

Relationship between stearoyl-CoA desaturase activity and plasma triglycerides in human and mouse hypertriglyceridemia

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Abstract Stearoyl-CoA desaturase (SCD) is expressed at high levels in several human tissues and is required for the biosynthesis of oleate (18:1) and palmitoleate (16:1). These monounsaturated fatty acids are the major components of phospholipids, triglycerides, wax esters, and cholesterol esters. Mice with a targeted disruption of the SCD1 gene have very low levels of VLDL and impaired triglyceride and cholesterol ester biosynthesis. In the HYPLIP mouse, a model of hyperlipidemia, there was a 4-fold increase in hepatic SCD activity, a 1.8-fold increase in the desaturation index, and a 2-fold increase in plasma triglycerides. We used the plasma ratio of 18:1/18:0 (the “desaturation index”) as an in vivo measure of SCD activity in human subjects. In human subjects with triglycerides ranging from 0.3 to 20 mM, the desaturation ratio accounted for one-third of the variance in plasma triglyceride levels. A 2-fold increase in the desaturation index was associated with a 4-fold increase in plasma triglycerides. In human subjects exposed to a high carbohydrate diet, the desaturation index explained 44% of the variance in triglycerides. We propose that many of the factors that influence plasma triglyceride levels do so by converging upon the regulation of SCD activity.—Attie, A. D., R. M. Krauss, M. P. Gray-Keller, A. Brownlie, M. Miyazaki, J. J. Kastelein, A. J. Lusis, A. F. H. Stalenhoef, J. P. Stoehr, M. R. Hayden, and J. M. Ntambi. **Relationship between stearoyl-CoA desaturase activity and plasma triglycerides in human and mouse hypertriglyceridemia.** *J. Lipid Res.* 2002. 43: 1899–1907.

Supplementary key words metabolic syndrome • hyperlipidemia • monounsaturated fatty acids

The rate of de novo fatty acid synthesis is low in human populations that consume diets high in polyunsaturated fatty acids (PUFA) and cholesterol, known repressors of lipogenic gene expression (1). However, one lipogenic gene that is highly expressed in various human tissues even in the presence of cholesterol and PUFA-rich diets is stearoyl-CoA desaturase (SCD) (2).

SCD catalyzes the introduction of the first *cis*-double bond in the $\Delta 9$ position (between carbons 9 and 10) in several fatty acyl-CoA substrates. The preferred substrates are palmitoyl- and stearoyl-CoA, which are converted into palmitoleoyl- and oleoyl-CoA, respectively (3). Changes in activity of SCD in tissues are reflected in the composition of cellular phospholipids, cholesterol esters and triglycerides.

SCD gene expression is highly regulated; it is very sensitive to dietary lipids (PUFAs, cholesterol, and vitamin A), hormones (insulin), developmental processes, temperature changes, thiazolidinediones, metals, alcohol, peroxisomal proliferators, and phenolic compounds (4). Consequently, genetic variability in numerous pathways might manifest itself in changes in SCD expression and thus have consequences on lipid metabolism.

The liver and adipose tissue are the principal sites of de novo lipogenesis. Both tissues have a high capacity to convert carbohydrate into fatty acids when glycolytic and lipogenic enzymes are induced and activated. Recently, the transcription factor sterol responsive element binding protein-1c (SREBP-1c) has emerged as a master regulator of these metabolic pathways (5). SREBP-1c activates the

Manuscript received 8 May 2002 and in revised form 1 July 2002.
Published, JLR Papers in Press, August 16, 2002.
DOI 10.1194/jlr.M200189JLR200

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expression of genes encoding enzymes necessary for conversion of carbohydrate to fat (e.g., glucokinase, acetyl-CoA carboxylase, ATP-citrate lyase, glucose-6-phosphate dehydrogenase, and malic enzyme). SCD is exquisitely sensitive to SREBP-1c regulation (4, 6–8). The discovery that insulin stimulates the transcription of the SREBP-1c gene readily explains how insulin exerts a global stimulatory effect on lipogenesis (9).

Using a naturally occurring mouse model of SCD1-deficiency, as well as a gene-targeted SCD1-knockout mouse, we have shown that triglyceride and cholesterol ester synthesis are greatly reduced in the absence of this enzyme (10). The triglyceride phenotype cannot be reversed with dietary monounsaturated fat, suggesting that de novo synthesized monounsaturated fat is essential for triglyceride synthesis (10, 11). The SCD1-deficient animals produce very low levels of VLDL, suggesting that the rate of VLDL production might itself be influenced by SCD1 activity in vivo (10, 11).

The role of SCD in human lipoprotein metabolism has never been explored. Hypertriglyceridemia (HTG) syndromes are among the most common lipid disorders in humans. Although there is strong evidence that many of these syndromes are heritable (12–14), the genetics of these disorders is not well understood. It is most likely that HTG is a complex trait; i.e., the expression of the phenotype is influenced by multiple genes. In addition, the penetrance of HTG is affected by diet, insulin sensitivity, and obesity (15).

Given the strong correlation between the ability of cells to synthesize triglycerides and cholesterol esters, and the activity of SCD, we validated and applied a simple plasma marker of SCD activity, the ratio of plasma oleate to stearate (18:1/18:0 ratio, the “desaturation index”), to test the hypothesis that in vivo SCD activity accounts for a large fraction of the variation in plasma triglycerides in human subjects. In addition, we studied the desaturation index in human subjects exposed to a regimen known to raise serum triglyceride levels, high-carbohydrate diets. Our results support an important role of SCD in determining human serum triglyceride levels.

METHODS

Animals and diets

Asebia homozygous (ab^J/ab^J or $-/-$) and heterozygous ($+/ab^J$ or $+/-$) mice were obtained from the Jackson Laboratory (Bar Harbor, ME) and bred at the University of Wisconsin Animal Care Facility.

In this study, comparisons are made between the homozygous ($-/-$) and the heterozygous ($+/-$) mice since the latter are indistinguishable from normal mice. The generation of targeted $SCD^{-/-}$ mice has been previously described (11). Prebred homozygous ($SCD1^{-/-}$) and wild-type ($SCD1^{+/+}$) mice on an SV129 background were used. C3H mice and Hc-B19 mice were bred at UCLA by A. Jake Lusis and shipped to Madison, WI for analysis. These mice were housed in a pathogen-free barrier facility operating on a 12-h light/12-h dark cycle. At 3-weeks-of-age, these mice were fed ad libitum on lab-

oratory chow diet (5008 test diet, PMI Nutrition International Inc., IN).

