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Effects of glutamate on fatty acid profiles and lipid dynamics of fat tissues in Shaziling pig

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Abstract

The fat deposition and lipid composition directly influence the meat quality and feed efficiency during pork production. Glutamate (Glu) is a major component of proteins and has been widely utilized in livestock production. However, the role of Glu in regulating lipid deposition and lipo-nutritional quality of porcine fat remains unclear. This study aimed to investigate the effects of Glu on fatty acid composition and lipid profiles in subcutaneous fat (SF) and perirenal fat (PF) from Shaziling pigs. Forty-eight finishing pigs aged 150 days (31.56 ± 0.95 kg) were divided into the control (Con) group and the Glu-supplemented group (1% Glu), each consisting of 6 pens (4 pigs per pen). After 51 days, 6 pigs (1 pig/pen) from each group were slaughtered for analysis. Fatty acid analysis detected 46 species in SF and 40 in PF. In SF, 1% Glu significantly increased the content of C18:3n3 (P<0.05), which was accompanied by an increase in n3 PUFA deposition (P<0.05) and a decreased n6/n3 ratio (P=0.06). In PF, Glu supplementation reduced the levels of C18:1n9t, C24:1, C22:6n3, and others (P<0.05). The content of monounsaturated fatty acids (MUFAs) and n9 unsaturated fatty acids (UFAs) was significantly decreased in Glu group (P<0.05). Similarly, Glu significantly reduced the n6/n3 PUFA ratio in PF (P<0.05). Lipidomics profiling identified 2264 unique lipids in fat tissues. Glu had minimal effects on lipid composition in SF but significantly reduced ceramides (Cer), phosphatidylserine (PS), and phosphatidylinositol (PIP) contents in PF (P<0.05) compared to the Con group. Additionally, Glu influenced the acyl chain saturation degree, fatty acyl chain length, and individual acyl chain composition in glycerophospholipid (GP) pools of PF. These results demonstrate the regulatory role of Glu on the lipid dynamics in porcine fat and provide insights into regulating fat deposition and liponutritional quality in indigenous Chinese pig breeds.

Keywords: amino acid, pig, adipose tissue, fatty acid, lipidomics

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1. Introduction

China is one of the largest producers and consumers of pork globally, with pig farming as the backbone of domestic animal husbandry (Li et al. 2022). Economic development has driven the increase in the consumption of high-quality pork products in China (Yu and Jensen 2022). The content and composition of fatty acids significantly influence the meat products' edible value and nutritional quality (Zhang et al. 2019, Yi et al. 2023). A previous study found that the fat from fat-type pigs exhibited a lower n-3 / n-6 polyunsaturated fatty acid (PUFA) ratio, with the proportion of unsaturated fatty acid (UFA) ranging from 42% to 65%. These characteristics suggest promising applications of fat from fat-type pigs in the production of functional foods (Chernukha et al. 2023). A recent study has revealed that lard derived from subcutaneous fat (SF) contains higher levels of phosphatidylserine (PS) and offers superior health benefits as a dietary fat compared to lard from perirenal fat (PF) (Zhou et al. 2024). Chinese indigenous pig breeds are renowned for their higher intramuscular fat (IMF) content, better taste, and nutritional value of pork. However, these breeds typically have a slower growth rate and higher fat percentage (Zhang et al. 2022). Therefore, how to regulate backfat thickness and lipid composition is crucial for enhancing the productivity and sustainability of the high-quality pork industry.

Dietary nutrient supplementation effectively regulates backfat thickness and fat percentage in pigs. Supplementation with glycine or betaine has been shown to significantly reduce the average backfat thickness of finishing Huan Jiang mini-pigs (Zhong et al. 2021). Dietary supplemented with 500 mg/kg of hyodeoxycholic acid (HDCA) enhanced lipolysis and reduced backfat thickness in finishing pigs (Hu et al. 2024). Similarly, a low dietary n-6 / n-3 PUFA ratio decreased total triglyceride and total cholesterol contents in SF of Heigai pigs (Nong et al. 2020). Conjugated linoleic acid (CLA) treatment markedly reduced fat deposition both *in vivo* and *in vitro* and altered lipid profiles in the *longissimus dorsi* (LD) muscle and serum of Heigai pigs (Wang et al. 2022, Wang et al. 2023). Additionally, dietary supplementation with 1% Glu and 1% arginine (Arg) significantly reduced backfat thickness and saturated fatty

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acids (SFA) content in the backfat of growing-finishing pigs (Hu et al. 2017). Our previous study found that dietary supplementation with 1% leucine (Leu) reduced the ceramide (Cer) content in the fat tissue of Shaziling pigs (Zhang et al. 2024). Additionally, dietary Glu supplementation improved carcass traits, growth performance and IMF contents while decreasing fat percentage in Shaziling pigs (Zheng et al. 2024). However, it remains unclear whether Glu regualtes fatty acids profile and lipids composition of porcine fats.

In this study, we aimed to investigate the effects of Glu supplementation on fatty acids and lipid composition in SF and PF of Shaziling pigs. Our findings may serve as a reference for developing nutritional strategies to improve the nutritional quality of fat in Shaziling pigs.

2.Meterial and Methods

2.1 Animals preparation and sample collection

The experiment was approved by the Zhejiang University Animal Care and Committee (ZJU20170466). A total of 48 150-day-old Shaziling pigs were randomly divided into two groups (control group and glutamate group), with each group has 6 replicate pens, with 4 pigs per pen. The control (Con) group was fed with a basal diet (corn-soybean meal type diet), and the glutamate (Glu) group was fed with basal diet + 1% Glu (L-Glu). The supplementation dosage was based on our previous studies (Zhang et al. 2024, Zheng et al. 2024, Huang et al. 2025). The Glu levels in Con and Glu-supplemented diets were 2.42 % and 3.25 % respectively, as reported in our earlier work (Zheng et al. 2024). The basal diet meets the nutritional requirements of pigs and the detailed composition is shown in Supplementary Table1. The trial period was a total of 51 days, and free food and water intake. At the end of the experiment, 6 pigs (1 pig per pen) were randomly selected from each group, and slaughtered after 12 hours of fasting. The SF and PF were quickly collected and used for subsequent experiments.

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2.2. Detection of carcass traits

After removing the head, legs, tail, and viscera, and retaining the suet and kidneys of the left half carcass, the left side of the carcass was weighed and then dissected into skeletal muscle and fat. The fat mass was weighed, and recorded to calculate the fat percentage. Backfat thickness was determined by the average of the backfat thickness at the back edge of the left shoulder blade, the last rib, and the lumbar-sacral junction.

2.3 Hematoxylin-eosin staining

The paraffin section and H&E staining were performed according to our previous study (Liu et al. 2025). The sample was fixed with polyformaldehyde fixative for more than 24 hours, embedded, and made paraffin sections. After dewaxing and rehydrating, the sections were added into a hematoxylin staining solution for 3-5 minutes and rinsed with tap water. Treating with hematoxylin differentiation solution for a few seconds, the sections used the blue returning solution to turn blue and rinsed with tap water. After sequentially placing in 85% and 95% alcohol and dehydrating for 5 minutes, the sections were stained with an eosin staining solution for 5 minutes. The sections were dehydrated in anhydrous ethanol and xylene sequentially and sealed with neutral gum. The sections were examined under an upright microscope. Adipocyte area was measured by imaging J (v 1.43), and 3 randomly selected fields of view on each slide were analyzed for cell size.

2.4 Fatty acid composition analysis

The fatty acid composition in adipose tissues was determined by Shanghai Applied Protein Technology Company and the main steps were as follows. Using 51 fatty acid methyl ester mixed standard solutions and n-hexane, working standard solutions with ten different mixed standard concentration gradients were prepared. Sample metabolites were extracted by using a chloroform-methanol mixed solution with a volume ratio of 2:1. Mass spectrometry analysis used the Thermo Trace

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1300/TSQ 9000 gas mass spectrometry. MSD Chemstation software was used to extract the chromatographic peak area and retention time. The standard curve was drawn and the content of fatty acid in the sample was calculated.

