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Postprandial triglyceride levels in familial combined hyperlipidemia. The role of apolipoprotein E and lipoprotein lipase polymorphisms

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Abstract

The effect of apolipoprotein E genotype and polymorphisms of lipoprotein lipase gene on plasma postprandial triglyceride levels in familial combined hyperlipidemic subjects and their relatives have not been sufficiently studied. This study included sixteen familial combined hyperlipidemic parents (G1): age: 52 ± 9 years with total-cholesterol: 7.2 ± 1.7 mmol/L, fasting triglycerides: 2.8 ± 1.4 mmol/L and sixteen children (G2) (twelve were normolipidemic): of age: 22 ± 5 years with total-cholesterol: 5.2 ± 1.1 mmol/L, fasting triglycerides: 2.06 ± 1.8 mmol/L and twelve normolipidemic, healthy controls. Blood samples were taken fasting and 2, 4, 6, 8, 10 hr postprandially after the standard fat rich test meal. We determined lipid parameters, apolipoprotein E and lipoprotein lipase HindIII and PvuII polymorphisms as well. The 6-hr critical postprandial triglyceride values were abnormal in both G1: 5.88 ± 2.7 mmol/L and G2: 3.53 ± 2.7 mmol/L ($p < 0.001$), respectively, and differed significantly ($p < 0.001$) from each other. The subjects of familial combined hyperlipidemic families with E4 allele in both generations exhibited significantly ($p < 0.001$) higher and extended postprandial lipemia. We did not find significant effects of lipoprotein lipase HindIII or PvuII polymorphisms on the fasting lipid values alone, however in normolipidemic subjects from the same families the homozygosity of HindIII variation was associated with higher triglyceride postprandial peak ($p < 0.01$). The main findings of our study are that i.) normolipidemic G2 subjects in familial combined hyperlipidemic families have already abnormal postprandial status, and ii.) the 6 h postprandial triglyceride values were correlated with fasting triglyceride levels, which showed association with the apolipoprotein E4 allele.

Keywords: Familial combined hyperlipidemia; Postprandial lipemia; Apolipoprotein E; Lipoprotein lipase; DNA polymorphism

1. Introduction

Familial combined hyperlipidemia (FCHL) is one of the most common genetic disorders in man. It has been reported that 7-9% of male patients with coronary heart disease (CHD) under 60 years of age are affected by this type of dyslipidemia, and its frequency in the normal population is estimated to range from 0.3% to 2.0% [1]. FCHL is characterized by elevated apolipoprotein B, triglyceride and/or cholesterol levels, excess of small dense LDL, decreased post-heparin lipoprotein lipase activity, impaired chylomicron remnant clearance and the presence of insulin resistance. Both hypersecretion and impaired elimination of VLDL are therefore thought to be causally involved in the increased risk of premature atherosclerosis in FCHL patients. The lipoprotein profile may differ within the family, a characteristic feature in FCHL that is known as the 'multiple lipoprotein phenotype' [1]. Because of the sophisticated determination of FCHL status finding typical families is difficult which explains the low number of families studied.

Several restriction fragment length polymorphisms (RFLPs) in the LPL gene have also been reported; including the PvuII RFLP in the intron between exons 6 and 7 and the HindIII RFLP in the intron between exons 8 and 9. Neither of these RFLPs alters any amino acids of the enzyme [2,3]. Some population studies have concluded that the PvuII and HindIII RFLPs are in association with lipid and lipoprotein

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Table 1a
Description of subpopulations

	normolipidemia (NL)	dyslipidemia (DL)		
		normotriglyceridemic-hypercholesterolemia (NT)	hypertriglyceridemic-normocholesterolemia (HT)	hypertriglyceridemic-hypercholesterolemia (HT)
G1	0	8	0	8
G2	10	4	1	1
Control	12	0	0	0

variables and coronary heart disease. Mitchell et al. [4] reported that H+/H+ homozygote had the highest plasma triglyceride levels in a sample of white healthy males age between 40-70. Chen et al. [5] observed that hypertriglyceridemic and hypercholesterolemic male patients (selected from the ARIC-Study population) possessed higher frequency (0.65) of the H+/H+ genotype than the normolipidemics (0.55). Heinzmann et al. [6] reported an association between HindIII polymorphism and HDL-cholesterol level, but failed to find correlation between the HindIII genotype and triglyceride levels. Thorn et al. [7] found that Caucasian patients with severe coronary atherosclerosis have a higher frequency of the H+ allele compared to healthy controls (0.77 vs. 0.57, $p < .001$). More recently the decreased HDL-cholesterol levels were associated with abdominal obesity in women [8]. Nevertheless, HindIII and PvuII polymorphisms have not been analyzed in FCHL despite associations with dyslipidemia.

