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Interrelationships between low density lipoprotein receptor defect, serum fatty acid composition, and serum cholesterol concentration

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It is known that, in the general human population, serum fatty acid composition is correlated with serum triacylglycerol and cholesterol concentrations. The goal of the present study was to analyze whether the same is true of individuals who have a low density lipoprotein receptor (LDL-R) defect. Concentrations of 16 different fatty acids, cholesterol, triacylglycerol, and major lipoproteins in serum were determined in eight individuals who had (FH-North Karelia), the most common LDL-R defect in Finland, which causes familial hypercholesterolemia, and in their 30 relatives belonging to a single large pedigree as controls. The average number of double bonds (i.e., degree of desaturation) in serum fatty acids correlated negatively with the concentrations of serum total cholesterol ($r = 0.27$, $P < 0.05$*) and total triacylglycerol (*$r = -0.71$, $P < 0.001$*) and positively with the number of fish meals per week (*$r = 0.50$, $P < 0.01$*), which was analyzed in all pedigree members jointly. These effects were similar in individuals having LDL-R defect, in which group the correlation coefficients were* -0.31 (P = *NS),* -0.99 ($P < 0.001$), *and 0.79 (*P = *NS) for serum total cholesterol, triacylglycerol, and weekly fish meals, respectively. Thus, LDL-R defect does not impair the correlation between serum fatty acid composition and serum triacylglycerol concentration. This result is in agreement with dietary studies that have shown that familial hypercholesterolemia patients respond very favorably to dietary therapy.* (J. Nutr. Biochem. 10:360–366, 1999)

Keywords: fatty acids; low density lipoprotein receptor; cholesterol; triacylglycerol; familial hypercholesterolemia

Introduction

A high concentration of cholesterol in human serum is one of the primary factors in the development of atherosclerosis.[1] This association between serum total cholesterol and the risk of atherosclerosis is strengthened when individual lipoprotein fractions are examined. The concentration of low density lipoprotein cholesterol (LDL-C) correlates positively whereas plasma high density lipoprotein cholesterol (HDL-C) correlates inversely to the development of coronary heart disease.[2] In addition to the dietary cholesterol itself, the ratio of dietary saturated fatty acids to polyunsaturated fatty acids influences the concentration of serum LDL-C but not HDL-C concentration.[3]

Fatty acids affect serum LDL-C concentration by regulating the production rate of very low density lipoproteins (VLDL) and the LDL-receptor (LDL-R) activity in liver.[4] Dietary medium-length saturated fatty acids such as 14:0 and longer chain unsaturated fatty acids, especially 18:1, have different effects on serum cholesterol and triacylglycerol levels. Based mainly on experimental animal studies, it has been postulated that this difference can be explained at least partially by the effects of fatty acids on the so-called intracellular cholesterol pool. The longer unsaturated fatty acids are better substrates for the acyl-coenzyme A (CoA) cholesterol acyltransferase (ACAT) and, by reducing the putative free cholesterol pool via generating cholesteryl esters, they signal for the hepatic cells to maintain high

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LDL-R activity.[5–7] In addition, the enzymatic activity of 3-hydroxy-3-methylglutaryl CoA (HMG CoA) reductase, which is the rate-limiting enzyme in cholesterol synthesis, is regulated by the unesterified cholesterol.[8,9] Very short chain fatty acids do not take part in the regulation of cholesterol pools because they are rapidly metabolized to acetyl-CoA and used for the synthesis of a variety of products.[7] The equilibrium of fatty acids in liver cells and in serum is modified by the fatty acids in the diet.[10,11]

Familial hypercholesterolemia (FH) is characterized by markedly elevated serum LDL-C concentration and premature coronary artery disease. It is an autosomal dominant disease defined at the molecular level by mutations in the LDL-R gene.[12] Although FH is a single gene disorder, it shows great phenotypic variation[13] that is attributable to the variability of the mutations,[14,15] other genetic factors such as apoE polymorphisms,[16] and environmental factors such as obesity, diet, and cigarette smoking.[17]

Because LDL-R is part of the suggested mechanism by which different fatty acids affect the serum cholesterol concentration, we found it important to analyze whether a LDL-R defect will change the relationship between serum fatty acid composition and levels of cholesterol and triacylglycerol in serum. A LDL-R defect also might have an effect on serum fatty acid composition due to changes in fatty acid synthesis and/or degradation. Thus, our secondary aim was to compare serum fatty acid composition between subjects with LDL-R defect and controls living in the same environment.