2.5 Untargeted lipidomics analysis

The untargeted lipidomics in fat tissue was provided by Shanghai Applied Protein Technology Company and the analysis was based on our previously published paper(Wang et al. 2022). To evaluate the system's stability and data reliability, a pooled QC sample was created by combining the separate samples taken equally from each group. 50 mg fat sample was weighted and lipids were extracted using a 90% isopropanol (Thermo Fisher Scientific, USA) / acetonitrile (Thermo Fisher Scientific, USA) solution. LC-MS/MS analysis was performed using a Q Exactive plus mass spectrometer (Thermo Fisher Scientific, USA) coupled with a UHPLC Nexera LC-30A (SHIMADZU). Positive and negative ion modes were investigated using full-scan spectroscopy with mass-to-charge ratio (m/z) ranges of 200-1800 and 250-1800 respectively. Next, the mass-to-charge ratios of lipid molecules and their fragments were acquired using the following procedure: after each full scan, 10 fragment patterns (MS2 scan, HCD) were recorded. For lipid identification, peak extraction, peak alignment, and quantification, LipidSearch software version 4.1 (Thermo Scientific™) was employed. The extracted ion features were restricted to include only those that exhibited at least 50% nonzero measurement values across at least one experimental group.

2.6 Statistical analysis

The experimental data were initially processed using Microsoft Excel 2019. A two-tailed Student’s t-test was performed with IBM SPSS statistics 20 to compare differences between groups. Data were represented as Mean ± SEM. Statistical significance was determined as follows: $*P < 0.05$, $**P < 0.01$ indicating significant

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differences. Differences were considered with trends at $0.05 \leq P < 0.10$. Data visualization was performed using GraphPad Prism (version 9.0.0) and R (version 4.4.0), with R packages ggplot2 (version 3.5.1) and ggprism (version 1.0.5).

3.Results

3.1 Effect of Glu on fatty acid composition in SF of Shaziling pigs

Dietary supplementation with 1% Glu significantly reduced the fat percentage in Shaziling pigs compared to the Con group (Zheng et al. 2024) (Fig. 1A-B). Consistently, Glu tended to reduce the size of fat cells (Fig. 1C). Cell size analysis showed a significant increase in the ratio of adipocyte with an area of 0-2500 μm^2 ($P<0.05$) and a trend toward a decrease ($P = 0.1$) in the ratio of adipocyte with an area of 7500-10000μm^2 in Glu group compared to Con group (Fig. 1D). To explore the role of Glu in modulating fatty acid composition of porcine fat, targeted fatty acid analysis was performed in SF and PF collected from Glu and Con group. The fatty acids analysis revealed a total of 12 SFAs, 20 MUFAsand 14 PUFAs in porcine SF (Fig.1 E). Among them, C16:0, C18:0, C18:1n9c, C19:1n9t, C18:1n12, C18:2n6 and C18:3n3 were the most abundant. The results showed that dietary Glu did not affect the total content of fatty acids, SFAs, MUFAs, and PUFAs in SF (Fig. 1F-G). Similarly, Glu supplementation did not influence the content of total odd fatty acids (Odd-FAs) (Fig. 1H, trans fatty acids (Trans-FAs) (Fig. 1I), or the MUFA / PUFA ratio (Fig. 1J). However, Glu supplementation significantly increased the content of n3-PUFA ($P<0.05$) compared with the Con group (Fig. 1K), and tended to reduce the n6 / n3 ratio ($P = 0.06$) (Fig. 1L). Analysis of individual fatty acids showed that Glu significantly increased the accumulation of C18:3n3 ($P<0.05$) while decreasing several other fatty acids, including C24:0 ($P<0.01$), C15:1t ($P<0.05$), C14:1 ($P<0.05$), C14:1t ($P<0.05$), C20:5n3 ($P<0.01$), C22:6n3, C15:1 ($P<0.05$), C22:2 ($P< 0.01$), C20:1t ($P<0.01$) and C18:1n7t ($P<0.01$)(Fig. 1M).

3.2 Influence of Glu on the fatty acid composition of PF in Shaziling pigs

We next examined the effects of Glu on the fatty acid composition of PF in

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Shaziling pigs. A total of 8 SFAs, 20 MUFAs, and 12 PUFAs were detected in porcine PF (Fig. 2A). Among them, C16:0, C18:0, C18:1n7t, C18:1n12, C18:1n9c, C18:1n7, and C18:2n6 were the most abundant (Fig. 2A). Although the total fatty acid content was not affected by dietary Glu supplementation, MUFA levels were significantly decreased in the Glu group compared to the Con group (P<0.05) (Fig. 2B-C). The contents of odd-FA, trans-FA, and the MUFA /PUFA ratio were not significantly influenced by Glu supplementation (Fig. 2D-F). Within the UFA pool, Glu supplementation significantly decreased the content of n-9 UFA (Fig. 2G) and reduced the n6 / n3 ratio (Fig. 2H). Additionally, we identified 8 fatty acids species that were reduced in PF by Glu treatment, including the medium-chain fatty acids C10:0 and several long-chain fatty acids: C18:1n9t, C18:1n12t, C20:1, C20:1t, C24:1, C22:6n3 and C22:5n6 (P<0.05) (Fig. 2I).

3.3 Glu affected the lipid composition in SF of Shaziling pigs

To further investigate the effects of Glu on lipo-nutritional quality, we analyzed lipid composition of SF from Glu and control pigs using mass spectrometry-based lipidomic analysis. The lipomics data showed that Glu had no substantial effect on total lipid content (Fig. 3A). However, the orthogonal projections to latent structure discriminant analysis (OPLS-DA) plot indicated a clear separation between Glu and Con groups (Fig. S1A). Quantitative lipid analysis identified a total of 2264 lipid molecules in 43 lipid subclasses, primarily including 777 triglycerides (TGs), 391 diglycerides (DGs), 168 phosphatidylcholines (PCs), 147 phosphatidylethanolamines (PEs), and 136 Cers (Fig. 3B). Lipid subclasses composition analysis revealed that TGs were the predominant lipid subclass in both groups, accounting for 95.766 % in the Con group and 96.039 % in the Glu group (Fig. S1B-C). The dynamic distribution of lipid content between the two groups showed that the most abundant lipid molecule was TG (16:0/18:1/18:1) in both groups, while the least abundant lipid molecule was DG (8:0e/22:2) in the Con group and PC (20:3/14:1) in the Glu group (Fig. S1D). We further analyzed the composition of lipid subclasses in the SF of Shaziling pigs. A total of 43 lipid subclasses were categorized into 5 groups: glycerolipids (GLs), serol lipids (SEs), fatty acyls (FAs), sphingolipids (SLs), and glycerophospholipids (GPs)

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(Figure 3C-G). Although there was no significant difference in lipid groups between the Con and Glu groups, the lipid subclasses of lysophosphatidylinositol (LPIs) and lysophosphatidylcholines (LPCs) trended to decrease in the Glu group (Fig. 3H-J).

3.4 Impact of Glu on the lipid composition of PF in Shaziling pigs

We further investigate the effect of Glu on the lipid composition of PF in Shaziling pigs. The OPLS-DA plot showed a clear separation between the Glu and Con groups (Fig. S2A). However, Glu had no significant effect on total lipid content (Figure 4A). Quantitative lipid analysis identified a total of 2382 lipid molecules across 40 lipid subclasses, including 787 TGs, 394 DGs, 169 Cers, 150 PEs, and 92 hexosyl-1-ceramides (Hex1Cers) (Fig. 4B). Analysis of lipid subclasses composition revealed that TGs were the predominant lipid subclass in both groups, accounting for 97.399 % in the Con group and 97.585 % in the Glu group (Fig. S2B-C). Dynamic distribution analysis of lipid content showed that the most abundant lipid molecule in both groups was TG (16:0/18:1/18:1). Meanwhile, the least abundant lipid molecule was PC (36:2) in the Con group and LPC (17:0) in the Glu group (Fig. S2D). Next, we explored the effects of Glu on lipid subclass composition in PF. A total of 40 lipid subclass were classified into 5 categories including GLs, SEs, FAs, SLs, and GPs (Fig. 4C-G). Dietary Glu significantly decreased the content of total FAs compared to the Con group ($P< 0.05$) (Fig. 4G). Additionally, Glu had no significant effect on the contents of lipids in GL pool (Fig4. H) while inducing significant reductions in Cers ($P< 0.05$) in the SL pool (Fig. 4I), and PS, PIP in the GP pool ($P<0.05$) (Fig. 4J).

