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ORIGINAL CONTRIBUTIONS

A Pilot Study of Serum Sphingomyelin Dynamics in Subjects with Severe Obesity and Non-alcoholic Steatohepatitis after Sleeve Gastrectomy

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Abstract

Background Non-alcoholic steatohepatitis (NASH) is present in a high percentage of obese patients undergoing bariatric surgery. A significant proportion of patients still present NASH even after considerable weight loss and metabolic improvements after surgery.

Objective To determine whether the changes in the serum lipidome after sleeve gastrectomy could be used to discriminate obese patients with NASH patients to those with non-alcoholic fatty liver (NAFL).

Methods This study involved 24 patients with grade 3 obesity diagnosed with either NAFL ($n = 8$) or NASH ($n = 16$) using the non-invasive OWLiver assay. All patients suffering from NASH were re-evaluated 6 months after bariatric surgery using the OWLiver test to confirm NASH resolution. Serum lipid extracts were assessed at baseline and 6 months post surgery and were analyzed in an ultra-performance liquid chromatography/time-of-flight mass spectrometry (UPLC-TOF-MS)-based platform.

Results Lipidomic analysis revealed a differential sphingomyelin profile in patients with NASH resolution after sleeve gastrectomy. Certain serum sphingomyelin species were significantly higher at baseline in NASH patients in comparison to those with NAFL. Sphingomyelin profile of subjects with NASH resolution was similar to that for obese subjects with NAFL before bariatric surgery.

Conclusion Our study indicates that the serum sphingomyelin levels could be related to the status of non-alcoholic fatty liver disease and that certain sphingomyelin species may be used for the follow-up of obese patients with NASH after sleeve gastrectomy.

Keywords Bariatric surgery · Sleeve gastrectomy · Non-alcoholic fatty liver disease · Non-alcoholic steatohepatitis · Obesity · Lipidomics · Sphingolipids · Sphingomyelins

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Introduction

Obesity is frequently associated with comorbidities such as type 2 diabetes, insulin resistance, hypertension, dyslipidemia, and non-alcoholic fatty liver disease. As a result of the obesity pandemic, non-alcoholic fatty liver disease has become one of the most common liver disorders worldwide with an estimated prevalence ranging from 25 to 30% in the general population and over 80 to 90% in obese subjects [1, 2]. Non-alcoholic fatty liver disease comprises a spectrum of diseases ranging from non-alcoholic fatty liver (NAFL) to non-alcoholic steatohepatitis (NASH) with inflammation and fibrosis, which can potentially progress to advanced liver disease, cirrhosis, and hepatocellular carcinoma. NASH has been reported to be present in a high percentage of obese patients undergoing bariatric surgery (BS) [3, 4]. Remarkably, BS has been shown to be an effective strategy for NASH resolution [5–7], although a significant percentage of patients still have NASH even after considerable weight loss after surgery.

Recently, an increasing number of lipidomic analyses have reported a different serum and hepatic lipid signature between obese subjects with NASH compared to those with NAFL. Remarkably, most of these studies showed differences in the levels of a number of phospholipid and sphingolipid species between NASH and NAFL, although the results were highly variable depending on the analysis performed [8–12]. Additionally, only a limited number of lipidomic studies have been performed to evaluate the effects of bariatric surgery on serum phospholipid and sphingolipid signatures in obese subjects with NASH [10].

In this study, we analyzed the serum lipidome of obese subjects with NASH before and after sleeve gastrectomy (SG). We also compared the serum phospholipid and sphingolipid levels at baseline and after SG between individuals who achieved NASH resolution with those who did not reach NASH resolution 6 months after surgery, in order to identify differences in lipid species that may be related to the response or not for NASH resolution. Finally, we investigated whether the lipid species that significantly changed in both study groups were also different in obese patients with NASH or NAFL before bariatric surgery.

Material and Methods

Subjects and Study Design

This study involved 24 patients with grade 3 obesity (body mass index (BMI) ≥ 40 kg/m^2) that were aged 18–60 years. The patients were diagnosed with either NAFL ($n = 8$) or NASH ($n = 16$) using the non-invasive OWLiver assay, consisting in the measurement of a panel of 20 triglycerides (TG) in the serum that discriminates between NAFL and NASH with sensitivity and specificity of 0.73 and 0.80, respectively [13]. All patients from the NASH group underwent sleeve gastrectomy (seven men/nine women, mean age: 46.6 ± 5.9 years). NASH was re-evaluated 6 months after surgery using the OWLiver test, and these patients were classified as resolved cases (from NASH to NAFL; NASH_RES) and not resolved (N-NASH_RES). Anthropometric and biochemical variables were evaluated at baseline and at 6 months after surgery.

