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Fatty liver–based identification of two distinct hypertriglyceridemic subgroups in familial combined hyperlipidemia

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Abstract

The present study was conducted to investigate whether the fatty liver phenotype could be helpful in the identification of subgroups with distinct metabolic properties and lipid profiles within familial combined hyperlipidemia (FCHL). One hundred eighty-five FCHL family members participated in the current study; 38 subjects were found to be hypertriglyceridemic, of whom 66% showed evidence of fatty liver as measured with ultrasound. A detailed comparison between the hypertriglyceridemic FCHL subjects with (n = 25) and without (n = 13) fatty liver revealed that, despite very similar plasma triglyceride levels (3.5 vs 3.2 mmol/L in subjects with and without fatty liver, respectively), the fatty liver subgroup presented with significantly higher body mass index, visceral adipose tissue (ultrasound), insulin, and alanine aminotransferase levels. Moreover, very low-density lipoprotein (VLDL) subclass analysis showed that the VLDL2 fraction of the fatty liver subgroup contained significantly less cholesterol and triglycerides ($P = .02$ for both parameters), which was likely explained by a decreased VLDL2 particle number because VLDL2 apolipoprotein B levels tended to be lower ($P = .08$). These data indicate that hypertriglyceridemic FCHL subjects may belong to metabolically distinct subgroups and suggest that a refinement of the hypertriglyceridemic FCHL phenotype by adding information on fatty liver will eventually facilitate the elucidation of its complex genetic background.

1. Introduction

Familial combined hyperlipidemia (FCHL) is the most prevalent (1:100) genetic hyperlipidemia in Western society [1] and is associated with a 2- to 5-fold increased risk to develop cardiovascular complications [2,3]. It is estimated that 10% of the survivors of a premature myocardial infarction are affected with FCHL [1]. Recent studies have revealed that FCHL patients, similar to patients with type 2 diabetes mellitus, share many features of the metabolic syndrome [4]: insulin resistance [5], (visceral) obesity [6], hypertension [7], elevated plasma triglycerides, low high-density lipoprotein cholesterol, and abundance of small-dense low-density lipoprotein particles [8].

We recently reported that another aspect of the metabolic syndrome, that is, fatty liver, is also highly prevalent in FCHL [9,10], which is in part explained by an increased genetic susceptibility [10]. A recent study in normal subjects and patients with type 2 diabetes mellitus demonstrated that an increased amount of hepatic fat is associated with the overproduction of very low-density lipoprotein (VLDL) particles [11]. This is of interest considering that VLDL overproduction is one of the best documented features of FCHL [12,13]. It is very likely that a similar relation is present in FCHL, given our previously reported relation between fatty liver and the amount of VLDL particles in plasma [9].

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However, FCHL is a complex, heterogeneous entity. It is anticipated that the hyperlipidemic state in FCHL is the consequence of not only the fatty liver-VLDL overproduction pathway, but also of an impaired catabolism, for example, a delayed remnant particle clearance [14].

Therefore, the aim of the present study was to explore whether it is possible to refine the FCHL phenotype, in particular the hypertriglyceridemic (HTG) phenotype, by using a relatively easily obtainable trait that is closely related to VLDL overproduction, that is, fatty liver. The primary goal was to evaluate whether it is possible to identify subjects with and without fatty liver within the HTG subgroup because this would implicate that there are indeed distinct subgroups within the HTG FCHL phenotype. For this, we performed ultrasound (US) scanning of the liver in our well-defined FCHL families and first determined the prevalence of HTG in spouses and FCHL family members with and without radiological evidence of fatty liver. Subsequently, subgroups were identified within the HTG phenotype based on the presence or absence of fatty liver to investigate whether differences in metabolic parameters and VLDL1 and VLDL2 particle properties could be discerned.

2. Methods

2.1. Subjects

Familial combined hyperlipidemic probands (n = 23), their relatives (n = 162), and their spouses (n = 72) participated in this study. The FCHL families were diagnosed as described previously [7]. Because FCHL family members and their spouses share a similar environment, observed differences are likely to be explained by genetic factors. It should, however, be noted that the spouses do not necessarily represent a random sample from the general population.

Subjects visited the research ward after an overnight fast, 3 days' abstinence from alcohol, and 2 weeks' withdrawal from lipid-lowering medication. Furthermore, subjects consumed no more than 20 g of alcohol daily, had a stable weight, and did not take any medication associated with the development of fatty liver [15]. One subject was found to be seropositive for hepatitis C and was therefore excluded from this study.

