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Exploring the role of fatty acid esters of hydroxy fatty acids in metabolic dysfunction-associated steatotic liver disease in morbidly obese women

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ABSTRACT

Background: Fatty acid esters of hydroxy fatty acids (FAHFAs) present potential beneficial effects that could offer valuable insights into metabolic and inflammatory diseases. However, few FAHFAs have been studied, and their role is unclear.

Aims: To assess FAHFA levels in individuals with metabolic dysfunction-associated steatotic liver disease (MASLD) associated with morbid obesity (MO) to explore the potential significance of FAHFAs under these conditions.

Methods: Using ultra-precise liquid chromatography, FAHFA serum levels were measured in 219 women, including 53 with normal weight (NW) and 166 with MO. The MO group was classified by histological diagnosis into 35 normal liver (NL), 38 simple steatosis (SS) and 93 metabolic dysfunction-associated steatohepatitis (MASH) groups.

Results: Thirty-two FAHFA isoforms from 11 families were identified. Most FAHFAs presented low levels in MO, but tLAHOAs, LAHOA-1 and OAHOA-1 increased. In MASLD, elevated tLAHPO, LAHPO-2, PAHLA-3 and PAHLA-4 levels were observed. In MASH, increased POHLA-1, tLAHPOs, LAHPO-2 and LAHLA-3 and decreased PAHSA-1, tOAHOAs, OAHSA-2 and OAHSA-3 levels were reported.

Conclusion: This study reveals novel insights into FAHFAs in a cohort of women with MO with MASLD. In MASLD, we reported only increased levels of certain FAHFAs. In MASH, we found a different profile that could be characteristic.

1. Introduction

While high levels of circulating fatty acids (FAs) have traditionally been associated with insulin resistance [1], certain FAs, such as dietary omega-3, have been recognized for their metabolically beneficial effects [2]. In 2014, Yore et al. introduced the term "fatty acid–hydroxy fatty acids," or FAHFAs, to describe molecules containing a hydroxylated fatty acid (HFA) esterified by another FA. They proposed that FAHFAs, given their potential insulin-sensitizing and anti-inflammatory effects, could offer valuable insights into metabolic and inflammatory diseases [3].

In individuals with obesity, the serum levels of total FAHFAs and the palmitic acid ester of the hydroxysteraic acid (PAHSA) family, particularly the stearic acid ester of 9-hydroxystearic acid (9-SAHSA) and the oleic acid ester of 9-hydroxystearic acid (9-OAHSA), are lower than those in nonobese controls [4]. Moreover, the PAHSA family has been shown to be positively associated with insulin sensitivity in mice and humans [3,5]. On the other hand, the OAHSA family mimics PAHSA concentration profiles and may serve as alternative markers for PAHSA effects [6].

In terms of liver metabolism, 5-PAHSA was found to increase lipolysis and stimulate de novo lipogenesis in adipocytes [7], while 9-PAHSA increased viability and decreased steatosis in HepG2 hepatocytes [8]. Furthermore, linoleic acid ester of 13-hydroxylinoleic acid (13-LAHLA) was shown to suppress lipopolysaccharide-stimulated cytokine secretion and the expression of proinflammatory genes in macrophages [6]. Additionally, PAHSAs, palmitoleic acid esters of hydroxysteraic acids (POHSAs), OAHSAs, SAHSAs, and LAHLAs have been suggested to exhibit anti-inflammatory effects [9–11].

In certain contexts, FAHFAs have been proposed as a potential therapeutic strategy for addressing metabolic and inflammatory diseases. However, the current findings are preliminary, and

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a deeper understanding of the physiological role of FAHFAs in humans is necessary [12–14].

Metabolic dysfunction-associated steatotic liver disease (MASLD) is defined as the presence of steatotic liver disease in the absence of other causative factors, accompanied by at least one of five cardiometabolic criteria: obesity, hypertension, elevated glucose or triglyceride levels, or reduced levels of high-density lipoprotein-cholesterol (HDL-C) [15]. Cardiovascular events are commonly related to patients with MASLD [16]. Metabolic dysfunction-associated steatohepatitis (MASH) represents the advanced stage of MASLD, characterized by fat-induced lipotoxicity and subsequent inflammatory damage to hepatocytes [17,18]. MASH can progress to fibrosis, eventually leading to cirrhosis, with an elevated risk of hepatocellular carcinoma, but also extrahepatic tumors [16,19]. Recent studies have highlighted the gut microbiota's significant role in metabolic health and diseases, particularly obesity and MASLD. The gut microbiota interacts with diet and host metabolism, producing metabolites that impact the host's metabolic state [20,21]. Functional analysis of the gut microbiota can uncover its metabolic capabilities and their influence on MASLD and obesity progression. Such analyses can identify specific microbial pathways and metabolites involved in these diseases, presenting potential targets for therapeutic intervention [22,23].

Overall, we designed a study to determine the circulating levels of different FAHFA families and their isoforms in a homogeneous cohort of women with MASLD and MASH syndrome associated with MO. This study aimed to evaluate the possible role of FAHFAs in the pathophysiology of these metabolic diseases.

2. Materials and methods

2.1. Study cohort

In this study, we recruited a homogeneous cohort of 219 fertile women. We included a control group of 53 volunteer women with NW (body mass index (BMI) = 19–25 kg/m^2) and 166 women with MO (BMI >40 kg/m^2) who were scheduled to undergo laparoscopic bariatric surgery. In this study, we included only women to avoid heterogeneity since men and women present differences in terms of metabolic parameters [24,25].

Using the GRANMO sample size calculator (v.7.04), which has an alpha risk of 0.05 and a beta risk of <0.2 in bilateral contrast, we needed at least 60 cases (MO) and 19 controls (NW) to detect a minimum odds ratio of 0.1. It is assumed that the exposure rate in the control group will be 0.21. Tracking loss taxa of 0 were estimated. The POISSON approximation was used.

