Oral administration of short chain fatty acids could attenuate fat deposition of pigs

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Abstract

Short chain fatty acids (SCFAs) are the main products of indigestible carbohydrates that are fermented by microbiota in the hindgut. This study was designed to investigate the effects of oral SCFAs administration on the lipid metabolism of weaned pigs. A total of 21 barrows were randomly allocated into three groups, including control group (orally infused with 200 mL physiological saline per day), low dose SCFAs group (orally infused with 200 mL SCFAs containing acetic acid 20.04 mM, propionic acid 7.71 mM and butyric acid 4.89 mM per day), and high dose SCFAs group (orally infused with 200 mL SCFAs containing acetic acid 40.08 mM, propionic acid 15.42 mM and butyric acid 9.78 mM per day). The results showed that the average daily feed intake of SCFAs groups were lower than that of control group (P<0.05). Oral administration of SCFAs decreased the concentrations of triglyceride (TG), total cholesterol (TC), high density lipoprotein-cholesterol and insulin (P<0.05), and increased the leptin concentration in serum (P<0.05). The total fat, as well as TC and TG levels in liver, was decreased by oral SCFAs administration (P<0.05). In addition, SCFAs down-regulated the mRNA expressions of fatty acid synthase (FAS) and sterol regulatory element binding protein 1c (P<0.05), and enhanced the mRNA expression of carnitine palmitoyltransferase-1α (CPT-1α) in liver (P<0.05). And in abdominal fat, SCFAs reduced FAS and ACC mRNA expressions (P<0.05), and increased CPT-1α mRNA expression (P<0.05). These results suggested that oral administration of SCFAs could attenuate fat deposition in weaned pigs via reducing lipogenesis and enhancing lipolysis of different tissues.

Introduction

In recent years, owing to decreased physical activities and increased energy intake, metabolic syndrome, especially obesity, has become a global epidemic [1, 2]. And the incidence of obesity in most western countries is over 20%, which prompts us to find new therapies that are more effective [3]. Nowadays, the role of dietary fiber in body weight management has caught more
and more attention [4, 5]. This comes from the fact that increased fermentable fiber intake is beneficial to obesity control through suppressing appetite and modulating energy metabolism [6]. Feeding rodents with dietary fiber inhibited high fat diet-induced body weight gain and fat accumulation [7, 8]. Diets supplemented with fermentable fiber also helped to improve glucose homeostasis in humans [9]. Thus, there have been renewed interests in investigating the mechanism that dietary fiber suppresses appetite and obesity.

The hindgut is colonized by a large amount of microorganisms, which we call microbiota. They can ferment some nondigestible carbohydrates that escape absorption in small intestine, including dietary fiber. And short chain fatty acids (SCFAs), especially acetate, propionate and butyrate, are the main products of bacteria fermentation [10]. SCFAs have been shown to play important roles in shaping gut environment, maintaining electrolyte balance and providing energy for host cells as well as gut microbiota [11]. A growing number of in vivo and in vitro studies have demonstrated that SCFAs contribute greatly to energy homeostasis and lipid metabolism through stimulating several hormonal and neural signals at multiple tissues [12, 13].

However, the systematic impacts of SCFAs on lipid metabolism have been rarely studied, and the further mechanisms need to be investigated. Importantly, pigs share high similarities with humans regarding genetics, anatomy and physiology, which are considered better models when studying human nutrition and disease compared with rodents. Accordingly, the present study was conducted to investigate the effects of oral SCFAs administration on lipid metabolism in pigs, which could provide some insights into the mechanism that dietary fiber modulated body weight gain and fat accumulation.

Materials and methods

Ethics approval and consent to participate

All experimental procedures and animal care were accomplished in accordance with the Guide for the Care and Use of Laboratory Animals provided by the Institutional Animal Care Advisory Committee for Sichuan Agricultural University. All animal protocols used in this study were approved by the Animal Care and Use Committee of Sichuan Agricultural University under permit number DKY-B20131704.

Animal, management and diet

All experimental procedures and animal care were accomplished in accordance with the Guide for the Care and Use of Laboratory Animals provided by the Institutional Animal Care Advisory Committee for Sichuan Agricultural University. All animal protocols used in this study were approved by the Animal Care and Use Committee of Sichuan Agricultural University under permit number DKY-B20131704. A total of 21 healthy barrows (Duroc × Landrace × Yorkshire) with average initial body weight (8.31 ± 0.06 kg) were randomly allocated into 3 groups (n = 7). The groups were: 1) control group (orally infused with 200 mL physiological saline per day), 2) Low dose SCFAs group (orally infused with 200 mL SCFAs containing acetic acid 20.04 mM, propionic acid 7.71 mM and butyric acid 4.89 mM per day), 3) High dose SCFAs group (orally infused with 200 mL SCFAs containing acetic acid 40.08 mM, propionic acid 15.42 mM and butyric acid 9.78 mM per day). All the pigs were individually penned in metabolism cages (1×0.5×0.8m) under temperature, humidity and light control during the 1 week experimental period. The pH of SCFAs were adjusted to around 6 with sodium hydroxide or hydrochloric acid. Diet was formulated to meet or exceed the nutrient requirement of NRC (2012) recommendation for 7–11 kg pigs, and the compositions were presented in Table 1. Pigs were provided with ad libitum access to water and feed during the study.
Growth performance

