Leucine supplementation modulates lipid metabolism and metabolic pathways in mice with type 1 diabetes: A metabolomics study

JENG-YUAN YAO[1], DE-TIAN-YU TZENG[2], CAI-HONG FENG[2], GONG-MAN XIAO[2], TIAN-NI YOU[2] and RU-YU ZHENG[2]

[1]Key Laboratory of Functional and Clinical Translational Medicine, Xiamen Medical College, Fujian Province University, Xiamen, Fujian 361023, P.R. China; [2]Department of Clinical Medicine, Xiamen Medical College, Xiamen, Fujian 361023, P.R. China

Received March 27, 2025; Accepted October 24, 2025

DOI: 10.3892/etm.2025.13012

Abstract. Type 1 diabetes mellitus (T1DM) is an autoimmune destruction of pancreatic β-cells that causes absolute insulin deficiency and lifelong insulin use. It leads to ketoacidosis and long-term micro-/macro-vascular complications. Globally, ~9 million individuals live with T1DM and ~0.5 million are newly diagnosed each year. Leucine (Leu), a branched-chain amino acid, improves blood-glucose control and increases the insulin sensitivity of peripheral tissues. However, its role in modulating lipid metabolism and metabolic pathways in T1DM is yet to be elucidated. In the present study, male C57BL/6J mice were randomly divided into four groups, namely Normal, Normal-Leu, T1DM and T1DM-Leu. T1DM was induced by five daily intraperitoneal injections of streptozotocin (50 mg/kg per day), and leucine was provided in the drinking water at 1.5% (w/v) for 6 weeks. After 6 weeks, plasma samples were collected for metabolomics and biochemical analysis. Untargeted LC-MS/MS profiling showed elevated metabolites mapping to steroid-hormone biosynthesis, arachidonic/linoleic-acid and retinol pathways in T1DM vs. Normal; Leu partly lowered these signals and shifted galactose and primary bile-acid metabolism toward control patterns. Leu supplementation downregulated galactose-pathway intermediates and reduced signals consistent with primary bile-acid biosynthesis, shifting both pathways toward control levels in T1DM-Leu vs. T1DM. Biochemically, T1DM lowered high-density lipoprotein (HDL) and raised low-density lipoprotein (LDL) vs. Normal (HDL, 0.597±0.292 mmol/l; LDL, 1.154±0.501 mmol/l); with Leu, these lipids approached control levels: T1DM-Leu (HDL, 0.958±0.224 mmol/l; LDL, 0.548±0.267 mmol/l) vs. Normal-Leu (HDL, 0.912±0.121 mmol/l; LDL, 0.392±0.175 mmol/l). In summary, the findings of the present study indicated that dietary Leu may be a useful adjuvant for T1DM due to potentially easing dyslipidemia and reversing key metabolic disturbances.

Correspondence to: Dr Jeng-Yuan Yao, Key Laboratory of Functional and Clinical Translational Medicine, Xiamen Medical College, Fujian Province University, 1999 Guan Kou Zhong Road, Jimei, Xiamen, Fujian 361023, P.R. China
E-mail: 201500080004@xmmc.edu.cn

Key words: type 1 diabetes, leucine, metabolomics, lipoprotein, metabolic pathways

Introduction

Type 1 diabetes mellitus (T1DM) is an autoimmune disorder in which pancreatic β-cells are destroyed, leading to low insulin production and chronic hyperglycemia. Although T1DM only accounts for 5-10% of all cases of diabetes, it still places a heavy burden on healthcare systems because of its progressive course and various complications such as diabetic ketoacidosis, retinopathy, nephropathy, neuropathy and cardiovascular disease (1). Achieving tight glycemic control remains challenging because intensive insulin therapy is limited by hypoglycemia risk and glycemic variability despite frequent monitoring (2). Achieving tight glycemic control remains challenging, and patients with T1DM are susceptible to microvascular and macrovascular complications (such as retinopathy, nephropathy, neuropathy and cardiovascular disease) (3,4). As the global incidence rate of T1DM continues to rise, ~8.4 million individuals were living with T1DM in 2021, with 510,000 incident cases and 175,000 deaths annually (5).

Dyslipidemia, usually characterized by an increase in the low-density lipoprotein (LDL) cholesterol and a decrease in the high-density lipoprotein (HDL) cholesterol, increases the cardiovascular risk in T1DM (6-8). These lipid changes are also associated with poor insulin signaling and oxidative stress (9,10). Therefore, treatments that normalize the lipid profile may help reduce the overall burden of T1DM. Leucine (Leu), a branched-chain amino acid, is known to support muscle health, glucose control and insulin sensitivity (11). Mechanistically, Leu activates the mammalian target of rapamycin (mTOR) pathway and stimulates AMPK signaling (e.g., via the SIRT1-AMPK axis), which promotes fatty-acid oxidation and glucose uptake (12,13). Leu supplementation has

been shown to improve lipid handling and relieve dyslipidemia in several animal and human studies (14-16). However, its exact effect on T1DM-associated lipid changes, especially on HDL and LDL, is yet to be elucidated.

Therefore, to fill the aforementioned gap in the knowledge, the present study used a metabolomics approach with a mouse model of T1DM. The present study investigated whether Leu supplementation could adjust the HDL/LDL levels and the associated downstream metabolic pathways. Male C57BL/6J mice were placed into four groups, namely Normal, Normal with Leu supplementation (Normal-Leu), T1DM and T1DM with Leu supplementation (T1DM-Leu). T1DM was induced using streptozotocin (STZ) administered intraperitoneally at 50 mg/kg/day for 5 consecutive days, and Leu (1.5%) was provided in drinking water for 6 weeks. Plasma samples were then used in liquid chromatography-tandem mass spectrometry (LC-MS/MS) for metabolomic profiling and biochemical assays of plasma triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C).