Women who had an acute illness, acute or chronic inflammatory or infective diseases, or end-stage malignant disease were excluded from this study. Menopausal women and women receiving contraceptive treatment were also excluded. In addition, women who consumed >10 g of alcohol per day and who were recurrent smokers were excluded from this study.

This study was approved by the Ethics Committee of IISPV (CEIm; 23c/2015). All participants provided written informed consent. This study was conducted retrospectively by accessing patient data for the first time on June 5, 2023, and for the last time on January 18, 2024. During the data collection and the data analysis, the authors did not have access to the identification of the patients with the case studies because they worked on a blind and encrypted database.

2.2. Anthropometric and biochemical variables of the study cohort

For the anthropometrical variables, weight, height, BMI, waist–hip ratio, systolic blood pressure (SBP) and diastolic blood pressure (DBP) were obtained from each participant. Biochemical variables were measured from blood samples obtained by specialized nurses through a BD Vacutainer® system after overnight fasting and just before bariatric surgery in the MO subjects. Blood samples were collected in ethylenediaminetetraacetic acid tubes, separated into plasma and serum aliquots using centrifugation (3500 rpm, 4 °C, 15 min) and then stored at −80 °C until processing. The biochemical variables included were the levels of glucose, insulin, glycosylated hemoglobin A1c (HbA1c), total cholesterol, HDL-C, low-density lipoprotein-cholesterol (LDL-C), triglycerides, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and ferritin, which were measured using a conventional automated analyzer.

2.3. Hepatic groups by histological diagnosis

During bariatric surgery, in the cohort of women with MO (n = 166), liver biopsies were collected in a tube with RNAlater solution (Qiagen, Hilden, Germany) at 4 °C and then preserved in a formaldehyde solution for histopathological diagnosis. This biopsy was always obtained after suspicion of liver pathology. Liver samples were scored and classified by an experienced hepatopathologist through eosin-hematoxylin staining according to the Kleiner criteria [26] into normal liver (NL; n = 35), simple steatosis (SS, n = 38) and MASH (n = 93) samples.

Using the GRANMO sample size calculator (v.7.04), which has an alpha risk of 0.05 and a beta risk of <0.2 in bilateral contrast, we needed at least 71 cases (SS and MASH) and 18 controls (NL) to detect a minimum odds ratio of 0.1. It is assumed that the exposure rate in the control group will be 0.21. A tracking loss of 0 taxa was estimated. The POISSON approximation was used.

2.4. Comprehensive profiling and quantification of FAHFAs in serum samples

The analysis of FAHFA levels in serum samples was performed at the Centre of Omic Sciences (EURECAT Company, Reus, Spain). The analytical standards, reagents and instrumentation used for this analysis are detailed in Table S1.

2.4.1. Sample preparation

For the total lipid extraction, a liquid–liquid extraction with chloroform:methanol (2:1) based on the Folch procedure was performed. To extract 150 μL of serum, citric acid buffer (100 mM sodium citrate dihydrate and 1 M sodium chloride, adjusted to pH 3.6), 0.3 mL of methanol and 0.6 mL of chloroform were successively added to the tube. The mixture was vortexed and centrifuged at 15,000 rpm for 6 min at 4 °C to induce phase separation. The lower phase was transferred to another tube and evaporated to dryness in a SpeedVac. The dried residue was redissolved in 300 μL of chloroform and further loaded on a silica cartridge (Sep-Pak 3 cc Vac cartridge, 200 mg of sorbent and 55–105 μm) to remove the neutral lipids as reported by Zhu et al. [27]. The FAHFA eluate was collected and evaporated to dryness in a SpeedVac. Finally, the dried sample was redissolved in 100 μL of methanol and used for instrumentation [4,27].

2.4.2. UHPLC-(-ESI)-M/MS methodology

Chromatographic separation was performed with the gradient detailed in Table S2. Mobile phase A was water, mobile phase B was acetonitrile, mobile phase C was 2-propanol, and mobile phase D was 40 mM ammonium acetate. The column temperature was set at 50 °C, and the injection volume was 5 μL. The source parameters used for negative electrospray ionization (ESI) are shown in Table S3, and the mass analyzer was operated on a multi reaction monitoring (MRM) system. The transitions and their retention times are listed in Table S4.

Table 1
Anthropometric and biochemical variables of the study cohort in accordance with BMI.

Variables	Normal – weight (BMI 19–25 kg/m^2) (n = 53)	Morbid obesity (BMI >40 kg/m^2) (n = 166)
Age (years)	43 (36 - 48)	45 (37 - 50)
BMI (kg/m^2)	22.4 (21. - 23.5)	45.4 (43 - 49.6)*
Waist-hip (m) ratio	0.81 (0.73 - 0.89)	0.92 (0.86 - 0.97)*
SBP (mm Hg)	116 (107 – 122)	125 (114 – 137)*
DBP (mm Hg)	70 (61 – 75)	66 (59 – 78)
Glucose (mg/dl)	80 (69 - 90.5)	102 (87 – 123.5)*
Insulin (mUI/L)	6 (4.8 – 8.1)	14.10 (9 – 25.2)*
HbA1c (%)	5.1 (5 – 5.3)	5.7 (5.1 – 6.3)*
Triglycerides (mg/dl)	64 (49.5 – 90.5)	136 (102.0 – 187.5)*
Cholesterol (mg/dl)	180.8 (161 – 210.5)	160 (142.9 – 183.2)*
HDL-C (mg/dl)	64.3 (54.5 – 70)	37.8 (32 – 43)*
LDL-C (mg/dl)	108 (83 – 123.7)	92.6 (76.1 – 114)*
AST (UI/L)	20 (15 – 24)	32 (23 – 46)*
ALT (UI/L)	16 (12.25 – 24)	33 (23 – 51)*
GGT (UI/L)	14 (11 – 21)	22 (14.7 – 38.2)*
ALP (UI/L)	60.50 (48 – 75.2)	64 (55 – 76)
LDH (UI/L)	300 (185 – 366)	399 (350 – 453)*
Ferritin (ng/ml)	39.3 (13 – 64)	50 (27 – 96.4)*

The data are expressed as medians and interquartile ranges.
* Differences between NW and MO tissues were considered significant when the p value was <0.05 according to the Mann–Whitney test. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, glycosylated hemoglobin A1c; HDL-C, high density lipoprotein – cholesterol; LDL-C, low density lipoprotein – cholesterol; AST, aspartate-amino transferase; ALT, alanine-aminotransferase; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase.