Plasma lipoprotein analysis. Mice were fasted a minimum of 4 h and sacrificed by CO_2 asphyxiation and/or cervical dislocation.

Blood was collected aseptically by direct cardiac puncture and centrifuged (13,000 g, 5 min, $4^\circ C$) to collect plasma. Lipoproteins were fractionated on a Superose 6HR 10/30 FPLC column (Pharmacia). Plasma samples were diluted 1:1 with PBS, filtered (Cameo 3AS syringe filter, $0.22 \mu m$), and injected onto the column that had been equilibrated with PBS containing 1 mM EDTA and 0.02% NaN_3 . The equivalent of 100 μl of plasma was injected onto the column. The flow rate was set constant at 0.3 ml/min. Five hundred microliter fractions were collected and used for total triglyceride measurements (Sigma). Values reported are for total triglyceride mass per fraction. The identities of the lipoproteins have been confirmed by utilizing anti-ApoB immunoreactivity for LDL and Anti-Apo AI immunoreactivity for HDL (not shown).

Fatty acid analysis. Total lipids were extracted from plasma according to the method of Bligh and Dyer (16). Heptadecanoic acid (Sigma) was added as an internal standard for the quantitation of fatty acids. The lipids were methylated and analyzed by gas-liquid chromatography on a capillary column coated with DB-225 (0.25 mm, 30 m length id, $0.25 \mu m$; Agilent Technologies, Inc. Wilmington, DE). Column temperature was kept at $70^\circ C$ for 1 min, then increased from $70^\circ C$ to $180^\circ C$ at a rate of $20^\circ C/min$ and then to $220^\circ C$ at a rate of $3^\circ C/min$, and was kept at $220^\circ C$ for 15 min. 20:1n-9 and 20:1n-7 fatty acids were identified by comparison of retention times with authentic standards (Sigma, St. Louis, MO).

Familial combined hyperlipidemia patients. One hundred patients with hypertriglyceridemia were selected from a Dutch cohort of 32 families (299 subjects) with well-defined familial combined hyperlipidemia (FCH) (17).

Carbohydrate feeding study. Human subjects were selected from a combined group of 429 healthy, nonsmoking Caucasian individuals aged ≥ 20 who had participated in previous dietary intervention protocols as described (18, 19). All subjects had been free of chronic disease during the previous 5 years and were not taking medication likely to interfere with lipid metabolism. In addition, they were required to have plasma total cholesterol concentrations < 6.74 mM (260 mg/dl), triglyceride < 5.65 mM (500 mg/dl), resting blood pressure $< 160/105$ mm Hg, and body weight $< 130\%$ of ideal. Each participant signed a consent form approved by the Committee for the Protection of Human Subjects at E. O. Lawrence Berkeley National Laboratory, University of California, Berkeley, and participated in a medical interview. Fasting blood samples were obtained after 4–6 weeks in which subjects consuming high-fat/low-carbohydrate diets (carbohydrate 39–45% en) and low-fat/high-carbohydrate diets (carbohydrate 61–65% en) (1, 2). In all diets, $\sim 50\%$ of the carbohydrate was consumed as simple sugars.

Plasma lipid and lipoprotein measurements were performed as previously described (1). We wished to compare results in two subgroups of subjects selected on the basis of triglyceride responses to a high carbohydrate diet greater than 1 SD above or below the mean for the entire group (29.2 ± 77.9 mg/dl). Within each category, subjects were matched within 20% for basal triglyceride level on the low carbohydrate. As a result, mean levels of basal triglyceride were similar in the two groups, as were BMI and age. Statistical analyses were performed using Statview (SAS Institute, Inc.). Diet differences were analyzed by paired *t*-test within group correlations by Spearman correlations and between group differences by Mann-Whitney tests. Logistic regression analysis was used to assess determinants of triglyceride response category.

Relationship between SCD1 gene dosage and the desaturation index

We measured the ratio of oleate (18:1) to stearate (18:0) and of palmitoleate (16:1) to palmitate (16:0) in two mouse models of SCD1 deficiency. The asebia mouse has a naturally-occurring deletion encompassing most of the SCD1 gene (20). Homozygotes have no SCD1 expression and express residual desaturase activity of <10% of normal, due to the expression of SCD2. The 18:1/18:0 ratio was reduced about 50% in homozygotes relative to heterozygotes (Fig. 1A). The 16:1/16:0 ratio was reduced by about two-thirds (Fig. 1B).

We recently generated mice deficient in SCD1 through targeted disruption of the SCD1 gene (11). In these animals, there is a relationship between SCD1 gene dosage and both the respective C-18 and C-16 fatty acid ratios; heterozygotes have a triglyceride level intermediate between that of the wild type and that of the homozygous-null animals (Fig. 1C, D). The asebia mutation occurred in a BALB/c-derived mouse strain background (20). The SCD1-knockout mice are 129 × C57BL/6 F1 mice. The 18:1/18:0 ratio in the asebia heterozygotes is about double that of the SCD1-knockout heterozygotes. Interestingly, the triglyceride levels in the asebia heterozygotes are also twice the levels in the knockout heterozygotes. Thus, although strain background changes triglyceride levels, the quantitative relationship between the desaturation index and plasma triglycerides is preserved.

Deficiency of plasma VLDL in animals deficient in SCD1

Analysis of the lipoprotein profiles of both strains of SCD1-knockout mice revealed a striking reduction in VLDL triglycerides in both the asebia (Fig. 2A) and the knockout (Fig. 2C) mice. The cholesterol profiles revealed no significant differences between wild type and SCD1-deficient mice (Fig. 2B, D).

Correlation between desaturation index and plasma triglycerides in a murine model of familial combined hyperlipidemia

Castellani et al. identified a spontaneous mutation (termed “hyplip”) in the HcB congenic strain background associated with a phenotype closely resembling familial combined hyperlipidemia (FCHL) (21). The mutation maps to mouse chromosome 1, in a region syntenic with a segment of human chromosome 1q21-q23 where familial combined hyperlipidemia has been mapped in a human population (14). The potential genetic parallel with FCHL suggested that it might be a useful model to explore the possible relevance of SCD to FCHL. Subsequent to these studies, the Hyplip gene was identified as thioredoxin interacting protein (*Txnip*) (22).

