Multi-omics profiling reveals altered mitochondrial metabolism in adipose tissue from patients with metabolic dysfunction-associated steatohepatitis

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Summary

Background Metabolic dysfunction-associated steatotic liver disease (MASLD) and its more severe form steatohepatitis (MASH) contribute to rising morbidity and mortality rates. The storage of fat in humans is closely associated with these diseases' progression. Thus, adipose tissue metabolic homeostasis could be key in both the onset and progression of MASH.

Methods We conducted a case-control observational research using a systems biology-based approach to analyse liver, abdominal subcutaneous adipose tissue (SAT), omental visceral adipose tissue (VAT), and blood of n = 100 patients undergoing bariatric surgery (NCT05554224). MASH was diagnosed through histologic assessment. Whole-slide image analysis, lipidomics, proteomics, and transcriptomics were performed on tissue samples. Lipidomics and proteomics profiles were determined on plasma samples.

Findings Liver transcriptomics, proteomics, and lipidomics revealed interconnected pathways associated with inflammation, mitochondrial dysfunction, and lipotoxicity in MASH. Paired adipose tissue biopsies had larger adipocyte areas in both fat depots in MASH. Enrichment analyses of proteomics and lipidomics data confirmed the association of liver lesions with mitochondrial dysfunction in VAT. Plasma lipidomics identified candidates with high diagnostic accuracy (AUC = 0.919, 95% CI 0.840–0.979) for screening MASH.

Abbreviations: ALT, alanine aminotransferase; ASP, aspartate aminotransferase; AUC, area under the curve; BMI, body mass index; CE, cholesterol ester; DG, diglycerides; FA, fatty acid; FGF, fibroblast growth factor; GGT, gamma glutamyl transferase; GL, glycerolipids; GP, glycerophospholipids; GWAS, genome-wide association study; HDL, high-density lipoprotein; HOMA-IR, homeostatic model assessment for insulin resistance; LDL, low-density lipoprotein; LPC, lysophosphatidylcholines; LPE, lysophosphatidylethanolamine; MAFLD, metabolic dysfunction-associated fatty liver disease; MASH, metabolic dysfunction-associated steatohepatitis; NMR, nuclear magnetic resonance; PC, phosphatidylcholine; PCA, principal component analysis; PE, phosphatidylethanolamine; PLS-DA, partial least square discriminant analysis; RNA, ribonucleic acid; ROC, receiver operating characteristic; SL, sterol lipid; SM, sphingomyelins; SNP, single nucleotide polymorphism; SP, sphingolipid; SVM, supervised vector machine; TG, triglyceride; VIP, variable importance in projection; VLDL, very low-density lipoprotein

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Interpretation Mitochondrial dysfunction is also present in VAT in patients with obesity-associated MASH. This may cause a disruption in the metabolic equilibrium of lipid processing and storage, which impacts the liver and accelerates detrimental adaptative responses.

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Keywords: Interorgan crosstalk; Lipidomics; MASLD; Multi-tissue; Multi-omics

Research in context

Evidence before this study

Metabolic dysfunction-associated steatotic liver disease (MASLD) is the most prevalent liver disease globally, affecting over 25% of the population and more than 80% of individuals with obesity. Metabolic dysfunction-associated steatohepatitis (MASH), a more severe form of MASLD, increases the risk of cirrhosis and hepatocarcinoma, making it imperative to identify the molecular mechanisms that drive disease progression. Current pharmacological strategies targeting MASLD and MASH have yielded moderate success, underscoring the complexity and heterogeneity of these conditions. Among patients with severe obesity, dysregulation in adipose tissue quantity and metabolism appears to be a key factor influencing the MASLD spectrum and progression to MASH.

Added value of this study

Utilising a systems biology approach, this study examines the multi-omics profiles of liver tissue and two distinct adipose tissue depots from patients with severe obesity, with and without MASH. By carefully selecting patients to minimise confounding factors, including adipose tissue amount and distribution, we identified significant differences in mitochondrial lipid metabolism in visceral adipose tissue. Additionally, our findings suggest that circulating lipids serve as potential biomarkers for MASH, offering a non-invasive alternative for diagnosis in patients with severe obesity.

Implications of all the available evidence

The current gold standard for diagnosing MASH remains the liver biopsy, highlighting the need for non-invasive diagnostic methods. Our study identifies circulating lipids as promising biomarkers for MASH in patients with severe obesity. Furthermore, the discovery of mitochondrial dysfunction in visceral adipose tissue provides new insights into the disease mechanisms underlying MASLD and MASH, supporting the idea that adipose tissue dysfunction plays a crucial role in these conditions. Continued research in this area could pave the way for precision medicine strategies, improving diagnostic and therapeutic approaches for MASH.

Introduction

As global obesity rates continue to rise, with an expected peak between 2026 and 2054,[1] the excessive accumulation of white adipose tissue poses significant medical and societal challenges. One of these challenges is the relationship between obesity-associated metabolic disturbances and liver disease. Metabolic dysfunction-associated steatotic liver disease (MASLD) is a widespread pandemic affecting up to 25% of the global population.[2] The lesions associated with this chronic liver disease come in different combinations and varying severity, closely related to obesity. Metabolic dysfunction-associated steatohepatitis (MASH) is the most severe form and affects 1.5–6.5% of the world's population in association with an increased mortality rate.[3–5] In patients with severe obesity or body mass index (BMI) > 40 kg/m^2, the expanded adipose tissue is so remarkable that bariatric surgery remains an integral part of the therapeutic options. The prevalence of MASLD in severe obesity can reach 80–92%, with MASH occurring in 15–30% of patients.[6] The reasons why only some patients develop MASH and the driving factors remain unclear. The necessary personalised approach in medical practice management requires a better understanding of the pathogenesis,[7,8] the goal of our research. Patients with severe obesity represent a unique human model to explore organ interactions. Among other considerations, surgical procedures offer the potential advantage of safely donating paired biopsies of multiple tissues. Our hypothesis considers that adipose tissue has been an underappreciated organ and assumes a significant failure in homeostatic mechanisms communicating the liver and adipose tissue. The design facilitates exploring whether MASH and adipose tissue biology mutually influence each other.

Adipose tissue is critical in lipid and lipoprotein metabolism, energy balance, and immune function.[9] The cells within the tissue, including adipocytes, communicate their status through different signalling molecules, such as adipokines, cytokines, and lipid species, which can have widespread effects on the body.[10–12] There is evidence indicating that severe obesity

is a multisystem disease with complex characteristics.[13,14] Assessing the relative impact of potential confounding factors, including genetic susceptibility, on MASH is a particularly challenging aspect.[15] It is possible that obesity-induced metabolic dysfunction of adipose tissue, rather than its mass or location, is responsible for the strong relationship with MASH.[9,10] However, white adipose tissue is not uniform, and in severe obesity, we expect constant remodelling in response to lifestyle variations, which may vary in different locations.[16] Although there are conflicting data, visceral obesity in humans is thought to correlate more strongly with an increased risk of metabolic disorders than increased subcutaneous adipose tissue.[17–19] Based on our hypothesis, a thorough understanding of the distinct characteristics of these depots and their potential dysfunction could provide insights into organ interactions leading to MASH. We conducted a study examining the liver, abdominal subcutaneous adipose tissue, omental visceral adipose tissue, and blood in patients with and without MASH using a systems biology-based approach to identify pathophysiological differences and propose potential metabolic signatures with prognostic value.

