Short-chain Fatty Acids can Improve Lipid and Glucose Metabolism Independently of the Gut Microbiota

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Research

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Abstract

Background: Previous research has shown that exogenously short-chain fatty acids (SCFAs) infusion attenuated the body fat deposition in both mice, humans, and pigs. However, limited studies have evaluated the effects of SCFAs on lipid and glucose metabolism independently of the gut microbiota. Therefore, the present study was conducted to investigate the effects of exogenous infusion of SCFAs on lipid and glucose metabolism in a germ-free (GF) pig model.

Methods: Twelve hysterectomy-derived newborn pigs were reared in six sterile isolators. All pigs were hand-fed Co60-γ-irradiated sterile milk powder for 21 days and switched from liquid to sterile solid feed for another 21 days. In the second 21-days period, GF pigs (n = 6) were orally infused with 25 mL/kg sterile saline per day, FA pigs (n = 6) were orally infused with 25 mL/kg SCFAs mixture (acetic, propionic, and butyric acids, 45, 15, and 11 mM, respectively) per day.

Results: Orally infused with SCFAs tended to increase the concentration of adiponectin in serum, the activity of CPT-1 in longissimus dorsi, and the ANGPTL4 mRNA expression in colon ($P < 0.10$). Meanwhile, the mRNA abundances of ACC, FAS, and SREBP-1C in liver and CD36 in longissimus dorsi were decreased ($P < 0.05$), and the concentrations of ATP, ADP, or AMP in liver and longissimus dorsi were reduced ($P < 0.05$) in FA group. Besides, the mRNA expressions of PGC-1α in liver and LPL in longissimus dorsi were tended to ($P < 0.10$) increase and downregulate in FA group respectively. Moreover, the protein level of GPR43 was tended to increase ($P < 0.10$), and ACC was tended to decrease ($P < 0.10$), while the p-AMPK/T-AMPK ratio ($P < 0.05$) was upregulated in liver of FA group. Also, oral administration of SCFAs upregulated the mRNA expressions of SLC2a and GYS2 in liver ($P < 0.05$). Furthermore, the metabolic pathway associated with the biosynthesis of unsaturated fatty acids was significantly promoted ($P < 0.05$) by orally infused with SCFAs.

Conclusions: Short-chain fatty acids could attenuate fat deposition and to some extent improve glucose control, and were occur independently of the gut microbiota.

Background

Emerging evidence indicated that gut microbiota plays critical contributors to host health [1]. Notably, the beneficial effects are usually partly attributed to the short-chain fatty acids (SCFAs), as the end-products produced from the fermentation of dietary fiber and resistant starch by gut microbes [2, 3]. Indeed, SCFAs act as pivotal roles in various of biological activities, such as lipid and glucose metabolism [4–6]. Recent studies have shown that oral administration of SCFAs increased energy expenditure and fat oxidation in obese mice [7], and oral infusion of SCFAs prevented body weight gains and enhanced insulin sensitivity in high-fat diet-fed mice [8]. In addition, previous studies showed that orally infused with SCFAs reduced fat deposition in weaned and growing pigs by decreasing lipogenesis and promoting lipolysis of different tissues [9, 10]. Human intervention reports found that SCFAs could regulate whole-body substrates and energy metabolism, with an increased in fasting fat oxidation and resting energy expenditure [11]. However, controversy still exists considering the role of SCFAs in lipid metabolism. Previous research demonstrated that SCFAs were thought to contribute additional calories via fermentation in the obese, thus result in additional weight gain [12]. Meanwhile, conflicting literature reported that enhanced acetate turnover aggravated the development of obesity and insulin resistance in rodents [13]. The inconsistent effects of SCFAs on lipid and glucose metabolism might influence by gut microbiota, which plays a vital role in the development and progression of obesity [14, 15]. Previous studies observed that *Christensenella* genus could prevent weight gain in germ-free mice [16], and *Akkermansia* was reported to correlate with lower visceral fat deposits [17]. Moreover, the numbers of microbiota are positively associated with SCFAs concentrations [18]. Collectively, the endogenous gut microbiota might interfere the effects of exogenous SCFAs on the host health. Herein, further well-controlled studies are urgently needed. Of note, germ-free (GF) animals are free from living microorganisms, including bacteria, viruses, fungi, protozoa, and parasites throughout their life and reared in sterile environments [19, 20]. The domestic pig (*Sus scrofa*) is a preferable model of human health, which lies in similar anatomy, physiology, and genetics to humans [21, 22]. Accordingly, the pig in which absent microbes is a suitable experimental model used to dissect the effects of exogenously infused with SCFAs. Whether exogenously infusion of SCFAs improves the lipid and glucose metabolism independently of the gut microbiota remains largely unknown. Therefore, the present study was conducted to use biochemistry and metabolomics analysis to investigate the effects of oral administration of SCFAs on the lipid and glucose metabolism in a GF pig model, which could provide some novel insights into the roles of SCFAs on fat deposition and host health.

Materials And Methods

Animals

Twelve neonatal GF pigs were delivered by hysterectomy from two multiparous Bama sows (a native breed of China). At 112 days of gestation (full-term, 114 days), pregnant Bama sows were anesthetized with 4% isoflurane, the uterus was excised from the anesthetized sow and was transferred into a sterile isolator (DOSSY Experimental Animals Co., Ltd, Chengdu, China) through a tank including 120 L of 0.1% peracetic acid for decontamination of the uterus. Then the pigs were taken from the uterus in the isolator and the twelve neonatal pigs were transferred to six rearing isolators (Class Biologically Clean Ltd., Madison, Wisconsin, USA) depending on the litter of origin and sex. The isolator has a checkboard and pigs were fed separately. The rearing isolators had been sterilized by spraying with 1% peracetic acid in advance and maintained in sterile environments as described previously [20]. The sterile environments, pig's skin, oral mucosa, and rectal swabs were checked by anaerobic (thioglycollate medium) and aerobic (brain-heart infusion broth) culture of samples at least every week as described by Chinese National Standard (GB/T 14926-41-2001). After 14 days of culture, microbial growth was assessed by gram dyeing microscopy and together with the culture in blood agar base for 48 h at 37°C of colonic digesta collected at the end of the experiment, further confirmed the sterile status.

Experimental treatments and diets

For the six isolators, three of them were treated as the GF group (n = 6), and the other three isolators were designated as the FA group (n = 6). These pigs in GF and FA group were hand-fed Co60-γ-irradiated sterile milk powder prepared by our laboratory (Table S1) and diluted with sterile water (1:4) for 21 days. A corn-soybean feed formulated according to NRC (2012) requirements and Chinese feeding standards for local pigs (2004) (Table S2), and sterilized by Co60-γ-radiation, then introduced to the GF and FA pigs for another 21 days. In the second 21-days period, the GF group orally infused with 25 mL/kg sterile saline per day, FA group orally infused with 25 mL/kg SCFAs mixture (acetic, propionic, and butyric acids, 45, 15, and 11 mM, respectively) per day. The concentrations and dose of acetic, propionic, and butyric acids conducted in present experiments were according to the preliminary test on conventional Bama pigs. In the preliminary test, we had observed that orally infused with excessive concentration (acetic, propionic, and butyric acids, 60, 20, and 15 mM, respectively) and dose (35 mL/kg) of SCFAs led to the death of pigs. In addition, the SCFAs mixture was prepared in the laminar airflow clean benches, the acetic, propionic, and butyric acid (analytically pure) were filtered by a 0.22 um membrane and mixed into sterile water. In the two 21-days periods, all pigs were allowed *ad libitum* access to sterile water. When the sterile milk, feed, and bottled water in the isolators were consumed, a replacement container was provided via the transfer port, in which containers for sterile feed, milk, or water, and the container were sterilized by spraying with 1% peracetic acid.

