**:614-624.
- 10 Venkatesan S, Cullen P, Pacy P, Halliday D, Scott J.(1993) Stable isotopes show a direct relation between VLDL apoB overproduction and serum triglycerides levels and indicate a metabolically

and biochemically coherent basis for familial combined hyperlipidemia. *Arterioscler Thromb* **13**:1110-1118

- 11 Teng B, Sniderman AD, Soutar AK, Thompson GR. (1986) Metabolic basis of hyperapobetalipoproteinemia: turnover of apolipoprotein B in low density lipoprotein and its precursors and subfractions compared with normal and familial hypercholesterolemia. *J Clin Invest* **77**:663-672
- 12 Chait A, Albers JJ, Brunzell JD (1980). Very low density overproduction in genetic forms of hypertriglyceridemia. *Eur J Clin Invest*. **10**:161-172
- 13 Cortner JA, Bennett MJ, Le NA, Coates PM (1991). Familial combined hyperlipidemia: use of stable isotopes to demonstrate overproduction of very low density lipoprotein apolipoprotein B by the liver. *J Inher Metab Dis*. **14**:915-922.
- 14 Cianflone KM, Yasruel Z, Rodriguez MA, Vas D, Sniderman AD. (1990) Regulation of apoB secretion from HepG2 cells: evidence for a critical role for cholesteryl ester synthesis in the response to a fatty acid challenge. *J Lipid Res* **31**, 2045-2055.
- 15 Watts GF, Naoumova R, Cummings MH, et al. (1995) Direct correlation between cholesterol synthesis and hepatic secretion of apolipoprotein B-100 in normolipidemic subjects. *Metabolism* **44**, 1052-1057.
- 16 Veerkamp MJ, de Graaf J, Bredie SJ, Hendriks JC, Demacker PN, Stalenhoef AF. (2002) Diagnosis 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.
- 17 Bredie SJ, Vogelaar JM, Demacker PN, Stalenhoef AF. (1996) Apolipoprotein E polymorphism influences lipid phenotype expression, but not the low density lipoprotein subfraction distribution in familial combined hyperlipidemia. *Atherosclerosis* **126**, 313-24.
- 18 Bachorik PS, Lovejoy KL, Carroll MD, Johnson CL. (1997) Apolipoprotein B and AI distributions in the United States, 1988-1991: results of the National Health and Nutrition Examination Survey III (NHANES III) *Clin Chem* **43**, 2364-2378.
- 19 Lamarche B, Tchernof A, Moorjani S, et al. (1997) Small, dense low-density lipoprotein particles as a predictor of the risk of ischemic heart disease in men. Prospective results from the Quebec Cardiovascular Study *Circulation* **95**, 69-75.



- 20 Contois JH, McNamara JR, Lammi-Keefe CJ, Wilson PW, Massov T, Schaefer EJ. (1996) Reference intervals for plasma apolipoprotein B determined with a standardized commercial immunoturbidimetric assay: results from the Framingham Offspring Study. *Clin Chem* **42**, 515-523.
- 21 Demacker PN, Hijmans AG, Vos-Janssen HE, van't Laar A, Jansen AP. (1980) A study of the use of polyethylene glycol in estimating cholesterol in high-density lipoprotein. *Clin Chem* **26**, 1775-1779.
- 22 Stalenhoef AF, de Graaf J, Wittekoek ME, Bredie SJ, Demacker PN, Kastelein JJ. (2000) The effect of concentrated n-3 fatty acids versus gemfibrozil on plasma lipoproteins, low density lipoprotein heterogeneity and oxidizability in patients with hypertriglyceridemia. *Atherosclerosis* **153**, 129-138.
- 23 Demacker PN, Veerkamp MJ, Bredie SJ, Marcovina SM, de Graaf J, Stalenhoef AF. (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.
- 24 De Galan BE, Tack CJ, Lenders JW, et al. (2002) Theophylline improves hypoglycemia unawareness in type 1 diabetes. *Diabetes* **51**, 790-796.
- 25 Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* **28**, 412-419 .
- 26 Assmann G, Schulte H. (1993) Results and conclusions of the prospective cardiovascular Munster (PROCAM) study. *Lipid metabolism disorders and cardiovascular disease*. MMV-Medizin-Verlag GmbH. pp 19-68.
- 27 Dattilo AM, Kris-Etherton PM. (1992) Effects of weight reduction on blood lipids and lipoproteins: a meta-analysis. *Am J Clin Nutr* **56**, 320-328
- 28 Noakes M, Clifton P. (2004) Weight loss, diet composition and cardiovascular risk. *Curr Opin Lipidol* **15**, 31-35.
- 29 Smith J, Al-Amri M, Dorairaj P, Sniderman AD. (2006) The adipocyte life cycle hypothesis. *Clinical Science* **110**, 1-9.