Materials and methods

Materials. Leu and STZ were purchased from Sigma-Aldrich; Merck KGaA. Isoflurane, acetonitrile and methanol were obtained from Shanghai Aladdin Biochemical Technology Co., Ltd. Assay kits for plasma glucose [Glucose (GOD-POD) Assay Kit], triglycerides [Triglyceride (GPO-PAP) Assay Kit], total cholesterol [Total Cholesterol (CHOD-PAP) Assay Kit], HDL-C [Direct HDL-C Assay Kit], LDL-C [Direct LDL-C Assay Kit], and whole-blood glycated hemoglobin (HbA1c) [Boronate-affinity HbA1c Assay Kit] were from Beijing Solarbio Science & Technology Co., Ltd., and were used according to the manufacturer's instructions.

Animals. A total of 40 male C57BL/6J mice (8 weeks old; 20-24 g at study start) were used. Mice were obtained from Shanghai SLAC Laboratory Animal Co., Ltd. and acclimatized for 1 week. Animals were randomly assigned to four groups (n=10/group): Normal, Normal-Leu, T1DM and T1DM-Leu. All procedures conformed to the Guide for the Care and Use of Laboratory Animals (8th edition) (17) and the ARRIVE guidelines 2.0 (18), and were approved by the IACUC of Xiamen University (approval no. 20210305042). Mice were housed in an SPF facility under 22±2˚C, 50±10% relative humidity, a 12:12 h light/dark cycle, with *ad libitum* access to standard chow and water; 4-5 mice per cage with corncob bedding and environmental enrichment. Anesthesia was induced using 4-5% isoflurane in 1 l/min oxygen via an induction chamber and maintained at 1.5-2% using a nose cone during blood collection. Euthanasia was carried out using ≥5% isoflurane to for ≥3 min. Death was confirmed by the absence of respiration, heartbeat and corneal reflex. Every effort was made to minimize the discomfort to the animals. Humane endpoints were predefined as ≥20% body-weight loss, severe dehydration/ketonuria with prostration, persistent hypothermia/recumbency or inability to eat/drink; animals reaching any endpoint would be euthanized by isoflurane overdose. No animals reached a humane endpoint prior to planned study termination.

Induction of T1DM. STZ (50 mg/kg/day) was freshly dissolved in cold 0.1 M sodium citrate buffer (pH 4.5) at 5 mg/ml. A volume of 10 ml/kg (0.20-0.25 ml per 20-25 g mouse) was administered intraperitoneally once daily for five consecutive days (17); control groups received equal-volume citrate buffer i.p. Additionally, the Normal-Leu and T1DM-Leu groups received 1.5% Leu in their drinking water, while the Normal and T1DM groups received drinking water without additions (18). Diabetes was confirmed 7 days after the last STZ injection when fasting blood glucose ≥12 mmol/l was recorded from tail-vein blood (~5 μl) using a handheld glucometer after a 6 h fast (water *ad libitum*); one sample per mouse was taken for confirmation.

Plasma preparation. On week 6 following STZ-induced T1DM, mice were anesthetized with isoflurane and euthanized. Blood (0.8-1.0 ml per mouse) was collected by cardiac puncture into pre-chilled K2-EDTA microtubes and the entire volume was centrifuged at 2,000 x g for 10 min at 4˚C to obtain plasma, which was stored at -20˚C. Plasma from six randomly selected mice per group was used for LC-MS/MS, and plasma from all animals was used for biochemical assays.

Metabolomics analysis. Plasma samples (100 μl) were mixed with 400 μl methanol, vortexed and sonicated to precipitate proteins. After centrifugation (15,000 x g for 10 min at 4˚C), the supernatants were dried under nitrogen (35˚C; nebuliser pressure 15 psi), reconstituted in 100 μl acetonitrile and centrifuged again at 15,000 x g for 10 min at 4˚C. A 2 μl aliquot was injected into a UPLC-QTOF-MS system (Waters Xevo G2-XS QTOF with an ACQUITY BEH C18 1.8 μm 2.1x50 mm column; Waters Corporation), using electrospray ionization in positive mode. The mobile phase consisted of 0.01% formic acid in water (solvent A) or acetonitrile (solvent B) with a flow rate of 0.4 ml/min. The gradient elution used was 0-1 min with 10% solvent B, 1-7.5 min with 10-65% solvent B, 7.5-10.5 min with 65-100% solvent B and 10.5-12 min with 100-10% solvent B. Leucine-enkephalin (m/z 556.2771) was infused for lock-mass calibration. A pooled quality control (QC) sample was prepared by combining 10 μl aliquots of each extract. Five QC injections were used at the start to condition the system; one QC was injected every eight study samples; and three QC injections were run at the end. All injections were randomized and interleaved with blank samples.

Raw data were processed using Progenesis QI (version 2.4; Nonlinear Dynamics; Waters Corporation). After alignment to the third QC injection, ion features were extracted, normalized with the total ion current and corrected for drift using QC-based locally estimated scatterplot smoothing. Features with a relative SD of >30% in QCs or present in <80% of QC samples were excluded. All detected ion features have been deposited in the MassIVE repository (accession no. MSV000099368). Multivariate and pathway analyses were conducted using MetaboAnalyst version 5.0 (https://www.metaboanalyst.ca). Principal component (PC) analysis was used for visualization. For metabolite set enrichment analysis (MSEA), differential ions were first selected using the volcano plot criteria (a fold change of more than two; $P<0.05$) and mapped to pathways in the Kyoto Encyclopedia

of Genes and Genomes (KEGG) database (https://www.kegg.jp). Pathways with a P-value of <0.05 and more than or equal to two matched metabolites were considered significant. Metabolite annotation was based on accurate m/z and MS/MS spectrum matches against the Human Metabolome Database (https://hmdb.ca).