In the past years, a substantial body of evidence has accumulated showing that blood TG levels [9], TG-rich lipoprotein (TRL) and their remnants promote atherogenesis [10,11]. Studies have usually identified a larger and delayed postprandial response curve to a fatty meal in patients with CHD than in controls, suggesting that the metabolism of TRL and/or of their remnants might be defective [12].

Apolipoprotein E has a crucial role in the clearance of TRL and their remnants. The natural isoforms of apo E differ with respect to their affinity to cellular lipoprotein receptors [13], proteoglycans and various lipoprotein fractions [14]. There is data suggesting that the apo E polymorphism determines the amplitude of postprandial lipemia response in humans [15,16]. Bredie et al. demonstrated that the apo E4 allele in FCHL populations is slightly over-represented [17].

Although there is some association between dyslipidemia and HindIII and PvuII polymorphisms of LPL gene and postprandial lipemia and apo E polymorphism, they were not incorporated in FCHL studies [18]. In addition to FCHL is characterized by variability of genotype, by the age dependence of the hyperlipidemia and by the lack of unequivocal diagnostic criteria. It is important to emphasize the need for a variety of approaches, both biochemical and genetic, and for studies of multiple populations. This explains our choice to analyze the postprandial triglyceride levels during fat loading test in hyperlipidemic probands and in their children from FCHL families stratified by simultaneous examination of three different polymorphisms.

2. Materials and methods

2.1. Patients

The study included sixteen FCHL parents (G1): age: 52 (38–64) years, total-cholesterol: 7.2±1.7 mmol/L, fasting triglycerides: 2.8±1.4 mmol/L and sixteen children (G2) (fourteen were normotriglyceridemic): age: 22 [17-28] years, total-cholesterol: 5.2±1.1 mmol/L, fasting triglycerides: 2.06±1.8 mmol/L and twelve normolipidemic, healthy controls (C). The FCHL parents met all the criteria: (a) a primary hyperlipidemia with varying phenotypic expression (this included a fasting plasma TG concentration >2.3 mmol/L and/or fasting plasma cholesterol concentration >5.2 mmol/L); (b) at least one first-degree relative who had a different hyperlipidemic

Table 1b
Distribution of apolipoprotein E genotypes in subpopulations*

apoE	G1		G2		Control	
	NL	DL	NL	DL	NL	DL
3/3	0	6	7	2	12	0
4/3	0	10	3	4	0	0

* 2/2, 2/3, 2/4, 4/4 genotypes were not found.