3.5 Glu induced depot-specific changes in the molecular composition of lipids in fat tissues of Shaziling pigs.

To further investigate the role of Glu in regulating the properties of PS, PIP, and Cer in porcine fats, we analyzed the individual acyl chain composition of these lipids. In the PS pool, Glu significantly decreased the content of C11:0 ($P<0.05$) in SF (Fig. 5A) while C18:0, C18:1, C19:0, C20:3, C40:5, C40:6 and C42:4 ($P<0.05$) (Fig. 5B) in PF, accompanied by decreased SFA and PUFA contents (Fig. 5C). For PIP pool, Glu did not affect the fatty acyl chain composition in SF (Fig. 5D), but it significantly reduced the contents of C54:5 and C54:6 in PF ($P<0.05$) (Fig. 5E). The saturation

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level of PIP was unaffected by Glu treatment in both SF and PF (Fig. 5F). In the Cer pool, Glu increased the contents of d36:2 in SF and d41:8 in PF (P<0.05), while reducing d43:5 in SF and d15:1 in PF (P<0.05) (Fig. 5G-H). Additionally, Glu significantly reduced SFA and PUFA contents in the Cer pool of PF (P< 0.05) (Fig. 5I). The heatmap of the top 30 differential lipid molecules in SF and PF revealed depot-specific effects induced by Glu supplementation (Fig. 5J-K). Glu broadly reduced GLs and increased GPs in SF, while reducing GPs and increasing GLs in PF.

3.6 Specific changes in fatty acyl chain composition of porcine fat tissues induced by Glu supplementation.

Next, we analyzed the individual fatty acyl chain composition associated with GLs, GPs and SLs (Fig. S3, Fig. 6 and Fig. S4) in the SF and PF of Shaziling pigs. In SF, compared to the Con group, Glu supplementation broadly increased lipid molecules with 0 to 1 double bonds in the GL pool (Fig. S3A), lipid molecules with 3 to 5 double bonds in the GP pool (Fig. 6A) and lipid molecules with 0, 5 and 7 double bonds in SL pool (Fig. S4A). Lipid molecules with 4 to 6 double bonds in the GL pool were decreased (Fig. S3A). Despite these changes, Glu had no significant effect on the total double bond composition in the GL, GP, and SL pools (Fig. S3B, Fig. 6B, and Fig. S4B). In PF, Glu had minimal effects on the double bond composition of individual lipid species in the GL and GP pools (Fig. S3A, Fig. 6A). However, Glu increased SLs with 0 double bonds and decreased SLs with 4 double bonds (Fig. S4A). For total double bond composition, Glu significantly reduced lipids with 3 double bonds in GP pool (P<0.05) (Fig. 6C). Next, we explored the influence of Glu on the length of acyl chains (Fig. 6D-F, Fig. S3 D-F, and Fig. S4 D-F). In SF, Glu induced a notable reduction in lipid molecules containing 25-50 carbon atoms in the GP pool. However, the total content did not reach statistical differences (P > 0.05) (Fig. 6D -6E). In PF, Glu induced a notable reduction in lipid molecules containing 30-45 carbon atoms in the GP pool (Fig. 6D). GLU significantly reduced the total contents of lipid molecules containing 32 and 38 carbon atoms (P < 0.05) in the GP pool (Fig. 6F).

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4.Discussion

Shaziling pig is characterized by high IMF content, superior meat quality, but a high fat percentage (Song et al. 2022). These characteristics underscore the need to evaluate the lean carcass percentage, fattening effect, reproductive performance, and other important traits (Liu et al. 2021). Nutritional strategies are effective for regulating fat percentage in pigs and improving fatty acid composition (Nong et al. 2020, Wang et al. 2022, Liu et al. 2023, Wang et al. 2023, Zhang et al. 2024), which directly influence the edible value and nutritional quality of meat (Zhang et al. 2019, Zhou et al. 2024). As a significant reservoir of lipids, the lipo-nutritional quality of porcine fat has become a growing concern. Glu, abundant in muscle, has been shown to promote muscle growth when supplemented exogenously or produced endogenously (Hou and Wu 2018). Our previous study demonstrated that dietary Glu supplementation significantly improved growth performance and IMF contents, while reducing the half-carcass fat percentage of Shaziling pigs (Zheng et al. 2024). However, the effect of Glu on the lipid profile of porcine fat remains unclear. In this study, we revealed the regulatory roles of Glu in enhancing n3 PUFA accumulation and reconfiguring the lipid profile in the fat tissues of Shaziling pigs. Furthermore, we clarified the depots-specific characteristics of porcine fat tissues in response to dietary Glu supplementation.

Nutritional strategies effectively remodel the fatty acid profile in the skeletal muscle and fat tissues of pigs. For example, dietary supplementation with 1% CLA induced SFA accumulation and MUFA reduction in the SF of Heigai pigs (Wang et al. 2023). Similarly, supplementation with 1% Glu and 1% Arg significantly reduced total SFA content in the SF of Duroc×Landrace×Yorkshire (DLY) growing-finishing pigs (Hu et al. 2017). In contrast, 1% Leu promoted the accumulation of SFA, MUFA, and PUFA in the LD muscle without affecting their contents in the SF and PF of Shaziling pigs (Zhang et al. 2024). In the current study, we observed that, in parallel with a reduced fat percentage, 1% Glu significantly decreased MUFA content in PF without altering SFA and UFA contents in the SF of Shaziling pigs. However, Glu demonstrated a superior ability to regulate the position of the first unsaturated bond in

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PUFA molecules. Specifically, Glu increased n3 PUFA in SF and decreased n9 PUFA content in PF. Additionally, Glu reduced the n3 / n6 ratio in both SF and PF. Both n3 and n6 PUFAs are crucial in promoting human health and reducing disease risk. Numerous studies have highlighted the health benefits of n3 PUFAs in mitigating cardiovascular disease, diabetes, cancer, and various mental diseases(Shahidi and Ambigaipalan 2018). Likewise, n6 PUFA is essential for preventing heart disease (Wang 2018). The n6/n3 PUFA ratio is particularly important in regulating lipid metabolism and deposition, ultimately influencing the composition of fatty acids (Duan et al. 2014). Research has demonstrated that a higher n3 PUFA content and a lower n6/n3 PUFA ratio are more beneficial for human health (Mariamenatu and Abdu 2021). For instance, Nong et al. reported that dietary supplementation with a lower n6/3 PUFA ratio significantly increased n3 PUFA content and reduced the n6/n3 PUFA ratio in the backfat of Heigai pigs (Nong et al. 2020). Our results showed that Glu tended to reduce the n6/n3 PUFA ratio in fat tissues, suggesting that Glu might enhance the nutritional quality of fat in Shaziling pigs.

Nutritional strategies play an important role in regulating lipid dynamics in porcine meats. In this study, we found that Glu supplementation tended to decrease the contents of LPG and LPI in SF, while significantly reducing Cer, PS, and PIP levels in PF compared to the Con group. As an important factor influencing meat nutritional quality, more research focuses on how dietary strategies regulate lipid composition in meat. For example, supplementation with 1% CLA significantly altered the content of TGs, LPGs, phosphatidic acid (PAs), and phytosphingosine (phSMs) in the LD muscle, while decreasing serum Cer level of Heigai pigs (Wang et al. 2022). Similarly, 5% *Bacillus subtilis* and *Enterococcus faecium* co-fermented feed increased the levels of PC and phosphatidylethanolamine (PE) in the LD muscle of finishing pigs (Liu et al. 2024), and an 8% mulberry leaves diet in Yuxi black pigs caused significant accumulation of TGs, Cers, while reducing PC and PS level in the LD muscle (Hou et al. 2024). In our previous study, we found that 1% Leu supplementation led to a reduction in PEs, cardiolipins (CLs), and phosphatidylglycerols (PGs) in the LD muscle, and in lysophosphatidylethanolamines

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(LPEs), Cers, and phosphatidylinositols (PIs) in adipose tissue of Shaziling pigs (Zhang et al. 2024). These findings suggested that lipid classes in GPs, SLs, and GLs are more sensitive to nutrients-induced remodeling in both muscle and fat tissues.

