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Hepatic Lipid Composition and Stearoyl-Coenzyme A Desaturase 1 mRNA Expression Can Be Estimated from Plasma VLDL Fatty Acid Ratios

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BACKGROUND: Stearoyl-coenzyme A desaturase 1 (SCD1) catalyzes the limiting step of monounsaturated fatty acid synthesis in humans and is an important player in triglyceride generation. SCD1 has been repeatedly implicated in the pathogenesis of metabolic and inflammatory diseases. Therefore it is of great importance to determine SCD1 activity in human samples. In this study we aimed to evaluate a hepatic SCD1 activity index derived from plasma VLDL triglyceride composition as a tool to estimate hepatic SCD1 expression in humans. Additionally, we further evaluated commonly used fatty acid ratios [elongase, de novo lipogenesis, and Δ5 and Δ6 desaturase] in plasma VLDL and hepatic lipid fractions.

DESIGN AND METHODS: Liver biopsies and plasma samples were simultaneously collected from 15 individuals. Plasma VLDL was obtained by ultracentrifugation. Hepatic and plasma VLDL lipids were fractionated by thin-layer chromatography, and the fatty acid composition of each fraction was analyzed by gas chromatography. Hepatic SCD1 expression was determined by real-time PCR.

RESULTS: Hepatic SCD1 mRNA expression was associated with the product/precursor ratios (16:1/16:0 and 18:1/18:0) of hepatic lipid fractions. The 16:1/16:0 ratio in hepatic and VLDL triglycerides as well as the 18:1/18:0 ratio in plasma VLDL were closely associated with hepatic SCD1 expression. The hepatic de novo lipogenesis index from triglycerides was associated with expression of lipogenic genes [fatty acid synthase (*FASN*), acetyl-Coenzyme A carboxylase alpha (*ACACA*), and sterol regulatory element binding transcription factor 1 (*SREBP-1*)] and is closely reflected by the de novo lipogenesis index in VLDL triglycerides.

CONCLUSION: We demonstrated for the first time that hepatic SCD1 expression can be estimated noninvasively from routine blood samples by measuring the SCD1 activity index in fasting plasma VLDL.

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Stearoyl-coenzyme A desaturase 1 (SCD1)[4] is a microsomal enzyme that catalyzes Δ9 desaturation as the limiting step of monounsaturated fatty acid synthesis *(1)*. The cellular activity of the short-lived enzyme SCD1 is highly regulated and determined by the amount of enzyme present in the cell. SCD1 mRNA expression is influenced by hormonal *(1)*, nutritional *(2)*, environmental, and genetic factors *(3, 4)* and rapidly adjusts enzyme levels to the demands of the organism. Rodent and cell culture studies have suggested an important role of SCD1 in the pathogenesis of obesity *(1, 5)*, insulin resistance *(1, 5, 6)*, diabetes mellitus *(7)*, and atherosclerosis *(8–11)* as well as inflammatory diseases *(3, 12–16)*. Therefore, there has recently been considerable interest in the determination of SCD1 activity in human samples to elucidate the role of SCD1 for health and disease in clinical studies and interventions *(3, 4, 13–16)*. Apart from elegant stable isotope techniques to directly determine SCD1 desaturation activity in vivo *(13)*, the ratios of SCD1 product and precursor fatty acids (18:1n9/18:0 and 16:1n7/16:0) in various accessible tissues (adipose tissue, skeletal muscle) have been used as a surrogate of enzyme activity in studies of small human cohorts *(3, 14, 17)*. In addition, desaturase activity indices have been investigated in various plasma lipid fractions, although which tissues' SCD1 activity is reflected by these plasma markers remains theoretical and a point of controversy *(18–20)*. It has been recommended

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[4] Nonstandard abbreviations: SCD1, stearoyl-coenzyme A desaturase 1; FFA, free fatty acid; CE, cholesterol esters; PL, phospholipids; TG, triacylglycerides; TLC, thin-layer chromatography; DNL, de novo lipogenesis; DG, diacylglycerides.

that fatty acid ratios be determined from defined plasma lipid fractions instead of whole plasma *(18, 19)*. Because of the specific fatty acid composition of the major fatty acid–containing plasma lipid fractions [free fatty acid (FFA), cholesterol esters (CE), phospholipids (PL), and triacylglycerides (TG)], determination of the SCD1 desaturation index (16:1/16:0 or 18:1/18:0) from whole plasma led to results that may not represent SCD1 activity at all, but strongly correlate with plasma triglyceride levels *(15, 18, 19, 21)*. So far, only the fasting plasma FFA 16:1n7/16:0 ratio has been shown to represent adipose tissue fatty acid composition in humans *(18)*.