2.4.3. Identification of FAHFAs

The identification of FAHFAs with the corresponding standard was limited because these standards are not commercially available. We therefore used various tools to identify the maximum number of FAHFAs. Previous reports on the chromatographic behavior of C18 and FAHFAs in the studied human matrices [4,27–29] were used for tentative identification purposes. Although some FAHFAs have very similar chemical compositions and structures, mass spectrometry confirmed FAHFA identification by determining the molecular weight through pseudomolecular ion [M-H]- and fragmentation patterns using electrospray ionization in negative ionization mode [28].

To comprehensively identify endogenous FAHFAs in serum samples without commercial standards by ultra-precise liquid chromatography (UPLC)/QQQ couplet to a mass spectrometer (MS), theoretical multiple reaction monitoring (MRM) transitions (including FAHFAs with carbon numbers ranging from 32 to 36 and double-bond numbers ranging from 0 to 8), MRM data from serum samples were collected in the first step. To improve sensitivity, the number of acquired transitions simultaneously was minimized (Table S4). Compared with previously reported work [30], due to a lack of sensitivity, the fragments corresponding to the hydroxylation position of hydroxy fatty acids cannot be observed in the MS/MS data. In this sense, the number behind each isoform does not indicate the position of the carbon that would precede the compound's name; rather, it indicates the number of identified isoforms. Isoforms are numbered according to their order of appearance in the MMR spectrum (ex. OAHOA-1, OAHOA-2, etc.) (Figure S1).

The total levels of the different FAHFA families (ex. tPOHLAs, tPAHSAs, etc.) were determined by the sum of the levels of all identified isoforms within that family. Moreover, the total levels of FAHFAs (tFAHFAs) were defined as the sum of the levels of all identified isoforms in the study.

2.5. Statistical analysis

The data were analyzed using SPSS/PC+ for Windows (version 27.0; SPSS, Chicago, Illinois, USA). The Kolmogorov–Smirnov test was employed to assess the distribution of variables. Variables are presented as the median and interquartile range. Different comparative analyses were conducted using a nonparametric Mann–Whitney U test. P values <0.05 were considered to indicate statistical significance. Any instances with missing values were removed, and the predictor variables were scaled using the min–max scaler.

3. Results

3.1. Baseline characteristics of the study cohort

First, we classified our cohort according to BMI into two groups: NW subjects (n = 53) as the control group and MO subjects (n = 166), as shown in Table 1. These subjects were comparable in terms of sex, age, DBP and ALP levels. In this sense, compared with women in the NW cohort, women in the MO cohort presented increased BMIs, waist-to-hip ratios, SBPs and levels of glucose, insulin, HbA1c, triglycerides, AST, ALT, GGT, LDH and ferritin. Conversely, the MO group showed decreased levels of HDL-C, cholesterol and LDL-C since 60.7 % of the MO subjects were treated with lipid-lowering agents.

Then, we subclassified our MO subjects according to their hepatic histology into NL (n = 35), SS (n = 38) and MASH (n = 93) groups, as shown in Table 2. In this case, the subjects were comparable in terms of sex, age, BMI, waist-to-hip ratio, DBP and levels of cholesterol, HDL-C, LDL-C, AST, ALP, LDH and ferritin. In this regard, compared with NL subjects, MASLD (SS or MASH) subjects presented increased levels of glucose, insulin, HbA1c, triglycerides and GGT. However, only the MASH group presented increased SBP and ALT levels. Unfortunately, we did not observe significant anthropometric or biochemical differences between the SS and MASH diet groups.

3.2. Circulating FAHFA levels identified in serum samples

The FAHFA analysis of the serum samples by UPLC revealed that 32 FAHFAs belonged to 11 different FAHFA family members, as shown in Fig. 1 (1 FAHFA 32:1-POHPA, 1 FAHFA 34:3-POHLA, 2 FAHFA 34:3-LAHPO, 5 FAHFA 34:2-PAHLA, 4 FAHFA 34:1-POHSA, 1 FAHFA 34:0-PAHSA, 4 FAHFA 36:4-LAHLA (and 1 FAHFA 36:5-LnAHLA), 3 FAHFA 36:3-LAHOA (and 1 FAHFA 36:4-LnAHOA), 1

Table 2
Anthropometric and biochemical variables of the morbidly obese cohort according to liver histology.

