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Hepatic and metabolic effects of SGLT2 inhibitors in type 2 diabetes and MASLD assessed by metabolomic and lipidomic analysis

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Sodium–glucose cotransporter-2 (SGLT-2) inhibitors show promising hepatic benefits in patients with metabolic dysfunction–associated steatotic liver disease (MASLD); however, their effects on underlying metabolic mechanisms remain unclear. This study included 29 patients with type 2 diabetes mellitus (T2DM) and MASLD who initiated SGLT-2 inhibitor therapy. Hepatic fat content was assessed using advanced ultrasound techniques, including tissue attenuation imaging (TAI) and tissue scatter distribution imaging (TSI). Biochemical analyses, gas chromatography–mass spectrometry (GC–MS)–based metabolomics, and liquid chromatography–mass spectrometry (LC–MS)–based lipidomics were performed at baseline and after six months of treatment. Treatment resulted in significant reductions in hepatic fat fraction (17.0 ± 5.5% to 13.7 ± 5.9%, $p = 0.002$), glycated hemoglobin (HbA1c; $p < 0.001$), and body mass index (BMI; $p < 0.001$). Metabolomic analysis identified 17 significantly altered metabolites, predominantly amino acids (β-alanine, glycine, valine, and isoleucine), with enrichment of aminoacyl-transfer RNA (tRNA) biosynthesis and adenosine triphosphate–binding cassette (ABC) transporter pathways. Lipidomic profiling revealed remodeling of 492 lipid species, particularly phosphatidylcholine, phosphatidylethanolamine, sphingomyelin, and triacylglycerol subclasses. SGLT-2 inhibitor therapy improved hepatic steatosis and induced metabolic remodeling in patients with T2DM and MASLD. Integrated metabolomic and lipidomic analyses suggest hepatic benefits beyond glycemic control, involving amino acid and lipid metabolic pathways.

Keywords Hepatic steatosis, Lipid Metabolism, Aminoacid metabolism, Glucose lowering therapy, Insulin resistance

Abbreviations

MASLD	Metabolic dysfunction-associated steatotic liver disease
MASH	metabolic dysfunction associated steatohepatitis
GC-MS	Gas chromatography-mass spectrometry
KEGG	Kyoto Encyclopedia of Genes and Genomes
ROI	Region of interest
TAI	Tissue Attenuation Imaging
TSI	Tissue Scatter Distribution Imaging
LDL	Low density lipoprotein
HDL	High-density lipoprotein

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ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
GGT	Gamma-glutamyl-transferase
ALP	Alkaline phosphatase
HbA1c	hemoglobin A1C
BMI	Body mass index
PLS-DA	Partial least squares discriminant analysis
VIP	Variable importance in projection
ABC	ATP-binding cassette
PC	Phosphatidylcholine
PE	Phosphatidylethanolamine
PI	Phosphatidylinositol
SM	Sphingomyelin
TAG	Triacylglycerol
DAG	Diacylglycerol
LPC	lysophosphatidylcholine
FFA	Free fatty acid
MRI-PDFF	MRI-proton density fat fraction

The pathogenesis of MASLD is multifactorial and involves alterations in lipid metabolism, mitochondrial function, inflammation, and oxidative stress[1]. Despite its increasing prevalence and potential to progress to metabolic dysfunction-associated steatohepatitis (MASH), cirrhosis, and hepatocellular carcinoma, pharmacological therapy options remain limited[2].

Sodium-glucose cotransporter-2 (SGLT-2) inhibitors are a novel class of antidiabetic agents that have gained prominence not only for their glucose-lowering properties but also for their cardiovascular and renoprotective effects[3]. Preclinical and clinical studies have reported improvements in liver enzymes, hepatic fat content, markers of inflammation, and even fibrosis following SGLT-2 inhibitor therapy[4–7]. Although there is increasing evidence supporting the hepatic benefits of SGLT-2 inhibitors, the exact molecular mechanisms underlying these effects are still not fully elucidated. While these drugs are commonly associated with reductions in liver fat through improved glycemic control and weight loss, a randomized, double-blind phase 4 study by Kahl et al. demonstrated that SGLT-2 inhibitors also reduced liver fat content in patients with well-controlled diabetes. This finding suggests that the beneficial effects on hepatic steatosis may extend beyond glycemic control and weight reduction alone[8].

Lipidomics and metabolomics offer powerful platforms for elucidating the biochemical pathways involved in disease states and therapeutic responses. By profiling a wide range of lipid and metabolic intermediates, these approaches can provide insights into the systemic and hepatic metabolic alterations induced by SGLT-2 inhibition[9]. Previous studies have indicated that SGLT-2 inhibitors modulate pathways related to lipolysis, ketogenesis, and mitochondrial function, all of which may be pertinent to the pathophysiology of MASLD[10,11].

In this study, we aimed to investigate the effects of SGLT-2 inhibitors on hepatic steatosis using a combination of imaging, biochemical markers, and comprehensive lipidomic and metabolomic profiling. By integrating clinical and molecular data, we aimed to elucidate the metabolic pathways modulated by SGLT-2 inhibition and their potential implications for the treatment of MASLD[7].

Materials and methods

Patient enrollment

This study included individuals diagnosed with type 2 diabetes mellitus (T2DM) and MASLD who visited the endocrinology outpatient clinic from 05.08.2023 to 03.04.2024 and were set to begin SGLT-2 therapy for the first time.

Patients were excluded if they had started any new antidiabetic, antilipidemic, or antihypertensive treatments within six months before SGLT-2 therapy, or if they had not maintained a consistent prescribed diet for at least six months prior. Additional exclusion criteria included the diagnosis of other liver diseases, congestive heart failure, chronic kidney disease, chronic inflammatory disorders, or active cancer. Patients who consumed alcohol, used steroids, underwent hormone replacement therapy, or took medications known to cause hepatic steatosis (such as tamoxifen or amiodarone) were also excluded. Study participants were excluded if they began new medications for diabetes, hyperlipidemia, or hypertension during the follow-up period. Additionally, those who discontinued their prescribed drugs because of adverse effects or other factors were also removed from the study.