Exclusion criteria included diabetes, cardiovascular disease, arthritis, acute inflammatory disease, and infectious disease or treatment with drugs that could alter the lipid profile (statins, fibrates, and nicotinic acid). All participants gave their written informed consent, and the study was reviewed and approved by the local ethics and research committee.

Laboratory Measurements

Blood samples were obtained from the antecubital vein and placed in vacutainer tubes (BDvacutainer™, London, UK) after an overnight fast. The serum was separated by centrifugation for 10 min at 4000 rpm and frozen at − 80 °C until analysis. Serum glucose, total cholesterol, triglycerides, high-density lipoprotein (HDL) cholesterol, and transaminases were measured in a Dimension autoanalyzer (DadeBehring Inc., Deerfield, IL) by enzymatic methods (Randox Laboratories Ltd., UK and Wako Bioproducts, Richmond, VA). Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald formula. Insulin concentrations were quantified by radioimmunoassay supplied by BioSource S.A. (Nivelles, Belgium). Insulin resistance (IR) was calculated from the homeostasis model assessment of IR (HOMA-IR) with the formula: HOMA-IR = [fasting serum insulin (μU/mL) × fasting blood glucose (mmol/L)]/22.5. For quantitative measurement of the caspase-generated neoepitope of CK-18, we used the M30-Apoptosense ELISA kit according to the manufacturer's instructions (Peviva, Bromma, Sweden).

Lipidomic Analysis

Serum lipidomic profiles were semi-quantified at baseline and 6 months after BS as previously described [12, 14–16]. Briefly, two separate ultra-performance liquid chromatography/time-of-flight mass spectrometry (UPLC-TOF-MS)-based platforms analyzing methanol and chloroform/methanol serum extracts were combined. Lipid nomenclature, classification, and shorthand notation follow the recommendations given elsewhere [17] and the recommendations of the LIPID MAPS convention (www.lipidmaps.org).

Data obtained were pre-processed with the TargetLynx application manager for MassLynx (Waters Corp., Milford, MA). The lipid features were identified prior to the analysis,

either by comparison of their accurate mass spectra and chromatographic retention times with those of available reference standards or, where these were not available, by accurate mass MS/MS fragment ion analysis [12]. Intra- and inter-batch normalization was performed by inclusion of multiple internal standards and pool calibration response correction [18]. The overall normalized data was referred with respect to the batch average quality control (QC) calibration samples, arbitrarily adjusted to one as described previously [12, 16, 18]. The lipid classes have been calculated as the sum of the normalized areas of all the metabolites with the same chemical characteristics.

Data pre-processing generated a list of chromatographic peak areas for the metabolites detected in each sample injection. An approximated linear detection range was defined for each identified metabolite, assuming similar detector response levels for all metabolites belonging to a given lipid class represented by a single-standard compound.

Statistical Analysis

Results are given as the mean ± standard deviation (SD). Comparisons of the anthropometric and biochemical characteristics before and after surgery and between study groups were performed with Wilcoxon and Mann-Whitney *U* non-parametric tests, respectively. Spearman correlation coefficients were calculated to evaluate the association between the study variables. Analyses were performed with SPSS, version 22.0 for Windows (SPSS Iberica, Spain). Values were considered to be statistically significant when $p < 0.05$. Heat map charts were created and clustered using the Heatmapper web server [19].

Results

Biochemical and anthropometric characteristics of the study groups undergoing SG at baseline and 6 months post surgery are shown in Table 1. No significant differences between both study groups (NASH_RES vs N-NASH_RES) were found in any of the evaluated parameters neither at baseline nor after surgery (Table 1). After 6 months of follow-up, similar improvements in BMI, percentage of total body weight loss (% TWL), and serum TG levels were observed in both study groups. However, improvements in total cholesterol, HDL, and aspartate aminotransferase (AST) levels were only significant in the NASH-RES group 6 months after surgery (Table 1).