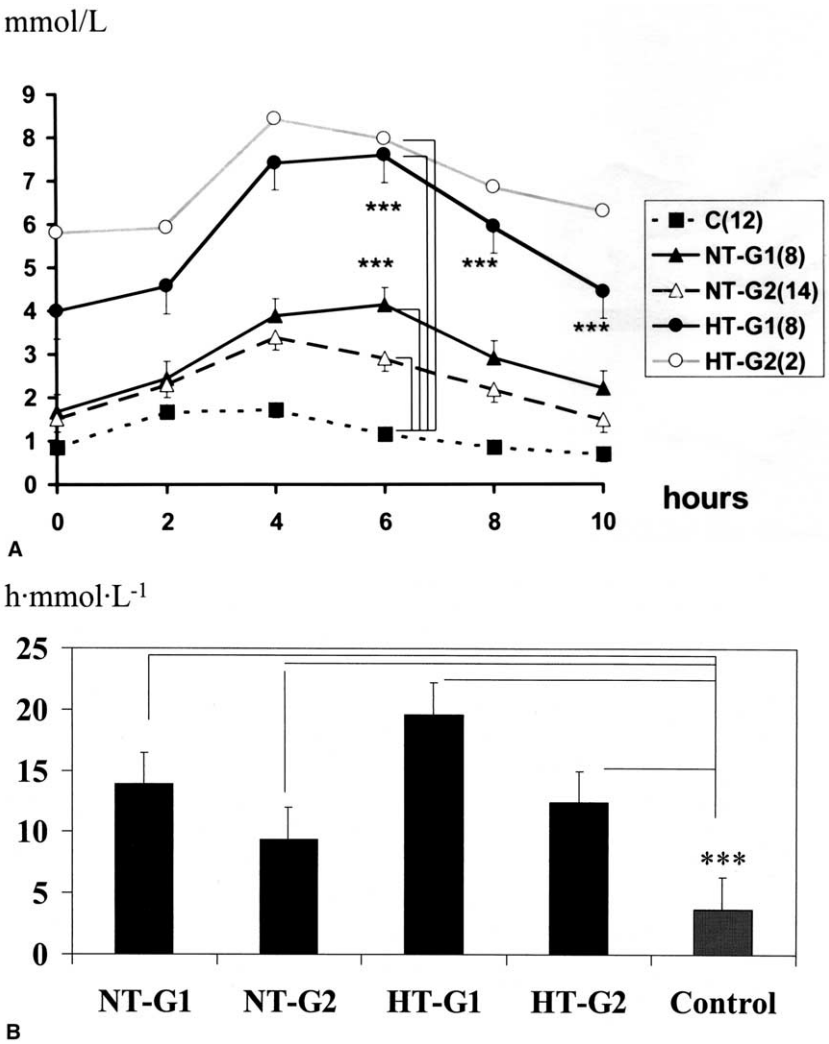

Fig. 1. a) Line plot of postprandial triglyceride levels (normotriglyceridemic-G1 and G2, hypertriglyceridemic-G1 and G2 vs. C, *** $p < 0.001$, p values evaluated by ANOVA test).b) Mass of Area Under Curve above the fasting triglyceride levels (*** $p < 0.001$).

genotype from the proband; (c) elevated fasting plasma apo B concentration (>1.2 g/L); (d) absence of xanthomas; and (e) a positive family history of premature coronary artery disease, defined as myocardial infarction before the age of 60 in at least one blood-related subject.

The local ethics committee approved the protocol. All subjects gave fully informed written consent.

3. Methods

3.1. Lipid and lipoprotein analysis

Venous blood samples for plasma lipid and lipoprotein determinations were collected into EDTA tubes from all subjects after a 12 h fasting (baseline). Cholesterol and

Table 2
Fasting triglyceride values and postprandial 6 h peak levels

Fasting TG (mmol/L)	6 h TG (mmol/L)
<1.7	2.69 ± 0.4
1.7–2.3	4.01 ± 0.6
2.3 <	7.68 ± 0.9**

**p< 0.01, p values evaluated by ANOVA test.

Table 3
Fasting triglyceride levels and ApoE genotypes in FCHL families

Fasting TG (mmol/L)	ApoE 3/3 (n)	ApoE 4/3 (n)
< 1.7	7	4
1.7–2.3	6	5
2.3 <	2	8

p< 0.05, p values evaluated by Chi^2 test.