The study protocol was approved by the Human Investigations Review Committee at Maastricht University/ Academic Hospital Maastricht. All subjects gave written informed consent.

2.2. Anthropometric measurements

Subjects were weighed in their underwear. Height was measured with a stadiometer, and body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared. Waist circumference was determined in supine position at the level midway between the lower rib and iliac crest.

2.3. Ultrasound

All US measurements were performed by the same researcher with an ATL 9 HDI US system (Bothell, WA) as described previously [9]. The thickness of both visceral adipose tissue (VAT-US) and subcutaneous adipose tissue (SAT-US) was determined at the same level as waist circumference. The SAT-US was measured at the midline as the distance between skin and linea alba using an L5-10 transducer. The VAT-US was determined as the distance between the anterior of the vertebrate body and the peritoneum, exactly as described by Stolk et al [16]. All measurements were done with minimal pressure exerted on the probe and at the end of a normal expiration. The intraobserver variability as determined in 30 subjects was 1.7%.

The presence of fatty liver was also detected by US using a C7-4 and C4-2 transducer. Standardized images of the liver and right kidney were recorded on videotape and examined by an independent radiologist unaware of the subject's clinical characteristics. Diagnosis of fatty liver was established by conventional criteria, that is, increased echogeneity ("bright liver"), posterior beam attenuation, and decreased visualization of hepatic blood vessels [17,18]. The different stages of fatty liver, that is, mild, moderate, and severe, have been described in detail elsewhere [9]. The intraobserver agreement expressed as κ, determined in 30 random scans, was substantial ($\kappa = 0.74$), which is in agreement with earlier studies [18].

2.4. Laboratory measurements

Blood was collected in precooled ethylenediaminetetraacetic acid tubes. After centrifugation at 3000 rpm for 15 minutes at 4°C, plasma was stored at −80°C for subsequent analyses. Measurements of plasma triglycerides, total cholesterol, glucose, insulin, and apolipoprotein B were done as previously described [19]. Alanine aminotransferase (ALT) levels were measured with a commercially available assay (Ecoline S+, DiaSys Diagnostic Systems, Holzheim, Germany). The degree of insulin resistance was estimated with the homeostasis model assessment (HOMA-IR; glucose × insulin/22.5) [20].

Isolation of VLDL was done as described by Redgrave et al [21]. Subsequently, VLDL1 and VLDL2 subfractions were separated by density gradient ultracentrifugation as described by Zhao et al [22], with minor modifications, which represents ultracentrifugation at 160 000g for 2.5 hours at 4°C in an SW40 Ti rotor (Beckman Instruments, Palo Alto, CA). Collection of fractions started from the top of the tube, where the upper 1.5 mL represents VLDL1 and the lower 5 mL represents VLDL2. Cholesterol and triglyceride concentrations in the VLDL1 and VLDL2 fractions were measured similarly as in total plasma. Apolipoprotein B concentration in these fractions was quantified by gel electrophoresis according to the method of Karpe and Hamsten [23].

2.5. Statistical analyses

Linear regression analyses were conducted to detect differences in general characteristics between FCHL probands, their relatives, and their spouses, with correction for age and sex. The different subgroups entered the models as dummy variables. Logistic regression was used in case of dichotomous variables (eg, fatty liver yes/no).

Differences in prevalence of HTG between spouses and FCHL family members (ie, probands and relatives combined) with and without fatty liver were tested with a χ^2 test.

General characteristics and lipoprotein particle properties were compared between HTG FCHL subjects with and without fatty liver by means of linear regression, with correction for age and sex. All analyses were conducted with SPSS 13.0 statistical package (SPSS, Chicago, IL).

3. Results

3.1. Population characteristics

The characteristics of FCHL probands, their relatives, and their spouses are summarized in Table 1. Both FCHL probands and their relatives were more (abdominally) obese than their spouses, which was predominantly explained by an increased amount of visceral fat as reflected by the VAT-US. The SAT-US, measured at the same level as waist circumference, was not significantly different among the 3 groups. Plasma triglycerides and total cholesterol are by definition elevated in FCHL probands and were also significantly higher in their relatives in comparison with spouses. In addition, both probands and relatives exhibited higher liver-specific ALT levels and were more insulin resistant than spouses, as suggested by the increased insulin and HOMA-IR levels. The prevalence of fatty liver, as estimated with US, was 2 times higher in both probands and relatives.