Methods

Participants and study design

This pilot case-control study included registered participants for the observational cross-sectional study (EOM) outlined at ClinicalTrials.gov number NCT05554224. The sample size was estimated following the formula of Fleiss et al.[20] The Type I error rate was set at 0.05, the power was at 0.8, and the case-to-control ratio was 1. We expected a 35% of MASH patients, therefore *p0* was set at 0.35. The estimation determined a sample size of n = 49 per group, and finally, n = 49 patients with MASH and n = 51 patients without MASH were included. The participants were ethnically homogeneous patients with severe obesity, defined as having a body mass index (BMI) > 40 kg/m^2 or a BMI of 35 kg/m^2 or higher experiencing obesity-related health conditions who met the inclusion criteria to undergo bariatric surgery. With a multi-omics approach, we examined the profiles of the liver, abdominal subcutaneous adipose tissue (SAT), omental visceral adipose tissue (VAT), and plasma from patients, both with and without MASH (Fig. 1a). Histological evaluation was performed using whole-slide imaging to determine steatosis, hepatocellular ballooning, lobular inflammation, and fibrosis. We established MASH in the traditional way, adding the scores of steatosis, hepatocellular ballooning, lobular inflammation, and fibrosis.[21,22] Scores of 5 or greater indicate MASH (cases, n = 49), while scores of 2 or less indicate non-MASH (controls, n = 51) (Fig. 1b). When necessary, non-MASH patients were further subclassified according to Bedossa et al. algorithm.[23] All participants provided written informed consent. The institutional ethics review board of the Institut d'Investigació Sanitària Pere Virgili and University Hospital Sant Joan (Reus, Spain) approved the protocol under the registration codes EPIMET083/2018 and PL4NASH112/2021, ensuring strict adherence to the ethical guidelines of the Declaration of Helsinki. Patients under 18 years of age, those with excessive alcohol consumption, acute illness, malignancies, previous or current major cardiovascular events, and viral hepatitis were excluded from the study.

Clinical assessment, biopsies sampling, and histologic studies

We diagnosed hyperlipidemia, type 2 diabetes mellitus, and hypertension using standard protocols and routine laboratory methods.[24] We gathered blood samples before surgery and took biopsies of hepatic, subcutaneous (abdomen), and visceral (omentum) adipose tissue during the operation. We stored a portion of the biopsies at −80 °C for further analysis. Simultaneously, another part was processed, sectioned, and stained for evaluation using Hematoxylin and Eosin, Masson's trichrome, and Sirius Red stainings and CD15 (760–2501) and CD68 (790–2931) immunostainings. These antibodies were purchased in ready to use format and immunostainings were performed on BenchMark ULTRA instrument (Ventana Medical Systems) following the manufacturer's instructions. We used established procedures to assess adipose tissue and liver biopsies, including examining fibrosis and the distribution of immune cells.[21,23,25] We analysed data separately for men and women but did not find significant sex-specific differences. For more details, see Extended methods.

Measurement of circulating lipoprotein profile and organokines

We collected plasma and serum samples and stored them at −80 °C until analysis. We used the Liposcale® software to analyse the ^{1}H-NMR spectra and calculate lipid concentrations and the number and sizes of particles in the main lipoprotein classes.[26] We also measured irisin (ref: DY9420-05), leptin (ref: DY398), adiponectin (ref: DY1065), fibroblast growth factor (FGF) 19 (ref: DY969), and FGF-21 (ref: DY2539) using ELISA kits from R&D Systems (Minneapolis, MN, USA).

Other laboratory procedures

We isolated DNA and RNA following the instructions of the Qiagen QIAmp DNA Micro Kit and the Qiagen RNeasy Lipid Tissue Mini Kit (Werfen, Barcelona, Spain). We measured the concentrations of DNA and RNA using a NanoDrop ND-1000 spectrophotometer (Nanodrop Technologies Inc., Wilmington, NC). The RNA integrity number (score > 8) was assessed using an RNA2100 Bioanalyzer (Agilent Technologies, Santa

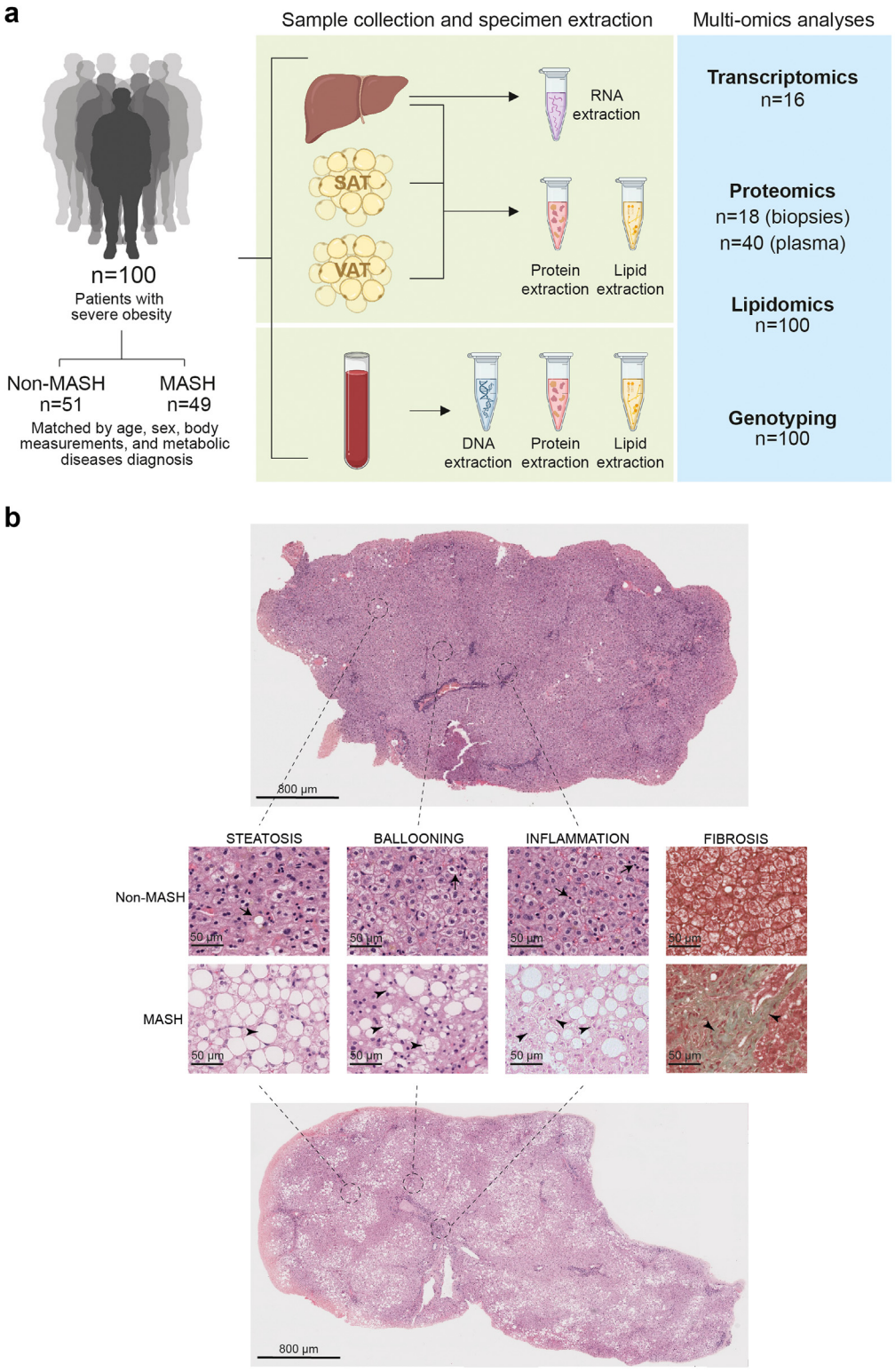

Fig. 1: **Patient selection and study design.** Hepatic and adipose tissues and blood were obtained from patients with obesity and with or without MASH for multi-omics analyses, created with BioRender.com (a). Digital pathology involves scanning standard biopsies to generate whole-slide images, breaking them into individual features, and assessing them on continuous or categorical scales. The total score obtained from these features helps in making a diagnosis. Ballooned hepatocytes and lobular inflammation distinguish between patients with and without MASH (b).