The liver is a central organ in lipid homeostasis, and hepatic lipid storage is associated with insulin resistance, diabetes mellitus, and obesity *(22)*. For ethical reasons liver tissue samples available for clinical studies are scarce; thus we aimed to establish a blood marker representing hepatic SCD1 expression and activity. Because VLDL are assembled in the liver and secreted into the circulation *(3, 16, 18)* we hypothesized that VLDL-TG may reflect hepatic fatty acid composition and SCD1 expression.

To address the above questions, we simultaneously obtained both liver and blood samples from 15 patients and determined the fatty acid pattern in plasma VLDL-TG and 5 hepatic lipid fractions separated by thin-layer chromatography (TLC). Next, we compared the SCD1 activity indices (16:1/16:0 and 18:1/18:0) between hepatic lipid fractions and plasma VLDL-TGs as well as hepatic SCD1 mRNA expression. We also evaluated the indices for Δ5 and Δ6 desaturase activity, as well as elongase and de novo lipogenesis (DNL) indices from the fatty acid patterns and mRNA expression of the respective enzymes.

Patients, Materials, and Methods

PATIENTS

A total of 15 individuals [6 female, 9 male, mean (SE) age 66 (9) years, body mass index 24 (4) kg/m^2] scheduled for liver surgery were included in the present study. Indications for the surgery were hepatic hemangioma *(1)*, curative resection of hepatic metastases of colorectal malignancies *(11)* or hepatocellular carcinoma *(2)*, and liver abscess *(1)*. Study patients had tested negative for viral hepatitis and had no liver cirrhosis. All study participants gave written informed consent to participate in this research study, which was approved by the Institutional Review Board of the University of Tübingen (protocol 168/2005) according to the Helsinki Declaration. Liver biopsy samples were taken from normal, nondiseased tissue during surgery, immediately frozen in liquid nitrogen, and stored at −80 °C. Fasting plasma samples were obtained on the same day and immediately stored at −80 °C for analysis of the fatty acid pattern.

FATTY ACID PATTERN

First, the plasma VLDL fraction was separated from the HDL and LDL fractions. For this procedure, equal volumes of NaCl solution (1.006 g/mL) and human plasma were subjected to ultracentrifugation at 817 480*g* at 10 °C for 18 h in a preparative ultracentrifuge (Optima; Beckman Coulter). Then the top layer (VLDL fraction) was removed and used for further analyses. A TissueLyser (Qiagen) was used to homogenize liver tissue for lipid analysis in PBS containing 1% Triton X-100.

We used TLC to separate the VLDL fraction and the liver homogenate into 5 subfractions containing phospholipids, diacylglycerol, FFA, TG, and CE. In detail, the samples were cleared from protein by use of 2-propanol, *n*-heptane and 2 mol/L phosphoric acid. Toluol, methanol, and water were added, and after centrifugation at 8175*g* the upper phase was dried under nitrogen. The lipids were dissolved in $CHCl_3$-CH_3OH and applied to a silica gel chromatography plate (Merck). The phospholipids, diacylglycerol, FFA, TG, and CE were separated by use of *n*-hexane and diethylether, with acetic acid as a solvent. To identify the localization of the fractions, pooled control plasma was also separated on each plate and lipid fractions were visualized by 2,7-dichlor-fluoresceine under ultraviolet light. The fractions were scraped off the TLC plate, transferred to screw-capped vials, and dissolved in a methanol/toluol mixture (4:1, vol/vol) containing *cis*-13,16,19-docosatrienoic acid as an internal standard. Trans-esterification was performed by incubation with acetyl chloride at 100 °C for 1 h. The cold sample was neutralized with K_2CO_3 and the upper phase was concentrated to 80 μL under nitrogen. The fatty acid methyl esters were quantified by using gas chromatography with a flame ionization detector, as previously described *(9)*.

CALCULATION OF ACTIVITY INDICES

The 18:1n9/18:0 and 16:1n7/16:0 ratios were calculated as indices of SCD1 (Δ9) desaturase activity. Furthermore, the 20:4n6/20:3*n*-6 and 20:3n6/18:2n6 ratios were calculated as indexes of Δ5 and Δ6 desaturase activities, respectively. The ratio of 18:0 to 16:0 was calculated as an elongase index and the ratio of 16:0 to 18:2 as the DNL index *(13, 23)*. Plasma triglycerides and tissue triglyceride content from the homogenate were quantified by use of the ADVIA 1650 clinical chemistry analyzer (Siemens Healthcare Diagnostics).