Table 1
Population characteristics

	Spouses	FCHL relatives	FCHL probands
Male/female	33/39	87/75	11/12
Age (y)	51.2 ± 11.8	45.6 ± 15.6 *	54.7 ± 9.5 †
BMI (kg/m^2)	24.6 (22.0-28.1)	25.6 (22.9-28.7) ‡	27.3 (25.6-29.5) ‡
Waist circumference (cm)	91.1 ± 12.7	93.3 ± 12.6 ‡	99.0 ± 7.8 ‡
SAT-US (cm)	2.3 ± 1.1	2.5 ± 1.2	2.8 ± 1.1
VAT-US (cm)	7.2 (5.6-9.3)	7.4 (6.0-9.5) ‡	8.4 (7.5-10.8) ‡
Triglycerides (mmol/L)	1.1 (0.9-1.2)	1.3 (1.0-1.9) ‡	3.2 (1.9-4.0) ‡, §
Total cholesterol (mmol/L)	5.4 (4.7-5.9)	5.5 (4.6-6.4) ‡	7.0 (5.7-8.7) ‡, §
Apolipoprotein B (g/L)	1.0 ± 0.2	1.1 ± 0.3 ‡	1.3 ± 0.3 ‡
Insulin (U/L)	5.1 (2.0-8.9)	6.7 (3.4-10.9) ‡	9.6 (7.7-14.9) ‡, §
HOMA-IR	1.0 (0.5-2.1)	1.5 (0.7-2.5) ‡	2.7 (1.8-4.0) ‡, §
ALT (U/L)	16.0 (13.2-19.1)	18.2 (13.9-25.3) ‡	24.0 (17.2-28.4) §
Fatty liver (%)	19	36 ‡	43 ‡

Data are presented as mean ± SD or as median (interquartile range). All analyses are Hochberg corrected.

* $P < .05$ vs spouses, linear regression.

† $P < .05$ vs FCHL relatives, linear regression.

‡ $P < .05$ vs FCHL relatives, linear regression with correction for age and sex.

§ $P < .05$ vs FCHL relatives, linear regression with correction for age and sex.

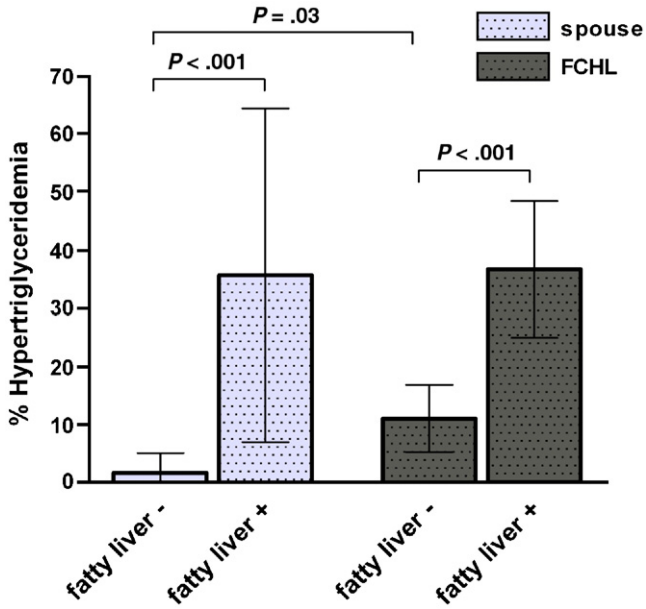

Fig. 1. Relation of fatty liver with the prevalence of HTG in spouses (n = 58 without fatty liver, n = 14 with fatty liver) and FCHL family members (probands and relatives combined; n = 117 without fatty liver, n = 68 with fatty liver). Error bars represent 95% CIs. Analyzed with χ^2 tests. Hochberg corrected.

3.2. Relation between fatty liver and HTG in spouses and FCHL

Numerous studies have shown that hepatic fat accumulation is associated with higher plasma triglyceride levels [24-26]. The increased prevalence of HTG among spouses and FCHL family members with fatty liver is in concordance with these studies: only 1 (2%) of 58 spouses without ultrasonographic evidence of fatty liver had elevated plasma triglycerides greater than 2.3 mmol/L, whereas HTG was observed in 5 (36%) of 14 spouses with fatty liver (odds ratio [OR], 31.7; 95% confidence interval [CI], 3.3-303.2; $P < .001$; Fig. 1). Similarly, HTG was present in 13 (11%) of 117 FCHL family members without fatty liver (probands and relatives combined), in contrast to 25 (37%) of 68 FCHL family members with fatty liver (OR, 4.7; 95% CI, 2.2-9.9; $P < .001$; Fig. 1).