Clara, CA) with the RNA 6000 Nano Kit. Genetic variants associated with MASLD and obesity are listed in Supplementary Table S1, and were identified through published genome-wide association studies. They were genotyped using the OpenArray technology (Thermo Fisher Scientific, Barcelona, Spain). We used 100 ng of total RNA from the livers and the Quick Amp Labelling kit to produce cyanine-labelled cRNA. The labelled cRNA was hybridised to the Sureprint G3 human gene expression 8 × 60 k v2 microarray according to the manufacturer's protocol (Agilent, Palo Alto, CA). The arrays were scanned in an Agilent Microarray Scanner, and the raw data were extracted using Agilent Feature Extraction as described.[27] For proteomic analysis, peptide labelling, chromatographic separation, mass spectrometry analysis, and protein identification and quantification, followed by protein extraction, quantification, and digestion. Tryptic peptides were injected into a nano liquid chromatography system coupled via nanoelectrospray ionisation source to Orbitrap Velos Pro mass spectrometer (Thermo Fisher Scientific). Annotated spectra are available via the MS-viewer tool in the PRIDE partner repository.[28,29] Semi-targeted lipidomics was performed essentially as described[30,31] using a 1290 Infinity ultra-high-pressure liquid chromatograph coupled with a dual electrospray ionisation source to a 6550 quadrupole-time-of-flight mass spectrometer (Agilent Technologies, Santa Clara, USA). More details on genotyping and omics methods are shown in Extended methods.

Statistical analyses

The data analysis was conducted using RStudio (version 2023.06.0.421), R (version 4.0.5), and Python (version 3.9.12). We used medians, interquartile ranges, number of cases, and percentages to represent quantitative and qualitative variables. Variables with more than 30% of missing values were excluded. Missing values of the remaining variables were imputed with the median. Non-parametric tests like the Mann–Whitney U test for quantitative variables and Fisher's exact test for qualitative variables were employed for comparisons. Spearman's correlation tests were used to assess correlations between quantitative variables, and multiple group comparisons were evaluated with ANOVA and ordinal logistic regression. The comparisons and assessments were performed using Tableone, tidyverse R packages,[32,33] and Panda's Python library.[34] For multivariate analyses, the MetaboAnalystR R package[35] was utilised for Principal Component Analysis (PCA), Partial Least Square Discriminant Analysis (PLS-DA), and Hierarchical clustering. Plots were generated using ggplot2 within the tidyverse R package, MetaboanalystR, and circle R packages.[36] P-values were adjusted for false discovery rate in the multivariate analyses. To explore the predictive ability of the variables to diagnose MASH non-invasively, we employed a machine learning approach using support vector machines (SVM) with MetaboanalystR package. This identified biomarker candidates, integrating combinations of 5–100 random variables through a Monte Carlo cross-validation (MCCV) model. Diagnostic accuracy was assessed using the area under the curve (AUC) of receiver operating characteristic (ROC) curves and confusion matrices. More detailed information and code sources can be found in Extended methods.

Role of the funding source

The funders of this study had no role in the design, data collection, data analysis, data interpretation, or writing of this manuscript.

Results

Selection of participants

We selected our patients to match based on age, sex, body measurements, and metabolic health conditions to minimise unbalanced disease risk from interfering factors. However, matching patients for insulin resistance and C-reactive protein levels was not feasible. In our cohort, only 17.6% of patients without MASH did not exhibit hepatic fibrosis, and only five patients presented a presumably healthy liver (Table 1). Additionally, in the non-MASH group, 22 patients (43.1%) met the criteria for MASLD, while 27 patients (52.9%) exhibited hepatic activity, characterised by inflammation or ballooning, without evidence of steatosis.

Inflammation, mitochondrial dysfunction, and lipotoxicity shape distinct liver phenotypes

Not all patients with severe obesity develop MASH. This finding suggests the role of complex adaptive responses and genetic variants, each with a minor impact, in causing distinct susceptibility. The selected variants were primarily associated with energy homeostasis, inflammation, and lipid metabolism. Logistic regression analysis revealed no discrimination between patients with and without MASH (Fig. 2a, Supplementary Table S2).

Transcriptome data highlighted differences in gene expression closely related to energy and lipid metabolism. Differentially expressed genes in livers with MASH indicated a significant upregulation associated with immune response pathways (Fig. 2b and c). Immunohistochemistry confirmed the increased gene expression of markers for human myeloid cell interactions in livers with MASH. CD15+ and CD68+ cells identified a strong correlation between inflammatory infiltrates and a distinct impact of neutrophils and hepatic crown-like structures that differentiate livers with and without MASH. Differences in histologic distribution suggest a close association between innate-adaptive immunity regulation and severe obesity-associated MASH (Supplementary Fig. S1a).

	Non-MASH (n = 51)	MASH (n = 49)	P-value
Anthropometric data			
Sex (woman), n (%)	38 (74.5)	31 (63.3)	0.318
Age (years)	51 (40–56)	48 (42–55)	0.953
BMI (kg/m^2)	43.4 (39.2–45.5)	46.4 (41.4–51.5)	0.053
Concomitant diseases			
Type 2 diabetes mellitus, n (%)	17 (33.3)	20 (40.8)	0.570
Hypertension, n (%)	27 (52.9)	35 (71.4)	0.090
Dyslipidemia, n (%)	17 (33.3)	22 (44.9)	0.327
Metabolic syndrome, n (%)	30 (58.8)	32 (65.3)	0.644
Biochemical characteristics			
Glucose (mmol/L)	6.5 (5.4–8.6)	7.6 (6.3–10.7)	0.052
Insulin (pmol/L)	75.7 (45.2–129.5)	104.2 (59.7–145.2)	0.125
HOMA-IR	3.3 (1.9–5.7)	5.0 (3.2–7.7)	0.051
Triglycerides (mmol/L)	1.5 (1.2–2.1)	1.7 (1.3–2.2)	0.11
Total cholesterol (mmol/L)	4.0 (3.4–4.7)	4.2 (3.5–5.0)	0.65
HDL-cholesterol (mmol/L)	1.0 (0.9–1.3)	0.9 (0.8–1.1)	0.130
LDL-cholesterol (mmol/L)	2.0 (1.8–2.7)	2.3 (1.8–3.1)	0.669
VLDL-cholesterol (mmol/L)	0.7 (0.6–1.0)	0.8 (0.6–1.0)	0.110
C reactive protein (mg/L)	0.3 (0.2–0.6)	0.9 (0.6–1.2)	0.024
Platelets (x10^9/L)	252 (215–297)	268 (215–321)	0.380
ALT (U/L)	25.8 (18.6–36.0)	52.8 (30.0–66.6)	<0.001
AST (U/L)	25.8 (20.4–36.0)	51.0 (33.0–81.0)	<0.001
GGT (U/L)	18.0 (13.2–28.8)	25.8 (16.2–55.8)	0.001
Hepatic histologic features			
Steatosis grade, n (%)	3 (0–10)	50 (40–70)	<0.001
Steatosis score, n (%)			<0.001
<5%	32 (62.7)	..	
5–33%	18 (35.3)	8 (16.3)	
34–66%	1 (2.0)	26 (53.1)	
>66%	..	15 (30.6)	
Lobular inflammation, n (%)			<0.001
No foci	17 (33.3)	..	
<2 foci	29 (56.9)	18 (36.7)	
2–4 foci	5 (9.8)	29 (59.2)	
>4 foci	..	2 (4.1)	
Ballooning, n (%)			<0.001
None	32 (62.7)	..	
Few cells	17 (33.3)	19 (38.8)	
Many cells	2 (3.9)	30 (61.2)	
Fibrosis, n (%)			<0.001
None	9 (17.6)	1 (2.1)	
Perisinusoidal or periportal fibrosis	23 (45.1)	8 (17.0)	
Perisinusoidal and periportal fibrosis	14 (27.5)	18 (38.3)	
Bridging fibrosis	5 (9.8)	18 (38.3)	
Cirrhosis	..	2 (4.3)	

ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MASH, metabolic associated steatohepatitis; VLDL, very low-density lipoprotein. Mann–Whitney U test for quantitative variables and Fisher's exact test for qualitative variables were employed for comparisons.