The body weight of each pig was measured on the morning of day 1 and 8 before feeding. The feed intake of pigs was recorded each day. These were used to calculate average daily weight gain (ADG), average daily feed intake (ADFI) and the ratio of feed to gain (F/G).

Slaughter and sample collection

On the morning of day 8, after weighing, blood samples were collected by acute jugular venipuncture, centrifuged at 3000 × g, and stored at -20˚C. And then, all pigs were slaughtered according to previously described procedures [14]. Liver, longissimus dorsi and abdominal fat were collected and stored at -80˚C for further analyses.

Biochemical analyses

About 0.6 g frozen liver sample of each pig was homogenized on ice with 5.4 mL physiological saline, and centrifuged at 3000 × g. Then, the supernatant was collected and stored at -20˚C for further biochemical analyses. The triglyceride (TG), total cholesterol (TC), high density lipoprotein-cholesterol (HDL-c), low density lipoprotein-cholesterol (LDL-c) and glucose of serum and liver were measured by commercial assay kits from Nanjing Jiancheng Biochemistry (Nanjing, China) according to the manufacturer’s instructions. The insulin, glucagon and leptin were measured by commercial enzyme-linked immunosorbent assay (ELISA) kits from Xinle Co. Ltd. (Shanghai, China) according to the manufacturer’s instructions. Liver total fat was measured according to previously described procedures [15].

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Table 1. Composition and nutrient level of experimental diets (air dry basis %).

| Ingredient                      | Content   | Calculated Composition | Nutrient content |
|---------------------------------|-----------|------------------------|-----------------|
| Corn                            | 28.79     | DE(MJ/kg)              | 3.55            |
| Extruded corn                   | 27.61     | CP                     | 19.59           |
| Dehulled soybean meal           | 10.33     | CF                     | 1.88            |
| Extruded soybean                | 4.50      | Ca                     | 0.81            |
| Fish meal                       | 0.50      | TP                     | 0.57            |
| Whey powder                     | 8.00      | AP                     | 0.37            |
| Soybean protein concentrate     | 12.00     | Lys                    | 1.36            |
| Soybean oil                     | 1.90      | Met+Cys                | 0.75            |
| Sucrose                         | 3.50      | Thr                    | 0.79            |
| Limestone                       | 0.91      | Trp                    | 0.23            |
| Dicalcium phosphate             | 0.74      |                        |                 |
| Nacl                            | 0.25      |                        |                 |
| 78% Lys                         | 0.38      |                        |                 |
| DL-Met                          | 0.17      |                        |                 |
| 98.5%Thr                        | 0.05      |                        |                 |
| 98%Trp                          | 0.02      |                        |                 |
| Chloride choline                | 0.10      |                        |                 |
| Vitamin premix\(^1\)           | 0.05      |                        |                 |
| Mineral premix\(^2\)           | 0.20      |                        |                 |
| Total                           | 100.00    |                        |                 |

\(^1\)The premix provides following per kg diet: VA 5512 IU, VD\(_3\) 2250 IU, VE 24 mg, VK\(_1\) 3 mg, VB\(_2\) 6 mg, VB\(_3\) 3 mg, VB\(_12\) 24 μg, folic acid 1.2 mg, nicotinic acid 14 mg, biotin 150 μg, D-pantothenic acid 15 mg.

\(^2\)The premix provides following per kg diet: Fe100 mg, Cu6 mg, Mn 4 mg, Zn100 mg, I 0.14 mg, Se 0.3 mg.
RNA isolation and reverse transcription

Total RNA was isolated from liver, longissimus dorsi and abdominal fat by using Trizol Reagent (TaKaRa Biotechnology, Dalian, China) according to the manufacturer’s protocols. The purity and concentration of total RNA were determined by spectrophotometer detection (Beckman Coulter DU800), and the OD\textsubscript{260}/OD\textsubscript{280} ratio ranged from 1.8 to 2.0 in all samples. The RNA integrity was analyzed by 1% agarose gel electrophoresis. The RNA samples were reversely transcribed into complementary DNA by using RT Reagents (TaKaRa Biotechnology, Dalian, China) according to the manufacturer’s protocols.

