Serum, urine and fecal metabolomic characterization of a zinc- deficient experimental rat model

Peng Wang
Harbin Medical University
Zhipeng Liu
Harbin Medical University
Yingfeng Zhang
Harbin Medical University
Maoqing Wang
Harbin Medical University
Ying Li (liying_helen@163.com)
Harbin Medical University

Research Article

Keywords: zinc deficiency1, metabolic disturbance2, metabolomics 3, multi-omics4, nutrition5

Posted Date: February 17th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1358581/v1

Abstract

Background

Zinc serves as a cofactor for over 10% of the human proteome, including transcription factors and enzymes, and maintains protein structure and function. This provides a strong evidence regarding the involvement of zinc in metabolic processes. Zinc deficiency is one of the most common nutrient deficiencies worldwide. Our objective was to comprehensively determine how zinc deficiency alters serum, urine, and fecal metabolic profiles.

Methods

Five-week-old growing Sprague–Dawley rats (n = 30) were housed individually in stainless steel metabolic cages, with ad libitum access to food and water. Isoenergetic and isonitrogenous diets were procured commercially. Serum, urine, and fecal samples were collected from zinc-sufficient (n = 15) and zinc-deficient (n = 15) rats after feeding a diet with an adequate amount of zinc (30 mg/kg) or a zinc-deficient diet (10 mg/kg), respectively, for four weeks. Samples from these three sources were metabolically profiled using ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry.

Results

A total of 33, 18, and 16 differential metabolites were identified in the serum, urine, and feces, respectively, between the zinc-sufficient and zinc-deficient rats ($P < 0.05$). The integrated analysis of altered serum, urine, and fecal metabolites revealed altered amino acid profiles in serum and urine, decreased acylcarnitine levels in serum and urine, increased bile acid excretion in feces, bile acid accumulation in serum, increased lysophospholipidcholine levels, and changes in arachidonic acid catabolites in the serum and feces of zinc-deficient rats.

Conclusions

In summary, our results confirm previous studies that altered metabolism of essential amino acids, glucose, lipid, and bile acid in rats in response to zinc deficiency. For the first time, we reported which improved the effect of zinc deficiency on metabolic homeostasis.

1 Introduction

Metabolic disturbance is the pathological basis of chronic diseases, such as cerebrovascular diseases and diabetes [1], and improving metabolic homeostasis is effective in preventing chronic diseases. Metabolic disturbance is a complex pathophysiological state caused by multiple factors, such as an

imbalance of calorie intake, predominance of a sedentary lifestyle over physical activity, genetics, and gut microbes [2]. However, these factors still cannot fully explain the causes of metabolic disorders, which hinders precise targeted intervention for metabolic disorders. Individuals suffering from metabolic disturbances often experience “hidden hunger,” which implies that individuals may have adequate energy consumption, but suboptimal micronutrient intake [3]. However, the state of “hidden hunger” also contributes to metabolic homeostasis. For example, three trace metals (selenium, vanadium, and chromium) may play crucial roles in controlling blood glucose concentrations, possibly through their insulin-mimetic effects [4]. Micronutrients, such as iron, zinc, and selenium, play a major role in regulating cardiovascular function [5]. This might yield novel biological insights for addressing metabolic disorders, and systematic studies on the effects of each trace element on metabolism, which require immediate investigation.

Zinc is an indispensable trace element for health and plays a critical role in numerous physiological functions. Zinc deficiency leads to growth retardation, delay in sexual development, impairment of neurological immune system, and acrodermatitis enteropathica [6–8]. Accumulating evidence has revealed the potential role of Zn2 + in maintaining metabolic homeostasis. Studies have confirmed the inverse association between zinc intake and the prevalence of obesity, insulin resistance, and metabolic syndrome [9–11]. A genome-wide association study also found that genetic variations in ZnT7/8, two Zn^{2+} transporters, play critical roles in fatty acid accumulation and insulin clearance [12, 13]. However, human genomic research has estimated that over 10% of the proteins in the human body require Zn^{2+} to maintain their structure and function [14]. This provides a strong evidence that zinc deficiency is more likely to be involved in metabolic disorders. Consequently, the existing knowledge regarding zinc deficiency-induced metabolic disturbances is insufficient. In addition, the transcriptomic and proteomic studies of zinc deficiency models also suggest that zinc further be involved in many other metabolic processes [15, 16] ; these gene- and protein-based findings need to be confirmed at the metabolic level. For the above reasons, it is necessary to systematically refine the effects of zinc deficiency on metabolism.

The levels of substances in the body are the result of their pharmacokinetics [17]. Zinc deficiency has pleiotropic effects on substance homeostasis, such as changing dietary patterns and material absorption, impairing substance metabolism, and altering excretion rates. Consequently, a comprehensive analysis of zinc deficiency in intestinal, blood, and urine metabolism would be helpful in revealing the effects of zinc deficiency on metabolic disorders. Metabolomics has been described as an efficient tool for identifying systemic metabolic variations by detecting the metabolic response of living systems to pathophysiological stimuli; it can be applied to explore metabolic changes associated with diseases [18]. Although metabolomics in different tissues and organs have been conducted in zinc-deficient models [19–21], few studies have combined serum, urine, and feces metabolomics to explore metabolic abnormalities caused by zinc deficiency. The current study is based on the use of ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-Q-TOF-MS/MS) for metabolomic analysis. This study aimed to comprehensively elucidate the changes in metabolic

phenotypes due to zinc deficiency, using the UPLC-Q-TOF-MS/MS platform, to detect changes in the serum, urine, and fecal metabolites; further these three metabolomic results were integrated to elucidate the metabolic events associated with zinc deficiency.

2 Materials And Methods

The main research contents included the stable establishment of a zinc-deficient rat model; metabolomic detection for serum, urine, and feces samples; and metabolite annotation in metabolic pathways. The overall research flowchart is presented in Supplementary Figure 1.

2.1 Animal model and samples preparation

Five-week-old growing Sprague–Dawley (110–130 g) rats (n=30) were obtained commercially (Beijing Vital River Laboratory Animal Technology Co., Ltd.). The average weight of the rats on initiation of the experiment was 160.78±10.29 g. All rats were housed individually in stainless steel metabolic cages. To minimize trace metal contamination, silicon plugs and stainless-steel drinking water pipes were used in water bottles. Before the experiment, a seven-day adaptive feeding period with a standard rat diet (AIN-93G, containing 30 mg Zn/kg) was conducted, with ad libitum access to distilled water. The animal room was controlled for temperature (21–23 °C), humidity (53 %), and light (12 h light/dark cycle). The 30 rats, which were numbered from 1 to 30 according to weight, were randomly divided into two groups (n=15/group) at day 8, and then 30 random numbers were read from any row or column in the random number table. All selected random numbers were numbered from small to large. Rats with odd and even numbers were assigned to the normal zinc diet group (NZG) and low-zinc diet group (LZG), respectively. The NZG and LZG were fed a diet containing 30 mg and 10 mg Zn/kg, respectively. The animal diets were obtained commercially from the Beijing KeAoLiXie Animal Food Co., Ltd., China. Simultaneously, the Zn2+ content of the diets was confirmed by mineral analysis using atomic absorption spectroscopy. The normal diet had 30.40±2.86 mg/kg of zinc and the low zinc diet had 9.70±1.50 mg/kg. The contents of minerals, trace elements, and compositions in the diets are detailed in Supplementary Table 5. Both groups were fed distilled water without metal ions. Feed intake was recorded daily, and body weight was measured weekly throughout the course of the study. After being fed the respective diets for four weeks, the animals were transferred to metabolic cages, using a sieve and funnel, to collect urine and feces samples. The 24 h urine and stool samples were collected twice. Stool samples of all the rats were collected and placed in non-ionic Eppendorf tubes. Urine samples were collected in 50 mL ion-free plastic centrifuge tubes (Corning Incorporated) and centrifuged at 3000 rpm for 10 min, and the supernatant was collected. Subsequently, all the rats were fasted for 12 h and euthanized using intraperitoneal injection of 10 % chloral hydrate (0.3 mL/kg body weight). Blood samples were collected from the abdominal aorta and placed at room temperature for 2 h. After centrifugation for 15 min at 3000 rpm, serum was collected. Serum, urine, and fecal samples were stored at -80 ℃ for further biochemical and metabolic profile analysis. The animal study was reviewed and approved by the Animal Protection and Use Committee of the Harbin Medical University.