DETERMINATION OF HEPATIC mRNA EXPRESSION BY QUANTITATIVE PCR ANALYSIS

Frozen tissue was homogenized in a TissueLyser (Qiagen) and RNA was extracted with the RNeasy Tissue Kit (Qiagen) according to the manufacturer's instructions. Reverse transcription of total RNA and quanti-

tative PCR of SCD1 and β-actin were performed on the LightCycler system (Roche) with SYBR® green as described previously *(9)*, and Δ5 and Δ6 desaturase mRNA expression was determined with a method similar to that described by Xiang et al. *(24)*.

Data are given as mean (SE). Linear regression analysis was performed to compare activity indices between lipid fractions. The statistical software package JMP 4.0 (SAS Institute) was used, and a P value ≤ 0.05 was considered statistically significant. P-values were not adjusted for multiple testing.

Results

FATTY ACID COMPOSITION OF HEPATIC AND PLASMA VLDL LIPID FRACTIONS

The fatty acid distributions in the hepatic and plasma VLDL lipid fractions are shown in Table 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol55/issue12. In the liver samples from our study participants, fatty acids were mainly derived from TG (52.4%), PL (34.6%), and FFA (6.5%), and only small amounts originated from CE and diacylglycerides (DG) (see online Supplemental Table 1, top lane). The hepatic TG fraction contained significantly less stearate (4-fold), eicosatrienoic acid C20:3n6 (26-fold), arachidonic acid C20:4n6 (17-fold), and docosahexaenoic acid C22:6n3 (22-fold) and contained significantly more oleate (4-fold) than PL (all $P < 10^{-10}$). Calculated from these values, the indices for elongase, DNL, and Δ9 desaturase activity showed strong differences between the hepatic lipid fractions (online Supplemental Table 1).

HEPATIC SCD1 mRNA EXPRESSION AND SCD1 ACTIVITY INDICES IN THE LIPID FRACTIONS OF LIVER, VLDL TRIGLYCERIDES AND TOTAL VLDL

Comparing the SCD1 activity index 16:1/16:0 in the hepatic lipid fractions with hepatic SCD1 mRNA expression levels, we found a strong association of SCD1 mRNA expression with activity indices of hepatic TG-, FFA-, CE-, and PL-SCD1 but not DG-SCD1 (Table 1, Fig. 1A). A much weaker association of the corresponding SCD1 activity indices 18:1/18:0 with SCD1 mRNA expression was observed. Only hepatic PL- and FFA-SCD1 but not TG-, DG-, and CE-SCD1 activity indices were significantly correlated with SCD1 mRNA expression (Table 2, Fig. 2A).

Because liver samples are not routinely available for quantification of SCD1 expression, we investigated the use of lipid fractions from simultaneously obtained VLDL as a surrogate for hepatic SCD1 expression. Therefore we compared hepatic SCD1 mRNA expression and SCD1 activity indices of hepatic lipid fractions

Table 1. 16:1/16:0 SCD1 activity index and SCD1 mRNA expression.[a]

	SCD1/β-actin mRNA		VLDL-TG 16:1/16:0		VLDL 16:1/16:0	
	r	*P*	*r*	*P*	*r*	*P*
SCD1/β-actin mRNA			0.67	**0.006**	0.57	**0.03**
Liver-TG 16:1/16:0	0.54	**0.04**	0.91	**<0.0001**	0.94	**<0.0001**
Liver-DG 16:1/16:0	0.42	***0.12***	0.51	**0.04**	0.56	**0.03**
Liver-FFA 16:1/16:0	0.71	**0.003**	0.82	**0.0001**	0.84	**<0.0001**
Liver-CE 16:1/16:0	0.60	**0.02**	0.87	**<0.0001**	0.90	**<0.0001**
Liver-PL 16:1/16:0	0.70	**0.005**	0.88	**<0.0001**	0.89	**<0.0001**
VLDL 16:1/16:0	0.57	**0.03**	0.99	**<0.0001**		

[a] Univariate relationships between hepatic fatty acid ratios, VLDL fatty acid ratios, and hepatic gene expression. Results of linear regression analysis of 15 individuals are displayed. Significant differences are highlighted in bold, trends in italic.

with the corresponding lipid fractions from plasma VLDL-TG. The SCD1 activity indices 16:1/16:0 in the VLDL-TG lipid fractions very closely reflected the activity index in all corresponding hepatic lipid fractions (Table 1, Fig. 1B), whereas the corresponding 18:1/18:0 activity indices showed a trend of association only with hepatic TG but not with other hepatic lipid fractions (Table 2, Fig. 2B). Accordingly we found a good correlation of hepatic SCD1 mRNA expression with the 16:1/16:0 activity index (Fig. 1C), but only a trend with the 18:1/18:0 activity index (Fig. 2C). Together these results indicate that the 16:1/16:0 activity indices obtained from plasma VLDL-TG reflected the corresponding lipid fractions in the liver and were also correlated with hepatic SCD1 mRNA expression.

