Exogenous butyrate regulates lipid metabolism through GPR41-ERK-AMPK pathway in rabbits

Bin Zhang, Hongli Liu, Mengqi Liu, Zhengkai Yue, Lei Liu and Li Fuchang

Shandong Provincial Key Laboratory of Animal Biotechnology and Disease Control and Prevention, Shandong, China; Department of Animal Science, Shandong Agricultural University, Taian, China

ABSTRACT

Diets containing higher levels of fat can lead to obesity, which is potentially harmful to health. Endogenous butyric acid is produced by intestinal microbial fermentation and participates in lipid metabolism. However, there is less butyric acid produced in the body, butyric acid has a shorter half-life in the blood. We used intraperitoneal injection of sodium butyrate to study the mechanism of butyrate involved in body lipid metabolism. Triglycerides in adipose tissue and liver were significantly reduced by butyrate. The content of triglyceride in plasma in butyrate group was also significantly decreased. In adipose tissue, butyrate up-regulated gene expression of hormone-sensitive lipase (HSL), carnitine palmitoyl transferase (CPT)1 and CPT2, and increased protein expression of G protein-coupled receptor (GPR)41, phosphorylated extracellular-signal-regulated kinase (P-ERK), adenosine 5'-monophosphate (AMP)-activated protein kinase (AMPK) and P-AMPK. Gene expression of lipoprotein lipase (LPL), differentiation-dependent factor 1 (ADD1) and fatty acid synthase (FAS) was down-regulated. In liver, butyrate up-regulated protein expression of GPR41, ERK, P-ERK, P-AMPK. Gene expression of ADD1 and FAS was down-regulated. In muscle tissue, butyrate significantly up-regulated the expression of gene LPL, fatty acid transport protein (FATP) and fatty acid-binding protein (FABP) and protein GPR41, GPR43. Thus, butyrate is involved in body lipid metabolism mainly through the GPR41-mediated ERK-AMPK pathway. Inhibits lipid synthesis in adipose tissue and the liver and promotes lipolysis, avoiding obesity caused by diets with higher fat content.

HIGHLIGHTS

- Butyrate affects lipid metabolism in the body by affecting lipid synthesis/decomposition in adipose tissue and liver.
- Butyrate mainly through GPR41-mediated ERK-AMPK pathway, inhibit lipid synthesis, promote lipid decomposition, and avoid a large amount of fat accumulation induced by higher-fat diet.

Introduction

Lipid metabolism, including fatty acid transport, uptake, catabolism, and storage (Shen et al. 2015; Liu et al. 2019). These processes are mainly carried out through coordination in the liver, muscle and adipose tissue. The liver plays a key role in lipid metabolism. Depending on species it is, more or less, the hub of fatty acid synthesis and lipid circulation through lipoprotein synthesis (Nguyen et al. 2008). Except in avian species in which de novo lipogenesis (DNL) occurs in the liver, DNL in many other animals is centred in the adipose tissues (Bergen and Mersmann 2005). Skeletal muscle is an important place for fatty acid oxidation, at the same time, the liver and adipose tissue can also consume some fatty acids.

Short-chain fatty acids (SCFAs), primarily acetate, propionate and butyrate, are organic acids produced within the intestinal lumen by bacterial fermentation of mainly undigested dietary carbohydrates (Koh et al. 2016). The addition of short-chain fatty acids to diets...
has shown to prevent obesity, making short-chain fatty acids promising candidates for preventing energy metabolism disorders (Lin et al. 2012; Yin et al. 2016; Liu et al. 2017). Among short-chain fatty acids, especially the addition of butyrate has been found to have multiple metabolic benefits, including prevention of higher-fat diet-induced obesity, insulin resistance, and liver steatosis. Sodium butyrate is also an effective regulator of insulin homeostasis (Gao et al. 2009; Guilloteau et al. 2010; Li et al. 2018). Intravenous injection of sodium butyrate in mammals could induce fatty acid oxidation and promote cholesterol metabolism, but has no significant effect on animal appetite (Li et al. 2018; Ren et al. 2018). In the cell culture model in vitro, butyrate reduced the concentration of 22:5 (n-6) in the cellular lipids and increased the rate of lipolysis in 3T3-L1 adipocytes (Awad et al. 1991; Rumberger et al. 2014). It impaired lipid transport by inhibiting microsomal triglyceride transfer protein in Caco-2 cells (Marcil et al. 2003), reduced the secretion of triglycerides and phospholipids (Marcil et al. 2002).

