

Lipidomic Analysis Reveals Alterations in Hepatic FA Profile Associated With MASLD Stage in Patients With Obesity

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Abstract

Context: Metabolic dysfunction-associated steatotic liver disease (MASLD) is characterized by the intracellular lipid accumulation in hepatocytes. Excess caloric intake and high-fat diets are considered to significantly contribute to MASLD development.

Objective: To evaluate the hepatic and serum fatty acid (FA) composition in patients with different stages of MASLD, and their relationship with FA dietary intake and MASLD-related risk factors.

Methods: This was a case-control study in patients with obesity undergoing bariatric surgery at a university hospital between January 2020 and December 2021. Participants were distributed in 3 groups: no MASLD (n = 26), steatotic liver disease (n = 33), and metabolic dysfunction-associated steatohepatitis (n = 32). Hepatic and serum FA levels were determined by gas chromatography-mass spectrometry. Nutritional status was evaluated using validated food frequency questionnaires. The hepatic expression of genes involved in FA metabolism was analyzed by reverse transcription quantitative polymerase chain reaction.

Results: The hepatic, but not serum, FA profiles were significantly altered in patients with MASLD compared with those without MASLD. No differences were observed in FA intake between the groups. Levels of C16:0, C18:1, and the C18:1/C18:0 ratio were higher, while C18:0 levels and C18:0/C16:0 ratio were lower in patients with MASLD, being significantly different between the 3 groups. Hepatic FA levels and ratios correlated with histopathological diagnosis and other MASLD-related parameters. The expression of genes involved in the FA metabolism was upregulated in patients with MASLD.

Conclusion: Alterations in hepatic FA levels in MASLD patients were due to enhancement of de novo lipogenesis in the liver.

Key Words: fatty acids, lipidomics, obesity, liver, metabolic dysfunction-associated steatotic liver disease, steatohepatitis

Abbreviations: ACC, acetyl-CoA carboxylase alpha; *ACLY*, ATP citrate lyase; ALT, alanine aminotransferase; ANOVA, analysis of variance; Apo-AI, apolipoprotein A-I; Apo-B, apolipoprotein B; AST, aspartate aminotransferase; BMI, body mass index; *DGAT1*, diacylglycerol O-acyltransferase 1; *DGAT2*, diacylglycerol O-acyltransferase 2; DNL, de novo lipogenesis; ECFA, even chain fatty acid; *ELOVL6*, fatty acid elongase 6; FA, fatty acids; *FASN*, fatty acid synthase; GC-MS, gas chromatography-mass spectrometry; GGT, gamma-glutamyltransferase; HbA1c, glycated hemoglobin; HDLc, high-density lipoprotein cholesterol; HOMA-IR, Homeostasis Model Assessment of Insulin Resistance; LDLc, low-density lipoprotein cholesterol; MASH, metabolic dysfunction-associated steatohepatitis; MASL, metabolic dysfunction-associated steatotic liver; MASLD, metabolic dysfunction-associated steatotic liver disease; MUFA, monounsaturated fatty acid; OCFA, odd chain fatty acid; PUFA, polyunsaturated fatty acid; SAF, Steatosis Activity Fibrosis; *SCD1*, stearoyl-CoA desaturase; SFA, saturated fatty acid; TC, total cholesterol; TG, triglyceride; VLDLc, very low-density lipoprotein cholesterol.

Metabolic dysfunction-associated steatotic liver disease (MASLD; formerly known as nonalcoholic fatty liver disease or metabolic dysfunction-associated fatty liver disease) is one of the leading causes of hepatic disease worldwide with an estimated prevalence of 30% and represents a major economic burden for health systems (1-3). MASLD includes metabolic dysfunction-associated steatotic liver (MASL) and metabolic dysfunction-associated steatohepatitis (MASH), which may evolve into fibrosis and cirrhosis and ultimately to hepatocellular carcinoma and liver failure (4, 5). MASLD is not only associated with the development of primary liver cancer but has also been linked to an increased risk of extrahepatic cancers such as gastrointestinal, breast, and genitourinary cancer in the long-term (6, 7).

MASLD is characterized by lipid accumulation in the liver together with metabolic and cardiovascular conditions including obesity, type 2 diabetes mellitus, dyslipidemia, or hypertension (3). Excessive caloric intake, obesity, insulin resistance, genetic modifiers, and, more recently, the gut microbiome have been described to play a critical role in the development and progression of MASLD (8, 9). The intracellular accumulation of lipids in hepatocytes represents the hallmark of MASLD. Previous studies have provided valuable information on the alterations in hepatic fatty acid (FA) levels associated with human MASLD pathogenesis. Thus, increased hepatic accumulation of saturated FAs (SFAs) and monounsaturated FAs (MUFAs), as well as reduced levels of polyunsaturated FAs (PUFAs), has been reported in several lipidomic studies performed in liver tissue samples of patients with MASLD (10). Remarkably, elevated levels of certain hepatic SFAs such as palmitic acid (C16:0) or stearic acid (C18:0) can promote liver damage by inducing lipotoxicity and insulin resistance, which may influence the development and progression of MASLD (11, 12). Hepatic alterations of FA content are a consequence of imbalances in lipid homeostasis in the liver with increased uptake of FAs and de novo lipogenesis (DNL), while export and oxidation are insufficient to normalize FA levels (13).

Epidemiological studies have also suggested that excessive calorie intake or diets rich in fats together with a sedentary lifestyle are major risk factors for MASLD development (14). Thus, high consumption of SFAs may promote insulin resistance and could be associated with a higher risk of MASLD development and progression (15-17). On the other hand, diets rich in n-3 PUFAs have been described to exert anti-inflammatory properties, suppress lipogenesis, and stimulate FA oxidation, thus preventing diet-induced hepatic steatosis (18). However, most of these studies have been performed using lean subjects as the control group and, thus, whether elevated FA consumption plays a key role in the accumulation of FA in liver remains unclear.

The purpose of this study was to determine the hepatic FA composition in patients with obesity and biopsy-proven MASLD and whether it relates to serum FA profiles and FA dietary intake. Furthermore, we also investigated if the hepatic FA composition associates with MASLD-related histopathological

and biochemical parameters. In addition, we evaluated the expression of genes encoding enzymes involved in the FA metabolism in the liver and explored the potential correlations that they might have with clinical parameters related to MASLD.

Materials and Methods

Study Design and Participants

In this observational cohort study, we enrolled 91 consecutive patients with obesity undergoing bariatric surgery at the Virgen de la Arrixaca University Hospital (Murcia, Spain) between January 2020 and December 2021. Inclusion criteria included adult subjects (men and women) with body mass index (BMI) ≥ 35 kg/m² or BMI ≥ 30 kg/m² with significant obesity-related comorbidities, duration of obesity ≥ 5 years, and age between 18 and 65 years old. Exclusion criteria were (1) evidence of liver disease other than MASLD; (2) history of alcohol abuse (>30 g daily for men and >20 g daily for women); (3) use of drugs known to promote steatosis; or (4) subjects who declined to participate. Patients were distributed in 3 groups depending on the histological findings in liver biopsies according to the SAF (Steatosis, Activity, Fibrosis) classification system (19): (1) patients with no MASLD (n = 26); (2) patients with MASL (n = 33) defined by the presence of at least grade 1 (5%) liver steatosis, with or without ballooning or lobular inflammation but not both at the same time; and (3) patients with MASH (n = 32), defined by the presence of at least grade 1 steatosis, ballooning, and lobular inflammation, with or without fibrosis.

The study was performed in agreement with the Declaration of Helsinki according to local and national laws and was approved by the Ethics and Clinical Research Committee of the Virgen de la Arrixaca University Hospital (ref. number 2020-2-4-HCUVA) and written informed consent was obtained from each participant.