Of the 98 patients who commenced SGLT-2 inhibitor therapy during the study period, 69 were excluded for the following reasons: chronic kidney disease ($n=30$), active malignancy ($n=5$), use of medications associated with hepatic steatosis ($n=2$), congestive heart failure ($n=15$), chronic inflammatory disease ($n=5$), treatment discontinuation during follow-up ($n=8$), and initiation of new medications during follow-up ($n=4$). A total of 29 patients met all the inclusion criteria and were included in the final analysis (Figure 1). SGLT-2 inhibitor therapy consisted of empagliflozin and dapagliflozin, prescribed as part of routine outpatient clinical care. Eight patients received empagliflozin 12.5 mg, four received empagliflozin 10 mg, and seventeen received dapagliflozin 10 mg. At baseline, 19 patients were receiving metformin monotherapy, 9 were treated with metformin in combination with a dipeptidyl peptidase-4 inhibitor, and 1 patient was receiving sulfonylurea monotherapy. Blood samples were collected at baseline (month 0) and month 6, centrifuged, and stored at − 80 °C. All samples were analyzed collectively at the end of the study period. All patients provided informed consent. Ethical approval for the study

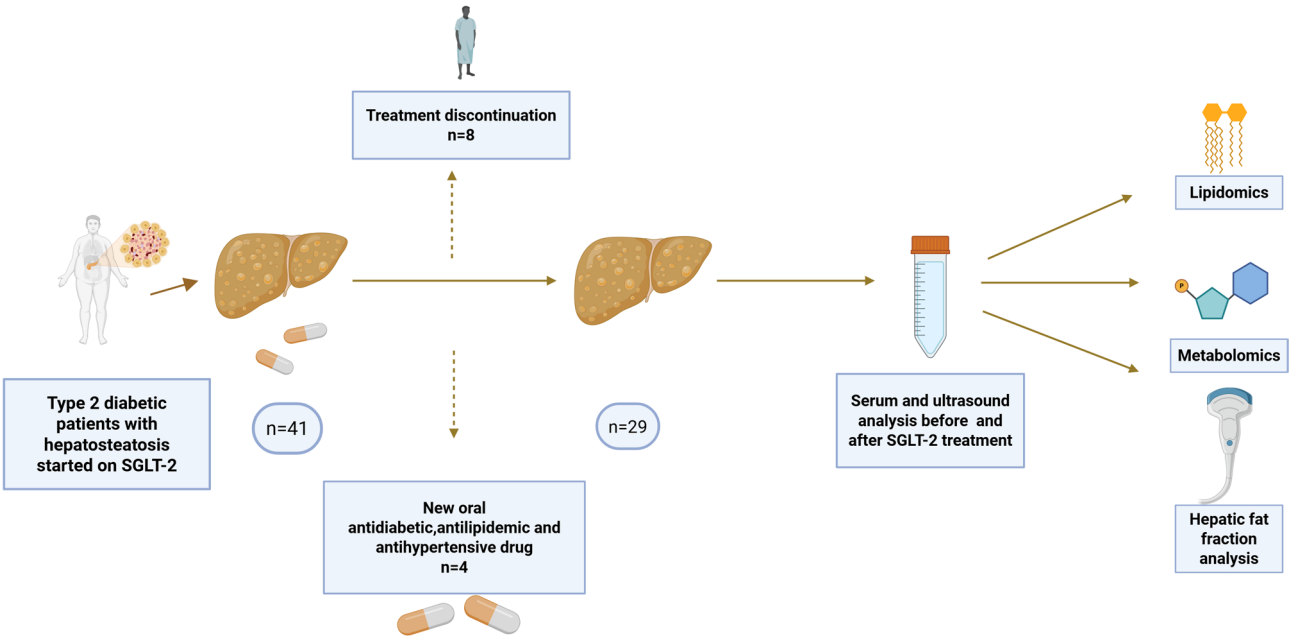

Fig. 1. Flow chart of the study.

was obtained from the Gazi University Ethics Commission (Date: 23.01.2023/Approval Code: 62). The study was in accordance with the Declaration of Helsinki.

GC/MS metabolomics analysis

The separated supernatant was evaporated overnight at 4 °C using a vacuum centrifuge. The dried samples were then methoxylated and derivatized, as previously described[12]. Following the addition of 20 µL of methoxyamine hydrochloride (20 mg/mL in pyridine), all samples were incubated at 90 °C for 30 min. Subsequently, N-methyl-N-(trimethylsilyl)trifluoroacetamide solution containing 1% trimethylchlorosilane was added, and the samples were incubated at 37 °C for 30 min. Metabolomic profiling was performed using gas chromatography-mass spectrometry (GC-MS) on a Shimadzu GCMS-QP2010 Ultra system (Kyoto, Japan) equipped with a DB-5MS column (30 m × 0.25 mm i.d., 0.25 µm film thickness). Samples were injected in splitless mode. The oven temperature program included an initial hold at 60 °C for 1 min, followed by a temperature ramp of 10 °C/min to 325 °C, and a final hold at 325 °C for 10 min. Electron ionization mass spectrometry was conducted at 70 eV, and data were acquired in full scan mode across an m/z range of 50–650. For metabolite identification, complex chromatograms were deconvoluted and peaks were aligned to generate a data matrix using MS-DIAL v.2.56. Mass detection ranged from 50 to 650 Da, with a minimum peak height of 1000 amplitude for reliable detection. The retention time tolerance was set at 0.05 min, and a recognition cut-off score of 70% was applied. Metabolite identification was performed by matching the results against the Fiehn Retention Index database. Statistical analysis was conducted using MetaboAnalyst 6.0 employing t-tests. Pathway analysis was performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) platform.[13] All QC samples were prepared and analyzed using the same procedure.

LC/MS lipidomics analysis

The separated supernatant was evaporated overnight at 4 °C using a vacuum centrifuge. The dried sample was reconstituted with 300 µL of IPA: ACN (7:3, v/v), carefully vortexed, and filtered. Lipids were analyzed using an LC/Q-TOF-MS system (Agilent 6530, California, USA). Chromatographic separation was achieved on a C18 column (100 × 2.1 mm, 2.7 µm, Poroshell HPH). The mobile phase consisted of water: ACN (6:4, v/v) with 0.1% formic acid (FA) and 10 mM ammonium formate (mobile phase A) and IPA: ACN (9:1, v/v) with 0.1% FA and 10 mM ammonium formate (mobile phase B). The flow rate was 0.25 mL/min, and the column temperature was maintained at 60 °C for lipidomics analysis. MS and MS/MS data were collected in both positive and negative ionization modes in the m/z range of 100–1700, with a medium isolation width. Collision energies of 10 V, 20 V, and 40 V were used for MS/MS fragmentation. All the quality control (QC) samples were prepared and analyzed using the same methodology. Feature detection, deconvolution, alignment, gap filling, and normalization were performed using MS-DIAL (ver. 4.92). Lipids were identified based on MS/MS data (10, 20, and 40 V) with a 70% cut-off value according to the MS-DIAL LipidBlast database. Statistical analysis was performed using the MetaboAnalyst 6.0 platform. All QC samples were prepared and analyzed using the same procedure.