Real-time quantitative PCR

Following reverse transcription, mRNA levels were analyzed by real-time quantitative PCR using SYBR Premix Ex Taq reagents (TaKaRa Biotechnology, Dalian, China) and CFX-96 Real-Time PCR Detection System (Bio-Rad Laboratories, Richmond, CA) as previously described [16]. The primers were purchased from TaKaRa Biotechnology (Dalian, China), which were shown in Table 2. The PCR condition was as follows: pre-denaturation 95°C for 30 s, followed by 40 cycles at 95°C for 5 s, then at annealing temperature for 30 s, finally at 72°C for 60 s. A melting curve was conducted to verify the specificity. Analysis of each sample was repeated in triplicate simultaneously on the same PCR plate. The average value of each triplicate was used for statistical analysis. The relative mRNA expression to the reference gene (β-actin) was determined in order to correct for the variance in amounts of RNA input in the reaction. In addition, the relative gene expressions compared to the reference gene were calculated with the previous method [17].

Table 2. Primer lists used for real time PCR assay.

| Gene name   | Sequence           |
|-------------|--------------------|
| β-actin     | Sense: TCTGGCACCACACCTTCT  |
|             | Antisense: TGATCTGGGTCATCTTCTTCTC  |
| FAS         | Sense: CTACGAGGCCATTGTTGACC  |
|             | Antisense: AGCCTATCACTGTAGTACCCC  |
| ACC         | Sense: AGCAAGGGCAGAGACGAGAAG  |
|             | Antisense: TAAGACCACCGCGGATAGA  |
| PPAR-α      | Sense: CGACCTGGAAGGCCGTTAT  |
|             | Antisense: GAGGCTTCTGCCCCACAGAT  |
| PPAR-γ      | Sense: CTCTTCTCAACAGAGAGGT  |
|             | Antisense: GCAGCCATCTATTAGAC  |
| PPAR—γ      | Sense: CCAGCATTTCCACTCCCACTACTA  |
|             | Antisense: GACACAGGGCCTCACTTTGATG  |
| SREBP-1c     | Sense: AAGCGGAGGCTCAACAATG  |
|             | Antisense: GCAAGAGGGGCTCTTTATCA  |
| LPL         | Sense: CACATCCCAAGAGGCGTC  |
|             | Antisense: TCAATGGAGCATTACCG  |
| CPT-1α      | Sense: GACAGTCCTCCACCCTCATC  |
|             | Antisense: GGGTTGTTGTGCCCAAACAG  |
| LIPE        | Sense: GCTTCTCTGAGAGCATCT  |
|             | Antisense: CACTGGGAGAAGGGAGCGT  |

FAS fatty acid synthase; ACC acetyl-CoA carboxylase; PPAR peroxisome proliferator activated receptor; SREBP-1c sterol regulatory element binding protein 1C; LPL lipoprotein lipase; CPT-1α carnitine palmitoyltransferase-1α; LIPE lipase hormone sensitive

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Statistical analysis

All data were analyzed by one-way ANOVA model followed by Duncan’s multiple-range tests using SPSS 20.0 for Windows statistical software package (Statistical Product and Service Solutions, Inc, USA). Results were presented as the mean and SE. *P* < 0.05 were used to assess statistical significance.

Results

Growth performance

According to Table 3, during the experimental period, pigs that were orally administered with SCFAs had lower ADFI compared with those in the control group (*P* < 0.05). However, no significant differences were observed among the three groups regarding ADG and F/G (*P* > 0.05).

Serum metabolites

As shown in Table 4, oral administration of SCFAs decreased the concentrations of TG, TC and HDL-C in serum (*P* < 0.05). Moreover, high dose SCFAs group had lower insulin concentration and greater leptin concentration in serum compared with control group (*P* < 0.05). We did not observe any differences in serum LDL-C, glucose and glucagon among the three groups (*P* > 0.05).

Table 3. Effects of SCFAs on the growth performance over the experimental period of weaned pigs.

|                | Control | SCFA (L) | SCFA (H) | SEM | *P*-value |
|----------------|---------|----------|----------|-----|-----------|
| Initial BW (kg)| 8.32    | 8.31     | 8.31     | 0.06| 0.994     |
| Final BW (kg)  | 9.33    | 9.13     | 9.30     | 0.27| 0.842     |
| ADFI (g/d)     | 258.10a | 218.69b  | 231.55ab | 8.21| 0.020     |
| ADG (g/d)      | 173.81  | 125.00   | 159.52   | 13.42| 0.071     |
| F/G            | 1.59    | 1.76     | 1.48     | 0.14| 0.389     |

SCFAs (L): acetic acid 20.04 mM, propionic acid 7.71 mM and butyric acid 4.89 mM; SCFAs (H): acetic acid 40.08 mM, propionic acid 15.42 mM and butyric acid 9.78 mM.