Anthropometric Measurements

Anthropometric and biochemical measurements were determined as previously described (20). Briefly, the measurements of weight, height, and waist ratio were performed to determine the nutritional status of the participants, and the BMI of the individual was calculated by Quetelet's formula:

$$\text{BMI} = \text{weight in kg}/(\text{height in meters})^2$$

Preoperative clinical data were collected on the day of the surgery after an overnight fast of at least 12 hours and serum was separated by centrifugation. Laboratory measurements including glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDLc), triglycerides (TGs), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), apolipoprotein A-I (Apo-AI), and apolipoprotein B (Apo-B) levels were performed using the Cobas Analyzer c702 (Roche) following standardized methods. Non-HDLc was calculated by subtracting HDLc values from TC. Low-density

lipoprotein cholesterol (LDLc) levels were calculated using Friedewald's formula ($\text{LDLc} = \text{TC} - \text{HDLc} - \text{TG}/5$). The levels of glycated hemoglobin (HbA1c) were measured in blood with the glycohemoglobin analyzer HLC-723G8 (Tosoh Bioscience). Insulin levels were measured using the Cobas Analyzer e801 (Roche). Insulin resistance was determined by the Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) index, and was calculated as $\text{insulin } (\mu\text{U/mL}) \times \text{glucose } (\text{mmol/L}) / 22.5$ (21).

Liver Biopsies Collection and Sample Processing

Liver biopsies of at least 1 cm^2 were obtained from patients on the day of the surgery. The biopsy specimens were divided into 3 parts: 1 part was fixed in 4% buffered neutral formalin (pH 7.4), processed, and paraffin-embedded for histological assessment; a second part was snap-frozen and stored at -80°C for FA profile determination; a third part was collected in 1.5 mL nuclease-free tubes containing 1 mL of RNA stabilization Solution (RNAlater, Sigma) for reverse transcription quantitative polymerase chain reaction analyses, maintained overnight at 4°C and kept in -80°C until use.

Paraffin-embedded 5- μm sections of liver biopsies were stained with hematoxylin and eosin, Masson's trichrome, Periodic acid-Schiff, Prussian blue, and reticulin staining. All biopsies were reviewed and scored by trained liver pathologists from the Virgen de la Arrixaca University Hospital and the Experimental Pathology Platform of the Biomedical Research Institute of Murcia to determine the SAF score (19).

Food Frequency Questionnaires

The dietary habits of the patients were recorded before surgery by trained dietitians using a semiquantitative food frequency questionnaire previously validated in the Spanish population (22, 23) and comprising 146 food items and 9 consumption frequencies ranging from never or rarely to more than 6 times/day. The recorded intakes were representative of their usual diets. Nutrient intakes were calculated with a computer program designed on the basis of Spanish food composition tables. Intakes of FAs were also calculated from Spanish food composition tables (24). Individual nutrient intakes were calculated as the mean daily intake. These calculations allow the estimation of the intake of the major SFAs, MUFAs, and PUFAs, including linoleic acid (18:2n-6), α -linolenic acid (18:3n-3), n-3 FAs of marine origin (20:5n-3 and 22:6n-3), and trans FA content.

Analysis of Fatty Acids

Preparation of FAs from serum included extraction of total lipids using the method described by Folch et al (25) with chloroform:methanol mixture (2:1, v/v) and hydrolysis of extracted lipids by KOH in methanol; 10% boron trifluoride in methanol solution was used to obtain FA methyl esters (FAMEs) and gas chromatography-mass spectrometry (GC-MS) analysis of FA methyl esters was conducted with a GC-MS QP-2010 SE (Shimadzu, Kyoto, Japan) as described previously (26).

Reverse Transcription Quantitative Polymerase Chain Reaction Analysis

Liver samples maintained in RNAlater were homogenized with Trizol (Life Technologies, Carlsbad, CA) using an IKA

T10 Ultra-Turrax equipment (Janke and Kunkel, IKA Lavortechnik, Germany) at 24 000 rpm at 4°C for 1 minute. Total RNA extraction and quality assessment were performed as previously described (26). Only samples with A 260/230 and 260/280 ratios between 1.8 and 2.2 were used for gene expression analysis. cDNA was obtained from RNA following reverse transcription according to the manufacturer's instructions (High-Capacity RNA-to-cDNA kit, Applied Biosystems, USA). Transcriptomic analyses were done using the Power SYBR Green Master mix (Applied Biosystems, USA) in a Fast 7500 Real-Time instrument (Applied Biosystems, USA). Primers are outlined elsewhere (Table S1 (27)), and 18S rRNA was used as housekeeping gene control. Relative expression levels were analyzed using the $2^{-\Delta\text{CT}}$ method. The amplification setup used was 40 runs in 96-well plates with SYBR green detection format. Product melting curve measurement was performed in continuous acquisition with 1°C every 30 seconds.

Statistical Analysis

Data were analyzed using the SPSS 28.0.1.1 software (IBM, NY, USA). All variables were assessed for normality using the Kolmogorov-Smirnov test. Pearson's chi-squared test was performed to evaluate intergroup differences in categorical data. For quantitative measures, the analysis of variance (ANOVA) test, or the Kruskal-Wallis 1-way analysis of variance on ranks were performed to evaluate group differences in those variables with and without a normal distribution respectively. The intergroup differences were adjusted by the Bonferroni post hoc analysis. Relationships between pairs of continuous variables were determined based on Spearman's correlation test. All values are given as mean $\pm$ SD. A level of $P < .05$ was considered statistically significant. Figures were represented using the Prism 9.0 program (GraphPad Software Inc., La Jolla, CA, USA).

Results

Characteristics of the Study Participants

The clinical, anthropometric, and laboratory measurements of the patients (26 without MASLD, 33 with MASL, and 32 with MASH) included in the study are shown in Table 1. Dietary intake of macronutrients are presented in Table 1 and dietary FAs are presented elsewhere (Table S2 (27)). Table 2 shows the main histopathological results obtained from liver biopsies (ie, SAF, steatosis, lobular inflammation, hepatocellular ballooning, and fibrosis).

There were no statistically significant differences between the groups regarding age, weight, or BMI. Of the 91 patients included in our study, 70 were females and 21 males with an uneven distribution among the groups ($P = .020$) (Table 1). Compared with those subjects without MASLD, patients with MASL and MASH showed significantly higher levels of HbA1c and lower HDLc. Levels of TG, VLDLc, and Apo-B as well as values of TC/HDLc and LDLc/HDLc ratios were elevated in patients with MASL in comparison to patients without MASLD. Similarly, patients with MASH had higher values of systolic blood pressure, glucose, and GGT than patients without MASLD. On the other hand, non-HDLc was higher in patients with MASL, and AST and ALT in patients with MASH, than in the other groups. Finally, no differences were observed in nutritional habits between any of the