G protein-coupled receptors (GPCRs) are large and diverse families of transmembrane proteins. In 2003, SCFAs were found to be ligands of orphan GPR41 and GPR43, and thus were named free fatty acids receptors 3 and 2, respectively (Brown et al. 2003). Butyrate may bind to its receptors G protein-coupled receptor (GPR41 and 43) to activate G protein-mediated second messenger signalling pathways (Nilsson et al. 2003). Among cell signalling pathways, MAPK pathway plays an important role in cell metabolism, proliferation, differentiation, apoptosis and other processes. In eukaryotic cells, there are mainly four MAPK signalling cascade circuits, namely ERK, JNK/stress-activated protein kinase, p38 MAPK and ERK5. ERK is a member of MAPK family, and ERK/MAPK pathway is the core of signal network involved in the regulation of cell metabolism, growth, development and division (Guo et al. 2020). Akt/PKB protein kinase is a serine/threonine kinase, belonging to the cAMP-dependent protein kinase A/protein kinase G/protein kinase C (AGC) superfamily, with structural homology in its catalytic domain and similar activation mechanisms (Song et al. 2005). In previous studies, Akt/PKB has become the core role of growth factor or insulin-activated signal transduction pathway and is believed to contribute to a variety of cellular functions, including nutritional metabolism, cell growth, transcriptional regulation and cell survival (Brazil and Hemmings 2001). The AMP-activated protein kinase (AMPK) is a key regulator of catabolism and anabolic processes. As an energy sensor, it can combine the energy state of the cell with the metabolic environment. These adaptations can occur not only through acute regulation of key metabolic enzymes directly phosphorylated but also through slower transcriptional adaptation responses (Cantó and Auwerx 2010). Peroxisome proliferator-activated receptor α (PPARα) is highly expressed in the liver, adipose and skeletal muscle tissues that use a lot of lipid-derived energy, where it regulates a set of enzymes crucial for fatty acid oxidation. Its primary role is to increase the cellular capacity to mobilise and catabolise fatty acids (Nguyen et al. 2008). Peroxisome proliferator-activated receptor γ (PPAR γ) participates in regulating energy metabolism, promoting fat formation and maintaining adipocyte differentiation (Rosen and MacDougald 2006; Sevane et al. 2013). The transcription factor sterol regulatory element binding protein-1c/differentiation-dependent factor 1 (SREBP-1c/ADD1) has an important role in the control of fatty acid synthase (FAS) expression (Horton et al. 2002). Typically SREBP-1c and FAS genes are both expressed and correlated in tissues that synthesise fatty acids de novo (Gondret et al. 2001). FAS and carnitine palmitoyltransferase 1/2 (CPT 1/2) are involved in the regulation of fatty acid synthesis and catabolism, respectively (Thupari et al. 2001). Hormone-sensitive lipase (HSL) is a multifunctional enzyme involved in fatty acid metabolism that hydrolyses triacylglycerols (TAGs), diacylglycerols (DAGs), monoacylglycerols (MAGs), retinyl esters (REs), cholesterol esters (CEs) and other lipids in adipose tissue (Kraemer and Shen 2002; Lampidonis et al. 2011). A major step in energy metabolism is hydrolysis of triacylglycerol-rich lipoproteins (TRLs) to release fatty acids that can be used or stored. This is accomplished by lipoprotein lipase (LPL) (Olivecrona 2016). The main functions of the fatty acid transport protein (FATP) and the fatty acid-binding protein (FABP) are to regulate cellular uptake and intracellular transport of fatty acids (Chmurzyńska 2006).

Our hypothesis is that intraperitoneal injection of sodium butyrate will affect fatty acid synthesis, transport and oxidation in rabbits, and have a positive effect on rabbits lipid metabolism. The aim of this study was, therefore, to analyse the effect of sodium butyrate injection on fat deposition, fat mobilisation and to investigate which regulatory factors and signalling pathways are involved in metabolism.

Materials and method

Animals

Male Hyla rabbits were housed individually in cages (60 × 40 × 40 cm). Rabbits house temperature is 23°C,
natural light, free to eat and drink. The basic feed formula was formulated according to NRC (United States National Research Council, 1977) and Nutrition of the rabbit (Blas and Wiseman 1998, 2010). Additional soya oil was added to the basal diet to prepare a test diet with a higher fat content. The composition and nutrient levels of the higher-fat diet are shown in Table 1. All rabbits were fed the same diet.

### Experimental protocol and sample collection

**Pre-experiment:** sixty male Hyla rabbits (40-day old) with similar body weight (1500 ± 10 g) were divided into 6 groups (10 rabbits per group). We selected six doses based on previous studies (Reolon et al. 2011; Khan and Jena 2014) and injected sodium butyrate intraperitoneally. The doses of sodium butyrate injected into the six groups were: 100 mg/kg/day, 300 mg/kg/day, 500 mg/kg/day, 750 mg/kg/day, 1000 mg/kg/day and 2000 mg/kg/day. After 5 days of the test, according to the rabbit’s feed intake, daily gain and health status after injection, we selected 500 mg/kg/day injection dose for the formal test.

**Formal experiment:** forty male Hyla rabbits (40-day old) with similar body weight (1500 ± 10 g) were divided into 2 groups (20 rabbits per group): control group with saline (0.9% NaCl, pH 7.4, 37 °C) intraperitoneal injection for 5 days, and the butyrate treated group received an intraperitoneal injection of Sodium butyrate (200 mg/mL, pH 7.4, 37 °C) for 5 days (500 mg/kg body weight per day). The control group was injected with the same volume of saline as the sodium butyrate group. The injection time is 8:00 a.m. every day. Body weight and feed intake were recorded daily during the experiment. On the last day of the experiment, 6 rabbits were randomly selected from each group. After injection at 8 o’clock in the morning, blood samples were collected at 08:05, 08:10, 08:15, 08:20, 08:30, 09:00 and 10:00. Blood collection needles and anticoagulant vacuum blood collection tubes were used to collect rabbits plasma from ear veins. Centrifuged at 3000 xg for 10 min, aspirated the supernatant, and stored at −80 °C. The samples of rabbits in each group were collected, weighed, counted and analysed separately. Rabbits were sacrificed by cervical dislocation, and the liver, skeletal muscle, shoulder fat, Subcutaneous fat, perirenal fat were collected, weighed and snap frozen in liquid nitrogen and stored at −80 °C.

