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Clinical and Population Studies

Familial Combined Hyperlipidemia Is Associated With Alterations in the Cholesterol Synthesis Pathway

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Objectives—Familial combined hyperlipidemia (FCH) is a common familial lipid disorder characterized by increases in plasma total cholesterol, triglyceride, and apolipoprotein B-100 levels. In light of prior metabolic and genetic research, our purpose was to ascertain whether FCH cases had significant abnormalities of plasma markers of cholesterol synthesis and absorption as compared to unaffected kindred members.

Methods and Results—Plasma levels of squalene, desmosterol, and lathosterol (cholesterol synthesis markers) and campesterol, sitosterol, and cholestanol (cholesterol absorption markers) were measured by gas-liquid chromatography in 103 FCH patients and 240 normolipidemic relatives (NLR). Squalene, desmosterol, and lathosterol levels were 6% (0.078), 31%, ($P<0.001$) and 51% ($P<0.001$) higher in FCH as compared to NLR, and these differences were especially pronounced in women. An interaction with obesity was also noted for a subset of these markers. We did not observe any apparent differences for the cholesterol absorption markers among FCH patients and NLR.

Conclusions—Our data indicate that both men and women with FCH have alterations in the cholesterol synthesis pathway, resulting in 51% higher levels of lathosterol (and additionally desmosterol in women). Plasma levels of the cholesterol precursor sterol squalene were only slightly increased (6%), suggesting enhanced conversion of squalene to lathosterol in this disorder. (*Arterioscler Thromb Vasc Biol*. 2010;30:113-120.)

Key words: cholesterol ■ lipids ■ sterols ■ familial combined hyperlipidemia

Familial combined hyperlipidemia (FCH) is a common genetic lipid disorder. Affected subjects characteristically have elevated levels of plasma total cholesterol, triglycerides, and apolipoprotein (apo) B, and are more prone to develop premature cardiovascular disease (CVD).[1,2] The prevalence of FCH in the general population has been reported to be up to about 6%,[3] whereas in families with premature coronary heart disease prevalence rates of 15% to 20% have been reported.[1,2,4,5]

The pathophysiological mechanism underlying FCH is believed to be hepatic overproduction of apoB-100 containing lipoprotein particles (ie, VLDL and LDL), resulting in increased plasma total cholesterol, triglycerides, and apoB levels.[6–8] In addition, lower levels of HDL cholesterol and increased amounts of small dense LDL (sdLDL) and remnant lipoprotein particles have been observed in patients with FCH.[2,9–14]

The heterogeneous lipid profile indicates that FCH is a multifactorial disease in which several genes affect the lipid and lipoprotein metabolism. To date, the most important genetic data has been generated by 2 genome-wide scan studies in Finnish and Dutch populations, respec-

tively, showing associations of the FCH phenotype with regions on chromosome 1 and 11.[15,16] When the same diagnostic criteria were used, both studies linked FCH to the 1q21–23 genomic region which codes for the upstream transcription factor 1 (USF1) gene.[17] USF1, however, transcribes a wide array of genes which play a role in the lipid and glucose metabolism,[17] making it a complex task to identify the exact genes involved in FCH.

Previously it has been postulated that the apoB secretory rate is related to cholesterol and cholesterol ester mass,[18] and studies show that there is a clear link between fatty acid flux to the liver and increased cholesterol synthesis.[19,20] The purpose of the current study was to investigate the underlying mechanisms leading to the lipid elevations seen in FCH patients by measuring plasma markers of cholesterol synthesis and absorption. Total body cholesterol pools represent a balance between endogenous synthesis of cholesterol on the one hand and dietary absorption of cholesterol on the other.[21] It has been documented using formal cholesterol balance studies, that plasma level of the cholesterol precursors squalene, lathosterol, and desmosterol serve as markers of cholesterol synthesis,[22,23]

Table 1. Study Characteristics of the Normolipidemic Relatives (NLR) and Patients With Familial Combined Hyperlipidemia (FCH) in the Population at Large and by Gender