- 30 Yusuf S, Hawken S, Ounpuu S, et al. (2005) Obesity and the risk of myocardial infarction in 27,000 participants from 52 countries: a case-control study. *Lancet* **366**, 1640-1649.
- 31 Cortner JA, Coates PM, Gallagher PR. (1990) Prevalence and expression of familial combined hyperlipidemia in childhood. *J Pediatr* **116**, 514-519.
- 32 Sniderman AD, Zhang XJ, Cianflone K. (2000) Governance of the concentration of plasma LDL: a reevaluation of the LDL receptor paradigm. *Atherosclerosis* **148**, 215-229.

**TABLE 1.** Anthropometric measurements, lipid and (apo)lipoprotein concentrations, and HOMA-indices of 194 young-adults recruited from FCH-families, stratified by apoB concentration.

|                                 | normoapoB<br><75 <sup>th</sup> percentile<br>(n=135) | hyperapoB<br>>75 <sup>th</sup> percentile<br>(n=59) | <i>p</i> <sup>*</sup> |
|---------------------------------|--|---|-----------------------|
| Age (y)                         | 18.9± 4.0  | 21.0±3.4  | 0.001                 |
| BMI (kg.m <sup>-2</sup> )       | 21.2±3.0   | 24.2±4.1  | 0.001                 |
| TC (mmol.l <sup>-1</sup> )      | 4.20±0.64  | 5.51±0.82   | <0.001                |
| TG (mmol.l <sup>-1</sup> )      | 0.86±0.36  | 1.66±1.45   | <0.001                |
| VLDL-c (mmol.l <sup>-1</sup> )  | 0.27±0.16  | 0.62±0.43   | <0.001                |
| VLDL-TG (mmol.l <sup>-1</sup> ) | 0.48±0.27  | 1.09±0.68   | <0.001                |
| VLDL-c/TG ratio                 | 0.29±0.09  | 0.35±0.08   | 0.003                 |
| LDL-c (mmol.l <sup>-1</sup> )   | 2.53±0.53  | 3.66±0.73   | <0.001                |
| HDL-c (mmol.l <sup>-1</sup> )   | 1.31±0.30  | 1.14±0.29   | 0.001                 |
| ApoB (mg.l <sup>-1</sup> )      | 752±130  | 1119±149  | <0.001                |
| HOMA-index                      | 2.36±0.96  | 2.86±0.30   | 0.342                 |
| K-value                         | 0.03±0.19  | -0.18±0.26  | <0.001                |

Values are estimated mean ± SD. \* two-way ANOVA with family-number as a random factor for difference between measurements of both groups (normoapoB vs hyperapoB). BMI, body mass index; TC total cholesterol; TG, triglycerides; VLDL-c, very low density lipoprotein-cholesterol; VLDL-TG, very low density lipoprotein-triglycerides; LDL-c, low density lipoprotein-cholesterol; HDL-c, high density lipoprotein-cholesterol; apoB, apolipoprotein B; HOMA, homeostasis model assessment; Values of K<-0.1 reflect the presence of small dense LDL.