Lipidomic analysis of the changes in serum phospholipid (glycerophospholipids and lysoglycerophospholipids) and sphingolipid (ceramides and sphingomyelins) levels in obese subjects with NASH before and after SG revealed no significant differences between study groups (data not shown). However, despite the lack of statistically significant differences, we observed that the levels of sphingomyelins (SM) tended to be lower after surgery in NASH_RES subjects, but they remained unchanged in N-NASH_RES subjects (fold changes of − 0.305 and 0.010 for NASH-RES and N-NASH_RES groups, respectively). Interestingly, when individual SM species were analyzed, we observed significant changes in the serum levels of SM 30:1 (d18:1/12:0), SM 36:0 (d18:0/18:0), SM 36:1 (d18:1/18:0), and SM 36:2 (d18:1/18:1) between NASH_RES vs N-NASH_RES subjects (Fig. 1a).

Comparison of serum SM levels between NAFL and NASH patients with obesity at baseline revealed that several SM species were higher in NASH compared to NAFL (Fig. 1b). Clinical parameters of both study groups are shown in Table 2. Interestingly, SM 36:0 (d18:0/18:0) was also significantly lowered in NASH_RES individuals after bariatric surgery, suggesting that the serum levels of this sphingomyelin could be used to discriminate between NAFL and NASH. An overall visualization of the serum levels of the identified SM species in all study groups at baseline (NAFL, NASH) and after surgery (NASH_RES, N-NASH_RES) is depicted in Fig. 2. As expected, we observed that serum SM species from NASH_RES patients clustered with those from NAFL (NAFL_BASAL), whereas SM values from NASH patients, remained similar before (NASH_BASAL) and after surgery (N-NASH_RES).

Discussion

In this work, we have established an association between the profile of SM species and NASH resolution in obese patients after sleeve gastrectomy. We also showed that the serum SM profile in patients with NAFL is similar to that observed in NASH patients who achieved resolution to NAFL after surgery.

Sphingolipids represent a significant fraction of total lipids in both the serum and liver, especially sphingomyelins that amount up to 5% of plasma lipids [20, 21]. Numerous studies have attributed a lipotoxic effect for sphingolipids in diverse metabolic tissues, including the liver [22, 23]. Thus, increased levels of ceramides (Cer) and SM species have been associated to obesity-related metabolic disorders such as insulin resistance, non-alcoholic fatty liver, and cardiovascular disease [24]. However, the association of the serum levels of sphingolipids with NASH has been less studied. In mouse models, it has been reported a significantly higher abundance of sphingolipids (both Cer and SM) in the liver of mice with NASH compared to controls (NAFL), and they remain unchanged in early-stages of NASH compared to advanced fibrosis-stages [25]. In humans, it has been

Table 1 Anthropometric, biochemical, and clinical characteristics of the patients with NASH at baseline and 6 months after sleeve gastrectomy

		Baseline	6-Month follow-up
Age, y	N-NASH_RES	47.2 (5.7)	–
	NASH_RES	46.0 (6.4)	–
Weight, kg	N-NASH_RES	134.8 (22.9)	96.7 (15.2)[#]
	NASH_RES	144.0 (27.1)	106.1 (18.7)[#]
BMI, kg/m^2	N-NASH_RES	48.9 (4.9)	35.1 (3.7)[#]
	NASH_RES	52.4 (7.3)	38.6 (4.9)[#]
% TWL	N-NASH_RES	–	26.7 (6.5)
	NASH_RES	–	24.5 (3.7)
% EBWL	N-NASH_RES	–	56.6 (14.3)
	NASH_RES	–	48.8 (6.3)
Glucose, mg/dL	N-NASH_RES	100.5 (18.7)	85.5 (9.7)
	NASH_RES	93.0 (10.3)	84.9 (4.4)
HOMA-IR	N-NASH_RES	5.8 (6.8)	1.7 (0.7)[#]
	NASH_RES	4.4 (3.4)	2.1 (0.8)[#]
SBP, mmHg	N-NASH_RES	144.1 (25.7)	133.6 (21.3)
	NASH_RES	135.0 (9.4)	135.7 (12.7)
DBP, mmHg	N-NASH_RES	85.4 (10.5)	84.7 (14.4)
	NASH_RES	88.2 (11.8)	88.5 (12.1)
Total cholesterol, mg/dL	N-NASH_RES	173.5 (37.7)	179.5 (41.1)
	NASH_RES	168.7 (25.2)	185.9 (26.1)[#]
HDL-cholesterol, mg/dL	N-NASH_RES	48.0 (13.4)	55.6 (21.8)
	NASH_RES	37.2 (9.2)	54.5 (6.7)[#]
LDL-cholesterol, mg/dL	N-NASH_RES	102.3 (39.5)	107.9 (42.3)
	NASH_RES	108.7 (22.4)	112.3 (22.7)
Triglycerides, mg/dL	N-NASH_RES	113.0 (42.1)	78.0 (26.2)[#]
	NASH_RES	119.9 (35.3)	88.6 (15.7)[#]
AST, units/L	N-NASH_RES	29.2 (6.1)	26.5 (19.0)
	NASH_RES	32.5 (17.0)	14.7 (5.4)
ALT, units/L	N-NASH_RES	42.7 (18.5)	34.7 (21.1)
	NASH_RES	48.0 (32.2)	21.0 (8.2)[#]
GGT, units/L	N-NASH_RES	54.5 (30.9)	36.2 (37.7)
	NASH_RES	39.0 (18.2)	28.2 (19.3)
CK-18 M30, units/L	N-NASH_RES	117.9 (44.1)	69.5 (28.8)[#]
	NASH_RES	357.7 (392.9)	76.3 (31.4)[#]