Materials and methods

Subjects

A large, previously unpublished pedigree came to our attention because of familial clustering of markedly elevated serum cholesterol values and of several fatal and nonfatal cardiovascular events. Over 70 individuals were included in a genealogic analysis through five generations. Informed consent to join the study was given by 39 individuals who answered a questionnaire concerning basic demographic data including age, gender, height, and weight. To be able to control the effects of diet on the fatty acid and lipoprotein profile, they also answered a diet questionnaire that included a question about their average number of fish meals per week. Blood samples for DNA isolation and for fatty acid and lipoprotein analyses were drawn after overnight fast. Serum samples were stored frozen at −70°C until analyzed. Subjects using lipid-lowering agents were asked to interrupt medication at least 1 week prior to blood sampling. One of the subjects did not interrupt medication due to very severe coronary heart disease. One subject was excluded from the study because of insulin-dependent diabetes mellitus. The study design was approved by the ethical committee of the National Public Health Institute.

Methods

Genetic testing of familial hypercholesterolemia. A phenol-chloroform extraction method was used to isolate DNA.[18] FH-North Karelia and FH-Helsinki, which are the two most common LDL-R mutations in Finland that cause FH,[14] were analyzed from the study subjects using the solid-phase minisequencing method.[19] In this method variable nucleotides are identified by a single nucleotide primer extension reaction catalyzed by DNA polymerase from a polymerase chain reaction (PCR) product on a solid support. Three different primers are used to study each polymorphism; each DNA fragment containing a nucleotide to be tested was first amplified by PCR and then the product was analyzed by a detection primer required in minisequencing. The primers, which were used to amplify the target sequence, had been used previously for this purpose.[20]

Biochemical analyses. For determination of plasma total esterified fatty acids, 100 μL of internal standard trinonadecanate (100 mg/L methanol) was added to 500 μL of plasma and mixed. The esterified fatty acids then were extracted with 1.9 mL of chloroform-methanol according to the standard procedure of Folch et al.[21] Aliquots of 1.5 mL of the lower phase were transferred to separate tubes and evaporated under a vacuum. The resulting dried lipid pellet was dissolved in petroleum ether. Methyl esters were prepared by transesterification with sodium methanolate as described previously.[22] The methyl derivatives were separated using OV-351 fused silica column (25 m, 0.32 mm inner diameter), mounted on a Dani HR 3800 PTV gas chromatograph, and determined with a FID detector. PTV-split technique was used for sample introduction. Seronorm lipid (Nycomed, Oslo, Norway) was used as a quality control sample in every batch of samples. Between and within batch imprecision was not more than 2%. Serum total and unesterified cholesterol and HDL-C were determined enzymatically after precipitation of apolipoprotein B (apoB)-containing lipoproteins with dextran sulfate and magnesium chloride.[23] Triacylglycerol concentration was determined with a fully enzymatic method (CHOD-PAP, Boehringer-Mannheim, Mannheim, Germany). LDL-C was calculated according to the formula of Friedewald et al.[24] Serum apolipoproteins A-I, A-II, and B were analyzed using an immunoturbidometric assays (Hoffmann-La Roche, Basel, Switzerland, and Orion Diagnostica, Espoo, Finland).