These results encouraged us to investigate a possible association of these hepatic parameters with total VLDL SCD1 activity indices, because VLDL consists mainly of triglycerides. We found that the 16:1/16:0 activity index is almost identical in total VLDL and VLDL-TG (Fig. 1D) and that the VLDL 16:1/16:0 activity index also reflects hepatic fatty acid composition in all subfractions as well as SCD1 mRNA expression (Table 1 and Fig. 1, E and F). The VLDL 18:1/18:0 activity index closely reflects hepatic PL fatty acid composition as well as SCD1 mRNA expression and shows a trend for correlation with hepatic FFA (Table 2 and Fig. 2, E and F).

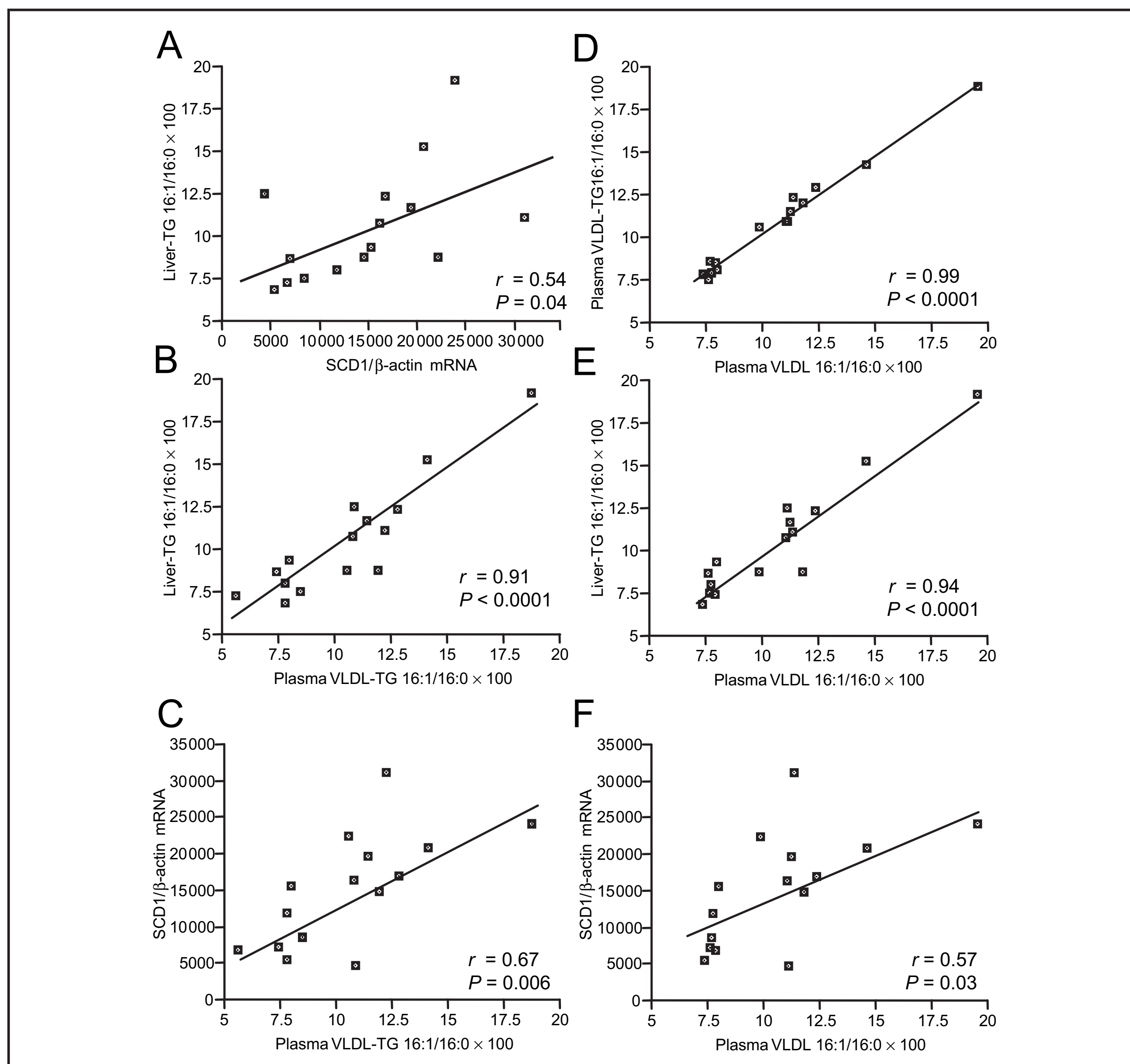

Fig. 1. Univariate relationships between the 16:1/16:0 ratio of hepatic and plasma VLDL fatty acid fractions and hepatic SCD1 mRNA expression are displayed.

(A), The 16:1/16:0 ratio in liver triglycerides is positively associated with hepatic SCD1 mRNA expression, normalized for β-actin. (B), The 16:1/16:0 ratio in liver triglycerides strongly correlates with the 16:1/16:0-ratio in VLDL triglycerides, which are assembled and secreted from the liver. (C), Hepatic SCD1 mRNA expression, normalized for β-actin is positively correlated with the 16:1/16:0-ratio in VLDL triglycerides. (D), 16:1/16:0 ratio in total VLDL triglycerides is very closely reflected in the 16:1/16:0 ratio of total VLDL fatty acids. (E), The 16:1/16:0 ratio in liver triglycerides strongly correlates with the 16:1/16:0 ratio in total VLDL fatty acids. (F), Hepatic SCD1 mRNA expression, normalized for β-actin, is positively correlated with the 16:1/16:0 ratio in total VLDL fatty acids.

FATTY ACID COMPOSITION OF THE HEPATIC AND VLDL LIPID FRACTIONS AND ACTIVITY INDICES AND mRNA EXPRESSION OF Δ5 AND Δ6 DESATURASES AND ELONGASE