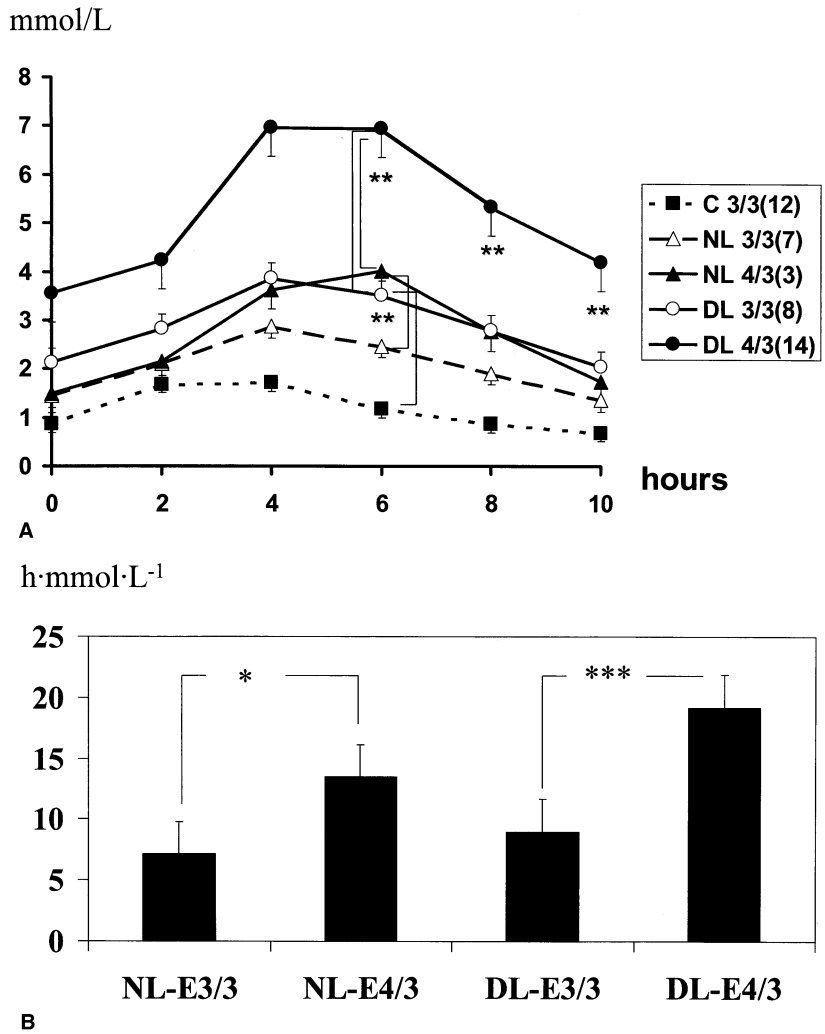

Fig. 2. a) Line plot of postprandial triglyceride levels according to apolipoprotein E genotypes (Dyslipidemic-E4/3 vs. normolipidemic-E4/3 and vs. dyslipidemic-E3/3. Dyslipidemic-E3/3 vs. normolipidemic-E3/3 vs. and C-E3/3, ** $p < 0.01$, p values evaluated by ANOVA test).b) Mass of Area Under Curve according to apolipoprotein E alleles and fasting lipid levels (*** $p < 0.001$, * $p < 0.05$).

triglycerides were measured with fully enzymatic methods (Roche Diagnostica) on a Hitachi 707 Clinical Chemistry Analyzer. HDL-cholesterol was determined by measuring cholesterol in the supernatant with an enzymatic colorimetric test, after precipitation with $MgCl_2$. Plasma apo B was determined by immunonephelometry on a Behring Nephelometer Analyzer with Behring reagents.

3.2. DNA analysis for detection of LPL Pvull and Hindlll RFLPs

DNA extraction was performed according to the method of Miller et al. [19]. The extracted DNA was stored at 4°C until analysis. The PCR was used to detect the LPL PvuII RFLP between exons 6 and 7 and HindIII RFLP between exons 8 and 9.

3.2.1. Pvull

The denaturation step of the PCR was carried out at 94°C for 4 min; annealing and extension at 72°C for 5 min. Thirty-five subsequent cycles were as follows; 95°C for 30 sec, 55°C for 1 min and 72°C for 1 min. The size of the PCR-amplified product is 440 bp (P-) [20]. After digestion 2 fragments of 330 bp and 110 bp are produced if the polymorphic site is present (P+).

3.2.2. Hindlll

The denaturation step of PCR was performed at 94°C for 4 min; annealing and extension at 72°C for 5 min. Thirty-five subsequent cycles were follows; 95°C for 30 sec, 58°C for 1 min and 72°C for 1 min. The size of the PCR-amplified product is 365 bp (H-) [21]. After digestion 2 fragments of 205 bp and 160 bp are produced if the polymorphic site is present (H+).