The HcB-19 strain was used to explore the relationship between hepatic SCD activity, the plasma desaturation index, and triglyceride levels. The triglyceride levels of the HcB-19 mice were about 2-fold higher than those of the C3H background strain (Fig. 3A). The SCD activity of liver microsomes was about 4-fold higher in the HcB-19 mice

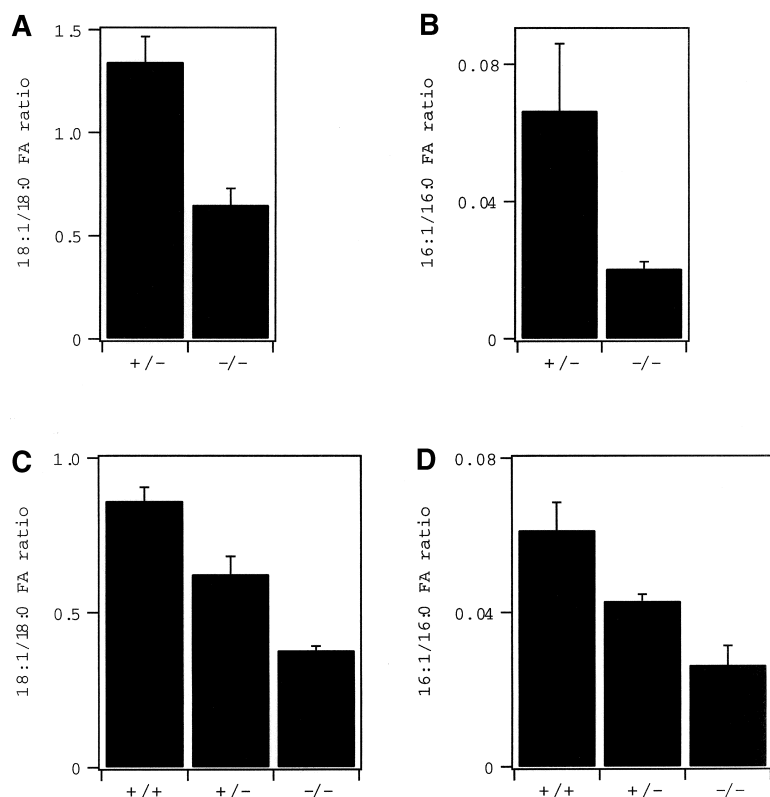


Fig. 1. The ratio of monounsaturated to saturated fatty acids in plasma (the desaturation index) is proportional to gene dosage in two mouse models of SCD1 deficiency. The steady-state levels of oleate (18:1), stearate (18:0), palmitoleate (16:1), and palmitate (16:0) were measured in fasting plasma collected from Asebia mice, a naturally occurring SCD1 deficient strain (A and B) and targeted SCD1 knockout mice (C and D). The data are expressed as the ratio of monounsaturated to saturated fatty acids for oleate/stearate (A and C) and palmitoleate/palmitate (B and D) and establish a “desaturation index” that is proportional to SCD1 gene dosage. The *P* values in the Asebia panels are derived from Student’s two-tailed *t*-tests. The *P* values in the SCD1-knockout panels are derived from single factor ANOVA tests.

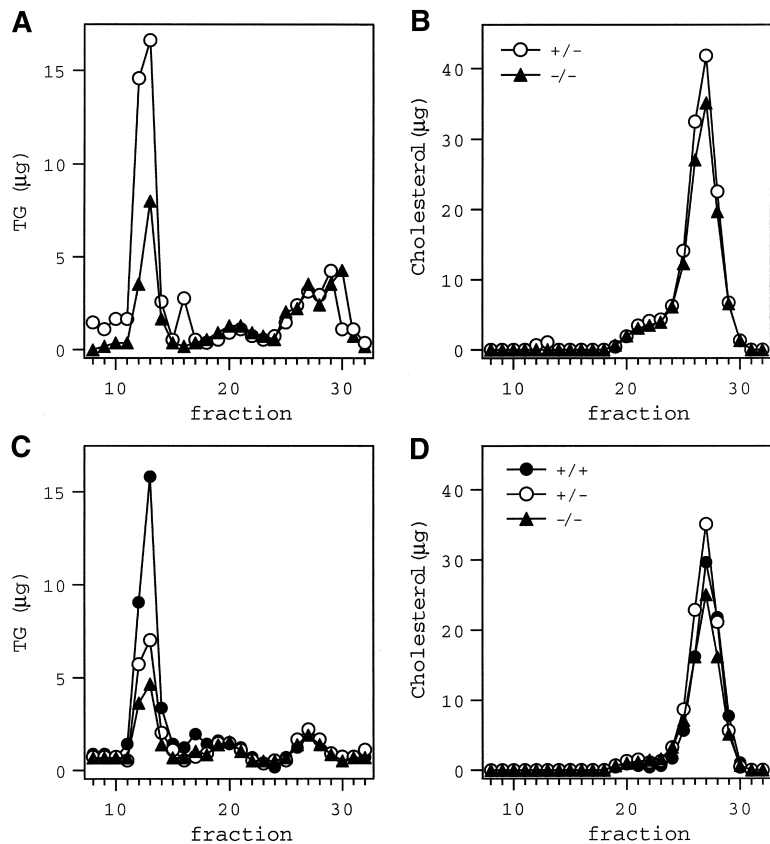


Fig. 2. Lipoprotein profiles in Asebia and SCD1 knockout mice demonstrate a selective loss of VLDL triglyceride. Plasma was collected from fasting Asebia (A and B) and SCD1 knockout mice (C and D) and lipoproteins isolated by fast performance liquid chromatography. Triglyceride (A and C) and cholesterol (B and D) levels were determined for each fraction and provide a distribution of these lipids among the various density fractions eluted from the column. The lipoprotein peaks for VLDL, LDL, and HDL are indicated.

than the controls (Fig. 3B). In this model, as in the SCD1 loss-of-function models described above, the desaturation index correlated with the SCD activity; the desaturation index was about 2-fold higher in the HcB-19 mice than in

the C3H mice (Fig. 3C). There was only a small difference in the 16:1/16:0 ratio between the two strains (Fig. 3D). Despite the fact that these are C3H mice, the magnitude of the relationship between the desaturation index and