Statistical analyses of the anthropometric and biochemical characteristics before and after surgery and between study groups were performed with Wilcoxon and Mann-Whitney *U* non-parametric tests, respectively. Results are given as the mean ± SD

N-NASH_RES, (NASH after sleeve gastrectomy, $n = 8$); NASH_RES (from NASH to NAFL after sleeve gastrectomy, $n = 8$); *BMI*, body mass index; *HOMA-IR*, insulin resistance index; *SBP*, systolic blood pressure; *DBP*, diastolic blood pressure; *HDL*, high-density lipoprotein; *LDL*, low-density lipoprotein; *AST*, aspartate aminotransferase; *ALT*, alanine aminotransferase; *GGT*, gamma-glutamyltransferase; *TWL*, total weight loss; *EBWL*, excess body weight loss; *CK-18/M30*, M30 epitope of cytokeratin 18

[#] $p \leq 0.05$ (comparison before and after surgery)

described as an increase in the hepatic content of SM (but not Cer) in NASH compared to NAFL [8]. Chiappini et al. previously reported an increase in the serum levels of certain sphingomyelins such as SM (40:1) and SM (40:2) in mice with NASH respect to those with NAFL [26]. Supporting the previous observations in mouse models, in this study, we have shown a different pattern in the serum levels of SM in both NASH and NAFL obese patients. In fact, we observed slightly higher SM values in NASH compared with NAFL in obese subjects. In particular, serum levels of SM 36:0 in NASH patients, who showed resolution to NAFL after SG, showed a

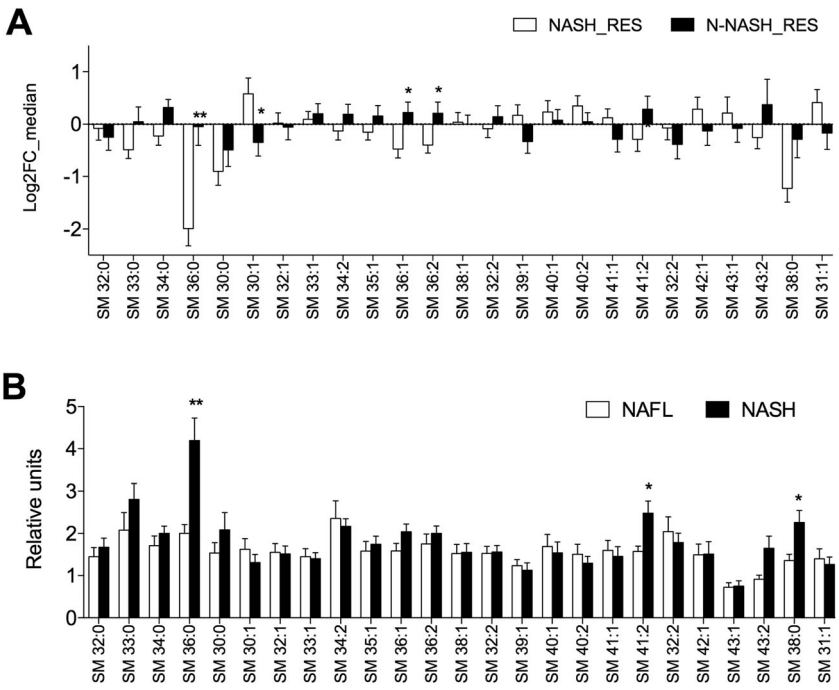

Fig. 1 Serum levels of sphingomyelins in obese subjects with NASH. **a** Changes in serum sphingomyelin (SM) levels in NASH_RES vs N-NASH_RES subjects 6 months after bariatric surgery. **b** Serum levels of SM species in NAFL vs NASH subjects with severe obesity

significant decrease, which might suggest that the analysis of the circulating levels of SM 36:0 could be used to monitor NASH resolution after surgery.