BW body weight; ADFI average daily feed intake; ADG average daily gain; F/G the ratio of feed to gain

Within a row, means without a common superscript differ (*P*<0.05).

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Table 4. Effects of SCFAs on the serum metabolites over the experimental period of weaned pigs.

|                 | Control | SCFA (L) | SCFA (H) | SEM | *P*-value |
|-----------------|---------|----------|----------|-----|-----------|
| TG mmol/L       | 0.56a   | 0.26b    | 0.24b    | 0.05| 0.004     |
| TC mmol/L       | 2.67a   | 1.63b    | 1.64b    | 0.19| 0.026     |
| HDL-c mmol/L    | 1.41a   | 0.88b    | 0.79b    | 0.09| 0.004     |
| LDL-c mmol/L    | 0.58    | 0.41     | 0.42     | 0.05| 0.378     |
| Glucose mmol/L  | 6.99    | 6.31     | 6.78     | 0.23| 0.499     |
| Insulin μU/mL   | 20.18a  | 14.11b   | 11.35b   | 1.51| 0.039     |
| Glucagon μU/mL  | 247.83  | 146.35   | 99.07    | 29.28| 0.100     |
| Leptin ng/mL    | 5.66b   | 5.79b    | 7.27a    | 0.27| 0.015     |

SCFAs (L): acetic acid 20.04 mM, propionic acid 7.71 mM and butyric acid 4.89 mM; SCFAs (H): acetic acid 40.08 mM, propionic acid 15.42 mM and butyric acid 9.78 mM.

TG triglyceride; TC total cholesterol; HDL-c high density lipoprotein-cholesterol; LDL-c low density lipoprotein-cholesterol

Within a row, means without a common superscript differ (*P*<0.05).

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Liver total fat and metabolites

As shown in Table 5, SCFAs treatments had lower total fat of liver than control group \( (P<0.05) \). Liver TG was also markedly decreased when treated with SCFAs \( (P<0.05) \). Furthermore, high dose SCFAs group had lower liver TC, compared with the other two groups \( (P<0.05) \). There were no differences in liver HDL-C, LDL-C, glucose, insulin, glucagon and leptin among the three groups \( (P>0.05) \).

The expressions of lipid metabolism related genes in liver, longissimus dorsi and abdominal fat

Corresponding to lower total fat in liver, oral administration of SCFAs down-regulated the mRNA expressions of fatty acid synthetize (FAS) and sterol regulatory element binding protein 1c (SREBP-1c), and enhanced the mRNA expression of carnitine palmitoyltransferase-1α (CPT-1α) in liver \( (P<0.05, \text{ Fig 1}) \). SCFAs also decreased FAS, acetyl-CoA carboxylase (ACC) and peroxisome proliferator activated receptor σ (PPARσ) mRNA levels in longissimus dorsi \( (P<0.05, \text{ Fig 2}) \). In abdominal fat, SCFAs down-regulated the mRNA expressions of FAS and ACC, and enhanced the mRNA expression of CPT-1α \( (P<0.05, \text{ Fig 3}) \).

Discussion

Obesity has become one of the most serious health problems all over the world, which contributes greatly to a complex of symptoms that are called metabolic syndrome [18]. And SCFAs, the main products of dietary fiber by bacterial fermentation, have been shown to play important roles in the prevention and cure of metabolic syndrome [19]. According to previous experiment in which beet pulp was used as a dietary fiber source, the average concentrations of SCFAs in colon were as follows: acetic acid 40.08 mM, propionic acid 15.42 mM and butyric acid 9.78 mM. SCFAs (L): acetic acid 20.04 mM, propionic acid 7.71 mM and butyric acid 4.89 mM; SCFAs (H) :acetic acid 40.08 mM, propionic acid 15.42 mM and butyric acid 9.78 mM. TG triglyceride; TC total cholesterol; HDL-c high density lipoprotein-cholesterol; LDL-c low density lipoprotein-cholesterol. a–b Within a row, means without a common superscript differ \( (P<0.05) \).