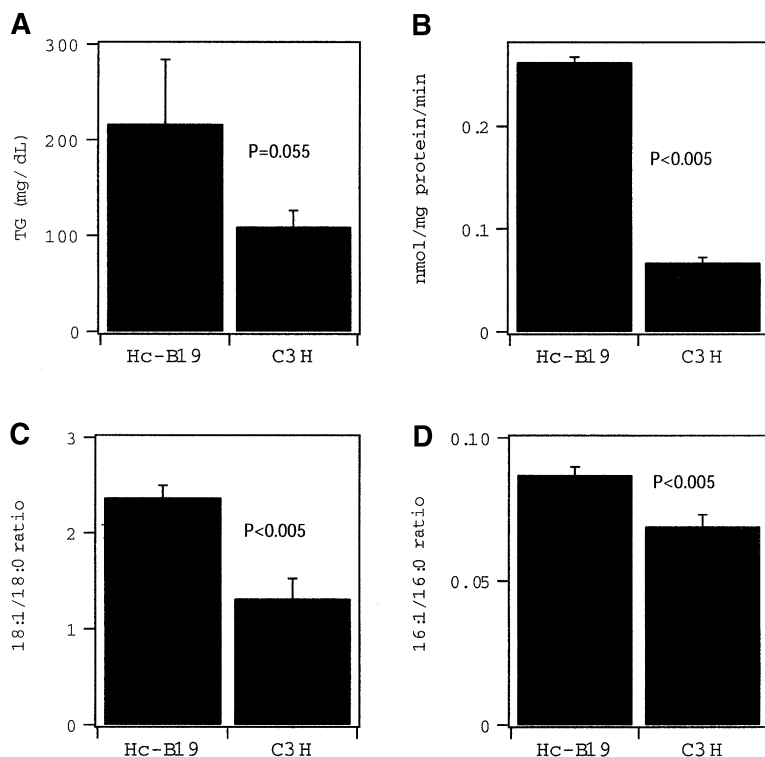


Fig. 3. A murine model for familial combined hyperlipidemia (FCHL) reveals elevated SCD1 activity, plasma triglycerides, and desaturation indices for plasma fatty acids. Fasting plasma from both mouse strains were used to determine triglyceride levels (A). SCD1 activity was determined in a liver microsomal fraction of normal (C3H) and hypertriglyceridemic (HcB19) mice (B). SCD1 desaturation index for oleate/stearate (C) and palmitoleate/palmitate (D).

triglyceride levels was similar to that of the other mouse strains we have assessed.

Correlation between desaturation index and plasma triglycerides in human subjects

We analyzed fasting plasma from 173 human subjects with plasma triglyceride levels ranging from 0.3 to 20 mM. One hundred of these subjects had familial combined hyperlipidemia (open circles, **Fig. 4**). The desaturation index correlated with triglyceride levels (**Fig. 4A**) and to a lesser extent, with HDL (**Fig. 4B**); the desaturation index explains 53% of the variation in plasma triglyceride and 17% of the variance in HDL. By contrast, there was no significant correlation between the 16:1/16:0 ratio and plasma triglycerides or HDL (**Fig. 4C, D**).

Responsiveness of desaturation index and plasma triglycerides to high-carbohydrate diet in human subjects

Human subjects were placed on a high carbohydrate diet (61–65% calories derived from carbohydrate) for 4–6

weeks. Out of 429 subjects, 20 whose triglycerides increased and 20 who did not show a triglyceride rise in response to the high-carbohydrate diet were chosen for measurement of the desaturation ratio. Prior to the start of the diet, the desaturation ratio explained 11% of the variance in plasma triglyceride (**Fig. 5A**). However, after consumption of the high-carbohydrate diet, the desaturation ratio accounted for 44% of the variance in plasma triglyceride (**Fig. 5B**). The correlation between these measurements was significant on both diets (Spearman $P < 0.0001$) in the group as a whole as well as in the subgroup with increased triglyceride on the high-carbohydrate diet ($P < 0.05$).

The plasma desaturation ratio increased significantly in both triglyceride response subgroups (**Table 1**). The increase was significantly greater, however, in the subgroup of subjects with increased versus decreased triglyceride in response to the high-carbohydrate diet. There was no significant correlation of the magnitude of triglyceride change

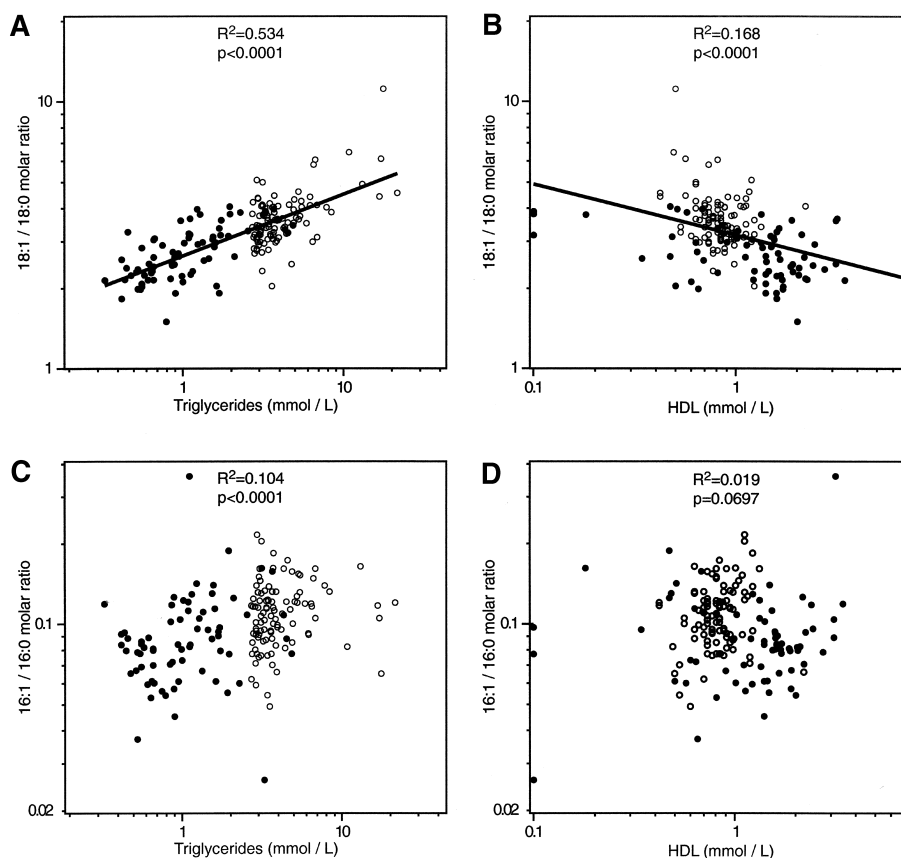


Fig. 4. The desaturation index correlates with plasma triglycerides and is inversely proportional to HDL levels in humans. Fasting plasma from 175 human subjects was used to determine triglyceride and HDL levels as well as determine the SCD1 desaturation index. The relationship between the 18:1/18:0 ratio and plasma triglyceride and HDL cholesterol is shown in A and B, respectively. The relationship between 16:1/16:0 ratio and plasma triglyceride and HDL cholesterol is shown in C and D, respectively. One hundred subjects (open circles) with familial combined hyperlipidemia had a mean plasma triglyceride of 4.6 ± 0.3 mmol/l and average HDL of 0.84 ± 0.02 mmol/l. The remaining subjects (closed circles) had a mean plasma triglyceride value of 1.3 ± 0.1 mmol/l and an average HDL of 1.36 ± 0.09 mmol/l. Three subjects shown in C were not included in the linear regression. For the nomolipidemic group alone the correlation remained significant ($R^2 = 0.371$, $P < 0.001$), whereas for the FCHL group alone, it was not significant ($R^2 = 0.023$; $P = 0.13$).