Table 2 Baseline clinical parameters of NAFL and NASH subjects with severe obesity

	NAFL ($n = 8$)	NASH (n = 16)	p value
Age, y	46.1 (6.5)	46.6 (5.9)	0.881
BMI, kg/m^2	46.5 (5.2)	49.7 (6.3)	0.172
Waist (cm)	124.8 (7.8)	137.6 (17.4)	0.061
SBP, mmHg	129.3 (14.4)	139.6 (19.3)	0.278
DBP, mmHg	77.9 (11.5)	86.8 (10.9)	0.089
Glucose, mg/dL	100.4 (14.4)	96.7 (15.1)	0.569
HOMA-IR	3.1 (1.9)	5.1 (5.2)	0.383
Total cholesterol, mg/dL	182.2 (33.4)	171.1 (31.0)	0.264
HDL-cholesterol, mg/dL	48.4 (9.3)	42.6 (12.4)	0.136
LDL-cholesterol, mg/dL	111.0 (26.8)	105.5 (31.2)	0.610
Triglycerides, mg/dL	116.4 (48.3)	116.4 (37.7)	0.834
AST, U/L	20.2 (3.5)	30.9 (12.5)	0.192
ALT, U/L	31.6 (8.9)	45.4 (25.5)	0.291
GGT, U/L	24.4 (7.3)	46.7 (25.8)	*0.038*
CK-18/M30, U/L	64.3 (49.9)	237.9 (297.1)	*0.002*

Statistical analysis between study groups was performed with Mann-Whitney *U* non-parametric tests. Results are given as the mean ± SD

Italic values meant statistical significance

NAFL, non-alcoholic fatty liver; *NASH*, non-alcoholic steatohepatitis; *BMI*, body mass index; *HOMA-IR*, insulin resistance index; *SBP*, systolic blood pressure; *DBP*, diastolic blood pressure; *HDL*, high-density lipoprotein; *LDL*, low-density lipoprotein; *AST*, aspartate aminotransferase; *ALT*, alanine aminotransferase; *GGT*, gamma-glutamyltransferase; *CK-18/M30*, M30 epitope of cytokeratin 18

The effect of bariatric surgery on the circulating levels of sphingolipids has been so far only mildly explored. Previous studies have described a decrease in the serum levels of certain

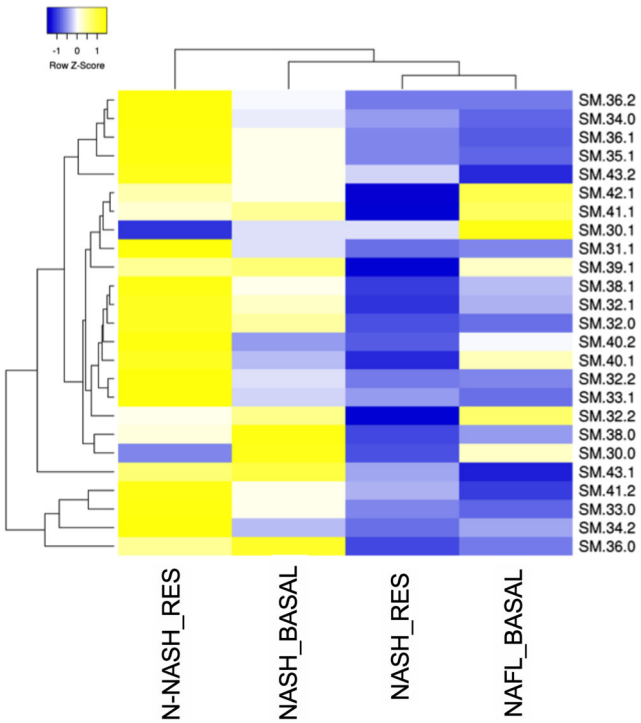

Fig. 2 Heat map of the serum sphingomyelin profile in obese patients with either NAFL or NASH. Average linkage was used as clustering method for sphingomyelin species. Blue squares indicate a decrease in relative lipid abundance while yellow squares represent an increase as indicated by the scale bar (Log2 of the fold change, M6/M0)

