Fatty acids in multiple circulating lipid fractions reflects the composition of liver triglycerides in humans

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Short Communication

Fatty acids in multiple circulating lipid fractions reflects the composition of liver triglycerides in humans

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SUMMARY

Background and aims: Fatty acids (e.g. 16:1n-7) and desaturase indices (e.g. stearoyl-CoA desaturase, SCD) in plasma cholesteryl esters (CE) and phospholipids (PL) are used as biomarkers of dietary fat quality and lipid metabolism and are associated with disease outcomes. Endogenously produced circulating fatty acids are believed to reflect composition of the liver, yet little data exist to support such relationship. We investigated associations between circulating fatty acids and fatty acids within the liver.
Methods: Liver biopsies and blood were collected from n = 60 patients with non-alcoholic fatty liver disease. Fatty acids in CE, PL and triglycerides (TG) in plasma and liver were analyzed using gas chromatography. Associations were assessed using Spearman rank correlations.
Results: Overall, fatty acids and desaturase indices in plasma PL and TG showed moderate–strong correlations with fatty acids and desaturase indices in corresponding lipid fractions in liver. For plasma CE, 16:1n-7 and SCD were correlated with 16:1n-7 and SCD in liver CE. Noteworthy, fatty acids in plasma CE and PL also showed moderate–strong correlations with fatty acids in liver TG (e.g. r = 0.82–0.87 for 16:1n-7 and r = 0.77 for SCD).
Conclusion: We demonstrate that fatty acids in circulating lipid fractions, including CE, TG and PL, reflects the composition of liver TG in humans, suggesting that circulating fatty acids might be useful biomarkers for the fatty acid composition of the liver. As liver tissue is rarely available in cohort studies, our findings could enhance our understanding of plasma fatty acids as markers of hepatic lipid metabolism and their links to metabolic diseases.

1. Introduction

Fatty acid composition in circulating lipid fractions is a useful biomarker of dietary intake and metabolism. Plasma phospholipids (PL) and/or cholesteryl esters (CE) are often used in large observational studies, and the proportion of individual fatty acids in these fractions have been robustly associated with disease outcomes. For example, the proportion of palmitoleic acid (16:1n-7) and the stearoyl-CoA desaturase (SCD) index in plasma PL and/or CE have been positively associated with cardiovascular disease (CVD) mortality, total mortality and incidence of type 2 diabetes and the metabolic syndrome [1,2]. Similarly, even-chained saturated fatty acids (SFA) in plasma PL have been positively associated with type 2 diabetes [3]. Fatty acids in circulating PL and CE are often assumed to reflect the composition of the liver, and for

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SFA and monounsaturated fatty acids in particular to reflect metabolic processes such as hepatic *de novo* lipogenesis (DNL) and fatty acid desaturation [1,2]. Desaturation of *de novo* synthesized palmitate into 16:1n-7 in the liver may protect the liver from excessive levels of harmful palmitate, explaining why SCD may be upregulated in parallel with DNL and thus a potential biomarker of DNL. However there is sparse data from human studies to substantiate these assumptions although the SCD index in total serum was strongly associated with the SCD index in the liver in eight severely obese subjects [4]. Liver biopsies are difficult to obtain, especially in larger cohorts and from healthy participants. Circulating biomarkers reflecting the composition of the liver would thus be highly valuable to enable larger-scale investigations of the importance of hepatic fatty acid profile for clinical outcomes. Thus, the objective of the present study was to investigate the associations between fatty acids in multiple circulating lipid fractions and fatty acids within the liver.

2. Material and methods

2.1. Subjects

Patients were recruited from the Departments of Gastroenterology and Hepatology Hospital and from the Swedish CArdio-Pulmonary BioImage Study "SCAPIS" (scapis.org). Inclusion criteria: individuals aged 18–70 with clinically suspected NAFLD and at least one of the following: imaging indicative of NAFLD, ALT more than 1,5 × upper limit of normal, CK18 M30 more than 180 U/L and/or biopsy showing NAFLD within three months prior to screening visit. Exclusion criteria included other liver diseases and high intake of alcohol. All patients provided written informed consent and the study was approved by the Swedish Ethical Review Authority.