2.2 Serum biochemical analysis

Serum, urine, and feces Zn^{2+} and serum metallothionein concentrations were used to ascertain zinc levels in the two groups. Samples were prepared by wet digestion, and the levels of serum, urine, and feces Zn^{2+} concentrations were measured using an atomic absorption spectrophotometer. Briefly, $HNO_{3:}HClO_4$ (4:1) solution (Guaranteed reagent, Xilong Scitenfic, China) was added to 200 μL serum, and the mixtures were digested at 100 ℃ until the solutions became colorless. To reduce the acid content, 3 mL deionized water was added to the digestive solution and dried twice with heat. The digested samples were dissolved in 5 mL 5 % HNO_3. The pretreatment methods for feces and urine samples were similar to those used for serum samples. The concentration of metallothionein was determined using enzyme-linked immunosorbent assay kits (Summus Biotechnology Development Co. Ltd.) and a Hitachi 7100 automatic biochemistry analyzer (Hitachi High-Technologies, Shanghai, China). We analyzed the differences between the two groups using independent sample t-tests. Data were considered statistically significant at $P<0.05$.

2.3 Metabolomics analysis of serum, urine, and feces

2.3.1 Sample preparation

To precipitate out protein from serum, 3× volume of methanol was added to the serum (350 mL). After vortexing for 2 min, the mixture was centrifuged at 10,000 rpm for 10 min at 4 °C, and the supernatant was collected. The supernatant was dried by evaporation using N_2 at 37 °C, and the residues were re-dissolved in 350 mL of acetonitrile/water (1:2), vortex-mixed for 2 min, and centrifuged at 14,000 rpm for 10 min at 4 °C. The pretreatment methods for urine samples were similar to those used for serum samples. The fecal metabolite was extracted at a ratio of 1:3 (weight of fresh feces-to-methanol) in methanol (chromatography grade). The samples were homogenized by whirl mixing for 2 min and then centrifuged at 10000 rpm for 10 min at 4 °C. Serum, urine, and fecal samples were filtered through a 0.22 μm ultrafiltration membrane (Millipore) for further removal of contaminants. The supernatants were transferred to Eppendorf tubes and stored at -80 °C.

2.3.2 UPLC–QTOF-MS/MS Analysis

UPLC-Q-TOF-MS/MS analysis was performed using a Waters Acquity UPLC system coupled to a Waters Micromass Q-TOF micro™ mass spectrometer, with electrospray ionization (ESI) in the positive and negative ion modes. Chromatographic (2 μL) separation was performed on an Acquity UPLC™ BEH C18 column (100 mm × 2.17 mm, 1.7 μm, Waters, Milford, MA, USA), maintained at a temperature of 35 °C and a flow rate of 0.35 mL/min. Analytes were eluted using a gradient of mobile phase A—water-formic acid (100: 0.1, v/v) and mobile phase B—acetonitrile. The elution gradient was as follows: 2 % B for 0.5 min, 2–20 % B over 0.5–6.0 min, 20–35 % B over 6.0–7.0 min; 35–70 % B over 7.0–9.0 min; 70–98 % B over 9.0–10.5 min; 98 % B for 2.0 min, and back to 2 % B for 6.0 min.

For mass spectrometry analysis, a Waters Micromass Q-TOF 13 spectrometer was used, with a full scan over 50–1000 m/z in positive and negative ion modes (Waters, Manchester, U.K.), source temperature of 110 °C, and a cone gas flow of 15 L/h. A desolvation gas temperature of 320 °C and gas flow of 650 L/h were used. The capillary voltage was set at 3.0 kV in the ESI+ mode and 2.8 kV in the ESI− mode, and the cone voltage was 35 V. All analyses were performed using a locking spray to ensure accuracy and reproducibility. A lock mass of leucine enkephalin for the ESI+ ([M+H] + = 556.2771) and ESI− modes ([M+H]− = 554.2615) were used *via* a lock spray interface. The lock spray frequency was set at 10 s, and the lock mass data were averaged over ten scans for correction.

UPLC-Q-TOF-MS/MS analysis of urine and feces samples was performed in a manner similar to the serum analysis, with minor modifications.

2.3.3 Data processing

The raw UPLC-TOF-MS/MS data were analyzed using MarkerLynx Application Manager 4.1 (Waters Corporation, Milford, MA, USA). MarkerLynx ApexTrack peak integration was used for peak detection and alignment. The peak parameters (peak width at 5 % height and peak-to-peak baseline noise) were automatically calculated by the system. The collection parameters were set as described in our previous study [22].

The data matrix of samples was imported to SIMCA-P 14.0 software (Umetrics, Umeå, Sweden) for multivariate analysis. Unsupervised principal component analysis was used for all the samples to determine the general separation. Supervised multivariate analyses of orthogonal partial least square discriminant analysis (OPLS-DA) were applied to highlight the maximal different metabolites associated with zinc deficiency.

2.3.4 Identification of differential metabolites

To ensure data availability, only metabolites with less than 20 % missing values were used for the analysis. With respect to the identification of differential metabolites, variables were selected based on a threshold of VIP value (VIP > 1.0) and S-plot from the OPLS-DA model. Simultaneously, these differential metabolites from the OPLS-DA model were validated at the univariate level using Student's t-test, with the critical P-value set to 0.05. Metabolites were identified based on the accurate mass, retention time, and matching MS spectra of the unknowns to the standard model compounds. The following databases were used to support metabolite identification: HMDB (http://www.hmdb.ca/) and METLIN (http://metlin-scripps.edu/). Finally, variables with fold change less than 0.8 and greater than 1.2 were included in the metabolic profile analysis to be considered as biologically significant.

2.3.5 Metabolite profiling

MetaboAnalyst (http://www.metaboanalyst.ca/) was used for the pathway analysis. Kyoto Encyclopedia of Genes and Genomes (https://www.kegg.jp/) and Small Molecule Pathway Database

(http://www.smpdb.ca/) were used for functional analysis and mapping significantly altered metabolites in the metabolic pathway.

2.3.6 Data analysis

The analysis of food intake and weight development was expressed as β (95 % confidence interval). Differences between groups were analyzed using linear mixed models. Data were analyzed using R software (version 4.1.2).

3 Results

3.1 The effects of zinc deficiency on the clinical characteristics of rats

In the current study, LZG rats exhibited slow weight gain with an estimated value of 33.82 (17.89–49.75, P=5.55e^{-5}) and reduced food intake at 12.48 (8.65–16.32, P=5.22e^{-09}). In addition, the total zinc content in the serum and zinc excretion in urine and feces were significantly decreased in LZG rats. The detailed Zn^{2+} concentrations in the serum, urine, and fecal samples are presented in Supplementary Table 1.