Variables	Normal liver (n = 35)	Simple Steatosis (n = 38)	MASH (n = 93)
Age (years)	44 (37 - 49)	45 (38 - 48)	46 (39 - 50)
BMI (kg/m^2)	44 (41.7 - 49.4)	45.9 (42.6 - 50.1)	46.1 (43.2 - 49.9)
Waist-hip (m) ratio	0.88 (0.84 - 0.93)	0.92 (0.86 - 0.97)	0.92 (0.87 - 0.98)
SBP (mm Hg)	118 (107.5 – 130)	123 (113 – 137)	125 (115 – 140)[#]
DBP (mm Hg)	62 (57.5 – 75.7)	65 (59 – 75)	70 (60 – 80)
Glucose (mg/dl)	90 (78.2 – 101.2)	107 (85 – 135) [#]	104.5 (89.7 – 129.7) [#]
Insulin (mUI/L)	9.2 (5.5 – 13.2)	14.1 (8.3 – 27.6) [#]	16 (11.7 – 29.2) [#]
HbA1c (%)	5.3 (5 – 5.7)	5.9 (5.4 – 6.7) [#]	5.8 (5.2 – 6.6) [#]
Triglycerides (mg/dl)	101.5 (73.5 – 136)	132 (108.5 – 176) [#]	146.5 (124 – 206.5) [#]
Cholesterol (mg/dl)	157.6 (133.8 – 195)	157 (143.5 – 171.1)	163.2 (146.4 – 183.9)
HDL-C (mg/dl)	39 (32 – 48.5)	35.1 (31 – 43)	38 (33.5 – 43)
LDL-C (mg/dl)	101 (74.2 – 122.6)	90.5 (77.1 – 112.8)	92.7 (74.7 – 119.2)
AST (UI/L)	29 (19.7 – 44.2)	35 (26 – 51)	32.5 (24 – 46.2)
ALT (UI/L)	24 (16 – 51.5)	38.5 (28.2 – 49)	33 (24.5 – 54.2) [#]
GGT (UI/L)	17 (11.2 – 23.5)	24 (16 – 35) [#]	22.2 (15 – 49.5) [#]
ALP (UI/L)	63 (50 – 73)	65 (56.5 – 78)	64 (57.5 – 78)
LDH (UI/L)	383.5 (339.2 – 424.2)	435 (375 – 466)	391 (345.7 – 479)
Ferritin (ng/ml)	35 (23 – 83.9)	65.6 (37.3 – 135.8)	54 (28.3 – 101.7)

The data are expressed as medians and interquartile ranges.
[#] Significant differences between NL and SS or between NL and MASH were considered significant when the p value was <0.05 according to the Mann–Whitney test. MASH, metabolic dysfunction-associated steatohepatitis; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HbA1c, glycosylated hemoglobin A1c; HDL-C, high-density lipoprotein–cholesterol; LDL-C, low-density lipoprotein–cholesterol; AST, aspartate-amino transferase; ALT, alanine-aminotransferase; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase; LDH, lactate dehydrogenase.

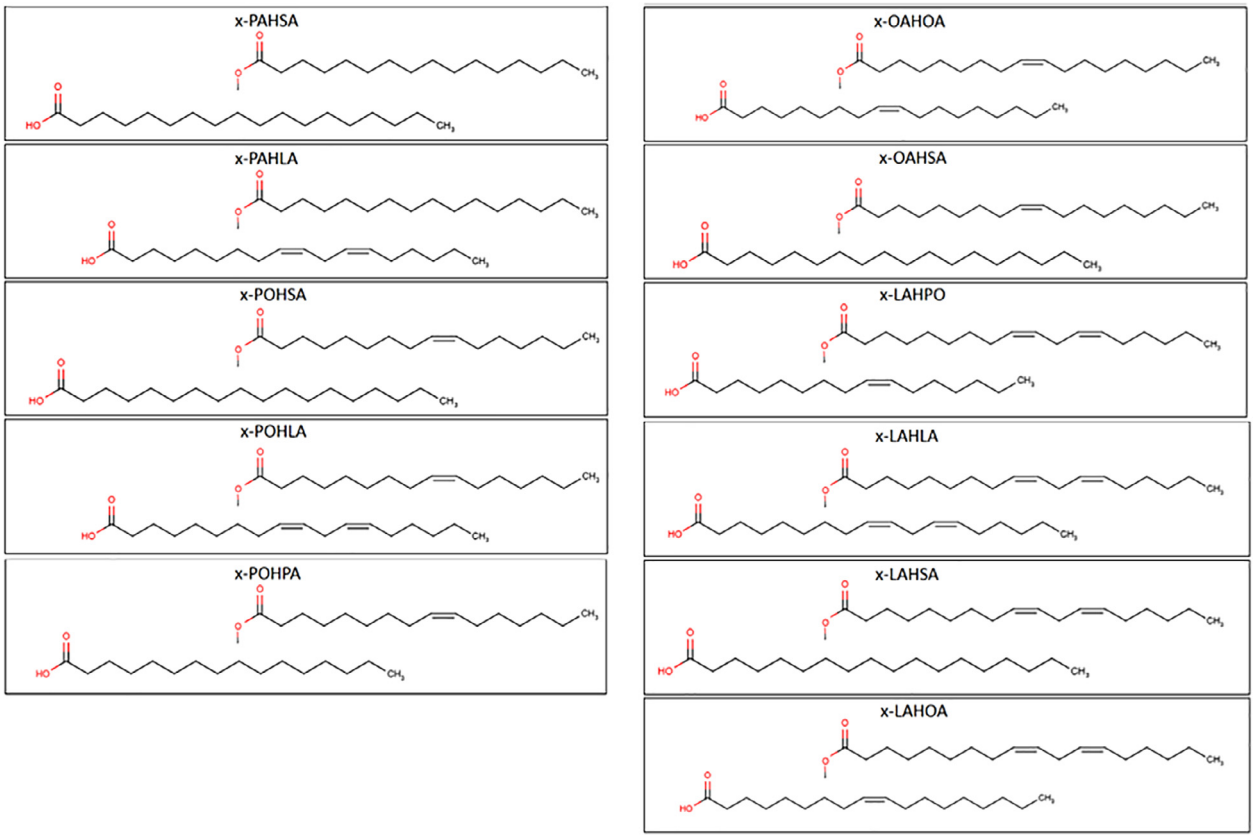

Fig. 1. FAHFA families identified in the serum samples of the study cohort. x: represents the isoform number; PA: palmitic acid; PO: palmitoleic acid; OA: oleic acid; LA: linoleic acid; SA: stearic acid; H: hydroxy bond.