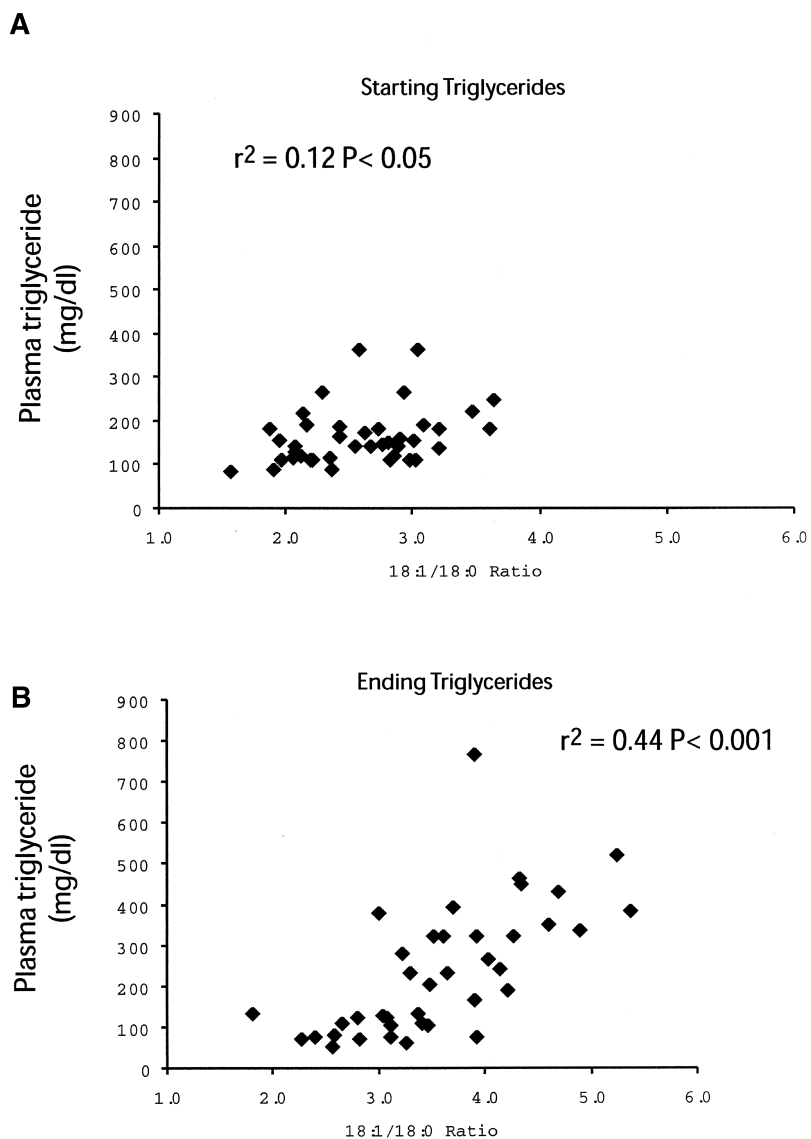


Fig. 5. Relationship between desaturation ratio and serum triglyceride concentration before (A) and after (B) 4–6 weeks on a high-carbohydrate diet. Subjects were placed on diets consisting of 61–65% calories derived from carbohydrate. Samples taken before and after the diet were analyzed for 18:1 and 18:0 fatty acids.

versus fatty acid ratio change in either group. However, both the desaturation ratio after the high-carbohydrate diet and the diet-induced change in this ratio were significant determinants of the triglyceride response category in logistic regression analysis ($P < 0.005$), accounting for 27% and 32% of the variance, respectively.

DISCUSSION

Despite 30 years of intense effort, the genetics of HTG syndromes is largely unsolved. The best-understood syndromes are those in which HTG results from defects in lipoprotein lipase (23). However, the most common forms of HTG involve overproduction of VLDL particles or the packaging of excess triglyceride on VLDL particles. With the exception of LDL receptor (LDLR) mutations (24,

25), no single gene defect has been shown to cause overproduction of lipoproteins in human subjects. Since the LDLR does not appear to be an agent in HTG and FCHL, new genes and perhaps new pathways must be identified.

The genetics of FCHL is especially challenging. As implied by the name, FCHL involves the transmission of elevated VLDL, LDL, or both within families. Until recently, it has been difficult to postulate regulatory mechanisms that might influence both cholesterol and triglyceride metabolism. Recent progress in understanding transcriptional regulation of lipogenic and cholesterogenic genes creates a new framework to bring to bear on these syndromes (26).

The SREBP genes, SREBP-1c and SREBP-2 regulate the lipogenic and cholesterogenic pathways, respectively (26). The abundance of the active forms of these transcription factors is regulated in two ways. First, the transcription of

TABLE 1. Lipid measurements (mean \pm SEM) in groups with triglyceride increase or decrease on low versus high carbohydrate diets

Group	Triglyceride Increase (n = 20)		Triglyceride Decrease (n = 20)	
	Low	High	Low	High
Dietary Carbohydrate				
Triglyceride mg/dl	168.2 \pm 15.0	368.3 \pm 27.7 ^{a,c}	160.0 \pm 14.4	111.2 \pm 10.1 ^a
18:0/18:1 fatty acids	2.64 \pm 0.13	4.10 \pm 0.15 ^{a,c}	2.56 \pm 0.10	3.12 \pm 0.15 ^b

^a $P < 0.0001$ versus low carbohydrate.

^b $P < 0.01$ versus low carbohydrate.

^c $P < 0.0001$ versus triglyceride decrease group.

SREBP-1c is regulated by insulin (9) and by the oxysterol-activated transcription factor, LXR (27). Second, the activation of the SREBP proteins, SREBP-1a, SREBP-1c, and SREBP-2, requires a proteolytic cleavage event that is inhibited by cholesterol (or an oxysterol) and polyunsaturated fatty acids (28). This web of regulatory mechanisms means that insulin sensitivity and its effect on insulin levels, dietary carbohydrate and fat, and cholesterol all interact to regulate SREBP-responsive genes. Given the range of potential genes that can conceivably be involved in these processes, it is not surprising that the genetics of these dyslipidemias is quite complex.