3.2 General alterations in the serum, urine, and feces metabolome after zinc deprivation

Based on the principal component analysis and OPLS-DA (Supplementary Figure 2–4) models, we observed significant differences between the LZG and NZG rats. The score plot from the OPLS-DA model identified the metabolites of the three metabolomes that contributed to the dissociation between the two groups under restrictive conditions (VIP >1, fold change < 0.8 and >1.2, P<0.05); 33 significantly altered serum metabolites, 18 differential urine metabolites, and 16 differential fecal metabolites were ultimately identified.

3.3 Metabolic profiling analysis of altered serum metabolites

Serum metabolomics revealed 33 significantly changed metabolites, which could be assigned to the following categories: amino acids, acylcarnitine and glucose metabolites, bile acids, lysophosphatidylcholine (LysoPC), and arachidonic acid catabolites (Figure 2, Supplementary Table 2). Among these, nine amino acids and their catabolites, including essential amino acids (threonine lysine, and methionine) and nonessential amino acids (glutamate, glycine, serine, and tyrosine), and tryptophan hydrolysates of kynurenine and hydroxykynurenine showed significantly lower concentrations in the LZG

rats than in the NZG rats. Conversely, serum saccharopine levels were elevated in the LZG rats. Serum glucose levels were increased in the LZG rats, while levels of glucose metabolites, L-lactic acid, and glycerate 3-phosphate were decreased. Further, four kinds of acylcarnitines were found to decline in the serum. In addition, the levels of four bile acids, seven LysoPC, and delta-12-prostaglandin J2 were increased in the serum. Levels of glycerophosphocholine, a hydrolysate of LysoPC, were reduced in the serum.

3.4 Metabolic profile analysis of altered urine metabolites

Eighteen differential metabolites were detected using urine metabolomics. Our metabolite classification results indicated changes in the urine metabolic profiles of amino acids, acylcarnitines, glucose metabolism-related metabolites, and several organic acids in LZG rats (Figure 3, Supplementary Table 3). Briefly, the urinary excretion rates of two amino acids (glycine and taurine) and amino acid metabolites (quinolinic acid, S-adenosylhomocysteine, homocarnosine, indole pyruvate, and indoxyl sulfate) decreased in the LZG rats. The urinary excretion of glucose was augmented, whereas its downstream metabolites, including acetic acid and alpha ketoglutarate, were decreased. Regarding lipid metabolism, we observed the augmented excretion of three kinds of organic acids related to lipid metabolism and decreased excretion of five acylcarnitines in LZG rats.

3.5 Metabolic profile analysis of altered fecal metabolites

Among the 16 fecal metabolites detected, excretion of seven bile acids was significantly augmented in feces. The augmented excretion of phosphatidic acid and monoglyceride in feces were also observed. In addition, the excretion of two hydrolysates of arachidonic acid, prostaglandin G1 and 12-oxo-20-d-LB4, and the tryptophan catabolite of indoleacetyl glutamine, were augmented in the LZG rats. In general, zinc-deficient rats showed increased excretion of fecal metabolites (Figure 4, Supplementary Table 4).

3.6 Integrated analysis of altered serum, urine, and fecal metabolites

To elucidate the association between metabolites and the possible exchange processes in serum, urine, and feces, we manually mapped all the altered metabolites of the three metabolomes against the Kyoto Encyclopedia of Genes and Genomes and Small Molecule Pathway databases (Figure 5). In the serum and urine, declined glycine and acetyl-carnitine levels were observed. In addition, serum glucose and urine glucose excretion were elevated in zinc-deficient rats. Bile acids and arachidonic acid-related metabolites were detected in the serum and feces. Elevated 7-sulfocholic acid levels were observed in both serum and feces. The same substance was not detected in the urine and feces.

4 Discussion

To our knowledge, the present study is the first to use serum, urine, and feces metabolomics to illustrate the adverse effects of zinc deficiency in rats. The results showed changes in the metabolic profile of amino acids in serum and urine, decreased acylcarnitine levels in serum and urine, increased bile acid excretion in feces, increased bile acid accumulation in serum, increased LysoPC levels, and zinc deficiency-induced changes in arachidonic acid catabolites in serum and feces.

4.1 Serum and urine metabolomic signatures indicated abnormally changed amino acid profiles

Zinc deprivation in food alters the intake pattern of self-selecting low protein food and impaired amino acid metabolism [17, 23-25]. Consistent with previous studies, we observed decreased levels of essential amino acids and their catabolites in serum and urine after dietary zinc deprivation. Saccharopine is a catabolite of lysine, and indoleacetyl glutamine, indole pyruvate, and indoxyl sulfate are all tryptophan catabolites. Notably, all four metabolites are derived from microflora [26-28], indicating that zinc deprivation may disturb the bacterial degradation of essential amino acids.

4.2 Serum and urine metabolome signature indicating abated glucose anaerobic metabolism and decreased enzymatic carnitine transfer system

Zinc deprivation is associated with glucolipid metabolism disorders. Previous studies have revealed that zinc deficiency induces the downregulation of glycolysis and free fatty acid degradation by reducing the expression and weakening the activities of enzymes associated with glucolipid metabolism [29]. Consistently, the current serum and urine metabolomics results also reflected impaired glycolysis, explained by the high glucose levels in serum and urine, accompanied by lowered levels of the intermediates of glycolysis, including serum glycerate 3-phosphate, L-lactic acid, and urine acetic acid, after zinc deprivation.

Regarding lipid metabolism, a change in tissue fatty acid composition and the ratio of saturated and monounsaturated to polyunsaturated fatty acids, depending on the zinc status, has been reported for a variety of tissues [30-32]. We observed a significant reduction in arachidonic acid concentration and a modest increase (20:5) in the ratio of saturated and monounsaturated to polyunsaturated fatty acids (n-3) in serum, similar to a previous study in rats fed a zinc-deficient diet [33, 34].

The altered fatty acid composition of hepatic lipids in zinc-deficient rats reflects the downregulation of enzymes required for fatty acid oxidation in peroxisomes and mitochondria [35]. In the current study, we observed increased levels of 4-aminohippuric acid and cis-5-decenedioic acid, which are fatty acid metabolites, in urine. The enrichment of these metabolites in urine was used to diagnose disorders associated with mitochondrial free fatty acid beta-oxidation [36, 37]. Thus, their increased urine

concentrations indirectly confirmed that the oxidation of fatty acids by mitochondrial pathways may be reduced in zinc-deficient rats.

In addition, we observed decreased urine dodecanedioic acid excretion. Urine dodecanedioic acid is an indicator of liver carnitine palmitoyltransferase-I (CPT-1) [38], and its decreased excretion indicate the lack of CPT-1 in the liver of zinc-deficient rats. This is consistent with the reduction in CPT-1 mRNA levels in the liver of zinc-deficient rats observed in a previous study [35]. CPT-1 is an essential enzyme, catalyzing the transfer of the acyl group of long-chain fatty acid-coenzyme A conjugates onto carnitine were decreased in the serum and urine [39]. In the current study, various short-, medium-, long-, and branched-chain acylcarnitine levels [40]. In particular, the serum and urine levels of L-acetyl carnitine, the quantitatively and functionally most important acylcarnitine [41], after zinc deprivation decreased to 0.54 and 0.71 times, respectively, of those in the NZG rats. These results confirm that the import and oxidation of fatty acids by mitochondrial pathways and the peroxisomal breakdown may also reduce due to the impaired fatty acid import system in the inner mitochondrial membrane, which provides a new insight for exploring the potential effect of zinc deficiency on lipid metabolism.