Sample collection

Before the pigs were euthanized, blood samples were obtained from anterior vena cava before euthanized via isoflurane anesthesia on the 42th day of the experiment, centrifuged at 3,000 g for 15 min, and stored at -80 °C for further analysis. The abdomen was opened in the laminar airflow clean benches, and the tissues of the colon, liver, and longissimus dorsi were immediately collected in liquid nitrogen and stored at -80 °C for further measurements.

Serum biochemical analyses

The concentrations of adiponectin, insulin, glucagon, glucagon-like peptide 1, and leptin in serum were detected by commercial enzyme-linked immunosorbent assay (ELISA) kits from Chenglin Co. Ltd. (Beijing, China) according to the manufacturer's instructions. The concentrations of total cholesterol (TC), triglyceride (TG), high density lipoprotein-cholesterol (HDL-c), low density lipoprotein-cholesterol (LDL-c), and glucose in serum were measured using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) following with the manufacturer's instructions. Each parameter was measured in triplicate simultaneously on the same plate. And the differences among parallels must be small (coefficient of variation was less than 10%) to guarantee the reproducibility of repeated measurements.

Determination of enzyme activity

For the enzyme activity assessment, about 1 g frozen sample of liver and longissimus dorsi were homogenized in ice-cold saline solution (1:9, wt/vol) and then centrifuged at 3,000 g for 15 min at 4 ℃. The supernatant was collected for further analysis. The activities of carnitine palmitoyltransferase 1 (CPT-1), lipoprotein lipase (LPL), hepatic lipase (HL), and malate dehydrogenase (MDH) in liver and longissimus dorsi were determined using commercial kits (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) according to the manufacturer's instructions. The total protein content of liver and longissimus dorsi homogenates was detected by Bradford brilliant blue method [23]. Each parameter was determined in triplicate simultaneously on the same plate.

Real-time quantitative PCR

Total RNA was isolated from frozen colon, liver, and longissimus dorsi, using Trizol reagent (TaKaRa) according to the manufacturer's instructions. The concentration and purity of the RNA were determined using a NanoDrop ND-2000 Spectrophotometer (NanoDrop, Germany). The ratio of OD_{260}:OD_{280} ranging from 1.8 to 2.0 in all samples was regarded as suitable for further analysis. The integrity of RNA was detected by agarose gel electrophoresis and the 28S:18S ribosomal RNA band ratio was determined as ≥1.8. RNA was reverse transcribed into cDNA using the PrimeScriptTM RT reagent kit (TaKaRa) according to the manufacturer's guidelines. Primers for the selected genes (Table S3) were designed by Primer 6 Software (PREMIER Biosoft International, Palo Alto, CA, USA) and synthesized commercially by Sangon Biotech Limited (Shanghai, China). The Quantitative real-time PCR was performed on an ABI Prism 7000 detection system in a two-step protocol with SYBR Green (Applied Biosystems, Foster City, CA, USA). Each reaction was performed in a volume of with 1 μL of cDNA, 5 μL of SYBR Premix Ex Taq TM (2×), 0.2 μL of ROX reference dye (50×), 0.4 μL of each forward and reverse primer, and 3 μL of PCR-grade water. The PCR conditions were as follows: initial denaturation at 95 °C for 30 s, followed by 40 cycles of denaturation at 95 °C for 10 s, annealing at 60 °C for 25 s, and a 72 °C extension step for 5 min. A melting curve analysis was generated following each Quantitative real-time PCR assay to verify the specificity of the reactions. The housekeeping gene β-actin was chosen as the reference gene to normalize mRNA expression of target genes. Gene expression data of replicate samples was calculated using the $2^{-\Delta\Delta CT}$ method [24]. The relative expression of the target genes in the GF group was set to be 1.0. Each sample was measured in triplicate.

Analysis of adenosine

The sample of liver or longissimus dorsi (100 mg) was homogenized with 5 mL of 0.4 M perchloric acid at 0 ℃ for 1 min, and with ultrasonic treatment for 30 min. Then, the mixture was centrifuged at 3,000 g for 10 min. and the supernatant immediately neutralized to pH 6.5 with 50 mM monopotassium phosphate. After that, monopotassium phosphate was removed by filtration through the sintered glass and stored at -80 ℃ for subsequent analysis. The high-performance liquid chromatography (HPLC, U3000, Thermo Fisher Scientific, USA) was used to determine the concentrations of adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine monophosphate (AMP) in liver and longissimus dorsi [25].

Determination of protein levels by western blot

The antibodies against β-actin, GPR43, p-AMPK, AMPK, CPT-1B, and ACC were obtained from Cell Signaling Technology (Davers, MA), Abcam (Cambridge, MA, USA), and Santa Cruz Biotechnology Inc. (Santa Cruz, CA, USA), respectively. Protein levels for the β-actin, GPR43, p-AMPK, AMPK, CPT-1B, and ACC in liver were measured by western blot analysis according to the instructions described by Suryawan et al. (2001) [26].

Ultrahigh-performance liquid chromatography equipped with quadrupole time of flight mass spectrometry (UHPLC-Q-TOF/MS) analysis

The UHPLC-Q-TOF/MS analysis was performed with an Agilent 1290 UHPLC system (Agilent, Palo Alto, USA) and combined with a Q-TOF mass spectrometer (ESI/Triple TOF 5600; AB Sciex, Concord, Canada) were used to measure the serum metabolites. For the

serum sample, the pretreatment, extraction, and identification were according to the procedure described by Hu et al. (2019) [27]. The raw data (whiff scan files) were converted into mzXML format using ProteoWizard [29], and were imported to the XCMS software for peak matching, retention time alignment, and peak area extraction [30]. Metabolite structure identification was performed by comparing the accuracy of m/z values (< 25 ppm), and MS/MS spectra were interpreted with an in-house database (Shanghai Applied Protein Technology Co. Ltd, China) established with authentic standards. For the XCM data, the ion peaks that were missing values greater than 50% in the group were filtered and excluded and data were normalized to total peak intensity. Then, statistical analyses were performed using SIMCA-P software (version 14.1, Umetrics, Umea, Sweden), where could subjected to multivariate data analysis, including partial least squares discriminant analysis (PLS-DA) and orthogonal partial least squares discriminant analysis (OPLS-DA), which were carried out to uncover and extract the statistically significant metabolite variations. The PLS-DA and OPLS-DA models were validated based on multiple correlation coefficient (R^2) and cross-validated (Q^2) in cross-validation and permutation test by applying 2000 iterations [31]. The R^2 value in the permutated plot described how well the data fit the derived model, whereas the Q^2 value described the predictive ability of the constructed model and was a measure of model quality [32]. The significance of the biomarkers was ranked using the variable importance in the projection (VIP) score (>1) from the OPLS-DA model. Metabolites with the highest VIP score are the most powerful group discriminators, VIP score > 1 are significant [33]. The procedure of metabolites identification and pathway analysis was according to Wang et al. (2017) [31].

Statistical analysis

Metabolites with a VIP score >1 was further analyzed by the Student's *t*-test at the univariate level to measure the significance of each metabolite. The univariate data analysis also included a fold-change analysis. Other data were analyzed in SAS 9.2 (SAS Institute, Inc., Cary, NC, USA) and analyzed using Student's *t*-test, and were presented as means ± SEMs. The individual pig as the statistical unit. $P < 0.05$ was considered to be statistically significant, and a tendency was declared with $0.05 < P < 0.10$.