In the backfat of Glu-supplemented Shaziling pigs, we observed a reduction in the level of LPC and LPI. LPCs are major components of oxidized low-density lipoprotein (oxLDL), which is involved in the development of atherosclerosis and inflammatory factors (Liu et al. 2020). LPC has also been identified as a potential biomarker for obesity, given its association with chronic low-grade inflammation (Rasouli et al. 2009, Zhu et al. 2014, Bellot et al. 2023). LPIs act as ligands for GPR55 and are implicated in conditions such as obesity and cancer (Alhouayek et al. 2018). The reduction of LPC and LPI levels through Glu supplementation could therefore have beneficial effects on lipid metabolism and inflammation. Furthermore, Cers in porcine fat tissues exhibited a strong response to dietary amino acids supplementation. Sphingolipids are ubiquitous building blocks of cell membranes that have key functions in membrane structure, cellular signaling and mitochondrial function (Maceyka and Spiegel 2014, Boyd et al. 2023). As the central molecule of sphingolipid metabolism, Cer regulates a diverse range of cellular processes that are important in immunity, chronic inflammation and inflammatory disorders (Maceyka and Spiegel 2014). Elevated Cer levels are characteristic of obesity (Huang et al. 2024). Cers in adipose tissues influence energy metabolism and nutrient regulation, and recent studies have shown that promoting Cer catabolism can reduce atherogenesis and inflammation (Chaurasia et al. 2016, Zhang et al. 2019). The decrease in Cer content in PF of Glu pigs suggested potential health benefits for the pigs and consumers.

Fat tissues, primarily divided into visceral adipose tissue (VAT) and subcutaneous adipose tissue (SAT), are highly flexible and heterogeneous organs with distinct metabolic properties (Nahmgoong et al. 2022, Sakers et al. 2022, Zhou et al. 2022). As the major component of fat tissue, the lipid profile plays a critical role in determining the nutrition quality of fat (Zhou et al. 2024). Recent studies have confirmed that fat tissues exhibit remarkable adaptability to dietary changes (Sárvári

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et al. 2021, Nilaweera and Cotter 2023). However, the effects of nutritional strategies on depot-specific alteration in lipid composition in porcine fat have not been thoroughly explored. Our previous study found that 1% Leu supplementation led to a significant reduction in lipid classes in PF but not in SF. Similarly, the current study demonstrated that lipid dynamics were more active in PF following Glu supplementation than in SF. Our previous research also showed that short-term cold exposure induced broad changes in fatty acid species in visceral fat compared to subcutaneous fat (Zhou et al. 2022). This evidence suggests that porcine visceral fat is not merely an inert energy storage tissue. Instead, it is highly active in lipid metabolism and capable of lipid remodeling in response to dietary and environmental changes. Additionally, we observed that after Glu treatment, the lipids that were differentially elevated in SF were mostly GP, while those that were differentially reduced were predominantly GL. In contrast, the opposite trend was observed in PF. We hypothesize that this could be due to the stronger glycerophospholipid metabolic activity in SF (Zhou et al. 2024). Recent studies showed that acyl chains in lipids exhibit diverse structures, which influence the elongation, desaturation, and transport of fatty acids (Vanni et al. 2019, Ho et al. 2022). In this study, we found that Glu specifically decreased the GP species containing three double bonds, 32 and 38 carbon atoms in PF. However, further research is needed to determine how this alteration affects the nutritional quality of fat.

5.Conclusion

In conclusion, this study reveals the effects of dietary Glu supplementation on fatty acids and lipid dynamics of fat tissue in Shaziling pigs. In SF, Glu significantly increased the deposition of n3 PUFA (P<0.05), especially C18:3n3 (P<0.05). In PF, Glu significantly decreased the content of MUFAs and n9 UFAB(P<0.05) with a tendency to decrease the n6/n3 PUFA ratio (P=0.06), and significantly decreased C18:1n9t, C24:1 and C22:6n3 content (P<0.05). The lipidomic analysis showed that Glu significantly reduced Cer, PS, and PIP content (P<0.05), and altered the acyl chain saturation, length, and composition within GP pools in PF. These results suggest that Glu supplementation regualtes lipid metabolism and alters the fatty acids and

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lipid composition in fat tissues of Shaziling pigs.

Author Contributions

Yanbing Zhou: data curation, investigation, methodology, visualization, writing-review & editing; Yuqing Huang: data curation, investigation, writing-original draft; Xien Xiang : investigation; Liyi Wang: writing-review & editing; Shu Zhang: investigation; Changbing Zheng: methodology; Yehui Duan: methodology; Tenhao Wang: methodology, supervision; Tizhong Shan: conceptualization, funding acquisition, project administration, resources, supervision, writing-review. All authors reviewed the results and approved the final version of the manuscript.

Conflict of Interest Statement

The authors declare no conflict of interest, financial or otherwise.

Ethical Statement

All procedures were reviewed and preapproved by the Zhejiang University Animal Care and Committee (ZJU20240229) and the Institute of Subtropical Agriculture, Chinese Academy of Sciences (ISA-2020-023).

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Reference

Alhouayek, M., J. Masquelier and G. G. Muccioli (2018). "Lysophosphatidylinositols, from cell membrane constituents to GPR55 ligands."Trends in Pharmacological Sciences **39**(6): 586-604.

Bellot, P., M. N. Moia, B. Z. Reis, et al. (2023). "Are phosphatidylcholine and lysophosphatidylcholine body levels potentially reliable biomarkers in obesity? A Review of Human Studies." Molecular Nutrition & Food Research **67**(7): e2200568.

Boyd, R. A., S. Majumder, J. Stiban, et al. (2023). "The heat shock protein Hsp27 controls mitochondrial function by modulating ceramide generation."Cell Reports **42**(9): 113081.

Chaurasia, B., V. A. Kaddai, G. I. Lancaster, et al. (2016). "Adipocyte ceramides regulate subcutaneous adipose browning, inflammation, and metabolism."Cell Metabolism **24**(6): 820-834.

Chernukha, I., E. Kotenkova, V. Pchelkina, et al. (2023). "Pork fat and meat: A balance between consumer expectations and nutrient composition of four pig breeds."Foods **12**(4).

Duan, Y., F. Li, L. Li, et al. (2014). "n-6:n-3 PUFA ratio is involved in regulating lipid metabolism and inflammation in pigs." British Journal of Nutrition **111**(3): 445-451.

Ho, Q. W. C., X. Zheng and Y. Ali (2022). "Ceramide acyl chain length and its relevance to intracellular lipid regulation."International Journal of Molecular Sciences **23**(17).

Hou, J., X. Ji, X. Chu, et al. (2024). "Mulberry leaf dietary supplementation can improve the lipo-nutritional quality of pork and regulate gut microbiota in pigs: A comprehensive multi-omics analysis."Animals **14**(8): 1233.

Hou, Y. and G. Wu (2018). "L-Glutamate nutrition and metabolism in swine."Amino Acids **50**(11): 1497-1510.

Hu, C. J., Q. Y. Jiang, T. Zhang, et al. (2017). "Dietary supplementation with arginine and glutamic acid modifies growth performance, carcass traits, and meat quality in growing-finishing pigs."Journal of Animal Science **95**(6): 2680-2689.

Hu, Y., N. Sang, A. Wu, et al. (2024). "Different types of bile acids exhibit opposite regulatory effects on lipid metabolism in finishing pigs through bile acid receptors."Animal Nutrition 21:25-36.

Huang, Y., K. Sulek, S. E. Stinson, et al. (2024). "Lipid profiling identifies modifiable signatures of cardiometabolic risk in children and adolescents with obesity."Nature Medicine **31**, 294–305.

Huang, Y., L. Wang, S. Zhang, et al. (2025). "Effect of dietary glutamate supplementation on the lipo-nutritional quality of pork in Shaziling pigs."Animal Advances 2: e006.

Li, C., B. Ozturk-Kerimoglu, L. He, et al. (2022). "Advanced lipidomics in the modern meat industry: Quality traceability, processing requirement, and health concerns."Frontiers in Nutrition **9**: 925846.