Table 1: **Clinical, biochemical, and histologic characteristics of the patients.**

Our study also identified specific protein signatures associated with inflammatory response, cell adhesion, and lipid metabolism pathways. Notably, proteins like CCR2, Gal3, and TREM2, expressed on macrophages, may regulate inflammation, suggesting potential targets for therapeutic intervention. ADAMTSL2 is likely associated with fibrosis, and ANNEXIN A2 is a pleiotropic phospholipid-binding protein that organises exocytosis

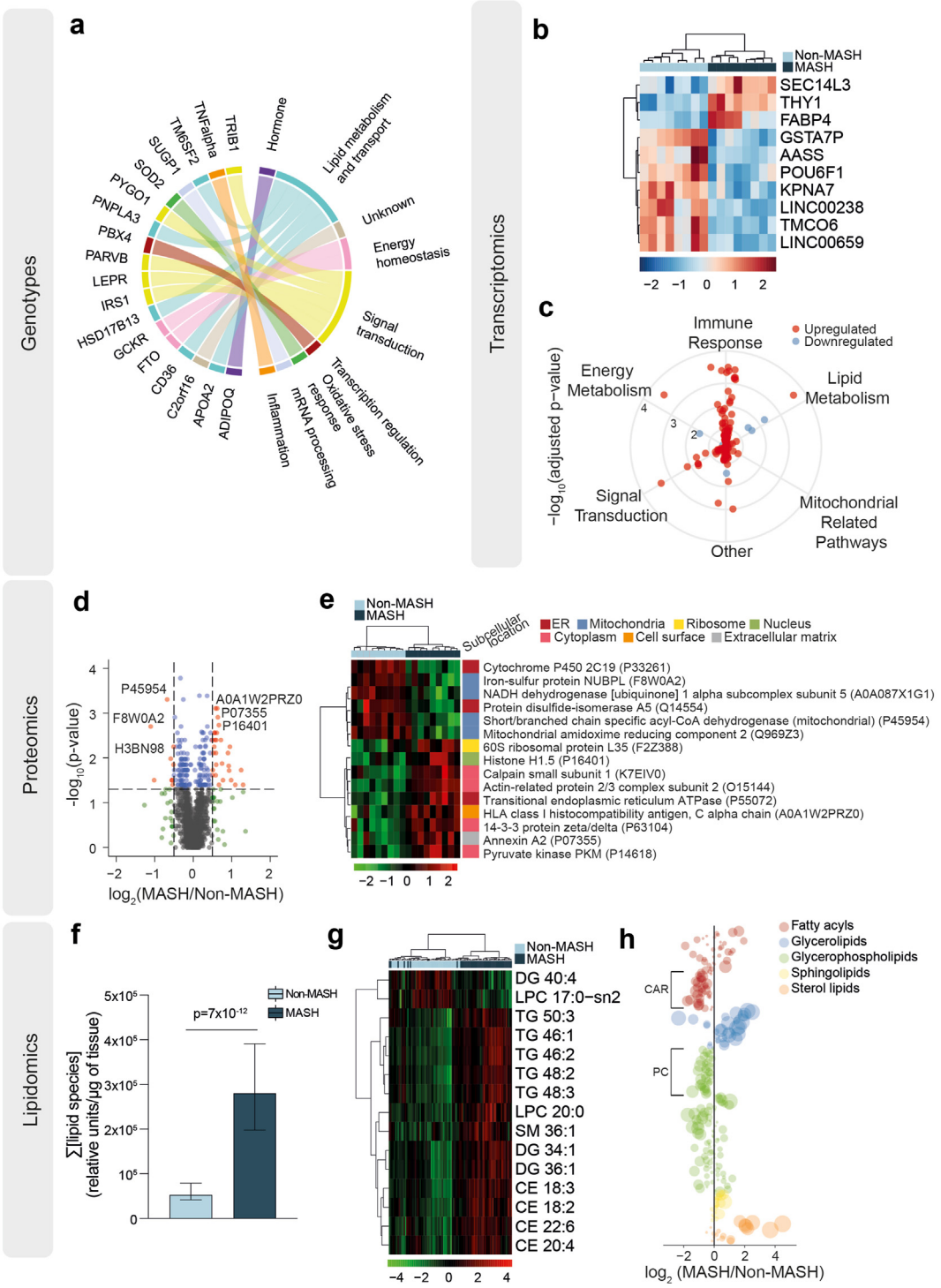

Fig. 2: **Understanding the mechanisms underlying the development of MASH requires a multi-omics approach, which involves investigating genetic susceptibility.** Genome-wide association studies have identified genetic variants in specific genes and facilitate calculation of polygenic scores. The panel shows a chord diagram representing the gene's association with their affected biological processes (a). Transcriptome data and pathway enrichment analysis have revealed differentially expressed genes in livers with and without MASH (b, c). Furthermore, mass spectrometry-based proteomics (d, e) and lipidomics (f–h) have provided distinct signatures that can differentiate between livers with and without MASH. Genotypes (n = 100), transcriptomics (n = 16), proteomics (n = 18), and lipidomics (n = 100) data were analysed using non-parametric statistical tests (Mann–Whitney U test) for univariate comparisons, and multivariate analyses were applied for multivariable data interpretation.

to the extracellular domain. Proteomics study confirmed mitochondrial dysfunction and endoplasmic reticulum stress in livers with MASH, and changes in mitochondrial acyl-CoA dehydrogenase and ubiquinone suggestive of lipotoxicity (see Fig. 2d, e). Lipidomics studies denoted the harmful effect of lipid accumulation, especially triglycerides and their products, such as long-chain fatty acids, ceramides, and diacylglycerols in MASH livers. Compared with livers without MASH, the decrease in carnitines and glycerophospholipids also indicated significant impairment in mitochondrial pathways associated with beta-oxidation and fatty acid biosynthesis (see Fig. 2f–h, Supplementary Fig. S1b and Table S3). Further analysis underscored the heterogeneity within hepatic lesions. When liver lipidomes were assessed without collapsing patients into binary MASH/non-MASH categories, a strong association emerged between specific lipid profiles and MASH severity. In this refined approach, most patients with MASH clustered together, indicating a distinct lipidomic signature (Supplementary Fig. S1c). The research emphasises the interconnectedness of developing MASH through complex pathogenic pathways.

Structural and functional differences between visceral and subcutaneous adipose tissue in severe obesity

SAT and VAT primarily consist of adipocytes with a unilocular lipid droplet of varying sizes. However, we found that the mean area of adipocytes and the percentage of fibrosis in SAT were significantly higher than in VAT (Fig. 3a-c). These differences suggest that SAT is more involved in fuel storage and extracellular matrix remodelling than VAT. Additionally, we observed higher expression of proteins associated with actin and microtubule network in SAT, confirming higher remodelling in this depot (Supplementary Fig. S2a). While there were no specific proteomic signatures to distinguish SAT from VAT significantly (Fig. 3d-e), lipidomic analysis revealed specific profiles that classified almost perfectly SAT from VAT. The differences in concentrations of fatty acid, carnitines, glycerophospholipids, and oxylipins between SAT and VAT suggested distinct lipid metabolism. Furthermore, we identified changes primarily associated with lysophosphatidylethanolamine metabolism and their potential role in cell signalling (Fig. 3d-h, Supplementary Fig. S2b). These findings indicate that the structural and functional differences between SAT and VAT in severe obesity are likely related to fat accumulation and metabolic dysfunction.