Results

No signs of illness, abnormal behavior, or mortality of pigs were observed in the current study. In addition, there were no differences in growth performance and relative weight of organs between the GF and FA group (data not shown).

Serum parameters

According to Table 1, oral infusion of SCFAs tended to increase the concentration of adiponectin in serum ($P < 0.10$).

Table 1

Effects of orally infused with SCFAs on the serum indicators in a germ-free pig model[1]

Items	GF	FA	*P-value*
Adiponectin, ug/L	74.48 ± 1.62	80.63 ± 2.32	0.06
Glucagon, pg/ml	28.01 ± 1.52	30.06 ± 1.23	0.12
GLP-1, pmol/L	2.51 ± 0.06	2.57 ± 0.08	0.52
Insulin, mIU/L	10.20 ± 0.38	10.76 ± 0.52	0.40
Leptin, ng/L	1403.2 ± 38.30	1368.4 ± 45.03	0.57
TC, mmol/L	1.45 ± 0.10	1.59 ± 0.11	0.38
TG, mmol/L	0.30 ± 0.03	0.50 ± 0.12	0.15
HDL, mmol/L	0.73 ± 0.08	0.80 ± 0.06	0.51
LDL, mmol/L	0.77 ± 0.05	0.81 ± 0.06	0.65
Glucose, mmol/L	5.31 ± 0.31	5.33 ± 0.58	0.97

[1] GF, germ-free; FA, short-chain fatty acids; GLP-1, Glucagon like peptide 1; TC, total cholesterol; TG, triglyceride; HDL-c, high density lipoprotein-cholesterol; LDL-c, low density lipoprotein-cholesterol. Values are means ± SEMs, n = 6/group.

The enzyme activity in liver and longissimus dorsi

As presented in Table 2, the activity of CPT-1 in longissimus dorsi of FA group was higher than the GF group ($P < 0.10$).

Table 2

Effects of orally infused with SCFAs on the activities associated with lipids metabolism in liver and longissimus dorsi of a germ-free pig model[1]

Items	GF	FA	*P-value*
Liver			
CPT-1, ng/L	192.40 ± 3.58	191.6 ± 7.27	0.92
LPL, U/mg protein	0.75 ± 0.02	0.73 ± 0.10	0.86
HL, U/mg protein	0.77 ± 0.11	0.78 ± 0.06	0.97
MDH, U/mg protein	43.56 ± 3.93	41.62 ± 2.48	0.68
Longissimus dorsi			
CPT-1 (ng/L)	197.70 ± 5.92	209.90 ± 1.91	0.08
LPL, U/mg protein	0.66 ± 0.26	0.34 ± 0.05	0.28
HL, U/mg protein	0.48 ± 0.05	0.39 ± 0.04	0.21
MDH, U/mg protein	4.08 ± 0.32	3.41 ± 0.16	0.10

[1] GF, germ-free; FA, short-chain fatty acids; CPT-1, carnitine palmitoyltransferase 1, LPL, lipoprotein lipase; HL, hepatic lipase; MDH, malate dehydrogenase. Values are means ± SEMs, n = 6/group.

The mRNA expressions of lipid metabolism-related genes in liver and longissimus dorsi

As shown in Table 3, the mRNA expressions of ANGPTL4 in colon and PGC-1α in liver were tended to upregulate in the FA group ($P < 0.10$). Moreover, the mRNA abundances of ACC, FAS, and SREBP-1C in liver of FA group were lower than the GF group ($P < 0.05$). As presented in Table 4, the oral infusion of SCFAs decreased the mRNA expression of CD36 ($P < 0.05$) and tended to downregulate the mRNA expression of LPL in longissimus dorsi ($P < 0.10$).

Table 3
Effects of orally infused with SCFAs on the mRNA abundances for key factors associated with lipid metabolism in colon and liver of a germ-free pig model[1]

Items	GF	FA	*P-value*
Colon			
ANGPTL4	1.00 ± 0.13	1.48 ± 0.17	0.06
PPAR-γ	1.00 ± 0.07	0.95 ± 0.14	0.75
Liver			
ACC	1.00 ± 0.17	0.63 ± 0.05	<0.01
FAS	1.00 ± 0.16	0.56 ± 0.03	0.03
CD36	1.00 ± 0.25	0.60 ± 0.17	0.19
LPL	1.00 ± 0.11	0.69 ± 0.13	0.11
SREBP-1C	1.00 ± 0.09	0.72 ± 0.08	0.04
PPKAA1	1.00 ± 0.10	1.10 ± 0.09	0.47
PPKAA2	1.00 ± 0.13	0.99 ± 0.08	0.95
CPT-1B	1.00 ± 0.14	0.98 ± 0.11	0.93
PGC-1α	1.00 ± 0.16	2.02 ± 0.43	0.06
PNPLA2	1.00 ± 0.11	0.86 ± 0.15	0.47

[1] GF, germ-free; FA, short-chain fatty acids; ANGPTL4, angiopoietin-like 4; PPAR-γ, peroxisome proliferator-activated receptor gamma; ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; CD36, atty acid transporter CD36; LPL, lipoprotein lipase; SREBP-1C, sterol regulatory element binding protein 1C; PRKAA1, AMP activated alpha 1; PRKAA2, AMP activated alpha 2; CPT-1B, carnitine palmitoyltransferase 1 B; PNPLA2, adipose triglyceride lipase. Values are means ± SEMs, n = 6/group.

Table 4
Effects of orally infused with SCFAs on the mRNA abundances for key factors associated with lipid metabolism in longissimus dorsi of a germ-free pig model[1]

Items	GF	FA	*P-value*
ACC	1.00 ± 0.14	0.79 ± 0.03	0.19
FAS	1.00 ± 0.33	0.68 ± 0.06	0.37
CD36	1.00 ± 0.07	0.71 ± 0.06	0.02
LPL	1.00 ± 0.09	0.81 ± 0.04	0.08
SREBP-1C	1.00 ± 0.18	0.65 ± 0.06	0.11
PPKAA1	1.00 ± 0.08	0.91 ± 0.07	0.44
PPKAA2	1.00 ± 0.03	1.07 ± 0.06	0.39
CPT-1B	1.00 ± 0.15	1.14 ± 0.10	0.44
PGC-1α	1.00 ± 0.08	1.58 ± 0.39	0.19
PNPLA2	1.00 ± 0.16	0.97 ± 0.14	0.90

[1] GF, germ-free; FA, short-chain fatty acids; ACC, acetyl-CoA carboxylase; FAS, fatty acid synthase; CD36, atty acid transporter CD36; LPL, lipoprotein lipase; SREBP-1C, sterol regulatory element binding protein 1C; PRKAA1, AMP activated alpha 1; PRKAA2, AMP activated alpha 2; CPT-1B, carnitine palmitoyltransferase 1 B; PNPLA2, adipose triglyceride lipase. Values are means ± SEMs, n = 6/group.

The mRNA expressions of glucose metabolism-related genes in liver and longissimus dorsi

According to Table 5, oral infusion of SCFAs upregulated the mRNA expressions of SLC2a and GYS2 in liver ($P < 0.05$). In addition, the mRNA abundances of genes related to glucose metabolism in longissimus dorsi were no differences between FA and GF groups ($P > 0.10$) (Table 6).