Radiological analysis of hepatic fat content and fibrosis

All patients underwent ultrasound examinations using a single ultrasound device (RS85 Prestige, Samsung) with a 1–7 MHz convex transducer. All examinations were performed by one of two radiologists with more than 15

years of experience in abdominal radiology. The patients were examined in the supine position and instructed to place their right hands above their heads. For elastography imaging, five measurements with 1 cm circular regions of interest (ROIs) were obtained for each patient, and the median value in kilopascal was noted. For all patients, the interquartile range/median value of the dataset was less than 30%. All measurements were obtained during the breath-hold situation of a normal respiration cycle. For hepatic fat quantification, five measurements with 3 cm fan-shaped ROIs were used for both Tissue Attenuation Imaging (TAI) and Tissue Scatter Distribution Imaging (TSI) techniques[14]. The median values for each technique were noted. The ultrasound fat fraction values were calculated based on a validated formula [USFF= (41.9xTAI) + (0.23xTSI) – 44.3][15].

Evaluation of blood, metabolomics and lipidomics parameters before (0.month) and after (6.month) treatment

Hepatobiliary ultrasound examinations were conducted before initiating SGLT-2 therapy (0 months) and after treatment (6 months). Hepatic fat was assessed using TAI and TSI analyses. Before and after the treatment period, various blood parameters were evaluated, including low density lipoprotein (LDL), high-density lipoprotein (HDL), triglyceride levels, blood glucose levels, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl-transferase (GGT), alkaline phosphatase (ALP), hemoglobin A1C (hba1c) and body mass index (BMI).

Statistical analysis

Data analysis was conducted using IBM SPSS Statistics for Windows version 26.0 (IBM Corp., Armonk, NY, USA). Categorical variables are presented as numbers and percentages, while normally distributed variables are expressed as means ± standard deviation. For non-normally distributed variables, the results are reported as median and interquartile range (25–75%).

Within-group comparisons between baseline and six months were performed using the paired Student's *t*-test or Wilcoxon signed-rank test, as appropriate. Associations between continuous variables were assessed using Pearson or Spearman's correlation analyses, as applicable. For metabolomics and lipidomics analyses, the MetaboAnalyst 6.0 platform was used. Partial least squares discriminant analysis (PLS-DA) was performed to evaluate the alterations in the metabolomic profiles across the experimental groups. A t-test was performed on both the metabolomics and lipidomics datasets. In metabolomics, a p-value < 0.05 and a fold-change cutoff of 1.2 were used, whereas in lipidomics, the fold-change cutoff was set at 2.

Metabolite enrichment analysis was performed using MetaboAnalyst, applying a p-value threshold of < 0.05, and requiring at least two metabolites per chemical class. Pathway analysis was conducted using the KEGG platform, with a minimum of three metabolites required for pathway identification.

Results

Demographic parameters of the patients

The average age of the study participants was 59 ± 8 years. Among the 29 patients, 48.3% ($n = 14$) were female. Hyperlipidemia was present in 52% (15/29) of patients, while 62% (18/29) had hypertension. Smokers comprised 31% (9/29) of the study population. The patients' liver enzyme levels, ferritin, and lipid profiles were within normal or slightly below normal ranges. The median HbA1c level was 8%, and the average body mass index was 30.4 kg/m^2. (Table 1).

Changes in blood parameters and hepatic fat content before and after therapy and correlation

Following six months of SGLT-2 inhibitor therapy, significant reductions were observed in HbA1c ($p < 0.001$), hepatic fat content ($p = 0.002$), and BMI ($p < 0.001$) (Table 2). However, no statistically significant correlation was identified between the change in hepatic fat content and the changes in either HbA1c or BMI (Table 3).

Metabolomics analysis

Global metabolic alterations were initially assessed using partial least squares discriminant analysis (PLS-DA) (Fig. 2) between O, which indicates baseline (month 0), and T, which indicates post-treatment (month 6). The PLS-DA score plot (Fig. 2A) demonstrated moderate separation between the pre- and post-treatment metabolomic profiles, accounting for a total variance of approximately 87.2%. This distinct clustering suggests that SGLT-2 inhibitor therapy induces significant physiological and metabolic shifts in the patient cohort.

Variable importance in projection (VIP) scores (Fig. 2B) highlighted specific metabolites that contributed most significantly to the discrimination between groups. To identify metabolites significantly altered by treatment, univariate statistical analyses were conducted, and those with statistically significant differences were listed (Table 4). A total of 17 metabolites were altered between the experimental groups. Metabolite enrichment analysis was performed to identify the sub-chemical classes of altered metabolites. The results indicated that the significantly altered metabolites pertained to various chemical classes (Fig. 2C). Mainly carbohydrate and amino acid-based classes changed with treatment in the plasma. Beta-Alanine, Glycine, L-Alanine, L-Asparagine, Isoleucine, L-Valine, and sarcosine were the amino acids that changed with SGLT-2 inhibitor treatment.

A total of 17 metabolites were significantly elevated following therapy and were subsequently subjected to pathway enrichment analysis using the KEGG database. The analysis revealed that these metabolites were enriched in several biological pathways, with the most prominent alterations observed in the ATP-binding cassette (ABC) transporter pathway (Table 5), suggesting a potential mechanistic link between SGLT-2 inhibition and membrane transport regulation.

Age*	59 ± 8
Female/Male	14/15
Hyperlipidemia	15/29
Hypertension	18/29
Smoking	9/29
Creatinine (mg/dl)	0.7 (0.8-1)
ALT (U/L)**	24 (22–38)
AST (U/L)**	24 (21–26)
GGT (U/L)*	41 ± 24
ALP (IU/L)*	74 ± 22
Ferritin (µg/L)**	29 (24–33)
Hba1c (%)**	8 (7.2–8.8)
LDL (mg/dL)**	100 (85–112)
HDL (mg/dl)*	47 ± 10
Trigliserid (mg/dl)**	125 (104–158)
BMI**	30.4 (27–33)

Table 1. Demographic and basic laboratory values of patients ($n = 29$). *(Mean±standard deviation) **(Median 25%-75%) ALT=Alanine aminotransferase, AST= Aspartate Aminotransferase, GGT= Gamma-glutamyl-transferase, ALP=Alkaline phosphatase, LDL=Low-density lipoprotein, HDL= High-density lipoprotein, BMI=Body mass index.