### Measurements

#### GC/MS
The two groups of plasma samples stored in −80 °C were melted and centrifuged for 60 s at 3000 xg, and the clear liquid of the upper layer was transferred to a new centrifuge tube. The content of plasma butyrate was detected using GC/MS method described by Zhang et al. (2019).

#### Oil red O staining
Adipose tissue, liver, muscle oil red O staining: The tissue samples were first made into frozen sections and then stained with oil red O (Mehlem et al. 2013). Image J software (NIH Image J system, Bethesda, MD) was used for quantitative analysis.

#### Plasma biochemical
Plasma glucose, triglyceride and total cholesterol were detected by Japanese automatic biochemical analyser HITACHI 7020. Plasma VLDL content, hepatic lipase and lipoprotein lipase enzyme activity was determined using the method described by previous study (Zhu 2018; Han et al. 2019).

#### Quantitative real-time PCR
Total RNA extraction and qRT-PCR were carried out according to the previous research description (Zhang et al. 2011; Du et al. 2013; Liu et al. 2019a, 2019b). The quality and quantity of RNA were determined by agarose gel electrophoresis and biophotometer (Eppendorf, Germany), respectively. The primers were designed for exon-intron junctions using Primer 6.0 software (Primer-E Ltd., Plymouth, UK). The primer

### Table 1. Composition and nutrient levels of higher-fat diet (air-dry basis).

| Ingredients       | Content (%) | Nutrient levels<sup>b</sup> | Content       |
|-------------------|-------------|-----------------------------|---------------|
| Corn              | 20          | DE (MJ/kg)                  | 11.41         |
| Soybean meal      | 13          | DM (%)                      | 91.33         |
| Wheat bran        | 17          | CP (%)                      | 16.28         |
| Alfalfa meal      | 20          | Ash (%)                     | 7.39          |
| Rice bran         | 4           | EE (%)                      | 5.01          |
| Wheat middlings   | 8           | CF (%)                      | 13.57         |
| Soybean husk powder | 5           | Ca (%)                      | 0.98          |
| Germ meal         | 3           | P (%)                       | 0.56          |
| Bean straw powder | 5           |                             |               |
| Soya-bean oil     | 1           |                             |               |
| Premix<sup>a</sup> | 4           |                             | 4             |
| Total             | 100         |                             |               |

<sup>a</sup>The premix provided the following per kg of diets: VA 12,000 IU, VD 900 IU, VE 50 mg, VK3 1 mg, VB1 1 mg, VB2 3 mg, VB6 1 mg, VB12 10 μg, choline chloride 500 mg, Fe (as ferrous sulfate) 100 mg, Zn (as Zinc) 50 mg, Se (as Selenium) 0.05 mg, I (as iodine) 0.6 mg, Lys 1500 mg, Met 1500 mg. The rest was miscellaneous meal carrier complement.

<sup>b</sup>Nutrient levels were measured values. DE: digestible energy; DM: dry matter; CP: crude protein; EE: ether extract; CF: crude fibre; Ca: calcium; P: phosphorus.
sequences are shown in Table 2. The PCR data were analysed with the $2^{-\Delta\Delta CT}$ method (Livak and Schmittgen 2001). The mRNA levels of target genes were normalised to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) and $\beta$-actin ($\Delta CT$) (Liu et al. 2016, 2019; Wu et al. 2019). Based on the cycle

| Gene     | Genebank accession number. | Primers sequences (5'→3') | Product size (bp) |
|----------|-----------------------------|---------------------------|-------------------|
| $\beta$-actin | NM_001101683.1 | F:GCAGAAAAGAGAAGCTTAT | 168 |
| ADD1     | XM_008249862.1         | F:GCTTAAGTACAACACATCAA | 141 |
| CPT1     | XM_002724092.2         | F:ATCTCACTGGTTTCTGAGC | 196 |
| CPT2     | XM_008265231.1         | F:AGGGGTCTTTCTAGGACAC | 101 |
| FABP     | XM_002716060           | F:AGCTGGTGAAGACAGAAT | 129 |
| FATP     | KF201292.1             | F:ACACGTTACCGCGATGACT | 112 |
| GAPDH    | NM_001082253           | F:TGACACCCACTCTCTACCTCG | 163 |
| HSL      | XM_008249691.2         | F:CCAGGTATGAGGTCTCCTA | 119 |
| LPL      | NM_001177330.1         | F:TTCAACCAAGACAGAAGAC | 141 |
| PPARα    | XM_002723354           | F:AGCCCTCTTCAGAAGCTGT | 122 |
| PPARγ    | NM_001082148.1         | F:GGAGCAAGGAAGAAGATCG | 111 |

