Propionate Promotes Intestinal Lipolysis and Metabolic Benefits via AMPK/LSD1 Pathway in Mice

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Abstract: Dietary fibers and the microbial fermentation products short chain fatty acids promote metabolic benefits, but the underlying mechanisms are poorly understood. Recent studies indicate that intestinal lipid handling is under regulatory control and has broad influence on whole body energy homeostasis. Here we report that dietary inulin and propionate significantly decrease whole body fat mass without affecting food intake in mice fed with chow diet. Inulin and propionate decrease intestinal triglyceride content and induce enterocyte lipolysis genes expression, such as adipose triglyceride lipase (ATGL), hormone sensitive lipase (HSL) and adipose triglyceride lipase (LAL). Both in vivo and in vitro studies show propionate can induce enterocyte lipolysis and decrease lipid content, via direct activation of phosphorylation of AMP-activated protein kinase (p-AMPK) and lysine-specific histone demethylase 1 (LSD1). Moreover, propionate and inulin can increase intestinal lipid lipolysis under high fat diet (HFD) fed condition and prevent from HFD-induced obesity. Our study suggests dietary fiber inulin and its microbial fermentation product propionate can induce intestinal lipolysis and decrease whole body fat mass via activation of p-AMPK and LSD1, which could provide a novel therapeutic target for both prevention and treatment of obesity.

Keywords: Inulin, propionate, intestinal lipid metabolism, AMP-activated protein kinase, lysine-specific histone demethylase 1, obesity

1. Introduction

Metabolic disorders such as obesity and type 2 diabetes have become major threats to human health worldwide [1]. It is well recognized that dietary fiber intervention is one of the most efficient strategies for improving metabolic health [2-5]. However, the mechanisms for dietary fiber on metabolic regulation still need further investigation.

These beneficial effects of dietary fiber are, at least partially, attributed to their fermentation end-products by gut microbiota, short chain fatty acids (SCFAs) [6,7]. Among SCFAs, propionate has been
identified as principal hepatic gluconeogenic substrate and studies showed that the total amount of fecal propionate was higher in the obese subjects compared with lean subjects [8,9]. However, emerging evidence shows propionate has beneficial effects on metabolic regulation [10-14]. When transplanting fecal microbiota from human twin donors discordant for obesity into germfree mice, results showed there was a significant negative correlation between adiposity and caecal propionate content [15]. Moreover, recent studies in obese humans suggested long-term propionate administration prevented body weight gain and reduced lipid accumulation [10,13]. Dietary propionate supplementation also protected against high-fat diet (HFD)-induced obesity and insulin resistance in mice [12]. This beneficial effect could attribute to appetite reduction and increased resting energy expenditure via promoting lipid oxidation by propionate [12,13]. Therefore, conflicting results suggest the mechanisms of propionate in metabolic regulation need further investigation.

Recent evidence suggests propionate can regulate metabolic homeostasis in gut. Vadder et al’s study showed propionate activated intestinal gluconeogenesis to improve whole body glucose metabolism [16]. Meanwhile, lipid handling by the gut is being recognized as under regulatory control and has broad ramifications for whole body energy homeostasis [17]. Recent studies showed that lipid storage might be present within enterocytes, primarily as triglyceride. Intestinal triglyceride goes through resynthesis, cytoplasmic storage and mobilization as well as secretion in chylomicron particles [18]. Regulation of intestinal lipid metabolism could affect enterocyte lipid content and whole body fat accumulation [19-22]. Catabolism of lipid in the intestine includes two aspects, cytoplasmic lipolysis and lipophagy [23,24]. Adipose triglyceride lipase (ATGL) is the first rate-limiting enzyme in lipolysis and evidence showed intestine-specific ATGL deficiency increased the accumulation of triglyceride in enterocyte [25]. Lysosomal acid lipase (LAL) is the enzyme that breaks down triglyceride via lipophagy [26]. LAL deficiency in mice also resulted in massive accumulation of triglyceride and cholesteryl ester in small intestine [27]. Whether SCFAs, especially propionate, has an effect on intestinal lipid metabolism is worth studying.