Table 1. Characteristics of the study population

	No MASLD (n = 26)	MASL (n = 33)	MASH (n = 32)	P
Gender (F/M)	25/1	22/11	23/9	.020
Age (years)	43.35 ± 12.3	48.2 ± 10.4	49.0 ± 10.3	.117
Weight (kg)	114.6 ± 9.5	117.1 ± 20.2	115.3 ± 23.2	.906
BMI (kg/m ²)	42.9 ± 4.4	43.4 ± 6.0	43.1 ± 6.3	.926
WC (cm)	121.6 ± 9.1	125.3 ± 12.3	126.9 ± 15.1	.286
SBP (mm Hg)	131.7 ± 18.8 ^a	140.6 ± 19.7 ^{a,b}	144.5 ± 17.9 ^b	.036
DBP (mm Hg)	83.3 ± 9.7	85.3 ± 12.1	83.4 ± 9.6	.536
Glucose (mg/dL)	90.2 ± 10.9 ^a	103.8 ± 25.7 ^{a,b}	122.7 ± 57.2 ^b	.016
HbA1c (%)	5.4 ± 0.4 ^a	6.1 ± 1.1 ^b	6.6 ± 1.7 ^b	< .001
Insulin (μIU/mL)	15.2 ± 12.9	14.3 ± 6.7	20.8 ± 23.2	.317
HOMA-IR	3.5 ± 3.0	3.7 ± 2.0	6.4 ± 8.2	.070
Cholesterol (mg/dL)	155.1 ± 26.9	172.3 ± 31.1	156.9 ± 30.4	.048
HDLc (mg/dL)	48.8 ± 11.9 ^a	40.1 ± 8.9 ^b	42.0 ± 12.3 ^b	.019
LDLc (mg/dL)	81.3 ± 22.7	93.7 ± 28.8	79.2 ± 27.6	.084
VLDLc (mg/dL)	27.8 ± 8.7 ^a	37.6 ± 12.3 ^b	34.8 ± 11.9 ^{a,b}	.009
Non-HDLc (mg/dL)	109.0 ± 21.9 ^a	131.4 ± 30.4 ^b	114.3 ± 29.5 ^a	.003
TC/HDLc	3.4 ± .7 ^a	4.4 ± 1.1 ^b	3.8 ± 0.9 ^{a,b}	.001
LDLc/HDLc	1.7 ± 0.6 ^a	2.4 ± 0.8 ^b	1.9 ± 0.8 ^{a,b}	.007
Triglycerides (mg/dL)	152.2 ± 87.7 ^a	234.2 ± 215.6 ^b	183.1 ± 73.0 ^{a,b}	.007
AST (U/L)	17.0 ± 5.0 ^a	18.4 ± 6.1 ^a	27.3 ± 11.0 ^b	< .001
ALT (U/L)	14.6 ± 4.4 ^a	21.7 ± 10.9 ^a	33.4 ± 15.4 ^b	< .001
GGT (U/L)	19.3 ± 15.7 ^a	23.1 ± 21.6 ^{a,b}	33.7 ± 32.6 ^b	.007
ApoA-I (mg/dL)	145.7 ± 38.1	139.4 ± 34.7	138.7 ± 30.1	.463
ApoB (mg/dL)	77.2 ± 15.7 ^a	98.3 ± 25.8 ^b	87.3 ± 19.9 ^{a,b}	.001
Platelets (10 ³ /μL)	245.4 ± 53.2	247.5 ± 61.3	272.8 ± 79.9	.202
Nutritional habits				
Energy (Kcal/day)	4232.3 ± 2073.8	4574.4 ± 3313.8	5064.7 ± 3479.9	.732
Carbohydrates (g/day)	377.9 ± 214.9	474.2 ± 413.6	502.2 ± 342.7	.379
Fiber (g/day)	51.1 ± 34.4	53.6 ± 41.8	54.8 ± 30.9	.608
Protein (g/day)	208.8 ± 95.1	199.9 ± 115.7	226.5 ± 119.4	.768
Total fat (g/day)	196.5 ± 110.1	193.9 ± 156.2	223.8 ± 185.4	.767

Values are presented as mean ± SD. All variables were assessed for normality using the Kolmogorov–Smirnov normality test. Statistical significance was calculated using 1-way ANOVA in those parameters with a normal distribution (ie, age, BMI, weight, WC, SBP, cholesterol, LDLc, TC/HDLc, LDLc/HDLc, ALT, and platelets), and Kruskal–Wallis for those parameters without normal distribution, considering $P < .05$ to be statistically significant (significant P values are represented in bold), followed by a Bonferroni post hoc analysis for the intergroup differences test in those parameters with a significant P value.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; DBP, diastolic blood pressure; F, female; GGT, gamma-glutamyltransferase; HbA1c, glycated hemoglobin; HDLc, high-density lipoprotein cholesterol; HOMA-IR, homeostatic model assessment of insulin resistance; LDLc, low-density lipoprotein cholesterol; M, male; MASH, metabolic dysfunction–associated steatohepatitis; MASL, metabolic dysfunction–associated liver; MASLD, metabolic dysfunction–associated steatotic liver disease; SBP, systolic blood pressure; TC, total cholesterol; VLDLc, very low density lipoprotein cholesterol; WC, waist circumference.

Different superscript letters indicate statistically significant difference at $P < .05$ within each row between the groups.

study groups neither in energy and macronutrient intake nor in FA consumption (Table 1 and Table S2 (27)).

Lipidomic Analysis of Hepatic and Serum Fatty Acid Levels.

The analysis of hepatic and serum composition of the different FAs according to histopathological diagnosis is shown in Fig. 1 and Fig. 2 (see also Tables S3, S4, S5, and S6 (27)). The analysis of hepatic FA composition revealed that the absolute concentration (expressed as μmol/g tissue) of both total and distinct families of FAs (ie, odd chain fatty acids [OCFAs], even chain fatty acids [ECFAs], MUFAs, and n-6 PUFAs), as well as the n-6/n-3 PUFA ratio, were significantly higher in

patients with MASL and MASH (Table S3 (27)). In addition, when the hepatic FA composition was analyzed as a percentage of total FAs, only MUFAs were significantly higher with the severity of MASLD (Fig. 1A; Table S4 (27)). Moreover, in contrast to the results obtained for relative abundance, the percentages of OCFAs, n-3, and n-6 PUFAs were significantly reduced in the MASL and MASH groups compared with subjects without MASLD.

Similarly, FA species followed a similar pattern as the FA families. Thus, while absolute concentrations of most of the specific FAs analyzed were different between groups, being higher in the MASH group (Table S3 (27)), such changes were not observed when analyzed as a percentage of total FAs (Table S4 (27)). In this regard, while C16:0 and C18:1

levels were higher in both relative abundance and percentage of total FAs, the percentage of C16:1 did not change between the groups (Fig. 2). Whereas C18:0 showed higher concentrations

Table 2. Histopathological findings in livers of the study population

	MASL (n = 33)	MASH (n = 32)	P
SAF score (0/1/2/3/4/5/6/7/8)	0/16/12/4/1/0/0/0/0	0/0/0/8/8/9/4/2/1	< .001
Steatosis (0/1/2/3)	0/25/8/0	0/14/9/9	< .001
Hepatocellular ballooning (0/1/2)	19/13/1	0/24/8	< .001
Lobular inflammation (0/1/2/3)	33/0/0/0	0/23/9/0	< .001
Fibrosis (0/1)	33/0	25/7	< .001

Values are presented as frequencies. Statistical significance was calculated using Pearson's chi-squared test, considering $P < .05$ to be statistically significant (significant P values are represented in bold). Abbreviations: MASH, metabolic dysfunction–associated steatohepatitis; MASL, metabolic dysfunction–associated liver; MASLD, metabolic dysfunction–associated steatotic liver disease; SAF, steatosis, activity, fibrosis.

in the MASL and MASH groups, the percentage to total FAs of C18:0 was drastically lowered with MASLD severity, as values were significantly lower in the MASH group than in the other groups (Fig. 2). On the other hand, no differences were observed in serum FAs between the 3 groups (Tables S5 and S6 (27)).

Correlations Between Selected Hepatic Fatty Acids and Histopathological/Biochemical Parameters.

Correlations analyses were performed to establish the relationship between the different FAs and the different histopathological and biochemical parameters altered (Table 3). We observed a strong positive correlation between hepatic concentrations of total MUFAs, C16:0, and C18:1, and the histopathological parameters including histopathological diagnosis, SAF score, steatosis, hepatocyte ballooning, lobular inflammation, and fibrosis. Furthermore, these FAs were also positively associated with different biochemical parameters related to MASLD such as VLDLc, non-HDLc, TC/HDLc ratio, AST, ALT, GGT, and TG. On the other hand, these correlations followed a negative trend between OCFAs, n-6 PUFAs, n-3 PUFAs, and C18:0 and the histopathological parameters mentioned above. However, the correlations between these FAs and the biochemical