Interactions between MASH and altered adipose tissue biology

The presence or absence of MASH leads to structural differences in adipose tissue. Fibrosis in SAT and VAT were similar in patients with and without MASH, and fat storage was significantly higher in patients with MASH, both in SAT and VAT (Fig. 4a, g). There were also differences in the lipid composition of adipose tissue associated with liver disease. In SAT, the presence of MASH did not alter the concentration of glycerolipids (Supplementary Table S4). However, we identified specific lipidomic signatures associated with an increase in the concentration of sterol lipids, increased levels of specific fatty acids and phospholipids, and a consistent decrease in carnitine concentration, with significant classification values to distinguish patients with MASH. Carnitine deficiency was the most discriminative factor in patients with MASH (Fig. 4b-f). In VAT, there were quantitative and qualitative differences related to MASH. Compared to patients without MASH, there was a significant reduction in the VAT concentration of glycerolipids, glycerophospholipids, and sphingolipids. The contribution of decreased carnitines and increased amounts of fatty acids and sterol lipids provided specific lipid signatures with discriminative values between patients with and without MASH (Fig. 4h-l). Notably, when stratifying patients considering the heterogeneity of the hepatic lesions, the SAT lipidome showed limited accuracy in distinguishing MASH from other categories. In contrast, VAT lipidomic profiles demonstrated higher classification accuracy for patients with MASH (Supplementary Fig. S3b). The functional enrichment analyses confirmed affected pathways in SAT and VAT associated with MASH. In both depots, low carnitine levels suggest a low transfer of fatty acids to mitochondria for their oxidation. The accumulation of biliary acids likely represents the potential role of microbiota-derived metabolites in regulating the metabolic activities of adipose tissue associated with MASH (refer to Supplementary Fig. S3c and review in[37]). The proteomic analysis supports that interconnections between liver lesions and adipose tissue dysfunction are more prominent in VAT. Indeed, the analysis showed poorly grouped and non-significant associations in SAT (Fig. 5a, b), and specific VAT profiles indicated significant differences between patients with and without MASH (Fig. 5c, d). Notably, in patients with MASH, we found a significant downregulation in adipose tissue proteins associated with mitochondrial β-oxidation and tricarboxylate transport and increased expression of extracellular matrix peptides, as illustrated in Fig. 5e and Supplementary Table S4.

ANOVA analysis and ordinal logistic regression revealed patterns in composition across liver steatosis, ballooning and inflammation. The changing trends in lipids are summarised as heat maps (Supplementary Fig. S4), and Supplementary Table S5 lists the identified proteins. For example, TGs and CEs correlate to all histologic features scored in the liver. The accumulation of biliary and fatty acids was similar in both fat depots, while carnitines in SAT and lysophospholipids in VAT

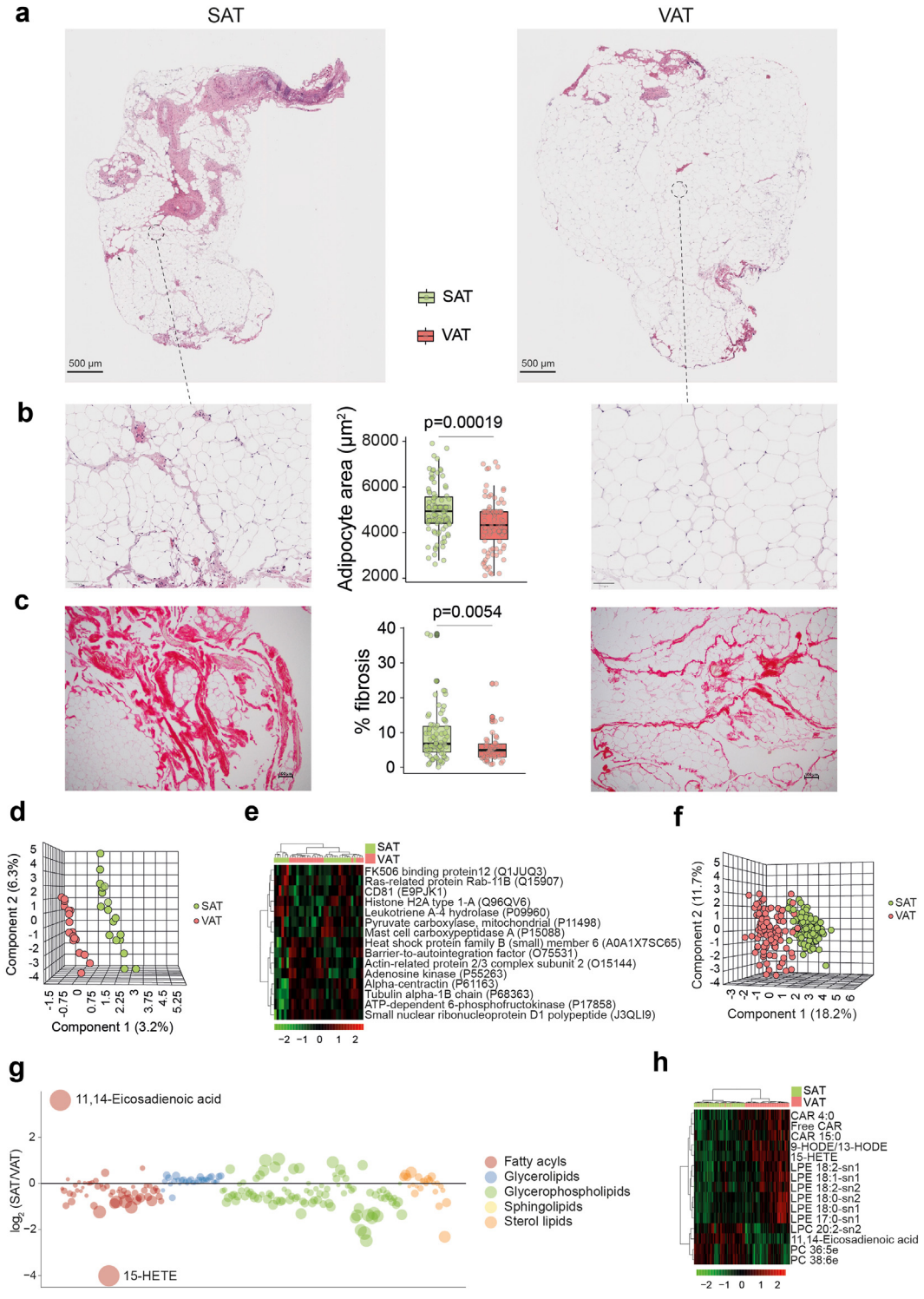

Fig. 3: **White adipose tissue shows anatomical and functional diversity in patients with severe obesity.** Using digital pathology, we examined subcutaneous (SAT) and omentum visceral (VAT) adipose tissue. We found structural differences in adipocyte size and fibrosis at the specific collection sites in all patients (a–c). Our proteomics data showed that immune response and cellular repair-related proteins were expressed differently in VAT compared to SAT (d, e). Additionally, our lipidomics analysis revealed distinct compositions for both fat depots (f) and highlighted significant differences in enrichment of oxylipins derived from various fatty acid sources and lysophoshpatidylethanolamines (LPE) (g, h), which provided the most distinctive value. Proteomics (n = 18, per tissue) and lipidomics (n = 100, per tissue) data were analysed using non-parametric statistical tests (Mann–Whitney U test) for univariate comparisons, and multivariate analyses were applied for multi-variable data interpretation.