	All Subjects			Men			Women		
	NLR	FCH	% Difference	NLR	FCH	% Difference	NLR	FCH	% Difference
n	240	103	…	117	45	…	123	58	…
Age, y	44 (15)	51 (15)	16*	44 (14)	52 (12)	18*	44 (15)	50 (17)	14‡
BMI, kg/m^2	25 (3)	29 (4)	16*	25.5 (3.3)	28.8 (3.5)	13*	24.8 (3.5)	28.5 (4.6)	15*
WHR	0.87 (0.08)	0.91 (0.08)	5†	0.92 (0.07)	0.97 (0.04)	5*	0.83 (0.06)	0.86 (0.06)	4‡
Glucose, mmol/L	4.8 (4.5, 5.2)	5.1 (4.6, 5.6)	6†	4.9 (4.6, 5.3)	5.4 (5.0, 5.9)	10‡	4.6 (4.4, 5.0)	4.9 (4.4, 5.3)	7‡
Insulin, μU/mL	5.9 (4.1, 8.1)	8.8 (6.8, 12.4)	49*	6.3 (3.9, 8.3)	8.7 (6.1, 9.5)	38†	5.8 (4.2, 7.8)	8.9 (7.4, 13.0)	53*
Triglycerides, mmol/L	1.2 (0.9, 1.6)	2.9 (2.0, 4.1)	142*	1.4 (1.0, 1.8)	3.4 (2.7, 4.8)	143*	1.1 (0.8, 1.3)	2.5 (1.7, 3.2)	127*
Total cholesterol	5.2 (1.0)	6.9 (1.2)	33*	5.4 (0.9)	7.0 (1.2)	30*	5.1 (0.9)	6.8 (1.2)	33*
HDL-C, mmol/L	1.3 (0.3)	1.1 (0.3)	−15*	1.2 (0.3)	1.0 (0.2)	−17*	1.4 (0.3)	1.3 (0.2)	−7*
LDL-C, mmol/L	3.5 (0.9)	4.2 (1.2)	20*	3.7 (0.9)	3.9 (1.3)	5	3.3 (0.9)	4.4 (1.1)	33*
Non HDL-C, mmol/L	3.9 (0.9)	5.7 (1.2)	46*	4.2 (0.9)	6.0 (1.1)	43*	3.7 (0.9)	5.5 (1.2)	49*
VLDL-C, mmol/L	0.4 (0.2, 0.6)	1.1 (0.7, 1.9)	175*	0.5 (0.3, 0.7)	1.5 (1.1, 2.5)	200*	0.3 (0.2, 0.5)	0.9 (0.6, 1.7)	200*
VLDL-TG, mmol/L	0.7 (0.4, 1.0)	2.2 (1.3, 3.2)	214*	0.9 (0.6, 1.2)	2.7 (2.1, 4.2)	200*	0.6 (0.3, 0.8)	1.6 (1.0, 2.6)	167*
sdLDL-C, mg/dL	37 (19)	57 (27)	54*	48 (19)	50 (24)	4	30 (14)	60 (27)	100*
RLPC, mmol/L	6 (4, 10)	20 (12, 35)	233*	8 (5, 12)	29 (19, 53)	263*	5 (4, 8)	16 (9, 26)	220*
ApoB, mg/L	960 (221)	1371 (256)	43*	1026 (214)	1372 (198)	34*	898 (210)	1371 (294)	53*
ApoB48, mg/dL	0.4 (0.2, 0.6)	0.8 (0.5, 1.6)	100*	0.4 (0.3, 0.6)	1.4 (1.0, 1.8)	250*	0.3 (0.2, 0.5)	0.7 (0.4, 1.1)	133*

Values are mean (SD) or median (interquartile range). *$P<0.001$, †$P<0.01$, ‡$P<0.05$. P values were adjusted for age and family structure. Because of limited sample availability insulin, sdLDL-C, RLPC, and ApoB48 were measured in 269 subjects.

whereas the plant sterols campesterol and sitosterol (both derived from the diet), and the bile acid residue cholestanol, are all absorbed in very low quantities in the intestine and their plasma levels have been demonstrated to serve as markers of fractional cholesterol absorption.[23] In support, treatment with cholesterol synthesis and absorption inhibitors (ie, statins and ezetimibe) not only lower total cholesterol levels, they also specifically lower plasma levels of these synthesis and absorption markers, respectively.[24,25]

For the current study we measured squalene, desmosterol, lathosterol (markers of synthesis) and campesterol, and sitosterol and cholestanol (markers of absorption) in a population of FCH patients and their normolipidemic relatives (NLR). In addition, we also performed an extensive characterization of the lipid profiles in these FCH families, measuring total cholesterol, triglycerides, LDL-C, HDL-C and VLDL-C, sdLDL-C, total apoB levels, apoB48 particles, and remnant lipoprotein cholesterol (RLPC), of which apoB-48 is a specific marker of intestinal lipid transport.