Table 5. Effects of SCFAs on the liver lipids and metabolites over the experimental period of weaned pigs.

|                     | Control | SCFA (L) | SCFA (H) | SEM | \( P\)-value |
|---------------------|---------|----------|----------|-----|--------------|
| Liver total fat (g/100g) | 3.34\(^a\) | 2.92\(^b\) | 2.79\(^b\) | 0.08 | 0.007        |
| TG mmol/L           | 1.05\(^a\) | 0.71\(^b\) | 0.71\(^b\) | 0.06 | 0.006        |
| TC mmol/L           | 0.93\(^a\) | 0.83\(^a\) | 0.51\(^b\) | 0.06 | 0.008        |
| HDL-c mmol/L        | 0.33     | 0.17     | 0.18     | 0.05 | 0.351        |
| LDL-c mmol/L        | 0.81     | 0.43     | 0.30     | 0.09 | 0.073        |
| Glucose mmol/L      | 10.72    | 9.87     | 9.35     | 0.39 | 0.714        |
| Insulin μIU/mL      | 31.01    | 29.65    | 29.73    | 0.77 | 0.743        |
| Glucagon μIU/mL     | 767.00   | 750.73   | 656.00   | 22.75| 0.092        |
| Leptin ng/mL        | 6.12     | 6.10     | 6.11     | 0.08 | 0.993        |

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Fig 1. Effects of SCFAs on mRNA expressions for key factors related to lipid metabolism in liver over the experimental period of weaned pigs. SCFAs (L): acetic acid 20.04 mM, propionic acid 7.71 mM and butyric acid 4.89 mM; SCFAs (H): acetic acid 40.08 mM, propionic acid 15.42 mM and butyric acid 9.78 mM. FAS fatty acid synthase; ACC acetyl-CoA carboxylase; PPAR peroxisome proliferator activated receptor; SREBP-1C sterol regulatory element binding protein 1C; LPL lipoprotein lipase; CPT-1α carnitine palmitoyltransferase-1α; LIPE lipase hormone-sensitive.

Within a row, means without a common superscript differ (P < 0.05).

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Fig 2. Effects of SCFAs on mRNA expressions for key factors related to lipid metabolism in longissimus dorsi over the experimental period of weaned pigs. SCFAs (L): acetic acid 20.04 mM, propionic acid 7.71 mM and butyric acid 4.89 mM; SCFAs (H): acetic acid 40.08 mM, propionic acid 15.42 mM and butyric acid 9.78 mM. FAS fatty acid synthase; ACC acetyl-CoA carboxylase; PPAR peroxisome proliferator activated receptor; SREBP-1C sterol regulatory element binding protein 1C; LPL lipoprotein lipase; CPT-1α carnitine palmitoyltransferase-1α; LIPE lipase hormone-sensitive.

Within a row, means without a common superscript differ (P < 0.05).

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Previous research has demonstrated that SCFAs suppress appetite through a central homeostatic mechanism [20]. This can be due to the fact that SCFAs stimulate the release of anorexigenic hormones such as peptide tyrosine tyrosine and glucagon-like peptide 1, and then delay gastric emptying [21]. In our study, oral administration of SCFAs significantly decreased ADFI and tended to decrease ADG, which were similar to the results of previous studies [22, 23].

Numerous changes in plasma lipid and lipoprotein are easily observed in obesity [24]. Indeed, obese individuals are often characterized by higher fasting plasma TG and TC compared with lean ones [25]. Here, we reported that oral administration of SCFAs decreased the concentrations of serum TG, TC and HDL-C, which were consistent with the results of previous studies about different SCFAs [26, 27]. These indicated that SCFAs might be beneficial to lowering some risk factors for obesity and diabetes.

Insulin is an important peptide hormone secreted by pancreatic beta cells, which modulates blood glucose level through inhibiting glycogen lysis. Thus, the balance between insulin production and insulin function is the key for maintaining glucose homeostasis [28]. Leptin, an adipose-derived hormone, can regulate energy homeostasis and several physiological processes (like feeding behavior and metabolic rate) [29], which is relative with inhibiting neuropeptide Y/agouti-related peptide neurons and activating POMC/cocaine and amphetamine-regulated transcript neurons [30]. In addition, leptin knock-out mice exhibited hyperphagia and obesity while leptin administration could reverse these effects [31]. In this study, SCFAs decreased insulin level, and increased leptin level in serum, which were consistent with previous in vivo and in vitro studies [32, 33]. Therefore, it was possible that SCFAs administration decreased ADFI and lipid metabolism via regulating the levels of relative hormones.