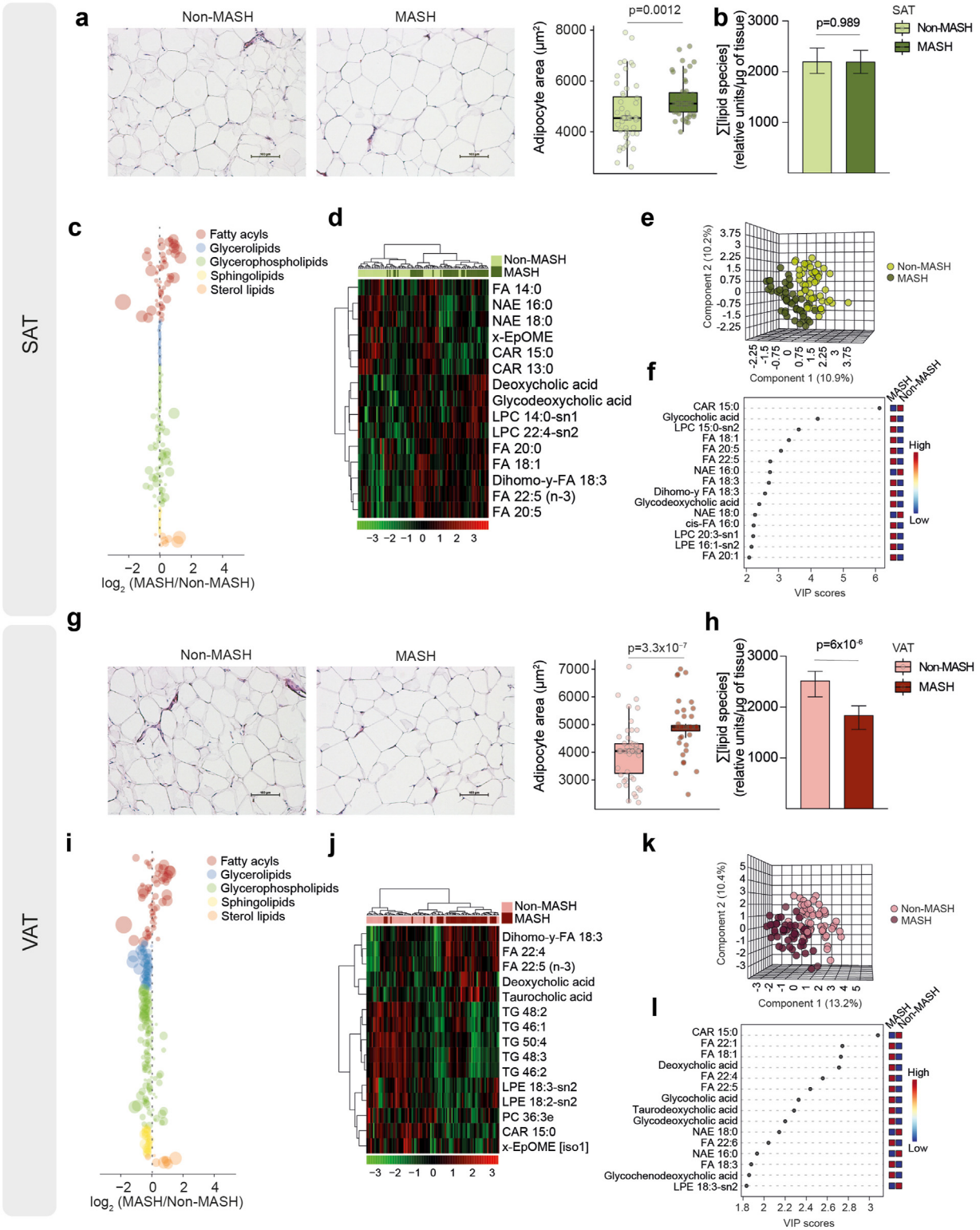

Fig. 4: **Patients with and without MASH exhibit distinct differences in the structure and lipid composition in both fat depots, with the most significant disparities in visceral adipose tissue.** Subcutaneous adipose tissue (SAT) from patients with MASH had increased adipocyte area and a similar amount of lipids (a, b). Lipidomics studies revealed differences in composition that included significant changes in the distribution of fatty acids, bile acids, and carnitines, which contributed to

showed a higher association with increasing histological scores. We also explored specific signatures associated with the fibrosis stage, a histologic feature not typically used in diagnosing MASH but likely associated with clinical outcomes (Supplementary Fig. S5a). We identified critical lipidomic signatures in adipose tissues at the liver F2 transition to F3. The liver, SAT, and VAT lipidomics and proteomics profiles provided information for stratification (Supplementary Fig. S5b–d). However, the relative importance of each molecular species varied among tissues (Supplementary Figs. S5 and S6). Attempts to find discriminative models using information on the identified species in plasma were ineffective in predicting hepatic fibrosis. Only plasma CE 18:0 levels differentiated patients with F0-2 from those with F3-4 (Supplementary Fig. S7a). Our findings provide connections between the liver and adipose tissue dysfunction, more remarkable in VAT, but potential use in clinical practice requires large studies of epidemiologic trends.

Plasma lipidomics predicts MASH in severe obesity

The search for plasma biomarkers that can distinguish patients with and without MASH in severe obesity remains challenging. Our analysis of plasma proteomics revealed some distinct patterns in protein abundance between patients with and without MASH. However, these patterns did not translate into effective discrimination between groups (Fig. 6a). We found significant differences between patients with and without MASH regarding circulating VLDL, including cholesterol and triglyceride concentrations, size, overall number of particles, and adiponectin and FGF-21. Despite these findings, as shown in Fig. 6b, there is a considerable overlap, limiting their potential for effective discrimination. Examining differences in circulating HDL and LDL particles, FGF-19, irisin, and leptin revealed poor or no association with liver disease in patients with severe obesity (Supplementary Fig. S7b).

However, our lipidomics analysis in blood has successfully identified discriminative molecular signatures for MASH diagnosis (Supplementary Table S6). By leveraging high dimensional datasets, supervised vector machines, and PLS-DA algorithms, we obtained a robust model for classifying MASH (Fig. 6c). To enhance reliability, we incorporated recursive feature elimination, visual inspection, and stratified K-fold cross-validation (Fig. 6d), and the approach led us to identify three lipid species with sufficient diagnostic accuracy (AUC = 0.919, 95% CI 0.840–0.979) when used in combination (Fig. 6e). These values significantly outperformed those described for other non-invasive tests, even when considering the hepatic heterogeneity of the non-MASH patients (Supplementary Fig. S8). Lastly, we used mass spectrometry data, tissue histology, and routinely available tests to construct correlation networks in patients with and without MASH (Supplementary Fig. S9a). Results confirmed variations in tissue interconnectedness regarding liver injury and showed the influence of lipid composition in organs on circulating lipids. Data integration between proteomics and lipidomics in the liver and VAT highlighted the putative role of dysfunctional lipid dynamics and mitochondrial metabolism in MASH patients again (Supplementary Fig. S9b).

Discussion

Mathematical models forecast a considerable increase in liver-related mortality in the coming years and a rapidly growing prevalence of obesity-associated MASH.[38] No single mechanism explains why only some patients with extreme fat accumulation develop MASH.[9,10] We examined the impact of expanded adipose tissue on liver disease in patients with severe obesity at opposite ends of the liver disease spectrum while accounting for differences in adiposity and the influence of genetic susceptibility and metabolic comorbidities.[39–42] In the liver, a comprehensive approach recognised alterations associated with inflammation, mitochondrial dysfunction, and lipotoxicity as significant factors affecting hepatic health. This interplay between metabolic and inflammatory disturbances may help to explain the variable degrees of hepatic injury observed among individuals. Insulin resistance exacerbates hepatic lipotoxicity and oxidative stress, creating a pro-inflammatory hepatic environment that may contribute to the onset of MASH.[14,43] Although we noted heterogeneity in hepatic lesions within both groups, which introduced complexity in our analyses and represented a limitation, MASH patients exhibited clear profiles in both omics analyses and in insulin resistance and CRP levels. Comparing specific lipidomic and proteomic signatures in blood, liver, and distinct adipose tissue depots indicates that adipose tissue biology modifies the likelihood of developing MASH. Molecular signatures in adipose tissue suggest a likely connection between mitochondrial dysfunction and extracellular matrix remodelling in MASH. We also identified potential candidates for MASH screening and follow-up among circulating lipid signals.