**TABLE 2.** The effect of 5-year follow-up on anthropometric measurements, lipid- and (apo)lipoprotein-concentrations and HOMA-index of 85 young-adults recruited from FCH-families, stratified by apoB concentration at their recruitment visit.

|                                 | NormoapoB<br>(n=62)    |                           | HyperapoB<br>(n=23)    |                         | <i>p</i> <sup>*</sup> |
|---------------------------------|------------------------|---------------------------|------------------------|-------------------------|-----------------------|
|                                 | mean at<br>recruitment | absolute<br>change        | mean at<br>recruitment | absolute<br>change      |                       |
| BMI (kg.m <sup>-2</sup> )       | 21.7±3.1               | +2.0 (0.5) <sup>†</sup>   | 25.9±4.3               | +1.4 (0.4) <sup>†</sup> | <0.001                |
| TC (mmol.l <sup>-1</sup> )      | 4.30±0.82              | +0.22(0.09) <sup>†</sup>  | 5.24±0.70              | +0.17(0.18)             | <0.001                |
| TG (mmol.l <sup>-1</sup> )      | 0.97±0.45              | +0.21 (0.06) <sup>†</sup> | 2.10±0.94              | -0.09 (0.20)            | NS                    |
| VLDL-c (mmol.l <sup>-1</sup> )  | 0.30±0.21              | +0.08(0.02) <sup>†</sup>  | 0.80±0.50              | -0.05(0.09)             | NS                    |
| VLDL-TG (mmol.l <sup>-1</sup> ) | 0.56±0.39              | +0.19 (0.06) <sup>†</sup> | 1.45±0.86              | +0.04 (0.17)            | NS                    |
| VLDL-c/TG ratio                 | 0.30±0.10              | -0.006 (0.014)            | 0.37±0.09              | -0.02 (0.12)            | NS                    |
| LDL-c (mmol.l <sup>-1</sup> )   | 2.58±0.64              | +0.24 (0.06) <sup>†</sup> | 3.29±0.69              | +0.28 (0.14)            | <0.001                |
| HDL-c (mmol.l <sup>-1</sup> )   | 1.29±0.27              | -0.008 (0.032)            | 1.09±0.48              | +0.01 (0.10)            | NS                    |
| ApoB, (mg l <sup>-1</sup> )     | 776±153                | +36 (15) <sup>†</sup>     | 1090±116               | +13 (46)                | NS                    |
| K-value                         | 0.065±0.20             | -0.13 (0.03) <sup>†</sup> | -0.23± 0.27            | 0.11 (0.05)             | 0.026                 |
| HOMA-index                      | 2.17±0.99              | -0.46(0.14) <sup>†</sup>  | 2.46±1.01              | -0.27(0.21)             | NS                    |