Fig 3. Effects of SCFAs on mRNA expressions for key factors related to lipid metabolism in abdominal fat over the experimental period of weaned pigs. SCFAs (L): acetic acid 20.04 mM, propionic acid 7.71 mM and butyric acid 4.89 mM; SCFAs (H): acetic acid 40.08 mM, propionic acid 15.42 mM and butyric acid 9.78 mM. FAS fatty acid synthase; ACC acetyl-CoA carboxylase; PPAR peroxisome proliferator activated receptor; SREBP-1C sterol regulatory element binding protein 1C; LPL lipoprotein lipase; CPT-1α carnitine palmitoyltransferase-1α; LIPE lipase hormone-sensitive. *Within a row, means without a common superscript differ (P < 0.05).
Nonalcoholic fatty liver has been regarded as one of the most common liver diseases in the world, which is associated with obesity, insulin resistance and metabolic syndrome [34]. And the storage of TG in non-adipose tissues (like liver and skeletal muscle) that are known as ectopic fat deposition can lead to metabolic disorder and impaired organ function [35, 36]. In current study, we were pleased to find that oral administration of SCFAs decreased total fat as well as the concentrations of TG and TC in liver of pigs. Similarly, propionate could reduce intrahepatocellular lipid contents that met the diagnostic criteria of non-alcoholic fatty liver disease [27]. Therefore, SCFAs administration could be recommended as the therapy of nonalcoholic fatty liver.

SREBPs are lipid synthetic transcription factors that regulate the synthesis of cholesterol and fatty acid in liver [37, 38]. Previous study reported SCFAs suppressed cholesterol synthesis in rat liver and intestine [39]. We also found that liver TC level was lower in SCFAs groups compared with control group, which could be due to the fact that SCFAs down-regulated the mRNA expression of SREBP-1c in liver. FAS is an enzyme that catalyzes fatty acid synthesis while CPT-1α is a rate-limiting enzyme that participates in fatty acid oxidation [40]. ACC is an enzyme that regulates the metabolism of fatty acids, and its product, malonyl-CoA, act as building block for de novo fatty acid synthesis [41]. PPAR is a member of the nuclear receptor superfamily and regulates adipocyte differentiation and fat depotation [40]. In this study, SCFAs treatment down-regulated FAS mRNA level in liver, decreased FAS, ACC and PPARσ mRNA levels in longissimus dorsi, inhibited FAS and ACC mRNA levels in abdominal fat, and enhanced CPT-1α mRNA level in liver and abdominal fat. There were some similar changes of muscles and adipose tissues in previous studies [26, 42]. In addition, recent study showed that butyrate and other SCFAs increased the rate of lipolysis in vitro [43]. These suggested that SCFAs could reduce lipid accumulation by decreasing fatty acid synthesis and increasing lipolysis in different tissues.

Conclusion

In summary, we demonstrated that SCFAs attenuated fat deposition in pigs via inhibiting feed intake, modulating endocrine and regulating lipid metabolism, which could provide some insights into the new therapies of obesity and nonalcoholic fatty liver.

Supporting information

S1 File. Minimal data set.
(XLSX)

