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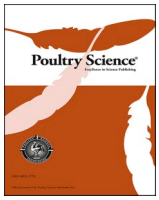

Full-Length Article

Lipidomics reveals lipid changes in the hepatic during the late chick embryonic development

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ABSTRACT

The liver undergoes significant metabolic changes during the late stage of chick embryonic development, particularly in lipid metabolism. Lipids are critical energy sources and structural components for the growth and development of chicken embryos. However, the dynamic changes in hepatic lipid composition during this critical developmental window remain unclear. To investigate the lipid composition changes and underlying mechanisms, we conducted a study on chick embryonic livers at two key developmental time points (E14 and D1). The results showed that the liver in the D1 group exhibited greater lipid droplet accumulation compared to the E14 group. Biochemical analysis revealed significantly elevated levels of triglycerides, total cholesterol, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol in the liver of the D1 group relative to the E14 group. Furthermore, we performed lipidomics analysis and identified 2274 lipid species in chicken liver, which were predominantly composed of glycerolipids and glycerophospholipids. Score plots of principal component analysis and partial least squares discriminant analysis revealed distinct lipid profiles between the E14 and D1 groups, suggesting potential remodeling of liver lipid composition and metabolism during the late stage of chick embryonic development. Meanwhile, a total of 105 differentially abundant lipid species were identified, with 91 significantly upregulated and 14 significantly downregulated in the D1 group compared to the E14 group. Pathway analysis revealed the enrichment of the glycerophospholipid metabolic pathway, which comprises 37 differentially abundant lipids that may play crucial roles in the growth and development of chick embryos during late stages. In summary, this study characterized the liver lipid profile and explored the changes in lipid composition and species of the developing liver, with the aim of identifying the nutritional requirements of chicken embryos in the late stages of development. These findings could be utilized for improving chick quality and broiler performance.

Introduction

In the past few decades, growing consumer awareness and demand for healthy diets have underscored the importance of chicken as a high-quality protein source for human consumption. In broiler production systems, the incubation period represents approximately 33 % of the total productive lifespan, as broilers are typically slaughtered at an average age of 42 days. This demonstrates that the embryonic period is crucial for the entire production cycle, with studies of dynamic changes in chick embryos serving as the fundamental basis. Unlike mammals, avian embryos develop in a closed system, relying solely on nutrients contained within the egg prior to hatching (Tompkins et al., 2023). Glucose and amino acids are the main energy sources for the chick embryo during the early stages of development, which represent the period when the amniotic, allantoic, and yolk sac membranes form (Freeman and Vince, 1974). As these extraembryonic membranes become fully developed, lipids are used as the primary energy substrate for growth and development of chick embryo (Givisiez et al., 2020), accounting for over 90 % of total energy production through oxidative metabolism of yolk lipids. In addition, it has been shown that less than

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10 % of yolk fat is absorbed by the chick embryo on the embryonic day 13 (**E13**), whereas at stages E15-E21, yolk lipids are absorbed rapidly (Wong and Uni, 2021).

The liver, present in all vertebrates, is a vital metabolic organ that governs many functions such as lipid and fatty acid metabolism, glucose metabolism, bile secretion, detoxification, and hematopoietic processes (Berasain et al., 2023; Xiong et al., 2020). The liver is the main site of lipid metabolism in chickens. During the late stage of chick embryo development, yolk-derived lipids are utilized by the liver to provide energy for chicken embryonic growth and development on one hand, and on the other hand, they are converted into cholesterol via cholesterol acyltransferase for storage in the liver (Meng et al., 2021). Therefore, we speculated that the hepatic lipid composition undergoes dynamic changes during chick embryonic development. While numerous studies have employed multi-omics approaches to characterize metabolic and functional transitions in the embryonic liver during late development, including transcriptomics (Cogburn et al., 2018; Hicks et al., 2017), proteomics (Na et al., 2018; Peng et al., 2018), and epigenetic changes (Sun et al., 2023), there remanins a lack of research on lipid composition alteration. A comprehensive profiling of hepatic lipid species during this critical developmental window could provide novel insights and compelling evidence to elucidate the precise lipid requirements during late chick embryonic development.

Lipidomic profiling employing liquid chromatography and mass spectrometry (**LC-MS**) has emerged as a powerful analytical platform for comprehensive detection and precise monitoring of the changes in various arrays of lipid species at the molecular level (Hou et al., 2020). This advanced methodology facilitates systematic investigation of dynamic alterations and functional roles exhibited by distinct lipid families and molecular species across different physiological states and various biological processes. Such capability holds significant implications for revealing the underlying mechanisms regulating relevant biological activities (Cao et al., 2024; Zhang et al., 2021). With advances in detection technology, lipid sequencing analysis has become well-established in liver research. However, current applications primarily focus on elucidating pathogenesis, diagnosis, and advancing drug development for various liver diseases, including fatty liver disease, hepatocellular carcinomas, and cirrhosis (Hall et al., 2021; Hove et al., 2020; Wei et al., 2023). In addition, numerous studies have employed lipidomic approaches to characterize dynamic changes in organ lipid profile during growth and development (Cao et al., 2024; Karnati et al., 2018; Yu et al., 2020). These investigations have identified the key lipid and metabolism pathways associated with organ development and functional maturation. Nevertheless, the comprehensive landscape of hepatic lipid dynamics during chick embryo development remains poorly understood.

In this study, we performed lipidomic profiling on liver tissue from E14 and post-hatch day 1 (**D1**) to comprehensively characterize the lipidome. At E14, the liver completes its structural development and becomes the metabolic center of the chick embryo. D1 is the time when the hatching process ends in the chick embryo. Additionally, we analyzed the differences in lipid composition and molecular species between E14 and D1 to gain a more detailed understanding of the change in lipid metabolism in the liver during chick embryonic development. This study fills the information gap regarding comprehensive alterations in hepatic lipid profile during the late stage of chick embryonic development. These findings may help determine the nutritional requirements during specific development stages of chick embryos, ultimately improve hatchability and chick quality.

Materials and methods

Animal ethic

The experimental animals followed the ethical guidelines for research in animal science and protocols approved by the Animal Care and Use Committee of Northwest A&F University (Permit Number: NWAFAC 1008).

Animal experiment design and sample collection

The fertilized eggs (Arbor Acres) employed in this study were obtained from Xianyang Dacheng Poultry Co., Ltd. (Xianyang, China). All eggs were sterilized and incubated in an automatic incubator under standard temperature and humidity conditions (Beijing LanTianJiao Electronic Technology Co., Ltd., Beijing, China) and harvested at predetermined time points. Liver tissues were aseptically collected from 3 chick embryos at E14 and D1 stages, respectively. At each time point, part of the liver tissues was snap-frozen in liquid nitrogen and then kept in a −80°C refrigerator for lipidomics and biochemical analyses. Part of the liver tissues was fixed in 4 % paraformaldehyde for paraffin embrdding and subsequent histochemical analysis.

Oil red staining

The lipid accumulation in liver tissues was measured by Oil Red O staining. Briefly, liver tissues were fixed with 4 % paraformaldehyde, paraffin-embedded, and sectioned at 3-5 μm slices. The sections were stained with Oil Red O solution, then observed and photographed under a microscope. The Oil Red O staining was quantitatively analyzed using Image-Pro Plus software (v6.0).