4.3 Zinc deprivation induced changes in bile acid profiles in serum and feces

The main sodium/bile acid cotransporter (SLC10A1) is liver-specific [42], and its decreased levels in the liver have been reported in zinc-deficient rats [16]. In addition,the bile acid content of feces is also increased during zinc deficiency [43]. Consistent with previous studies, in the current study, we observed increased bile acids in the serum and feces of LZG rats. Chenodeoxycholic acid glycine conjugate and lithocholic acid, mainly bile acid and secondary bile acid in rats [44], respectively, increased 3.40-fold and 3.59-fold in feces, respectively. The primary defect in lipid absorptive processes in zinc-deficient rats occurs during the formation of chylomicrons [45]. Meanwhile, the vectorial transport of bile salts from the blood into bile is essential for the emulsification of lipids in the intestine[46]. Thus, the increased excretion of monoglyceride and phosphatidic acid may be associated with zinc deficiency-induced abnormal bile acid metabolism. These results reflect the direct involvement of zinc in the regulation of bile acid metabolism.

4.4 Zinc deprivation increased inflammation-related serum LysoPC levels and caused changes in arachidonic acid metabolism

LysoPC is a major lipid component of the plasma membrane and has a broad spectrum of proinflammatory activities [47]. LysoPC is mainly derived from the turnover of phosphatidylcholine by secretory phospholipase A [48]. Previous research has suggested that the luminal hydrolysis of phosphatidylcholine to LysoPC by phospholipase 2 may be impaired in zinc-deficient rats [49]. In the

current study, we observed augmented levels of seven LysoPCs in the serum of zinc-deficient rats. This reflects the disturbance of phosphatidylcholine metabolism due to zinc deprivation. Additionally, decreased serum levels of glycerophosphocholine, the hydrolysate of LysoPC, were also observed in the current study. Lysophospholipase participates in the hydrolysis of LysoPC to glycerophosphocholine. A complementary DNA-based array showed significantly decreased transcriptional levels of lysophospholipase in zinc-deficient rats [50]. This indicates that zinc deficiency may induce the systemic accumulation of LysoPC in the following two ways: augmented production and suppression of LysoPC catabolism.

Previous studies have also reported disturbed arachidonic acid metabolism in zinc-deficient conditions. The involved metabolism included the induced expression of cyclooxygenase-2 and the increased activity of arachidonate 5-lipoxygenase, two key enzymes involved in the production of prostaglandin and leukotriene B4 from arachidonic acid [51-53]. Prostaglandin and leukotriene B4 are both inflammatory mediators, involved in triggering different inflammatory responses [54]. In the current study, we observed increased prostaglandin and leukotriene B4 catabolite levels in serum and feces, rather than prostaglandin and leukotriene B4. This could be because prostaglandin and leukotriene B4 are unstable and hydrolyze rapidly [55, 56].

Conclusions

In summary, integrated metabolomics of serum, urine, and feces provided a comprehensive platform to interrogate the metabolic profile associated with zinc deficiency. In the current study, we confirmed the previous studies that zinc deficiency impaired amino acid metabolism, glucose anaerobic metabolism, lipid metabolism, changed bile acid circulation,arachidonic acid metabolism and induced systemic accumulation of LysoPC. In addition, a novel aspect, a depleted enzymatic carnitine transfer system was also revealed in the current study. These findings highlight the critical role of trace element in maintaining metabolic homeostasis. The current study also has several limitations. First, the zinc-deficient model used in the current study was a severe deficiency, which could lead to obviously impaired feed intake and weight loss. However, people are often suffered from a state of “hidden hunger”, namely individuals who may have adequate energy consumption, but suboptimal micronutrient intakes. This condition does not produce obvious clinical symptoms but is a potential threat to health. Thus, future research on the metabolic changes under subclinical Zn deficiency conditions, which are more relevant from a practical perspective. Second, the present study lacks resolution since only one low and one high dose were compared, which is not in favor of the well-known non-linear adaption of Zn homeostasis and associated metabolic pathways. Third, lack of joint research on proteomics and metabolomics, resulting in limitations in mechanism interpretation. Thus, these findings are presented with caution, and further studies on subclinical zinc deficiency using a dose-response models and more dimensional omics research should be conducted.

Abbreviations

MD	Metabolic disturbance
VIP	Variable importance in the projection
PCA	Principal component analysis
OPLS-DA	Orthogonal partial least squared discriminant analysis
LysoPC	Lysophosphatidylcholine
TCA cycle	Citric acid cycle
ECTS	Enzymatic carnitine transfer system

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This research was supported by funds from the National Nature Science Foundation of China (81573147, 82173514, and 81973036).

Author Contributions

YL and MW contributed to the conception and design of the study. ZL methodology and software. PW and YZ performed the statistical analysis. PW wrote the first draft of the manuscript. YL and MW wrote sections of the manuscript. All authors read and approved the final manuscript.

Acknowledgments

The authors would like to thank the constructive comments and suggestions from the anonymous reviewers and the editor for improving the manuscript. The authors would like to thank Editage

(app.editage.com) for providing linguistic assistance.

References

1. Eckel RH, Grundy SM, Zimmet PZ: **The metabolic syndrome.** *Lancet* 2005, **365:**1415-1428.
2. Saklayen MG: **The Global Epidemic of the Metabolic Syndrome.** *Curr Hypertens Rep* 2018, **20:**12.
3. Eggersdorfer M, Akobundu U, Bailey RL, Shlisky J, Beaudreault AR, Bergeron G, Blancato RB, Blumberg JB, Bourassa MW, Gomes F, et al: **Hidden Hunger: Solutions for America's Aging Populations.** *Nutrients* 2018, **10**.
4. Panchal SK, Wanyonyi S, Brown L: **Selenium, Vanadium, and Chromium as Micronutrients to Improve Metabolic Syndrome.** *Curr Hypertens Rep* 2017, **19:**10.
5. Dubey P, Thakur V, Chattopadhyay M: **Role of Minerals and Trace Elements in Diabetes and Insulin Resistance.** *Nutrients* 2020, **12**.
6. Maares M, Haase H: **Zinc and immunity: An essential interrelation.** *Arch Biochem Biophys* 2016, **611:**58-65.
7. Prasad AS: **Impact of the discovery of human zinc deficiency on health.** *J Trace Elem Med Biol* 2014, **28:**357-363.
8. Prasad AS: **Impact of the discovery of human zinc deficiency on health.** *J Am Coll Nutr* 2009, **28:**257-265.
9. Ahn BI, Kim MJ, Koo HS, Seo N, Joo NS, Kim YS: **Serum zinc concentration is inversely associated with insulin resistance but not related with metabolic syndrome in nondiabetic Korean adults.** *Biol Trace Elem Res* 2014, **160:**169-175.
10. Adachi Y, Yoshida J, Kodera Y, Kiss T, Jakusch T, Enyedy EA, Yoshikawa Y, Sakurai H: **Oral administration of a zinc complex improves type 2 diabetes and metabolic syndromes.** *Biochem Biophys Res Commun* 2006, **351:**165-170.
11. Yary T, Virtanen JK, Ruusunen A, Tuomainen TP, Voutilainen S: **Association between serum zinc and later development of metabolic syndrome in middle aged and older men: The Kuopio Ischaemic Heart Disease Risk Factor Study.** *Nutrition* 2017, **37:**43-47.
12. Tepaamorndech S, Kirschke CP, Pedersen TL, Keyes WR, Newman JW, Huang L: **Zinc transporter 7 deficiency affects lipid synthesis in adipocytes by inhibiting insulin-dependent Akt activation and glucose uptake.** *Febs j* 2016, **283:**378-394.
13. Tamaki M, Fujitani Y, Hara A, Uchida T, Tamura Y, Takeno K, Kawaguchi M, Watanabe T, Ogihara T, Fukunaka A, et al: **The diabetes-susceptible gene SLC30A8/ZnT8 regulates hepatic insulin clearance.** *J Clin Invest* 2013, **123:**4513-4524.
14. Andreini C, Banci L, Bertini I, Rosato A: **Counting the zinc-proteins encoded in the human genome.** *J Proteome Res* 2006, **5:**196-201.
15. Ryu MS, Langkamp-Henken B, Chang SM, Shankar MN, Cousins RJ: **Genomic analysis, cytokine expression, and microRNA profiling reveal biomarkers of human dietary zinc depletion and**