Statistical analyses. Subjects were divided into LDL-R deficiency and control groups after genetic minisequencing test. For both groups the means ± SD for basic demographic and biochemical characteristics were calculated. The average number of fish meals per week and the presence of medication for elevated blood pressure were registered from the questionnaires. To define how the groups differed for these characteristics, analysis of variance (ANOVA) was performed. Partial Pearson correlation coefficients were used to measure the association between the length or average number of double bonds in serum esterified fatty acids and other characteristics in the subjects with or without LDL-R defects. To describe the quantitative changes in serum total cholesterol and triacylglycerol, the groups were divided in tertiles according to the number of double bonds in serum esterified fatty acids. In statistical analyses, age, gender, and body mass index (BMI) were used as covariates and the triacylglycerol values were log-transformed to remove positive skewness.

Results

Basic characteristics of the study subjects

Of the 39 individuals tested, 8 individuals carried a heterozygous *LDL-R* mutation FH-North Karelia. The mean serum cholesterol concentration in subjects having the LDL-R defect was 8.27 ± 2.1 mmol/L (range 5.49–12.3 mmol/L). The subject with the lowest serum cholesterol concentration received medication; the next lowest value in this group was 6.9 mmol/L. In the control group, the mean serum cholesterol concentration was 5.3 ± 1.0 mmol/L (range 2.81–7.45 mmol/L). The groups also differed in serum triacylglycerol, free cholesterol, HDL-C, LDL-C, and

Table 1 Selected characteristics of individuals with and without LDL-R defect

Characteristics	With LDL-R defect (n = 8)	Without (n = 30)
Age (years)	46.6 ± 15	41.1 ± 15
Gender (♂/♀)	6/2	16/14
BMI (kg/m^2)	25.87 ± 4.3	25.51 ± 3.3
Cholesterol (mmol/L)	8.27 ± 2.1[c]	5.3 ± 1.0
Triacylglycerol (mmol/L)	2.51 ± 2.1	1.40 ± 0.89
Free cholesterol (mmol/L)	0.80 ± 0.19[c]	0.51 ± 0.11
HDL-C (mmol/L)	1.10 ± 0.27[a]	1.37 ± 0.29
LDL-C (mmol/L)	6.52 ± 2.0[c]	3.39 ± 0.94
ApoB (g/L)	1.71 ± 0.43[c]	0.99 ± 0.26
ApoA-I (g/L)	1.30 ± 0.17[b]	1.59 ± 0.22
ApoA-II (g/L)	0.38 ± 0.07	0.41 ± 0.05
Average chain length	17.68 ± 0.16	17.73 ± 0.09
Average double bonds	1.34 ± 0.17	1.41 ± 0.11
Eating fish (times/week)	1.6 ± 1.6 (0–5)	1.3 ± 1.1 (0–4)
Elevated blood pressure*	2/8	4/30

Note: Covariates were age, gender and body mass index (BMI), except only age and gender when testing BMI.
Analysis of variance: [a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$.
*Number of individuals who receive medication for elevated blood pressure. Mean ± SD.
LDL-R–low density lipoprotein receptor. HDL-C, high density lipoprotein cholesterol.

apoB concentrations (*Table 1*). The groups were similar with regard to age, gender, and BMI, and there were no statistically significant differences in the average fatty acid chain length, number of double bonds in fatty acids, presence of elevated blood pressure, or the number of fish meals per week (*Table 1*).

The concentrations of fatty acids in serum

Serum esterified fatty acid composition was very similar in both study groups. Specifically, there was no significant differences in the proportions of the long chain n-3 fatty acids. Palmitic acid (16:0) was the main saturated fatty acid and linoleic acid (18:2) the main unsaturated fatty acid. Only the proportion of eicosenoic acid (20:1) was slightly higher in subjects with LDL-R defect, and the proportion of docosapentaenoic acid (22:5) was slightly lower in these individuals ($P < 0.05$) (*Table 2*).