One important mechanism for SCFAs-mediated lipid metabolism is via free fatty acid receptor (FFAR2 and FFAR3, preferentially activated by propionate) [28,29]. However, evidence suggested FFAR-independent mechanisms could also be involved for propionate since FFAR3-deficiency mice was still sensitive to SCFAs metabolic regulation [14,30]. Propionate, as an energy substrate, could affect intracellular energy status. In vitro studies showed propionate activated the phosphorylation of AMPK, a master sensor of energy in HepG2, 3T3-L1, Caco-2 as well as HCT116 cells [12,31,32]. AMPK is a vital regulator of lipid metabolism in multiple organs [33-35]. Meanwhile, propionate also acts as a modulator of epigenome through altering histone acetylation [36]. Lysine-specific histone demethylase 1 (LSD1), an epigenetic regulator by altering histone methylation, particularly plays a key role in lipid metabolism in adipose tissue [37,38]. Recent study demonstrated that intestinal AMPK deficiency in mice and Caco-2 cell regulated LSD1 activity [39], indicating an intrinsic link between AMPK and LSD1 in intestines.

In this study, we show that SCFA propionate and inulin decreased whole body fat mass in chow diet fed mice without affecting food intake and lipid absorption. Meanwhile, propionate and inulin induced intestinal enterocyte lipolysis and decreased intracellular lipid content. Propionate increased enterocyte lipolysis genes independent of FFARs, instead, via direct activation of phosphorylation of AMPK and LSD1. This beneficial effect of propionate on lipid metabolism was also present in HFD...
fed mice. Thus, we report a mechanism linking microbial fermentation of dietary fiber into SCFAs, especially propionate, and host energy homeostasis through intestinal lipid handling, which could provide novel therapeutic target for both prevention and treatment of metabolic diseases such as obesity.

2. Materials and Methods

2.1. Animals Experiments

All procedures were performed in accordance with the Guidelines for Care and Use of Laboratory Animals of South China Agricultural University (Guangzhou, China) and experiments were approved by the Animal Ethics Committee of South China Agricultural University (Guangzhou, China). Three-week-old male specific pathogen-free (SPF) C57BL/6J mice were purchased from Guangdong Medical Laboratory Animal Center and housed in a light-and temperature-controlled facility (12 h light/12 h dark, 22-24°C) with free access to water and food. For SCFAs treatment studies, 8 weeks old male C57Bl/6J mice were fed on chow diet or HFD (D12451, 45% fat) and were dividedly gavaged with sodium acetate (S2889, Sigma), sodium propionate (P1880, Sigma), sodium butyrate (303410, Sigma) or inulin (BENE0-Oralit, Belgium) at 2000 mg/kg body weight in saline solution every day for 2 weeks. Food intake and body weight were monitored every other day. Final body composition was determined in mice using quantitative magnetic resonance (QMR, Niumag Corporation, Shanghai, China). By the end of the experiment, all mice were sacrificed using isofoflurane anesthesia after 6 h fasting and tissue samples and plasma were collected for further analysis.

2.2. Tissue, Fecal and Serum Lipid Analysis

Lipids were extracted from tissues following the method of Folch et al by a minor modification [40]. Briefly, tissues are homogenized with chloroform: methanol (2:1) to a final volume 20 times the volume of the tissue sample. After dispersion, the whole mixture is agitated for a minimum of overnight at room temperature. Then, the solvent is washed with 0.2 vols of 0.9% NaCl. After vortex and spin at 2000 g for 10 min, the bottom chloroform phase containing lipids is evaporated under a nitrogen stream. Finally, the dry content is dissolved in butanol: TritonX-100: methanol (0.6: 0.267: 0.133). Final lipid content was normalized to protein concentrations. To determine the amount of fecal lipid, feces was collected 3 days before the end of the experiment and measured following the method of Kraus et al [41]. Triglyceride contents in the tissues, feces and blood were assayed using a triglyceride assay kit (Nanjing Jiancheng, A110-2).