We have recently shown that SCD1 expression is essential and rate-limiting for the synthesis of triglycerides and cholesterol esters in mice (10). The supply of free fatty acids to the liver is rate limiting for the secretion of VLDL triglycerides (29). In rodents, high carbohydrate diets greatly enhance the synthesis and secretion of triglycerides by the liver (30). However, in the SCD1-knockout mice, carbohydrate feeding fails to induce triglyceride synthesis and secretion (11), implying that SCD1 represents a crucial bottleneck in this pathway.

In this manuscript, we show that SCD1 activity correlates with plasma triglyceride levels in several different mouse strains. We use these strains to validate the plasma desaturation index (the 18:1/18:0 ratio) as an *in vivo* surrogate of SCD activity and show that in human subjects, like the mouse strains, plasma triglyceride levels correlate with SCD activity. The desaturation ratio is closely related to plasma triglyceride levels in human subjects. This relationship extends throughout the normal range of triglyceride values. In addition, we found that a low-fat, high-carbohydrate diet induces an increase in the desaturation ratio, and that this increase is greater in individuals who exhibit a hypertriglyceridemic response to the diet than in those whose triglyceride is reduced. Moreover, although the magnitude of the changes in triglyceride and fatty acid ratios with diet were not significantly correlated, the increase in the fatty acid ratio was a significant determinant of triglyceride response category. Although the results are from a post hoc analysis and should be confirmed in other studies, they suggest that SCD activity is stimulated in conjunction with, and may contribute to, carbohydrate-induced lipemia. The finding that the mean 18:1/18:0 ratio increased in the group with a triglyceride reduc-

tion on the high carbohydrate diet suggests that other metabolic responses may have acted to reduce triglyceride production and/or increase triglyceride clearance in these individuals.

The desaturation ratio also explained a substantial proportion of the variance in triglycerides in subjects with hypertriglyceridemia. Although the HTG of these various subjects is undoubtedly due to various genetic and environmental causes, our most striking finding is that in most of the subjects, elevated triglycerides are accompanied by elevated SCD activity. Within the FCHL group (open circles, Fig. 4), the correlation between the desaturation ratio is lost ($R^2 = 0.023$, $P < 0.0001$ for the FCHL patients; $R^2 = 0.371$, $P < 0.0001$ for the non-FCHL patients). The lack of a significant correlation between the 18:1/18:0 FA ratio and triglyceride levels among FCHL subjects in our study (Fig. 4) may be because once triglyceride level is elevated in conjunction with increased SCD, the contribution of SCD to the overall variance of triglyceride levels is diminished. This would be the case, for example, if one were to study the relationship between cholesterol level and LDLR expression in people who already have a mutation in the LDLR.


We propose that there are numerous causes of HTG that converge upon and exert their triglyceride elevating effects through SCD. For example, HTG is frequently associated with insulin resistance. Recent studies suggest that in the liver, insulin resistance is selective; it involves impaired suppression of gluconeogenesis without an impairment in insulin-stimulated SREBP-1c gene transcription (31). Thus, the hyperinsulinemia that usually accompanies insulin resistance syndromes might lead to enhanced SREBP-1c expression and thus enhanced SCD expression. A failure to suppress SREBP-1c activation by cholesterol or polyunsaturated fatty acids might cause SCD activity to rise above the critical threshold that causes HTG.

In humans, there is recent evidence that adipose tissue might be the major source of the carbohydrate-derived fatty acids that end up as VLDL triglycerides (32). Therefore, it is possible that plasma triglycerides correlate with liver SCD in mice and with adipose tissue SCD activity in humans. Recent evidence from mouse (33) and human (34) studies shows that in obesity, the expression of SREBP-1c goes down in adipose tissue, and in mice and humans (35, 36) goes up in the liver (37). This redistribution in SREBP-1c gene expression orchestrates a shift in the lipogenic burden away from the adipose tissue (38). SCD expression is tightly linked to SREBP-1c expression (8). If SCD expression in the liver is more closely associated with VLDL production than is SCD expression in adipose tissue, then the shift in SREBP-1c expression from adipose tissue to liver (if it also occurs in obese humans) might form the basis for the relationship between HTG and obesity.

The rate of bile acid synthesis is correlated with the rate of VLDL triglyceride secretion in humans (39) and in animal models (40). Bile acid sequestrant therapy up-regulates cholesterol-7 α -hydroxylase (CYP-7A1), the rate-limit-

ing enzyme in bile acid production, and leads to increased VLDL triglyceride secretion (41). Impaired bile acid absorption due to reduced expression of the intestinal bile acid transporter is also associated with increased plasma triglyceride levels (42). Overexpression of CYP-7A1 in hepatoma cells (43) or in transgenic mice also leads to increased VLDL secretion. Recently, Miyake et al. reported that in the transgenic mice overexpressing CYP-7A1, SREBP1 and SREBP2 were upregulated as were genes regulated by these transcription factors; SCD1 mRNA abundance was increased 4.7-fold (40). Together with the results presented herein, it is likely that the mechanistic link between bile acid production and triglyceride synthesis is through the regulation of SCD expression. This link likely involves the CYP-7A1 oxidation of sterol-derived ligands of LXR, a transcription factor that regulates SREBP expression (27).

A limitation in genetic studies of complex traits such as HTG is that variation at numerous gene loci can contribute to the same phenotype. If plasma triglyceride concentration is the sole phenotype used to carry out linkage studies, then the power to detect single major genes will be lost in populations with biologically significant variation at many genes that contribute to the same phenotype. One way to overcome this problem is to sub-divide families based on sub-phenotypes. For example, in the search for a major gene responsible for familial hypoalphalipoproteinemia, it was crucial to separate low-HDL families with demonstrable cholesterol efflux defects from those with normal cholesterol efflux (44). In the present case, we note that there are HTG individuals with apparently normal SCD activity and others who fit the linear trend of high SCD activity with HTG. The difference in SCD activity might therefore be a useful sub-phenotype that can be used to identify major genes for HTG and FCHL.

Because of the convergence of several types of HTG syndromes on SCD expression, we hypothesize that SCD activity might be rate-limiting for triglyceride production in a wide array of dyslipidemias. This, together with the lipid profiles of the SCD-deficient mice, suggests that SCD might be an attractive target for triglyceride-lowering drugs. 

This work was supported by Xenon Genetics, Burnaby, BC, Canada and National Institutes of Health Grant HL56593.