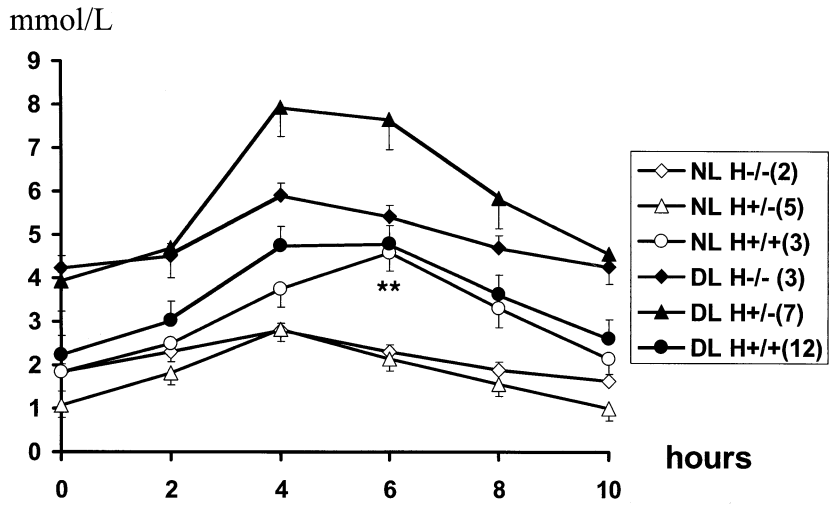

Fig. 3. Line plot of postprandial triglyceride levels according to lipoprotein lipase Hindlll genotypes (NL-H+/+ vs. NL-H+/- and NL-H-/-, ** p <0.01).

3.3. Apolipoprotein E polymorphism

Apolipoprotein E genotyping was achieved with PCR followed by cleavage with HhaI restriction enzyme as described by Hixson et al. [22].

3.4. Oral fat loading test

After 12 hr overnight fasting the subjects were given standard test meal containing 83% fat, 14% carbohydrates, 2.8% protein and obtained 1.362 kcal and 480 mg cholesterol [23]. Subjects ate the meal within 15 min and were instructed to keep physical activity to a minimum, refrain from smoking and fasting during the 10 hr of the test. Blood was obtained after an overnight fasting and at 2, 4, 6, 8 and 10 hr after ingestion of the fat meal for determination of triglycerides. The postprandial lipid status can be characterized by different ways. We preferred the use of TG levels at 6 hr and the magnitude of area under the curve (AUC) for comparison as suggested by the erlier investigations [12,23].

3.5. Statistics

Lipid levels according to genotype were studied by ANOVA adjusted for age.

The postprandial response was compared across genotypes by ANOVA for each time point, for the AUC above fasting TG concentration. For distributions positively skewed, a power transformation (log or square-root) was applied for tests, but untransformed values are given in the graphs. Correlations between fasting TG and at 6 hr TG levels were determined by calculation of Pearson correlation coefficients. The SPSS-PC 4.0 computer program was used for statistical calculations.

3.4.1. Results

In dyslipidemic status, dominantly in hypertriglyceridemia, the abnormal postprandial reaction has been already proved. Therefore we analyzed the family members according to their lipid values separately. The subjects were divided in two groups according to triglyceride level: normotriglyceridemic (NT): TG <2.3 mmol/L and hypertriglyceridemic (HT): TG >2.3 mmol/L. We also used the term of normolipidemic (NL) and dyslipidemic (DL), where the latter concerns any type of hypertriglyceridemia and/or hypercholesterolemia (Table 1a).

As expected the most abnormal postprandial lipid status was observed in the HT G1 and G2 persons (n = 8 and 2) (Fig. 1a). We found also extended postprandial lipemia in NT G1 (n = 8) and NT G2 (n = 14) subjects by regular comparison to control values (Fig. 1a). Postprandial 6 h plasma triglyceride values correlated positively with the fasting TG levels (r = 0.865, p <0.001) (Table 2).

Distribution of apolipoprotein E genotypes in different subgroups is shown in Table 1b. In DL group of the investigated FCHL population the apo E4/3 genotype was nearly twice as frequent as apo E3/3 genotype. Subjects carrying apo E4/3 alleles showed higher fasting TG concentrations (Table 3). The postprandial results across the apo E genotype distribution according to DL (n = 22) and NL (n = 10) members of family are in Fig. 2a where the influence of apo E4 allele is clearly shown.

We were not able to find consistent association of the LPL gene Hind III polymorphism and the fasting TG levels. In the NL group the subjects with H+/+ had TG peak at 6 hr. It is significantly later (p <0.01) than that of subjects with H-/- and H+/- genotypes (Fig. 3). In the DL group we did not find differences among the genotypes. By combining individuals with apo E alleles and HindIII genotypes into a single group than the most favorable fat load results