FAHFA 36:2-LAHSA, 5 FAHFA 36:2-OAHOA and 3 FAHFA 36:1-OAHSA). FAHFA 36:5-LnAHLA was deliberately excluded from further analysis due to its high incidence of missing values, thus ensuring data integrity. The MRM chromatograms from a pool of samples are shown in Figure S1.

On the other hand, for the PAHSA and SAHSA families, the acquired transitions were disrupted. Unfortunately, as shown in Figure S1, in this work, the MRM transitions for PAHSAs and SAHSAs presented high background levels, which meant that only one PAHSA and no compound SAHSA could be detected.

Then, we conducted a correlation analysis between the levels of identified FAHFAs and the anthropometric and biochemical variables of our patients (Table S5). In this regard, we identified significant negative correlations between a large number of FAHFAs and

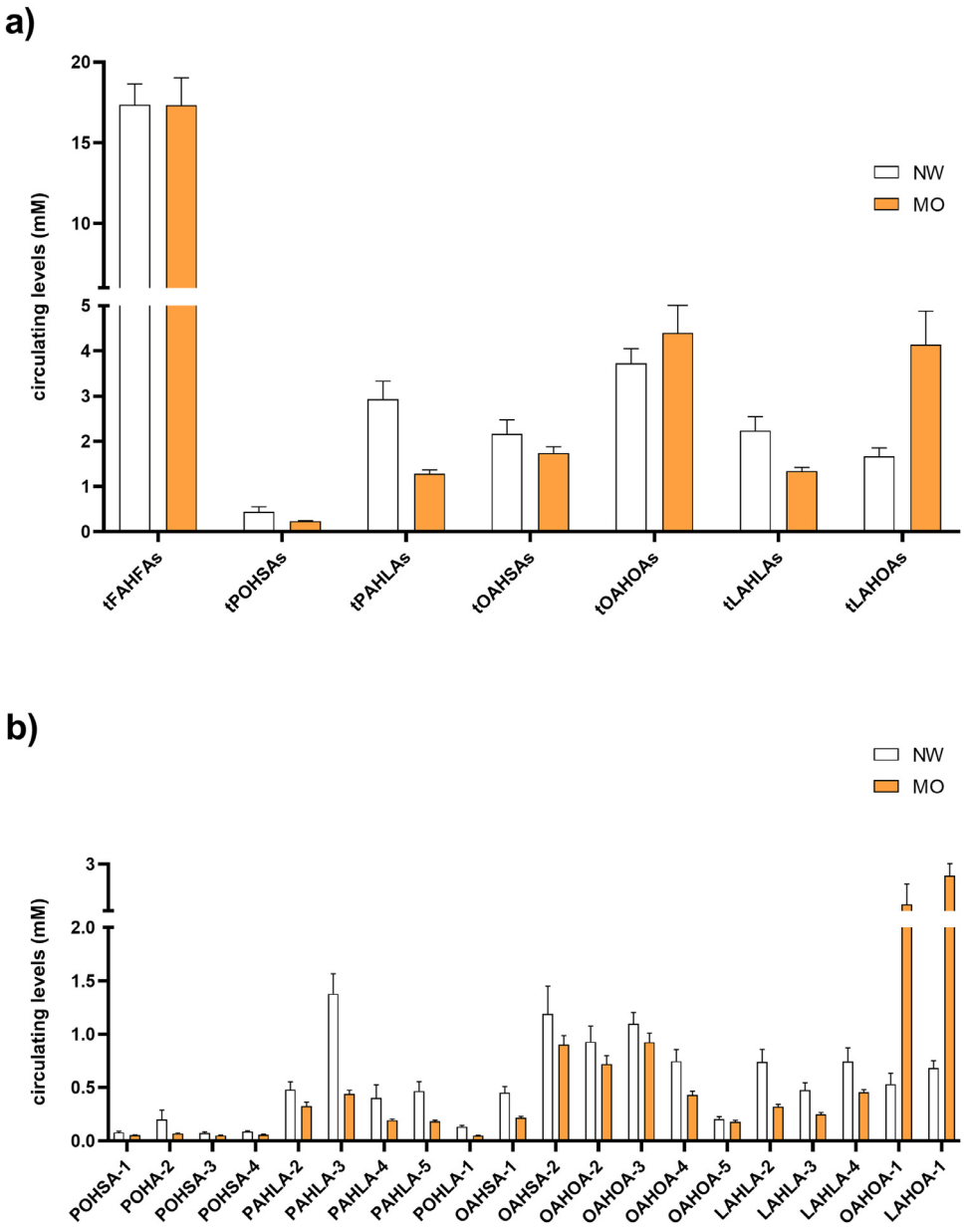

Fig. 2. Circulating FAHFA levels with significant differences between the MO and NW cohorts: a) families; b) isoforms. PO, palmitoleic acid; PA, palmitic acid; OA, oleic acid; LA, linoleic acid; SA, stearic acid; H, hydroxy bond. The number at the end indicates the isoform. NW, normal weight; MO, morbid obesity. Differences between groups were calculated using the Mann–Whitney test, and $p < 0.05$ was considered to indicate statistical significance. P values and group descriptions are given in Table S5. Bar graphs were generated using GraphPad Prism (v8).

variables such as BMI, HbA1c levels and triglycerides and between FAHFAs and liver enzymes such as AST, ALT, GGT and LDH. On the other hand, we found significant positive associations of the majority of FAHFAs with cholesterol levels and HDL-C.

3.3. Circulating FAHFA levels according to the presence of MO

First, we aimed to evaluate tFAHFA levels, families and individual isoforms based on whether the patient had NW or presented with MO. In this regard, as shown in Fig. 2a, we observed that MO patients exhibited decreased levels of tFAHFAs and some FAHFAs but increased levels of only the tLAHOAs. Furthermore, regarding individual isoforms, MO patients exhibited decreased levels of a significant number of isoforms, as shown in Fig. 2b, while demonstrating increased levels of OAHOA-1 and LAHOA-1 compared to NW patients. The results of the comparative test between the FAHFA levels of the NW and MO groups (p values) and group descriptions are given in Table S6.