Methods

Study Population

The study population consisted of 343 subjects, 103 FCH patients and 240 NLR, from 32 well-defined FCH families.[26] The diagnosis of FCH was based on a previously established nomogram.[27] Briefly, plasma triglycerides and total cholesterol levels, adjusted for age and gender, and absolute apoB levels were included in a nomogram to calculate the likelihood of having FCH. A subject was defined as being affected with FCH if the probability was above 60%, provided that the diagnostic phenotype was also present in at least 1 first-degree relative and premature CVD (ie, before the age of 60) was present in at least 1 individual in the

family. Families were excluded when a secondary cause of the hyperlipidemia was diagnosed in the proband (ie, diabetes mellitus, hypothyroidism, and hepatic or renal impairment). Before the start of the study all participants withdrew from using lipid-lowering medication for at least 4 weeks. Blood was drawn after an overnight fast for laboratory analyses and the isolation of DNA. The ethics committee of the Radboud University Nijmegen Medical Center approved the study protocol and all subjects gave informed consent.

Laboratory Measurements

Plasma total cholesterol, triglycerides, LDL-C, VLDL-C, HDL-C, apoB, and glucose concentrations in all subjects were determined using routine laboratory procedures as previously described.[28] Insulin was measured directly by an immunoassay obtained from the Otsuka Corporation.[29] Small dense (sd) LDL-C was measured using kits obtained from the Denka Seiken Company.[30] RLPC was measured using kits obtained from the Kyowa Medex Corporation,[31] and plasma apoB48 was measured by an enzyme linked immunoassay obtained from the Shibayagi Company as previously described.[32] All assays had within and between run coefficients of variation of less than 5%, and the nonroutine assays were only performed on a subset of subjects because of limited sample availability (see footnote to Table 1).

Measurements of Plasma Sterols

Plasma concentrations of squalene, lathosterol, desmosterol, campesterol, sitosterol and cholestanol were measured using gas-liquid chromatography according to methods previously described in all subjects and were expressed as μmol/L as well as normalized to 10^2 mmol/mol total cholesterol.[33]

Statistical Analyses

All continuous variables were checked for their distribution and expressed as means±SD, or in the case of nonlinear distributions,

Table 2.   Correlations of the Cholesterol Synthesis and Absorption Markers With BMI, WHR, Glucose, and Insulin in All Subjects (n=343)

	BMI	WHR	Glucose	Insulin
Squalene	0.062	0.059	−0.013	−0.011
Lathosterol	0.482*	0.471*	0.389*	0.336*
Desmosterol	0.337*	0.320*	0.360*	0.304*
Campesterol	−0.082	0.081	0.076	−0.074
Sitosterol	−0.128†	0.030	0.074	−0.148†
Cholestanol	−0.063	0.032	−0.013	−0.110

Values represent Spearman Rank correlation coefficients. *$P<0.001$ and †$P<0.05$.

as medians and interquartile ranges. Spearman correlation coefficients were calculated for BMI, WHR, glucose and insulin levels, and the cholesterol synthesis and absorption markers. For the comparisons among the NLR and FCH group, probability values were adjusted for age, BMI, family structure by logistic regression, using FCH status as dependent variable and age, BMI, and the family identification number and the variable under investigation as independent variables. A probability value less than 0.05 was considered statistically significant and all calculations were performed using the STATA software package, version 10.

Results

The characteristics of the 103 patients with FCH and the 240 NLR are presented in the study population at large, as well as stratified by gender (Table 1). There was no difference in the gender distribution among FCH patients and their NLR, although the FCH group was significantly older and had higher BMIs and WHRs ($P<0.05$ for all comparisons). FCH patients had higher glucose ($P<0.01$) and insulin ($P<0.001$) levels and as expected, higher levels of total cholesterol, triglycerides, non–HDL-C, VLDL-C and VLDL-TG ($P<0.001$, for all parameters) and HDL-C levels were significantly lower. In addition, RLPC, apoB and apoB48 (the intestinal apoB), were significantly higher ($P<0.001$, for all comparisons) in FCH as compared to the NLR. There were interesting gender differences among FCH patients and the NLR. In women, FCH was associated with 33% higher LDL-C levels and 100% higher sdLDL-C levels ($P<0.001$, for both comparisons), whereas these difference were not observed in the men. As mentioned above, apoB48 levels were higher in the FCH group when compared to the NLR, however the percentile difference observed in men was almost twice as high as the difference seen in women (250% higher versus +133%).