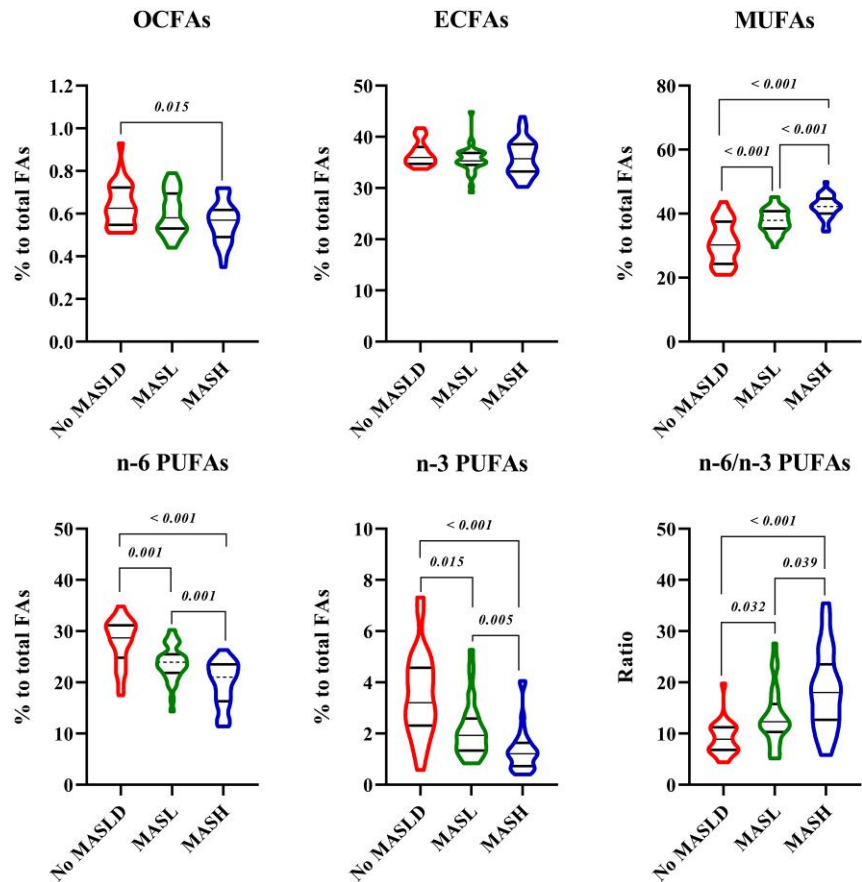


Figure 1. Hepatic levels of fatty acid families quantified by GC-MS and n-6/n-3 polyunsaturated ratio, presented as % of total fatty acids. All variables were assessed for normality using the Kolmogorov–Smirnov normality test. Statistical differences were calculated using ANOVA in those parameters with normal distribution (MUFAs and n-6 PUFAs) or the Kruskal–Wallis test in those parameters without normal distribution, considering $P < .05$ to be statistically significant. Bonferroni post hoc analysis was performed for the intergroup differences test in those parameters with a significant P value. ECFAs, even chain fatty acid; FA, fatty acid; GC-MS, gas chromatography–mass spectrometry; MASH, metabolic dysfunction–associated steatohepatitis; MASL, metabolic dysfunction–associated steatotic liver; MASLD, metabolic dysfunction–associated steatotic liver disease; MUFA, monounsaturated fatty acid; OCFAs, odd chain fatty acid; PUFA, polyunsaturated fatty acid. Medians and quartiles are represented in violin plots.

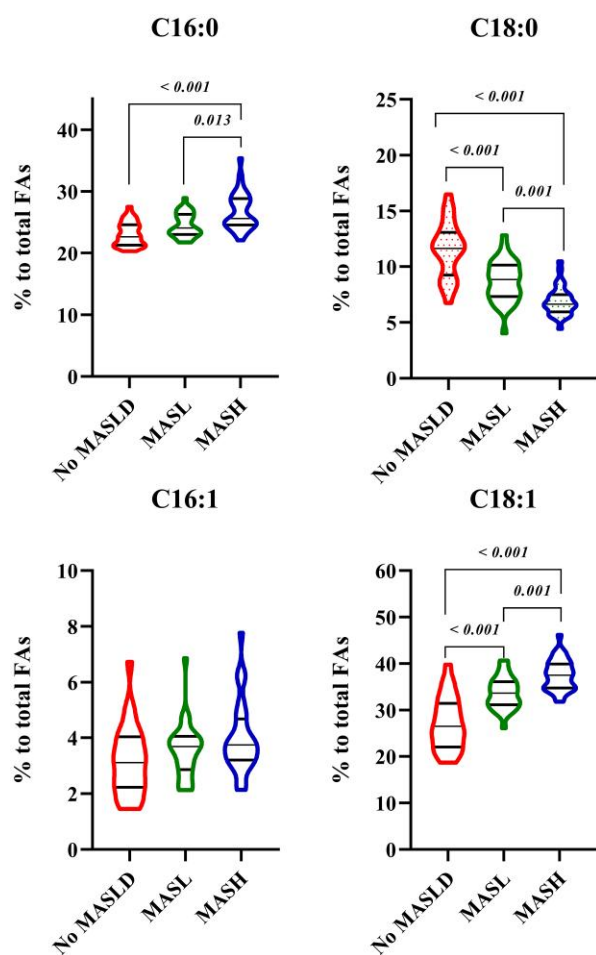


Figure 2. Hepatic levels of individual fatty acids quantified by GC-MS. Represented as % to total FAs. All variables were assessed for normality using the Kolmogorov–Smirnov normality test. Statistical differences were calculated using ANOVA in those parameters with normal distribution (C18:0 and C18:1) or Kruskal–Wallis test in those parameters without normal distribution, considering $P < .05$ to be statistically significant. Bonferroni post hoc analysis was performed for the intergroup differences test in those parameters with a significant P value. FA; fatty acid; GC-MS, gas chromatography–mass spectrometry; MASH, metabolic dysfunction–associated steatohepatitis; MASL, metabolic dysfunction–associated steatotic liver; MASLD, metabolic dysfunction–associated steatotic liver disease. Medians and quartiles are represented in violin plots.

parameters included showed that, while n-6 PUFAs, n-3 PUFAs, and C18:0 were negatively associated with the majority of biochemical parameters included, changes in OCFAs only correlated moderately with HbA1c and ALT. Interestingly, lower levels of n-3 PUFAs and C18:0 were also correlated with higher cholesterol, LDLc/HDLc ratio, and GGT levels (Table 3). No associations were found between serum FA levels and the MASLD-related histopathological and biochemical parameters (Table S7 (27)).

Hepatic Fatty Acid Desaturation and Elongation Indexes and Correlations With Histopathological/Biochemical Parameters.

The C18:0/C16:0, C16:1/C16:0, and C18:1/C18:0 ratios were studied to evaluate the elongation index and the desaturation index (Fig. 3). Results showed that the C18:0/C16:0 ratio

was lower with MASLD severity, being significantly different between the 3 groups. On the other hand, the C18:1/C18:0 ratio, but not the C16:1/C16:0 ratio, was elevated in patients with MASH compared with the MASL and no MASLD groups. Furthermore, such differences were also significant between patients with MASL and those without MASLD.

Correlations between hepatic C18:0/C16:0, C16:1/C16:0, and C18:1/C18:0 ratios and the histopathological/biochemical parameters are shown in Table 4. As expected, except for cholesterol and non-HDLc levels, no correlations were observed between the parameters included and the C16:1/C16:0 ratio. In contrast, the C18:0/C16:0 ratio was negatively and the C18:1/C16:1 positively correlated with all the histopathological parameters included. Also, except for HDLc, the C18:1/C16:1 ratio correlated with the MASLD-biochemical parameters selected. Regarding the C18:0/C16:0 ratio, there was a significant negative correlation with HbA1c, cholesterol, VLDLc, non-HDLc, TC/HDLc, AST, ALT GGT, and TGs.

Expression of Genes Involved in Fatty Acid Synthesis.

The expression of genes involved in FA synthesis was determined in liver biopsies (Fig. 4). The levels of fatty acid synthase (FASN) were significantly upregulated in patients with MASL and MASH compared with patients without MASLD ($P = .032$ and $P = .005$ respectively). In addition, the expression of FA elongase 6 (ELOVL6), diacylglycerol O-acyltransferase 1 and 2 (DGAT1 and DGAT2), and ATP citrate lyase (ACLY) were significantly higher in patients with MASH compared with patients with MASL or without MASLD.

On the other hand, the expression of stearoyl-CoA desaturase (SCD1) was higher in the MASH group compared with those patients without MASLD but not compared with patients with MASL. No significant differences were observed among the groups in the expression levels of the acetyl-CoA carboxylase alpha (ACC) (Fig. 4).

Finally, significant correlations were observed between gene expression of some of the enzymes involved in FA metabolism and histopathological parameters and the FA ratios (Table 5; Table S8, respectively) (27).

Discussion

In this study, we have evaluated the FA composition in hepatic and serum samples of patients with obesity and different grades of MASLD and examined the relationship between the FA composition and different histopathological/biochemical parameters associated with MASLD. Our results revealed that the hepatic FA composition was significantly altered in patients with MASL and MASH compared with a control group without MASLD. Furthermore, we observed a strong correlation between the relative amount of different hepatic FAs (% to total FAs) and histopathological diagnosis as well as those parameters related to MASLD progression including SAF score, hepatocyte ballooning, inflammation, fibrosis, or levels of AST, ALT, and GGT. We also evaluated the serum composition of FA as well as the dietary habits of the studied population, finding no significant differences between study groups.