REFERENCES

- Hellerstein, M. K. 1999. De novo lipogenesis in humans: metabolic and regulatory aspects. *Eur. J. Clin. Nutr.* **53**(Suppl 1): S53–65.
- Zhang, L., L. Ge, S. Parimoo, K. Stenn, and S. M. Prouty. 1999. Human stearoyl-CoA desaturase: alternative transcripts generated from a single gene by usage of tandem polyadenylation sites. *Biochem. J.* **340**: 255–264.
- Enoch, H. G., A. Catala, and P. Strittmatter. 1976. Mechanism of rat liver microsomal stearoyl-CoA desaturase. Studies of the substrate specificity, enzyme-substrate interactions, and the function of lipid. *J. Biol. Chem.* **251**: 5095–5103.
- Ntambi, J. M. 1999. Regulation of stearoyl-CoA desaturase by polyunsaturated fatty acids and cholesterol. *J. Lipid Res.* **40**: 1549–1558.
- Horton, J. D., J. L. Goldstein, and M. S. Brown. 2002. SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J. Clin. Invest.* **109**: 1125–1131.
- Tabor, D. E., J. B. Kim, B. M. Spiegelman, and P. A. Edwards. 1998. Transcriptional activation of the stearoyl-CoA desaturase 2 gene by sterol regulatory element-binding protein/adipocyte determination and differentiation factor 1. *J. Biol. Chem.* **273**: 22052–22058.
- Tabor, D. E., J. B. Kim, B. M. Spiegelman, and P. A. Edwards. 1999. Identification of conserved cis-elements and transcription factors required for sterol-regulated transcription of stearoyl-CoA desaturase 1 and 2. *J. Biol. Chem.* **274**: 20603–20610.
- Shimomura, I., H. Shimano, B. S. Korn, Y. Bashmakov, and J. D. Horton. 1998. Nuclear sterol regulatory element-binding proteins activate genes responsible for the entire program of unsaturated fatty acid biosynthesis in transgenic mouse liver. *J. Biol. Chem.* **273**: 35299–35306.
- Shimomura, I., Y. Bashmakov, S. Ikemoto, J. D. Horton, M. S. Brown, and J. L. Goldstein. 1999. Insulin selectively increases SREBP-1c mRNA in the livers of rats with streptozotocin-induced diabetes. *Proc. Natl. Acad. Sci. USA.* **96**: 13656–13661.
- Miyazaki, M., Y. C. Kim, M. P. Gray-Keller, A. D. Attie, and J. M. Ntambi. 2000. The biosynthesis of hepatic cholesterol esters and triglycerides is impaired in mice with a disruption of the gene for stearoyl-CoA desaturase 1. *J. Biol. Chem.* **275**: 30132–30138.
- Miyazaki, M., Y. C. Kim, and J. M. Ntambi. 2001. A lipogenic diet in mice with a disruption of the stearoyl-CoA desaturase 1 gene reveals a stringent requirement of endogenous monounsaturated fatty acids for triglyceride synthesis. *J. Lipid Res.* **42**: 1018–1024.
- Goldstein, J. L., H. G. Schrott, W. R. Hazzard, E. L. Bierman, and A. G. Motulsky. 1973. Hyperlipidemia in coronary heart disease. II. Genetic analysis of lipid levels in 176 families and delineation of a new inherited disorder, combined hyperlipidemia. *J. Clin. Invest.* **52**: 1544–1568.
- Pajukanta, P., J. D. Terwilliger, M. Perola, T. Hiekkalinna, I. Nuotio, P. Ellonen, M. Parkkonen, J. Hartiala, K. Ylitalo, J. Pihlajamaki, K. Porkka, M. Laakso, J. Viikari, C. Ehnholm, M. R. Taskinen, and L. Peltonen. 1999. Genome-wide scan for familial combined hyperlipidemia genes in Finnish families, suggesting multiple susceptibility loci influencing triglyceride, cholesterol, and apolipoprotein B levels. *Am. J. Hum. Genet.* **64**: 1453–1463.
- Pajukanta, P., I. Nuotio, J. D. Terwilliger, K. V. Porkka, K. Ylitalo, J. Pihlajamaki, A. J. Suomalainen, A. C. Syvanen, T. Lehtimaki, J. S. Viikari, M. Laakso, M. R. Taskinen, C. Ehnholm, and L. Peltonen. 1998. Linkage of familial combined hyperlipidaemia to chromosome 1q21-q23. *Nat. Genet.* **18**: 369–373.
- Reaven, G. M. 1993. Role of insulin resistance in human disease (syndrome X): an expanded definition. *Annu. Rev. Med.* **44**: 121–131.
- Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37**: 911–917.
- Veerkamp, M. J., J. de Graaf, S. J. Bredie, J. C. Hendriks, P. N. Demacker, and A. F. Stalenhoef. 2002. Diagnosis of familial combined hyperlipidemia based on lipid phenotype expression in 32 families: results of a 5-year follow-up study. *Arterioscler. Thromb. Vasc. Biol.* **22**: 274–282.
- Krauss, R. M., and D. M. Dreon. 1995. Low-density-lipoprotein subclasses and response to a low-fat diet in healthy men. *Am. J. Clin. Nutr.* **62**: 478S–487S.
- Pennacchio, L. A., M. Olivier, J. A. Hubacek, J. C. Cohen, D. R. Cox, J. C. Fruchart, R. M. Krauss, and E. M. Rubin. 2001. An apolipoprotein influencing triglycerides in humans and mice revealed by comparative sequencing. *Science.* **294**: 169–173.
- Zheng, Y., K. J. Eilertsen, L. Ge, L. Zhang, J. P. Sundberg, S. M. Prouty, K. S. Stenn, and S. Parimoo. 1999. Scd1 is expressed in sebaceous glands and is disrupted in the asebia mouse. *Nat. Genet.* **23**: 268–270.
- Castellani, L. W., A. Weinreb, J. Bodnar, A. M. Goto, M. Doolittle, M. Mehrabian, P. Demant, and A. J. Lusis. 1998. Mapping a gene for combined hyperlipidaemia in a mutant mouse strain. *Nat. Genet.* **18**: 374–377.
- Bodnar, J. S., A. Chatterjee, L. W. Castellani, D. A. Ross, J. Ohmen, J. Cavalcoli, C. Wu, K. M. Dains, J. Catanese, M. Chu, S. S. Sheth, K. Charugundla, P. Dement, D. B. West, P. de Jong, and A. J. Lusis. 2002. Positional cloning of the combined hyperlipidemia gene *Hyp1l1*. *Nat. Genet.* **30**: 110–116.