The correlation of average number of double bonds in serum fatty acids with serum lipids

Many of the clinical, dietary, and biochemical characteristics were correlated with the composition of serum esterified fatty acids. The average chain length and the number of double bonds were strongly correlated ($r > 0.97$, $P < 0.01$) in both study groups together or separately (*Table 3*). Only the results related to the number of double bonds are reported because the results were essentially the same when the average chain length was used in the correlation analysis. The average number of double bonds in serum fatty acids strongly correlated with the total serum triacylglycerol; the correlation coefficient was -0.99 ($P < 0.001$) in the LDL-R deficiency group and -0.58 ($P < 0.001$) in the control group (*Table 3* and *Figure 1*). There was significant

Table 2 The concentrations of 16 fatty acids measured from total serum esterified fatty acid pool in individuals with and without LDL-R defect

Fatty acid	Abbreviation	Percent (weight percent) of serum total esterified fatty acids: FH-patients	Controls
Saturated		31.7 ± 7.4	31.1 ± 4.4
Myristic 14:0	MA	0.90 ± 0.39	0.93 ± 0.39
Palmitic 16:0	PA	23.0 ± 3.8	22.7 ± 1.5
Stearic 18:0	SA	7.80 ± 0.92	7.8 ± 1.0
Monounsaturated		25.1 ± 4.1	22.8 ± 3.5
Palmitoleic 16:1	PO	2.50 ± 1.1	2.38 ± 0.79
Oleic 18:1	OA	23.9 ± 3.6	21.8 ± 3.2
Eicosenoic 20:1	EA	0.29 ± 0.21	0.19 ± 0.04[a]
Omega-6 unsaturated		36.0 ± 8.0	37.7 ± 5.3
Linoleic 18:2	LA	27.5 ± 7.7	28.1 ± 4.4
Gammalinoleic 18:3	GLA	0.41 ± 0.15	0.43 ± 0.17
Eicosadienoic 20:2	EDA	0.37 ± 0.10	0.39 ± 0.08
Dihomogammalinoleic 20:3	HGLA	1.61 ± 0.37	1.83 ± 0.43
Arachidonic 20:4	AA	6.12 ± 1.4	6.63 ± 1.4
Omega-3 unsaturated		5.68 ± 1.12	6.75 ± 2.22
α-Linoleic 18:3	LLA	0.99 ± 0.19	0.97 ± 0.33
Octadecatetraenoic 18:4	ODT	0.22 ± 0.06	0.27 ± 0.08
Eicosapentaenoic 20:5	EPA	1.11 ± 0.36	1.52 ± 1.02
Docosapentaenoic 22:5	DPA	0.67 ± 0.13	0.82 ± 0.18[a]
Docosahexaenoic 22:6	DHA	2.70 ± 0.72	3.21 ± 1.18
Average chain length		17.68 ± 0.16	17.73 ± 0.09
Average double bonds		1.34 ± 0.17	1.41 ± 0.11

Note: Covariates were age, gender, and body mass index.
[a]$P < 0.05$.
LDL-R–low density lipoprotein receptor.

correlation between the average number of double bonds in serum esterified fatty acids and total serum cholesterol ($r = -0.27$, $P < 0.05$), free cholesterol ($r = -0.40$, $P < 0.05$), apoB ($r = -0.34$, $P < 0.05$), apoA-II ($r = -0.41$, $P < 0.01$), and the number of fish meals per week ($r = 0.50$, $P < 0.01$) when the analysis was performed on both groups combined (*Table 3*). The correlation coefficient between LDL-C and the average number of double bonds in serum esterified fatty acids was -0.97 in FH patients and 0.03 in controls. The relationship between the average number of double bonds in serum fatty acids and serum LDL-C is shown in *Figure 2*. When the groups were divided in tertiles according to the number of double bonds in fatty acids, the serum total cholesterol was 5.6 mmol/L and 8.4 mmol/L in the lowest and 5.3 mmol/L (P = NS) and 6.4 mmol/L (P = NS) in the highest tertile for controls and FH patients, respectively. In the lowest tertile serum triacylglycerol concentrations were 1.9 g/L and 5.0 g/L, and in the highest tertile only 1.2 g/L ($P < 0.05$) and 2.2 g/L ($P < 0.001$) for the control and FH patient groups, respectively. The concentrations of unsaturated and polyunsaturated fatty acids in serum did not correlate significantly with the blood pressure, serum LDL-C, or serum HDL-C concentration.