ADD1: differentiation-dependent factor 1 (sterol regulatory element binding protein-1c; SREBP-1c); CPT1/2: carnitine palmitoyltransferase 1/2; FABP: fatty acid binding protein; FAS: fatty acid synthase; FATP: fatty acid transporter; GAPDH: glyceraldehyde 3-phosphate dehydrogenase; HSL: hormone-sensitive lipase; LPL: lipoprotein lipase; PPARα: peroxisome proliferator-activated receptor α; PPARγ: peroxisome proliferator-activated receptor γ

Figure 1. Feed intake (a, c), daily gain (d) and the content of butyrate in plasma (b). (a) Changes of average daily feed intake during the trial period. (b) The content of butyrate in plasma after injection of butyrate on the fifth day of the experiment. (c) Average daily feed intake = total feed intake/5; (d) average daily gain = total weight gain/5; values are means ± SEM (n = 6). *p < .05 compared with the control.
threshold (CT) values, GAPDH and β-actin mRNA expression were stable across treatments in this study ($p > .1$).

**Western blot**
Six samples of adipose tissue, liver and muscle were selected, and WB was used to detect the relative expression levels of AMPK and p-AMPK in their respective tissues. The specific test procedures refer to the previous studies in Mahmood and Yang (2012).

Monoclonal mouse anti-GAPDH antibody was used as a loading control. Western blots were developed and quantified using BioSpectrum 810 with VisionWorksLS 7.1 software (UVP LLC, Upland, CA). Image J software was used for quantitative analysis (NIH Image J system, Bethesda, MD).

**Statistical analysis**
Use SPSS 26 software (SPSS, Chicago, IL) for data statistics. Results are presented as mean ± SEM. Statistical

![Box plots of tissue and organ weight](image)

**Figure 2.** Tissue and organ weight at the end of the experiment. Reference carcass weight: weight of the chilled carcass (carcase after chilling for 24 h in a ventilated cold room (0–4 °C) about 1 h after slaughter) minus the head and the above mentioned organs (liver, kidney, organs of chest and neck). Total fat weight: shoulder fat + subcutaneous fat + perirenal fat. *$p < .05$ compared with the control ($n = 6$).
significance was determined at \( p < .05 \). Student’s t-test was used to compare differences between two groups. GraphPad Prism 7 software (GraphPad Software, La Jolla, CA) is used for drawing.

**Results**

There were no significant changes \( (p > .05) \) in feed intake, average daily feed intake and average daily gain in the butyrate group injected with sodium butyrate intraperitoneally compared with the control group injected with normal saline (Figure 1(a, c, d)). From 5 to 30 min after injection of sodium butyrate, the content of plasma butyrate in the butyrate group was significantly higher \( (p < .05) \) than that in the control group (Figure 1(b)).

Compared to the control group, the butyrate group significantly reduced \( (p < .05) \) subcutaneous fat and total fat content, while having no significant effect \( (p > .05) \) on reference carcass weight, liver weight, shoulder fat weight and perirenal fat weight (Figure 2). As shown in Figure 3, the oil red staining results of adipose tissue, liver and muscle tissue showed that sodium butyrate group significantly reduced \( (p < .05) \) the content of triglycerides in adipose tissue and liver (Figure 3(A, a, (a), B, b, (b))). However, there was no effect on triglyceride content in muscle between the two groups \( (p > .05); \) Figure 3(C, c, (c))).

![Figure 3](image_url)

**Figure 3.** Oil red O staining of adipose (A, a), liver (B, b) and muscle (C, c) tissue sections. (a), (b), (c): Image J software was used to make statistics on the results of the dyed pictures, and SPSS was used for analysis. Values are means ± SEM \( (n = 3) \). * \( p < .05 \) compared with the control.
**Figure 4.** The contents of glucose (a), triglyceride (b), total cholesterol (c), very low density lipoprotein (d), hepatic lipase (e) and lipoprotein lipase (f) in plasma of rabbits. *p < .05 compared with the control (n = 6).

**Figure 5.** The protein expression of GPR41 and GPR43 in adipose tissue (a), liver (b) and muscle (c). Image J software was used to make statistics on the results of the western blot (WB), and SPSS (SPSS Inc., Chicago, IL) was used for analysis. Values are means ± SEM (n = 6). *p < .05 compared with the control.
It was found that the contents of triglyceride and very low density lipoprotein in plasma of butyrate group decreased significantly (Figure 4(b, d); \( p < .05 \)). Plasma hepatic lipase and lipoprotein lipase activities were significantly increased (Figure 4(e, f); \( p < .05 \)). There was no significant change (\( p > .05 \)) in the contents of glucose and total cholesterol in plasma (Figure 4(a, c)).

As shown in Figure 5, compared with the control group, butyrate significantly up-regulated (\( p < .05 \)) the protein expression of GPR41 in adipose tissue, liver and muscle. At the same time, the protein expression of GPR43 was significantly up-regulated (\( p < .05 \)) in muscle, but had no significant (\( p > .05 \)) effect in adipose tissue and liver.