Liu, F., C. J. Brewster, S. L. Gilmour, et al. (2021). "Relationship between energy intake and growth performance and body composition in pigs selected for low backfat thickness."Journal of Animal Science **99**(12).

https://doi.org/10.1017/anr.2025.10007 Published online by Cambridge University Press

Liu, P., W. Zhu, C. Chen, et al. (2020). "The mechanisms of lysophosphatidylcholine in the development of diseases."Life Science **247**: 117443.
Liu, S., Cai, P.,.You,W., et al. (2025). " Enhancement of gut barrier integrity by a *Bacillus subtilis* secreted metabolite through the GADD45A-Wnt/β-Catenin pathway." *iMeta* e70005.
Liu, S., M. Du, J. Sun, et al. (2024). "Bacillus subtilis and Enterococcus faecium co-fermented feed alters antioxidant capacity, muscle fibre characteristics and lipid profiles of finishing pigs."British Journal of Nutrition **131**(8): 1298-1307.
Liu, S., M. Du, Y. Tu, et al. (2023). "Fermented mixed feed alters growth performance, carcass traits, meat quality and muscle fatty acid and amino acid profiles in finishing pigs."Animal Nutrition **12**: 87-95.
Maceyka, M. and S. Spiegel (2014). "Sphingolipid metabolites in inflammatory disease."Nature **510**(7503): 58-67.
Mariamenatu, A. H. and E. M. Abdu (2021). "Overconsumption of Omega-6 polyunsaturated fatty acids (PUFAs) versus deficiency of Omega-3 PUFAs in modern-day diets: The disturbing factor for their "Balanced Antagonistic Metabolic Functions" in the Human Body."Journal of Lipids **2021**: 8848161.
Nahmgoong, H., Y. G. Jeon, E. S. Park, et al. (2022). "Distinct properties of adipose stem cell subpopulations determine fat depot-specific characteristics."Cell Metabolism **34**(3): 458-472.e456.
Nilaweera, K. N. and P. D. Cotter (2023). "Can dietary proteins selectively reduce either the visceral or subcutaneous adipose tissues?" Obesity Reviews **24**(11): e13613.
Nong, Q., L. Wang, Y. Zhou, et al. (2020). "Low dietary n-6/n-3 PUFA ratio regulates meat quality, reduces triglyceride content, and improves fatty acid composition of meat in Heigai pigs."Animals (Basel) **10**(9).
Rasouli, N., A. Yao-Borengasser, V. Varma, et al. (2009). "Association of scavenger receptors in adipose tissue with insulin resistance in nondiabetic humans." Arteriosclerosis Thrombosis and Vascular Biology **29**(9): 1328-1335.
Sakers, A., M. K. De Siqueira, P. Seale, et al. (2022). "Adipose-tissue plasticity in health and disease."Cell **185**(3): 419-446.
Sárvári, A. K., E. L. Van Hauwaert, L. K. Markussen, et al. (2021). "Plasticity of epididymal adipose tissue in response to diet-induced obesity at single-nucleus resolution."Cell Metabolism **33**(2): 437-453.e435.
Shahidi, F. and P. Ambigaipalan (2018). "Omega-3 polyunsaturated fatty acids and their health benefits." The Annual Review of Food Science and Technology **9**: 345-381.
Song, B., C. Zheng, J. Zheng, et al. (2022). "Comparisons of carcass traits, meat quality, and serum metabolome between Shaziling and Yorkshire pigs."Animal Nutrition **8**(1): 125-134.
Vanni, S., L. Riccardi, G. Palermo, et al. (2019). "Structure and dynamics of the acyl chains in the membrane trafficking and enzymatic processing of lipids." Accounts of Chemical Research Journal **52**(11): 3087-3096.

https://doi.org/10.1017/anr.2025.10007 Published online by Cambridge University Press

Wang, D. D. (2018). "Dietary n-6 polyunsaturated fatty acids and cardiovascular disease: Epidemiologic evidence." Prostaglandins, Leukotrienes and Essential Fatty Acids **135**: 5-9.
Wang, L., S. Zhang, Y. Huang, et al. (2022). "CLA improves the lipo-nutritional quality of pork and regulates the gut microbiota in Heigai pigs."Food Function **13**(23): 12093-12104.
Wang, L., S. Zhang, Y. Huang, et al. (2023). "Conjugated linoleic acids inhibit lipid deposition in subcutaneous adipose tissue and alter lipid profiles in serum of pigs."Journal of Animal Science **101**.
Yi, W., Q. Huang, Y. Wang, et al. (2023). "Lipo-nutritional quality of pork: The lipid composition, regulation, and molecular mechanisms of fatty acid deposition."Animal Nutrition **13**: 373-385.
Yu, W. and J. D. Jensen (2022). "Sustainability implications of rising global pork demand."Animal Frontiers **12**(6): 56-60.
Zhang, J., J. Wang, C. Ma, et al. (2022). "Comparative transcriptomic analysis of mRNAs, miRNAs and lncRNAs in the longissimus dorsi muscles between fat-type and lean-type pigs."Biomolecules **12**(9).
Zhang, L. S., P. Xu, M. Y. Chu, et al. (2019). "Using 1-propanol to significantly enhance the production of valuable odd-chain fatty acids by Rhodococcus opacus PD630." World Journal of Microbiology and Biotechnology **35**(11): 164.
Zhang, S., Y. Huang, C. Zheng, et al. (2024). "Leucine improves the growth performance, carcass traits, and lipid nutritional quality of pork in Shaziling pigs."Meat Science **210**: 109435.
Zhang, X., Y. Zhang, P. Wang, et al. (2019). "Adipocyte hypoxia-inducible factor 2α suppresses atherosclerosis by promoting adipose ceramide catabolism."Cell Metabolism **30**(5): 937-951.e935.
Zheng, C., M. Wan, Q. Guo, et al. (2024). "Glutamate increases the lean percentage and intramuscular fat content and alters gut microbiota in Shaziling pigs."Animal Nutrition **20**:110-119.
Zhong, Y., Z. Yan, B. Song, et al. (2021). "Dietary supplementation with betaine or glycine improves the carcass trait, meat quality and lipid metabolism of finishing mini-pigs."Animal Nutrition **7**(2): 376-383.
Zhou, Y., D. Ling, L. Wang, et al. (2024). "Dietary "beigeing" fat contains more phosphatidylserine and enhances mitochondrial function while counteracting obesity."Research (Wash D C) **7**: 0492.
Zhou, Y., Z. Xu, L. Wang, et al. (2022). "Cold exposure induces depot-specific alterations in fatty acid composition and transcriptional profile in adipose tissues of pigs." Frontiers in endocrinology (Lausanne) **13**: 827523.
Zhu, X., G. Zong, L. Zhu, et al. (2014). "Deletion of class A scavenger receptor deteriorates obesity-induced insulin resistance in adipose tissue."Diabetes **63**(2): 562-577.

https://doi.org/10.1017/anr.2025.10007 Published online by Cambridge University Press

Figure 1 The effect of Glu on the fatty acid composition of the subcutaneous fat in Shaziling pigs.

(A) The backfat thickness of Shaziling pigs, n=6. (B) The fat percentage Shaziling pigs, n=6. (C) Representative section of dorsal subcutaneous fat. (D) Frequency of adipocytes, n=3. (E) Individual fatty acids proportion in SF, n=5, the size of the circle represents the average percentage of fatty acid molecules in the total fatty acids. (F) The content of total fatty acid. (G) The content of fatty acids with different saturations. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. (H) The content of Odd fatty acids. (I) The content of trans fatty acids. (J) The ratio of monounsaturated fatty acids to polyunsaturated fatty acids. (K) The content of n3, n6, n7, n9 unsaturated fatty acids. (L) The ratio of n6 and n3 PUFA. (M) Heat map of differential fatty acids. The $P<0.05$, * indicates a statistically significant difference.

Figure 2 The effect of Glu on the fatty acid composition of the perirenal fat in Shaziling pigs.

(A) Individual fatty acids proportion in PF, n=5, the size of the circle represents the average percentage of fatty acid molecules in the total fatty acids. (B) The content of total fatty acid. (C) The content of fatty acids with different saturations. SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid. (D) The content of Odd fatty acids. (E) The content of trans fatty acids. (F) The ratio of monounsaturated fatty acids to polyunsaturated fatty acids. (G) The content of n3, n6, n7, n9 unsaturated fatty acids. (H) The ratio of n6 and n3 PUFA. (I) Heat map of differential fatty acids. The $P<0.05$, * indicates a statistically significant difference.