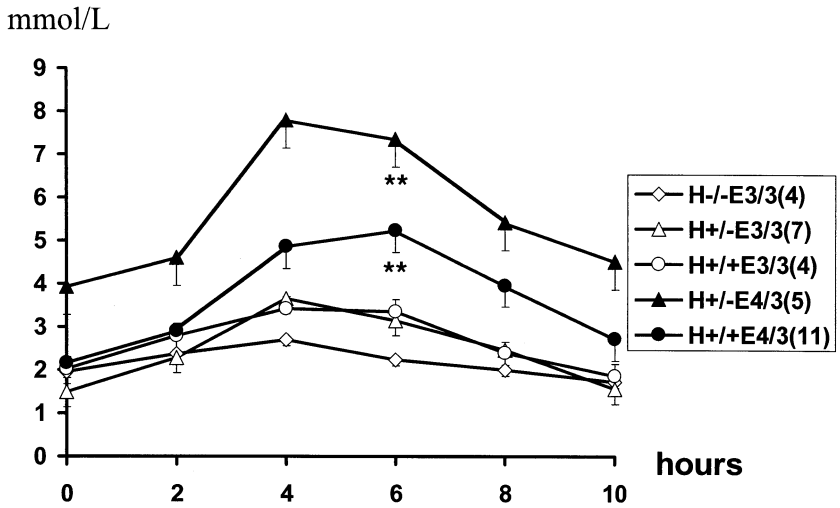

Fig. 4. Line plot of apolipoprotein E alleles and HindIII genotypes combined effect(H+/-E4/3 vs. H+/-E3/3 and H+/+E4/3 vs. H-/-E3/3, ** $p < 0.01$).

were observed in subjects with apo E3/3H-/- alleles. Subjects with apo E4/3 and H+/+ or H+/- had the most abnormal postprandial lipid status (Fig. 4).

Pvull genotypes did not show any effects on postprandial status in NL and DL groups.

All the AUC calculations showed similar results to 6 h peak values

4. Discussion

The FCHL genotype seems to be determined by one or more unknown "major" genes. The function of these putative genes is to increase the secretion of VLDL. A number of "modifier" genes could also influence the levels of plasma lipids. One such "major" gene has already been mapped [24]. Several "modifier" genes have also been identified including the LPL gene and apo AI-CIII-AIV gene cluster.

The primary goal of this work was to investigate the postprandial effect of apo E variants and of HindIII and PvuII polymorphisms of LPL gene in probands and in their children of FCHL families.

Reports about the correlations between lipid variables and LPL HindIII and PvuII polymorphisms are contradictory. Several studies reported significant associations between H+ allele and/or H+/H+ genotype and higher triglyceride and lower HDL cholesterol levels [25,26,27]. On the other hand, Wang et al [28] found significant dosage-dependent relationship between elevated triglycerides and P+ allele.

Data on the impact of apo E genotype on plasma TG levels are not totally consistent. In a meta-analysis it was concluded that the apo E2 and apo E4 alleles result in moderately higher fasting TG levels in the general population [29]. The latest and largest survey (EARS II) also demonstrated that apo E polymorphism explains part of postprandial TG variability in normal young adults [30]. Postprandial triglyceridemia was the highest in subjects with E2 allele. Subjects with E4 isoform showed somewhat lower level and subjects being homozygous for E3 showed the least extent level of elevation. These finding were independent of baseline TG values.

Similarly, we found clear association between the apo E allele polymorphism and the magnitude of postprandial lipemia in FCHL families. The most expressed and extended postprandial lipemia occurred in 13 DL family members with one apo E4 allele. In the 3 NL members of FCHL families with one apo E 4 allele the postprandial TG peak was at 6 hr, which was 2 hr later than in 8 DL members with two apo E3 alleles. Considering the predictive role of fasting TG levels in postprandial answer and its association with apo E4 allele, the peak of TG in both DL and NL group was also influenced by apo E4 allele. Therefore it seems fair to suppose a decreased and extended catabolism of the remnants in FCHL caused by apo E4 isoform.

The only significant association respecting lipase gene polymorphisms was in NL group in which we detected extended lipemia (TG peak at 6 h) in subjects with H+/+ as compared with H+/- and H-/- genotypes. We propose that the structural variation of LPL caused by the Hind III polymorphism may decrease the binding capacity of the TRL remnant particles to the remnant receptors. The other influence of HindIII polymorphism could be on LPL activity. Recent evidence suggests that the LPL HindIII polymorphism may be in linkage disequilibrium with functional LPL variants such as the Ser447-stop mutation [31].