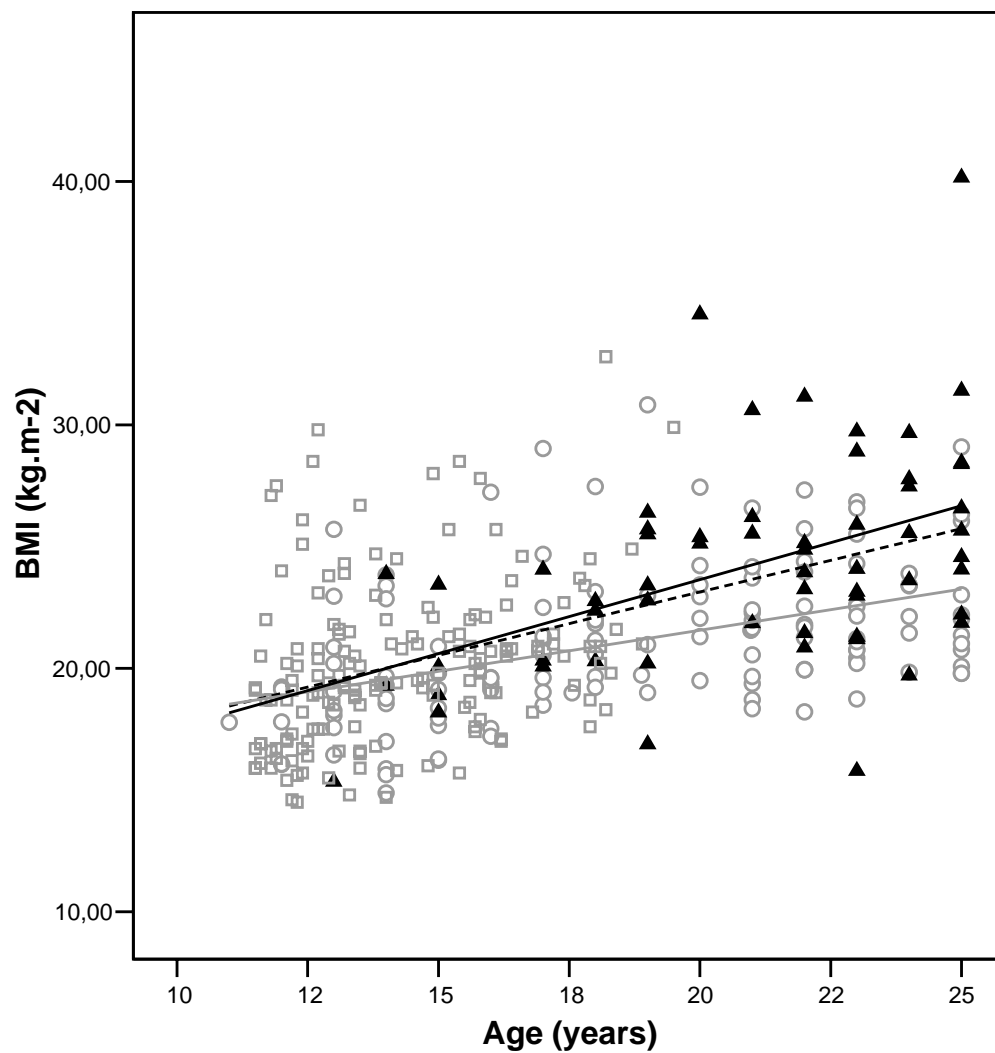
Values are estimated mean ± SD at recruitment visit and mean ± SEM changes after 5-year follow-up. Abbreviations as in table 1. <sup>†</sup> = statistically significant change (*p*<0.05) from recruitment by paired t-test. \* = *p*-value for fixed effect of the independent variable “time” (5year follow-up) on the dependent variable in the linear mixed model with “age” as a co-variate.

**TABLE 3** Spearman's rank correlation coefficients representing the correlation between intra-individual changes in respectively plasma ApoB-level and BMI on the one hand and changes in the plasma concentration of the various (apo)lipoproteins, triglycerides and HOMA-index after 5 year follow-up on the other hand (n=85).

|                                  | ApoB   | BMI     |
|----------------------------------|--------|---------|
| BMI, (kg.m <sup>-2</sup> )       | 0.27 * |         |
| LDL-C, (mmol. l <sup>-1</sup> )  | 0.59 ‡ | 0.05    |
| TC, (mmol. l <sup>-1</sup> )     | 0.64 ‡ | -0.06   |
| ApoB, (mg.l <sup>-1</sup> )      |        | 0.27 *  |
| VLDL-c, (mmol. l <sup>-1</sup> ) | 0.36 ‡ | 0.22*   |
| VLDL-TG, (mmol l <sup>-1</sup> ) | 0.29 † | 0.25 *  |
| VLDL-c/TG ratio                  | 0.08   | 0.07    |
| HDL-C, (mmol. l <sup>-1</sup> )  | 0.09   | -0.27 * |
| TG, (mmol. l <sup>-1</sup> )     | 0.39 ‡ | 0.30 †  |
| HOMA-index                       | 0.24   | 0.31    |