Both MASH and severe obesity are complex conditions with multiple connections. Since not all treatment options work for everyone, combining

the classification of patients with and without MASH (c–f). These effects were more apparent in visceral adipose tissue (VAT) (g–i) with differences in the concentration of lipid species that could predict MASH. In VAT, decreased glycerolipids and glycerophospholipids accompanied a significant decrease in carnitines and increased bile acids, also observed in SAT (j–l). Histological determinations and lipidomics (n = 100, per tissue) data were analysed using non-parametric statistical test (Mann–Whitney U test) for univariate comparisons, and multivariate analyses were applied for multivariable data interpretation.

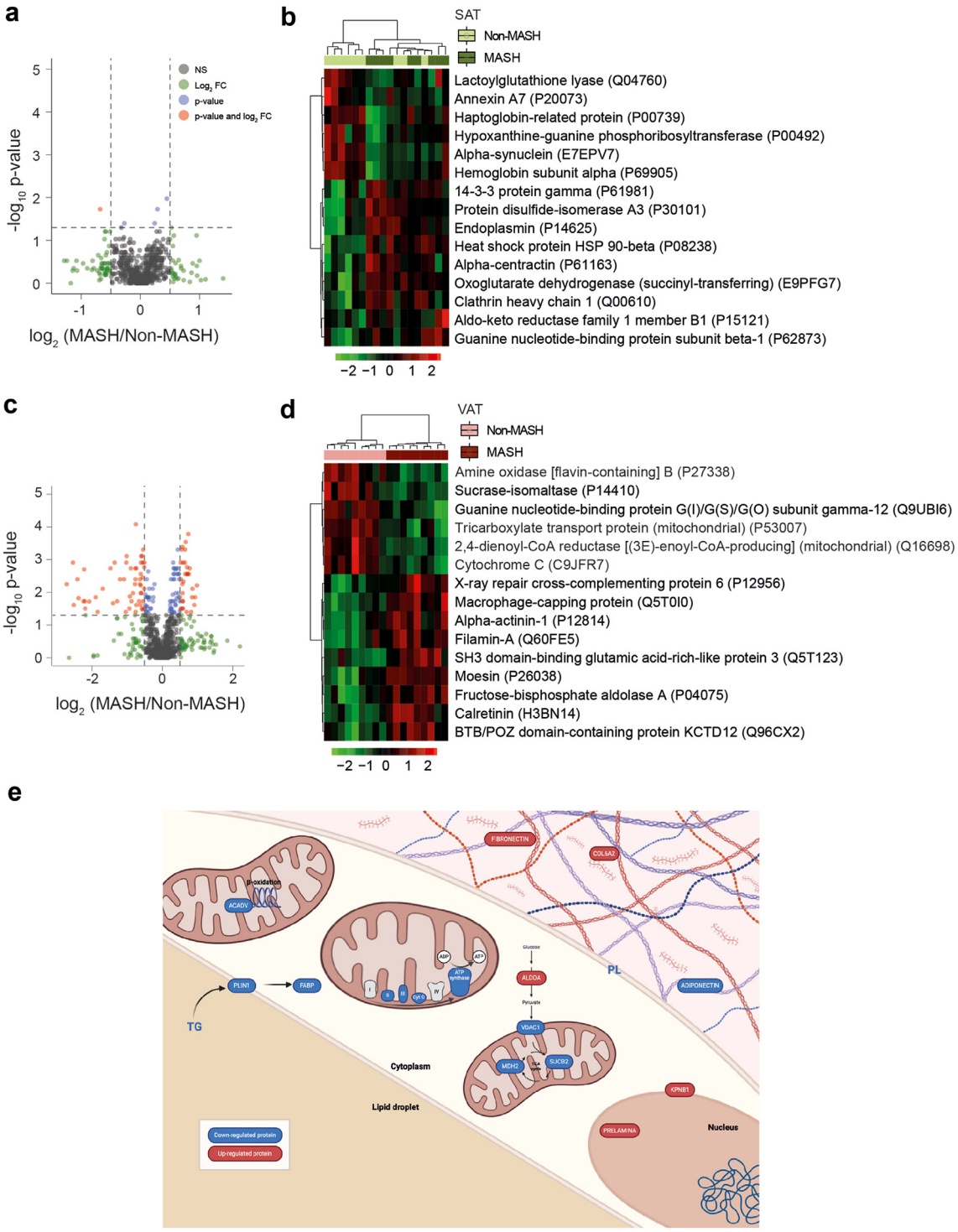

Fig. 5: **Proteomics of visceral adipose tissue in patients with MASH unveils mitochondrial dysfunction and an increased extracellular matrix response.** In subcutaneous adipose tissue (SAT), specific proteomic signatures were poorly clustered and did not differentiate patients with and without MASH (a, b). However, in visceral adipose tissue (VAT) there were significant MASH-related responses (c–d). In patients with MASH, we observed a significant decrease in proteins associated with mitochondrial beta-oxidation and tricarboxylate transport and an increase in the expression of extracellular matrix peptides in VAT (e). Proteomics (n = 18, per tissue) data were analysed by multivariate statistics.