Determination of liver lipid indices

Liver samples were weighed and homogenized with pre-cooled saline or anhydrous ethanol at a ratio of 1:9 (w/v g/mL) using a tissue homogenizer. The homogenate was centrifuged at 1,000 rpm for 10 min at 4°C to collect the supernatant. Then, the supernatant was used to measure the contents of triglyceride (**TG**), cholesterol (**TC**), high-density lipoprotein-cholesterol (**HDL-C**), and low-density lipoprotein-cholesterol (**LDL-C**) using commercial kits (Nanjing Jiancheng Biotechnology Co., Ltd, Nanjing, China) according to the manufacturer's instructions. Protein concentration was determined using a total protein quantitative assay kit (Nanjing Jiancheng Biotechnology Co., Ltd, Nanjing, China). TG, TC, HDL-C and LDL-C levels were expressed by mmol/g of protein.

Lipid extraction

Total lipid extraction was performed as described by Norheim et al. (2021) with slight modifications. Briefly, liver samples (approximately 100 mg) were homogenized, and lipids were extracted with 750 μl of chloroform: methanol (2:1 v/v). The mixture was centrifuged at 12,000 rpm for 5 min, and then the lower phase was collected and evaporated under vacuum. The dried samples were re-dissolved in 200 μl of isopropanol for liquid chromatography-mass spectrometry analysis.

Lipidomic analysis

Lipidomic analysis was performed on liver extracts using high-performance liquid chromatography (**HPLC**) coupled with a 6460 triple quadruple electrospray ionization mass spectrometer (**ESI/MS**). Full-scan spectra of positive ion mode and negative ion were acquired across a mass-to-charge ratio (m/z) range of 150-2000. The m/z values of precursor ions and their corresponding fragments were collected using a data-dependent acquisition method: after each full scan, 10 fragment patterns were collected via higher-energy collisional dissociation. Lipid identification (secondary identification), retention time, peak intensity, and peak alignment were processed using the LipidSearch software (V4.0; Thermo Fisher Scientific, Waltham, MA, USA). All peak intensities were normalized to the total spectral intensity to enable comparison of data across different orders of magnitude. Partial Least Squares Discriminant Analysis (**PLS-DA**) was applied using R package

models (http://www.r-project.org/) to initially visualize inter-group differences. Lipid species with variable significance in the projection (**VIP**) of > 1, |fold change (**FC**)| > 2 and *P*-values ≤ 0.05 were identified as statistically significant. MetaboAnalyst 5.0 (https://www.metaboanalyst.ca/) was used for the pathway enrichment analysis of the differential lipid species.

RNA extraction and RT-PCR

Total RNA was extracted from liver tissues using TRIzol reagent according to the manufacturer's instruction. The extracted RNA was reverse transcribed into cDNA using Evo M-MLV RT Premix, following the manufacturer's instruction. RT-qPCR was carried out on a Roche LightCycel 96 PCR system (F. Hoffmann-La Roche AG, Swiss) with SYBR green. β-actin was used as an internal reference gene. The expression levels of target genes were calculated by $2^{-\Delta\Delta Ct}$ method. The primer sequences are shown in Table S1.

Statistical analysis

All statistical analyses of the lipidomic data described in this work were based on relative abundance. Experiment data were expressed as the mean ± standard error of the mean (SEM). Unpaired two-tailed Student's t-tests was used to evaluate the difference between the two groups, and data normality was confirmed by Shapiro-Wilk tests ($P > 0.05$ for all groups). A *P*-value < 0.05 was considered significant.

Results

Liver lipid analysis

To visually observe the changes in liver lipid content between E14 and D1, we measured liver lipid parameters at these developmental stages. Oil Red O staining showed an excess of red lipid droplets in the D1 group compared to the E14 group (Fig. 1**A and B**), indicating a significant increase in lipid content in the D1 group. In addition, as shown in Fig. 1**C–F**, the concentrations of TG, TC, HDL-C, and LDL-C in D1 liver tissues were significantly higher than those in E14 ($P < 0.05$). These results revealed dynamic changes in hepatic lipid metabolism between E14 and D1.

Liver lipid composition

To comprehensively understand changes in liver lipids during the late developmental stages of chick embryos, the liver lipid profiles of E14 and D1 were analyzed (Fig. 2**A**). A total of 2274 lipids from 39 lipid subclasses were identified and classified into 6 categories, including fatty acids (**FAs**), glycerolipids (**GLs**), glycerophospholipids (**GPs**), prenol lipids (**PRs**), sterol lipids (**STs**), and sphingolipids (**SPs**) (Fig. 2**B**). Among them, GPs represented the largest category with 992 lipid species, while the TG subclass had the highest number of lipid species with 382 species, followed by phosphatidylcholine (**PC**, 307 species), phosphatidylethanolamine (**PE**, 242 species), and diacylglycerol (**DG**, 241 species). Furthermore, the relative abundance of lipid subclasses was analyzed. As illustrated in Fig. 2**C**, PC, TG, and PE were the predominant lipids in embryonic livers at both stages (E14 and D1), accounting for more than 85 % of total hepatic lipids.

Multivariate statistical analysis of lipid in liver

To further compare the lipidomic features and differences in lipids between the two groups, unsupervised PCA was applied to determine significant variations in principal component space between chicken embryo livers from E14 and D1 groups in both positive and negative ion modes (Fig. 3**A and B**). In addition, a supervised PLS-DA was employed for each comparison group. After 7-fold cross-validation, the model evaluation parameters for the positive ion mode were R2Y = 1 and Q2Y = 0.997, while those for the negative ion mode were R2Y = 0.999 and