3.4. Circulating FAHFA levels according to the presence of MASLD

Later, focusing solely on the cohort of patients with MO, we aimed to assess whether the levels of FAHFAs were significantly different between patients suffering from MASLD and those with NL according to histopathological evaluation. In this regard, we did not find decreased levels of any FAHFA, but we did find increased levels of the t-LAHPO family in MASLD subjects (Fig. 3a) and, specifically, higher levels of the isoforms PAHLA-3, PAHLA-4 and LAHPO-2 in MASLD subjects than in NL subjects (Fig. 3b).

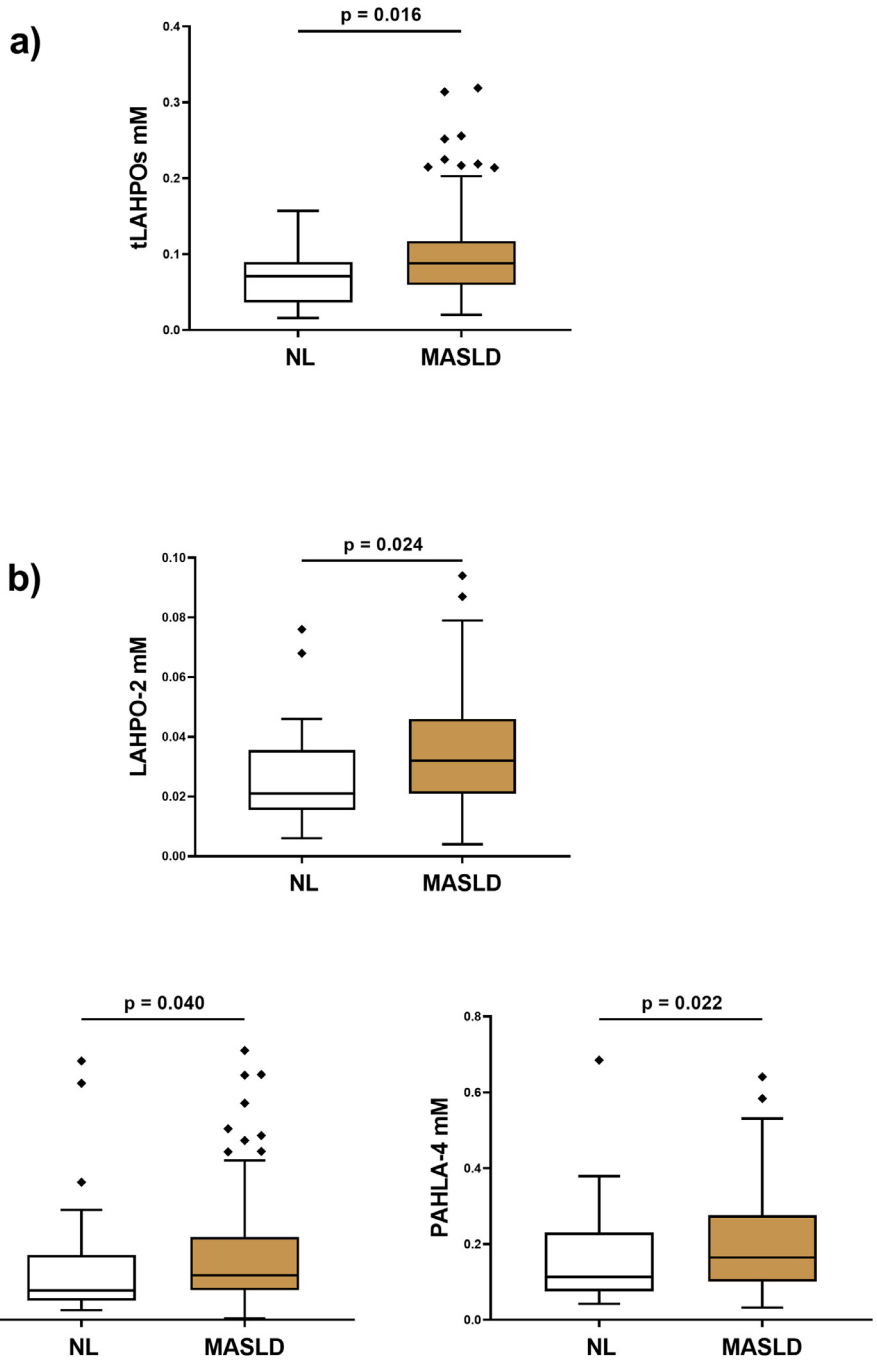

Fig. 3. Circulating FAHFA levels according to the presence of MASLD: a) families; b) isoforms. LAHPO, linoleic acid ester of hydroxy palmitoleic acid; PAHLAs, palmitoleic acid ester of hydroxy linoleic acid; the number at the end indicates the isoform. NL, normal liver; MASLD, metabolic dysfunction-associated steatotic liver disease. Differences between groups were calculated using the Mann–Whitney test, and $p < 0.05$ was considered to indicate statistical significance. Box plots were generated using GraphPad Prism (v8). The diamonds represent outliers.

3.5. Circulating FAHFA levels according to liver histopathological classification

Later, we aimed to study the levels of FAHFAs and their families and isoforms based on whether the patient had NL, SS or MASH. In this analysis, on the one hand, we found that tLAHPOs exhibited increased levels in MASH patients compared to NL patients, while tOAHOAs showed increased levels in MASH patients compared to those in the SS and NL groups (Fig. 4a). On the other hand, as shown in Fig. 4b, we reported that the PAHSA-1 isoform presented increased levels in the SS group compared to those in the NL and MASH groups. We also found that the levels of the POHLA-1 and LAHLA-3 isoforms were greater in the MASH group than in the SS cohort. Then, we detected increased levels of LAHPO-2 in MASH compared to NL and SS. Finally, we observed decreased levels of the OAHOA-2 and OAHOA-3 isoforms in the MASH group compared to those in the NL and SS groups.