Individual correlations between markers cholesterol synthesis and absorption in relation to markers of obesity and insulin resistance are presented in Table 2. In the combined study groups, lathosterol and desmosterol levels correlated (all at $P<0.001$) with BMI and WHR and with glucose and insulin levels. In addition, there was a small inverse correlation for sitosterol with BMI and insulin levels (both $P<0.05$, Table 2). Supplemental Table I (available online at http://atvb.ahajournals.org) provides data showing a better correlation for lathosterol and desmosterol with glucose levels and a less pronounced correlation with insulin levels in the FCH patients compared to the NLR. The nature of these correlations remained the same in gender subgroups (data not shown).

We observed a strong correlation between both lathosterol and desmosterol in relation to apoB, triglycerides, and LDL-C ($P<0.001$ for all), which appeared to be stronger in women than in men (supplemental Table II). In addition, apoB and LDL-C correlated with campesterol and sitosterol in both men and women and with cholestanol in men only (supplemental Table II).

The FCH phenotype was associated with increased markers of cholesterol synthesis (Table 3). Lathosterol levels were elevated by 51% ($P<0.001$) in FCH, squalene tended to be higher in FCH (+6%, $P=0.125$), however, this effect was observed in women only (+15%, $P<0.001$) and not in men. In addition, desmosterol levels were 54% higher in female FCH patients ($P<0.001$) and only 11% higher in male FCH patients ($P=0.069$) when compared to their NLR counterparts. To account for the fact that plasma sterols are mainly transported in the LDL and HDL particles,[34] values were also presented as ratio to total cholesterol (/C). Although squalene concentrations were increased in female FCH patients 15% ($P=0.001$), squalene/C levels were actually reduced in both male and female FCH patients by −21% ($P=0.010$) and −14% ($P=0.050$), respectively, independent of BMI. Desmosterol/C and lathosterol/C overall remained comparable to the unadjusted values, desmosterol/C being elevated in women by 24% ($P=0.002$) and lathosterol/C in both men and women by 17% ($P=0.013$) and 22% ($P<0.001$), respectively. Adjusting the lathosterol and desmosterol associations for BMI did not affect the level of significance of the sterol markers when expressed in absolute terms, however the associations were no longer significant when using the sterol values normalized to total cholesterol.

Markers of cholesterol absorption in FCH patients and NLR are presented in Table 4. With regard to these markers there was evidence for a gender interaction. In men with FCH, absolute concentrations of campesterol and sitosterol tended to be elevated by 21% ($P=0.065$), while remaining constant in women. After adjusting for BMI, the differences were significant ($P=0.012$ and $P=0.027$). Normalized to total cholesterol, campesterol/C and sitosterol/C were no longer elevated in men and significantly lower in women with FCH (−26%, $P=0.003$ and −25%, $P=0.004$, respectively). Absolute cholestanol concentrations were not different in FCH, but cholestanol/C was 16% lower ($P<0.001$) in both men and women with FCH and independent of BMI.

Although we did not observe correlations between the markers of synthesis and absorption when expressed in absolute terms (data not show), there was a negative correlation between lathosterol/C and campesterol/C in the NLR ($r=-0.271$, $P<0.001$) not observed in the FCH patients ($r=-0.156$, $P=0.120$). Furthermore, the lathosterol/campesterol ratio was 37% higher in FCH patients than in NLR, and this was predominantly attributable to the elevations observed in women (66%) rather than in men (19%) (Table 5).

Table 3. Cholesterol Synthesis Markers, Expressed in Absolute Concentrations as Well as Normalized to Total Cholesterol, in the Normolipidemic Relatives (NLR) and Patients With Familial Combined Hyperlipidemia (FCH)