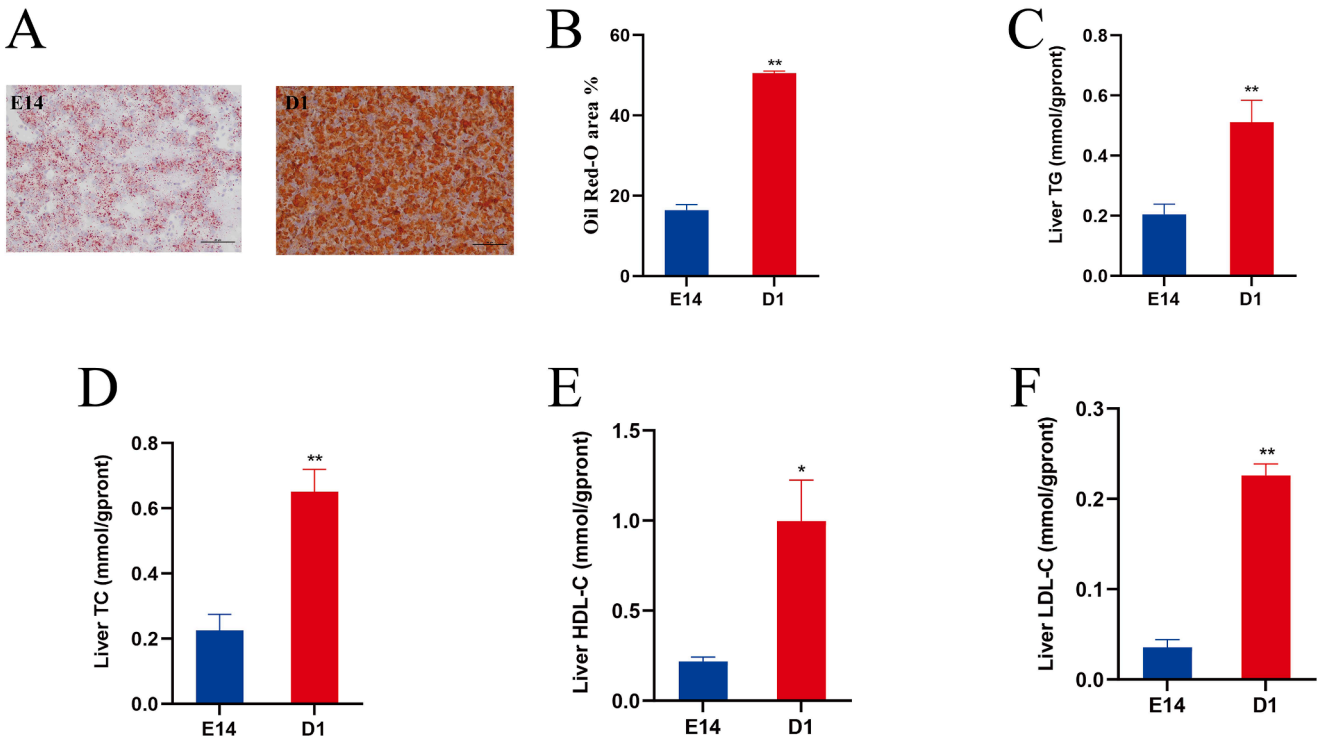

Fig. 1. Liver lipid analysis of liver tissue from the E14 and D1. (A) Observation of Oil red O staining slice of liver. (B) Quantification of Oil red O staining. The concentration of TG (C), TC (D), HDL-C (E), and VDL-C (F) in the liver. TG=Triglyceride, TC=Total Cholesterol, HDL-C=High density lipoprotein cholesterol, VDL-C=Low density lipoprotein cholesterol. Data are presented as means ± SEM ($n = 6$). Compared to E14 group, $^*P < 0.05$, $^{**}P < 0.01$.

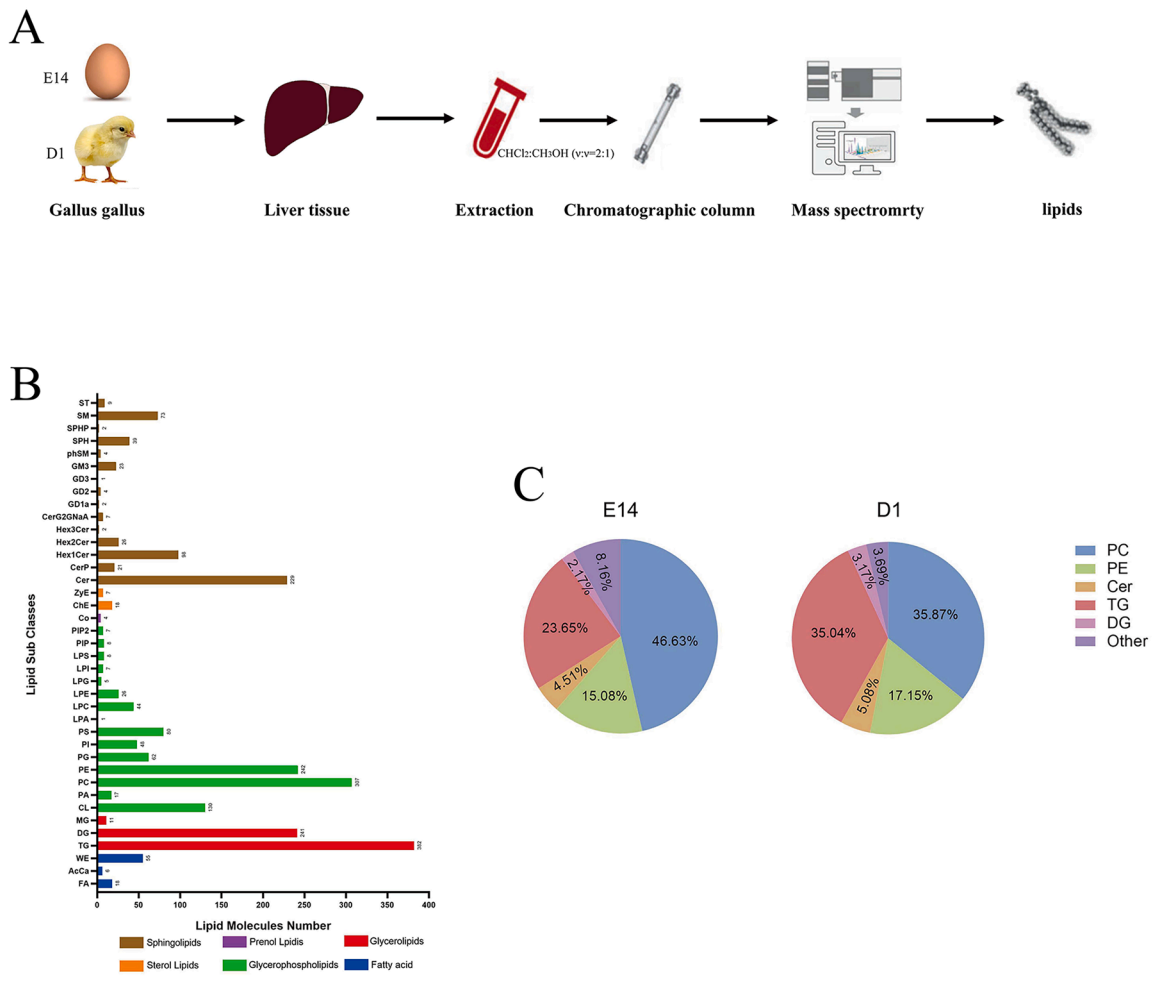

Fig. 2. Overview of lipidomic detection in chicken embryo liver. (A) A schematic summarizing the workflow for lipidomic profiling. (B) The lipid subclasses/categories and the corresponding number of lipid species per category. (C) Percentage of each lipid subclass in the liver from the E14 and D1 groups.

Q2Y = 0.984. The R2 and Q2 values for both ion modes were close to 1, indicating that these modes were stable and reliable (Fig. 3**C and D**). Furthermore, permutation tests were conducted to assess model quality and evaluate potential overfitting. After 200 rounds of permutation and modeling, the R2 and Q2 intercept values for the positive ion mode were 0.77 and −0.12, respectively, while the R2 and Q2 intercept values for the negative ion mode were 0.79 and 0.03, respectively (Fig. 3**E and** 3**F**). In permutation tests, all Q2 values were less than 0.05, confirming the validity of the PLS-DA models for identifying inter-group differences and supporting downstream analyses. Multivariate statistical analysis revealed clear separation between the two groups, indicating distinct lipidome signatures for E14 and D1, respectively.