sphingomyelin species in obese patients who underwent several bariatric procedures [27–30] and that the change in serum lipidome after BS is highly dependent on the bariatric technique [29, 30]. More recently, it has been described a decrease of urinary sphingomyelins in adolescents with severe obesity after Roux-en-Y gastric bypass (RYGB) [31]. Here, we described for the first time the effect of SG in the serum levels of phospholipids and sphingolipids in individuals with NASH. This bariatric technique has been previously shown to be more effective than RYGB for improving liver function in obese patients with NASH [32]. Moreover, we show that obese individuals with NASH have a different pattern of sphingomyelins in the circulation compared to those with NAFL. Altogether, these results may suggest that these lipid species could be associated with the grade of liver disease in obese subjects.

The results of this pilot study are limited to only a 6-month follow-up after sleeve gastrectomy and need to be validated in long-term follow-up studies utilizing larger cohorts. In addition, diabetic patients are needed to be excluded from this study since the OWLiver test has more limited diagnostic capability in this study population [33].

In conclusion, we have demonstrated that the profile of circulating sphingomyelins could be associated with the severity of non-alcoholic fatty liver disease in obese subjects and that may be used for the follow-up of obese subjects with NASH after bariatric surgery. Our finding might suggest that strategies that specifically lower SM species could be used for the treatment of NASH in addition to weight loss and other metabolic improvements in patients with severe obesity.

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Compliance with Ethical Standards All participants gave their written informed consent, and the study was reviewed and approved by the local ethics and research committee.

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Younossi ZM, Koenig AB, Abdelatif D, et al. Global epidemiology of nonalcoholic fatty liver disease-meta-analytic assessment of prevalence, incidence, and outcomes. Hepatology. 2016;64:73–84.
2. Younossi Z, Anstee QM, Marietti M, et al. Global burden of NAFLD and NASH: trends, predictions, risk factors and prevention. Nat Rev Gastroenterol Hepatol. 2018;15:11–20.
3. Machado M, Marques-Vidal P, Cortez-Pinto H. Hepatic histology in obese patients undergoing bariatric surgery. J Hepatol. 2006;45:600–6.
4. Alqahtani A, Elahmedi M, Alswat K, et al. Features of nonalcoholic steatohepatitis in severely obese children and adolescents undergoing sleeve gastrectomy. Surg Obes Relat Dis. 2017;13:1599–609.
5. Lassailly G, Caiazzo R, Buob D, et al. Bariatric surgery reduces features of nonalcoholic steatohepatitis in morbidly obese patients. Gastroenterology. 2015;149:379–88. quiz e15–6
6. Klebanoff MJ, Corey KE, Chhatwal J, et al. Bariatric surgery for nonalcoholic steatohepatitis: a clinical and cost-effectiveness analysis. Hepatology. 2017;65:1156–64.
7. Caravatto PP, Cohen R. The role of metabolic surgery in nonalcoholic steatohepatitis improvement. Curr Atheroscler Rep. 2017;19:45.
8. Puri P, Baillie RA, Wiest MM, et al. A lipidomic analysis of nonalcoholic fatty liver disease. Hepatology. 2007;46:1081–90.
9. Puri P, Wiest MM, Cheung O, et al. The plasma lipidomic signature of nonalcoholic steatohepatitis. Hepatology. 2009;50:1827–38.
10. Anjani K, Lhomme M, Sokolovska N, et al. Circulating phospholipid profiling identifies portal contribution to NASH signature in obesity. J Hepatol. 2015;62:905–12.
11. Chiappini F, Coilly A, Kadar H, et al. Metabolism dysregulation induces a specific lipid signature of nonalcoholic steatohepatitis in patients. Sci Rep. 2017;7:46658.
12. Barr J, Caballeria J, Martinez-Arranz I, et al. Obesity-dependent metabolic signatures associated with nonalcoholic fatty liver disease progression. J Proteome Res. 2012;11:2521–32.
13. Mayo R, Crespo J, Martínez-Arranz I, et al. Metabolomic-based noninvasive serum test to diagnose nonalcoholic steatohepatitis: results from discovery and validation cohorts. Hepatol Commun. 2018;2:807–20.
14. Barr J, Vazquez-Chantada M, Alonso C, et al. Liquid chromatography-mass spectrometry-based parallel metabolic profiling of human and mouse model serum reveals putative biomarkers associated with the progression of nonalcoholic fatty liver disease. J Proteome Res. 2010;9:4501–12.
15. Alonso C, Fernandez-Ramos D, Varela-Rey M, et al. Metabolomic identification of subtypes of nonalcoholic steatohepatitis. Gastroenterology. 2017;152:1449–61 e7.
16. Cano A, Marino Z, Millet O, et al. A metabolomics signature linked to liver fibrosis in the serum of transplanted hepatitis C patients. Sci Rep. 2017;7:10497.
17. Liebisch G, Vizcaino JA, Kofeler H, et al. Shorthand notation for lipid structures derived from mass spectrometry. J Lipid Res. 2013;54:1523–30.
18. Martinez-Arranz I, Mayo R, Perez-Cormenzana M, et al. Enhancing metabolomics research through data mining. J Proteome. 2015;127:275–88.
19. Babicki S, Arndt D, Marcu A, et al. Heatmapper: web-enabled heat mapping for all. Nucleic Acids Res. 2016;44:W147–53.
20. Kotronen A, Seppanen-Laakso T, Westerbacka J, et al. Comparison of lipid and fatty acid composition of the liver, subcutaneous and intra-abdominal adipose tissue, and serum. Obesity (Silver Spring). 2010;18:937–44.
21. Quehenberger O, Armando AM, Brown AH, et al. Lipidomics reveals a remarkable diversity of lipids in human plasma. J Lipid Res. 2010;51:3299–305.
22. Rodriguez-Cuenca S, Pellegrinelli V, Campbell M, et al. Sphingolipids and glycerophospholipids - the "ying and yang" of lipotoxicity in metabolic diseases. Prog Lipid Res. 2017;66:14–29.
23. Pagadala M, Kasumov T, McCullough AJ, et al. Role of ceramides in nonalcoholic fatty liver disease. Trends Endocrinol Metab. 2012;23:365–71.