Table 5
Effects of orally infused with SCFAs on the mRNA abundances for key factors associated with glucose metabolism in liver of a germ-free pig model[1]

Items	GF	FA	*P-value*
FOXO-1	1.00 ± 0.05	1.09 ± 0.04	0.22
INSR	1.00 ± 0.06	0.81 ± 0.06	0.16
INS1	1.00 ± 0.10	1.02 ± 0.04	0.80
PIK3	1.00 ± 0.09	0.72 ± 0.02	0.12
GLU-2	1.00 ± 0.12	0.58 ± 0.08	<0.01
PCK1	1.00 ± 0.05	1.08 ± 0.01	0.14
GSK3	1.00 ± 0.04	1.10 ± 0.05	0.17
GYS2	1.00 ± 0.01	1.67 ± 0.20	<0.01

[1] GF, germ-free; FA, short-chain fatty acids; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator-1α; FOXO-1, foxo1 forkhead box O1; INSR, insulin receptor; IRS1, insulin receptor substrate 1; PIK3, phosphatidylinositol 3-kinase catalytic subunit type 3; SLC-2α, solute carrier family 2 member; G6PC, glucose-6-phosphatase; PCK 1, phosphoenolpyruvate carboxykinase 1; GSK 3, glycogen synthase kinase 3; GYS2, glycogen synthase 2. Values are means ± SEMs, n = 6/group.

Table 6
Effects of orally infused with SCFAs on the mRNA abundances for key factors associated with glucose metabolism in longissimus dorsi of a germ-free pig model[1]

Items	GF	FA	*P-value*
FOXO-1	1.00 ± 0.44	1.35 ± 0.34	0.20
Sirt1	1.00 ± 0.02	0.86 ± 0.03	0.11
INSR	1.00 ± 0.09	0.99 ± 0.21	0.97
INS1	1.00 ± 0.15	1.08 ± 0.09	0.64
PIK3	1.00 ± 0.09	0.96 ± 0.07	0.73
GLU-2	1.00 ± 0.13	1.05 ± 0.06	0.73
PCK1	1.00 ± 0.19	1.37 ± 0.22	0.24
GSK3	1.00 ± 0.04	1.01 ± 0.08	0.91

[1] GF, germ-free; FA, short-chain fatty acids; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator-1α; FOX-1, foxo1 forkhead box O1; Sirt1, silent information regulator 1; INSR, insulin receptor; IRS1, insulin receptor substrate 1; PIK3, phosphatidylinositol 3-kinase catalytic subunit type 3; SLC-2α, solute carrier family 2 member; G6PC, glucose-6-phosphatase; PCK 1, phosphoenolpyruvate carboxykinase 1; GSK 3, glycogen synthase kinase 3. Values are means ± SEMs, n = 6/group.

The concentrations of adenosine in liver and longissimus dorsi

As presented in Table 7, the concentrations of ATP and ADP in liver, and the concentrations of ATP and AMP in longissimus dorsi of FA group were lower than the GF group ($P < 0.05$).

Table 7
Effects of orally infused with SCFAs on the concentrations of adenosine in liver and longissimus dorsi of a germ-free pig model[1]

Items	GF	FA	*P-value*
Liver			
ATP (ug/g)	17.91 ± 0.34	16.80 ± 0.19	0.02
ADP (ug/g)	1836.8 ± 181.7	1293.9 ± 135.5	0.04
AMP (ug/g)	147.60 ± 13.87	117.50 ± 8.83	0.10
AMP/ATP	8.24 ± 0.76	6.98 ± 0.49	0.20
Longissimus dorsi			
ATP (ug/g)	681.3 ± 37.32	471.50 ± 42.95	<0.01
ADP (ug/g)	1261.0 ± 128.0	980.2 ± 125.4	0.16
AMP (ug/g)	357.9 ± 32.6	264.2 ± 18.58	0.03
AMP/ATP	0.52 ± 0.04	0.57 ± 0.03	0.46

[1] GF, germ-free; FA, short-chain fatty acids; ATP, adenosine triphosphate; ADP, adenosine diphosphate; AMP, adenosine monophosphate. Values are means ± SEMs, n = 6/group.

The protein level associated with lipid metabolism

As presented in Figure 1, the protein level of GPR43 was tended to increase ($P < 0.10$) and ACC was tended to decrease ($P < 0.10$) in FA group. Moreover, oral administration of SCFAs upregulated the p-AMPK/AMPK ratio in liver ($P < 0.05$).

Metabolomic analysis of the serum

To further predict the difference in metabolite profiles related to lipid and glucose metabolism in serum, GC-TOF/MS was used to analyze the metabolite profiles. The PLS-DA (Figure 2) and OPLS-DA (Figure 3) models showed that the two groups were well-separated in serum. To assess which compounds were responsible for the differences between the two groups, the parameters of VIP > 1.0 and adjusted $P < 0.10$ were used as key lineages for separating the serum compounds between the two groups (Figure 4 and Table 8). In total, thirty-three compounds with a VIP > 1.0 and adjusted $P < 0.10$ were identified. Among these, seventeen metabolites (choline, hypoxanthine, glycerophosphocholine, N1-Methyl-2-pyridone-5-carboxamide, L-malic acid, 1-Oleoyl-L-alpha-lysophosphatidic acid, arachidonic acid, stearic acid, ketoisocaproic acid, hypoxanthine, 9R,10S-EpOME, phosphorylcholine, succinate, docosahexaenoic acid, docosatrienoic acid, and palmitic acid) were enriched ($P < 0.05$) and four metabolites (D-mannose, L-pyroglutamic acid, 4-nitrophenol, and stavudine) were reduced ($P < 0.05$) in FA group compared with GF group. Overall, these results suggested that orally infused with SCFAs markedly increased lipids related compounds (arachidonic acid, stearic acid, docosahexaenoic acid, palmitic acid, glycerophosphocholine), indicating that SCFAs had a strong effect on lipid metabolism characteristics in serum.To comprehensively understand the physiological change induced by oral infusion of SCFAs, the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database was utilized for analyzing related metabolic pathways of 33 metabolites observed in serum. According to Figure 5, these metabolites were involved in multiple biochemical pathways, while the metabolic pathway of biosynthesis of unsaturated fatty acids was the most significantly affected ($P < 0.05$) by oral infusion of SCFAs.

Table 8

Altered metabolites in serum between GF and FA group[1]