Discussion

This study was borne from a practical medical problem. A number of individuals that belonged to a same pedigree with a high incidence of cardiovascular events but without a clear

Table 3 The partial Pearson correlation coefficients between the average number of fatty acid double bonds and selected characteristics in individuals with and without LDL-R defect and in both groups combined

Characteristic	LDL-R defect	No LDL-R defect	Both groups
Age	0.49	0.10	0.12
Gender	0.26	0.07	0.03
BMI	−0.70	0.07	−0.15
Cholesterol	−0.31	−0.18	−0.27[a]
Triacylglycerol	−0.99[c]	−0.58[b]	−0.71[c]
Free cholesterol	−0.69	−0.26	−0.40[a]
HDL-C	0.43	0.11	0.03
LDL-C	−0.97	0.03	−0.22
ApoB	−0.48	−0.24	−0.34[a]
ApoA-I	0.11	−0.20	−0.25
ApoA-II	−0.15	−0.34	−0.41[a]
ApoA-I/apoA-II (molarity)	0.35	0.11	0.16
ApoA-I/apoB (molarity)	0.62	0.04	0.02
HDL-C/LDL-C	0.97	−0.03	−0.01
Average chain length	0.98[b]	0.97[c]	0.98[c]
Eating fish (times/week)	0.79	0.52[b]	0.50[b]
Elevated blood pressure*	−0.81	−0.27	−0.25

Note: Age, gender, and body mass index (BMI) were used as covariates; only age and gender were used when BMI was tested. When both groups were combined, the low density lipoprotein receptor (LDL-R) status was added to covariates.
[a]$P < 0.05$; [b]$P < 0.01$; [c]$P < 0.001$.
*Individuals who receive medication for elevated blood pressure.
HDL-C–high density lipoprotein cholesterol.

diagnosis came to our attention. Fatty acid composition and lipoproteins were studied from their serum samples and the two most common LDL-R gene mutations in Finland were tested using a minisequencing method. Eight subjects having a mutated LDL-R gene allele were found. The finding that the individuals with LDL-R defect had markedly higher total cholesterol than controls was not surprising. However, it may be of some practical value to note that some of the individuals with LDL-R defect had serum total cholesterol concentration of only approximately 7 mmol/L and that the distribution of cholesterol values in the patient and control groups overlapped. Environmental factors, which have been shown to have a strong effect on lipid and lipoprotein profiles, are often shared between family members in a household.[25] To conduct a study about the effects of LDL-R gene defect on lipid metabolism, we found it advantageous to have both study groups from a single large pedigree. The participants did not know about their possible LDL-R defect before the study.

Starting from the studies of Dyerberg et al.[26] with Greenland Inuits, it has been known that different fatty acids have varying effects on serum cholesterol concentration and on blood pressure, even so that fatty acids with apparent similarities may have very different effects.[27] In humans fish oils are the major source of 20:5 and 22:6 n-3 fatty acids whereas in plants α-linolenic acid is the primary fatty acid that is produced in this fatty acid class. After the initial findings, several epidemiologic studies have confirmed that a diet containing fish oils is associated with reduced coronary mortality.[28,29] Whether the cardioprotective effects are due to changes in serum cholesterol and/or triac-