	NLR	FCH	% Difference FCH vs NLR	P Value†	BMI Adj. P Value‡
Sterols expressed in μmol/L					
Squalene					
Total	1.26 (0.37)	1.33 (0.43)	6	0.078	0.433
Men	1.36 (0.44)	1.31 (0.40)	−4	0.706	0.355
Women	1.17 (0.27)*	1.35 (0.44)	15	0.001	0.011
Desmosterol					
Total	1.6 (1.1, 2.4)	2.1 (1.6, 3.1)	31	<0.001	0.018
Men	1.9 (1.3, 2.8)	2.1 (1.6, 3.3)	11	0.069	0.444
Women	1.3 (1.0, 1.9)*	2.0 (1.5, 3.1)	54	<0.001	0.001
Lathosterol					
Total	7.4 (3.1)	11.2 (4.0)	51	<0.001	<0.001
Men	8.3 (3.6)	11.9 (3.7)	43	<0.001	0.006
Women	6.6 (2.4)*	10.7 (4.1)	62	<0.001	<0.001
Sterols expressed as 10^2 mmol/mol cholesterol					
Squalene/C					
Total	26.5 (8.9)	22.0 (7.4)	−17	0.001	0.001
Men	27.1 (9.6)	21.4 (7.3)	−21	0.010	0.007
Women	25.9 (8.1)	22.4 (7.6)	−14	0.050	0.021
Desmosterol/C					
Total	34 (24, 46)	36 (27, 52)	6	0.267	0.714
Men	39 (28, 54)	34 (25, 52)	−13	0.821	0.319
Women	29 (22, 42)*	36 (28, 53)	24	0.029	0.290
Lathosterol/C					
Total	150 (55)	179 (61)	19	<0.001	0.155
Men	160 (59)	187 (64)	17	0.010	0.369
Women	142 (49)*	173 (59)	22	0.001	0.098

*Significantly different from men at $P<0.05$ within the subgroup. †P values adjusted for age and family structure. ‡P values adjusted for age and family structure and BMI.

Discussion

Since the description and characterization of the FCH phenotype more than 30 years ago,[1] many studies have been undertaken to uncover the mechanisms causing FCH. Both the results of biochemical investigations as well as the results from candidate gene studies and genome wide scan studies have been of great interest, but the actual cause of the elevated total cholesterol, triglycerides, and apoB levels as well as the underlying genetic defect has not yet been elucidated. This study shows that both men and women with FCH have higher levels of the cholesterol precursor lathosterol when compared to their NLR. Based on these findings we hypothesize that patients with FCH have an underlying defect in sterol metabolism resulting in overproduction of lathosterol and cholesterol.

The higher sterol levels observed in the FCH may suggest that this is the result of impaired plasma disposal of these sterols or an impaired conversion to cholesterol. Alternatively, lathosterol and desmosterol are both formed from squalene, each via a different pathway (see Figure).

Although we found marginally higher levels of plasma squalene (6%), this effect was predominantly attributable to increased squalene levels in women (15%). When the squalene levels were expressed as a ratio over total cholesterol, we found them to be lower in both men and women. The cholesterol precursor lathosterol was elevated in men and women with FCH (both in absolute terms as well as adjusted for total cholesterol), suggesting that there is either enhanced conversion of squalene to lathosterol or increased direct production of lathosterol in these patients. Although a limitation of our study is that the design is cross-sectional and does not establish causation, in FCH we find increased levels of lathosterol which correlate with apoB, triglycerides and LDL-C, suggesting that FCH is (partly) the result of an imbalance in the cholesterol synthesis pathway and future research should focus on regulators within this pathway.

In contrast to the markers of cholesterol synthesis, the differences among FCH and NLR regarding the markers of cholesterol absorption are less clear. Both campesterol and sitosterol tended to be higher in FCH patients, but these

Table 4. Cholesterol Absorption Markers, Expressed in Absolute Concentrations as Well as Normalized to Total Cholesterol, in the Normolipidemic Relatives (NLR) and Patients With Familial Combined Hyperlipidemia (FCH)