Adduct	Metabolites	Metabolic pathway	M-to-Z	Rt(s)[2]	VIP[3]	FC[4]	*P*-value[5]
M+	Choline	Glycerophospholipid metabolism	104.11	559.22	3.47	1.25	0.0042
(M+H)+	Hypoxanthine	Purine metabolism	137.05	349.60	6.37	1.33	0.0058
M+	Glycerophosphocholine	Lipid metabolism	258.11	781.81	8.80	1.53	0.0124
(M+H)+	N1-Methyl-2-pyridone-5-carboxamide	Nicotinate and nicotinamide metabolism	153.07	127.37	1.36	1.32	0.0212
(M+Na)+	D-Mannose	Lysosome	203.05	574.98	1.70	0.65	0.0314
(M+H)+	L-Pyroglutamic acid	Glutathione metabolism	130.05	751.76	1.85	0.65	0.0344
(M+Na)+	1-Palmitoyl-sn-glycero-3-phosphocholine	—	518.32	386.60	1.49	1.24	0.0575
(M-H+2Na)+	1-Stearoyl-2-hydroxy-sn-glycero-3-phosphocholine	—	568.34	367.11	4.30	1.74	0.0699
(M+H)+	5-Methylcytosine	Pyrimidine metabolism	126.07	403.47	1.05	0.66	0.0741
(M+H)+	1-Palmitoyl-2-hydroxy-sn-glycero-3-phosphoethanolamine	—	454.29	396.52	1.27	1.57	0.0790
(M+H-2H2O)+	2-Amino-2-methyl-1,3-propanediol	—	70.06	541.31	1.15	0.78	0.0801
(M+H)+	2-Hydroxyadenine	Purine metabolism	152.06	457.91	1.50	1.32	0.0813
(M+H)+	Deoxycytidine	Pyrimidine metabolism	228.10	421.57	1.02	0.74	0.0881
(M-H)-	L-Malic acid	Renal cell carcinoma	133.01	727.91	1.80	1.85	0.0009
(M+Na-2H)-	1-Oleoyl-L-alpha-lysophosphatidic acid	—	457.23	438.26	1.67	1.63	0.0027
(M-H)-	4-Nitrophenol	—	138.02	59.08	1.05	0.82	0.0167
(M-H)-	Arachidonic acid	Biosynthesis of unsaturated fatty acids	303.23	67.07	16.18	1.77	0.0170
(M-H)-	Stearic acid	Biosynthesis of unsaturated fatty acids	283.26	349.68	1.11	1.58	0.0183
(M-H)-	Ketoisocaproic acid	Biosynthesis of amino acids	129.06	94.17	5.75	1.20	0.0218
(M+Na-2H)-	Stavudine	—	245.05	539.43	1.16	0.30	0.0281
(M-H)-	Hypoxanthine	Purine metabolism	135.03	298.92	7.13	1.25	0.0335
(M-H)-	9R,10S-EpOME	—	295.23	75.32	1.46	1.68	0.0373
(M+CH3COO)-	Phosphorylcholine	Glycerophospholipid metabolism	242.08	721.65	1.32	2.28	0.0387
(M-H)-	Succinate	Citrate cycle (TCA cycle)	117.02	697.09	1.81	1.30	0.0409

(M-H)-	(4Z,7Z,10Z,13Z,16Z,19Z)-4,7,10,13,16,19-Docosahexaenoic acid	Biosynthesis of unsaturated fatty acids	327.23	67.13	8.88	1.87	0.0413
(M-H)-	Docosatrienoic acid	—	333.28	64.84	1.15	1.44	0.0459
(M-H)-	Palmitic acid	Biosynthesis of unsaturated fatty acids	255.23	89.61	7.25	2.51	0.0478
(M-H)-	Eicosapentaenoic acid	Biosynthesis of unsaturated fatty acids	301.22	68.36	4.80	1.90	0.0505
(M-H)-	Dihomo-gamma-linolenic acid	Lipid metabolism	305.25	66.69	4.36	1.67	0.0543
(M-H)-	L-Ascorbic acid	Glutathione metabolism	175.02	660.84	2.19	2.01	0.0617
(M-H)-	Norethindrone acetate	—	339.20	1152.64	1.82	0.64	0.0852
(M-H)-	Glycocholic acid	Lipid metabolism	464.31	322.78	1.41	1.43	0.0884
(M-H)-	Nnamecis-9,10-epoxystearic acid	—	297.24	75.71	1.42	1.82	0.0895

[1] GF, germ-free pigs; FA, short chain fatty acids pigs.

[2] M-to-Z, mass-to-charge ratio

[3] Rt, retention time.

[4] The significance *P*-value was obtained from Student's test with a threshold of 0.10.

[5] FC, foldchange, was calculated by dividing the mean intensity of FA group pig' plasma metabolites by the mean intensity of GF group pig' plasma metabolites.

[6] VIP=variable importance projection. This value was obtained from OPLS-DA model with a threshold of 1.0.

Discussion

As is known to us, obesity has become one of the most serious public health problems all over the world [34]. Consequently, it is very important to develop effective measures to prevent obesity. Noteworthy, it has been reported that exogenously SCFAs infusion attenuated the body fat deposition in both mice, humans, and pigs [7, 10, 11]. The SCFAs can be produced naturally by host metabolic pathways particularly in the liver, while the major site of SCFAs production is the colon which requires the presence of specific bacteria [35]. The numbers and diversity of microbiota are positively associated with SCFAs concentrations [18], and several gut microbes were closely related to host lipid metabolism [16, 17]. However, whether the SCFAs regulate lipid and glucose metabolism independent of the gut microbiota are still unknown. In the present study, we conducted to investigate that exogenous infusion of SCFAs on the lipid and glucose metabolism in a GF pig model, and further dissect the underlying mechanisms of SCFAs on lipid and glucose metabolism.

The concentration of adiponectin in serum was reduced in individuals with obesity and obesity-related diseases [36]. Our results showed that oral infusion of SCFAs tended to increase the content of adiponectin in serum. Noteworthy, acting in peripheral tissues, adiponectin could regulate lipid metabolism and influence energy expenditure [37]. Moreover, we observed the activity of CPT-1 in longissimus dorsi was tended to increase in FA group. Importantly, CPT-1 is the rate-limiting enzyme that determines fatty acid oxidation [38]. ANGPTL4 is a valid inhibitor of lipoprotein lipase to regulate cellular uptake of triglycerides and promote fatty acid oxidation [39, 40]. In the present work, orally infused with SCFAs tended to increase the mRNA abundance of ANGPTL4 in colon. In addition, we found that oral infusion of SCFAs markedly downregulated the mRNA expressions of FAS, ACC, and SREBP-1C in liver. Consistently, previous studies reported similar results in liver, longissimus dorsi, and adipose tissues of conventional

pigs [9, 10, 41]. Remarkably, FAS is the pivotal enzyme that catalyzes fatty acid synthesis [42]. ACC modulates fatty acids metabolism, and its product (e.g. malonyl-CoA) serves as a building block for de novo fatty acid synthesis [43]. The SREBPs increases the transcription of genes that encode the enzymes of fatty acid biosynthesis and cholesterol uptake [44]. Additionally, the function of LPL catalyzes the hydrolysis of triglycerides residing in chylomicrons, and providing free fatty acid for tissue utilization [38]. CD36, the fatty acid translocase, regulates the uptake of long-chain fatty acids into cells [45], and elevated expression of CD36 in various tissues results in lipid overload and lipotoxicity [46]. Interestingly, we detected the mRNA abundance of CD36 was apparently decreased, and LPL was tended to reduce in longissimus dorsi of FA group. The PGC-1α was measured as a vital regulator of fatty acid metabolism [47], and increasing the PGC-1α expression in liver was a negative association with body fat [48, 49]. In the present study, oral infusion of SCFAs tended to upregulate the mRNA expression of PGC-1α in liver, in agreement with the previous studies in conventional pigs and mice [7, 10]. In addition to associated with a reduction in fat deposition, SCFAs have been demonstrated to enhance the rates of oxygen consumption, and increase both fat oxidation and adaptive thermogenesis in rodents [7, 50]. Indeed, in our study, both the concentrations of ATP and ADP in liver, and the concentrations of ATP and AMP in longissimus dorsi were apparently reduced in FA group. Collectively, these demonstrated that the SCFAs could decrease lipid accumulation via downregulating the mRNA expressions of genes related to fatty acid synthesis and enhancing energy expenditure in liver and longissimus dorsi, and was occur independently of the gut microbiota.