Compared with the control group, butyrate significantly up-regulated (\( p < .05 \)) the protein expression of ERK in liver, while no significant change in adipose tissue and muscle (\( p > .05 \)). AKT expression was increased in adipose tissue, liver and muscle of rabbits in the sodium butyrate group, but not significantly (\( p > .05 \)). Butyrate group up-regulated (\( p < .05 \)) the
protein expression of P-ERK, but had no significant effect \( (p > 0.05) \) on the expression of protein P-AKT in adipose tissue, liver and muscle (Figure 6). In the absence of significant changes in total protein expression, sodium butyrate significantly up-regulated \( (p < 0.05) \) the ratio of phosphorylated protein to total protein in adipose tissue and muscle.

As shown in Figures 7 and 8, butyrate significantly upregulated \( (p < 0.05) \) the protein expression of AMPK and P-AMPK in adipose tissue and P-AMPK in the liver. Meanwhile, the butyrate group significantly increased \( (p < 0.05) \) the ratio of phosphorylated AMPK to total AMPK protein. However, there was no significant effect \( (p > 0.05) \) on protein expression in muscle (Figure 9).

Compared with the control group, the expression of gene LPL, ADD1 and FAS in the adipose tissue of the butyrate group was significantly down-regulated \( (p < 0.05) \), HSL, CPT1 and CPT2 were significantly up-regulated \( (p < 0.05) \), and no significant effect on PPAR\(_{y}\), PPAR\(_{x}\) \( (p > 0.05) \). In the liver, butyrate significantly down-regulated \( (p < 0.05) \) gene expression of ADD1 and FAS, and up-regulated \( (p < 0.05) \) CPT1 and CPT2, but had no significant effect \( (p > 0.05) \) on PPAR\(_{x}\). In muscle, butyrate significantly up-regulated \( (p < 0.05) \) gene expression of LPL, FATP and FABP, but PPAR\(_{x}\), CPT1 and CPT2 were not significantly altered \( (p > 0.05) \), Figure 10).

**Discussion**

Since 1980, the global prevalence of overweight and obesity has doubled, and now almost one-third of the
world’s population is classified as overweight or obese. Obesity has an adverse effect on almost all physiological functions of the body and poses a serious public health threat, increasing the risk of patients with many diseases (Singh et al. 2013). Obesity is a multifactorial disease caused by chronic positive energy balance, that is, when dietary energy intake exceeds energy consumption. The excess energy is converted into triglycerides and stored in adipose tissue storage, which increases the volume of the storage, which increases body fat and leads to weight gain (Rogge and Gautam 2017). In our study, it was found that plasma hepatic lipase and lipoprotein lipase enzyme activities were significantly increased after intraperitoneal administration of sodium butyrate. Plasma very low-density lipoproteins and triglycerides are significantly reduced, reducing lipid droplet deposition in adipose tissue and the liver, ultimately leading to a reduction in body fat mass. Consistent with previous studies (Mattace Raso et al. 2013; Matheus et al. 2017), butyrate alleviated obesity or lipid deposition caused by higher-fat diets.

Butyrate is the ligand for metabolite-sensing G-protein coupled receptors (GPCRs), such as GPR43, GPR41 (Coppola et al. 2021). Our study also found that the expression of GPR41 in adipose tissue, liver and muscle of rabbits in the sodium butyrate group was significantly up-regulated, and the expression of GPR43 protein in muscle was significantly increased. Therefore, after injection of sodium butyrate in rabbits,
G protein-coupled receptors may be activated, especially GPR41. A variety of stimulators, such as cytokines, viruses, G-protein-coupled receptor ligands and oncogenes play regulatory roles by activating the ERK/MAPK signalling pathway (Guo et al. 2020). As a downstream effector of PI3-kinase, Akt/PKB is activated by Class 1A and Class 1B PI3-Kinase. Class 1A and Class 1B PI3-kinase are activated by tyrosine kinase and G-protein-coupled receptors, respectively (Song et al. 2005). It was found that the expression of P-ERK in the adipose tissue, liver and muscle of the sodium butyrate group and ERK in the liver were significantly increased, while the expression of AKT and P-AKT in the tissue did not change significantly. Moreover, the ratio of P-ERK to total ERK was increased in the butyrate group. Therefore, GPR41 activated by sodium butyrate could affect the body’s metabolism by activating ERK.

Disorders of the phosphatidylinositol-3-kinase (PI3K)/Akt, ERK, and AMPK pathways are critical to the body’s energy homeostasis and often lead to diseases such as obesity (Schultze et al. 2012). Phosphorylation of AMPK may be mediated indirectly/directly by Akt and ERK in some cellular metabolic processes (López-Cotarelo et al. 2015). In metabolic tissues, AMPK has a regulatory effect on factors related to lipid metabolism. When AMPK is activated, it can inhibit cell active factors such as ADD1 and FAS to inhibit lipid synthesis, and activate HSL, CPT1 and CPT2 to promote lipid decomposition (Hardie et al. 2012; Herzig and Shaw 2018). Consistent with this, butyrate significantly increased the expression of P-AMPK in adipose tissue.