Variable	0.month	6.month	*p*-value
ALT (U/L)**	24 (22–38)	24 (20.5–28)	0.182
AST (U/L)**	24 (21–26)	23 (21–28)	0.973
GGT (U/L)*	41 ± 24	35 ± 22	0.621
ALP (IU/L)*	74 ± 22	78 ± 18	0.887
Ferritin (µg/L)**	29 (24–33)	28 (31–31)	0.065
Hba1c (%)*	8 (7.2–8.8)	7.2 (6.7–7.8)	**< 0.001**
LDL (mg/dL)**	100 (85–112)	99 (86–111)	0.675
HDL(mg/dL)*	47 ± 10	50 ± 11	0.088
Trigliserid(mg/dL)**	125 (104–158)	121 (105–121)	0.078
BMI**	30.4 (27–33)	29.9 (27–31)	**< 0.001**
Hepatic fat ratio*	17 ± 5.5	13.7 ± 5.9	**0.002**
SWE**	5.7 (4.8–6.35)	5 (4.5–5.6)	**0.008**

Table 2. Comparison of the basic laboratory parameters, hepatic fat quantification and SWE values before SGLT-2 therapy and after SGLT-2 therapy. *t-test was used **Wilcoxon test was used ALT=Alanine aminotransferase, AST= Aspartate Aminotransferase, GGT= Gamma-glutamyl-transferase, ALP=Alkaline phosphatase, LDL=Low-density lipoprotein, HDL= High-density lipoprotein, BMI=Body mass index, SWE= Shear wave elastography.

	Hepatic fat ratio 0.month (ρ, *p*-Value)	Hepatic fat ratio 6.month (ρ, *p*-Value)
Hba1c (%) 0.month	0.066 $p = 0.732$	-0.063 $p = 0.745$
Hba1c (%) 6.month	0.087 $p = 0.655$	0.089 $p = 0.645$
BMI 0.month	0.356 $p = 0.058$	0.132 $p = 0.495$
BMI 6.month	0.346 $p = 0.066$	0.163 $p = 0.398$

Table 3. Spearman correlation coefficients (ρ) for hepatic fat ratio and BMI and Hba1c.

Lipidomics analysis

LC/MS-based lipidomics analysis was performed to evaluate the plasma lipid profile. PLS-DA demonstrated distinct alterations in the lipidomic profile after SGLT-2 inhibitor therapy (Fig. 3). VIP scores demonstrated the most effective lipid species that contributed to the differentiation of the experimental groups (Fig. 3B). Volcano plot analysis illustrated the distribution of all lipid species between the experimental groups. (Fig. 3C).