The liver acts as a central role in regulating blood glucose homeostasis by uptake of glucose in the postprandial state and conversion to glycogen and triglyceride, and by the production of glucose in the postabsorptive state through glycogenolysis and gluconeogenesis [51, 52]. The rate-limiting enzyme for glycogen synthesis is glycogen synthase (GS), in mammals, there are two GS isoforms: muscle GS (encoded by GYS1), which is abundantly expressed in skeletal and cardiac muscles, and the liver-restricted isoform (encoded by GYS2) [53]. Previous work indicated that mice lacked GYS2 had a severe decrease in their ability to store glycogen in hepatocytes [53]. It is well established that insulin resistance and hepatic steatosis lead to compromised glycogen synthesis [54, 55]. Conversely, increased liver glycogen synthesis improves glucose tolerance [56]. Of note, we observed the mRNA expression of GYS2 in liver was significantly increased in FA group. Similarly, it has been shown that SCFAs supplementation reduced adiposity and improved glucose homeostasis compared to the control group [57, 58]. The GLUT-2 transports glucose in the liver across the membrane in a bi-directional manner for glycolysis and gluconeogenesis and was identified as a major contributor to glucose and fructose homeostasis in the liver [59, 60]. Increased expression of GLUT-2 in liver may be associated with insulin resistance and type-2 diabetes mellitus [61, 62]. In the current study, we found that oral infusion of SCFAs markedly downregulated the mRNA abundance of GLU-2 in liver. Hence, these suggested that the metabolic benefits of SCFAs administration to some extent improved the glucose control in liver of GF pigs.

Although numerous researches have demonstrated the beneficial functions of SCFAs administration on energy homeostasis. Unfortunately, until now, the underlying mechanisms of SCFAs on lipid and glucose metabolism are not fully understood. Importantly, G-protein-coupled receptors (GPCRs) GPR41 (also known as FFAR3) and GPR43 (also known as FFAR2) have been demonstrated to be indispensable for a range of SCFA-mediated effects [63, 64]. It has been indicated that GPR43 knockout mice exhibit a reduction in energy expenditure, while overexpression of GPR43 exhibited an increase in energy expenditure [65]. The SCFAs have been shown to promote energy consumption and fat tissue combustion when the activation of GPCRs [66]. Moreover, the effects of SCFAs involves improved insulin response also mediated by GPR43, which induces enhanced glucose control [67]. Of note, we observed the protein expression of GPR43 in liver tended to upregulate in FA group. In addition to SCFAs- GPCRs pathways turn out to be involved in the regulation of lipid and glucose metabolism, adenosine monophosphate-activated protein kinase (AMPK) also plays an important role in this regulation. The SCFAs have been shown to increase the AMPK activity in liver and muscle tissue [7, 68]. In the present study, the ratio of p-AMPK/AMPK was significantly increased in FA group. Additionally, SCFAs were found to mediate liver lipid and glucose homeostasis via PPAR dependent AMPK-ACC pathway activation, which regulated the effects on gluconeogenesis and lipogenesis [8]. Similarly, we observed the protein expression of ACC in liver of FA group was tended to higher than the GF group. Taken together, these findings suggested that SCFAs might increase fat oxidation and decrease fat storage by binding to the GPCRs and activating the AMPK-ACC pathway.

The metabolomics is a pyramidally used tool for exhaustive research of all metabolites comprised in an organism [69], which exploits high-throughput analytical measures to quantify and identify metabolites. It can allow the characterization of the dynamic changes in phenotype and system homeostasis [70], and describe a direct biochemical picture of the metabolites [71]. Hence, metabolomics offers a novel strategy to identify the potential metabolite markers, explore molecular mechanisms and metabolic

pathways response to specific nutritional interventions. It is well known that serum can be regarded as a metabolic fingerprint that provides a visual result of the metabolic events and reveals changes in metabolic pathways under various nutritional or physiological conditions [72]. In the present study, PLS-DA and OPLS-DA analyses demonstrated a clear separation of serum metabolites due to orally infused with SCFAs, suggesting marked differences in the metabolic profiles. Indeed, several fatty acids, such as stearic acid, arachidonic acid, docosahexaenoic acid, and palmitic acid in serum of FA group were apparently increased compared with GF group. An increased serum fatty acids levels implied that lipid or energy metabolisms have been altered. Besides, taken KEGG pathway analysis, we observed these fatty acids (stearic acid, arachidonic acid, docosahexaenoic acid, palmitic acid) were involved in the biosynthesis of unsaturated fatty acids metabolic pathway, and this metabolic pathway was markedly affected by oral infusion of SCFAs. Of note, intake of unsaturated fatty acids, which consist of monounsaturated fatty acids and polyunsaturated fatty acids, has recently been associated with favorable body composition and cardiac diastolic function in patients with obesity [73]. Moreover, a high unsaturated fatty acids diet also prevented weight gain and cardiac dysfunction in a mouse model [74]. Consequently, these indicated that exogenously infusion of SCFAs might reduce the lipid deposition via activating the biosynthesis of unsaturated fatty acids metabolic pathways in GF pigs.

Conclusions

In summary, the present study demonstrated that the SCFAs could attenuate the fat deposition and to some extent improve the glucose control in liver and longissimus dorsi, and were occur independently of the gut microbiota. Moreover, the possible mechanisms of orally infused with SCFAs on lipid reduction was by binding to the GPCRs, activating the AMPK-ACC pathway, and stimulating the metabolic pathway of biosynthesis of unsaturated fatty acids. To our knowledge, the SCFAs are mainly produced by anaerobic microbial fermentation of indigestible carbohydrates in the gastrointestinal tract. Herein, the current work further suggests the importance of the presence of gut microbes. Moreover, these findings may provide some novel evidence for the potential application of modulating SCFAs as a possible therapeutic strategy to prevent metabolic disorders and to counteract the gut microbiota deficiency or imbalance.

Abbreviations

ACC, acetyl-CoA carboxylase; ADP, adenosine diphosphate; AMP, adenosine monophosphate; AMPK, adenosine monophosphate-activated protein kinase; ANGPTL4, angiopoietin-like 4; ATP, adenosine triphosphate; CD36, atty acid transporter CD36; CPT-1B, carnitine palmitoyltransferase 1 B; FA, short-chain fatty acids; FAS, fatty acid synthase; FOXO-1, foxo1 forkhead box O1; GF, germ-free; GPR43, G-protein-coupled receptors 43; G6PC, glucose-6-phosphatase; GSK 3, glycogen synthase kinase 3; GYS2, glycogen synthase 2; HDL-c, high density lipoprotein-cholesterol; HL, hepatic lipase; INSR, insulin receptor; IRS1, insulin receptor substrate 1; LDL-c, low density lipoprotein-cholesterol; LPL, lipoprotein lipase; MDH, malate dehydrogenase; OPLS-DA, orthogonal partial least squares discriminant analysis; p-AMPK, phosphorylated adenosine monophosphate-activated protein kinase; PCK 1, phosphoenolpyruvate carboxykinase 1; PIK3, phosphatidylinositol 3-kinase catalytic subunit type 3; PGC-1α, peroxisome proliferator-activated receptor gamma coactivator-1α; PLS-DA, partial least squares discriminant analysis; PNPLA2, adipose triglyceride lipase; PPAR-γ, peroxisome proliferator-activated receptor gamma; PRKAA1, AMP activated alpha 1; PRKAA2, AMP activated alpha 2; Sirt1, silent information regulator 1; SLC-2α, solute carrier family 2 member; SREBP-1C, sterol regulatory element binding protein 1C; TC, total cholesterol; TG, triglyceride; UHPLC-Q-TOF/MS, ultrahigh-performance liquid chromatography equipped with quadrupole time of-flight mass spectrometry; VIP, variable importance in the projection.

Declarations

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Not applicable.

Authors' contributions

H.Z. conducted the animal work and the laboratory work, and wrote the manuscript. H.Z., L.G. and D.C. designed the experiment. B.Y. and J.S. gave advice on the experiment design. H.Z. analyzed the study data and wrote the manuscript. Z.L. and H.C. helped to revise the manuscript. All the authors have read and approved the final manuscript.

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Availability of data and material

The data were exhibited in the main manuscript and supplemental materials.