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References

1. Vernarelli JA, Mitchell DC, Rolls BJ, Hartman TJ. Dietary energy density is associated with obesity and other biomarkers of chronic disease in US adults. European Journal of Nutrition. 2015; 54(1):59–65. https://doi.org/10.1007/s00394-014-0685-0 PMID: 24664188
2. Sanz Y, Santacruz A, Gauffin P. Gut microbiota in obesity and metabolic disorders. Proceedings of the Nutrition Society. 2010; 69(3):434–41. https://doi.org/10.1017/S0029665510001813 PMID: 20540826
3. Swinburn BA, Sacks G, Hall KD, Mcpherson K, Finegood DT, Moodie ML, et al. The global obesity pandemic: shaped by global drivers and local environments. Lancet. 2011; 378(9793):804–14. https://doi.org/10.1016/S0140-6736(11)60813-1 PMID: 21872749
4. Chambers ES, Morrison DJ, Gary F. Control of appetite and energy intake by SCFA: what are the potential underlying mechanisms? Proc Nutr Soc. 2015; 74(3):328–36. https://doi.org/10.1017/S0029665114001657 PMID: 25497601
5. Du H, van der A DL, Boshuizen HC, Forouhi NG, Wareham NJ, Halkjaer J, et al. Dietary fiber and subsequent changes in body weight and waist circumference in European men and women. American Journal of Clinical Nutrition. 2010; 91(2):329. https://doi.org/10.3945/ajcn.2009.28191 PMID: 20016015
6. Liu S, Willett WC, Manson JAE, Hu FB, Rosner B, Colditz G. Relation between changes in intakes of dietary fiber and grain products and changes in weight and development of obesity among middle-aged women. American Journal of Clinical Nutrition. 2003; 78(5):920. https://doi.org/10.1093/ajcn/78.5.920 PMID: 14594777
7. Po-Wah S, Yu WS, Yu-Ting K, Clive W, Goldstone AP, Bell JD, et al. Impact of Resistant Starch on Body Fat Pattern and Central Appetite Regulation. Plos One. 2007; 2(12):e1309. https://doi.org/10.1371/journal.pone.0001309 PMID: 18074032
8. Cani PD, Knauf C, Iglesias MA, Drucker DJ, Delzenne NM, Burcelin R. Improvement of glucose tolerance and hepatic insulin sensitivity by oligofructose requires a functional glucagon-like peptide 1 receptor. Diabetes. 2006; 55(5):1484. PMID: 16644709
9. Parnell JA, Reimer RA. Weight loss during oligofructose supplementation is associated with decreased ghrelin and increased peptide YY in overweight and obese adults. American Journal of Clinical Nutrition. 2009; 89(6):1751–9. https://doi.org/10.3945/ajcn.2009.27465 PMID: 19387471
10. Topping DL, Clifton PM. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. Physiological Reviews. 2001; 81(3):1031–64. https://doi.org/10.1152/physrev.2001.81.3.1031 PMID: 11427681
11. Rioscovián D, Ruasmadiedo P, Margolles A, Gueimonde M, Cg RG, Salazar N. Intestinal Short Chain Fatty Acids and their Link with Diet and Human Frontiers in Microbiology. 2016; 7:185. https://doi.org/10.3389/fmicb.2016.00185 PMID: 26925050
12. Gao Z, Yin J, Zhang J, Ward RE, Martin RJ, Lefevre M, et al. Butyrate improves insulin sensitivity and increases energy expenditure in mice. Diabetes. 2009; 58(7):1509. https://doi.org/10.2337/db08-1637 PMID: 19368664
13. Kimura I, Ozawa K, Inoue D, Immamura T, Kimura K, Maeda T, et al. The gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. Nature Communications. 2013; 4(11):1829.
14. Wan J, Jiang F, Xu Q, Chen D, Yu B, Huang Z, et al. New insights into the role of chitosan oligosaccharide in enhancing growth performance, antioxidant capacity, immunity and intestinal development of weaned pigs. Rsc Advances. 2017; 7(16):9669–79.
15. Sukhija PS, Palmquist DL. Rapid method for determination of total fatty acid content and composition of feedstuffs and feces. Journal of Agricultural & Food Chemistry. 1988; 36(6):1202–6.
16. Mao X, Gu C, Hu H, Tang J, Chen D, Yu B, et al. Dietary Lactobacillus rhamnosus GG Supplementation Improves the Mucosal Barrier Function in the Intestine of Weaned Piglets Challenged by Porcine Rotavirus. Plos One. 2016; 11(1):e0146312. https://doi.org/10.1371/journal.pone.0146312 PMID: 26727003
17. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2012; 25(4):402–8.
18. Kahn SE, Hull RL, Utzschneider KM. Mechanisms linking obesity to insulin resistance and type 2 diabetes. Nature. 2006; 444(7121):840. https://doi.org/10.1038/nature05482 PMID: 1716471
19. Donohoe DR, Garge N, Zhang X, Sun W, O’Connell TM, Bunger MK, et al. The Microbiome and Butyrate Regulate Energy Metabolism and Autophagy in the Mammalian Colon. Cell Metabolism. 2011; 13(5):517–26. https://doi.org/10.1016/j.cmet.2011.02.018 PMID: 21531334
20. Frost G, Sleeth ML, Sahuri-Ardakani M, Lizarbe B, Cerdan X, Brody L, et al. The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. Nature Communications. 2014; 5(3611):3611.
21. Gwenn T, Helen H, Shan LY, Parker HE, Habib AM, Eleftheria D, et al. Short-Chain Fatty Acids Stimulate Glucagon-Like Peptide-1 Secretion via the G-Protein–Coupled Receptor FFAR2. Diabetes. 2012; 61(2):364–71. https://doi.org/10.2337/db11-1019 PMID: 22190648