Figure 9. The expression of AMPK (A) and P-AMPK (B) in muscle. (C, D) Image J software was used to make statistics on the results of the western blot (WB), and SPSS (SPSS Inc., Chicago, IL) was used for analysis. (E) Ratio of phosphorylated protein to total protein greyscale values. Values are means ± SEM (n = 6). *p < .05 compared with the control.
and liver, and significantly down-regulated the expressions of FAS and ADD1, and significantly up-regulated the expressions of HSL, CPT and CPT2. Therefore, butyrate regulation of lipid metabolism may be related to AMPK pathway. PPARs is a nuclear hormone receptor that acts as a ligand-activated transcription factor. Originally identified as an orphan receptor, it is now known to be activated by binding to fatty acids and to play an important role in maintaining lipid homeostasis and glucose metabolism (Darbre 2015). However, we did not find that the expression of PPARs was affected by sodium butyrate in the experiment, so we speculated that PPARs was not involved in the regulation of butyrate on lipid metabolism.

Figure 10. The expression of AMPK downstream gene in adipose tissue (a, b), liver (c, d) and muscle (e). Values are means ± SEM (n = 6). *p < .05 compared with the control.
Conclusion
Butyrate mainly affects lipid metabolism in the body by affecting lipid synthesis/decomposition in adipose tissue and liver. And, mainly through GPR41-mediated ERK-AMPK pathway, inhibit lipid synthesis, promote lipid decomposition, and avoid a large amount of fat accumulation induced by higher-fat diet.

Ethical approval
All study procedures were approved by the Shandong Agriculture University Animal Care and Use Committee and were in accordance with the Guidelines for Experimental Animals established by the Ministry of Science and Technology (Beijing, China).

Acknowledgement
Appreciate the help given by the animal nutrition and metabolism research team of the College of Animal Science and Technology of Shandong Agricultural University in animal feeding, drug injection, sample collection and sample analysis.

Disclosure statement
The authors declare no conflict of interest.

Data availability description
The data that support the findings of this study are available from the corresponding author, upon reasonable request.

References
Awad AB, Horvath PJ, Andersen MS. 1991. Influence of butyrate on lipid metabolism, survival, and differentiation of colon cancer cells. Nutr Cancer. 16(2):125–133.
Bergen WG, Mersmann HJ. 2005. Comparative aspects of lipid metabolism: impact on contemporary research and use of animal models. J Nutr. 135(11):2499–2502.
Blas CD, Wiseman J. 1998. Feed formulation. In: Nutrition of the rabbit. CAB International, Wallingford, UK. p. 222–232.
Blas CD, Wiseman J. 2010. Nutrition of the rabbit. Cabi Bookshop. (6): 657–666.
Brazil DP, Hemmings BA. 2001. Ten years of protein kinase B signalling: a hard Akt to follow. Trends Biochem Sci. 26(11):657–664.
Brown AJ, Goldsworthy SM, Barnes AA, Eilert MM, Tcheang L, Daniels D, Muir AI, Wigglesworth MJ, Kinghorn I, Fraser NJ, et al. 2003. The orphan G protein-coupled receptors GPR41 and GPR43 are activated by propionate and other short chain carboxylic acids. J Biol Chem. 278(13):11312–11319.
Cantó C, Auwerx J. 2010. AMP-activated protein kinase and its downstream transcriptional pathways. Cell Mol Life Sci. 67(20):3407–3423.
Chmuryzinska A. 2006. The multigene family of fatty acid-binding proteins (FABPs): function, structure and polymorphism. J Appl Genet. 47(1):39–48.
Coppola S, Avagliano C, Calignano A, Canani RB. 2021. The protective role of butyrate against obesity and obesity-related diseases. Molecules. 26(3):682.
Darbre PD. 2015. Disruption of other receptor systems: progesterone and glucocorticoid receptors, peroxisome proliferator-activated receptors, pregnane X receptor, and aryl hydrocarbon receptor. Endocrine Disruption and Human Health. London: Academic Press. p. 111–122.
Du HT, Wang CY, Wang XP, Ma MW, Li FC. 2013. The effects of dietary α-linolenic acid on growth performance, meat quality, fatty acid composition, and liver relative enzyme mRNA expression of growing meat rabbits. J Anim Feed Sci. 22(2):122–129.
Gao Z, Yin J, Zhang J, Ward RE, Martin RJ, Lefevre M, Cefalu WT, Ye J. 2009. Butyrate improves insulin sensitivity and increases energy expenditure in mice. Diabetes. 58(7):1509–1517.
Gondret F, Ferre P, Dugail I. 2001. ADD-1/SREBP-1 is a major determinant of tissue differential lipogenic capacity in mammalian and avian species. J Lipid Res. 42(1):106–113.
Guilloteau P, Martin L, Eeckhaut V, Ducatelle R, Zabielski R, Van Immerseel F. 2010. From the gut to the peripheral tissues: the multiple effects of butyrate. Nutr Res Rev. 23(2):366–384.
Guo Y-J, Pan W-W, Liu S-B, Shen Z-F, Xu Y, Hu L-L. 2020. ERK/AMPK signalling pathway and tumorigenesis. Exp Ther Med. 19(3):1997–2007.
Han H, Dai D, Wang W, Zhu J, Zhu Z, Lu L, Zhang R. 2019. Impact of serum levels of lipoprotein lipase, hepatic lipase, and endothelial lipase on the progression of coronary artery disease. J Interv Med. 2(1):16–20.
Hardie DG, Ross FA, Hawley SA. 2012. AMPK: a nutrient and energy sensor that maintains energy homeostasis. Nat Rev Mol Cell Biol. 13(4):251–262.
Herzig S, Shaw RJ. 2018. AMPK: guardian of metabolism and mitochondrial homeostasis. Nat Rev Mol Cell Biol. 19(2):121–135.
Horton JD, Goldstein JL, Brown MS. 2002. SREBP-1: activators of the complete program for cholesterol and fatty acid synthesis in liver. J Clin Invest. 109(9):1125–1131.
Khan S, Jena GB. 2014. Protective role of sodium butyrate, a HDAC inhibitor on beta-cell proliferation, function and glucose homeostasis through modulation of p38/ERK MAPK and apoptotic pathways: study in juvenile diabetic rat. Chem Biol Interact. 213:1–12.
Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F. 2016. From dietary fiber to host physiology: short-chain fatty acids as key bacterial metabolites. Cell. 165(6):1332–1345.
Kraemer FB, Shen WJ. 2002. Hormone-sensitive lipase: control of intracellular tri-(di-)acylglycerol and cholesteryl ester hydrolysis. J Lipid Res. 43(10):1585–1594.
Lampidonis AD, Rogdakis E, Voutsinas GE, Stravopodis DJ. 2011. The resurgence of hormone-sensitive lipase (HSL) in mammalian lipolysis. Gene. 477(1–2):1–11.
Li Z, Yi C-X, Katariae S, Kooijman S, Zhou E, Chung CK, Gao Y, van den Heuvel JK, Meijer OC, Berbée JFP, et al. 2018. Butyrate reduces appetite and activates brown adipose tissue via the gut-brain neural circuit. Gut. 67(7):1269–1279.