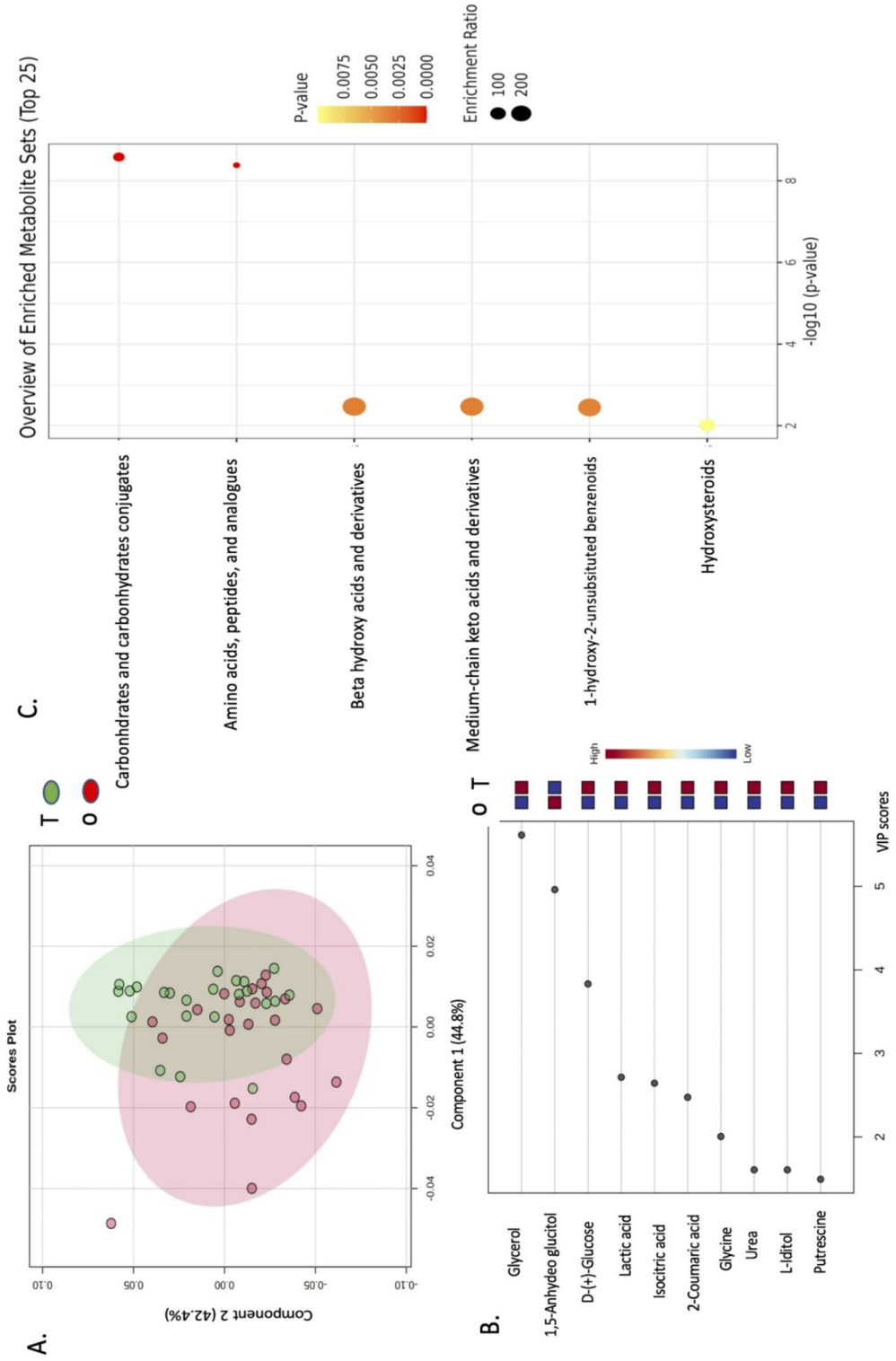

Fig. 2. Global metabolomic alterations induced by SGLT-2 inhibitor therapy (**A**) PLS-DA score plot showing partial separation between baseline (O, month 0) and post treatment (T, month 6) metabolomic profiles. (**B**) Variable importance in projection (VIP) scores of the top metabolites contributing to group discrimination. (**C**) Chemical class enrichment analysis of significantly altered metabolites ($p < 0.05$). O: baseline (month 0); T: post-treatment (month 6).

Metabolit Name	KEGG ID	Fold Change (O/T)	Log2 Fold Change	*p* value
beta-Alanine	C00099	0.15998	-2.6441	0.00019855
Galactinol	C01235	0.46389	-1.1081	0.00052248
L-Alanine	C00041	0.69954	-0.51552	0.0005435
Sarcosine	C00213	0.6999	-0.51478	0.00083581
L-Asparagine	C00152	0.43249	-1.2093	0.0017521
Glycine	C00037	0.78822	-0.34333	0.0064172
D-Fructose	C00095	0.61048	-0.71199	0.0081218
3-Oxoadipic acid	C00846	0.76208	-0.392	0.013738
Glycerol	C00116	0.71723	-0.47949	0.014915
D-Ribose	C00121	0.77569	-0.36645	0.023002
Pectin	C00714	0.8158	-0.29371	0.030111
Threonic acid	C01620	0.77049	-0.37615	0.031042
L-Valine	C00183	0.75369	-0.40796	0.03441
Malic acid	C00149	0.77082	-0.37554	0.039883
p-Hydroxyphenylacetic acid	C00642	0.71317	-0.48767	0.046496
Isoleucine	C00407	0.78917	-0.34159	0.049203
1,5-anhydroglucitol	C07326	2.224	1.1532	0.0062508

Table 4. Altered metabolic pathways under SGLT-2 treatment.

hsa02010 ABC transporters - Homo sapiens (human) (7)
hsa04974 Protein digestion and absorption - Homo sapiens (human) (6)
hsa01230 Biosynthesis of amino acids - Homo sapiens (human) (5)
hsa00970 Aminoacyl-tRNA biosynthesis - Homo sapiens (human) (5)
hsa04978 Mineral absorption - Homo sapiens (human) (5)
hsa01210 2-Oxocarboxylic acid metabolism - Homo sapiens (human) (3)
hsa00052 Galactose metabolism - Homo sapiens (human) (3)
hsa01240 Biosynthesis of cofactors - Homo sapiens (human) (3)

Table 5. Altered metabolic pathways under SGLT-2 treatment.

A heatmap was used for the top 50 lipid species (Fig. 3D). Statistical analysis showed that 492 lipid species were significantly altered after treatment (Supplementary information-1).

The number of upregulated and downregulated main lipid species is presented in Table 6. The results showed that the phospholipid profiles of phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylinositol (PI) changed significantly with treatment. Moreover, we observed that the plasma sphingolipid profile was altered by SGLT-2 inhibitor treatment. The sphingomyelin (SM) profile, which is a subclass of sphingolipids, changed dramatically. A total of 190 SM species were altered by the treatment (Table 6). Another important point is the differentiation of the glycerolipid profile. A total of 88 species of triacylglycerol (TAG) and diacylglycerol (DAG) were significantly altered (Table 6). In addition to PCs, significant alterations were observed in lysophosphatidylcholine (LPC) (Fig. 4A), PE, and PI subclasses, indicating widespread remodeling of membrane-associated lipid species in response to the therapy. Furthermore, we observed that the levels of various oxidized lipids decreased with treatment (Fig. 4B). Moreover, the treatment also affected the plasma free fatty acid profile (Fig. 4C).

Discussion

In this study, we evaluated the effects of SGLT-2 inhibitor therapy on hepatic steatosis and serum lipidomic and metabolomic profiles in patients with T2DM and MASLD. Six months of treatment resulted in significant improvements in hepatic fat content, glycemic control, and BMI. Comprehensive metabolomic and lipidomic analyses further revealed distinct molecular alterations, providing mechanistic insights into the metabolic benefits of SGLT-2 inhibition.

Consistent with previous reports, we observed significant reductions in HbA1c, BMI, and hepatic fat content following treatment[16]. Interestingly, improvements in hepatic fat content were not significantly correlated with changes in HbA1c or BMI, implying that the reduction in steatosis may occur partly independently of glycemic control or weight loss. This aligns with the findings of Kahl et al., who demonstrated that SGLT-2 inhibitors reduced liver fat in patients with well-controlled diabetes, indicating that additional metabolic mechanisms may be involved[8]. Although the sample size limited the statistical power to detect correlations, the observed direction of change could suggest a clinically meaningful trend in the results.