Biochemical measurements. Plasma glucose, TG, total cholesterol, HDL and LDL were quantified in plasma samples, whereas HbA1c was determined from whole blood (erythrocyte hemolysates), using the respective Beijing Solarbio Science & Technology Co., Ltd. kits according to the manufacturer's protocols.

Statistical analysis. Analyses were carried out using SPSS version 16.0 (SPSS, Inc.). One-way ANOVA followed by Tukey's post hoc test was used for multiple-group comparisons. Data are presented as mean ± SD or as box-and-whisker plots (median, interquartile range and 10-90th percentiles) as specified in the figure legends. P<0.05 was considered to indicate a statistically significant difference. Biochemical outcomes (glucose, TG, total cholesterol, HDL, LDL and HbA1c) were measured in 10 mice per group (biological replicates); each assay was run in duplicate and the mean was used for statistics. Untargeted metabolomics used plasma from 6 mice per group.

Results

Metabolite profiling and pathway analysis. Untargeted LC-MS/MS detected 1,692 ion features in the plasma of mice. After excluding peaks with a QC relative SD >30% in QCs (Fig. 1A) and applying an interquartile range filter, 888 features remained for statistical evaluation. One-way ANOVA followed by Tukey's post hoc test identified ions with significant differences among the four groups (FDR-adjusted P<0.05; full list provided in the public MassIVE dataset) (Fig. 1A). PC analysis further revealed a clear metabolic separation between the Normal, Normal-Leu, T1DM and T1DM-Leu group clusters. Where PC1 and PC2 accounted for 38.2 and 19.9% of the total variance, respectively (Fig. 1B).

Pairwise analyses were then carried out. In the T1DM vs. Normal comparison, 227 features were higher in T1DM (upregulated) and 186 were lower in T1DM (downregulated) relative to Normal (Fig. 1C). KEGG-based MSEA (using a criteria of P<0.05 and more than or equal to two hits) pointed to marked disturbances in steroid-hormone biosynthesis, arachidonic-acid metabolism, retinol metabolism, linoleic-acid metabolism and a number of lipid-related pathways (Fig. 1C). Compared with mice in the untreated T1DM group, Leu supplementation in the T1DM-Leu group resulted in an increase in 75 features and a decrease in 251 features (Fig. 1D). Enrichment analysis indicated that Leu modulated steroid-hormone biosynthesis, arachidonic-acid metabolism, galactose metabolism, pentose-and-glucuronate interconversions and primary bile-acid biosynthesis (Fig. 1D). In summary, these results indicated that Leu treatment may partially reverse the metabolic disruptions of untreated T1DM, especially within pathways associated with lipid and bile-acid metabolism.

Key metabolites affected by Leu supplementation. Fig. 2 indicates that eicosapentaenoic acid, 8,11,14-eicosatrienoic acid, arachidonic acid, retinyl ester, vitamin A and norepinephrine levels were significantly higher in the T1DM group compared with the Normal control group, and Leu supplementation (T1DM-Leu) reduced these metabolites toward control levels. After 6 weeks of treatment with 1.5% Leu, there was a significant decrease in each of these metabolites in the mice in the T1DM-Leu group. Across all six metabolites (eicosapentaenoic acid, 8,11,14-eicosatrienoic acid, arachidonic acid, retinyl ester, vitamin A and norepinephrine), levels in the T1DM group were higher than those in the Normal group (P<0.01), whereas leucine supplementation lowered these levels in the T1DM-Leu group compared with T1DM (one-way ANOVA with Tukey's post hoc test; P<0.05). In most cases, T1DM-Leu values were intermediate between T1DM and Normal/Normal-Leu, and Leu alone did not differ from Normal. These changes indicated that Leu may potentially oppose the lipid-inflammatory and retinoid disturbances associated with early-stage T1DM.

Biochemical analyses. HbA1c and fasting blood glucose were significantly higher in the T1DM group compared with the Normal group. In the T1DM-Leu group, both indices remained elevated and were not significantly different from the T1DM group (Fig. 3A and B). TG levels were significantly higher than in the Normal group in all three intervention groups; however, there were no significant differences among the intervention groups (Fig. 3C). There were no significant differences in the total cholesterol levels between the Normal and the treatment groups (Fig. 3D). HDL levels were significantly reduced in the T1DM group compared with the Normal group; however, in the T1DM-Leu group, the HDL levels were similar to the levels in the Normal group (Fig. 3E). By contrast, LDL levels were significantly increased in the T1DM group compared with the Normal group, whereas the LDL levels in the T1DM-Leu group were similar to the levels in the Normal and Normal-Leu groups (Fig. 3F).

Discussion

The findings of the present study suggested that Leu supplementation may mitigate the metabolic disturbances in T1DM, particularly in lipid metabolism. In the plasma metabolomics data, eicosapentaenoic acid, 8,11,14-eicosatrienoic acid, arachidonic acid, retinyl ester, vitamin A and norepinephrine were lower in T1DM-Leu than in T1DM mice. Taken together with the pathway-enrichment results (MetaboAnalyst/KEGG), these findings suggest a partial amelioration of lipid-related pathways, including arachidonic-acid metabolism (KEGG map00590), linoleic-acid metabolism (map00591), and biosynthesis of unsaturated fatty acids (map01040), as well as retinol metabolism (map00830).