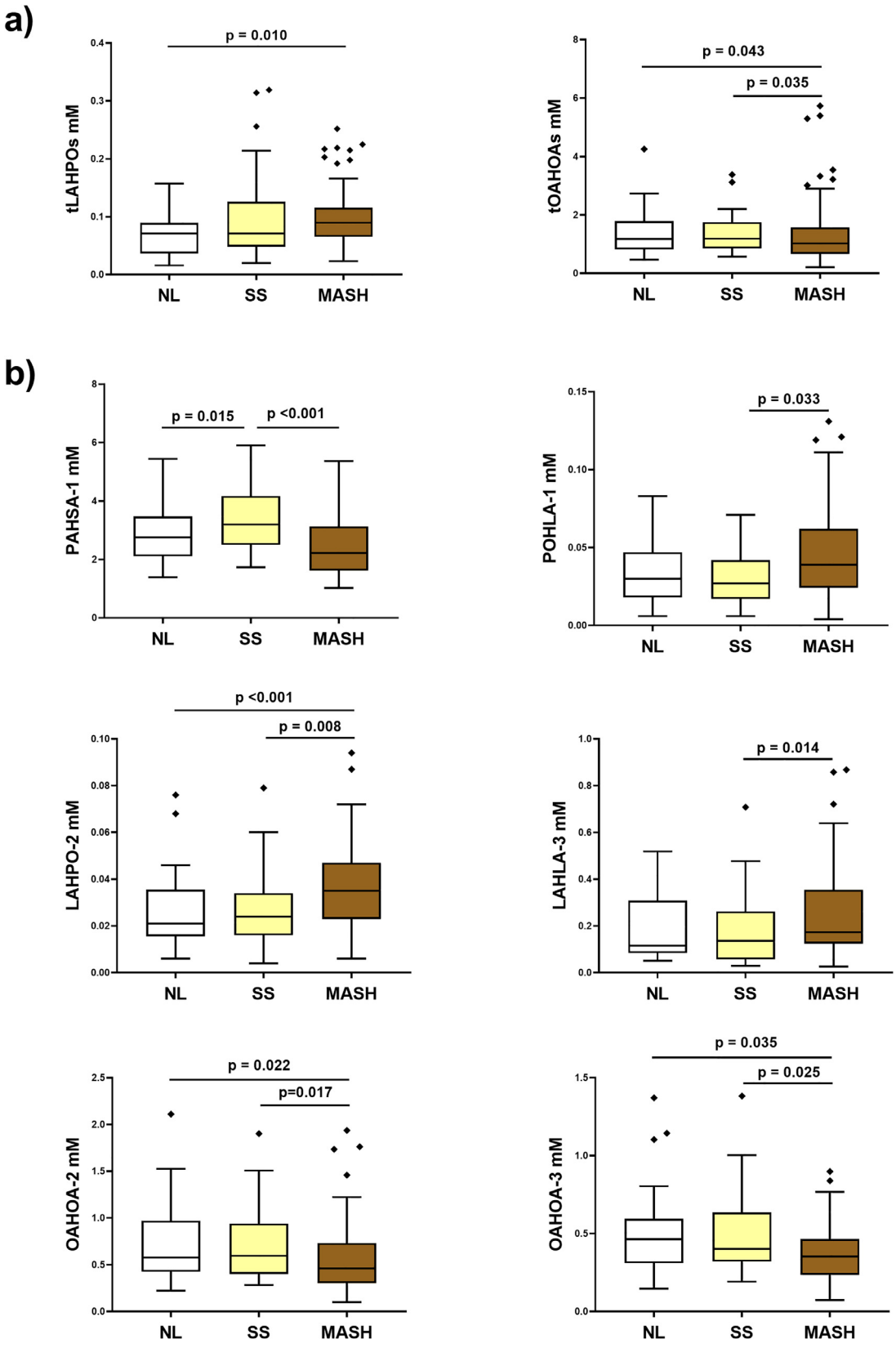

Fig. 4. Circulating FAHFA levels according to histopathological group: a) families; b) isoforms. LAHPO, linoleic acid esters of hydroxy palmitoleic acid; OAHOA, oleic acid esters of hydroxy oleic acid; PAHSA, palmitic acid esters of hydroxy stearic acid; POHLA, palmitoleic acid esters of hydroxy linoleic acid; LAHLA, linoleic acid esters of hydroxy linoleic acid; The number at the end identifies the isoform. NL, normal liver; SS, simple steatosis; MASH, metabolic dysfunction-associated steatohepatitis. Differences between groups were calculated using the Mann–Whitney test, and $p < 0.05$ was considered to indicate statistical significance. Box plots were generated using GraphPad Prism (v8). The diamonds represent outliers.

4. Discussion

This study presents novel findings regarding serum FAHFA levels in individuals with obesity and MASLD. Thirty-two FAHFA isoforms from eleven different families were identified, including POHPA, POHLA, LAHPO, PAHLA, POHSA, PAHSA, LAHLA, LAHOA, LAHSA, OAHOA and OAHSA.