24. Iqbal J, Walsh MT, Hammad SM, et al. Sphingolipids and lipoproteins in health and metabolic disorders. Trends Endocrinol Metab. 2017;28:506–18.
25. Saito K, Uebanso T, Maekawa K, et al. Characterization of hepatic lipid profiles in a mouse model with nonalcoholic steatohepatitis and subsequent fibrosis. Sci Rep. 2015;5:12466.
26. Chiappini F, Desterke C, Bertrand-Michel J, et al. Hepatic and serum lipid signatures specific to nonalcoholic steatohepatitis in murine models. Sci Rep. 2016;6:31587.
27. Graessler J, Bornstein TD, Goel D, et al. Lipidomic profiling before and after Roux-en-Y gastric bypass in obese patients with diabetes. Pharmacogenomics J. 2014;14:201–7.
28. Arora T, Velagapudi V, Pournaras DJ, et al. Roux-en-Y gastric bypass surgery induces early plasma metabolomic and lipidomic alterations in humans associated with diabetes remission. PLoS One. 2015;10:e0126401.
29. Kayser BD, Lhomme M, Dao MC, et al. Serum lipidomics reveals early differential effects of gastric bypass compared with banding on phospholipids and sphingolipids independent of differences in weight loss. Int J Obes. 2017;41:917–25.
30. Ramos-Molina B, Castellano-Castillo D, Alcaide Torres J, Pastor O, de Luna Diaz R, Salas-Salvado J, et al. Differential effects of restrictive and malabsorptive bariatric surgery procedures on the serum lipidome in obese subjects. J Clin Lipidol. 2018;S1933-2874(18)30307-6. https://doi.org/10.1016/j.jacl.2018.07.006.
31. Davis S, Nehus E, Inge T, et al. Effect of bariatric surgery on urinary sphingolipids in adolescents with severe obesity. Surg Obes Relat Dis. 2018;14:446–51.
32. Kalinowski P, Paluszkiewicz R, Ziarkiewicz-Wroblewska B, et al. Liver function in patients with nonalcoholic fatty liver disease randomized to Roux-en-Y gastric bypass versus sleeve gastrectomy: a secondary analysis of a randomized clinical trial. Ann Surg. 2017;266:738–45.
33. Bril F, Millan L, Kalavalpalli S, et al. Use of a metabolomic approach to non-invasively diagnose nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus. Diabetes Obes Metab. 2018;20:1702–9.