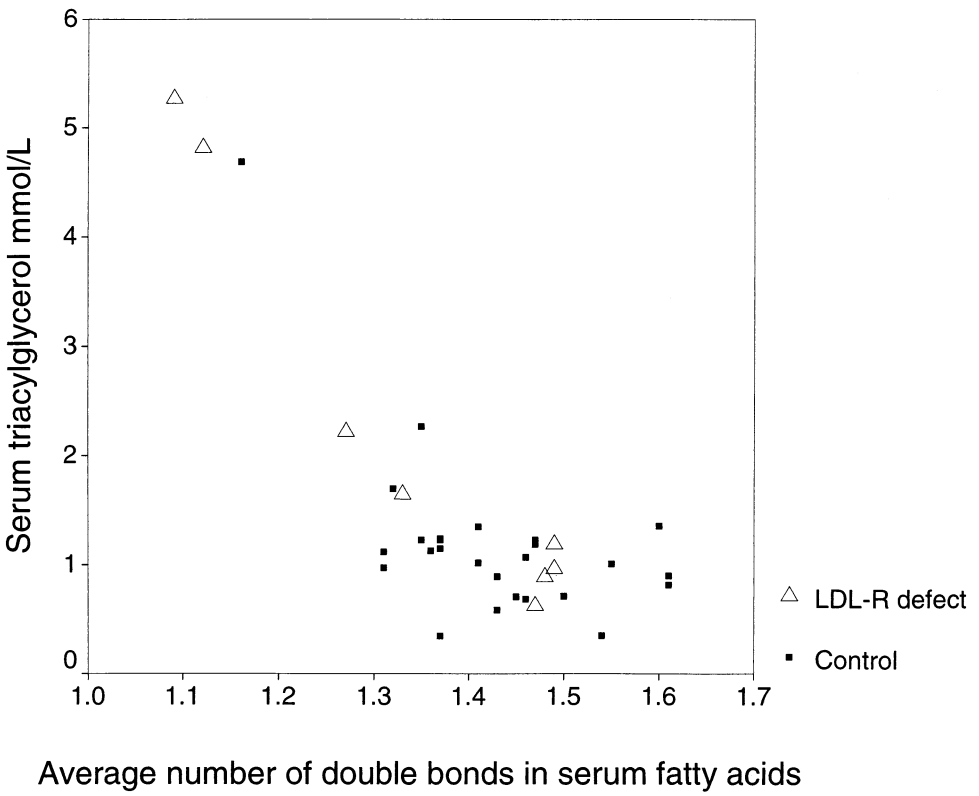

Figure 1 The correlation between the average number of double bonds in serum fatty acids and serum triacylglycerol concentration. LDL-R, low density lipoprotein receptor.