homeostasis. *Proc Natl Acad Sci U S A* 2011, **108:**20970-20975.

16. tom Dieck H, Döring F, Fuchs D, Roth HP, Daniel H: **Transcriptome and proteome analysis identifies the pathways that increase hepatic lipid accumulation in zinc-deficient rats.** *J Nutr* 2005, **135:**199-205.
17. Reeves PG: **Patterns of food intake and self-selection of macronutrients in rats during short-term deprivation of dietary zinc.** *J Nutr Biochem* 2003, **14:**232-243.
18. Liu L, Wang M, Yang X, Bi M, Na L, Niu Y, Li Y, Sun C: **Fasting serum lipid and dehydroepiandrosterone sulfate as important metabolites for detecting isolated postchallenge diabetes: serum metabolomics via ultra-high-performance LC-MS.** *Clin Chem* 2013, **59:**1338-1348.
19. Huang Q, Teng H, Chang M, Wang Y, He D, Chen L, Song H: **Mass spectrometry-based metabolomics identifies the effects of dietary oligosaccharide-zinc complex on serum and liver of zinc deficiency mice.** *Journal of Functional Foods* 2020, **65:**103777.
20. Mayneris-Perxachs J, Bolick DT, Leng J, Medlock GL, Kolling GL, Papin JA, Swann JR, Guerrant RL: **Protein- and zinc-deficient diets modulate the murine microbiome and metabolic phenotype.** *Am J Clin Nutr* 2016, **104:**1253-1262.
21. Sauer AK, Grabrucker AM: **Zinc Deficiency During Pregnancy Leads to Altered Microbiome and Elevated Inflammatory Markers in Mice.** *Front Neurosci* 2019, **13:**1295.
22. Wang M, Yang X, Ren L, Li S, He X, Wu X, Liu T, Lin L, Li Y, Sun C: **Biomarkers identified by urinary metabonomics for noninvasive diagnosis of nutritional rickets.** *J Proteome Res* 2014, **13:**4131-4142.
23. Hsu JM, Rubenstein B: **Effect of Zinc Deficiency on Histidine Metabolism in Rats.** *The Journal of Nutrition* 1982, **112:**461-467.
24. Hsu JM, Woosley RL: **Metabolism of L-Methionine-35S in Zinc-deficient Rats.** *The Journal of Nutrition* 1972, **102:**1181-1186.
25. Wallwork JC, Duerre JA: **Effect of Zinc Deficiency on Methionine Metabolism, Methylation Reactions and Protein Synthesis in Isolated Perfused Rat Liver.** *The Journal of Nutrition* 1985, **115:**252-262.
26. Goedert JJ, Sampson JN, Moore SC, Xiao Q, Xiong X, Hayes RB, Ahn J, Shi J, Sinha R: **Fecal metabolomics: assay performance and association with colorectal cancer.** *Carcinogenesis* 2014, **35:**2089-2096.
27. Dodd D, Spitzer MH, Van Treuren W, Merrill BD, Hryckowian AJ, Higginbottom SK, Le A, Cowan TM, Nolan GP, Fischbach MA, Sonnenburg JL: **A gut bacterial pathway metabolizes aromatic amino acids into nine circulating metabolites.** *Nature* 2017, **551:**648-652.
28. Evenepoel P, Meijers BK, Bammens BR, Verbeke K: **Uremic toxins originating from colonic microbial metabolism.** *Kidney Int Suppl* 2009**:**S12-19.
29. Eder K, Kirchgessner M: **Zinc deficiency and activities of lipogenic and glycolytic enzymes in liver of rats fed coconut oil or linseed oil.** *Lipids* 1995, **30:**63-69.
30. Cunnane SC, Horrobin DF, Manku MS: **Essential fatty acids in tissue phospholipids and triglycerides of the zinc-deficient rat.** *Proc Soc Exp Biol Med* 1984, **177:**441-446.

31. Cunnane SC: **Evidence that adverse effects of zinc deficiency on essential fatty acid composition in rats are independent of food intake.** *Br J Nutr* 1988, **59:**273-278.
32. Ayala S, Brenner RR: **Essential fatty acid status in zinc deficiency. Effect on lipid and fatty acid composition, desaturation activity and structure of microsomal membranes of rat liver and testes.** *Acta Physiol Lat Am* 1983, **33:**193-204.
33. Burke JP, Owens K, Fenton MR: **Effect of a zinc-deficient diet on mitochondrial and microsomal lipid composition in TEPC-183 plasmacytoma.** *Biochem Med Metab Biol* 1987, **37:**148-156.
34. Eder K, Kirchgessner M: **[The effect of zinc depletion on the fat content and fatty acid composition of the liver and brain in forcibly fed rats].** *Z Ernahrungswiss* 1993, **32:**187-197.
35. Dieck Ht, Döring F, Fuchs D, Roth H-P, Daniel H: **Transcriptome and Proteome Analysis Identifies the Pathways That Increase Hepatic Lipid Accumulation in Zinc-Deficient Rats.** *The Journal of Nutrition* 2005, **135:**199-205.
36. Bonham Carter SM, Watson DG, Midgley JM, Logan RW: **Synthesis and characterisation of acyl glycines. Their measurement in single blood spots by gas chromatography-mass spectrometry to diagnose inborn errors of metabolism.** *J Chromatogr B Biomed Appl* 1996, **677:**29-35.
37. Tserng KY, Jin SJ, Kerr DS, Hoppel CL: **Abnormal urinary excretion of unsaturated dicarboxylic acids in patients with medium-chain acyl-CoA dehydrogenase deficiency.** *J Lipid Res* 1990, **31:**763-771.
38. Korman SH, Waterham HR, Gutman A, Jakobs C, Wanders RJ: **Novel metabolic and molecular findings in hepatic carnitine palmitoyltransferase I deficiency.** *Mol Genet Metab* 2005, **86:**337-343.
39. Stefanovic-Racic M, Perdomo G, Mantell BS, Sipula IJ, Brown NF, O'Doherty RM: **A moderate increase in carnitine palmitoyltransferase 1a activity is sufficient to substantially reduce hepatic triglyceride levels.** *Am J Physiol Endocrinol Metab* 2008, **294:**E969-977.
40. Reuter SE, Evans AM: **Carnitine and acylcarnitines: pharmacokinetic, pharmacological and clinical aspects.** *Clin Pharmacokinet* 2012, **51:**553-572.
41. Rebouche CJ: **Kinetics, pharmacokinetics, and regulation of L-carnitine and acetyl-L-carnitine metabolism.** *Ann N Y Acad Sci* 2004, **1033:**30-41.
42. Fagerberg L, Hallström BM, Oksvold P, Kampf C, Djureinovic D, Odeberg J, Habuka M, Tahmasebpoor S, Danielsson A, Edlund K, et al: **Analysis of the human tissue-specific expression by genome-wide integration of transcriptomics and antibody-based proteomics.** *Mol Cell Proteomics* 2014, **13:**397-406.
43. Topping DL, Illman RJ, Dreosti IE, Trimble RP, Record IR: **Effects of zinc deficiency on bile acid secretion in the rat.** dec1978, **v. 18**.
44. Yang T, Shu T, Liu G, Mei H, Zhu X, Huang X, Zhang L, Jiang Z: **Quantitative profiling of 19 bile acids in rat plasma, liver, bile and different intestinal section contents to investigate bile acid homeostasis and the application of temporal variation of endogenous bile acids.** *J Steroid Biochem Mol Biol* 2017, **172:**69-78.
45. Alrefai WA, Gill RK: **Bile acid transporters: structure, function, regulation and pathophysiological implications.** *Pharm Res* 2007, **24:**1803-1823.