Altered levels of arachidonic acid and associated lipid metabolites are implicated in inflammation and insulin resistance, and their dysregulation contributes to diabetic complications such as cardiovascular disease and nephropathy (19,20). Reduced levels of retinyl ester and vitamin A are observed in mice with diabetes and may exacerbate oxidative stress-for example, mitochondrial ROS overproduction, NADPH oxidase/AGE-RAGE-driven

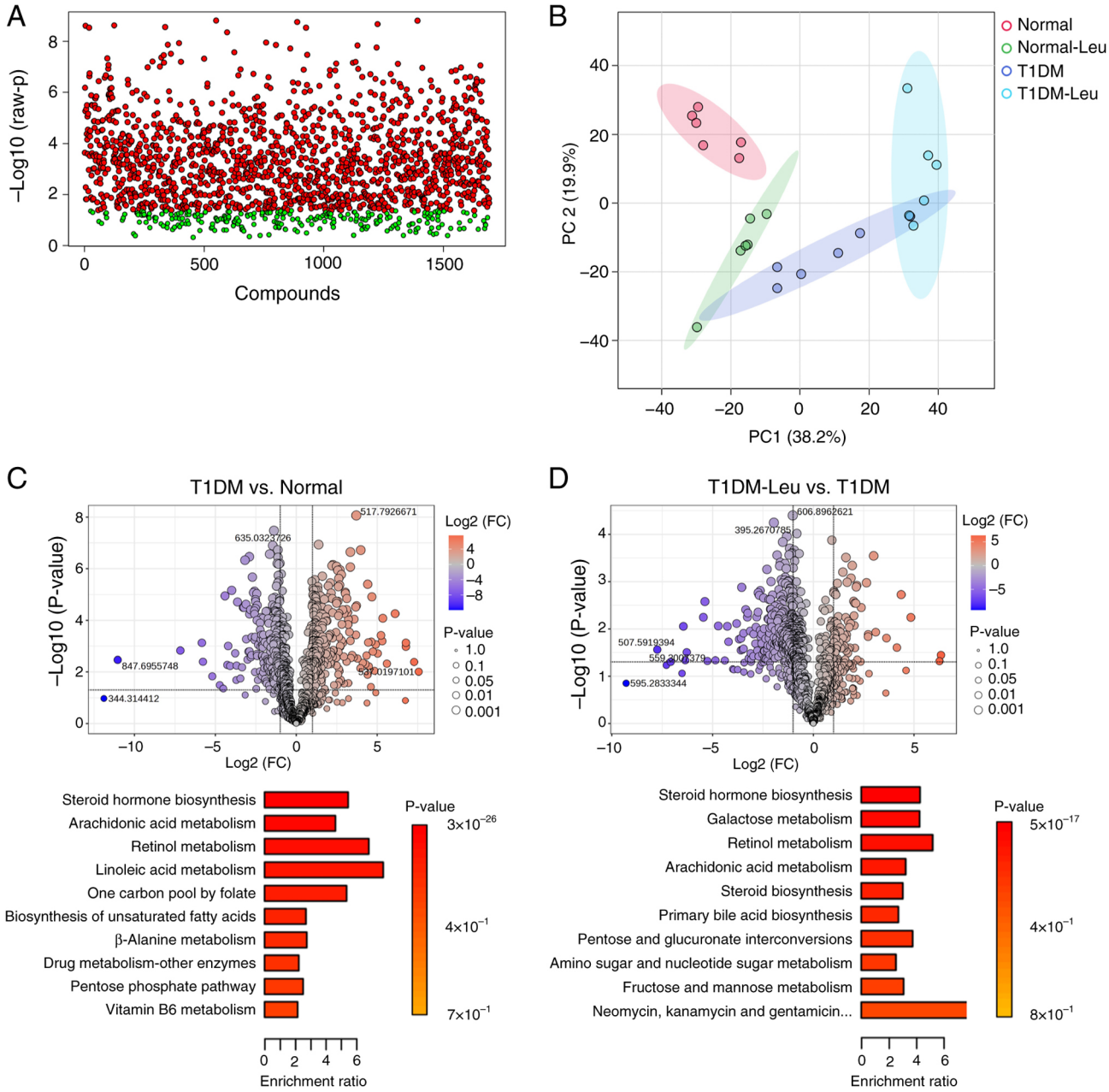

Figure 1. Metabolomic overview of plasma samples. (A) One-way ANOVA of 1,692 detected ion features after quality-control filtering (relative SD >30%), followed by Tukey's post hoc test. Red dots represent ions with P-values of <0.05; green dots represent non-significant ions. (B) PC analysis scores plot illustrating the separation of the Normal, Normal-Leu, T1DM and T1DM-Leu groups. PC1 and PC2 explain 38.2 and 19.9% of the variance, respectively. (C) The top panel shows a volcano plot of the differential features in the T1DM vs. Normal group comparison (FC, >2; P<0.05). The bottom panel shows the KEGG MSEA of the top 10 pathways altered in the mice in the T1DM group compared with those in the Normal group. (D) The top panel shows a volcano plot of the differential features in the T1DM-Leu vs. T1DM group comparison (FC, >2; P<0.05). The bottom panel shows the KEGG MSEA of the top 10 pathways modulated by Leu supplementation in mice with T1DM. Leu, leucine; Normal-Leu, Normal with 1.5% Leu supplementation; T1DM, type 1 diabetes mellitus; T1DM-Leu, T1DM with 1.5% Leu supplementation; PC, principal component; FC, fold-change; KEGG, Kyoto Encyclopedia of Genes and Genomes; MSEA, metabolite-set enrichment analysis.

lipid peroxidation- and weaken antioxidant defenses such as superoxide dismutase, catalase and glutathione peroxidase (21). Therefore, the Leu-mediated reduction of these metabolites in the mice with T1DM may potentially represent a protective metabolic shift. Additionally, norepinephrine dysregulation is associated with an increase in the risk of cardiovascular issues in patients with diabetes (22). This suggests that the reduction of the norepinephrine levels in the mice with T1DM following Leu supplementation may be of clinical importance.