Changes of lipid subclass profile in liver

Lipid subclasses are essential for the comprehensive exploration of metabolic pathways involved in lipids conversion. Therefore, the relative abundance of lipid subclasses was compared between the E14 and D1 group. The relative abundances of TG, DG, monoglyceride (**MG**), PC, PE, phosphatidylserine (**PS**), phosphatidylglycerol (**PG**), ceramides (**Cer**), and sphingomyelin (**SM**) were significantly higher in D1 compared to E14 ($P < 0.05$) (Fig. 4**A–D**). In contrast, the relative abundances of lysophosphatidylethanolamine (**LPE**) and lysophosphatidylserine (**LPS**) were significantly lower in the D1 group than in the E14 group ($P < 0.05$) (Fig. 4**B and** 4**D**). Correlation analyses between lipid subclasses further validated these metabolic shifts. As depicted in Fig. 4**E**, negative correlations were observed between TG, DG, PC, PE and the metabolites LPE and LPS, whereas metabolites TG, DG, PC, and PE exhibited positive correlations among themselves. A significant positive correlation was also identified between LPE and LPS. These patterns may reflect opposing regulatory roles of phospholipids (e.g., PC, PE) and lysophospholipids (e.g., LPE, LPS) in hepatic development and function during the late stages of chick embryonic development.

Differential lipid molecules identification and metabolic pathway enriched

To identify lipid species with significant changes in the liver during the late stages of chicken embryonic development, metabolites meeting the criteria of VIP > 1, *P*-value < 0.05, and |FC| > 2 were selected as differential lipids. After screening, a total of 105 lipid species exhibiting significant differences were identified in the liver of the two groups (Fig. 5**A and Table S2**). Based on the metabolic profile, compared to the E14 group, the D1 group showed upregulation of 91 lipids, including 15 lipid subclasses, most of which belonged to TG, DG, and Cer subclasses. In contrast, the D1 group displayed downregulation of 14 lipids including 7 lipid subclasses, predominantly from PE and LPE (Fig. 5**B**). VIP scoring was used to identify representative lipids as potential biomarkers for group differentiation. Of these, the top 5 lipid species with the highest VIP scores and significant abundance differences were selected. Among these candidate biomarkers, TG (18:0_18:1_18:1), PC (16:0_20:3), TG (16:0_18:1_18:1), TG (18:1_18:1_18:1), and Cer (d18:1_23:0) were all upregulated in the liver of D1 chicken as compared to E14 chicken embryo (Fig. 5**C**). These results suggested that the differential lipids between E14 and D1 may be associated with rapid

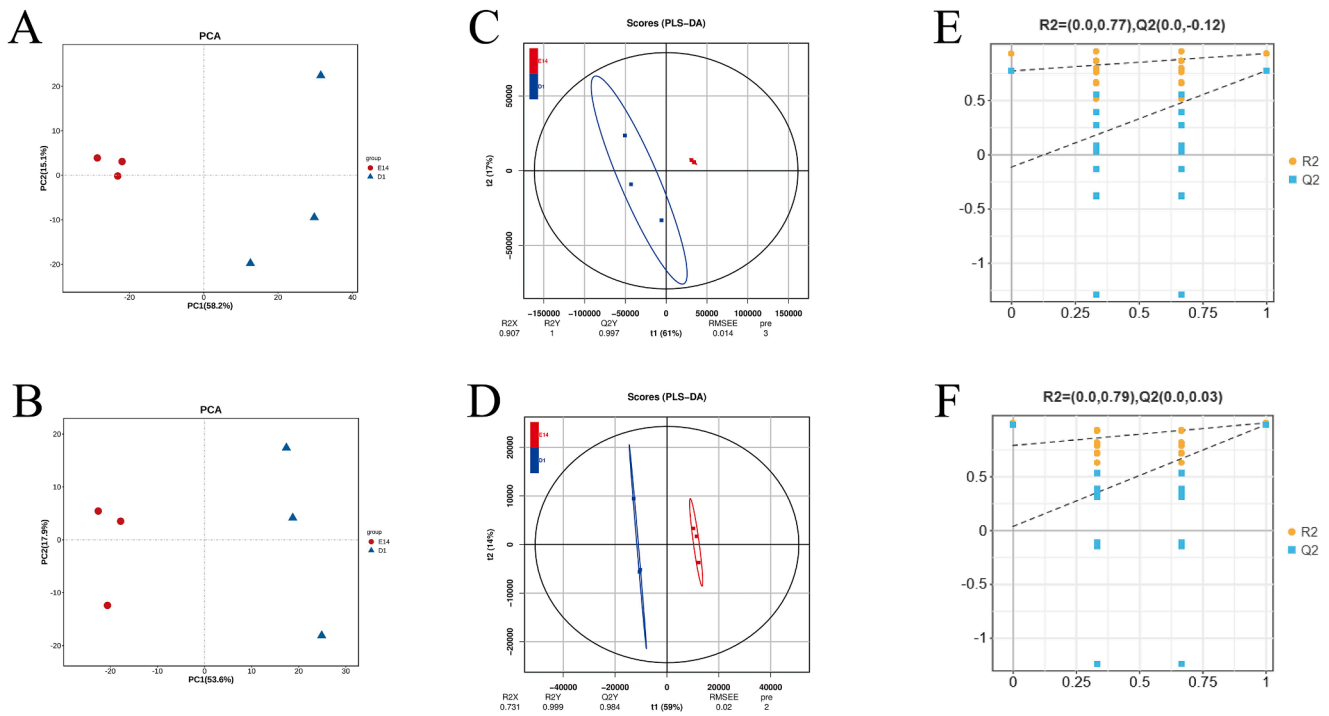

Fig. 3. Lipidome profile analysis of the livers in the chicken embryo. (A) PCA score plot, (C) PLS-DA score plot, and (E) verification of the PLS-DA model based on permutation test in position ion mode. (B) PCA score plot, (D) PLS-DA score plot, and (F) verification of the PLS-DA model based on permutation test in negative ion mode.

metabolism processes during chick embryonic growth and development.

To further elucidate the biological functions of differential lipid species, we performed pathway enrichment analysis by MetaboAnalyst 5.0. As shown in Fig. 5**D**, seven metabolic pathways were enriched in the liver, including glycerophospholipid metabolism, linoleic acid metabolism, α-linolenic acid metabolism, glycerolipid metabolism, glycerophosphatidylinositol-anchor biosynthesis, sphingolipid metabolism, and arachidonic acid metabolism. Among these, glycerophospholipid metabolism with P-value < 0.05 and pathway impact > 0.1 was identified as significantly enriched pathways.

Gene expression dynamics underlying lipid metabolic shift

According to the pathway analysis, glycerophospholipid metabolism was significantly enriched. The expression levels of key genes related to glycerophospholipid metabolism in the liver were shown in Fig. 6. The expression levels of hepatic lysophosphatidylglycerol acyltransferase 1 (***LPGAT1)***, choline/ethanolamine phosphotransferase 1 (***CEPT1)***, and phosphatidylethanolamine cytidyltransferase (***PCYT2***) in the D1 group were significantly higher than those in the E14 group ($P < 0.05$). Conversely, the expression levels of ethanolamine-phosphate phospholyase (***ETNPPL)***, phospholipid phosphatase type 3 (***PLPP3)***, and phosphatidylserine-specific phospholipase A1-alpha (***PLA1A)*** in the livers of the D1 group were significantly lower than those in the E14 group ($P < 0.05$).