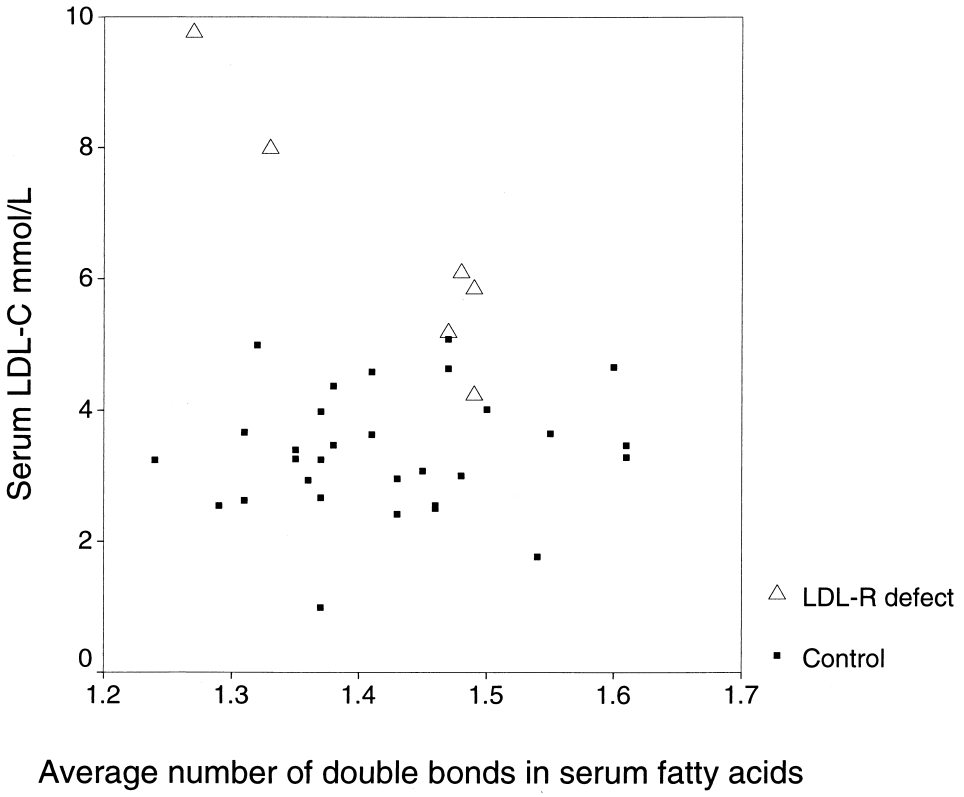

Figure 2 The correlation between the average number of double bonds in serum fatty acids and serum low density lipoprotein cholesterol (LDL-C). The serum LDL-C was calculated by the Friedewald formula; thus, individuals with serum triacylglycerol over 2.5 mmol/L were excluded. LDL-R, low density lipoprotein receptor.

ylglycerol concentrations is not clear yet. In some studies fish oils have had no lowering effect on the serum total or LDL cholesterol concentrations. In contrast, even some increase in the LDL concentrations in hypertriacylglycerolemic subjects has been observed.[30] There are numerous unanswered questions remaining about the role of fatty acids in atherosclerosis. For example, it is not known which specific fatty acids are responsible for the "fish oil effect."[31] Because the mechanisms of how serum fatty acid composition is correlated with serum cholesterol and triacylglycerol concentrations are largely unknown, we found it interesting to analyze whether the relationships between serum fatty acid composition and serum cholesterol or triacylglycerol concentrations are different in FH patients.

Some parameters of serum fatty acid composition showed strong correlation with parameters of lipid metabolism both in controls and in FH patients. The strongest correlation was seen between the number of double bonds in the serum esterified fatty acids and the serum triacylglycerol concentration, but to some extent also with the serum cholesterol concentration. It is quite interesting that the correlation coefficients seemed to be larger in the group of FH patients. For example, there was a very strong correlation ($r = -0.99$, $P < 0.001$) between the average number of double bonds in serum esterified fatty acids and serum triacylglycerol concentration compared with that of controls ($r = -0.58$, $P < 0.01$). Although not statistically significant, the correlation between the average number of double bonds in serum fatty acids and serum cholesterol or LDL-C concentrations also seemed to be similar or even stronger in FH patients. A similar result has been obtained in studies concerning the effects of HMG CoA reductase inhibitors in FH patients, in which it also has been shown that the single operating LDL-R gene can be further activated.[32] It also may be that fatty acids have direct effects on expression of other genes involved in lipid metabolism; such effects have been shown, for example, for lipoprotein lipase[33] and microsomal triacylglycerol transfer protein.[34]

Little is known about the effects of the LDL-R defect on fatty acid synthesis. Fatty acid elongation was increased in cultured fibroblasts with LDL-R defect compared with control cells,[35] but the effect on fatty acid synthesis was comparatively less than the effect on cholesterol synthesis. That effect may be due to the synchronous regulation of fatty acid synthesis by steroid regulatory element binding protein.[36] In the present study, we did not find any drastic effects of LDL-R deficiency on any of the 16 fatty acids measured. There were also no significant differences in the mean chain length or mean number of double bonds between these groups.

It has been shown that the serum cholesterol level in FH patients is amenable to dietary interventions,[37,38] and it also has been suggested that a cholesterol-lowering diet has greater effects in hypercholesterolemic than in normolipidemic subjects, even after adjustment for regression to the mean.[39] Our results encourage the use of dietary means for treatment of hyperlipidemia in FH patients. The LDL-R defect does not impair the correlation between serum fatty acid composition and serum triacylglycerol and cholesterol concentrations in FH patients.

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