46. Kullak-Ublick GA, Stieger B, Meier PJ: **Enterohepatic bile salt transporters in normal physiology and liver disease.** *Gastroenterology* 2004, **126:**322-342.
47. Corrêa R, Silva LFF, Ribeiro DJS, Almeida RDN, Santos IO, Corrêa LH, de Sant'Ana LP, Assunção LS, Bozza PT, Magalhães KG: **Lysophosphatidylcholine Induces NLRP3 Inflammasome-Mediated Foam Cell Formation and Pyroptosis in Human Monocytes and Endothelial Cells.** *Front Immunol* 2019, **10:**2927.
48. Law SH, Chan ML, Marathe GK, Parveen F, Chen CH, Ke LY: **An Updated Review of Lysophosphatidylcholine Metabolism in Human Diseases.** *Int J Mol Sci* 2019, **20**.
49. Noh SK, Koo SI: **Intraduodenal infusion of lysophosphatidylcholine restores the intestinal absorption of vitamins A and E in rats fed a low-zinc diet.** *Exp Biol Med (Maywood)* 2001, **226:**342-348.
50. tom Dieck H, Doring F, Roth HP, Daniel H: **Changes in rat hepatic gene expression in response to zinc deficiency as assessed by DNA arrays.** *J Nutr* 2003, **133:**1004-1010.
51. Fong LY, Zhang L, Jiang Y, Farber JL: **Dietary zinc modulation of COX-2 expression and lingual and esophageal carcinogenesis in rats.** *J Natl Cancer Inst* 2005, **97:**40-50.
52. Gomez NN, Davicino RC, Biaggio VS, Bianco GA, Alvarez SM, Fischer P, Masnatta L, Rabinovich GA, Gimenez MS: **Overexpression of inducible nitric oxide synthase and cyclooxygenase-2 in rat zinc-deficient lung: Involvement of a NF-kappaB dependent pathway.** *Nitric Oxide* 2006, **14:**30-38.
53. Wetterholm A, Macchia L, Haeggstrom JZ: **Zinc and other divalent cations inhibit purified leukotriene A4 hydrolase and leukotriene B4 biosynthesis in human polymorphonuclear leukocytes.** *Arch Biochem Biophys* 1994, **311:**263-271.
54. Tsai CC, Hong YC, Chen CC, Wu YM: **Measurement of prostaglandin E2 and leukotriene B4 in the gingival crevicular fluid.** *J Dent* 1998, **26:**97-103.
55. Bedrick AD, Britton JR, Johnson S, Koldovský O: **Prostaglandin stability in human milk and infant gastric fluid.** *Biol Neonate* 1989, **56:**192-197.
56. Dickinson Zimmer JS, Voelker DR, Bernlohr DA, Murphy RC: **Stabilization of leukotriene A4 by epithelial fatty acid-binding protein in the rat basophilic leukemia cell.** *J Biol Chem* 2004, **279:**7420-7426.

Figures

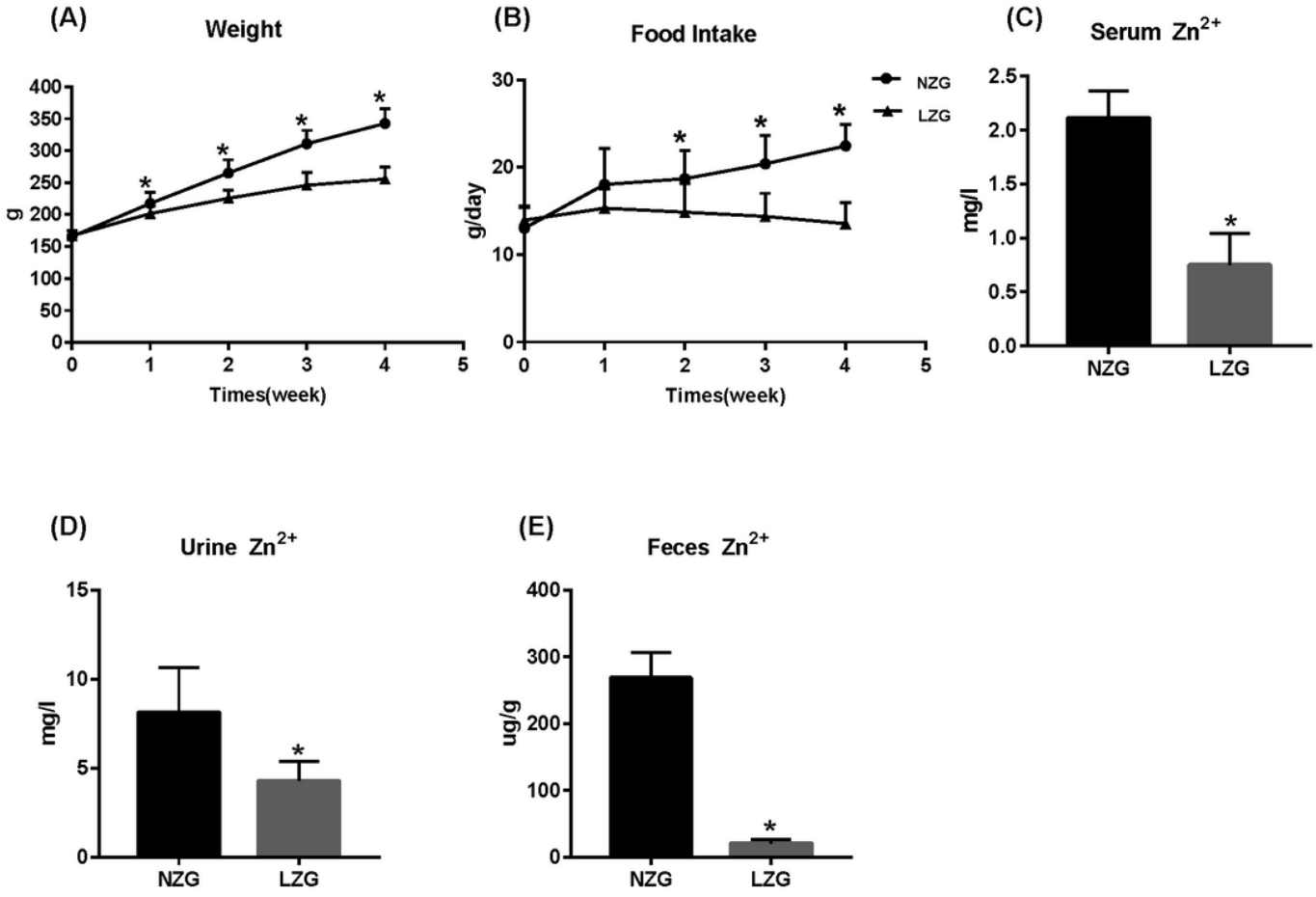

Figure 1

Change trends in food intakes, body weight, food intake, serum Zn^{2+}, urine Zn^{2+}, and fecal Zn^{2+} of two groups. *: $P < 0.05$, LZG vs NZG. LZG, low zinc group; NZG, normal zinc group.