Discussion

The liver, a multifunctional organ, orchestrates diverse metabolic and immune-related processes, including lipid metabolism, bile secretion, and detoxification, all of which are essential for the growth and development of the body (Wang et al., 2024). In chickens, the liver is the main site of lipid metabolism. Previous studies have reported a gradual increase in hepatic lipid content during the late chick embryonic development (Liu et al., 2020; Sun et al., 2023), a trend corroborated by our findings, with the D1 group exhibiting significantly higher lipid content than E14. To further elucidate the molecular basis of these lipid dynamics, we performed comprehensive profiling of hepatic lipid molecules during the late chick embryonic development.

Lipidomic analysis is a powerful approach to directly illustrate the interrelationships between mechanism and phenotype (Han and Gross, 2022). To the best of our knowledge, there are no data on developmental changes in lipid species in the chicken liver, which play roles in membranes construction, neutral lipid depots formation, and gene expression regulation. In the current study, we characterized the basal lipid composition of the E14 and D1 chicken liver tissues using a lipidomics approach. Detailed lipid profiles were generated, including 2274 distinct lipid species from 39 major lipid subclasses. In addition to the neutral lipids glycerolipid and sterol lipid, phospholipid and sphingolipid classes were also systematically identified. Multivariate analysis revealed a clear separation between E14 and D1 groups in both PCA and PLS-DA models, demonstrating significant remodeling of hepatic lipid profiles during this developmental transition.

In chicken liver tissue, TG emerged as the most abundant lipid class, with 382 distinct species identified. This finding aligns with a previous lipidomics study of laying hen liver, which also identified TG as the lipid subclass with the highest molecular diversity (You et al., 2023). TG is the major component of lipid droplets, and its conventional function is energy storage in metabolic tissues (Zhang et al., 2024). In the current study, TG levels were significantly higher in the D1 group compared to the E14 group, reflecting normal physiological fat deposition in the chick-embryonic liver The reason for this phenomenon may be that lipids were taken up from the yolk by the yolk sac membrane and then transferred into the liver for growth during the late phase of chicken embryonic development (Cogburn et al., 2003; Feast et al., 1998). The increase in TG likely served to support the enhanced energy demands and biosynthesis activity necessary for chick embryonic growth and development. However, excessive TG also increases the liver burden in newborn chicks, causing lipid peroxidation and increasing the risk of chick death.

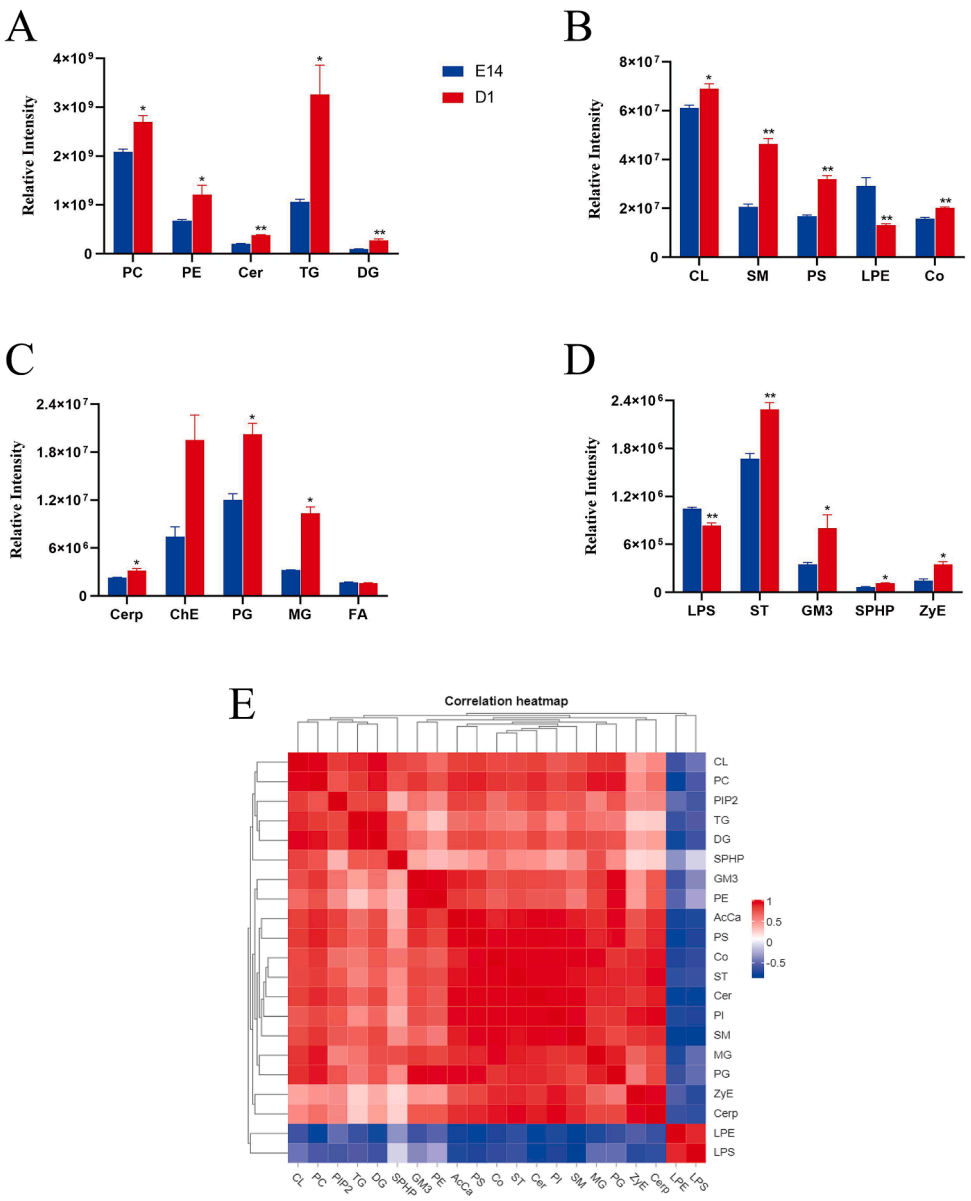

Fig. 4. Lipid subclass analysis of the chicken embryonic liver on E14 and D1. (A-D) Relative mass spectrometry intensity of lipid subclasses of liver tissue. (E) Heatmap of correlation between different metabolite subclasses.

Glycerophospholipids are the main components of cell membrane, including PC, PE, phosphatidylinositol (**PI**), PS, and cardiolipin (**CL**) (Wang and Tontonoz, 2019). They play crucial roles in various biological processes such as cell growth, substance metabolism, information transduction, and molecular recognition (Farooqui et al., 2000; Shevchenko and Simons, 2010). In the present study, PC was the most dominant glycerophospholipid in chicken liver, followed by PE, CL, PS, PG and PI. As the most abundant phospholipid in cell membranes, PC constitutes nearly 50 % of total membrane phospholipids content. It is also known to improve brain function, promote liver repair, exert anti-ageing, and enhance antioxidant capacity (Kim et al., 2019; Li et al., 2022). PE, the second most abundant phospholipid, is not simply a passive membrane constituent but plays critical roles in protein biogenesis and activity, oxidative phosphorylation, oxidative stress, autophagy, and mitochondrial stability (Calzada et al., 2016; Zhao et al., 2022). In addition, it has been reported that PC acts as a mitogen for DNA synthesis induced by growth factors, while PE plays a pivotal role during cell division to ensure the proper progression of cytokines (Gibellini et al., 2010). Lipidomic analysis of liver tissues revealed that the relative abundance of both PC and PE was significantly higher in the D1 group than in the E14 group, further supporting their critical roles in chicken embryonic growth and development. This may result from hepatic cell proliferation during chicken embryogenesis. Our previous study also found a significant increase in the expression levels of genes associated with DNA synthesis and cell division in the liver during the later stages of chick embryo growth and development (Wang et al., 2024), consistent with the results of lipid profile alterations. These findings highlight the importance of PC and PE in the growth and development of chick embryo. However, whether in ovo injection of PC or PE can improve the quality of chick and broiler performance requires further research.