22. Yamashita H, Fujisawa K, Ito E, Idei S, Kawaguchi N, Kimoto M, et al. Improvement of obesity and glucose tolerance by acetate in Type 2 diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats. Bioscience Biotechnology & Biochemistry. 2007; 71(5):1236–43.
23. Lin HV, Frassetto A, KEJ, Nawrocki AR, Lu MM, Kosinski JR, et al. Butyrate and Propionate Protect against Diet-Induced Obesity and Regulate Gut Hormones via Free Fatty Acid Receptor 3-Independent Mechanisms. Plos One. 2012; 7(4):e35240. https://doi.org/10.1371/journal.pone.0035240 PMID: 22506074
24. Kissebah AH, Krakower GR. Regional adiposity and morbidity. Physiological Reviews. 1994; 74(4):761–811. https://doi.org/10.1152/physrev.1994.74.4.761 PMID: 7938225
25. Couillard C, Bergeron N, H D, Bergeron J, Tremblay A, Bouchard C, Mauriege P, et al. Postprandial triglyceride response in visceral obesity in men. Diabetes. 1998; 47(6):953–60. PMID: 9604874
26. Yu S, Ren E, Xu J, Su Y, Zhu W. Effects of early intervention with sodium butyrate on lipid metabolism-related gene expression and liver metabolite profiles in neonatal piglets. Livestock Science. 2017; 195:80–6.
27. Chambers ES, Viardot A, Psichas A, Morrison DJ, Murphy KG, Zacvarghese SEK, et al. Effects of targeted delivery of propionate to the human colon on appetite regulation, body weight maintenance and adiposity in overweight adults. Gut. 2015; 64(11):1744. https://doi.org/10.1136/gutjnl-2014-307913 PMID: 25500202
28. Puddu A, Sanguineti R, Montecucco F, Viviani GL. Evidence for the gut microbiota short-chain fatty acids as key pathophysiological molecules improving diabetes. Mediators Inflamm. 2014; 2014 (6):162021.
29. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature. 1994; 372(6505):425–32. https://doi.org/10.1038/372425a0 PMID: 7624777
30. Baskin DG, Breiningier JF, Schwartz MW. Leptin receptor mRNA identifies a subpopulation of neuro-peptide Y neurons activated by fasting in rat hypothalamus. Diabetes. 1999; 48(4):828. PMID: 10102700
31. Halaas JL, Gajiwala KS, Maffei M, Cohen SL, Chait BT, Rabinowitz D, et al. Weight-reducing effects of the plasma protein encoded by the obese gene. Science. 1995; 269(5223):543–6. PMID: 7624777
32. Xiong Y, Miyamoto N, Shibata K, Valasek MA, Motoike T, Kedzierski RM, et al. Short-chain fatty acids stimulate leptin production in adipocytes through the G protein-coupled receptor GPR41. Proceedings of the National Academy of Sciences of the United States of America. 2004; 101(4):1045–50. https://doi.org/10.1073/pnas.2637002100 PMID: 14722361
33. Zaibi MS, Stocker CJ, O’Dowd J, Davies A, Bellahcene M, Cawthorne MA, et al. Roles of GPR41 and GPR43 in leptin secretory responses of murine adipocytes to short chain fatty acids. Fedics Letters. 2010; 584(11):2381–6. https://doi.org/10.1016/j.fedlets.2010.04.027 PMID: 20399779
34. Angulo P. Nonalcoholic fatty liver disease. Journal of Gastroenterology & Hepatology. 2007; 65(Supplement s1):S57–S63.
35. Shulman GI. Cellular mechanisms of insulin resistance. Journal of Clinical Investigation. 2000; 106(2):171–6. https://doi.org/10.1172/JCI10583 PMID: 10903330
36. Malecha S. Ectopic Fat and Insulin Resistance: Pathophysiology and Effect of Diet and Lifestyle Interventions. International Journal of Endocrinology,2012,(2012-05-24). 2012:2012(7):983814.
37. Shimano H. Sterol regulatory element-binding protein family as global regulators of lipid synthetic genes in energy metabolism. Vitamins & Hormones-advances in Research & Applications. 2002; 65(4):167–94.
38. Horton JD, Shimomura I. Sterol regulatory element-binding proteins: activators of cholesterol and fatty acid biosynthesis. Current Opinion in Lipidology. 1999; 10(2):143. PMID: 10327282

39. Hara H, Haga S, Y, Kiriyama S. Short-chain fatty acids suppress cholesterol synthesis in rat liver and intestine. Journal of Nutrition. 1999; 129(5):942. https://doi.org/10.1093/jn/129.5.942 PMID: 10222383

40. Yan H, Zheng P, Yu B, Yu J, Mao X, He J, et al. Postnatal high-fat diet enhances ectopic fat deposition in pigs with intrauterine growth retardation. European Journal of Nutrition. 2017; 56(2):1–8.

41. Kim KH. Regulation of mammalian acetyl-coenzyme A carboxylase. Annual Review of Nutrition. 1997; 17(1):77.

42. Den BG, Bleeker A, Gerding A, Van EK, Havinga R, van Dijk TH, et al. Short-Chain Fatty Acids Protect Against High-Fat Diet-Induced Obesity via a PPAR-dependent Switch From Lipogenesis to Fat Oxidation. Diabetes. 2015; 64(7):2398–408. https://doi.org/10.2337/db14-1213 PMID: 25695945

43. Rumberger JM, Arch JR, Green A. Butyrate and other short-chain fatty acids increase the rate of lipolysis in 3T3-L1 adipocytes. Peerj. 2014; 2(Suppl 1):e611.