Figure 3 The effect of Glu on the lipid composition of the subcutaneous fat in Shaziling pigs.

(A) The content of total lipid. (B) The types and quantities of different lipid molecules.

(C-G) Content of different lipid classes in SF of Shaziling pigs. (H) The content of glycerolipids subclasses. (I) The content of sphingolipids subclasses. (J) The content of glycerophospholipids subclasses. n = 5. Error bars represent SEM. *P < 0.05,

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two-tailed Student's t-test.

Figure 4 The effect of Glu on the lipid composition of the perirenal fat in Shaziling pigs.

(A) The content of total lipid. (B) The types and quantities of different lipid molecules.

(C-G) Content of different lipid classes in PF of Shaziling pigs. (H) The content of glycerolipids subclasses. (I) The content of sphingolipids subclasses. (J) The content of glycerophospholipids subclasses. n = 5. Error bars represent SEM. *P < 0.05, two-tailed Student's t-test.

Figure 5 The effect of Glu on the lipid molecules of the fat tissues in Shaziling pigs.

(A-B) PS acyl chain contents at different saturation levels in SF(A) and PF(B). (C) Log2 foldchange (Glu vs Con) of SFA, MUFA, and PUFA contents in the PS pool. (D-E) PIP acyl chain contents at different saturation levels in SF(D) and PF(E). (F) Log2 foldchange (Glu vs Con) of SFA, MUFA, and PUFA contents in the PIP pool. (G-H) Cer acyl chain contents at different saturation levels in SF(G) and PF(H). (I) Log2 foldchange (Glu vs Con) of SFA, MUFA, and PUFA contents in the Cer pool. (J-K) Heat map of differential lipid species in SF (J) and PF (K) of Shaziling pigs. n = 5. Error bars represent SEM. *P < 0.05, two-tailed Student's t-test.

Figure 6 Glu affected the lipid acyl chain compositions in the GP pool of fat tissues.

(A) GPs with double bond contents. (B-C) Heatmap of acyl chain double bond contents in GP pools from SF(B) and PF(C) in the control and Glu groups. (D) GPs with different numbers of carbon atoms. (E-F) Heatmap of acyl chain carbon atoms a in GP pools from SF(B) and PF(C) in the control and Glu groups. n = 5. Error bars represent SEM. *P < 0.05, two-tailed Student's t-test.

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Figure 1:

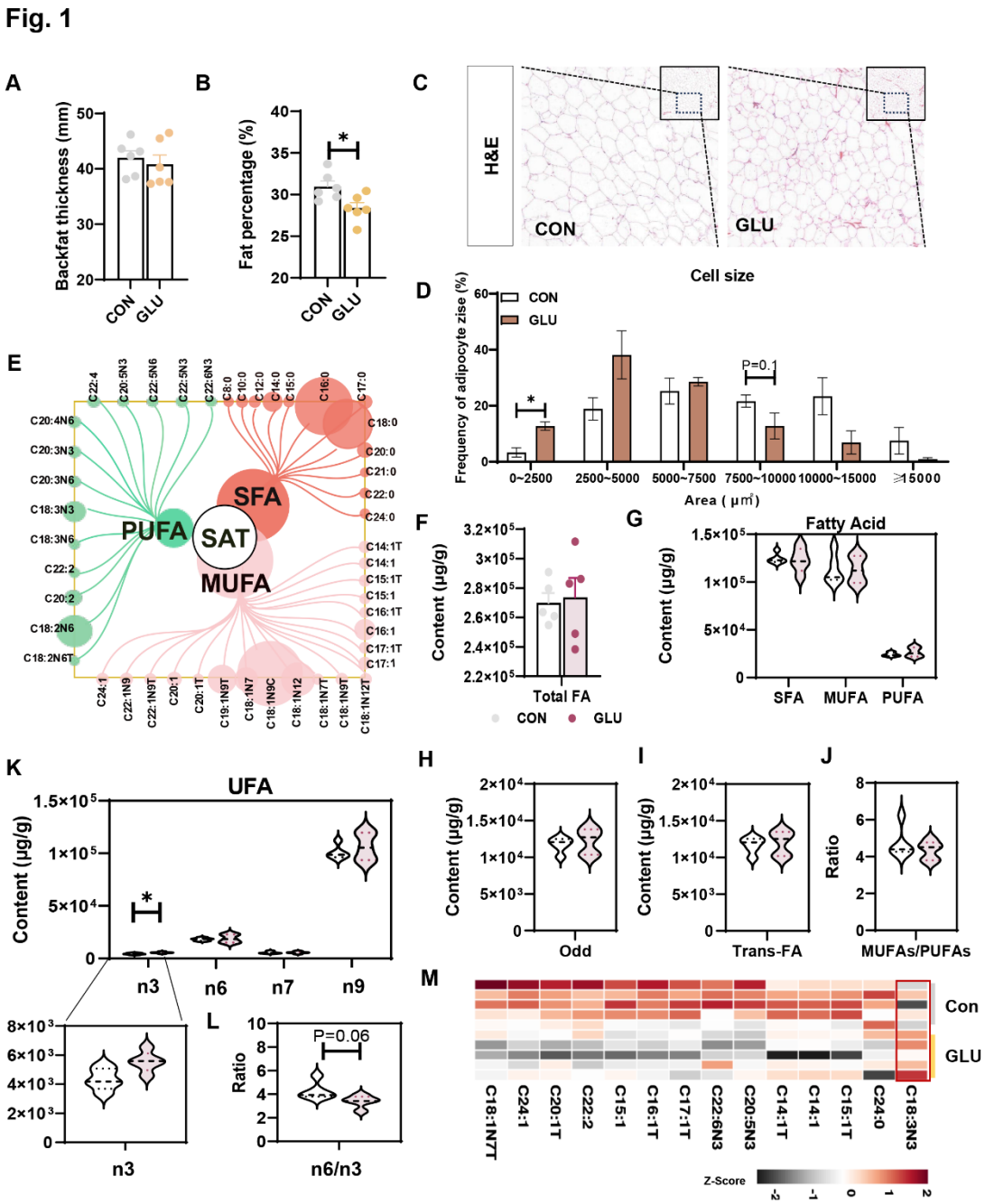

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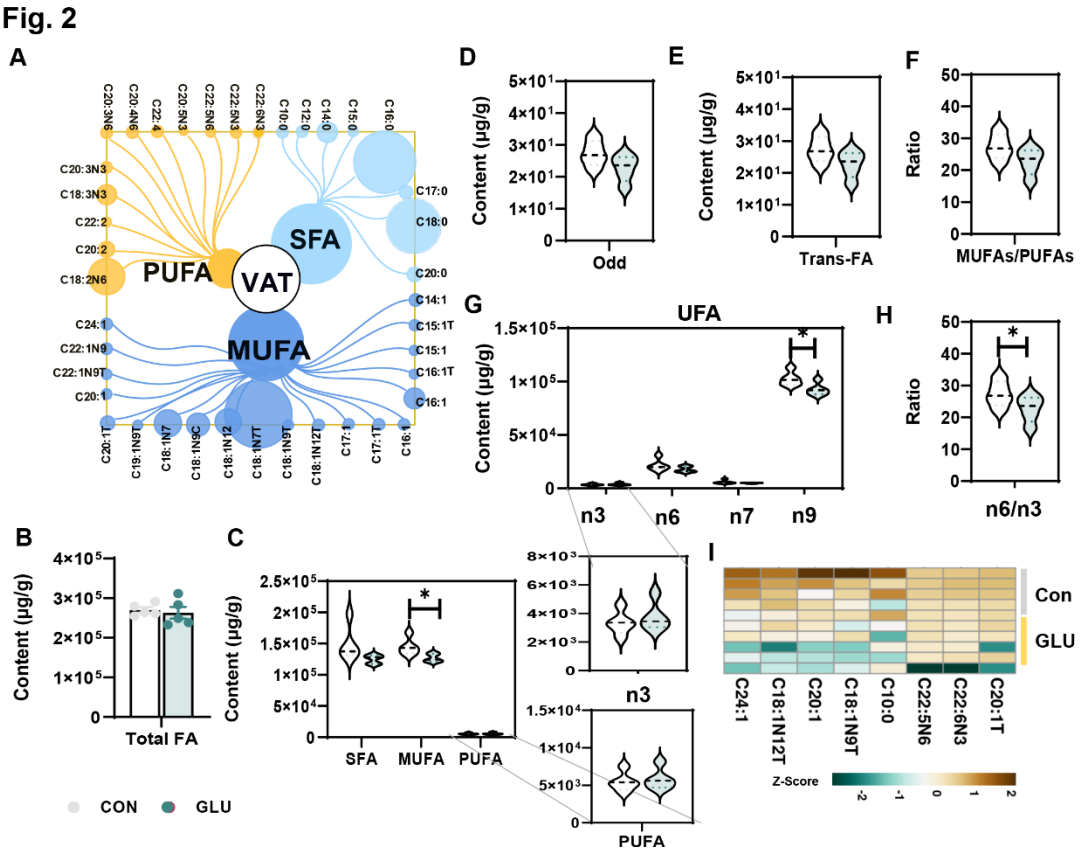
Figure 2:



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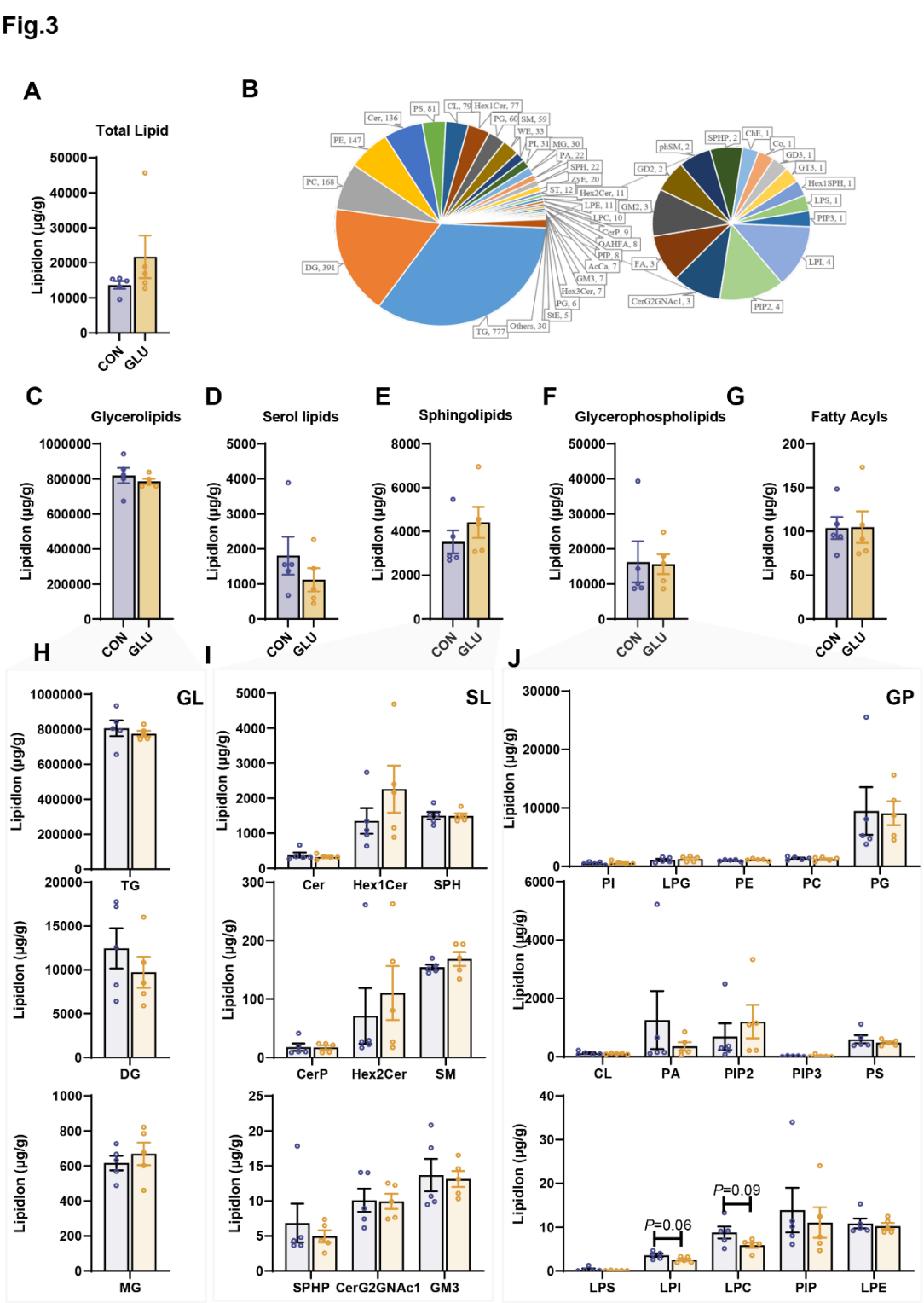

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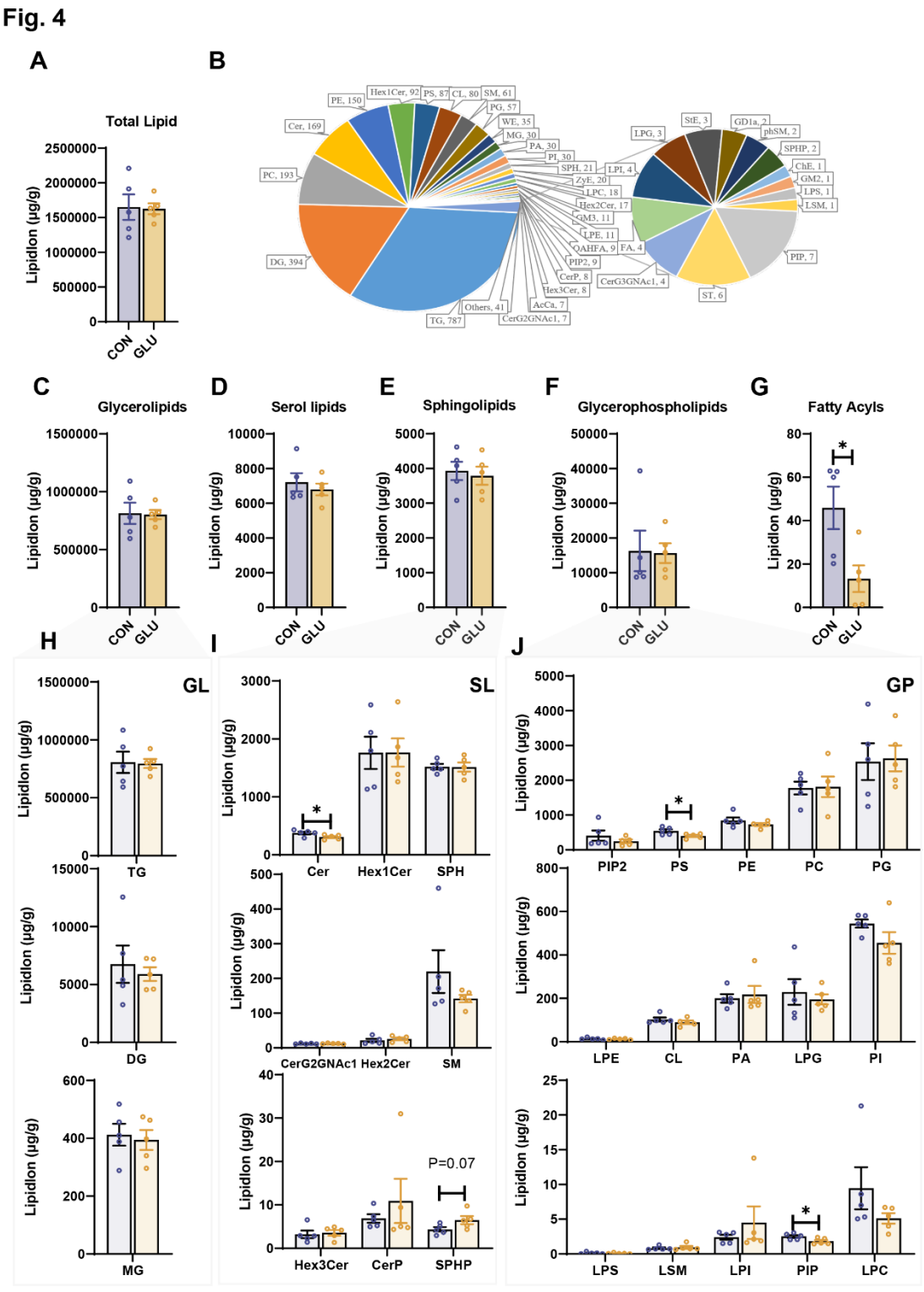