Furthermore, we observed an increase in the relative abundance of CL in the D1 group liver. CL, a specific phospholipid that resides within the mitochondrial inner membrane, is critical for several mitochondrial functions, including mitochondrial respiration, mitochondrial dynamics, and oxidative phosphorylation (Chen et al., 2022). Elevated CL levels may enhance ATP production (Senoo et al., 2020), thereby providing energy for various activities in the chicken embryos during

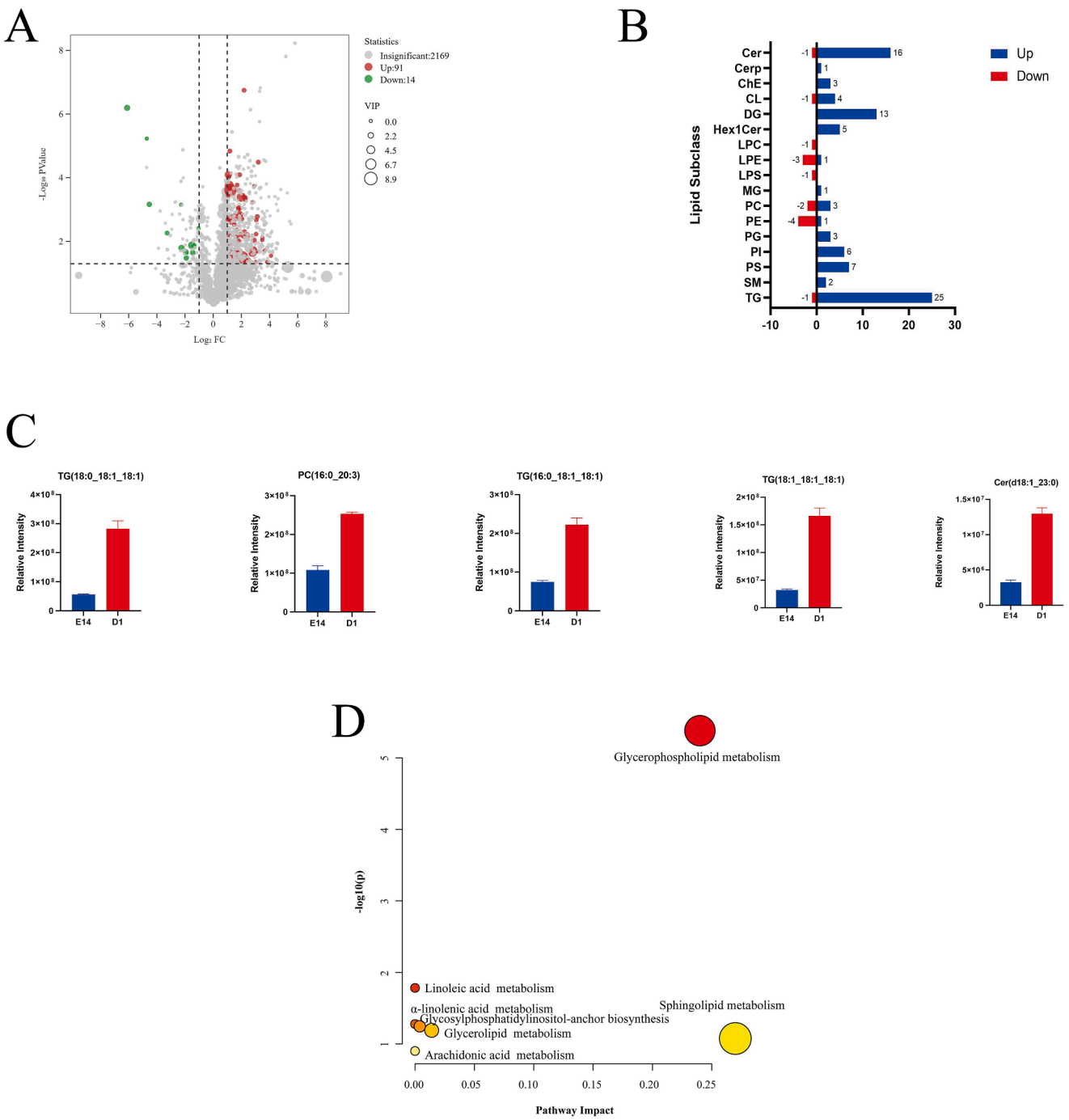

Fig. 5. Lipid species analysis of the chick embryonic liver on E14 and D1. (A) Volcanic map of differential lipid species. (B) The distribution of differential lipid species. (C) Candidate lipid species used as a marker. (D) Pathway analysis of differential lipid species.

the late stage chick embryonic development. In contrast, there were significant reductions in the relative abundance of LPE and LPS in the liver of the D1groups. LPE and LPS are hydrolysis products of PE and PS mediated by phospholipase A1 and phospholipase A2, respectively (Yamamoto et al., 2021), elevated PE and PS levels in D1 likely explain the observed decline in LPE and LPS.

Furthermore, 105 different lipid molecules were identified between E14 and D1, and glycerophospholipid metabolism was identified as the key pathway. PC, PE, PS, LPE, and LPS were mainly involved in glycerophospholipid metabolism, indicating that glycerophospholipid may play an important role in liver development and functional changes during the late chick embryonic development. Then, we selected some key genes in this pathway and validated them by combining with differential lipid subclasses. ETNPPL encodes ethanolamine phosphate phospholyase, which irreversibly converts phosphoethanolamine into ammonia, inorganic phosphate, and acetaldehyde, thus inhibiting the production of PE (Leventoux et al., 2020). PCYT2 and CEPT1 are key enzymes in the production of PE, increased PCYT2 and CEPT1 expression also promotes PC production (Liu et al., 2024; Pavlovic and Bakovic, 2013). PLA1A-encoded phosphatidylserine-specific phospholipase A1 specifically acts on PS to produce LPS (Zhou et al., 2022). In this study, the expression of PCYT2 and CEPT1 was significantly higher in the D1 group than that in the E14 group, while the expression of ETNPPL and PLA1A was significantly lower than that in the E14 group.

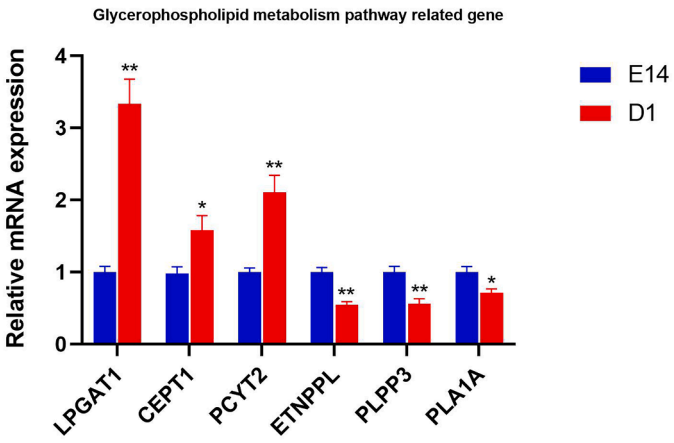

Fig. 6. The expression levels of glycerophospholipid metabolism related genes.