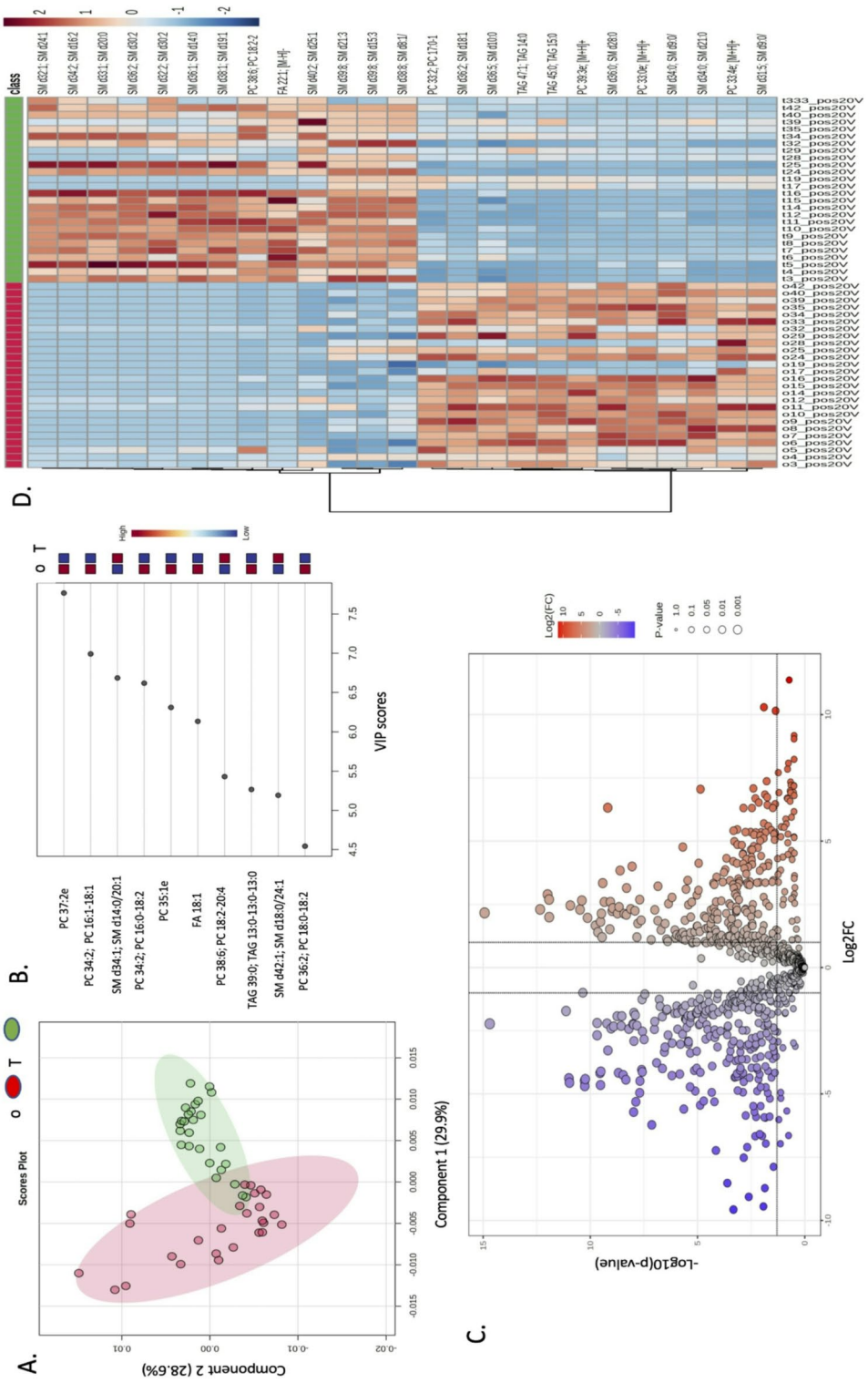

Fig. 3. Plasma lipidomic remodeling following SGLT-2 inhibitor therapy. (**A**) Partial least squares discriminant analysis (PLS-DA) score plot demonstrating global differences in plasma lipidomic profiles between baseline (O, month 0) and post-treatment (T, month 6) groups. (**B**) Variable importance in projection (VIP) scores of the lipid species contributing most strongly to group discrimination. (**C**) Volcano plot illustrating significantly altered lipid species following treatment ($p < 0.05$, fold change > 2). (D) Heatmap of the top 25 differentially altered lipid species across experimental groups, showing relative abundance patterns.

Lipid Class	Number of upregulated lipids with treatment	Number of down regulated lipids with treatment
PC	62	64
PE	16	18
PI	3	10
SM	103	87
TAG	54	25
FA	5	13
CE	5	3
DAG	5	4
LPC	1	3
Oxidized phospholipids	0	4

Table 6. The number of altered lipid species in PC, PE, PI, FA, TAG, DAG, CE class.

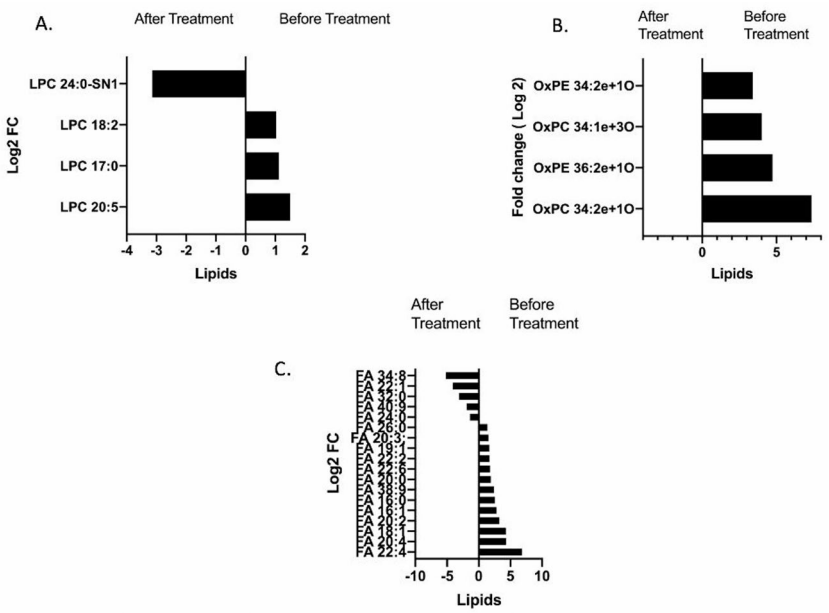

Fig. 4. Altered lipid subclasses following SGLT-2 inhibitor therapy (**A**) LPC lipids, (**B**) Oxidized lipids (**C**) FA lipids. (LPC: lysophosphatidylcholine, PC: phosphatidylcholine, PE: phosphatidylethanolamine, FA: Fatty acids)(p value < 0.05).

Although PLS-DA analysis demonstrated a discernible shift in the global metabolomic and lipidomic profiles following SGLT-2 inhibitor therapy, a partial overlap of confidence ellipses was observed. This finding indicates that the metabolic differences between groups are moderate rather than absolute, which is not unexpected, given the relatively small sample size and biological heterogeneity inherent to clinical cohorts. PLS-DA was applied as an exploratory multivariate approach to visualize overall metabolic trends, while the identification of treatment-associated metabolites relied primarily on univariate statistical testing and pathway enrichment analyses. The concordance between the univariate results and biologically meaningful pathway modulation supports the robustness of the observed metabolic remodeling, despite limited multivariate separation.