Activity indices of Δ5 and Δ6 desaturases calculated from tissue product/precursor ratios, as well as fatty acid elongase activity *(8, 25, 26)* have been implicated in insulin resistance. We tested whether hepatic activity indices of these enzymes are also reflected by the VLDL fatty acid composition in humans. We found no association between the indices of fatty acid elongase (18:0/16:0, $P \geq 0.2$ and $R \leq 0.3$), Δ5 desaturase (20:4n6/20:3n6, $P \geq 0.7$ and $R \leq 0.1$), and Δ6 desaturase (20:3n6/

Table 2. 18:1/18:0 SCD1 activity index and SCD1 mRNA expression.[a]

	SCD1/ β-actin mRNA		VLDL-TG 18:1/18:0		VLDL 18:1/18:0	
	r	*P*	*r*	*P*	*r*	*P*
SCD1/β-actin mRNA			0.38	***0.16***	0.72	**0.004**
Liver-TG 18:1/18:0	0.10	0.72	0.41	***0.12***	0.28	0.32
Liver-DG 18:1/18:0	0.19	0.49	0.13	0.62	0.14	0.61
Liver-FFA 18:1/18:0	0.56	**0.03**	0.18	0.51	0.46	***0.08***
Liver-CE 18:1/18:0	0.24	0.38	0.25	0.36	0.27	0.99
Liver-PL 18:1/18:0	0.59	**0.03**	0.34	0.22	0.70	**0.005**
VLDL 18:1/18:0	0.72	**0.004**	0.63	**0.01**		

[a] Univariate relationships between hepatic fatty acid ratios, VLDL fatty acid ratios, and hepatic gene expression. Results of linear regression analysis of 15 individuals are displayed. Significant differences are highlighted in bold, trends in italic.

18:2n6, $P \geq 0.3$ and $R \leq 0.38$) in VLDL and hepatic lipid fractions (data not shown). Furthermore, neither hepatic nor VLDL Δ5 and Δ6 desaturase activity indices showed a significant correlation with Δ5 and Δ6 desaturase mRNA expression in liver tissue samples (data not shown). Only the Δ6 desaturase activity index in hepatic PL was associated with Δ6 desaturase mRNA expression ($P = 0.014$, $R = 0.61$).