The results were in accordance with the changes in lipid subclasses, i.e. an increase in the relative abundance of PC, PE and PS and a decrease in the relative abundance of LPE and LPS in the D1 groups, These findings not only validate the reliability of the results of lipidomics but also identifie PC, PE, LPE, and LPS as crucial role in .changes of liver functions during the late of chick embryonic development

Conclusion

In summary, this is the first study of lipidomics to describe the lipid characteristics of chicken liver at different embryonic development stages. Lipids serve as important energy sources and structural components during embryonic development. The observed changes in lipid molecules revealed significant differences in lipid composition and metabolic pathways in the liver during the late chick embryonic development, particularly in glycerides and glycerophospholipids. These differentially expressed lipids may play an important role in chick embryo development and could hold potential for regulating liver function. Although the specific roles of different lipids in this process need to be further verified, the present study provides new insights into nutritional requirements in the late chick embryonic development and established a theoretical basis for new strategies to promote chick embryo healthy development and growth.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.psj.2025.105218.

References

Berasain, C., Arechederra, M., Argemí, J., Fernández-Barrena, M.G., Avila, M.A., 2023. Loss of liver function in chronic liver disease: an identity crisis. J. Hepatol. 78 (2), 401–414.
Calzada, E., Onguka, O., Claypool, S.M., 2016. Chapter two-phosphatidylethanolamine metabolism in health and disease. Int. Rev. Cell Mol. Biol. 321, 29–88.
Cao, Z., Xu, M.D., Qi, S.Z., Xu, X.L., Liu, W., Liu, L.Y., Bao, Q., Zhang, Y., Xu, Q., Zhao, W. M., Chen, G.H., 2024. Lipidomics reveals lipid changes in the intramuscular fat of geese at different growth stages. Poult. Sci. 1, 10312.
Chen, L., Dong, J., Liao, S.Y., Wang, S.Y., Wu, Z.D., Zuo, M.L., Liu, B., Yan, C.J., Chen, Y., He, H., Meng, Q.T., Song, Z.Y., 2022. Loss of Sam50 in hepatocytes induces cardiolipin-dependent mitochondrial membrane remodeling to trigger mtDNA release and liver injury. Hepatology 76 (5), 1389–1408.
Cogburn, L.A., Trakooljul, N.., Chen, C., Huang, H., Wu, C.H., Carré, W., Wang, X.F., White 3rd, H.B., 2018. Transcriptional profiling of liver during the critical embryo-to-hatching transition period in the chicken (Gallus gallus). BMC Genomics 19, 695.
Cogburn, L.A., Wang, X.., Carre, W., Rejto, L., Porter, T.E., Aggrey, S.E., Simon, J., 2003. Systems-wide chicken DNA microarrays, gene expression profiling, and discovery of functional gene. Poult. Sci. 82 (6), 939–951.
Farooqui, A.A., Horrocks, L..A., Farooqui, T., 2000. Glycerophospholipids in brain: their metabolism, incorporation into membranes, functions, and involvement in neurological disorders. Chem. Phys. Lipids. 106 (1), 1–29.
Feast, M., Noble, R.C., Speake, B.K., Ferguson, M.W., 1998. The effect of temporary reductions in incubation temperature on growth characteristics and lipid utilization in chick embryo. J. Anat. 193 (pt3), 383–390.
Freeman, B.M., Vince, M.A., 1974. Development of the avain Embryo. Abehavioural and Physiological Study. John Wiley and Sons, New York.
Gibellini, F., Smith, T.K., 2010. The Kennedy pathway–de novo synthesis of phosphatidylethanolamine and phosphatidylcholine. IUBMB Life 62 (6), 414–428.
Givisiez, P.E.N., Moreira Filho, A.L.B., Santos, M.R.B., Oliveira, H.B., Ferket, P.R., Oliveira, C.J.B., Malheiros, R.D., 2020. Chick embryo development: metabolic and morphological basis for in ovo feeding technology. Poult. Sci. 99 (12), 6774–6782.
Hall, Z., Chiarugi, D., Charidemou, E., Leslie, J., Scott, E., Pellegrinet, L., Allison, M., Mocciaro, G., Anstee, Q.M., Evan, G.I., Hoare, M., Vidal-Puig, A., Oakley, F., Vacca, M., Griffin, J.L., 2021. Lipid remodeling in hepatocyte proliferation and hepatocellular carcinoma. Hepatology 73, 1028–1044.
Han, X.L., Gross, R.W., 2022. The foundations and development of lipidomics. J. Lipid. Res. 63, 100164.
Hicks, J.A., Porter, T..E., Liu, H.C., 2017. Identification of microRNAs controlling hepatic mRNA levels for metabolic genes during the metabolic transition from embryonic to posthatch development in the chicken. BMC Genomics 18, 687.
Hou, B.Y., Zhao, Y.., He, P., Xu, C.Y., Ma, P., Lam, S.M., Li, B.W., Gil, V., Shui, G.H., Qiang, G.F., Liew, C.W., Du, G.H., 2020. Targeted lipidomics and transcriptomics profiling reveal the heterogeneity of visceral and subcutaneous white adipose tissue. Life Sci 245, 117352.
Hove, M.T., Pater, L.., Storm, G., Weiskirchen, S., Weiskirchen, R., Lammers, T., Bansal, R., 2020. The hepatic lipidome: from basic science to clinical translation. Adv. Drgu. Deliver. Rev. 189, 180–197.
Karnati, S., Garikapati, V., Liebisch, G., Van Veldhoven, P.P., Spengler, B., Schmitz, G., Baumgart-Vogt, E., 2018. Quantitative lipidomic analysis of mouse lung during postnatal development by electrospray ionization tandem mass spectrometry. PLoS One 13 (9), e0203464.
Kim, S.H., Kim, B..K., Park, S., Park, S.K., 2019. Phosphatidylcholine extends lifespan via DAF-16 and reduces amyloid-beta-induced toxicity in Caenorhabditis elegans. Oxid. Med. Cell Longev. 2019, 2860642.
Leventoux, X., Augustus, M., Azar, S., Riquier, S., Villemin, J.P., Guelfi, S., Falha, L., Bauchet, L., Gozé, C., Ritchie, W., Commes, T., Duffau, H., Rigau, V., Hugnot, J.P., 2020. Transformation foci in IDH1-mutated gliomas show STAT3 phosphorylation and downregulate the metabolic enzyme ETNPPL, a negative regulator of glioma growth. Sci. Rep. 10, 5504.
Li, H.Y., Zou, Y.., Xu, Y., Cai, L., Xie, S.C., Zhu, X.Q., Zheng, W.B., 2022. Lung lipidomic alteration in beagle dogs infected with Toxocara canis. Animals 12 (22), 3080.
Liu, X.G., Chen, Z.., Yan, Y.L., Zandkarimi, F., Nie, L.T., Li, Q.D., Horbath, A., Olszewski, K., Kondiparthi, L., Mao, C., Lee, H., Zhuang, L., Poyurovsky, M., Stockwell, B.R., Chen, J.J., Gan, B.Y., 2024. Proteomic analysis of ferroptosis pathways reveals a role of CEPT1 in suppressing ferroptosis. Protein & Cell 2024, pwae004.
Liu, Y.L., Zhou, J..H., Musa, B.B., Khawar, H., Yang, X., Cao, Y.L., Yang, X.J., 2020. Development changes in hepatic lipid metabolism of chicks during the embryonic periods and the first week of posthatch. Poult. Sci. 99, 1655–1662.
Meng, Y.Q., Qiu, N.., Mine, Y., Keast, R., 2021. Comparative lipidomics of chick yolk sac during the embryogenesis provides insight into understanding the development-related lipid supply. J. Agric. Food Chem. 26, 7647. -7477.
Na, W., Wu, Y.Y., Gong, P.F., Wu, C.Y., Cheng, B.H., Wang, Y.X., Wang, N., Du, Z.Q., Li, H., 2018. Embryonic transcriptome and proteome analyses on hepatic lipid metabolism in chickens divergently selected for abdominal fat content. BMC Genomics 19, 384–401.
Norheim, F., Chella Krishnan, K., Bjellaas, T., Vergnes, L., Pan, C., Parks, B.W., Meng, Y., Lang, J., Ward, J.A., Reue, K., Mehrabian, M., Gundersen, T.E., Péterfy, M., Dalen, K. T., Drevon, C.A., Hui, S.T., Lusis, A.J., Seldin, M.M., 2021. Genetic regulation of liver lipids in a mouse model of insulin resistance and hepatic steatosis. Mol. Syst. Biol. 17 (1), e9684.
Pavlovic, Z., Bakovic, M., 2013. Regulation of phosphatidylethanolamine homeostasis—The critical role of CTP:phosphoethanolamine cytidylyltransferase (Pcyt2). Int. J. Mol. Sci. 14 (2), 2529–2550.
Peng, M.L., Li, S..N., He, Q.N., Zhao, J.L., Li, L.L., Ma, H.T., 2018. Proteomics reveals changes in hepatic protein during chicken embryonic development: an alternative model to study human obesity. BMC Genomics 19, 21.
Senoo, N., Kandasamy, S., Ogunbona, O.B., Baile, M.G., Lu, Y., Claypool, S.M., 2020. Cardiolipin, conformation, and respiratory complex-dependent oligomerization of the major mitochondrial ADP/ATP carrier in yeast. Sci. Adv. 6 (35), eabb0780.