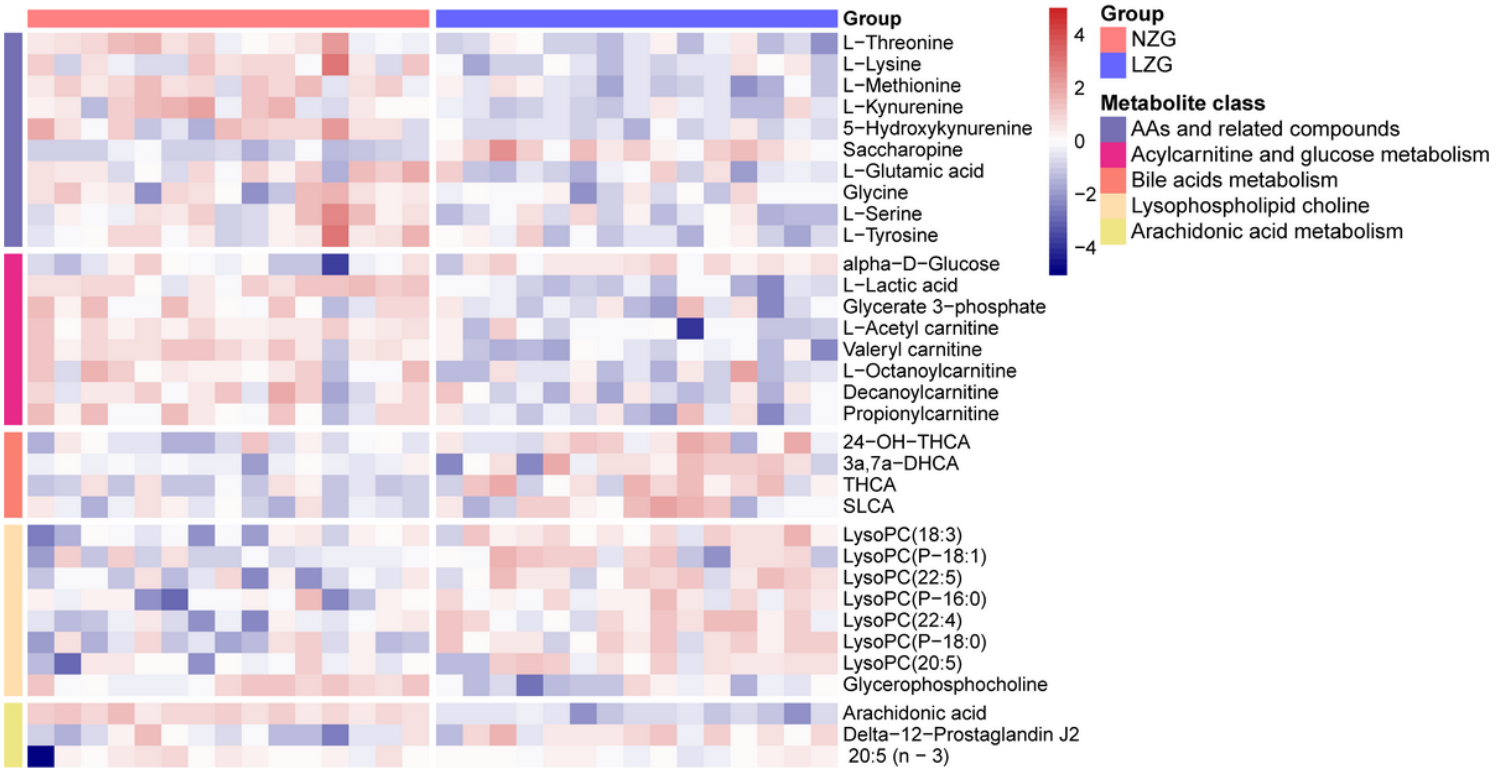

Figure 2

Heatmap of metabolite class significantly altered in serum in zinc deficiency rat model (P<0.05, T-test). The map displays the value of the mean log2-ratio from NZG and LZG group. Red shading denotes an increase and blue shading a decrease in LZG compared to NZG, respectively. 24-OH-THCA, Varanic acid; 3a,7a-DHCA, 3a,7a-Dihydroxycoprostanic acid; THCA, Coprocholic acid; SLCA, Sulfolithocholic acid; LysoPC, Lysophosphatidylcholine. Derived physiological clustering are labeled on the right.

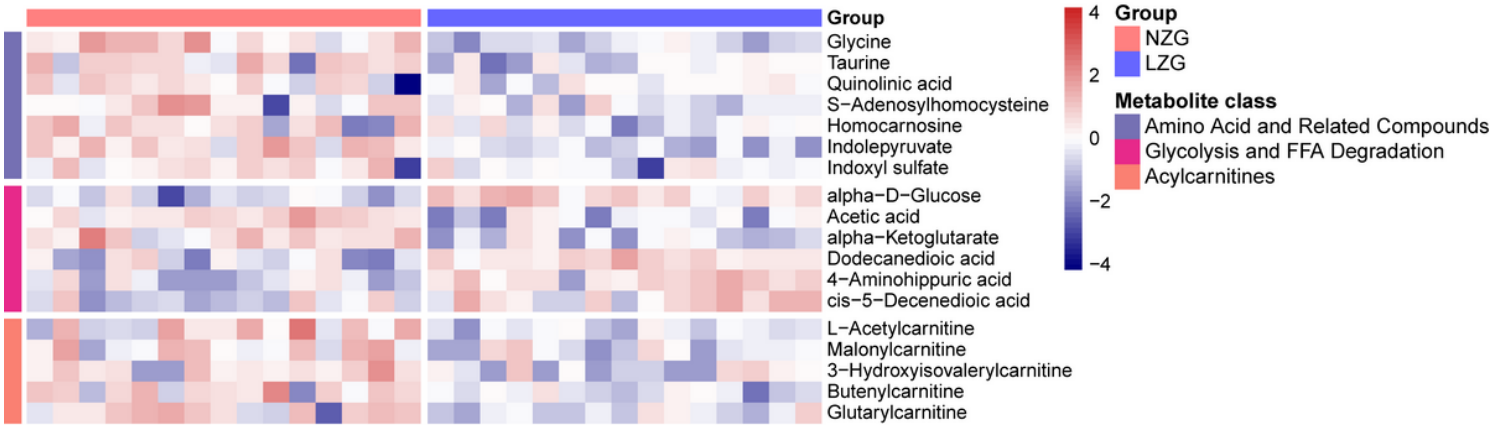

Figure 3

Heatmap of metabolite class significantly altered in urine in zinc deficiency rat model (P<0.05, T-test). The map displays the value of the mean log2-ratio from NZG and LZG group. Red shading denotes an increase and blue shading a decrease in LZG compared to NZG, respectively. Derived physiological clustering are labeled on the right.