2.2. Liver biopsy and blood sample

The liver biopsies were obtained from the right liver lobe under ultrasound guidance, and were assessed by two liver pathologists using the 'steatosis, activity, fibrosis (SAF)' histological scoring system. A venous blood sample was obtained from the arm and plasma was separated by centrifugation. Both liver biopsies and blood samples were collected after ≥6 h of fasting.

2.3. Analysis of fatty acids

Fatty acids in liver and plasma lipid fractions were analyzed using gas chromatography. CE, PL and TG fractions were separated by thin-layer chromatography. The level of individual fatty acids in the respective fractions are presented as the proportion of total fatty acids (area %). Desaturase activity indices were calculated as product-to-precursor ratios as follows: SCD (16:1n-7/16:0), delta 5 desaturase (D5D, 20:4n-6/20:3n-6), delta 6 desaturase (D6D, 18:3n-6/18:2n-6) in the respective lipid fractions.

2.4. Statistical analysis

Non-parametric correlations between fatty acids in plasma CE, PL and TG with fatty acids in liver CE, PL and TG are presented as Spearman rho. Sensitivity analyses were performed, adjusting for age, sex and hyperlipidemia. JMP version 14.1.0 (SAS) was used to analyze the data.

3. Results

Patient characteristics are presented in Table 1. Patients were obese and the majority (77%) had non-alcoholic steatohepatitis (NASH).

3.1. Correlations between fatty acids in liver and plasma

Table 2 shows the correlations between fatty acids in plasma TG, PL and CE and fatty acids in the corresponding fractions in the liver. All fatty acids and desaturase indices in plasma TG showed moderate to strong correlations with fatty acids and desaturase indices in liver TG; the strongest correlation was observed for 16:1n-7 ($r = 0.82$, $p < 0.0001$). Similarly, all fatty acids and desaturase indices in plasma PL, except for the very-long chain SFAs 20:0, 22:0 and 24:0, showed moderate to strong correlation with fatty acids and desaturase indices in liver PL; again the strongest correlation was observed for 16:1n-7 ($r = 0.92$, $p < 0.0001$). In contrast, most fatty acids in plasma CE were weakly correlated with fatty acids in liver CE; a notable exception was 16:1n-7 which was strongly correlated ($r = 0.73$, $p < 0.0001$). Associations were not appreciably affected by adjustment for age, sex and hyperlipidemia (Supplementary Table 1).

As many studies have observed robust associations between fatty acids and desaturase indices in plasma CE and PL with disease outcomes and metabolic disturbances, we also investigated how well plasma fatty acids and desaturase indices in these fractions correlated with fatty acids and desaturase indices in liver TG. Table 3 demonstrates that multiple fatty acids and desaturase indices in plasma CE and PL are strongly correlated with the corresponding fatty acids and desaturase indices in liver TG. The strongest correlation was observed for 16:1n-7 in both plasma CE ($r = 0.87$, $p < 0.0001$) and plasma PL ($r = 0.82$, $p < 0.0001$). Figure 1 illustrates the strong correlations for both 16:1n-7 and SCD in all plasma lipid fractions with 16:1n-7 and SCD in liver TG. Associations were not appreciably affected by adjustment for age, sex and hyperlipidemia (Supplementary Table 2).

4. Discussion

We demonstrate that fatty acids and desaturase indices in plasma CE, PL and TG are moderately to strongly associated with fatty acids in liver TG in humans. The strongest associations were observed for 16:1n-7, the desaturation product of SCD in all lipid fractions. This finding is compatible with the fact that dietary levels

Table 1
Population characteristics.

n, (M/F)	60 (36/24)
Age, years	58 (49–65)
BMI	30.5 ± 3.5
NASH, n (%)	46 (77)
Fibrosis stage 0/1/2/3/4	4/32/19/3/2
SAF ballooning (% 0/1/2)	5/83/12
SAF lobular inflammation (% 0/1/2)	22/77/2
Liver fat (%)	15.9 (9.9–23.4)
Platelets (10^9/L)	234 ± 56
Albumin (g/L)	39.6 ± 2.9
Systolic BP, mmHg	138 ± 12
Diastolic BP, mmHg	83 ± 7
Diabetes[a], n (%)	23 (38)
Hypertension[a], n (%)	30 (50)
Hyperlipidemia[a], n (%)	12 (20)

Data presented as n, mean ± SD or median (Q1-Q3). Abbreviations: BP, blood pressure.
[a] n = 52.