DNL INDEX IN HEPATIC AND VLDL LIPID FRACTIONS AND mRNA EXPRESSION OF LIPOGENIC GENES

Increased lipogenesis has been implicated in the pathogenesis of hepatic steatosis and insulin resistance *(27)*. The DNL index (16:0/18:2), which represents the main product of DNL and an essential fatty acid that originates from dietary lipids, has been proposed as a tool to assess fatty acid synthesis in humans *(13, 23)*. The DNL index was calculated from hepatic and VLDL-TG fatty acid composition. The ratio of 16:0 to 18:2 in hepatic TG is very closely mirrored by the corresponding ratio in VLDL-TG and total VLDL (Table 3, online Supplemental Fig. 3A). The DNL index has been established under strictly controlled conditions *(13, 23)* matching the content of dietary 18:2 to the adipose tissue. To investigate whether the DNL index provides information on fatty acid synthesis in individuals on their habitual diet, we measured hepatic mRNA expression of the lipogenic genes fatty acid synthase (*FASN*),[5] acetyl-Coenzyme A carboxylase alpha (*ACACA*), and sterol regulatory element binding transcription factor 1 (*SREBP-1*). Even in individuals who were consuming a habitual diet, the hepatic TG DNL index was significantly correlated with FASN and ACC expression, and showed a trend for SREBP-1 expression (Table 3, online Supplemental Fig. 3, B–D). The VLDL-TG, but not the VLDL DNL index, was positively correlated with *ACACA* and *SREBP-1* expression and showed a trend for *FASN* (Table 3, online Supplemental Fig. 3, E–G). These results indicate that the hepatic DNL may be estimated from the VLDL-TG DNL index.

Discussion

The determination of hepatic SCD1 activity is important for advancing our understanding of metabolic, vascular, and inflammatory diseases. To establish a marker of hepatic SCD1 activity, we examined human liver biopsy samples and simultaneously drawn plasma samples. We were able to demonstrate for the first time that the ratio of 16:1 to 16:0 in fasting VLDL-TG closely reflects the hepatic activity index in multiple lipid species, as well as hepatic SCD1 mRNA expression in humans. The measurement of this ratio therefore provides a tool to noninvasively estimate hepatic SCD1 activity. Data supporting this finding were recently reported by Chong et al. *(13)*, who observed a parallel activation of SCD activity estimated from fatty acid ratios as well as conversion of an intravenously infused stable isotope of C16:0 to C16:1 in VLDL-TG with DNL. Because stearate is preferred to palmitate as a substrate of SCD1 *(28, 29)*, it is surprising that the desaturation activity index of palmitate (16:1/16:0) and not stearate (18:1/18:0) reflects hepatic SCD1 expression more closely. A reason for this result could be the presence of 2 separate triglyceride pools in the liver, as suggested by Donelly et al. *(30)*. The majority of hepatic fatty acids derived from DNL or FFA uptake are incorporated into triglycerides and exported as VLDL within less than 1 h *(31)*. However, these exogenous and de novo synthesized fatty acids partially rotate through the large cellular triglyceride storage pool, which has a turnover time of several days to weeks *(23, 30)*. These cellular triglycerides may have been generated days ago when the SCD1 activity was different. Fatty acids from this storage pool may dilute SCD1 products at the time of measurement and therefore may also influence the SCD1 activity index in secreted VLDL-TGs. Because oleate is enriched in hepatic TG (42.5% vs 39.1%, hepatic TG vs VLDL-TG, $P < 0.0001$, online Supplemental Table 1) and 13 times more prevalent than palmitoleate, this dilution would mainly affect the ratio of 18:1 to 18:0. Additionally, the 18:1/18:0 ratio may be more susceptible to influences by dietary

[5] Human genes: *FASN*, fatty acid synthase; *ACACA*, acetyl-Coenzyme A carboxylase alpha; *SREBF1*, sterol regulatory element binding transcription factor 1 *SCD*, stearoyl-CoA desaturase (delta-9-desaturase).