The results of the present study revealed that Leu supplementation also helped restore the HDL and LDL levels in mice with T1DM, which potentially suggested a favorable effect on lipoprotein profiles. HDL-cholesterol is well known to have protective cardiovascular effects by promoting reverse cholesterol transport, reducing inflammation and improving endothelial function. However, elevated LDL-cholesterol levels accelerates atherogenesis, which notably increases the risk of cardiovascular complications in diabetes (23). Therefore, restoring the balance of HDL and LDL with Leu

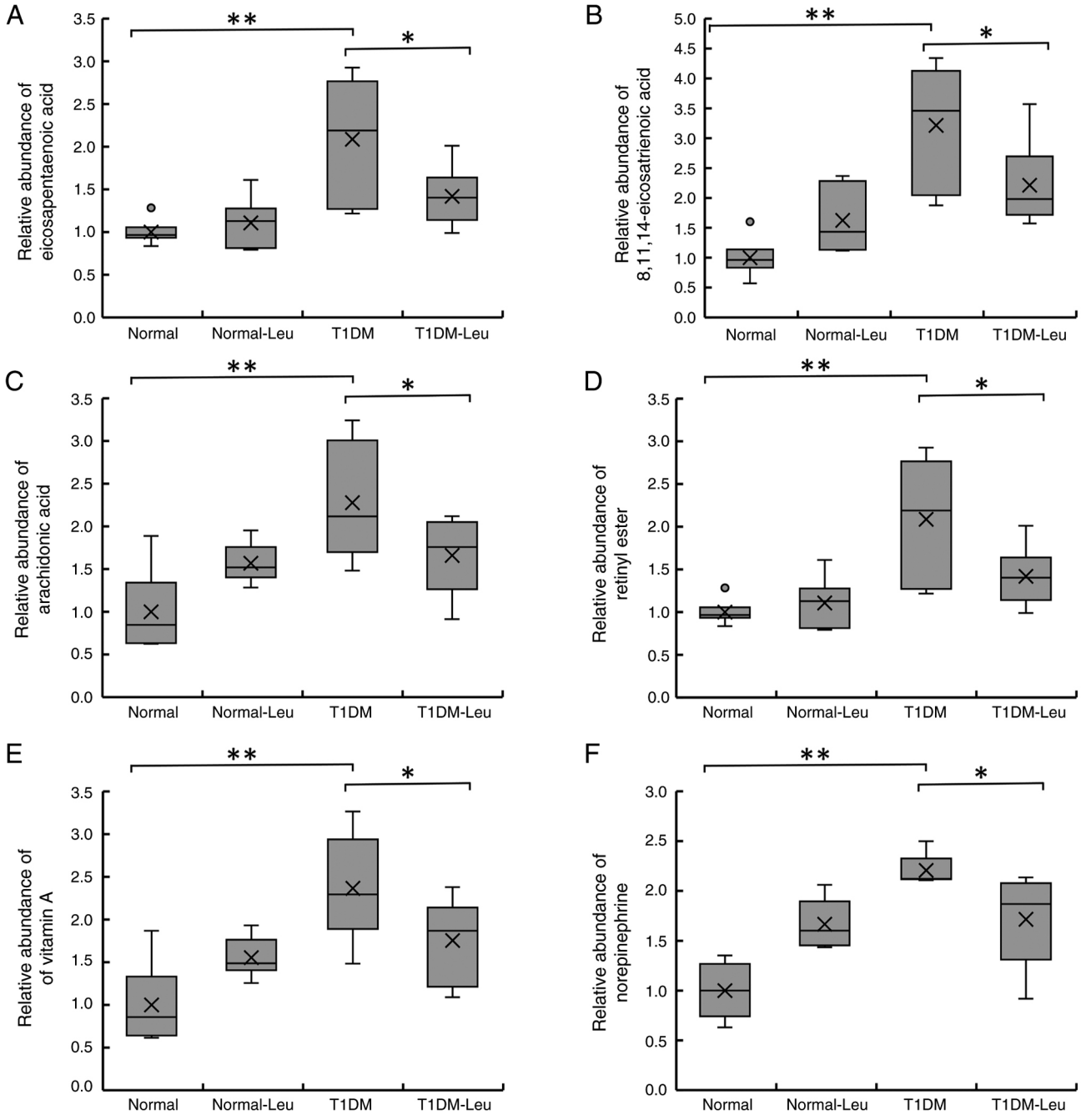

Figure 2. Relative abundance of selected metabolites affected by T1DM and Leu supplementation. (A) Eicosapentaenoic acid; (B) 8,11,14-eicosatrienoic acid; (C) arachidonic acid; (D) retinyl ester; (E) vitamin A and (F) norepinephrine. Data are presented as box-and-whisker plots (median, interquartile range and 10-90th percentiles). *P<0.05 and **P<0.01 indicate the significance level of the pairwise comparisons that were obtained using one-way ANOVA followed by Tukey's post hoc test. Leu, leucine; Normal-Leu, Normal with 1.5% Leu supplementation; T1DM, type 1 diabetes mellitus; T1DM-Leu, T1DM with 1.5% Leu supplementation.