Table 2
Correlations between fatty acids in corresponding fractions in liver and plasma.

Plasma CE			Plasma PL			Plasma TG		
Liver CE	r_s	p-value	**Liver PL**	r_s	p-value	**Liver TG**	r_s	p-value
14:0	0.11	0.41	14:0	0.5	<0.0001	14:0	0.66	<0.0001
15:0	0.02	0.89	15:0	0.59	<0.0001	15:0	–	–
16:0	0.16	0.23	16:0	0.42	0.0008	16:0	0.76	<0.0001
16:1n-7	0.73	<0.0001	16:1n-7	0.92	<0.0001	16:1n-7	0.82	<0.0001
17:0	–	–	17:0	0.72	<0.0001	17:0	–	–
18:0	0.06	0.67	18:0	0.55	<0.0001	18:0	0.57	<0.0001
18:1n-9	0.26	0.044	18:1n-9	0.72	<0.0001	18:1n-9	0.73	<0.0001
18:1n-7	0.09	0.48	18:1n-7	0.77	<0.0001	18:1n-7	0.59	<0.0001
18:2n-6	0.27	0.035	18:2n-6	0.84	<0.0001	18:2n-6	0.79	<0.0001
18:3n-6	−0.01	0.94	18:3n-6	0.68	<0.0001	18:3n-6	0.58	<0.0001
18:3n-3	0.14	0.28	18:3n-3	0.71	<0.0001	18:3n-3	0.67	<0.0001
20:3n-6	0.27	0.038	20:3n-6	0.84	<0.0001	20:3n-6	0.43	0.0007
20:4n-6	0.27	0.034	20:4n-6	0.85	<0.0001	20:4n-6	0.54	<0.0001
20:5n-3	0.11	0.42	20:5n-3	0.9	<0.0001	20:5n-3	0.5	<0.0001
22:5n-3	–	–	22:5n-3	0.54	<0.0001	22:5n-3	0.6	<0.0001
22:6n-3	−0.07	0.61	22:6n-3	0.79	<0.0001	22:6n-3	0.53	<0.0001
20:0	–	–	20:0	0.16	0.22	20:0	–	–
22:0	–	–	22:0	0.07	0.58	22:0	–	–
24:0	–	–	24:0	0.09	0.47	24:0	–	–
SCD	0.44	0.0004	SCD	0.92	<0.0001	SCD	0.78	<0.0001
D5D	0.42	0.013	D5D	0.92	<0.0001	D5D	0.49	<0.0001
D6D	0.07	0.58	D6D	0.66	<0.0001	D6D	0.56	<0.0001

Abbreviations: CE, cholesteryl esters; PL, phospholipids; TG, triglycerides; SCD, stearoyl-CoA desaturase; D5D, delta 5 desaturase; D6D, delta 6 desaturase.

of 16:1n-7 is very low, and thus the plasma levels mainly reflect desaturation of 16:0 to 16:1n-7 in the liver [2]. Noteworthy, these associations were equally strong in plasma CE and PL as in plasma TG. Our findings have implications for the interpretation of fatty acid data in observational studies and suggest that multiple fatty acids in circulating lipid fractions, including CE and PL, reflects the composition of liver TG and may thus be used as easily obtainable biomarkers. A strong correlation between SCD in plasma CE and liver TG has previously been demonstrated in rats [5] and in a small set ($n = 8$) of severely obese human subjects [4]. We can now confirm and expand this in a larger human population with NAFLD.

Data on fatty acid composition in plasma, most commonly in CE and PL, is frequently used in both observational and interventional settings and there is ample evidence linking e.g. 16:1n-7 and SCD indices to increased cardiometabolic risk [2]. Our findings are important as they suggest that multiple fatty acids and desaturase indices, also in circulating CE and PL, may reflect liver TG and thus a way to assess fatty acid profile in the liver without the need for liver biopsies. However, it should be noted that apart from circulating fatty acids reflecting liver fatty acids, there are also significant correlations between circulating fatty acids (e.g. 16:1n-7 and SCD index in CE) and adipose tissue TG (r~0.5) [6]. Thus, this points towards apparent inter-correlations between different lipid compartments and tissues which are to some extent fatty acid-dependent and determined by dietary intake as well as desaturation and elongation rates. By using a lipidomic methodology the liver was shown to contain more 16:0 and long-chain PUFA than adipose tissue in eight severely obese subjects [4]. Furthermore, although 16:1n-7 proportions were not higher in liver than adipose tissue, the serum SCD index (16:1n-7/16:0) was closely associated to that in the liver ($r = 0.86$) but not adipose tissue [4], thus in line with our study. Although adipose tissue biopsies were not available in the current study and thus no direct comparison could be made, when the current and previous evidence are taken together it suggests that fatty acids (at least e.g. 16:1n-7 and SCD) in circulating CE and PL are more strongly associated with liver TG than adipose tissue TG.