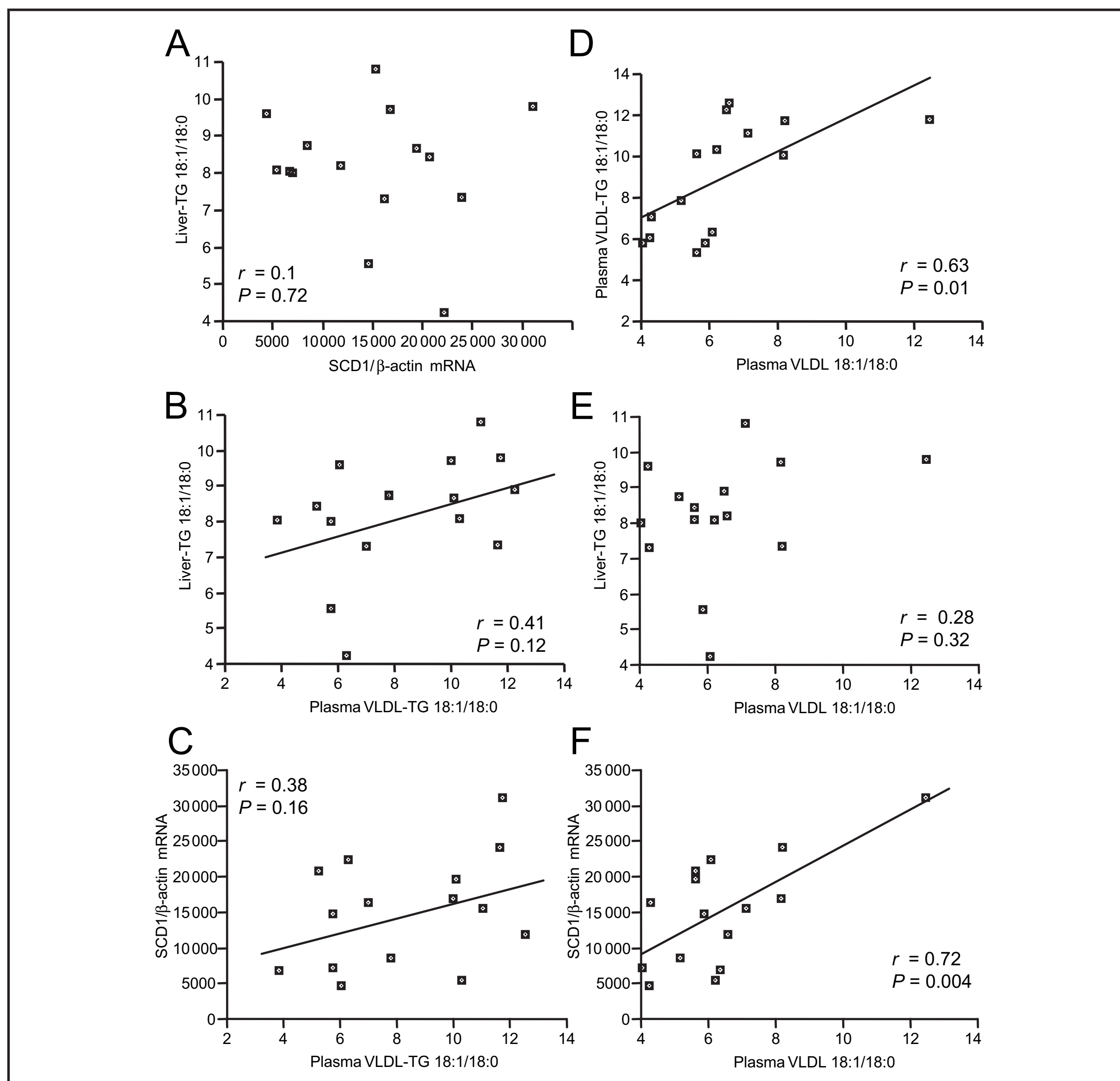

Fig. 2. Univariate relationships between the 18:1/18:0 ratio of hepatic and plasma VLDL fatty acid fractions and hepatic SCD1 mRNA expression are displayed.

(A), The 18:1/18:0 ratio in liver triglycerides is not correlated with hepatic SCD1 mRNA expression, normalized for β-actin. (B), The 18:1/18:0 ratio in liver triglycerides shows a trend towards a positive correlation with the 18:1/18:0 ratio in VLDL triglycerides, which are assembled and secreted from the liver. (C), Hepatic SCD1 mRNA expression, normalized for β-actin, tends to a positive correlation with the 18:1/18:0 ratio in VLDL triglycerides. (D), 18:1/18:0 ratio in VLDL triglycerides is closely associated with the 18:1/18:0 ratio of total VLDL fatty acids. (E), The 18:1/18:0 ratio in liver triglycerides strongly correlates with the 18:1/18:0 ratio in total VLDL fatty acids. (F), Hepatic SCD1 mRNA expression, normalized for β-actin is positively correlated with the 18:1/18:0 ratio in total VLDL fatty acids.

fat than the 16:1/16:0 ratio. Dietary lipids only contain small amounts of palmitoleate, whereas oleate is the most abundant dietary fatty acid and could primarily influence the 18:1/18:0 ratio *(18)*. Interestingly, in the short-lived hepatic lipid fractions PL *(32)* and FFA, the 18:1/18:0 ratio is significantly associated with hepatic SCD1 mRNA expression and the VLDL-TG 18:1/18:0 ratio (Table 2). It can be speculated that the 18:1/18:0 ratio in VLDL-TG more closely reflects long-term average hepatic SCD1 activity.