MASLD is known to be associated with increased lipogenesis and FAs accumulate in the liver entailing a major cause of cell injury and death (10). Previous reports have described that

Table 3. Correlations between hepatic fatty acids and histopathological/biochemical parameters

	OCFAs	MUFAs	n-6 PUFAs	n-3 PUFAs	C16:0	C18:0	C18:1
Histopathological diagnosis	-0.298**	0.690**	-0.617**	-0.604**	0.479**	-0.705**	0.693**
SAF score	-0.331**	0.724**	-0.634**	-0.710**	0.518**	-0.750**	0.726**
Steatosis	-0.293**	0.696**	-0.586**	-0.710**	0.413**	-0.714**	0.719**
Hepatocyte ballooning	-0.299**	0.587**	-0.566**	-0.608**	0.533**	-0.615**	0.563**
Lobular inflammation	-0.268*	0.596**	-0.509**	-0.521**	0.414**	-0.603**	0.592**
Fibrosis	-0.057	0.355**	-0.304**	-0.276**	0.217*	-0.293**	0.360**
HbA1c (%)	-0.222*	0.184	-0.189	-0.203	0.181	-0.225*	0.250*
Cholesterol (mg/dL)	-0.162	0.212*	-0.196	-0.227*	0.164	-0.257*	0.174
HDLc (mg/dL)	-0.011	-0.144	0.094	0.209*	-0.169	0.117	-0.196
VLDLc (mg/dL)	-0.118	0.324**	-0.380**	-0.377**	0.383**	-0.292**	0.289**
non-HDLc (mg/dL)	-0.143	0.262*	-0.223*	-0.328**	0.261*	-0.316**	0.249*
TC/HDLc	-0.154	0.286**	-0.213*	-0.384**	0.287**	-0.306**	0.249*
LDLc/HDLc	-0.092	0.190	-0.112	-0.269*	0.163	-0.228*	0.206
AST (U/L)	-0.047	0.501**	-0.419**	-0.493**	0.289**	-0.508**	0.469**
ALT (U/L)	-0.288**	0.587**	-0.453**	-0.586**	0.292**	-0.587**	0.589**
GGT (U/L)	-0.105	0.297**	-0.206	-0.332**	0.163	-0.255*	0.293**
Triglycerides (mg/dL)	-0.206	0.314**	-0.364**	-0.375**	0.405**	-0.309**	0.288**

Values are presented as the Spearman correlation coefficient. Values in bold represent statistical significance. Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ECFAs, even chain fatty acids; GGT, gamma-glutamyltransferase; HbA1c, glycated hemoglobin; HDLc, high-density lipoprotein cholesterol; LDLc, low-density lipoprotein cholesterol; MUFA, monounsaturated fatty acid; OCFA, odd chain fatty acid; PUFA, polyunsaturated fatty acid; SAF, steatosis, activity, fibrosis; TC, total cholesterol; VLDLc, very low-density lipoprotein cholesterol. **P* < .05. ***P* < .01.

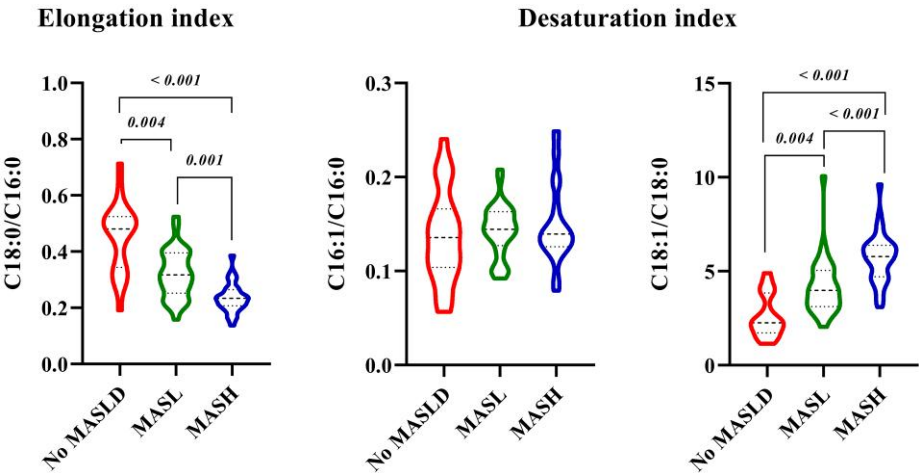


Figure 3. Elongation index and desaturation index in liver samples. All variables were assessed for normality using the Kolmogorov–Smirnov normality test. Statistical differences were calculated using the Kruskal–Wallis test, considering *P* < .05 to be statistically significant. Bonferroni post hoc analysis was performed for the intergroup differences test in those parameters with a significant *P* value. MASH, metabolic dysfunction–associated steatohepatitis; MASL, metabolic dysfunction–associated steatotic liver; MASLD, metabolic dysfunction–associated steatotic liver disease. Medians and quartiles are represented in violin plots.

elevated SFAs such as C18:0 or reduced PUFA consumption is associated with MASLD severity (12, 28). Similarly, several studies have described alterations in the serum FA profile in patients with MASLD compared with healthy subjects (29, 30). However, we did not find any differences between the groups regarding energy intake, macronutrient, and micro-nutrient consumption, and serum levels of FAs, probably because our control group is formed by patients with obesity. Nevertheless, our results agree with previous studies where no alterations were observed in dietary FAs intake or serum

FA levels suggesting that such alterations in hepatic FAs might be due to DNL rather than higher FA consumption (31, 32). A detailed analysis of the hepatic FA composition in our samples showed that absolute concentrations (μmol/g tissue) of all FAs (Table S3 (27)) were higher in patients with MASLD. However, here we performed the analysis based on the relative amount to total FAs (% of total FAs) as it has been suggested to be more useful for the prognosis of MASLD (11, 33). Thus, our results showed that patients with MASL and MASH had an elevated proportion of

Table 4. Correlations between hepatic fatty acid ratios and histopathological/biochemical parameters

	C18:0/ C16:0	C16:1/ C16:0	C18:1/ C18:0
Histopathological diagnosis	−0.686**	0.080	0.729**
SAF score	−0.734**	0.118	0.773**
Steatosis	−0.673**	0.156	0.742**
Hepatocyte ballooning	−0.639**	0.079	0.629**
Lobular inflammation	−0.588**	0.003	0.629**
Fibrosis	−0.305**	0.008	0.342**
HbA1c (%)	−0.221*	−0.210	0.250*
Cholesterol (mg/dL)	−0.257*	0.326**	0.222*
HDLc (mg/dL)	0.116	0.134	−0.149
VLDLc (mg/dL)	−0.313**	0.184	0.300**
non-HDLc (mg/dL)	−0.317**	0.292**	0.289**
TC/HDLc	−0.304**	0.129	0.306**
LDLc/HDLc	−0.212	0.163	0.216*
AST (U/L)	−0.507**	0.151	0.524**
ALT (U/L)	−0.570**	0.133	0.622**
GGT (U/L)	−0.238*	0.147	0.283**
Triglycerides (mg/dL)	−0.336**	0.177	0.310**

Values are presented as the Spearman correlation coefficient. Values in bold represent statistical significance.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyltransferase; HbA1c, glycated hemoglobin; HDLc, high-density lipoprotein cholesterol; LDLc, low-density lipoprotein cholesterol; SAF, steatosis, activity, fibrosis; TC, total cholesterol; VLDLc, very low-density lipoprotein cholesterol.

* $P < .05$.

** $P < .01$.

MUFAs while the levels of SFAs were similar among the groups. We have also observed lower n-6 and n-3 PUFAs levels with higher n-6/n-3 ratio. Elevated n-6/n-3 ratios in both liver and serum have been already described to be associated with MASLD severity. This is probably due to reduced FA oxidation and increased liver triacylglycerol storage as n-3 PUFAs are efficient activators of PPAR- α which in turn induces the expression of genes involved in FA oxidation and degradation and, thus, favoring the accumulation of fat in the liver (16, 34, 35). Interestingly, when the analysis was performed at the species level, we observed that the proportion of C16:0 was significantly elevated in patients with MASH compared with the MASL and no MASLD groups. Furthermore, the levels of C18:0 were lower and C18:1 higher in the MASLD groups (MASL and MASH), being significantly different between the 3 groups. Notably, we did not observe differences in the levels of C16:1 between the groups, in contrast to previous studies where higher levels of C16:1, both in serum and liver, were associated with liver disease development including MASLD (29, 36, 37). Such a discrepancy might be explained by the fact that our control group was composed of patients with morbid obesity, a condition that is already characterized by the abnormal accumulation of lipids in the liver (11), whereas in the other studies the control group included lean subjects. Also, it has to be considered that such studies were performed analyzing the concentrations of FAs, not the % composition.