	NLR	FCH	% Difference FCH vs NLR	P Value†	BMI Adj. P Value‡
Sterols expressed in μmol/L					
Campesterol					
Total	9.7 (7.1, 13.2)	11.0 (7.8, 15.3)	13	0.191	0.022
Men	10.2 (7.7, 14.2)	12.3 (9.7, 17.3)	21	0.065	0.012
Women	9.1 (6.8, 12.3)*	9.5 (7.1, 13.0)*	4	0.726	0.243
Sitosterol					
Total	6.2 (4.7, 8.1)	7.1 (4.7, 10.1)	15	0.167	0.006
Men	6.2 (4.9, 8.3)	7.5 (5.5, 10.4)	21	0.131	0.027
Women	6.2 (4.6, 8.0)	6.1 (4.5, 9.5)	−2	0.571	0.068
Cholestanol					
Total	7.8 (2.8)	8.1 (3.0)	4	0.387	0.303
Men	8.3 (3.1)	8.3 (2.3)	0	0.912	0.887
Women	7.3 (2.4)*	7.9 (3.4)	8	0.180	0.125
Sterols expressed as 10^2 mmol/mol cholesterol					
Campesterol/C					
Total	203 (155, 264)	169 (135, 241)	−17	0.011	0.302
Men	200 (152, 286)	181 (158, 273)	−10	0.839	0.369
Women	207 (158, 259)	154 (113, 211)†	−26	0.003	0.052
Sitosterol/C					
Total	128 (98, 165)	109 (82, 152)	−15	0.004	0.396
Men	121 (96, 157)	119 (86, 163)	−2	0.347	0.710
Women	135 (100, 170)	101 (78, 148)	−25	0.004	0.164
Cholestanol/C					
Total	160 (53)	134 (48)	−16	0.001	0.010
Men	162 (56)	135 (40)	−17	0.030	0.067
Women	158 (50)	133 (53)	−16	0.015	0.059

*Significantly different from men at $P<0.05$ within the subgroup. †P values adjusted for age and family structure.
‡P values adjusted for age and family structure and BMI.

findings were restricted to men and were only significant when taking into account BMI. When the absorption markers were expressed relative to total cholesterol, we found significantly lower levels in women for campesterol/C and sitosterol/C. Cholestanol/C was lower in both men and women. The question remains however whether the association of FCH with lower values for the absorption markers over cholesterol is a true effect (ie, the result of lower cholesterol absorption), or whether it is the result of dividing a constant concentration of absorption markers by a higher total cholesterol concentration caused by increased cholesterol synthesis. Furthermore, it is known that dietary intake of plant sterols can affect plasma concentrations of campesterol and sitosterol.[35] Because no dietary information was collected, we cannot rule out that FCH patients were consuming greater amounts of dietary

Table 5. Ratios of Lathosterol Over Campesterol in the Normolipidemic Relatives (NLR) and Patients With Familial Combined Hyperlipidemia (FCH)

	NLR	FCH	% Difference FCH vs NLR	P Value†	BMI Adj. P Value‡
Lathosterol/ campesterol ratio					
Total	0.71 (0.43, 1.11)	0.97 (0.67, 1.42)	37	<0.001	0.194
Men	0.77 (0.39, 1.27)	0.92 (0.71, 1.18)	19	0.097	0.305
Women	0.62 (0.44, 1.01)*	1.03 (0.66, 1.71)*	66	<0.001	0.018

*Significantly different from men at $P<0.05$ within the subgroup. †P values adjusted for age and family structure.
‡P values adjusted for age and family structure and BMI.
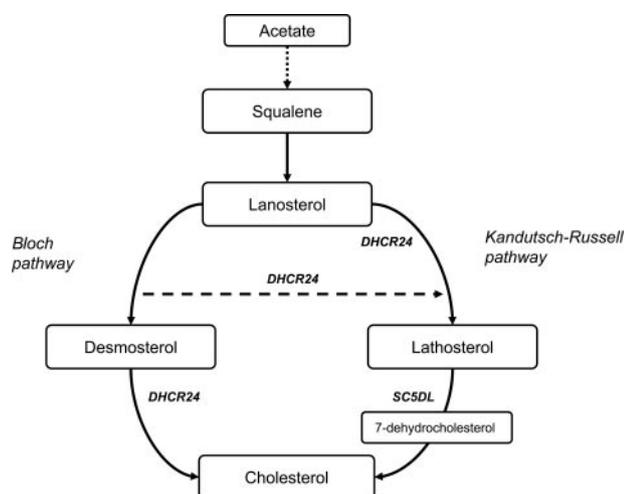
Figure. An overview of the cholesterol synthesis from squalene. Intermediate reactions from lanosterol to desmosterol and lathosterol in the Bloch pathway and Kandutsch–Russell pathway, respectively, are not shown. Sterol-C5-desaturase-like (SC5DL) converts lathosterol into 7-dehydrocholesterol. The 24-dehydrocholesterol reductase (DHCR24) catalyzes the reduction of the δ-24 double bond of sterol intermediates in the Bloch pathway forming a sterol which is part of the Kandutsch–Russell pathway. DHCR24 also catalyzes the formation of cholesterol from desmosterol.

plant sterol margarines and other sterols as compared to controls. Finally, even though we noted elevated levels of apoB48 in FCH patients, these elevations are likely a reflection of delayed clearance of intestinal remnant lipoproteins as previously described in FCH,[36] rather than attributable to increased absorption, and overall, these findings suggest that FCH is not associated with altered cholesterol absorption.