Combining the examination of the apo E genotypes and the LPL gene Hind III polymorphism we found the most favorable postprandial status in subjects with apoE 3/3H-/- homozygotes. On the other hand, subjects with apo E4/3/H+/+ alleles showed the highly abnormal fat load results. Recognizing the limitation of our data caused by low number of subjects investigated we suggest that normolipidemic

FCHL family members with combined existence of the unfavorable LPL structure (HindIII cutting site) and the inadequate receptor clearing caused by apo E genotypes (E4) would be first candidates for later development of dyslipidemia.

References

[1] de Bruin TW, Mailly HH, van Barlingen R, Fisher M, Castro Cabezas P, Talmud GM, Dallinga-Thie SE, Humphries SE. Lipoprotein lipase gene mutations D9N and N291S in four pedigrees with familial combined hyperlipidaemia. Eur J Clin Invest 1996;26:631–9.

[2] Funke H, Klug J, Assmann G. Hind III RFLP in the lipoprotein lipase gene, (LPL). Nuc Acids Res 1987;15:9102.

[3] Regis-Bailly A, Visvikis S, Steinmetz J, Feldmann L, Briancon S, Danchin N, Zannad F, Siest F. Frequencies of five genetic polymorphisms in coronarographed patients and effects on lipid levels in a supposedly healthy population. Clin Genet 1996;50:339–47.

[4] Mitchell RJ, Earl L, Bray P, Fripp YJ, Williams J. DNA polymorphisms at the lipoprotein lipase gene and their association with quantitative variation in plasma high-density lipoproteins and triacylglycerides. Hum Biol 1994;66:383–97.

[5] Chen L, Patsch W, Boerwinkle E. Hindlll DNA polymorphism in the lipoprotein lipase gene and plasma lipid genotypes and carotid artery atherosclerosis. Hum Genet 1996;98:551–6.

[6] Heinzmann C, Ladias J, Antonarakis S, Kirchgessner T, Schotz M, Lusis AJ. RFLP for the human lipoprotein lipase (LPL) gene: Hindlll. Nuc Acids Res 1987;5:6763.

[7] Thorn JA, Chamberlain JC, Alcolado JC, Oka K, Chan L, Stocks J, Galton DJ. Lipoprotein and hepatic lipase gene variants in coronary atherosclerosis. Atherosclerosis 1990;85:55–60.

[8] The REGICOR investigators, Senti M, Bosch M, Aubo C, Elosua R, Masia R, Marrugat J. Relationship of abdominal adiposity and dyslipemic status in women with a common mutation in the lipoprotein lipase gene. Atherosclerosis 2000;150:135–41.

[9] Hokanson JE, Austin MA. Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta- analysis of population-based prospective studies. J Cardiovasc Risk 1996;3:213–9.

[10] Havel RJ. Postprandial hyperlipidemia and remnant lipoproteins. Curr Opin Lipidol 1994;5:102–109.

[11] Zilversmit DB. Atherogenesis: a postprandial phenomenon, Circulation 1979;60:473–85.

[12] Patsch JR, Miesenbock G, Hopferwieser T, Muhlberger V, Knapp E, Dunn JK, Gotto AM, Patsch W. Relation of triglyceride metabolism and coronary artery disease. Studies in the postprandial state. Arterioscler Thromb 1992;12:1336–45.

[13] Schneider WJ, Kovanen PT, Brown MS, Goldstein JL, Utermann G, Weber W, Havel RJ, Kotite L, Kane JP, Innerarity TL, Mahley RW. Familial dysbetalipoproteinemia. Abnormal binding of mutant apoprotein E to low density lipoprotein receptors of human fibroblasts and membranes from liver and adrenal of rats, rabbits, and cows. J Clin Invest 1981;68:1075–85.

[14] Steinmetz A, Jakobs C, Motzny S, Kaffarnik H. Differential distribution of apolipoprotein E isoforms in human plasma lipoproteins. Arteriosclerosis 1989;9:405–11.

[15] Dart A, Sherrard B, Simpson H. Influence of apo E genotype on postprandial triglyceride and glucose responses in subjects with and without coronary heart disease. Atherosclerosis 1997;130:161–70.

[16] Nikkila M, Solakivi T, Lehtimaki T, Koivula T, Laippala P, Astrom B. Postprandial plasma lipoprotein changes in relation to apolipoprotein E genotypes and low density lipoprotein size in men with and without coronary artery disease. Atherosclerosis 1994;106:149–57.