We have also assessed the associations between the different FAs in both serum and liver samples and MASLD occurrence

and severity in patients with obesity. As expected, circulating levels of FAs showed no associations with any of the parameters analyzed (ie, histopathological diagnosis, SAF score, steatosis, hepatocyte ballooning, lobular inflammation, or fibrosis). Previous studies have described higher levels of circulating levels of FAs. Indeed, the potential use of serum C14:0 and C16:1 as possible biomarkers for MASLD has been previously addressed (38). However, this study was performed comparing lean to overweight and obese patients with MASLD while all our participants were subjects with obesity which suggests that these changes in FA profile suggested by these other studies in serum might be due to obesity grade rather than MASLD stage.

On the other hand, associations between relative concentrations of different hepatic FAs (as relative concentrations and ratios) and MASLD severity parameters were observed. Thus, our results showed a strong positive correlation between MUFAs and the histopathological findings including SAF score, steatosis, hepatocyte ballooning, lobular inflammation, and fibrosis. At the species level, such correlations were also observed for C16:0 and C18:1 levels. Conversely, levels of OCFAs, n-6 PUFAs, n-3 PUFAs, and C18:0 were negatively correlated with the histopathological findings with C18:0 showing the strongest correlation with all the parameters included except for fibrosis. Regarding biochemical parameters, the correlations between FAs and such parameters were less clear as, except for ALT and AST, correlations were weaker, although a certain degree of significance was observed. Insulin resistance and HOMA-IR index are hallmarks of MASLD (9, 39). However, even though we observed a positive trend with MASLD severity, we did not find significant differences between our groups in the levels of insulin or HOMA-IR index, and therefore, no correlations were observed between FAs and these parameters which might be due to the characteristics of our study population.

We next evaluated the FA desaturation and elongation indexes by analyzing different hepatic FA ratios. The analysis showed a lower C18:0/C16:0 ratio and a higher C18:1/C18:0 ratio in patients with either MASL or MASH compared with those without MASLD. These results are consistent with those previously reported by others (36, 40, 41), highlighting that even though the content of FAs is higher in patients with MASL and MASH, not all the FAs increase in the same proportion. Furthermore, contrary to what has been described in the above-mentioned studies (36, 40, 41), the C16:1/C16:0 ratio was not altered in patients with MASL or MASH in our study population. It must be considered that our control group was made up of patients with obesity while these studies used lean volunteers as controls. Regarding the alterations in the C18:0/C16:0 and C18:1/C18:0 ratios, we observed that these strongly correlated with MASLD severity including SAF score, hepatocyte ballooning, lobular inflammation, and fibrosis negatively and positively, respectively, confirming that the composition ratio of FAs is closely associated with the progression of early MASLD to MASH (41).

Finally, we performed the analysis of the gene expression of enzymes involved in FA metabolism in the liver including *FASN*, *SCD1*, *ELOVL6*, *DGAT1*, *DGAT2*, *ACC*, and *ACLY*. The increase in DNL has been previously linked to steatohepatitis due to the accumulation of FAs such as C16:0 and C18:0 (13, 42). In this regard, the alteration of the elongase *ELOVL6* and the desaturase *SCD1* have been reported to be involved in MASH development (43, 44).

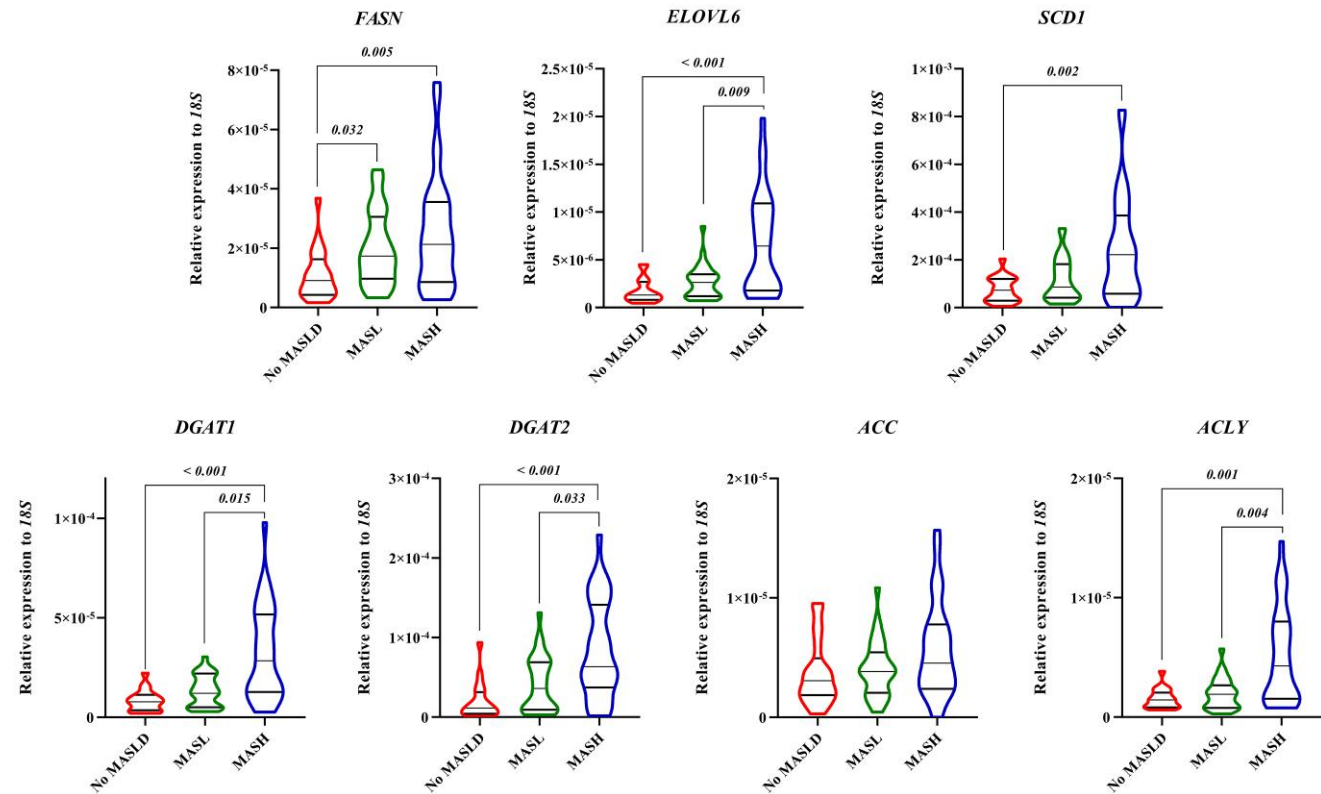


Figure 4. Hepatic expression of genes involved in fatty acid metabolism. The results are represented as mRNA expression levels in liver tissue normalized to 18S. All variables were assessed for normality using the Kolmogorov–Smirnov normality test. Statistical analyses were performed using the Kruskal–Wallis test, considering $P < .05$ to be statistically significant, followed by a Bonferroni post hoc analysis for the intergroup differences test in those parameters with a significant P value. ACC, acetyl-CoA carboxylase; ACLY, ATP citrate synthase; DGAT1, diacylglycerol acyltransferase-1; DGAT2, diacylglycerol acyltransferase-2; ELOVL6, fatty acid elongase 6; FASN, fatty acid synthase; MASH, metabolic dysfunction–associated steatohepatitis; MASL, metabolic dysfunction–associated steatotic liver; MASLD, metabolic dysfunction-associated liver disease; SCD1, stearyl-CoA desaturase-1.