By comparing FAHFA levels between women with MO and women with NW, we detected decreased levels of tFAHFAs, tPOHSAs (POHSA-1, 2, 3, and 4), tPAHLAs (PAHLA-2, 3, 4, and 5), POHLA-1, tOAHSAs (OAHSA-1 and 2), tOAHOAs (OAHOHA-2, 3, 4, and 5) and tLAHLAs (LAHLA-2, 3, and 4) and increased levels of tLAHOAs (LAHOA-1) and OAHOA-1. This aligns with findings by Kellerer et al., who also reported low levels of tFAHFAs, tPAHSAs, 9-SAHSA and 9-OAHSA in obese subjects compared to nonobese controls, but their cohort was limited in number and included both sexes [4]. Conversely, SAHSAs were not identified in this study's serum samples, and no significant differences were found in PAHSA between the MO and NW cohorts. Additionally, FAHFAs such as PAHSAs, POHSAs, OAHSAs, SAHSAs and LAHLAs have been suggested to have anti-inflammatory effects [9–11,31], which corresponds to the decreased levels observed in MO subjects and could be associated with the low-grade chronic inflammatory state of obesity [32]. However, for the first time, we found decreased levels of other FAHFAs, such as PAHLA, POHLA and OAHOA, and increased levels of LAHOAs and OAHOA in the context of obesity and metabolic alterations, warranting further investigation. This study requires a deeper examination of OAHOA due to its varied isoform responses in individuals with obesity.

Furthermore, we examined FAHFA levels in relation to the presence of MASLD, revealing elevated levels of tLAHPOs and their isoforms, such as LAHPO-2, PAHLA-3 and PAHLA-4. Notably, this study represents the first investigation into FAHFAs within a MASLD cohort, requesting future comparison with research on these FAHFAs that are currently unavailable. However, the literature suggests potential therapeutic benefits of 9-PAHSA in mitigating lipotoxicity in cultures of steatotic primary hepatocytes and HepG2 cells [8]. Additionally, previous studies have suggested that PAHSAs can suppress the production of inflammatory cytokines such as interleukin 1-β and tumor necrosis factor-α in macrophages [3,33]. In our findings, while we observed increased levels of PAHSA-1 in subjects with SS compared to those with NL, the PAHSA-1 levels were decreased in those with MASH compared to those in the SS group, consistent with the anti-inflammatory role suggested in the literature. On the other hand, it has been suggested that 9-OAHSA may exert a protective effect against liver apoptosis by preserving mitochondrial function and stability in animal models [34], which concurs with our observation of decreased PAHSA levels in MASH subjects, where apoptosis is frequently observed [35].

The biosynthesis of FAHFA molecules is not well understood. While de novo lipogenesis is the main process for producing fatty acid precursors, the carbohydrate response element-binding protein plays a crucial role in regulating FAHFA synthesis [36]. Although the synthesis of fatty acids is well described, the biosynthesis of hydroxy fatty acid precursors is more complex, involving independent pathways such as unsaturation and esterification that require different enzymes. While some enzymes involved in the biosynthetic pathway of saturated FAHFAs have been identified [37–39], little is known about the process. It is suggested that oxidative stress, a process present in MASH [40], can modulate FAHFA synthesis [37]. In this case, it makes sense that we found decreased PAHSA levels in our MASH patients, given that oxidative stress is likely altering its production. Furthermore, the involvement of lipases, which have been shown to be altered in MASLD [41], in the release of FAHFAs and remodeling of triacylglycerol estolides suggests tight control over the unesterified levels of these lipid messengers [42].

This study highlights the potential clinical relevance of circulating FAHFAs in MASLD and obesity. Our findings suggest that specific FAHFA families could serve as diagnostic biomarkers for assessing metabolic risk and disease progression, providing clinicians with valuable tools for tailoring interventions. Moreover, these FAHFAs represent promising therapeutic targets, as modulating their levels could improve insulin sensitivity and reduce inflammation, offering a novel approach to managing metabolic dysfunction. The study also emphasizes the importance of gut microbiota modulation, suggesting that dietary or probiotic interventions could correct dysbiosis patterns, enhancing traditional metabolic disorder treatments. Overall, these insights contribute to the field of personalized medicine by informing customized treatment plans based on individual variations in FAHFA levels and gut microbiota composition, potentially optimizing therapeutic outcomes and improving patient care in MASLD and obesity. Future research should focus on validating these findings in larger populations and exploring the mechanisms through which FAHFAs and gut microbiota exert their effects on metabolic diseases.

A limitation of this study is its focus on a cohort comprising fertile women with MO. However, this decision was made to mitigate biases associated with sex and age in the metabolic profile. Additionally, the necessity for severe obesity arises from ethical considerations surrounding the acquisition of liver biopsies by laparoscopy during bariatric surgery when liver disease is suspected. Liver biopsy during laparoscopic bariatric surgery poses a reduced risk to patients [43]. It is also important to note that 60 % of our MO patients were prescribed lipid-lowering agents, reflecting common comorbidities such as diabetes or dyslipidemia in this population, which necessitate treatment.

Another limitation of this study is methodological. This difference stems from the low sensitivity and decreased FAHFA levels observed in serum samples, making it challenging to determine the exact position at which hydroxy FA is hydroxylated. Furthermore, the MRM transitions, especially for the PAHSA and SAHSA families, which were previously studied in FAHFAs literature [14], exhibited elevated background levels. Previous research has indicated that the use of silica SPE columns and certain buffers can contribute to this background contamination [6,44], which can vary between different lots and columns. While this background contamination is negligible in tissues with high FAHFA levels, such as white adipose tissue and liver tissue, it poses significant challenges when analyzing PAHSAs and SAHSAs in serum samples. Consequently, in this analysis, we detected only one PAHSA and no SAHSA compounds.

In summary, this study revealed novel findings associated with MO in women with MASLD and MASH. In MASLD, we reported increased levels of tLAHPOs, LAHPO-2, PAHLA-3 and PAHLA-4. In addition, in individuals with MASH, we detected decreased levels of PAHSA-1, tOAHOAs, OAHSA-2 and OAHSA-3 and elevated levels of POHLA-1, tLAHPOs, LAHPO-2 and LAHLA-3. This study demonstrated a possible relationship between FAHFAs and MASLD/MASH pathophysiology, which can be useful for the diagnosis, follow-up and therapeutic target identification of these processes. Further studies are necessary to confirm these preliminary findings.

Conflict of interest

None

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.dld.2024.09.026.

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