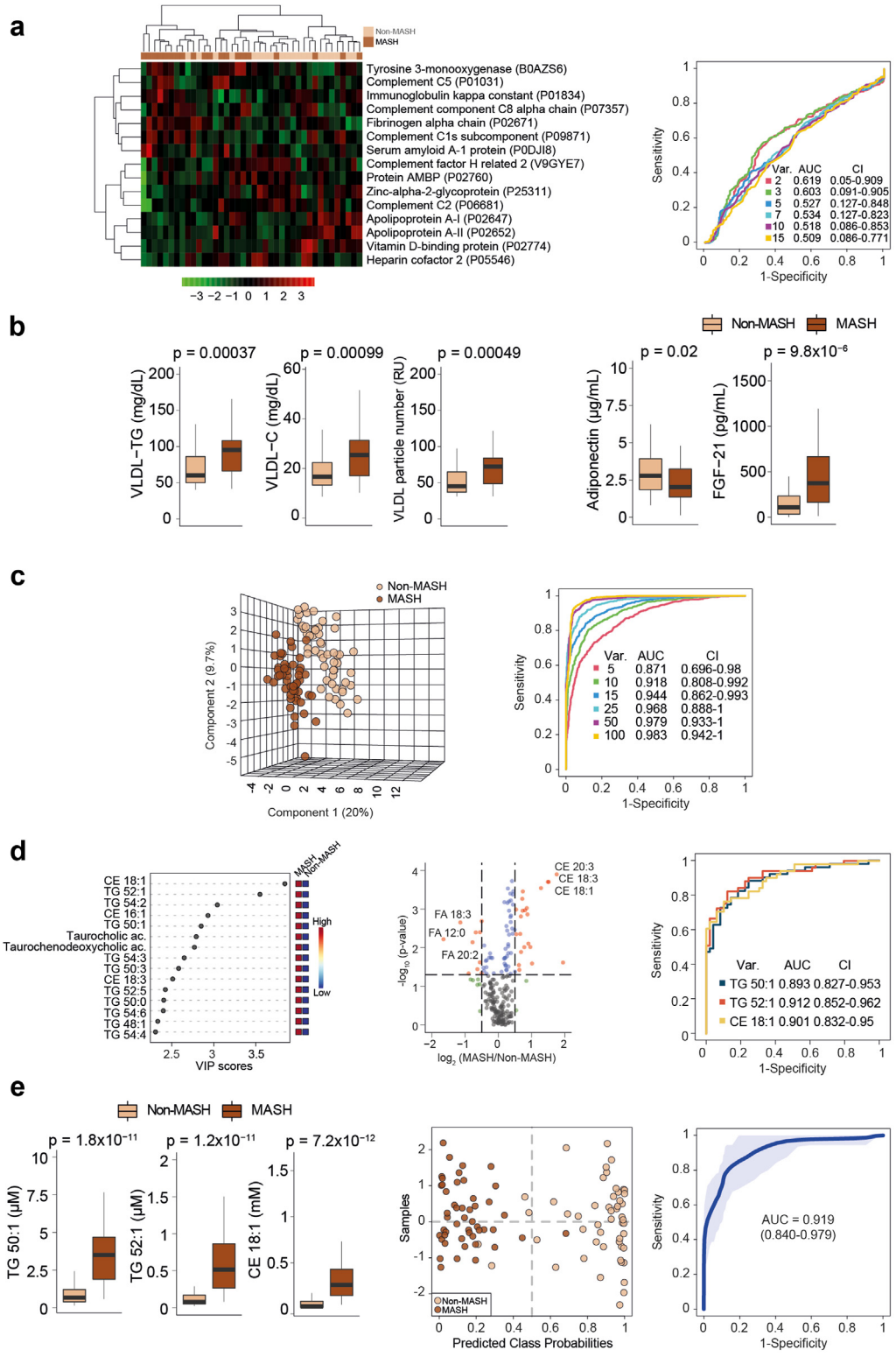

***Fig. 6:* Plasma biomarkers for MASH screening.** Plasma proteomics do not provide distinct patterns to distinguish patients with and without MASH (a). There were MASH-related differences in circulating VLDL, adiponectin and FGF-21, without practical implementation in clinical practice (b). However, plasma lipidomics delivered robust models for classifying MASH (c, d). Mathematical models indicate that circulating TG 50:1, TG 52:1 and CE 18:1 used in combination yield sufficient diagnostic accuracy to propose a candidate biomarker (e). Proteomics (n = 40), lipidomics (n = 100), and other laboratory assessments (n = 100) data were analysed using non-parametric statistical test (Mann–Whitney U test) for univariate comparisons, and multivariate analyses were applied for multivariable data interpretation.

therapies that influence weight loss and have independent metabolic effects may be more beneficial. The positive impact of weight reduction through lifestyle changes or bariatric surgeries on MASH is well documented.[11,44–46] There are now potential strategies for using medications to treat MASH. For example, drugs that target the glucagon-like peptide 1 and glucagon receptors show promise in changing the course of obesity-related cardiometabolic diseases.[47–49] While effective for only a small percentage of patients, drugs targeting inflammation are an active area of development.[50] Peroxisome proliferator-activated receptor agonists can reduce the formation of harmful lipid species, and lanifibranor is showing potential in targeting liver disease.[51–53] Many of these drugs also positively affect adiponectin levels, and FGF-21 analogues are being explored for treating MASH in clinical trials.[53,54] Although the links between severe obesity, altered cholesterol, and bile acid metabolism are not fully understood, novel interventions and statin treatment are appealing strategies for the early stages of diseases, mimicking metabolic changes seen with surgically induced weight loss.[55,56] Efforts to treat mitochondrial dysfunction and fibrosis are limited to animal studies, while liver-targeted thyroid hormone receptor β agonists have shown promise in resolving MASH and improving liver fibrosis.[57] The putative mechanisms of action include reduced fatty acid uptake and synthesis and stimulation of mitochondrial β-oxidation.

The effectiveness of procedures and drugs can vary across clinical trials. Of note, variability includes different response rates in the placebo group. Our data emphasise the need to assess outcomes in MASH by considering the impact of adipose tissue on the liver.[11,58] However, the way in which adipose tissue dysfunction leads to MASH still needs to be clarified. Structural and functional differences between SAT and VAT and the dynamic endocrine nature of different fat depots are associated with MASH.[9,59–61] For example, in our study group, we observed that changes in plasma adiponectin and FGF-21 levels are associated with increased liver secretion of VLDL and can distinguish patients with and without MASH. Fibrosis is an essential consideration in evaluating MASH and is likely influenced by increased extracellular matrix deposition in adipose tissue.[62] Our findings reveal lipid and protein expression patterns in adipose tissue associated with liver fibrosis during the transition from stage F2 to F3. Therefore, it is plausible that disruptions in adipose tissue metabolism, probably in mitochondrial pathways, cause structural and functional changes. This may impact other organs through signalling molecules derived from adipose tissue. Therefore, the significance of lipid signals in MASH and hepatic fibrosis warrants further investigation.[12,63–65] A blood-based test in routine care that considers both adipose tissue biology and liver injury would benefit follow-up in MASH, reducing screening failures and the limitations of liver biopsy. Indeed, by analysing mass spectrometry lipidomics data and employing machine-learning algorithms,[66–68] we identified several lipid species that could effectively screen for MASH in patients with severe obesity. These biomarkers require further validation in a separate cohort, but developing simple and affordable biosensors for these or other lipid species is feasible in future research.[69]

While our findings highlight potential diagnostic biomarkers and mechanisms in MASH, several limitations must be acknowledged. The study's unicentric design, limited to a specific geographic region, restricts the generalizability of our results. Small sample sizes in our transcriptomics and proteomics analyses limit statistical power and could affect the reproducibility of these findings. The inherent heterogeneity of hepatic lesions in patients with severe obesity also poses challenges. Lastly, as an observational study, our design limits causal inference.

In conclusion, a disruption in the metabolic equilibrium of lipid processing and storage in adipose tissue accelerates detrimental adaptive responses in the liver. Although our major limitation is the study's observational nature, it is clear that the differences in the structure and function of adipose tissue are relevant and influential in maintaining liver health. As we advance, we must conduct extensive studies to validate proposed biomarkers across the full spectrum of MASLD and associated complications in severe obesity, incorporating estimates of the composition and distribution of fat depots.

Contributors

Helena Castañé: Conceptualization, Methodology, Software, Formal analysis, Investigation, Writing—Original draft, Writing—Review and editing. **Andrea Jiménez-Franco:** Methodology, Software, Investigation, Writing—Review and editing. **Anna Hernández-Aguilera:** Methodology, Investigation, Writing—Review and editing. **Cristian Martínez-Navidad:** Methodology, Software, Writing—Review and editing. **Vicente Cambra-Cortés:** Methodology, Software, Writing—Review and editing. **Alina-Iuliana Onoiu:** Methodology, Software, Writing—Review and editing. **Juan Manuel Jiménez-Aguilar:** Methodology, Software, Writing—Review and editing. **Marta París:** Methodology, Investigation, Writing—Review and editing. **Mercè Hernández:** Methodology, Investigation, Writing—Review and editing. **David Parada:** Methodology, Investigation, Writing—Review and editing. **Carmen Guilarte:** Methodology, Investigation, Writing—Review and editing. **Antonio Zorzano:** Validation, Resources, Funding acquisition, Writing—Review and editing. **María Isabel Hernández-Álvarez:** Validation, Resources, Funding acquisition, Writing—Review and editing. **Jordi Camps:** Conceptualization, Validation, Data curation, Supervision, Writing—Original draft, Writing—Review and editing. **Jorge Joven:** Conceptualization, Validation, Resources, Data curation, Supervision, Writing—Original draft, Writing—Review and editing, Funding acquisition.

All authors read and approved the final version of the manuscript.

Data sharing statement

The analytical data that support the findings of this study are available from the corresponding authors and throughout the MTBLS10532 repository.

Declaration of interests
The authors declare that they have no competing interests.

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Appendix A. Supplementary data
Supplementary data related to this article can be found at https://doi.org/10.1016/j.ebiom.2024.105532.

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