Table 5. Correlations between gene expression levels of fatty acid metabolism enzymes and histopathological parameters

	<i>FASN</i>	<i>ELOVL6</i>	<i>SCD1</i>	<i>DGAT1</i>	<i>DGAT2</i>	<i>ACC</i>	<i>ACLY</i>
Histopathological diagnosis	0.282**	0.339**	0.349**	0.364**	0.320**	0.235*	0.268*
SAF score	0.235*	0.239*	0.266*	0.278**	0.254*	0.161	0.178
Steatosis	0.202	0.163	0.149	0.226*	0.193	0.138	0.097
Hepatocyte ballooning	0.204	0.168	0.232*	0.199	0.169	0.095	0.137
Lobular inflammation	0.214*	0.369**	0.359**	0.333**	0.331**	0.240*	0.324**
Fibrosis	0.203	0.178	0.212	0.175	0.227*	0.155	0.183

Values are presented as the Spearman correlation coefficient. Values in bold represent statistical significance.
Abbreviations: ACC, acetyl-CoA carboxylase; ACLY, ATP citrate synthase; DGAT1, diacylglycerol acyltransferase-1; DGAT2, diacylglycerol acyltransferase-2; ELOVL6, fatty acid elongase 6; FAS, fatty acid synthase; SAF, steatosis, activity, and fibrosis.; SCD1, stearyl-CoA desaturase-1.
* $P < .05$.
** $P < .01$.

ELOVL6, an enzyme involved in the elongation of C16:0 to C18:0 FAs, has been identified as a major player in the progression of MASH by promoting hepatic oxidative stress, inflammatory damage, liver injury, and fibrosis (36, 44). Furthermore, ELOVL6 expression has been positively correlated with steatosis and inflammation (44), which agrees with our observations where overexpression of ELOVL6 was associated with the histopathological diagnosis, SAF score, and inflammation. This increase in ELOVL6 expression might be considered contradictory to our previous statement about

the decreased elongation ratio. However, it should not be neglected that although the C18:0/C16:0 ratio is reduced, the absolute concentrations ($\mu\text{mol/g}$ tissue) concentrations of both C16:0 and C18:0 in patients with MASLD, which explains this potential discrepancy. In addition, we also detected an overexpression of SCD1 mRNA. SCD1 is the enzyme responsible for the desaturation of SFAs C16:0 and C18:0 to their respective MUFAs C16:1 and C18:1 (45). In addition, the inhibition of SCD1 has been described to increase FA oxidation and decrease de novo synthesis and thus steatosis (46).

In this study, we observed a noticeable increase in the SCD1 activity, mainly reflected in the conversion of C18:0 to C18:1, which would support that MASLD is mainly promoted by DNL in our study population. Lastly, we also demonstrated an increase in DGAT activity. Previous studies have described C18:1 to be the preferred substrate for the hepatic synthesis of TGs (31). After several enzymatic steps, DGAT1 and DGAT2 are responsible for the final step of TG synthesis which are either stored in the liver or assembled into VLDL and released into the circulatory system (45, 47). In our study population, the increased expression of both *DGAT1* and *DGAT2* in MASL and MASH patients was strongly correlated with histopathological diagnosis and inflammation and was concomitant with higher circulatory levels of VLDL.

We acknowledge that our study has certain limitations. One of the limitations is the relatively small sample size although the distribution of groups is well-balanced. As this represents a cross-sectional design, it has also inherent limitations related to the nature of the study where causality cannot be elucidated. Also, gender imbalance is another limitation that arises in bariatric surgery studies where the majority of the subjects are females. Nevertheless, the inclusion of patients with obesity in the control group, rather than lean subjects strengthens our study, since it minimizes any possible bias related to BMI differences between the groups. In addition, our careful design, the liver biopsy-driven MASLD definition, the use of gas chromatography-mass spectrometry for FA determination, and the gene expression analysis of FA-metabolizing enzymes in liver tissue samples are also important strengths in this study. Finally, it is also important to highlight that previous studies have mainly based on nonalcoholic fatty liver disease rather than MASLD classification. However, given that such classifications differ on small nuances, we do not expect any significant impact on the results obtained.

Conclusions

In this study, we have shown that the alterations in hepatic FA composition are associated with MASLD severity in patients with obesity. Furthermore, we also have described that neither dietary nor serum FA levels were altered within the groups. At individual levels, changes in all FA families analyzed (except for ECFAs), as well as changes in C16:0, C18:0, and C18:1, were associated with MASLD histopathological and biochemical parameters. The alterations in the FA desaturation and elongation ratios together with the gene expression analysis showed an enhancement of the DNL in the liver mainly driven by C18:1, albeit further research is needed to confirm these results in larger populations. Elucidating how these alterations in the FA synthesis and how C18:1 contributes to the progression of MASLD severity may lead to the development of future prevention and treatment strategies.

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Author Contributions

M.Á.N.-S.: Methodology, Validation, Formal analysis, Investigation, Data curation, Writing—original draft, Writing—review & editing. M.A.M.-S.: Methodology, Validation, Formal analysis, Investigation, Data curation, Writing—review & editing. J.I.M.-M.: Methodology, Validation, Formal analysis, Investigation, Data curation, Writing—review & editing. Andrés Balaguer-Román: Methodology, Validation, Investigation, Writing—review & editing. E.M.-G.: Methodology, Validation, Investigation, Writing—review & editing. V.E.F.-R.: Methodology, Validation, Investigation, Writing—review & editing. M.F.-G.: Methodology, Validation, Formal analysis, Investigation, Data curation, Writing—review & editing. C.M.M.-C.: Methodology, Validation, Formal analysis, Data curation, Investigation, Writing—review & editing. T.S.: Methodology, Validation, Formal analysis, Data curation, Investigation, Writing—review & editing. M.D.F.: Methodology, Validation, Formal analysis, Data curation, Investigation, Writing—review & editing. J.J.H.-M.: Methodology, Validation, Formal analysis, Data curation, Investigation, Writing—review & editing. J.C.F.-G.: Methodology, Validation, Formal analysis, Data curation, Investigation, Writing—review & editing. M.I.Q.-O.: Methodology, Validation, Formal analysis, Data curation, Investigation, Writing—review & editing. A.J.R.-A.: Methodology, Validation, Formal analysis, Data curation, Investigation, Writing—review & editing. A.M.: Conceptualization, Methodology, Validation, Formal analysis, Data curation, Investigation, Writing—review & editing, Visualization, Supervision. B.R.M.: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Data curation, Writing—review & editing, Supervision, Funding acquisition.

Disclosures

The authors declare no conflict of interests.

Data Availability

Some or all datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Ethics Approval

Ethics committee/IRB of Virgen de la Arrixaca University Hospital gave ethical approval for this work (ref. number 2020-2-4-HCUVA).