Metabolomics analysis showed that different classes of metabolites were altered by treatment. In particular, carbohydrates and their analogs and amino acid-based metabolites significantly changed during the treatment period.

Pathway analysis identified significant modulation of amino acid metabolism, including pathways related to protein digestion, absorption, and amino acid biosynthesis. Amino acid metabolism is critical in the pathogenesis of T2DM, metabolic disorders and cancer[17,18]. Previous studies have reported inconsistent effects of SGLT-2 inhibition on circulating amino acid profiles. Horibe et al. reported no significant changes in T2DM patients amino acid profile after 24 weeks of dapagliflozin treatment[19]. However, Furuya et al. observed elevated plasma levels of branched-chain amino acids following dapagliflozin administration[20]. In the present study, we observed an overall upregulation of amino acid metabolism after six months of SGLT-2 inhibitor therapy. Several amino acid–related metabolites, including branched-chain and glycine-related amino acids, were significantly increased following treatment.

This upward shift in amino acid–related metabolites may reflect altered amino acid turnover and enhanced metabolic flexibility, potentially linked to improved mitochondrial oxidative capacity and adaptive energy metabolism induced by SGLT-2 inhibition. The discrepancy between our findings and those reported by Horibe et al. may be explained by methodological and clinical differences between the studies, despite similar treatment durations. Notably, differences in study populations and baseline metabolic characteristics are likely to influence amino acid metabolism and its responsiveness to SGLT-2 inhibitors.

Importantly, our study specifically included patients with metabolic dysfunction–associated steatotic liver disease, a condition characterized by impaired amino acid handling and disrupted nitrogen metabolism. This disease-specific metabolic context may amplify amino acid–related changes in response to SGLT-2 inhibition that are not readily detectable in more metabolically heterogeneous patient cohorts.

Among the most prominently affected pathways were the ABC transporter system and aminoacyl-tRNA biosynthesis. Park et al.[21] reported associations between altered aminoacyl-tRNA biosynthesis and metabolic disorders. López-Soldado et al. highlighted reduced expression of aminoacyl-tRNA synthetases in T2DM development[22]. Other studies have shown that the downregulation of ABCA1 in pancreatic β-cells is associated with reduced HDL levels and impaired insulin secretion, contributing to glucose intolerance and T2DM[23]. ABCG1 also plays a role in β-cell function and lipid regulation[24]. SGLT-2 inhibitors may influence the expression and activity of ABC transporters, modulating metabolic pathways in T2DM and MASLD[25]. Our results corroborate the findings of Lu et al., who reported enhanced aminoacyl-tRNA biosynthesis following empagliflozin treatment, suggesting a link between SGLT-2 inhibitors and improved metabolic function[26]. Collectively, the upregulation of pathways related to ABC transporters and aminoacyl-tRNA biosynthesis following SGLT-2 inhibitor therapy points toward coordinated remodeling of amino acid handling and protein synthesis machinery, which may contribute to improved metabolic flexibility and cellular homeostasis in T2DM and MASLD. Further targeted metabolomics and transcriptomics studies are needed to elucidate the role of this pathway in SGLT-2 inhibition.

Lipidomic profiling revealed extensive remodeling of the lipid metabolism. A total of 492 lipid species were significantly altered post-treatment, with prominent changes across phospholipid classes, including PC, LPC, PE, and PI. Razquin et al. highlighted the significance of phospholipid profiles in the context of diabetes. Specifically, alterations in phospholipid composition LPC and PE have been identified as risk factors for the development of the disease[27]. PE biosynthesis has emerged as a critical factor in metabolic disorders, and disruptions in PE metabolism contribute to insulin resistance[28]. Furthermore, the PE lipid profile has been implicated in liver health, with the PC/PE ratio serving as a relevant marker of MASH progression[29]. In our study, we observed that 34 PE species were altered during the treatment period. LPC is one of the most important subclasses of phospholipids, and several studies have demonstrated that the dysregulation of LPC metabolism is associated with both T2DM and MASH. LPC lipids are generally considered to have protective roles in T2DM, as lower levels are often observed in affected individuals[30]. Conversely, elevated LPC concentrations in liver tissue have been linked to hepatic lipotoxicity in patients with MASH[31]. Our results showed that three LPC species were downregulated and one was upregulated following SGLT-2 inhibitor treatment suggesting a lipid class-specific response to therapy.

Alterations in amino acid and phospholipid metabolism may indicate enhanced mitochondrial oxidative capacity and improved energy homeostasis, consistent with evidence that SGLT-2 inhibition restores mitochondrial function and reduces oxidative stress. Elevated levels of oxidized lipids contribute to the progression and poor prognosis of type 2 diabetes and promote cardiovascular and neurodegenerative complications[32].

SGLT-2 inhibitor treatment markedly reduced oxidized PC and PE species suggesting the attenuation of lipid peroxidation and inflammation through the modulation of lipid metabolism. Further targeted lipidomic analyses of oxidized lipid species could provide deeper insights into the effects of SGLT-2 inhibitors on oxidative stress and inflammatory status.

Several studies have characterized the plasma free fatty acid (FFA) composition in individuals with T2DM compared to healthy controls using both targeted and untargeted lipidomic approaches[33]. Zhang et al. showed that FFA composition is also an important parameter, especially for predicting advanced fibrosis in MASLD patients[34]. In our study, treatment with SGLT-2 inhibitors resulted in significant alterations in the plasma FFA profile. Notably, 13 FFA species were decreased, whereas five species were increased following treatment. These findings suggest that SGLT-2 inhibitors modulate fatty acid metabolism, potentially exerting secondary effects on systemic energy homeostasis.

Sokołowska et al. demonstrated that SM levels are significantly altered in individuals with T2DM, suggesting a potential link between SM metabolism and the pathophysiology of the disease[35]. In our study, treatment with SGLT-2 inhibitors influenced plasma SM lipid levels.

Another important finding was that SGLT-2 inhibitors induced significant alterations in the glycerolipid profile, particularly affecting triacylglycerol (TAG) and diacylglycerol (DAG) species, as detailed in Supplementary Information 1.A total of 79 TAG and 9 DAG species were significantly modulated by the treatment. Previous studies have demonstrated that the composition of TAG species plays a critical role in the pathophysiology of insulin resistance[36]. Furthermore, hepatic accumulation of TAG is a key biomarker associated with hepatic steatosis and the development of T2DM[37]. DAGs are important lipid intermediates that influence insulin signaling pathways. Alterations in DAG composition have been implicated in insulin resistance and Type 2 DM. In vitro studies have shown that DAG species are crucial mediators of pancreatic β-cell function[38]. Clinical investigations further support the alteration of plasma DAG profiles in individuals with prediabetes and diabetes. Moreover, DAG concentrations in hepatic tissue and systemic circulation have been identified as critical factors in the progression of MASLD and other metabolic disorders[27,39].