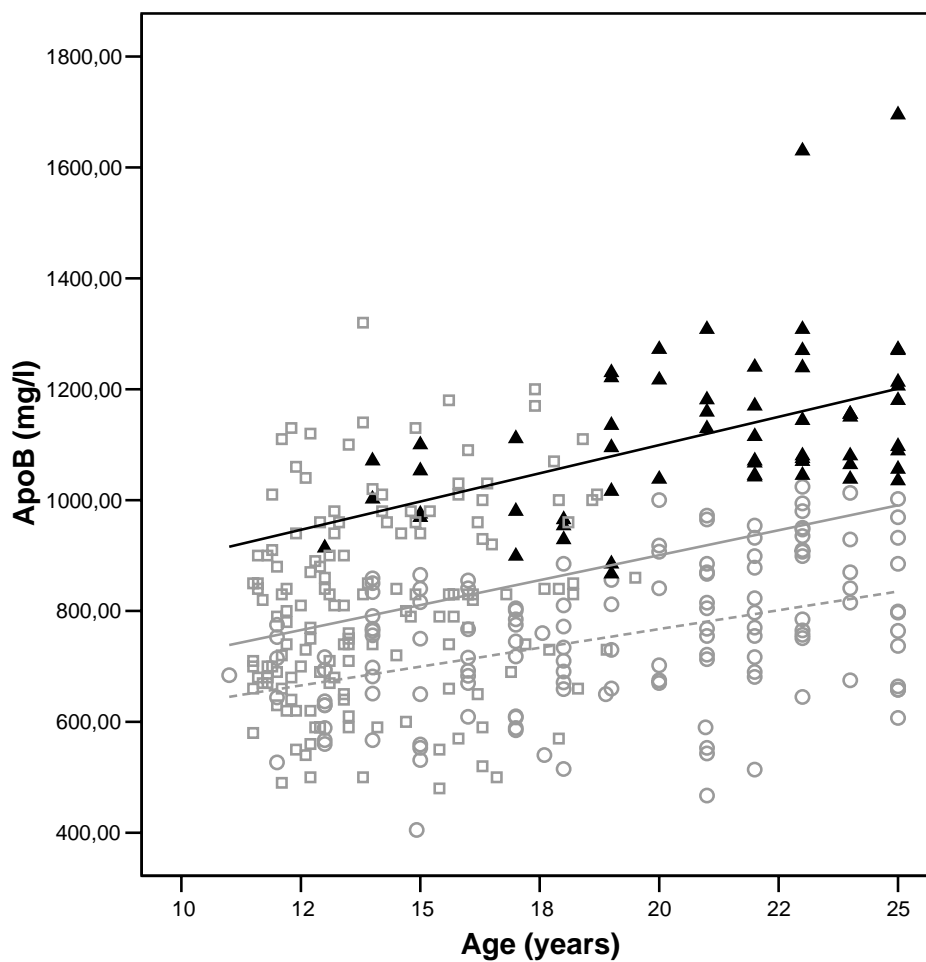
Abbreviations as in table 1. \*=  $p < 0.05$ ; †= $p < 0.01$ ; ‡= $p < 0.001$

**FIGURE 1.** Association of BMI with age in 194 subjects recruited from families with FCH (stratified by apoB phenotype) and 177 control subjects.