References

- Rinella ME, Lazarus JV, Ratziu V, *et al.* A multi-society delphi consensus statement on new fatty liver disease nomenclature. *J Hepatol.* 2023;78:1966-1986.
- Riazi K, Azhari H, Charette JH, *et al.* The prevalence and incidence of NAFLD worldwide: a systematic review and meta-analysis. *Lancet Gastroenterol Hepatol.* 2022;7(9):851-861.
- Eslam M, Sanyal AJ, George J, International Consensus Panel. MAFLD: a consensus-driven proposed Nomenclature for metabolic associated fatty liver disease. *Gastroenterology.* 2020;158(7):1999-2014 e1991.
- Anstee QM, Reeves HL, Kotsiliti E, Govaere O, Heikenwalder M. From NASH to HCC: current concepts and future challenges. *Nat Rev Gastroenterol Hepatol.* 2019;16(7):411-428.
- Kumar S, Duan Q, Wu R, Harris EN, Su Q. Pathophysiological communication between hepatocytes and non-parenchymal cells in liver injury from NAFLD to liver fibrosis. *Adv Drug Deliv Rev.* 2021;176:113869.
- Mantovani A, Petracca G, Beatrice G, *et al.* Non-alcoholic fatty liver disease and increased risk of incident extrahepatic cancers: a meta-analysis of observational cohort studies. *Gut.* 2022;71(4):778-788.
- Tarantino G, Crocetto F, Di Vito C, *et al.* Association of NAFLD and insulin resistance with non metastatic bladder cancer patients: a cross-sectional retrospective study. *J Clin Med.* 2021;10(2):346.
- Safari Z, Gerard P. The links between the gut microbiome and non-alcoholic fatty liver disease (NAFLD). *Cell Mol Life Sci.* 2019;76(8):1541-1558.
- Sakurai Y, Kubota N, Yamauchi T, Kadowaki T. Role of insulin resistance in MAFLD. *Int J Mol Sci.* 2021;22(8):4156.
- Hliwa A, Ramos-Molina B, Laski D, Mika A, Sledzinski T. The role of fatty acids in non-alcoholic fatty liver disease progression: an update. *Int J Mol Sci.* 2021;22(13):6900.
- Willis SA, Bawden SJ, Malaikah S, *et al.* The role of hepatic lipid composition in obesity-related metabolic disease. *Liver Int.* 2021;41(12):2819-2835.
- Monserat-Mesquida M, Quetglas-Llabrés MM, Bouzas C, *et al.* Plasma fatty acid composition, oxidative and inflammatory Status, and adherence to the Mediterranean diet of patients with non-alcoholic fatty liver disease. *Antioxidants.* 2023;12(8):1554.
- Ipsen DH, Lykkesfeldt J, Tveden-Nyborg P. Molecular mechanisms of hepatic lipid accumulation in non-alcoholic fatty liver disease. *Cell Mol Life Sci.* 2018;75(18):3313-3327.
- Duwaerts CC, Maher JJ. Macronutrients and the adipose-liver axis in obesity and fatty liver. *Cell Mol Gastroenterol Hepatol.* 2019;7(4):749-761.
- Maciejewska D, Drozd A, Ossowski P, *et al.* Fatty acid changes help to better understand regression of nonalcoholic fatty liver disease. *World J Gastroenterol.* 2015;21(1):301-310.
- Araya J, Rodrigo R, Videla LA, *et al.* Increase in long-chain polyunsaturated fatty acid n—6/n—3 ratio in relation to hepatic steatosis in patients with non-alcoholic fatty liver disease. *Clin Sci (Lond).* 2004;106(6):635-643.
- Perdomo CM, Frühbeck G, Escalada J. Impact of nutritional changes on nonalcoholic fatty liver disease. *Nutrients.* 2019;11(3):677.
- Kalupahana NS, Claycombe K, Newman SJ, *et al.* Eicosapentaenoic acid prevents and reverses insulin resistance in high-fat diet-induced obese mice via modulation of adipose tissue inflammation. *J Nutr.* 2010;140(11):1915-1922.
- Bedossa P, Poitou C, Veyrie N, *et al.* Histopathological algorithm and scoring system for evaluation of liver lesions in morbidly obese patients. *Hepatology.* 2012;56(5):1751-1759.
- Martinez-Sanchez MA, Balaguer-Roman A, Fernandez-Ruiz VE, *et al.* Plasma short-chain fatty acid changes after bariatric surgery in patients with severe obesity. *Surg Obes Relat Dis.* 2023;19(7):727-734.
- Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia.* 1985;28(7):412-419.
- Martin-Moreno JM, Boyle P, Gorgojo L, *et al.* Development and validation of a food frequency questionnaire in Spain. *Int J Epidemiol.* 1993;22(3):512-519.
- Hernandez-Morante JJ, Larque E, Luján JA, Zamora S, Garaulet M. N-6 from different sources protect from metabolic alterations to obese patients: a factor analysis. *Obesity (Silver Spring).* 2009;17(3):452-459.
- Mataix J, Mañas M, Llopis J, de Victoria E M. *Tablas de Composición de Alimentos Españoles.* Instituto de Nutrición y Tecnología: Universidad de Granada; 1998.
- Folch J, Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues. *J Biol Chem.* 1957;226(1):497-509.
- Martinez-Montoro JI, Nunez-Sanchez MA, Martinez-Sanchez MA, *et al.* Hepatic and serum branched-chain fatty acid profile in patients with nonalcoholic fatty liver disease: a case-control study. *Obesity (Silver Spring).* 2023;31(4):1064-1074.
- Núñez-Sánchez MA, Martínez-Sánchez MA, Martínez-Montoro JI, *et al.* Supplementary Material from Lipidomic analysis reveals alterations in hepatic FA profile associated with MASLD stage in patients with obesity. ZENODO. 2024. <https://zenodo.org/records/10448497>.
- López-Bautista F, Barbero-Becerra VJ, Rios MY, *et al.* Dietary consumption and serum pattern of bioactive fatty acids in NAFLD patients. *Ann Hepatol.* 2020;19(5):482-488.
- Puri P, Wiest MM, Cheung O, *et al.* The plasma lipidomic signature of nonalcoholic steatohepatitis. *Hepatology.* 2009;50(6):1827-1838.
- Miyake T, Furukawa S, Matsuura B, *et al.* Plasma fatty acid composition is associated with histological findings of nonalcoholic steatohepatitis. *Biomedicine.* 2022;10(10):2540.
- Allard JP, Aghdassi E, Mohammed S, *et al.* Nutritional assessment and hepatic fatty acid composition in non-alcoholic fatty liver disease (NAFLD): a cross-sectional study. *J Hepatol.* 2008;48(2):300-307.
- Ni Y, Zhao L, Yu H, *et al.* Circulating unsaturated fatty acids delineate the metabolic Status of obese individuals. *EBioMedicine.* 2015;2(10):1513-1522.
- Sergeant S, Ruczinski I, Ivester P, *et al.* Impact of methods used to express levels of circulating fatty acids on the degree and direction of associations with blood lipids in humans. *Br J Nutr.* 2016;115(2):251-261.
- Puri P, Baillie RA, Wiest MM, *et al.* A lipidomic analysis of non-alcoholic fatty liver disease. *Hepatology.* 2007;46(4):1081-1090.
- El-Badry AM, Graf R, Clavien PA. Omega 3—omega 6: what is right for the liver? *J Hepatol.* 2007;47(5):718-725.
- Chiappini F, Coilly A, Kadar H, *et al.* Metabolism dysregulation induces a specific lipid signature of nonalcoholic steatohepatitis in patients. *Sci Rep.* 2017;7(1):46658.
- Lee JJ, Lambert JE, Hovhannisyan Y, *et al.* Palmitoleic acid is elevated in fatty liver disease and reflects hepatic lipogenesis. *Am J Clin Nutr.* 2015;101(1):34-43.
- Feng R, Luo C, Li C, *et al.* Free fatty acids profile among lean, overweight and obese non-alcoholic fatty liver disease patients: a case—control study. *Lipids Health Dis.* 2017;16(1):165.
- Heeren J, Scheja L. Metabolic-associated fatty liver disease and lipoprotein metabolism. *Mol Metab.* 2021;50:101238.
- Yamada K, Mizukoshi E, Sunagozaka H, *et al.* Characteristics of hepatic fatty acid compositions in patients with nonalcoholic steatohepatitis. *Liver Int.* 2015;35(2):582-590.
- Yamada K, Mizukoshi E, Seike T, *et al.* Serum C16:1n7/C16:0 ratio as a diagnostic marker for non-alcoholic steatohepatitis. *J Gastroenterol Hepatol.* 2019;34(10):1829-1835.

42. Roumans KHM, Lindeboom L, Veeraiyah P, *et al.* Hepatic saturated fatty acid fraction is associated with de novo lipogenesis and hepatic insulin resistance. *Nat Commun.* 2020;11(1):1891.
43. Kotronen A, Seppänen-Laakso T, Westerbacka J, *et al.* Hepatic stearoyl-CoA desaturase (SCD)-1 activity and diacylglycerol but not ceramide concentrations are increased in the nonalcoholic human fatty liver. *Diabetes.* 2009;58(1):203-208.
44. Matsuzaka T, Atsumi A, Matsumori R, *et al.* Elovl6 promotes non-alcoholic steatohepatitis. *Hepatology.* 2012;56(6):2199-2208.
45. Jeyakumar SM, Vajreswari A. Stearoyl-CoA desaturase 1: a potential target for non-alcoholic fatty liver disease? -perspective on emerging experimental evidence. *World J Hepatol.* 2022;14(1):168-179.
46. Jiang G, Li Z, Liu F, *et al.* Prevention of obesity in mice by antisense oligonucleotide inhibitors of stearoyl-CoA desaturase-1. *J Clin Invest.* 2005;115(4):1030-1038.
47. Bhatt-Wessel B, Jordan TW, Miller JH, Peng L. Role of DGAT enzymes in triacylglycerol metabolism. *Arch Biochem Biophys.* 2018;655:1-11.