Lin HV, Frassetto A, Kowalik EJ, Nawrocki AR, Lu MM, Kosinski JR, Hubert JA, Szeto D, Yao X, Forrest G, et al. 2012. Butyrate and propionate protect against diet-induced obesity and regulate gut hormones via free fatty acid receptor 3-independent mechanisms. PLoS One. 7(4):e35240.

Liu L, Fu C, Li F. 2019. Acetate affects the process of lipid metabolism in rabbit liver, skeletal muscle and adipose tissue. Animals. 9(10):799.

Liu L, Liu H, Fu C, Li C, Li F. 2017. Acetate induces anorexia via up-regulating the hypothalamic pro-opiomelanocortin (POMC) gene expression in rabbits. J Anim Feed Sci. 26:266–273.

Liu L, Liu H, Ning L, Li F. 2019. Rabbit SLC15A1, SLC7A1 and SLC1A1 genes are affected by site of digestion, stage of development and dietary protein content. Animal. 13(2):326–332.

Liu L, Xu S, Wang X, Jiao H, Lin H. 2016. Peripheral insulin doesn’t alter appetite of broiler chicks. Asian Australas J Anim Sci. 29(9):1294–1299.

Liu L, Zhao X, Liu Y, Zhao H, Li F. 2019b. Dietary addition of garlic straw improved the intestinal barrier in rabbits.1. J Anim Sci. 97(10):4248–4255.

Liu L, Zuo W, Li F. 2019a. Dietary addition of Artemisia argyi reduces diarrhea and modulates the gut immune function without affecting growth performances of rabbits after weaning. J Anim Sci. 97(4):1693–1700.

Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods. 25(4):402–408.

López-Cotarelo P, Escribano-Díaz C, González-Bethencourt IL, Gómez-Moreira C, Duguiz ML, Torres-Bacete J, Gómez-Cabañas L, Fernández-Barrera J, Delgado-Martín C, Mellado M, et al. 2015. A novel MEK-ERK-AMPK signaling axis controls chemokine receptor CCR7-dependent survival in human mature dendritic cells. J Biol Chem. 290(2):827–840.

Marcil V, Delvin E, Garofalo C, Levy E. 2003. Butyrate impairs lipid transport by inhibiting microsomal triglyceride transfer protein in caco-2 cells. J Nutr. 133(7):2180–2183.

Marcil V, Delvin E, Seidman E, Poitras L, Zoltowska M, Garofalo C. 2002. Modulation of lipid synthesis, apolipoprotein biogenesis, and lipoprotein assembly by butyrate. Am J Physiol-Gastr L. 283(G340–G346).

Mahmood T, Yang PC. 2012. Western blot: technique, theory, and trouble shooting. N Am J Med Sci. 409(4):429–434.

Matheus VA, Monteiro LCS, Oliveira RB, Maschio DA, Collares-Buzato CB. 2017. Butyrate reduces higher-fat diet-induced metabolic alterations, hepatic steatosis and pancreatic beta cell and intestinal barrier dysfunctions in prediabetic mice. Exp Biol Med (Maywood). 242(12):1214–1226.