Previous studies suggest a central role for fat mass in the onset of FCH.[37] In agreement with this concept, we found associations indicating that there is an interaction between plasma sterols and BMI which may play a central role in the onset of FCH. The relationship between obesity and cholesterol production is not straightforward because more fat mass does not always cause excess cholesterol production. This concept was illustrated by a recent study from our laboratory in 19 severely obese women undergoing bypass surgery, showing a markedly reduction in fat mass without a significant reduction in total cholesterol levels.[38] A possible mechanism linking plasma sterols to fat metabolism may come from the demonstration that sterol intermediates from the cholesterol synthesis pathway, especially desmosterol, act as liver x receptor (LXR) ligands.[39] LXR stimulates hepatic triglyceride synthesis and fat storage and has been described as a master regulator of fat metabolism.[40] Further studies are necessary to determine a possible interaction between LXR, desmosterol and the other plasma sterols in cases with FCH versus controls.

We have performed extensive characterization of the lipid and apolipoprotein profiles in FCH patients and their NLR, as well as in subgroups of men and women, and found that within the FCH group LDL-C and sdLDL-C were elevated in women only. This may suggest that in

female FCH patients there is even greater cholesterol synthesis than in male FCH patients. In agreement, the cholesterol synthesis data also suggest that there are gender specific mechanisms associated with FCH. Although lathosterol was elevated in both male and female FCH patients, the effect seemed to be more prominent in women than in men (62% versus 42%). The other cholesterol precursors, squalene and desmosterol, were significantly higher (15% and 54%, respectively) in FCH women while remaining constant in men. These increases observed in FCH women were attributable to the fact that in the NLR group the women had lower sterol levels than the men. This was especially true for desmosterol. In women, desmosterol levels were higher in the FCH compared to the NLR group, but similar to the levels seen in the FCH men. To speculate on a possible underlying mechanism, recent studies demonstrate that the enzyme responsible for the conversion of desmosterol to cholesterol (ie, 24-dehydrocholesterol reductase, DHCR24 also known as Seladin-1), is activated by estrogens.[41] In theory the activation of the DHCR24 by estrogens may be reduced in women with FCH, resulting in elevated desmosterol levels. In support of this concept, a post hoc analysis in this study population revealed that desmosterol levels (but not squalene levels) were significantly higher in postmenopausal women when compared to premenopausal women (57% higher, $P<0.001$).

To our knowledge this is the second study to measure synthesis and absorption markers in FCH patients. Previously, Garcia-Otin and colleagues measured cholesterol synthesis and absorption markers among autosomal dominant hypercholesterolemia (ADH) patients, including 31 patients with familial hypercholesterolemia and 38 patients with FCH.[42] In contrast to our study, which was designed to gain insight into the mechanism underlying FCH, the primary objective of the Garcia-Otin group was to use these markers to differentiate between the different forms of ADH. They noted that the campesterol/C and sitosterol/C ratios were lower in the FCH patients when compared to controls, which is in line with our cholesterol adjusted data, but they observed no differences for the lathosterol/C ratio, suggesting that alterations in absorption markers were associated with FCH. Their study, however, was conducted in a FCH group consisting of predominantly men (27 versus 11 women), and limited numbers prevented them from analyzing subgroups. Because this is the second study to measure synthesis and absorption markers in a FCH population, replication of these findings is necessary in different study populations, preferably using the same standardized diagnostic criteria for FCH.[27]

Finally, we would like to speculate on possible novel candidate genes, taking into account our findings that the FCH phenotype was associated with elevated cholesterol synthesis markers. USF1 has been suggested to be the most important candidate gene for FCH.[17] However, in these families USF1 was not a major gene for FCH, but rather a modifier gene contributing to related lipid traits.[43] Recently, a whole genome study mapped all the target regions of USF1 and identified multiple regions involved in the