Table 3
Correlations between fatty acids in plasma CE and PL and liver TG.

Plasma CE			Plasma PL	
Liver TG	r_s	p-value	r_s	p-value
14:0	0.81	<0.0001	0.64	<0.0001
16:0	0.51	<0.0001	0.5	<0.0001
16:1n-7	0.87	<0.0001	0.82	<0.0001
18:0	0.04	0.77	−0.09	0.5
18:1n-9	−0.14	0.3	−0.13	0.33
18:1n-7	0.17	0.19	0.38	0.003
18:2n-6	0.67	<0.0001	0.42	0.0007
18:3n-6	0.43	0.0007	0.19	0.14
18:3n-3	0.51	<0.0001	0.52	<0.0001
20:3n-6	0.18	0.17	0.06	0.64
20:4n-6	0.24	0.07	0.23	0.08
20:5n-3	0.29	0.03	0.34	0.008
22:5n-3	–	–	0.21	0.11
22:6n-3	0.44	0.0005	0.48	<0.0001
SCD	0.77	<0.0001	0.77	<0.0001
D5D	0.61	<0.0001	0.57	<0.0001
D6D	0.72	<0.0001	0.59	<0.0001

Abbreviations: CE, cholesteryl esters; PL, phospholipids; TG, triglycerides; SCD, stearoyl-CoA desaturase; D5D, delta 5 desaturase; D6D, delta 6 desaturase.