Of more interest, when spouses and FCHL family members without fatty liver were compared, the proportion of subjects who were HTG was significantly higher in FCHL (13 of 117, 11%) than that in spouses (1 of 58, 2%; $P = .03$; Fig. 1). The prevalence of HTG did not differ between spouses and FCHL family members with fatty liver (5 of 14,

Table 2
Characteristics of HTG FCHL patients with and without fatty liver

	Fatty liver absent	Fatty liver present
Male/female	8/5	10/15
Age (y)	53.0 (43.5-54.5)	55.0 (45.0-59.5)
BMI (kg/m^2)	26.3 (24.4-28.4)	30.1 (28.7-32.5)*
Waist circumference (cm)	96.2 (90.1-104.0)	104.7 (95.4-106.5)*
SAT-US (cm)	2.9 (2.4-3.5)	3.1 (2.1-3.8)
VAT-US (cm)	8.4 (7.7-9.3)	10.7 (9.7-12.0)*
Triglycerides (mmol/L)	3.2 (3.1-3.8)	3.5 (2.7-4.9)
Total cholesterol (mmol/L)	6.9 (5.4-7.5)	5.8 (5.5-8.7)
Apolipoprotein B (g/L)	1.4 (1.0-1.5)	1.1 (1.0-1.4)
Insulin (mU/L)	8.1 (6.5-12.4)	12.8 (9.4-17.7)*
HOMA-IR	2.2 (1.5-3.7)	3.0 (2.0-4.8)*
ALT (U/L)	22.4 (17.2-26.5)	31.9 (21.2-40.7)*

Data are expressed as median (interquartile range).
* $P < .05$, linear regression with correction for age and sex.

36% vs 25 of 68, 37%; $P = .94$; Fig. 1). Similar trends were observed when the data were stratified by sex (results not shown).

In our further analyses, we concentrated on the HTG FCHL subgroups with (n = 25) and without fatty liver (n = 13) to investigate whether distinct properties could be discerned.

3.3. Characteristics of HTG FCHL subjects with and without fatty liver

The characteristics of both HTG groups with and without fatty liver are displayed in Table 2. Of all subjects with fatty liver, 8 presented with mild fatty liver, 9 with moderate fatty liver, and 8 with severe fatty liver. Hypertriglyceridemic FCHL subjects without fatty liver were only moderately overweight, in contrast to the HTG FCHL subjects with fatty liver who were, on average, obese ($P < .001$). The amount of visceral fat was significantly higher in the HTG FCHL subjects with fatty liver, whereas no differences were observed for the amount of abdominal subcutaneous fat. Of interest, plasma triglycerides were very similar between both groups (Table 2); and although the median values (but not the interquartile ranges) of total cholesterol and apolipoprotein B seemed to be different between the 2 groups (Table 2), age- and sex-corrected analyses revealed no significant differences ($P = .62$ and $P = .18$, respectively). The HOMA-IR and plasma insulin and ALT levels were significantly higher in HTG FCHL subjects with fatty liver.