[17] Bredie SJ, Vogelaar JM, Demacker PN, Stalenhoef AF. Apolipoprotein E polymorphism influences lipid phenotypic expression, but not the low density lipoprotein subfraction distribution in familial combined hyperlipidemia. Atherosclerosis 1996;126:313–24.

[18] Humphries SE, Nicaud V, Margalef J, Tiret L, Talmud PJ. Lipoprotein lipase gene variation is associated with a paternal history of premature coronary artery disease and fasting and postprandial plasma triglycerides: the European Atherosclerosis Research Study (EARS). Arterioscler Thromb Vasc Biol 1998;18:526–34.

[19] Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;6:1215.

[20] Peacock RE, Hamsten A, Nilsson-Ehle P, Humphries SE. Associations between lipoprotein lipase gene polymorphisms and plasma correlations of lipids, lipoproteins and lipase activities in young myocardial infarction survivors and age-matched healthy individuals from Sweden. Atherosclerosis 1992;97:171–85.

[21] Jemaa R, Tuzet S, Portos C, Betoulle D, Apfelbaum M, Fumeron F. Lipoprotein lipase gene polymorphisms: associations with hypertriglyceridemia and body mass index in obese people. Int J Obes Relat Metab Disord 1995;19:270–4.

[22] Hixson JE, Vernier DT. Restriction isotyping of human apolipoprotein E by gene amplification and cleavage with Hhal. J Lipid Res 1990;31:545–8.

[23] Patsch JR. Postprandial lipaemia. Baillieres Clin Endocrinol Metab 1987;1:551–80.

[24] Pajukanta P, Nuotio I, Terwilliger JD, Porkka KV, Ylitalo K, Pihlajamaki J, Suomalainen AJ, Syvanen AC, Lehtimaki T, Viikari JS, Laakso M, Taskinen MR, Ehnholm C, Peltonen L. Linkage of familial combined hyperlipidaemia to chromosome 1q21-q23. Nat Genet 1998;18:369–73.

[25] Ahn YI, Kamboh MI, Hamman RF, Cole SA, Ferrell RE. Two DNA polymorphisms in the lipoprotein lipase gene and their associations with factors related to cardiovascular disease. J Lipid Res 1993;34:421–8.

[26] Gerdes C, Gerdes LU, Hansen PS, Faergeman O. Polymorphisms in the lipoprotein lipase gene and their associations with plasma lipid concentrations in 40-year-old Danish men. Circulation 1995;92:1765–9.

[27] Vohl MC, Lamarche B, Moorjani S, Prud'homme D, Nadeau A, Bouchard C, Lupien PJ, Despres JP. The lipoprotein lipase HindIII polymorphism modulates plasma triglyceride levels in visceral obesity. Arterioscler Thromb Vasc Biol 1995;15:714–20.

[28] Wang XL, McCredie RM, Wilcken DE. Common DNA polymorphisms at the lipoprotein lipase gene. Association with severity of coronary artery disease and diabetes. Circulation 1996;93:1339–45.

[29] Dallongeville J, Lussier-Cacan S, Davignon J. Modulation of plasma triglyceride levels by apoE genotype: a meta- analysis. J Lipid Res 1992;33:447–54.

[30] European Atherosclerosis Research Study, Dallongeville L, Tiret S, Visvikis DS, O'Reilly M, Saava G, Tsitouris M, Rosseneu G, DeBacker SE], Humphries SE, Beisiegel U. Effect of apo E phenotype on plasma postprandial triglyceride levels in young male adults with and without a familial history of myocardial infarction: the EARS II study. Atherosclerosis 1999;145:381–8.

[31] Groenemeijer MD, Hallman PW, Reymer E, Gagne JA, Groenemeijer BE, Hallman MD, Reymer PW, Gagne E, Kuivenhoven JA, Bruin T, Jansen H, Lie KI, Bruschke AV, Boenwinkle E, Hayden MR, Kastelein JJ. Genetic variant showing a positive interaction with beta-blocking agents with a beneficial influence on lipoprotein lipase activity, HDL cholesterol, and triglyceride levels in coronary artery disease patients. The Ser447-stop substitution in the lipoprotein lipase gene. REGRESS Study Group, Circulation 1997;95:2628–35.