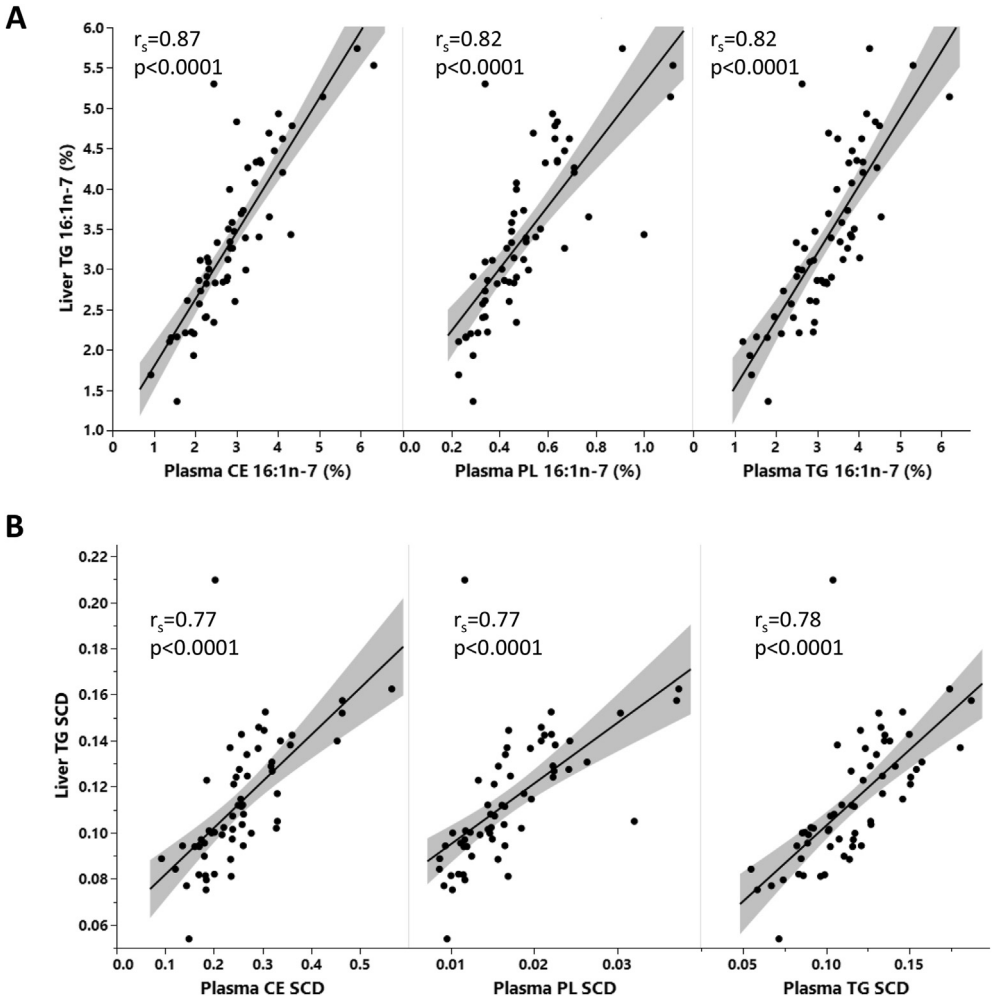

Fig. 1. Correlations between 16:1n-7 and SCD in plasma CE, PL and TG with 16:1n-7 and SCD in liver TG. Abbreviations: CE, cholesteryl esters; PL, phospholipids; TG, triglycerides; SCD, stearoyl-CoA desaturase.

Both 16:1n-7 and SCD has been suggested to reflect hepatic DNL [7]. The end product of hepatic DNL is the saturated fatty acid palmitate (16:0), which is also a substrate for ceramide synthesis. Circulating ceramides are linked to both insulin resistance and CVD and changes in circulating ceramide species is positively associated with changes in both 16:1n-7 and SCD in plasma CE in humans [8]. Furthermore, the insulin resistant human liver is characterized by elevated levels of both ceramides and saturated- and mono-unsaturated fatty acids [9]. Thus, speculatively, elevated levels of 16:1n-7 and SCD in circulating lipid fractions may indicate both elevated hepatic DNL and ceramide synthesis, partly explaining the link to cardiometabolic diseases.

The current strong correlations with 16:1n-7 in particular accord with controlled intervention studies showing that changes in plasma fatty acids such as the SCD index are closely associated with the changes in total liver TG [10].

Strengths of our work includes the availability of both liver biopsies and plasma samples (obtained at the same clinical visit), the analysis of fatty acid composition in multiple fractions in both compartments, the inclusion of both males and females and that the population consisted both of subjects having NASH as well as fatty liver without NASH, thus increasing generalizability across the NAFLD spectrum. Limitations include the cross-sectional design and that all subjects had NAFLD; caution is thus advised if extrapolating the results to populations without NAFLD. Furthermore, the associations between fatty acids in different fractions should be interpreted cautiously due to the inherent differences in fatty acid profiles between fractions; i.e. CE is dominated by 18:2n-6 (~50%) whereas PL is dominated by 16:0 (~30%) and TG by 18:1n-9 (~40%).

In conclusion, the proportions of multiple fatty acids and desaturase indices in circulating CE, PL and TG reflect the composition of liver TG in humans with NAFLD, suggesting that circulating fatty acids may be used as easily obtainable biomarkers for the fatty acid composition of the liver. This enables and facilitates larger-scale cohort studies of the importance of hepatic fatty acid profile for clinical outcomes.

Author contributions

Fredrik Rosqvist: Formal analysis, Writing – Original Draft, Visualization, **Michael Fridén**: Writing – Review & Editing, **Johan Vessby:** Investigation, Resources, Writing – Review & Editing, **Paul Hockings:** Writing – Review & Editing, **Johannes Hulthe:** Writing – Review & Editing, **Anders Gummesson:** Writing – Review & Editing, **Heiko G. Niessen:** Writing – Review & Editing, **Christian Schultheis:** Writing – Review & Editing, **Alkwin Wanders:** Investigation, Writing – Review & Editing, **Håkan Ahlström:** Investigation, Resources, Writing – Review & Editing, **Fredrik Rorsman:** Investigation, Resources, Writing – Review & Editing, **Ulf Risérus:** Writing – Review & Editing, Funding acquisition, Conceptualization, Resources.

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Conflicts of interest

Heiko G. Niessen and Christian Schultheis are employees of Boehringer Ingelheim Pharma GmbH & Co. KG. The other authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2022.02.005.

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