Table 3. 16:0/18:2 DNL index and expression of fatty acid synthesis genes *FASN*, *ACACA*, and *SREBP-1*.[a]

	Liver-TG DNL index		VLDL-TG DNL index		VLDL DNL index	
	r	*P*	*r*	*P*	*r*	*P*
VLDL-TG DNL	0.89	**<0.0001**			0.91	**<0.0001**
VLDL DNL	0.88	**<0.0001**	0.91	**<0.0001**		
FASN/β-actin mRNA	0.51	**0.05**	0.44	***0.10***	0.33	0.24
ACACA/β-actin mRNA	0.59	**0.02**	0.62	**0.01**	0.49	***0.08***
SREBP-1/β-actin mRNA	0.49	***0.07***	0.61	**0.02**	0.47	***0.09***

[a] Univariate relationships between hepatic fatty acid-ratios, VLDL fatty acid-ratios and hepatic gene expression. Results of linear regression analysis of 15 individuals are displayed. Significant differences are highlighted in bold, trends in italic.

The determination of fatty acid indices in VLDL-TG requires a long sample preparation time that involves ultracentrifugation, TLC separation, fatty acid transesterification, and gas chromatography. This tedious and time-consuming procedure limits this analysis to small sample sizes. Because TG are the main source of fatty acids in VLDL *(18, 30)*, we compared the VLDL-TG to the total VLDL fatty acid composition. The activity indices in total VLDL were almost identical, and the total VLDL SCD1 activity index also reflected hepatic fatty acid composition and mRNA expression. Therefore it may be feasible to use this parameter in larger studies.

Our data show, however, that unfractionated fatty acids from tissue samples must be interpreted with great caution and that the analysis of defined lipid fractions is preferable. Each of the 5 analyzed TLC lipid fractions from liver tissue showed clear signs of fatty acid partitioning and had a characteristic fatty acid composition. In agreement with earlier reports *(33)* the amount of fatty acids from the TG fraction increased ($r = 0.96$, $P < 0.0001$) with increasing liver TG content (0.6%–9%), whereas the fatty acids from the PL fraction remained unchanged ($r = -0.13$, $P = 0.28$). Because the TG fraction contained significantly less stearate and polyunsaturated fatty acids (C20:3N6, C20:4N6, C22:6N3) and more oleate than the PL fraction, the indices of elongase, DNL, and SCD1 activity show strong differences between the hepatic lipid fractions. Such differences may have a major influence if total, unfractionated fatty acids are analyzed in tissue samples with different TG content *(34)*. An increase in the triglyceride fraction would lead to a positive correlation between TG content and the desaturase and DNL indices as well as a negative correlation between TG content and elongase index and polyunsaturated fatty acid content solely due to the increasing proportion of TG-derived fatty acids. Similar difficulties may occur when fatty acid ratios from total plasma lipids are analyzed *(15, 18, 19)*. Therefore the analysis of defined lipid fractions is important to avoid misinterpretation of the fatty acid ratios as enzyme activity indices.

The determination of Δ5 and Δ6 desaturases and elongase indices in VLDL-TG or total VLDL does not provide any information on the activity indices of these enzymes in hepatic lipids and should not be interpreted. The ratio of 16:0/18:2 in hepatic TG has been suggested as a readout of DNL and was evaluated under strictly controlled nutritional conditions *(13, 23)*. In the individuals included in this study we were able to demonstrate that this DNL index in VLDL closely mirrored the hepatic TG fraction and is associated with mRNA expression levels of key hepatic lipogenic enzymes FAS, ACC, and SREBP-1. The determination of the plasma VLDL-TG DNL index may therefore also provide useful information on hepatic fatty acid synthesis in individuals on a habitual diet.

Although our study yielded a number of interesting and highly significant observations, one clear limitation of the study is the small sample size and therefore limited power to detect weaker associations. Accordingly, we were unable to detect a clear correlation between hepatic SCD1 expression and the VLDL-TG 18:1/18:0 ratio that may be detectable only in a larger study. However, simultaneously obtained hepatic tissue and plasma samples are rarely available, and obtaining liver biopsy samples from healthy individuals for research purposes is ethically not acceptable. Additionally, we did not specifically study a group of individuals who suffered from obesity-related diseases and therefore we cannot exclude the possibility that different results may have been obtained with severely obese study participants.

In conclusion, we demonstrated for the first time that the SCD1 activity index in fasting plasma VLDL may be used as a marker of hepatic SCD1 expression in humans and can be determined noninvasively from routine blood samples in clinical studies.

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