supplementation may potentially offer cardiovascular benefits in the management of T1DM. The findings of the present study align with those of previous studies that demonstrate beneficial effects of Leu on glucose and lipid metabolism (18,24,25). Mechanistically, Leu may benefit T1DM via the activation of mTOR and AMPK, which are pathways that regulate metabolic processes (26-31). For example, AMPK activation inhibits lipogenesis, promotes fatty acid oxidation and improves lipid homeostasis, processes that are often impaired in diabetes (32). Furthermore, mTOR activation by Leu modulates lipid metabolism through the regulation of lipid synthesis genes and mitochondrial function (12). These previously identified mechanisms align with the findings of the present study, which indicated that Leu supplementation corrected T1DM-induced metabolic dysregulation of lipid pathways. In addition, enrichment analysis showed shifts in galactose metabolism and primary bile-acid biosynthesis, which suggested that Leu may also influence carbohydrate metabolism and bile-acid signaling in mice with T1DM, both of which can influence lipid homeostasis. Therefore, these secondary pathways may warrant further follow-up experiments in the future.

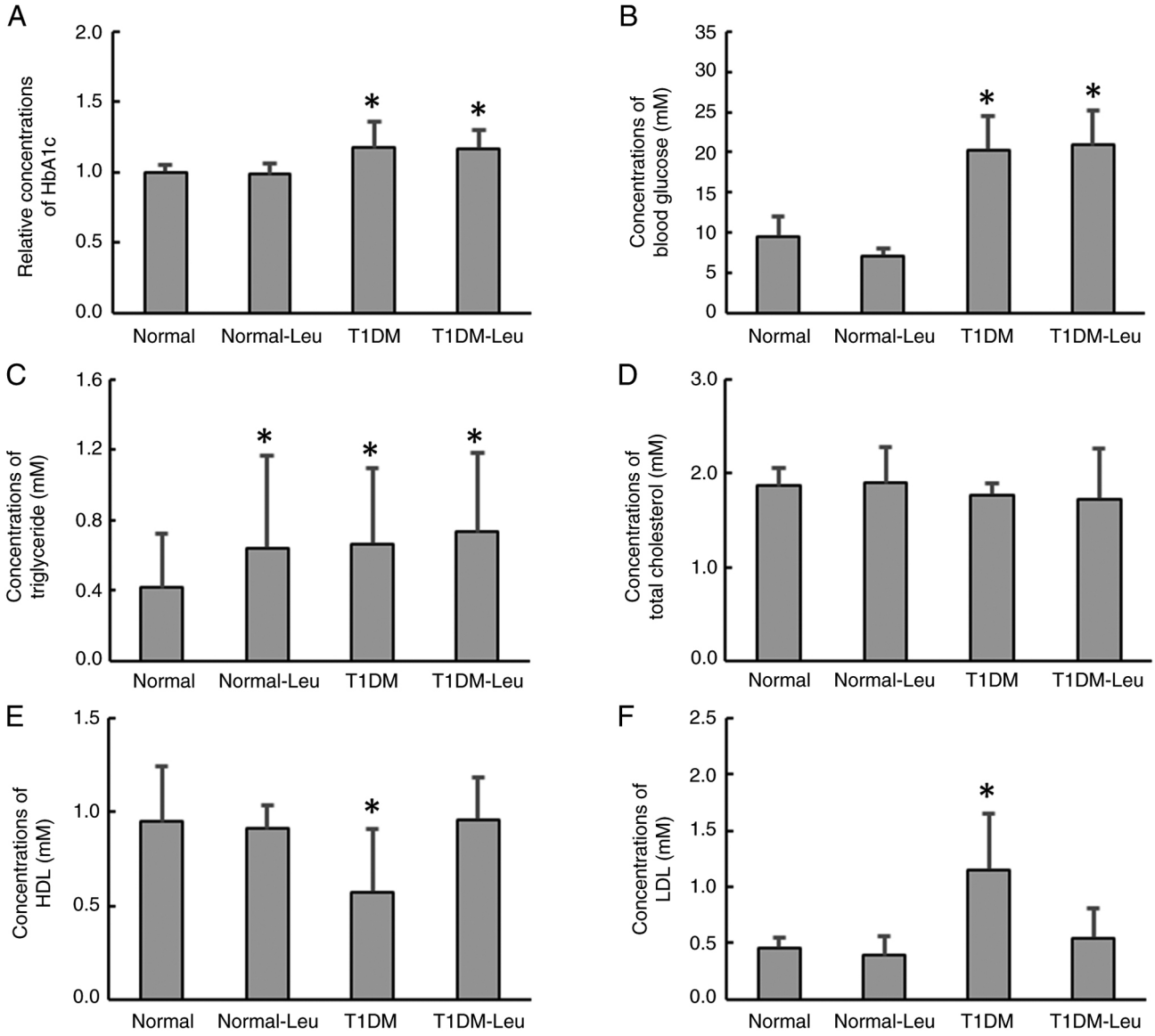

Figure 3. Biochemical parameters in plasma or whole blood. (A) Relative concentrations of HbA1c. Concentrations (mM) of (B) fasting blood glucose; (C) triglyceride; (D) total cholesterol; (E) high-density lipoprotein cholesterol and (F) low-density lipoprotein cholesterol. Data is presented as mean ± SD (n=10). *P<0.05 vs. the Normal group (one-way ANOVA followed by Tukey's post hoc test). Leu, leucine; Normal-Leu, Normal with 1.5% Leu supplementation; T1DM, type 1 diabetes mellitus; T1DM-Leu, T1DM with 1.5% Leu supplementation.