A more detailed evaluation of the supplementary lipidomic data indicates that alterations in TAG and DAG subclasses following SGLT-2 inhibitor therapy were not uniform but depended on lipid species composition.

Both TAG and DAG classes exhibited bidirectional changes, with subsets of species increasing and others decreasing, highlighting selective remodeling rather than global upregulation or downregulation.

Ether-linked diacylglycerol species consistently decreased after SGLT-2 inhibitor therapy. Ether-linked glycerolipids have been implicated in altered membrane dynamics, oxidative stress responses, and dysregulated lipid signaling in metabolic disorders. Their accumulation has been associated with perturbed redox balance and maladaptive lipid remodeling under conditions of metabolic stress.[40] The observed reduction in ether-linked DAG species suggests the attenuation of aberrant lipid signaling and normalization of glycerolipid composition. This finding aligns with the proposed metabolic benefits of SGLT-2 inhibitors, including improved mitochondrial function, reduced oxidative stress, and restoration of lipid homeostasis. This selective decrease further supports the concept that SGLT-2 inhibition induces qualitative lipid remodeling rather than nonspecific changes in total lipid abundance.

Interestingly, two polyunsaturated DAG species were increased after SGLT-2 inhibitor therapy. Polyunsaturated DAG species are generally considered less lipotoxic than their saturated or monounsaturated counterparts and may exert distinct biological effects on lipid signaling and membrane dynamics.[41] Their selective increase, along with reductions in other DAG species, further supports qualitative remodeling of DAG composition rather than uniform changes in total DAG abundance.

Analysis of TAG subclasses according to degree of unsaturation did not reveal a consistent or preferential directional shift in saturated, monounsaturated, or polyunsaturated TAG species following SGLT-2 inhibitor therapy. Instead, TAG species across all degrees of unsaturation exhibited mixed increases and decreases, indicating global remodeling of TAG composition rather than selective enrichment or depletion of a specific unsaturation class. This pattern suggests that SGLT-2 inhibition influences TAG metabolism in a broad and integrative manner, likely reflecting altered lipid turnover and redistribution among TAG pools rather than the targeted modulation of specific fatty acid saturation profiles.

Despite these promising findings, this study has several limitations. Hepatic steatosis in this study was assessed using an ultrasound-based quantitative approach, reflecting the growing clinical use of non-invasive tools for hepatic fat estimation. Ultrasound-based fat quantification techniques have been shown to be highly reproducible, demonstrate good diagnostic performance, and correlate well with MRI-proton density fat fraction (MRI-PDFF).[15,42,43].

However, because the standardization of acquisition protocols, analytical methods, and cut-off values is still evolving, further validation in larger cohorts is needed. Accordingly, ultrasound-based fat measurements should not be interpreted solely as absolute quantitative values but rather integrated in the overall clinical context. In this study, changes in hepatic fat content were best interpreted as relative treatment-associated differences over time.

The relatively small sample size restricts the generalizability of the results and limits statistical power, especially for correlation analyses. Additionally, omics analyses were conducted exclusively on blood samples, as none of the participants underwent liver biopsies. While this noninvasive approach provides valuable insights, metabolomic and lipidomic profiling directly from hepatic tissue would offer greater specificity and mechanistic clarity. Another major limitation of this study is the absence of a control group. Without a parallel untreated or alternative-treatment arm, the observed improvements in hepatic fat content, glycemic control, BMI, and metabolomic and lipidomic profiles cannot be attributed solely to SGLT-2 inhibitor therapy. Natural disease variability, regression to the mean, and unmeasured temporal effects may have contributed to the observed changes.

In addition, lifestyle factors such as diet and physical activity were not systematically monitored or standardized during therapy. As these factors substantially influence hepatic steatosis, metabolic control, and circulating omics profiles, concurrent lifestyle modifications following therapy initiation may have confounded the results. The lack of lifestyle data during therapy, together with the absence of a placebo-controlled group, limits the ability to disentangle the independent effects of SGLT-2 inhibition from behavioral or environmental influences.

Accordingly, while the integrated clinical, metabolomic, and lipidomic findings suggest biologically plausible metabolic remodeling associated with SGLT-2 inhibitor therapy, confirmation in larger, controlled, and ideally randomized studies with standardized lifestyle monitoring is required to establish causality.

Conclusion

Overall, these findings highlight the multifactorial metabolic effects of SGLT-2 inhibitors and lay the groundwork for future large-scale controlled studies that incorporate liver tissue-based omics.

Data availability

Anonymized and summarised data will be made available to other re-searchers upon publication after reasonable requests have been made tot he corresponding author.

Received: 4 November 2025; Accepted: 13 March 2026
Published online: 02 May 2026

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Acknowledgements
Paperpal 2.4.4 version was used in the main-text article to correct grammatical errors .

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Kenan Moral: Integrity of work, Methodology, Conceptualization, Writing original draft, Data Analysis, Engin Koçak: Methodology, Data analysis, Writing original Draft, Sevilay Erdoğan Kablan: Data analysis, methodology Emirhan Nemutlu: Data analysis, methodologyHalit Nahit Şendur: Data analysis, conceptualization, visualizationMahinur Cerit: Data analysis, conceptualization, visualizationMehmet Muhittin Yalçın: Data analysis, conceptualization, visualization, methodologyGülden Bilican: Data analysis, conceptualization, Mehmet Cindoruk : Data analysis, conceptualization, methodologyTarkan Karakan: Methodology, conceptualization, supervisionKübra Vardar: writing draft, methodology, conceptualizationNergiz Ekmen: Supervision, writing original draft, conceptualization, methodology, data analysis.

Funding
This project received a research grant from Turkish Society of Gastroenterology (Number: 94-TGD-2023) but had no influence on data collection, analysis, interpretation or reporting.

Declarations

Competing interests
The authors declare no competing interests.

Additional information
Supplementary Information The online version contains supplementary material available at https://doi.org/10.1038/s41598-026-44786-w.

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