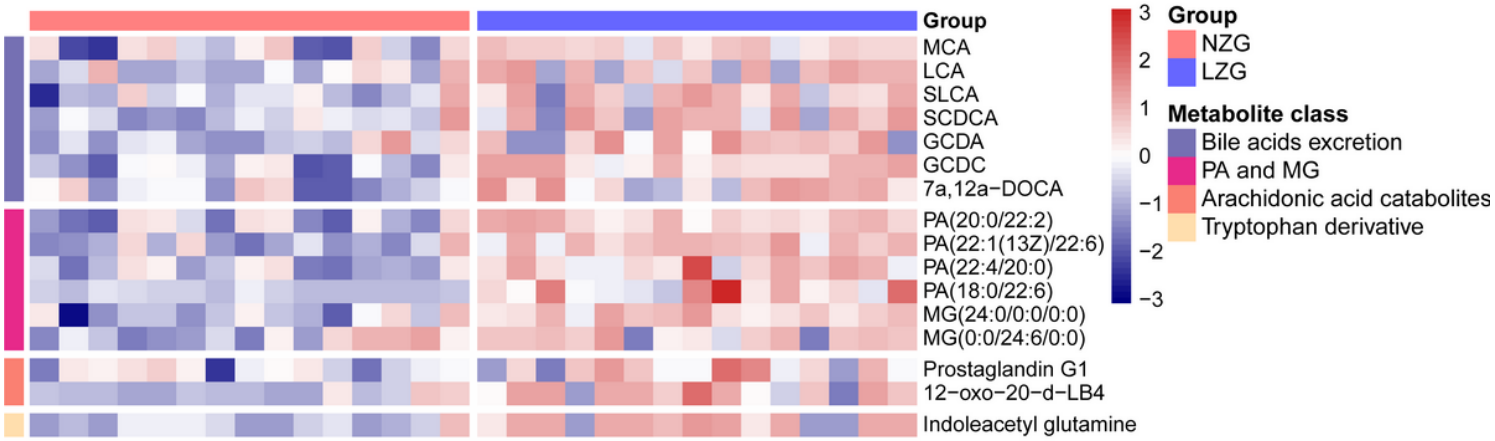

Figure 4

Heatmap of metabolites significantly altered in feces in zinc deficiency rats model (P<0.05, T-test). The map displays the value of the mean log2-ratio from NZG and LZG group. Red shading denotes an increase and blue shading a decrease in LZG compared to NZG, respectively. MCA, Muricholic acid; LCA, Lithocholic acid; SLCA, 7-Sulfocholic acid; SCDCA, Chenodeoxycholic acid sulfate; GCDA, Chenodeoxycholic acid glycine conjugate; GCDC, Glycochenodeoxycholic acid; 7a,12a-DOCA, 7a,12a-Dihydroxy-3-oxo-4-cholenoic acid; PA, phosphatidic acid; MG, Monoglyceride. Derived physiological clustering are labeled on the right.

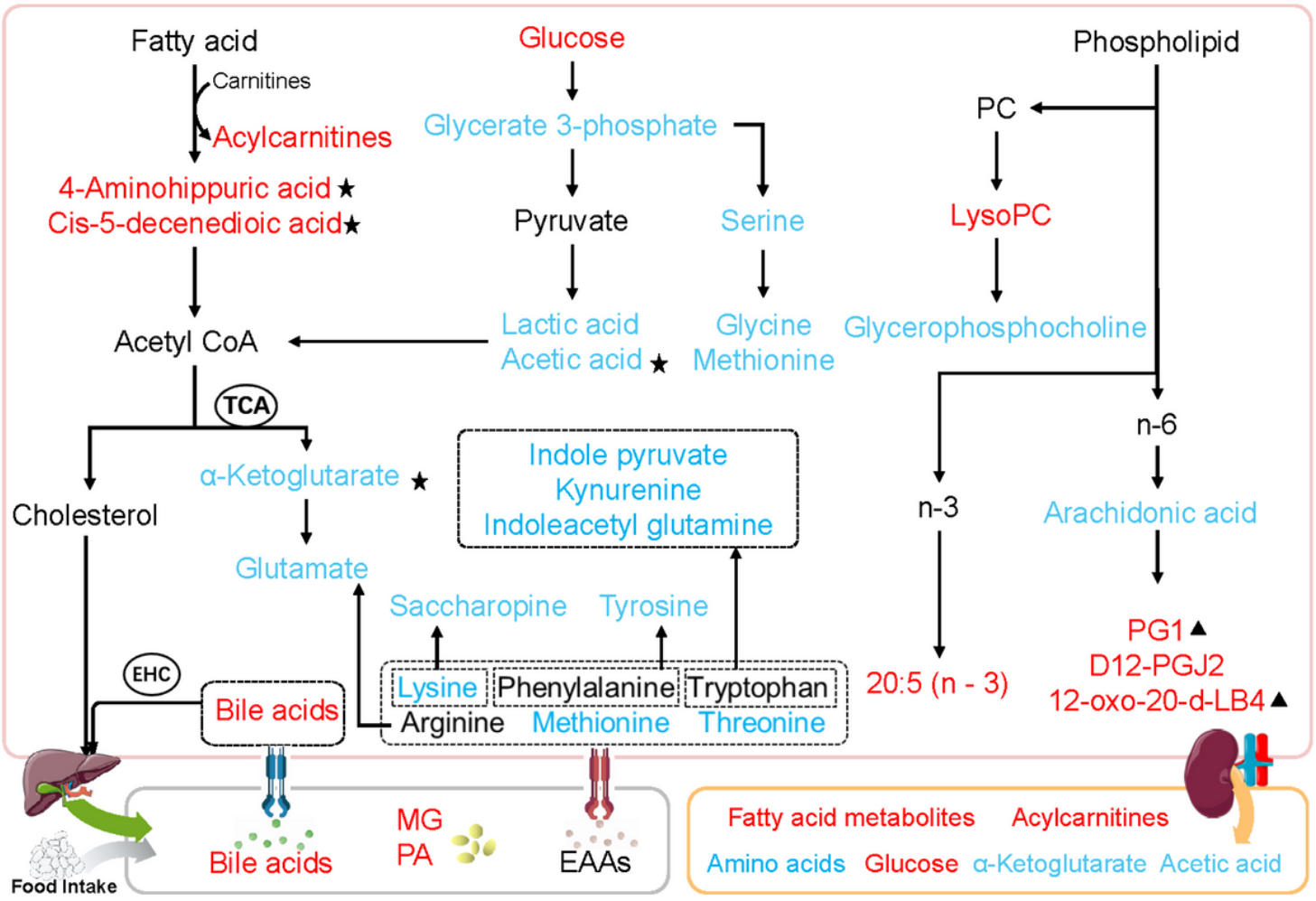

Figure 5

Potential metabolic pathways and metabolites flux associated with zinc deprivation. Red: increased after zinc deprivation; blue: decreased metabolites after zinc deprivation. Pentagram denotes metabolites were detected by urine metabolomics, and triangle denotes metabolites were detected by feces metabolomics. Pink box represents serum; gray box represents feces; yellow box represents urine.PA, Phosphatidic acid; MG, Monoglyceride; EAAs, Essential amino acids; LysoPC, lysophosphatidylcholine; PC, phosphatidylcholine; PG1, Prostaglandin G1; D12-PGJ2, Delta-12-rpostaglandin J2. TCA, Citric acid cycle; EHC, Enterohepatic circulation.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFigures.docx
- SupplementaryTables.xlsx