However, not all metabolites investigated in the present study exhibited identical degrees of modulation. For example, after Leu supplementation, the levels of arachidonic acid, 8,11,14-eicosatrienoic acid (20:3 n-6) and eicosapentaenoic acid (20:5 n-3) were significantly reduced in T1DM-Leu vs. T1DM mice, although the magnitude of change varied across metabolites, indicating a possible selective modulation of inflammatory and lipid pathways. Furthermore, vitamin A levels were partially, but significantly, restored. These differential responses may reflect distinct underlying regulatory mechanisms or pathway-specific sensitivities to Leu intervention.

In the present study, although supplementary Leu did not significantly lower blood glucose or HbA1c levels compared with those in the T1DM-only group, the selective improvement in lipid parameters suggested the potential utility of Leu in addressing dyslipidemia in T1DM. These results align with previous findings in which Leu supplementation is associated with improved lipid metabolism and cardiovascular health (7,15,33-35).

However, there were limitations of the present study. For example, the present study only focused on early-stage T1DM without examining advanced disease settings. Additionally, the present study did not fully elucidate the molecular mechanisms through which Leu exerted its effects. Therefore, future studies are needed for a more comprehensive evaluation. Finally, the safety and potential adverse effects of chronic Leu use are yet to be assessed. Overall, the findings of the present study supported the hypothesis that Leu supplementation may offer therapeutic benefits in T1DM by correcting imbalances in key lipids (for example, arachidonic acid, 8,11,14-eicosatrienoic acid, eicosapentaenoic acid, retinyl ester/vitamin A and HDL/LDL) and by modulating metabolic pathways highlighted by our analysis, including arachidonic/linoleic-acid and retinol

metabolism, steroid-hormone biosynthesis, galactose metabolism and primary bile-acid biosynthesis.

Acknowledgements

Not applicable.

Funding

The present study was supported by the Natural Science Foundation of Fujian Province, China (grant no. 2022D024) and the Xiamen Municipal Health Commission Guiding Project (grant no. 3502Z20244ZD1151).

Availability of data and materials

The data generated in the present study may be found in the MassIVE database under accession number MSV000099368 or at the following URL: https://doi.org/10.25345/C5MG7G83D.

Authors' contributions

JY contributed to the conception and design of the present study. DT, CF, GX, TY and RZ contributed to the data collection, analysis and interpretation of results. DT and CF confirm the authenticity of all the raw data generated and analyzed in this study. JY prepared the draft of the manuscript. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

All animal procedures were performed in accordance with the guidelines and regulations approved by the Institutional Animal Care and Use Committee of Xiamen University (Xiamen, China; approval no. 20210305042).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