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Figure 5:

Fig. 5

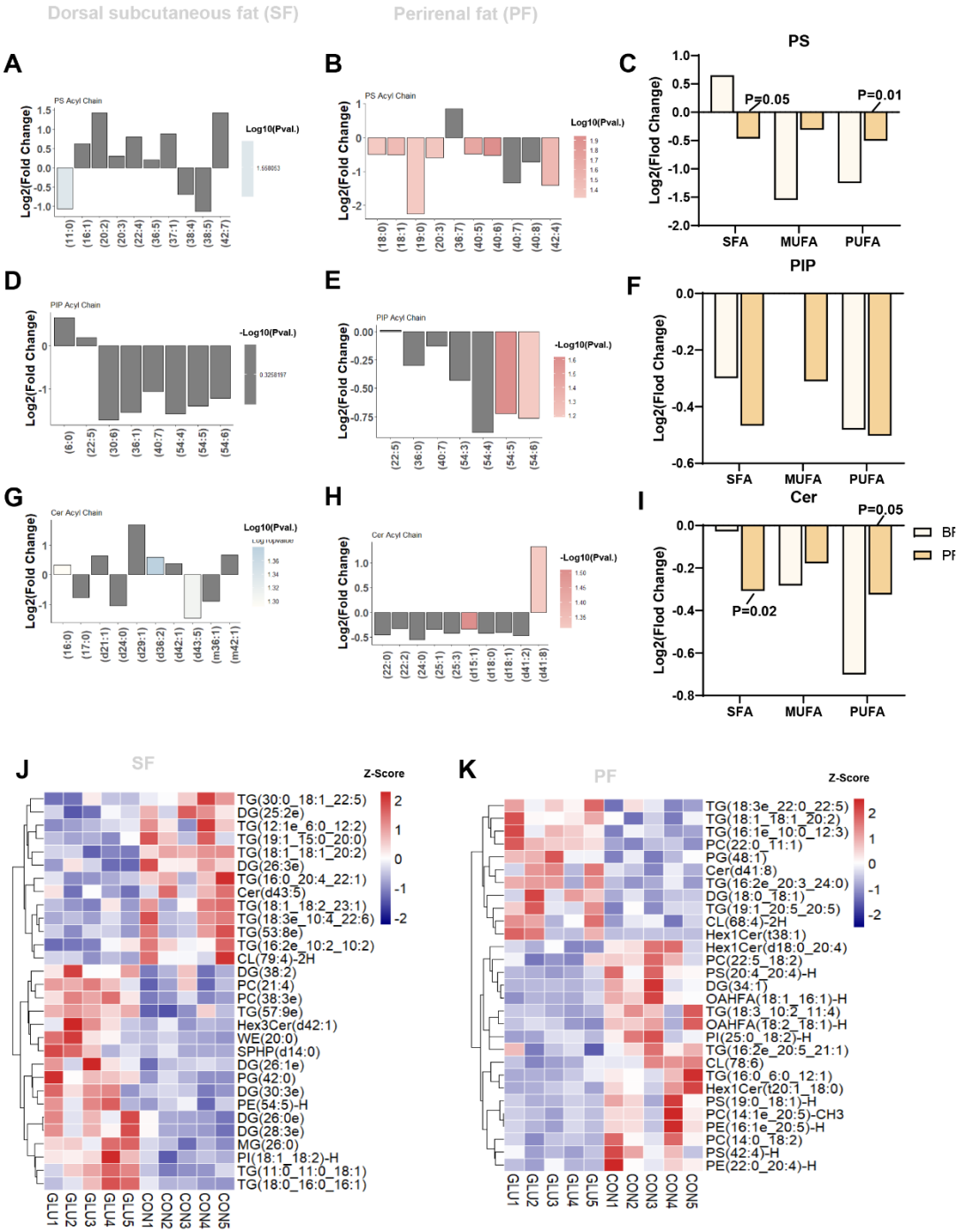

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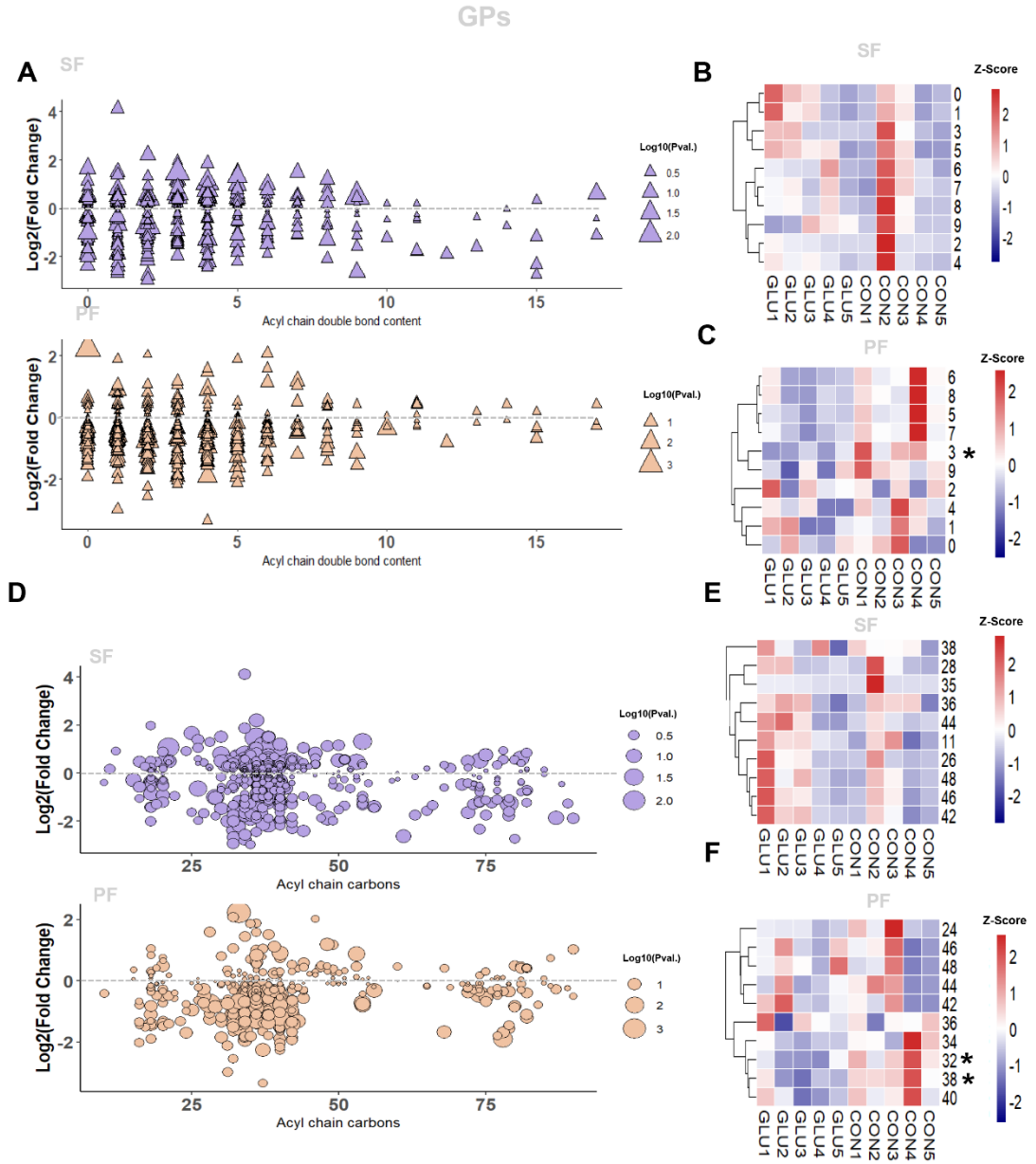
Figure 6:

Fig. 6



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