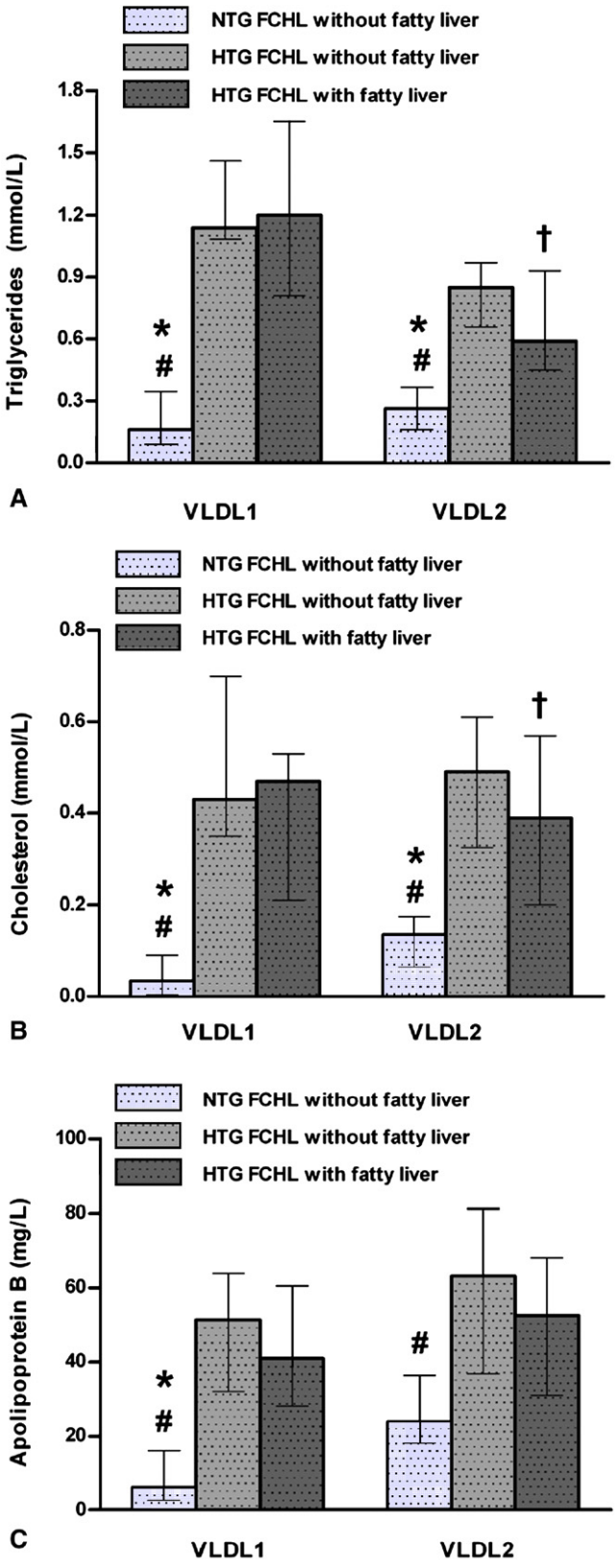

Fig. 2. Concentrations of triglycerides (A), cholesterol (B), and apolipoprotein B (C) in VLDL1 and VLDL2 fractions in normotriglyceridemic FCHL family members without fatty liver (n = 8), HTG FCHL subjects without fatty liver (n = 13), and HTG FCHL subjects with fatty liver (n = 25). Median values are displayed. Error bars represent interquartile range. *$P < .01$, normotriglyceridemic relatives vs HTG FCHL without fatty liver; #$P < .01$, normotriglyceridemic relatives vs HTG FCHL with fatty liver; †$P < .05$, HTG FCHL without fatty liver vs HTG FCHL with fatty liver. Analyzed with linear regression, with correction for age and sex. Hochberg corrected. NTG indicates normotriglyceridemic.

Of note, the FCHL subjects with and without fatty liver were derived from 17 and 10 different pedigrees, respectively, indicating that genetic dependency is not likely to account for the observed differences.

3.4. Lipoprotein particle properties in HTG FCHL subjects with and without fatty liver

The production of VLDL1 and VLDL2 particles, the main constituents of plasma triglycerides, is, among others, driven by the amount of fat in the liver [11]. The current study also showed an association of fatty liver with HTG. However, an FCHL subgroup had been identified with HTG despite the absence of fatty liver. We realized that HTG in the absence of fatty liver could represent a different causality; and we asked whether this could correspond to differences in lipoprotein amounts and composition, in particular VLDL particles. Therefore, we determined the concentrations of triglycerides, cholesterol, and apolipoprotein B in the VLDL1 and VLDL2 fractions in the HTG FCHL subgroups with and without fatty liver. Eight randomly selected normotriglyceridemic FCHL family members without fatty liver were included as a reference group (mean triglycerides, 1.2 mmol/L; total cholesterol, 5.6 mmol/L; apolipoprotein B, 1.1 g/L).

As shown in Fig. 2, both HTG subgroups showed increased concentrations of triglycerides and cholesterol in both the VLDL1 and VLDL2 fractions in comparison with the reference group. This was likely caused by an increased amount of particles because apolipoprotein B concentrations were generally increased in both fractions.

The VLDL1 apolipoprotein B, cholesterol, and triglyceride concentrations were not significantly different between both HTG groups (Fig. 2). In contrast, in the VLDL2 fraction, both triglyceride and cholesterol concentrations were significantly higher in the HTG subgroup without fatty liver compared with those in the HTG FCHL subjects with fatty liver ($P = .02$ for both parameters, Table 2). Because VLDL2 apolipoprotein B concentrations tended to be higher in the HTG subjects without fatty liver ($P = .08$) and the composition of the particles was not different ($P = .58$ for ratio of VLDL2 triglycerides to apolipoprotein B, $P = .88$ for ratio of VLDL2 cholesterol to apolipoprotein B), it is likely that an increased VLDL2 particle number, rather than an increased particle size, accounts for the significantly higher VLDL2 triglycerides and cholesterol.