References

1. Atkinson MA, Eisenbarth GS and Michels AW: Type 1 diabetes. Lancet 383: 69-82, 2014.
2. Maahs DM, West NA, Lawrence JM and Mayer-Davis EJ: Epidemiology of type 1 diabetes. Endocrinol Metab Clin North Am 39: 481-497, 2010.
3. de Ferranti SD, de Boer IH, Fonseca V, Fox CS, Golden SH, Lavie CJ, Magge SN, Marx N, McGuire DK, Orchard TJ, *et al*: Type 1 diabetes mellitus and cardiovascular disease: A scientific statement from the American heart association and American diabetes association. Diabetes Care 37: 2843-2863, 2014.
4. Forbes JM and Cooper ME: Mechanisms of diabetic complications. Physiol Rev 93: 137-188, 2013.
5. Gregory GA, Robinson TIG, Linklater SE, Wang F, Colagiuri S, de Beaufort C, Donaghue KC; International Diabetes Federation Diabetes Atlas Type 1 Diabetes in Adults Special Interest Group; Magliano DJ, Maniam J, *et al*: Global incidence, prevalence, and mortality of type 1 diabetes in 2021 with projection to 2040: A modelling study. Lancet Diabetes Endocrinol 10: 741-760, 2022.
6. Tell S, Nadeau KJ and Eckel RH: Lipid management for cardiovascular risk reduction in type 1 diabetes. Curr Opin Endocrinol Diabetes Obes 27: 207-214, 2020.
7. Wang X, Pei J, Zheng K and Hu X: High-density lipoprotein cholesterol levels are associated with major adverse cardiovascular events in male but not female patients with hypertension. Clin Cardiol 44: 723-730, 2021.
8. Sniderman AD, Williams K, Contois JH, Monroe HM, McQueen MJ, de Graaf J and Furberg CD: A meta-analysis of low-density lipoprotein cholesterol, non-high-density lipoprotein cholesterol, and apolipoprotein B as markers of cardiovascular risk. Circ Cardiovasc Qual Outcomes 4: 337-345, 2011.
9. Wu X and Xiao B: TDAG51 attenuates impaired lipid metabolism and insulin resistance in gestational diabetes mellitus through SREBP-1/ANGPTL8 pathway. Balkan Med J 40: 175-181, 2023.
10. Marques CL, Beretta MV, Prates RE, de Almeida JC and da Costa Rodrigues T: Body adiposity markers and insulin resistance in patients with type 1 diabetes. Arch Endocrinol Metab 67: 401-407, 2023.
11. Mero A: Leucine supplementation and intensive training. Sports Med 27: 347-358, 1999.
12. Laplante M and Sabatini DM: mTOR signaling in growth control and disease. Cell 149: 274-293, 2012.
13. Horman S, Browne G, Krause U, Patel J, Vertommen D, Bertrand L, Lavoinne A, Hue L, Proud C and Rider M: Activation of AMP-activated protein kinase leads to the phosphorylation of elongation factor 2 and an inhibition of protein synthesis. Curr Biol 12: 1419-1423, 2002.
14. Zhang Y, Guo K, LeBlanc RE, Loh D, Schwartz GJ and Yu YH: Increasing dietary leucine intake reduces diet-induced obesity and improves glucose and cholesterol metabolism in mice via multimechanisms. Diabetes 56: 1647-1654, 2007.
15. Zhang L, Li F, Guo Q, Duan Y, Wang W, Zhong Y, Yang Y and Yin Y: Leucine supplementation: A novel strategy for modulating lipid metabolism and energy homeostasis. Nutrients 12: 1299, 2020.
16. Bishop CA, Schulze MB, Klaus S and Weitkunat K: The branched-chain amino acids valine and leucine have differential effects on hepatic lipid metabolism. FASEB J 34: 9727-9739, 2020.
17. Chaudhry ZZ, Morris DL, Moss DR, Sims EK, Chiong Y, Kono T and Evans-Molina C: Streptozotocin is equally diabetogenic whether administered to fed or fasted mice. Lab Anim 47: 257-265, 2013.
18. Jiao J, Han SF, Zhang W, Xu JY, Tong X, Yin XB, Yuan LX and Qin LQ: Chronic leucine supplementation improves lipid metabolism in C57BL/6J mice fed with a high-fat/cholesterol diet. Food Nutr Res 60: 31304, 2016.
19. Wang T, Fu X, Chen Q, Patra JK, Wang D, Wang Z and Gai Z: Arachidonic acid metabolism and kidney inflammation. Int J Mol Sci 20: 3683, 2019.
20. Sears B and Perry M: The role of fatty acids in insulin resistance. Lipids Health Dis 14: 121, 2015.
21. Brun PJ, Yang KJZ, Lee SA, Yuen JJ and Blaner WS: Retinoids: Potent regulators of metabolism. Biofactors 39: 151-163, 2013.
22. Tentolouris N, Argyrakopoulou G and Katsilambros N: Perturbed autonomic nervous system function in metabolic syndrome. Neuromolecular Med 10: 169-178, 2008.
23. Vergès B: Pathophysiology of diabetic dyslipidaemia: Where are we? Diabetologia 58: 886-899, 2015.
24. Rivera ME, Lyon ES, Johnson MA and Vaughan RA: Leucine increases mitochondrial metabolism and lipid content without altering insulin signaling in myotubes. Biochimie 168: 124-133, 2020.
25. Fu L, Li F, Bruckbauer A, Cao Q, Cui X, Wu R, Shi H, Xue B and Zemel MB: Interaction between leucine and phosphodiesterase 5 inhibition in modulating insulin sensitivity and lipid metabolism. Diabetes Metab Syndr Obes 8: 227-239, 2015.
26. Holowaty MNH, Lees MJ, Abou Sawan S, Paulussen KJM, Jäger R, Purpura M, Paluska SA, Burd NA, Hodson N and Moore DR: Leucine ingestion promotes mTOR translocation to the periphery and enhances total and peripheral RPS6 phosphorylation in human skeletal muscle. Amino Acids 55: 253-261, 2023.
27. Azzout-Marniche D: New insight into the understanding of muscle glycolysis: Sestrins, key pivotal proteins integrating glucose and leucine to control mTOR activation. J Nutr 153: 915-916, 2023.
28. Cui C, Wu C, Wang J, Zheng X, Ma Z, Zhu P, Guan W, Zhang S and Chen F: Leucine supplementation during late gestation globally alters placental metabolism and nutrient transport via modulation of the PI3K/AKT/mTOR signaling pathway in sows. Food Funct 13: 2083-2097, 2022.

29. Wang MM, Guo HX, Huang YY, Liu WB, Wang X, Xiao K, Xiong W, Hua HK, Li XF and Jiang GZ: Dietary leucine supplementation improves muscle fiber growth and development by activating AMPK/Sirt1 pathway in blunt snout bream (megalobrama amblycephala). Aquac Nutr 2022: 7285851, 2022.
30. Martin SB, Reiche WS, Fifelski NA, Schultz AJ, Stanford SJ, Martin AA, Nack DL, Radlwimmer B, Boyer MP and Ananieva EA: Leucine and branched-chain amino acid metabolism contribute to the growth of bone sarcomas by regulating AMPK and mTORC1 signaling. Biochem J 477: 1579-1599, 2020.
31. Chen X, Xiang L, Jia G, Liu G, Zhao H and Huang Z: Leucine regulates slow-twitch muscle fibers expression and mitochondrial function by Sirt1/AMPK signaling in porcine skeletal muscle satellite cells. Anim Sci J 90: 255-263, 2019.
32. Herzig S and Shaw RJ: AMPK: Guardian of metabolism and mitochondrial homeostasis. Nat Rev Mol Cell Biol 19: 121-135, 2018.
33. Yun SY, Rim JH, Kang H, Lee SG and Lim JB: Associations of LDL cholesterol, non-HDL cholesterol, and apolipoprotein B with cardiovascular disease occurrence in adults: Korean genome and epidemiology study. Ann Lab Med 43: 237-243, 2023.
34. Lynch CJ and Adams SH: Branched-chain amino acids in metabolic signalling and insulin resistance. Nat Rev Endocrinol 10: 723-736, 2014.
35. McCormack SE, Shaham O, McCarthy MA, Deik AA, Wang TJ, Gerszten RE, Clish CB, Mootha VK, Grinspoon SK and Fleischman A: Circulating branched-chain amino acid concentrations are associated with obesity and future insulin resistance in children and adolescents. Pediatr Obes 8: 52-61, 2013.