Mattace Raso G, Simeoli R, Russo R, Iacono A, Santoro A, Paciello O, Ferrante MC, Canani RB, Calignano A, Meli R. 2013. Effects of sodium butyrate and its synthetic amide derivative on liver inflammation and glucose tolerance in an animal model of steatosis induced by high fat diet. PLoS One. 8(7):e68626.

Mehlem A, Hagberg CE, Muhl L, Eriksson U, Falkevall A. 2013. Imaging of neutral lipids by oil red O for analyzing the metabolic status in health and disease. Nat Protoc. 8(6):1149–1154.

Nguyen P, Leray V, Diez M, Serisier S, Le Bloc’h J, Sillart B, Dumon H. 2008. Liver lipid metabolism. J Anim Physiol Anim Nutr (Berl). 92(3):272–283.

Nilsson NE, Kotarsky K, Owman C, Olde B. 2003. Identification of a free fatty acid receptor, ffa 2 r, expressed on leukocytes and activated by short-chain fatty acids. Biochem Biophys Res Co. 303(4):1047–1052.

Olivecrona G. 2016. Role of lipoprotein lipase in lipid metabolism. Curr Opin Lipidol. 27(3):233–241.

Ren E, Chen X, Yu S, Xu J, Su Y, Zhu W. 2018. Transcriptomic and metabolomic responses induced in the livers of growing pigs by a short-term intravenous infusion of sodium butyrate. Animal. 12(11):2318–2326.

Realon GK, Maurmann N, Werencicz A, Garcia VA, Schröder N, Wood MA, Roesler R. 2011. Posttraining systemic administration of the histone deacetylase inhibitor sodium butyrate ameliorates aging-related memory decline in rats. Behav Brain Res. 221(1):329–332.

Rogge MM, Gautam B. 2017. Biology of obesity and weight regain: implications for clinical practice. J Am Assoc Nurse Pract. 29(5):515–529.

Rosen ED, MacDougald OA. 2006. Adipocyte differentiation from the inside out. Nat Rev Mol Cell Biol. 7(12):885–896.

Rumberger JM, Arch JRS, Green A. 2014. Butyrate and other short-chain fatty acids increase the rate of lipolysis in 3T3-L1 adipocytes. Peer J. 2:e611.

Schultze SM, Hemmings BA, Niessen M, Tschopp O. 2012. PI3K/AKT, MAPK and AMPK signalling: protein kinases in glucose homeostasis. Expert Rev Mol Med. 14(article e1):.-.

Sevane N, Armstrong E, Cortes O, Wiener P, Wong RP, Dunner S, GemQual Consortium. 2013. Association of bovine meat quality traits with genes included in the PPARG and PPARGC1A networks. Meat Sci. 94(3):328–335.

Shen Y, Xu X, Yue K, Xu G. 2015. Effect of different exercise protocols on metabolic profiles and fatty acid metabolism in skeletal muscle in high-fat diet-fed rats . Obesity (Silver Spring). 23(5):1000–1006.

Singh GM, Danai G, Farzadfar F, Stevens GA, Woodward M, Wormer D, Kaptoge S, Whitleck G, Qiao O, Lewington S, Prospective Studies Collaboration (PSC), et al. 2013. The age-specific quantitative effects of metabolic risk factors on cardiovascular diseases and diabetes: a pooled analysis. PLoS One. 8(7):e65174.

Song G, Ouyang G, Bao S. 2005. The activation of Akt/PKB signaling pathway and cell survival. J Cell Mol Med. 9(1):59–71.

Thupari JN, Pinn ML, Kuhajda FP. 2001. Fatty acid synthase inhibition in human breast cancer cells leads to malonyl-coa-induced inhibition of fatty acid oxidation and cytotoxicity. Biochem Biophys Res Commun. 285(2):217–223.

Wu Z, Sun L, Liu G, Liu H, Liu H, Yu Z, Xu S, Li F, Qin Y. 2019. Hair follicle development and related gene and protein expression of skins in Rex rabbits during the first 8 weeks of life. Asian-Australas J Anim. 32:477.

Yin F, Yu H, Lepp D, Shi X, Yang X, Hu J, Leeson S, Yang C, Nie S, Hou Y, et al. 2016. Transcriptome analysis reveals...
regulation of gene expression for lipid catabolism in young broilers by butyrate glycerides. PLoS One. 11(8): e0162150.

Zhang S, Wang H, Zhu MJ. 2019. A sensitive GC/MS detection method for analyzing microbial metabolites short chain fatty acids in fecal and serum samples. Talanta. 196: 249–254.

Zhang Y, Zhu S, Wang X, Wang C, Li F. 2011. The effect of dietary selenium levels on growth performance, antioxidant capacity and glutathione peroxidase 1 (GSHPx1) mRNA expression in growing meat rabbits. Anim Feed Sci Tech. 169(3–4):259–264.

Zhu X, Xiong T, Liu P, Guo X, Xiao L, Zhou F, Tang Y, Yao P. 2018. Quercetin ameliorates HFD-induced NAFLD by promoting hepatic VLDL assembly and lipophagy via the IRE1a/XBP1s pathway. Food Chem Toxicol. 114: 52–60.