Ethics approval and consent to participate

Experimental protocols and procedures used in the present experiment were approved by the Animal Care and Use Committee of Sichuan Agricultural University (Chengdu, China) under permit number DKY-B20131704. The experiment was carried out at the Experimental Swine Engineering Center of the Chongqing Academy of Animal Sciences (CMA No. 162221340234; Chongqing, China).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no conflict of interest.

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Figures

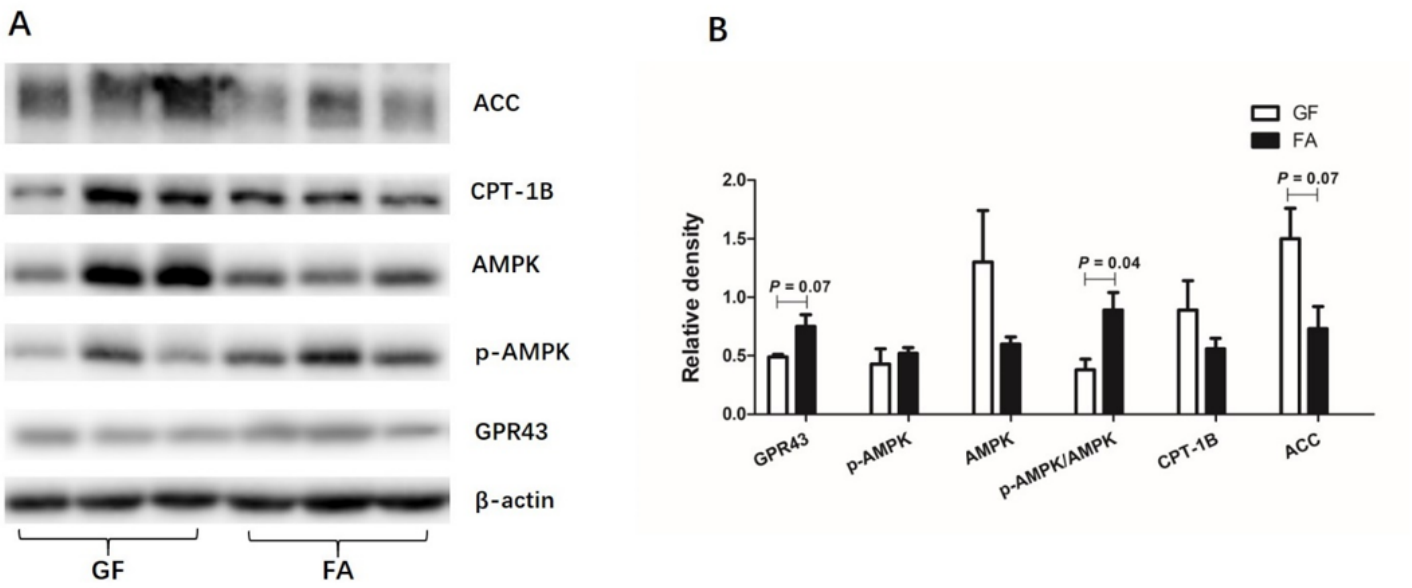

Figure 1

Effect of orally infused with SCFA on the protein levels of GPR43, p-AMPK, T-AMPK, CPT-1B, and ACC in liver of germ-free pigs. GF, germ-free pigs; FA, short chain fatty acids pigs; ACC, acetyl-CoA carboxylase; CPT-1B, carnitine palmitoyltransferase 1 B; p-AMPK, phosphorylated adenosine monophosphate-activated protein kinase; AMPK, adenosine monophosphate-activated protein kinase; GPR43, G-protein-coupled receptors 43.

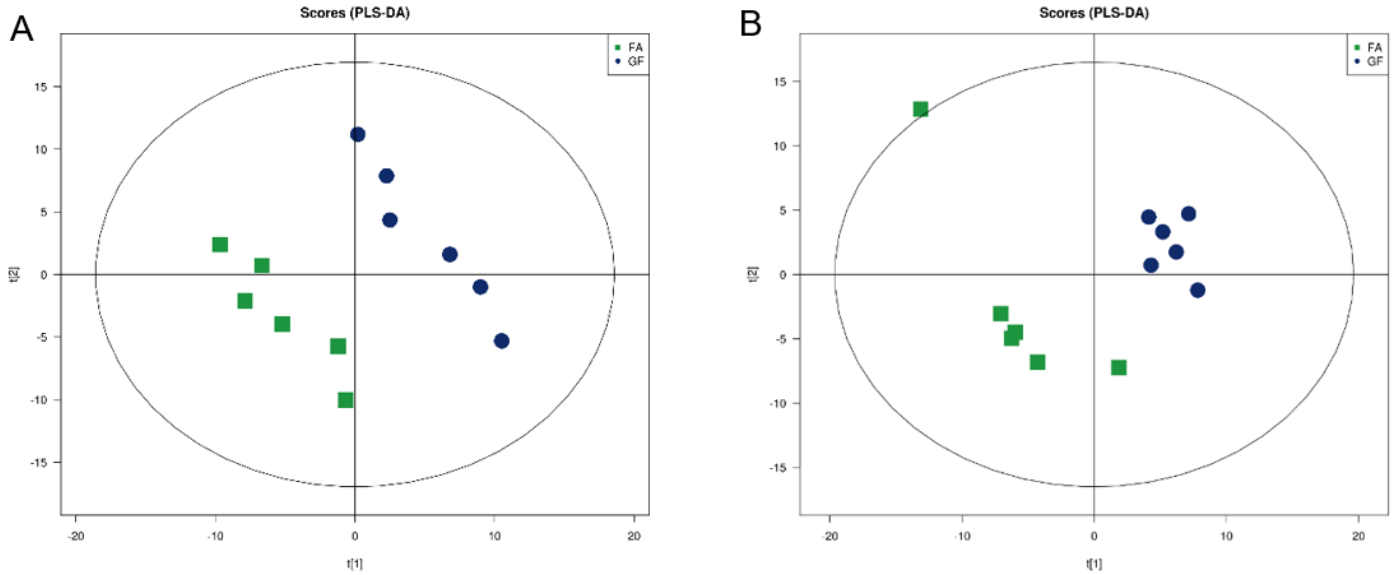

Figure 2

PLS-DA score map derived from UHPLC-Q-TOF/MS spectra concerning GF (blue rotundities) and FA (green squares) serum of pigs in the positive mode (R2X = 0.459, R2Y = 0.996, Q2 = 0.649; (A)) and negative mode (R2X = 0.514, R2Y = 0.999, Q2 = 0.818; (B)). PLS-DA, Partial least squares discriminant; GF, germ-free pigs; FA, short chain fatty acids pigs.