lipid metabolism,[44] including a number of vital genes in the synthesis of cholesterol summarized in supplemental Table III. Of these genes, sterol-C5-desaturase-like (SC5DL) plays a crucial role in the formation of cholesterol from lathosterol and the (earlier mentioned) DHCR24 is involved in many steps of the synthesis of cholesterol, one of which is the conversion of desmosterol to cholesterol. Our findings of elevated lathosterol in both men and women with FCH, and the association of lathosterol with LDL-C, triglycerides, and apoB in our entire study population, raises the possibility that the SC5DL gene is a candidate gene for FCH. In addition, when the chromosome 1 data were reanalyzed in the Dutch population, it revealed that 1p31 was strongly linked to elevated apoB levels.[45] The authors hypothesized that the nearby leptin receptor (LEPR) gene was involved, but the data proved to be negative.[45] Although speculative, the DHCR24 gene is also located in this region (Table 4) and based on our findings of increased desmosterol in women and the association of desmosterol with apoB levels, we hypothesize that alterations in this gene may cause elevated apoB rather than the LEPR gene. Future studies, sequencing the SC5DL as well as the DHCR24 genes in FCH patients, appear to be warranted.

In conclusion, our study indicates that FCH patients have alterations in the cholesterol synthesis pathway, resulting in higher levels of lathosterol (and additionally desmosterol in women). In the cholesterol synthesis pathway there are 2 enzymes that warrant further investigation: SC5DL and DHCR24. The genes for these enzymes are located in exactly those chromosomal regions previous linked to FCH in genome wide scans, and code for the key enzymes involved in the metabolism of lathosterol and desmosterol, the sterols we found to be elevated in FCH patients. Our data indicate that FCH patients appear to have enhanced conversion of squalene to lathosterol, and further examination of the SC5DL and DHCR24 genes and their gene products appear to be justified and may ultimately lead us to a precise understanding of the defects underlying FCH.

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Disclosures

None.

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Supplement Material.

Supplemental Table I. Correlations of the cholesterol synthesis and absorption markers with Glucose and Insulin in NRL and FCH patients.

	NLR		FCH	
	Glucose	Insulin	Glucose	Insulin
Squalene	0.060	-0.031	-0.152	-0.049
Lathosterol	**0.261***	**0.217†**	**0.456***	0.112
Desmosterol	**0.269***	**0.239†**	**0.382***	0.187
Campesterol	-0.001	**-0.150‡**	0.087	-0.145
Sitosterol	0.026	**-0.225†**	0.008	**-0.241‡**
Cholestanol	0.010	-0.132	-0.067	0.070

Values represent Spearman Rank correlation coefficients. *p<0.001, †p<0.01 and ‡p<0.05.

Supplemental Table II. Correlations of the cholesterol synthesis and absorption markers with apoB, triglycerides and LDL-C in all subjects (n=343) and by gender.

	ApoB	Triglycerides	LDL-C
Squalene			
Total	0.001	-0.010	0.038
Men	-0.003	-0.151	0.126
Women	-0.038	0.046	-0.042
Lathosterol			
Total	**0.577***	**0.534***	**0.385***
Men	**0.484***	**0.514***	**0.209‡**
Women	**0.610***	**0.498***	**0.505***
Desmosterol			
Total	**0.452***	**0.383***	**0.407***
Men	**0.236†**	**0.236†**	**0.231†**
Women	**0.550***	**0.430***	**0.530***
Campesterol			
Total	**0.270***	0.010	**0.248***
Men	**0.326***	-0.033	**0.276***
Women	**0.201†**	-0.005	**0.230†**
Sitosterol			
Total	**0.275***	-0.038	**0.267***
Men	**0.393***	0.000	**0.334***
Women	**0.172‡**	-0.098	**0.212†**
Cholestanol			
Total	**0.153†**	-0.040	**0.192***
Men	**0.212†**	-0.055	**0.265†**
Women	0.066	-0.086	0.127

Values represent Spearman Rank correlation coefficients. *p<0.001, †p<0.01 and ‡p<0.05.

Supplemental Table III. Key enzymes in the cholesterol metabolism pathway of which the genes posses a USF1 binding site

Gene	Chromosome	Protein	Metabolic Function
MVK	12q24	mevalonate kinase	Converts Mevalonic acid into mevalonate 5-phosphate.
PMVK	1q22	phosphomevalonate kinase	Converts mevalonate 5-phosphate into mevalonate 5-diphosphate.
SC5DL	11q23.3	sterol-C5-desaturase-like	Converts of lathosterol into 7-dehydrocholesterol.
DHCR24	1p33-p31.1	24-dehydrocholesterol reductase (also known as Seladin-1)	Catalyzes the reduction of the delta-24 double bond of sterol intermediates during cholesterol biosynthesis.