23. Hayden, M. R., and Y. Ma. 1992. Molecular genetics of human lipoprotein lipase deficiency. *Mol. Cell. Biochem.* **113**: 171–176.
24. Packard, C. J., J. L. H. C. Third, J. Shepherd, A. R. Lorimer, H. G. Morgan, and T. D. V. Lawrie. 1976. Low density lipoprotein metabolism in a family of familial hypercholesterolemic patients. *Metabolism.* **25**: 995–1006.
25. Soutar, A. K., N. B. Myant, and G. R. Thompson. 1977. Simultaneous measurement of apolipoprotein B turnover in very-low- and low-density lipoproteins in familial hypercholesterolaemia. *Atherosclerosis.* **28**: 247–256.
26. Brown, M. S., and J. L. Goldstein. 1997. The SREBP pathway: regulation of cholesterol metabolism by proteolysis of a membrane-bound transcription factor. *Cell.* **89**: 331–340.
27. Repa, J. J., G. Liang, J. Ou, Y. Bashmakov, J. M. Lobaccaro, I. Shimomura, B. Shan, M. S. Brown, J. L. Goldstein, and D. J. Mangelsdorf. 2000. Regulation of mouse sterol regulatory element-binding protein-1c gene (SREBP-1c) by oxysterol receptors, LXRalpha and LXRbeta. *Genes Dev.* **14**: 2819–2830.
28. Ou, J., H. Tu, B. Shan, A. Luk, R. A. DeBose-Boyd, Y. Bashmakov, J. L. Goldstein, and M. S. Brown. 2001. Unsaturated fatty acids inhibit transcription of the sterol regulatory element-binding protein-1c (SREBP-1c) gene by antagonizing ligand-dependent activation of the LXR. *Proc. Natl. Acad. Sci. USA.* **98**: 6027–6032.
29. Dixon, J. L., and H. N. Ginsberg. 1993. Regulation of hepatic secretion of apolipoprotein B-containing lipoproteins: Information obtained from cultured liver cells. *J. Lipid Res.* **34**: 167–179.
30. Boogaerts, J. R., M. Malone-McNeal, J. Archambault-Schexnayder, and R. A. Davis. 1984. Dietary carbohydrate induces lipogenesis and very-low-density lipoprotein synthesis. *Am. J. Physiol.* **246**: E77–83.
31. Shimomura, I., M. Matsuda, R. E. Hammer, Y. Bashmakov, M. S. Brown, and J. L. Goldstein. 2000. Decreased IRS-2 and increased SREBP-1c lead to mixed insulin resistance and sensitivity in livers of lipodystrophic and ob/ob mice. *Mol. Cell.* **6**: 77–86.
32. Parks, E. J., R. M. Krauss, M. P. Christiansen, R. A. Neese, and M. K. Hellerstein. 1999. Effects of a low-fat, high-carbohydrate diet on VLDL-triglyceride assembly, production, and clearance. *J. Clin. Invest.* **104**: 1087–1096.
33. Nadler, S. T., J. P. Stoehr, K. L. Schueler, G. Tanimoto, B. S. Yandell, and A. D. Attie. 2000. The expression of adipogenic genes is decreased in obesity and diabetes mellitus. *Proc. Natl. Acad. Sci. USA.* **97**: 11371–11376.
34. Ducluzeau, P. H., N. Perretti, M. Laville, F. Andreelli, N. Vega, J. P. Riou, and H. Vidal. 2001. Regulation by insulin of gene expression in human skeletal muscle and adipose tissue. Evidence for specific defects in type 2 diabetes. *Diabetes.* **50**: 1134–1142.
35. Diraison, F., E. Dusserre, H. Vidal, M. Sothier, and M. Beylot. 2002. Increased hepatic lipogenesis but decreased expression of lipogenic gene in adipose tissue in human obesity. *Am. J. Physiol. Endocrinol. Metab.* **282**: E46–51.
36. Kolehmainen, M., H. Vidal, E. Alhava, and M. I. Uusitupa. 2001. Sterol regulatory element binding protein 1c (SREBP-1c) expression in human obesity. *Obes. Res.* **9**: 706–712.
37. Shimomura, I., Y. Bashmakov, and J. D. Horton. 1999. Increased levels of nuclear SREBP-1c associated with fatty livers in two mouse models of diabetes mellitus. *J. Biol. Chem.* **274**: 30028–30032.
38. Nadler, S. T., and A. D. Attie. 2001. Please pass the chips: genomic insights into obesity and diabetes. *J. Nutr.* **131**: 2078–2081.
39. Angelin, B., K. Einarsson, K. Hellstrom, and B. Leijed. 1978. Bile acid kinetics in relation to endogenous tryglyceride metabolism in various types of hyperlipoproteinemia. *J. Lipid Res.* **19**: 1004–1016.
40. Miyake, J. H., X. D. Doung, W. Strauss, G. L. Moore, L. W. Castellani, L. K. Curtiss, J. M. Taylor, and R. A. Davis. 2001. Increased production of apolipoprotein B-containing lipoproteins in the absence of hyperlipidemia in transgenic mice expressing cholesterol 7alpha-hydroxylase. *J. Biol. Chem.* **276**: 23304–23311.
41. Witztum, J. L., G. Schonfeld, and S. W. Weidman. 1976. The effects of colestipol on the metabolism of very-low-density lipoproteins in man. *J. Lab. Clin. Med.* **88**: 1008–1018.
42. Duane, W. C., L. A. Hartich, A. E. Bartman, and S. B. Ho. 2000. Diminished gene expression of ileal apical sodium bile acid transporter explains impaired absorption of bile acid in patients with hypertriglyceridemia. *J. Lipid Res.* **41**: 1384–1389.
43. Wang, S. L., E. Z. Du, T. D. Martin, and R. A. Davis. 1997. Coordinate regulation of lipogenesis, the assembly and secretion of apolipoprotein B-containing lipoproteins by sterol response element binding protein 1. *J. Biol. Chem.* **272**: 19351–19358.
44. Brooks-Wilson, A., M. Marcil, S. M. Clee, L. H. Zhang, K. Roomp, M. van Dam, L. Yu, C. Brewer, J. A. Collins, H. O. Molhuizen, O. Loubser, B. F. Ouellette, K. Fichter, K. J. Ashbourne-Excoffon, C. W. Sensen, S. Scherer, S. Mott, M. Denis, D. Martindale, J. Frohlick, K. Morgan, B. Koop, S. Pimstone, J. Kastelein, M. R. Hayden, et al. 1999. Mutations in ABC1 in Tangier disease and familial high-density lipoprotein deficiency. *Nat. Genet.* **22**: 336–345.