Shevchenko, A., Simons, K., 2010. Lipidomics: coming to grips with lipid diversity. Nat. Rev. Mol. Cell Biol. 11, 593–598.
Sun, X., Wang, Y.M., Wang, C.H., Wang, Y.B., Ren, Z.Z., Yang, X., Yang, X.J., Liu, Y.L., 2023. Genome analysis reveals hepatic transcriptional reprogramming changes mediated by enhancers during chick embryonic development. Poult. Sci. 4, 102516.
Tompkins, Y.G., Liu, G..C., Marshall, B., Sharma, M.K., Kim, W.K., 2023. Effect of hydrogen oxide-induced oxidative stress on bone formation in the early embryonic development stage of chicken. Biomolecules 13, 154.
Wang, B., Tontonoz, P., 2019. Phospholipid remodeling in physiology and disease. Annu. Rev. Physiol. 81, 165–188.
Wang, H.M., Liu, J..Y., Wang, P.H., Shi, L., Liu, Y.L., Yang, X.J., Yang, X., 2024. Single-nucleus transcriptome reveals cell dynamic response of liver during the late chick embryonic development. Poult. Sci. 9, 103979.
Wei, R.X., Ning, R.., Han, C.C., Wei, S.H., Teng, Y.Q., Li, L., Li, H.H., Hu, S.Q., Kang, B., Xu, H.Y., 2023. Lipidomics analysis reveals new insights into the goose fatty liver formation. Poult. Sci. 102, 102428.
Wong, E.A., Uni, Z., 2021. Centennial Review: the chicken yolk sac is a multifunctional organ. Poult Sci 100 (3), 100821.
Xiong, X.L., Kuang, H.., Liu, T.Y., Lin, J.D., 2020. A single-cell perspective of the mammalian liver in health and disease. Hepatology 71, 1467–1473.
Yamamoto, Y., Sakurai, T., Chen, Z., Furukawa, T., Gowda, S..G.B., Wu, Y., Nouso, K., Fujii, Y., Yoshikawa, Y., Chiba, H., Hui, S.P., 2021. Analysis of serum lysophosphatidylethanolamine levels in patients with non-alcoholic fatty liver disease by liquid chromatography-tandem mass spectrometry. Anal. Bioanal. Chem. 413, 245–254.
You, M.H., Zhang, S..B., Shen, Y.M., Zhao, X.H., Chen, L.G., Liu, J.X., Ma, N., 2023. Quantitative lipidomics reveals lipid perturbation in the liver of fatty liver hemorrhagic syndrome in laying hens. Poult. Sci. 2, 102352.
Yu, Q.H., He, Z..S., Zubkov, D., Huang, S.Y., Kurochkin, I., Yang, X.D., Halene, T., Willmitzer, L., Giavalisco, P., Akbarian, S., Khaitovich, P., 2020. Lipidome alterations in human prefrontal cortex during development, aging, and cognitive disorders. Mol. Psychiatry. 25 (11), 2952–2969.
Zhang, H.Y., Wang, J..Y., Zhao, J., Sun, C.Q., Wang, J., Wang, Q., Qu, F., Yun, X.D., Feng, Z.W., 2021. Integrated lipidomic and transcriptomic analysis reveals lipid metabolism in foxtail millet (Setaria italica). Front. Genet. 12, 758003.
Zhang, L., Zhao, J., M.Lam, S., Chen, L., Gao, Y.Z., Wang, W.J., Xu, Y.Y., Tan, T.Y., Yu, H., Zhang, M., Liao, X.F., Wu, M.C., Zhang, T.Y., Huang, J., Li, B., Zhou, Q.D., Shen, N., Lee, H.J., Ye, C.Q., Li, D., Shui, G.H., Zhang, J., 2024. Low-input lipidomics reveals lipid metabolism remodelling during early mammalian embryo development. Nat. Cell Biol. 26 (2), 278–293.
Zhao, Z.J., Zheng, R..Z., Wang, X.J., Li, T.Q., Dong, X.H., Zhao, C.Z., Li, X.Y., 2022. Integrating lipidomics and transcriptomics reveals the crosstalk between oxidative stress and neuroinflammation in central nervous system demyelination. Front. Aging Neurosci. 14, 870957.
Zhou, Y., Chang, M.J., Wang, N., Zhuang, Y., Wang, F., Zhang, Z., Guo, M., Lin, N., Li, J. Z., Wang, Q., 2022. Phosphatidylserine-specific phospholipase A1 limits aggressiveness of lung adenocarcinoma by lysophosphatidylserine and pProtein kinase A-dependent pathway. Am. J. Pathol. 192 (6), 970–983.