4. Discussion

The heterogeneous nature of the dyslipidemia that is characteristic of FCHL has hampered researchers in their work to unravel its complex genetic background. The present study was conducted to investigate whether the application of the fatty liver trait could result in the identification of more homogeneous subgroups within FCHL and hence a refinement of the FCHL phenotype. The study was triggered by the recent observation of Adiels et al [11], who showed that the production of VLDL particles, a process that is increased in FCHL [12,13], is driven by the amount of fat that is accumulated in the liver. Furthermore, our laboratory recently demonstrated that the amount of hepatic fat is related to the amount of VLDL particles in plasma in FCHL [9].

The prevalence of HTG was associated with the presence of fatty liver in both FCHL family members and spouses, analogous to other reports [24-26]. However, several observations clearly distinguished FCHL family members from spouses. First, although the prevalence of HTG was similar in spouses and FCHL family members when fatty liver was present, fatty liver itself was twice as prevalent in FCHL probands and their relatives in comparison with their spouses. In other words, FCHL family members seem to be more prone to develop fatty liver than spouses; but as soon as fatty liver is present, the risk of developing HTG is very similar. This observation further emphasizes the central role of fatty liver in the expression of FCHL, as recently reported [9,10]. Second, the prevalence of HTG was significantly higher in FCHL family members without fatty liver than that in spouses without evidence of hepatic fat accumulation, indicating that fatty liver is not a prerequisite for the development of HTG in a subgroup of FCHL patients.

A direct comparison between the HTG FCHL subjects with and without fatty liver suggested that the underlying mechanisms for elevated plasma triglycerides are different. The subgroup with fatty liver was more (viscerally) obese and insulin resistant, and had higher ALT levels. These parameters have indeed been associated with hepatic fat accumulation [24,26]. Interestingly, plasma triglyceride, a variable that has also been linked to fatty liver [24-26], was very similar between both HTG subgroups. Because ALT levels were within the reference range in both subgroups, it was ethically not acceptable to perform liver biopsies to investigate whether both subgroups also differ in the degree of inflammation or fibrosis.

Analysis of the VLDL1 and VLDL2 particle profiles, particles that mainly determine plasma triglyceride concentrations, revealed that the HTG subgroup with fatty liver had less VLDL2 particles compared with the HTG subgroup without fatty liver, as shown by the lower triglyceride and cholesterol concentrations and a trend toward lower apolipoprotein B concentration in the VLDL2 fraction. Of note, the VLDL2 cholesterol, triglyceride, and apolipoprotein B levels were still substantially higher than those in the normotriglyceridemic reference group.

Because only 6 spouses were found to be HTG, it was not feasible to investigate whether the existence of HTG subgroups with and without fatty liver is specific for FCHL or whether similar outcomes could be obtained within the spouses as well.

The current data support the existence of 2 metabolically distinct subgroups within the HTG FCHL phenotype; but

studies, for example, using stable isotopes, are needed to provide the exact mechanistic difference. Because both insulin resistance and hepatic fat accumulation have been associated with VLDL overproduction [11,27,28], one can speculate that overproduction may be responsible for the HTG in those FCHL patients with fatty liver. Of note, given the significantly higher HOMA-IR levels in this subgroup, one may suggest that increased insulin resistance is actually the discriminating factor between HTG subjects with and without fatty liver. However, hepatic fat accumulation and insulin resistance are highly correlated; and current literature suggests that the former may precede the latter [29,30]. The underlying mechanism for the HTG of the FCHL subgroup without fatty liver is however much less obvious, and it can be speculated that mixed causes including delayed elimination of remnants are involved.

One can conclude that the current study has demonstrated that it is feasible to identify subgroups within the HTG FCHL phenotype based on the presence or absence of radiological evidence of fatty liver. Not only would researchers benefit from the identification of more homogeneous subgroups within FCHL, but clinicians also could use the fatty liver trait to select for the most optimal treatment strategy for an individual FCHL patient. The discovery and development of new therapeutic agents that target fatty liver and its associated metabolic abnormalities [31,32] are in that respect of particular interest.

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