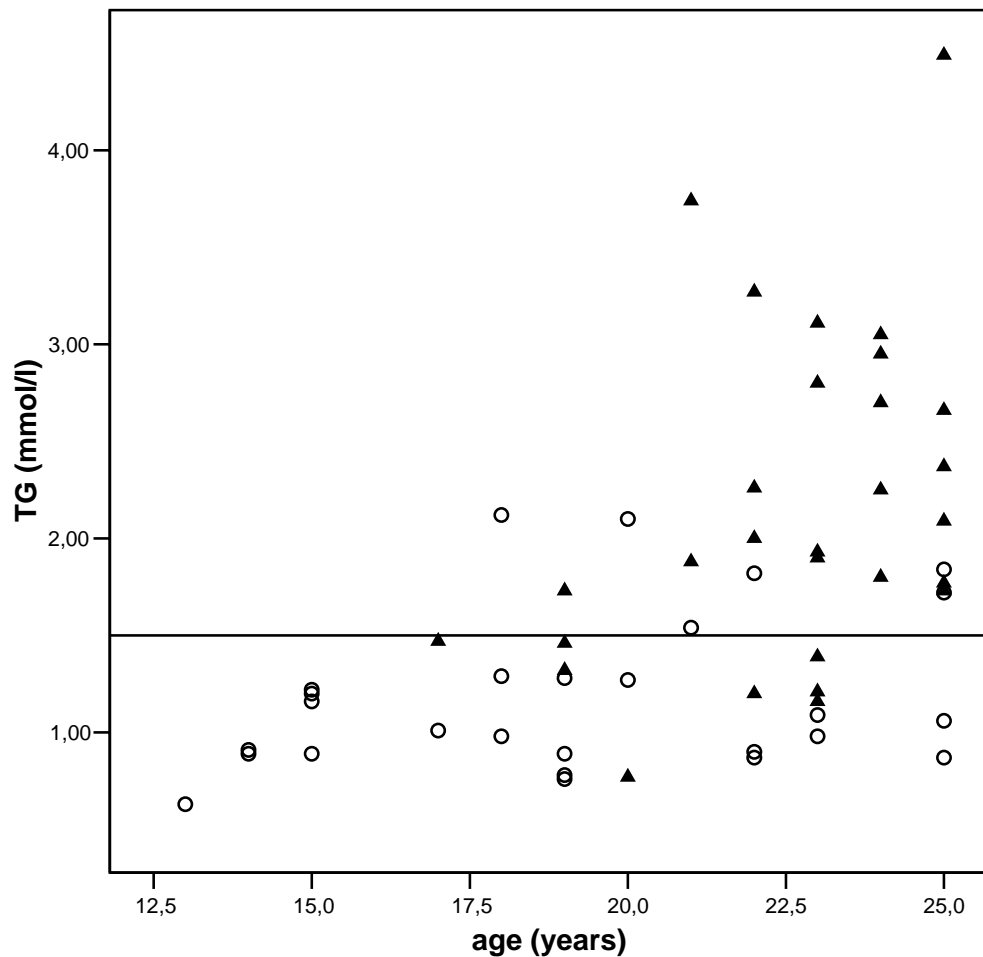
**Legend:** ▲ / — = subject (s) with apoB >75<sup>th</sup> percentile adjusted for age and gender, ○ / — = subject (s) with apoB <75<sup>th</sup> percentile adjusted for age and gender, □ / ... = unaffected FH siblings.

**FIGURE 2.** Association of ApoB with age in 194 young adults recruited from families with FCH (stratified by apoB phenotype) and 177 control subjects.



**Legend.** ▲ /— = subject (s) with apoB >75<sup>th</sup> percentile adjusted for age and gender, ○ /— = subject (s) with apoB < 75<sup>th</sup> percentile adjusted for age and gender, □ / ... = unaffected FH siblings.

**FIGURE 3.** Association of plasma triglyceride-concentration with age in 59 young adults with the hyperapoB-phenotype (apoB>75<sup>th</sup> percentile for age and gender) recruited from FCH-families.



**Legend:** ▲ = subject with K-value <-0.10, reflecting the presence of small dense LDL. ○ = subject with K-value >-0.10. Horizontal line denotes TG-concentration of 1.5 mmol.l<sup>-1</sup>.