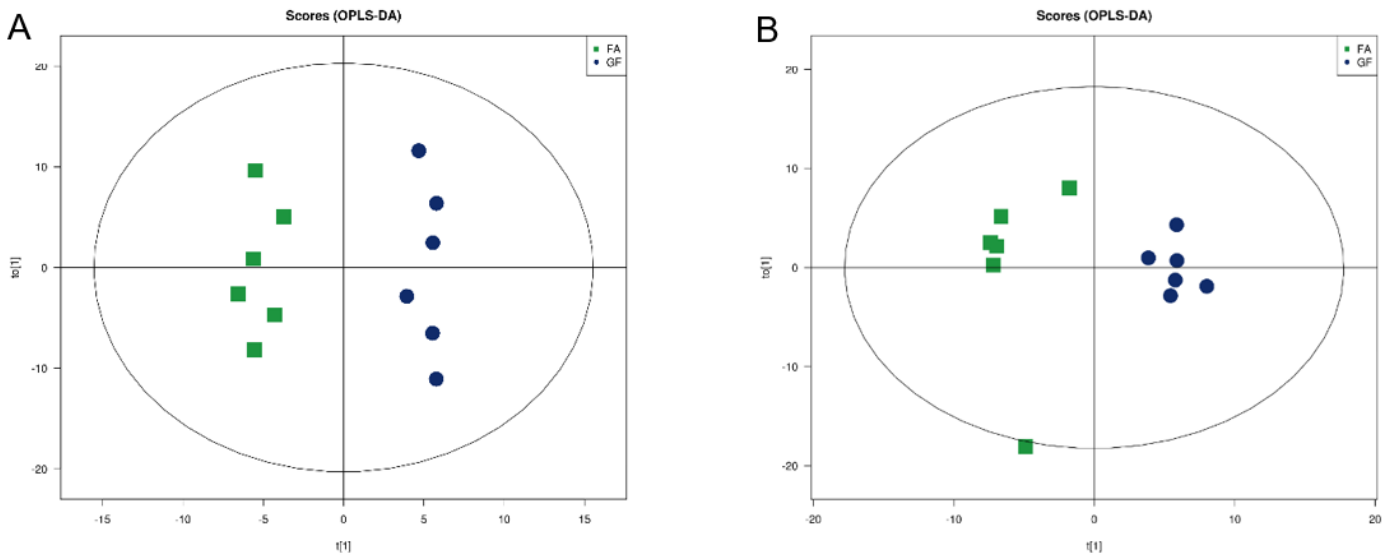

Figure 3

OPLS-DA score map derived from UHPLC-Q-TOF/MS spectra concerning GF (blue circles) and FA (green squares) serum of pigs in the positive mode (R2X = 0.317, R2Y = 0.976, Q2 = 0.009; (A)) and negative mode (R2X = 0.307, R2Y = 0.926, Q2 = 0.201; (B)) OPLS-DA, orthogonal partial least-squares discriminant. GF, germ-free pigs; FA, short chain fatty acids pigs.

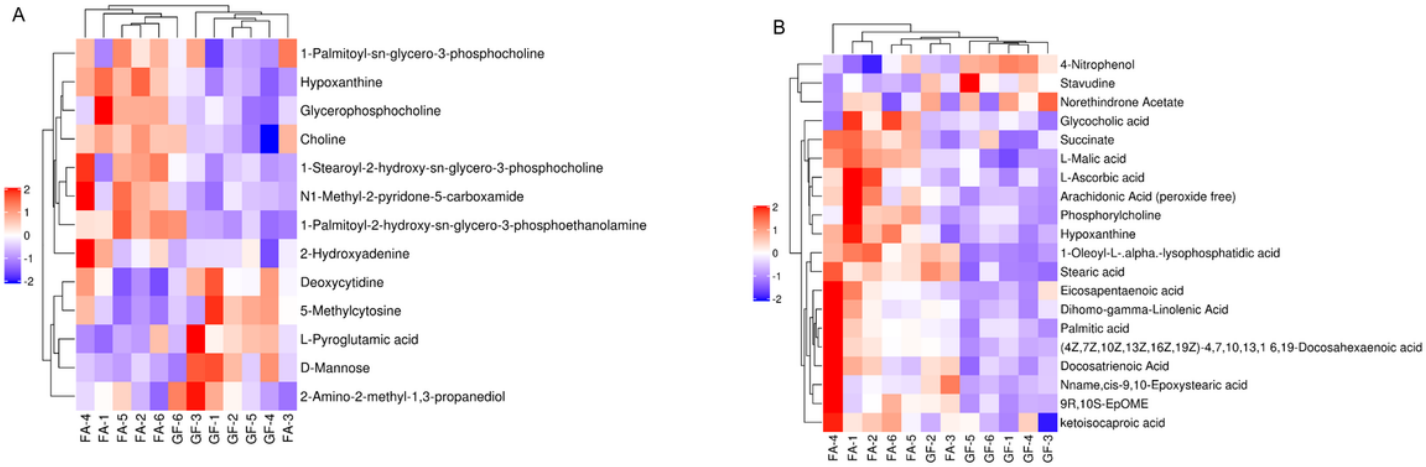

Figure 4

Hierarchical clustering heat map of significantly different metabolites from serum of pig in the positive mode (A) and negative mode (B). Metabolites peak area were Z score transformed. Warm color and cold color indicate increased and decreased expression of the metabolites, respectively. GF, germ-free pigs; FA, short chain fatty acids pigs.

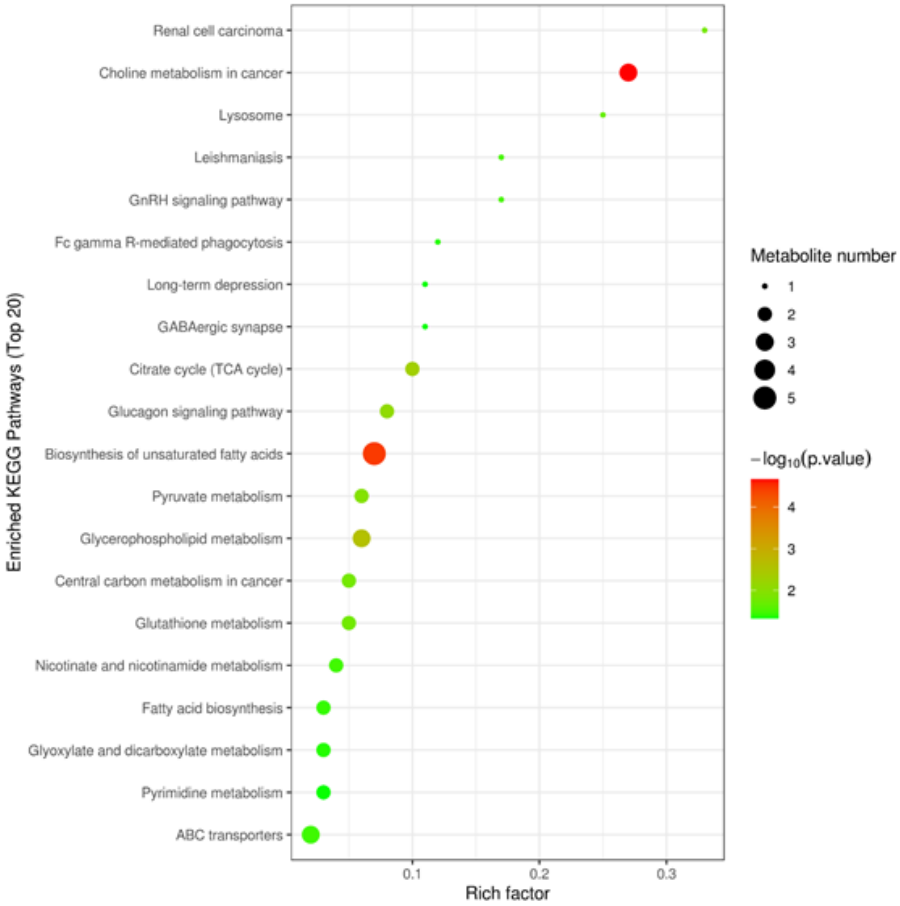

Figure 5

Topology analysis of metabolic pathways identified between GF and FA pigs. The X-axis represents the rich factor, and the Y-axis represents the pathway. Larger sizes and darker colors represent greater pathway enrichment and higher pathway impact values, respectively. GF, germ-free pigs; FA, short chain fatty acids pigs.

Supplementary Files

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- 4SupplementarymaterialJASB.docx