2.3. Cell Culture

The human adenocarcinoma cell line Caco-2 cells were purchased from the Type Culture Collection of the Chinese Academy of Sciences (Shanghai, China). Caco-2 cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum (Gibco), 50 μg/ml penicillin and 4 μg/ml streptomycin in a humidified atmosphere of 5% CO2 at 37 °C. Cells were passaged with 0.25% trypsin-EDTA (Gibco) every 3-4 days and then plated at a density of 20,000 cells/cm2. For SCFAs treatment experiments, Caco-2 cells were treated with sodium acetate, sodium propionate, sodium butyrate, compound C (HY-13418A, MCE) or GSK2879552 (HY-18632, MCE). After 24 h incubation, cells were stained with Oil-Red-O or were lysed for molecular analysis.

2.4. RNA Extraction and Quantitative RT-PCR
Total RNA was extracted from frozen tissues and cells using Trizol reagent (Invitrogen), according to the manufacturer’s instructions. Total RNA (1 μg) was reverse transcribed with RevertAid first strand cDNA synthesis kit for quantitative RT-PCR (Thermo Scientific; #K1622). The cDNA was diluted and used in real-time PCR with Power SYBR Green PCR master mix (Applied Biosystems, 1708040) on a Q6 real-time PCR system (Applied Biosystems). β-Actin or TATAbox binding protein (TBP) was used as a housekeeping gene. Calculations were made based on the comparative cycle threshold method (2-DDCt). Primer sequences are given in Table S1.

2.5. Western Blotting

The tissues and cells were homogenized in the lysis buffer (RIPA, BioTeke) containing 1 mmol/L protease inhibitor PMSF (P7626, Sigma), centrifuged at 12,000 rpm for 15 min. The supernatant was collected and protein concentration was determined by BCA Protein Assay Kit (Thermo Fisher Scientific, 23227). Equal amounts of protein were separated on 10% SDS-PAGE gels and blotted onto PVDF membranes. Membranes were probed using specific antibodies: anti-AMPK (#2532, 1/1000), anti-phospho-AMPK (#2535, 1/1000), anti-LSD1 (#2184, 1/1000) were purchased from Cell Signaling Technology; rabbit anti-β-actin (bs-0061R) and rabbit-anti IgG (BS13278) were obtained from Biosynthesis Technology (Beijing, China). The proteins were visualized with the Clarity Max Western ECL Substrate (Bio-Rad, #1705062S) and quantified in the Image Lab system (Bio-Rad).

2.6. Oil Red O Staining

Caco-2 cells were washed with phosphate buffered saline three times and fixed with 3.7% formaldehyde for 30 min. 0.3% oil Red O (Sigma, O0625) in isopropanol was then added directly to the fixed cells and incubated for 1 h. The red lipid droplets were visualized by microscopy (NIS-Elements, Nikon, Japan) and dissolved in isopropanol to quantify by a microplate reader (Thermo Fisher Scientific).

2.7. Statistical Analysis

The data were processed with SPSS software version 19.0 (SPSS Inc., Chicago, USA). The results were analyzed with Student’s t-test and One-Way ANOVA by a Least Significant Difference test. Differences among groups were considered statistically significant if P<0.05. Data are expressed as the mean ± SEM.

3. Results

3.1. Dietary Propionate and Inulin Reduce Whole Body Fat Mass and Intestinal Triglyceride Content in Chow Diet Fed Mice

We first confirmed the effects of dietary SCFAs and inulin, which is a fiber source that results in a rapid, extensive generation of propionate after fermentation, on body weight and fat mass in chow diet fed mice. The dose of 2000 mg/kg for SCFAs challenge was according to previous described by Xiong et al [42]. Our results showed that body weight gain was not affected after 2 weeks gavage-feeding with SCFAs or inulin comparing to control group (Figure S1A), yet whole body fat mass was decreased by propionate or inulin only (30.2% and 15.9%, respectively) (Figure 1A and Figure S1B). Importantly, there was no change of food intake (Figure 1B) and fecal triglyceride level (Figure 1C) by propionate or inulin treatment, suggesting dietary lipid absorption was not affected. Then we examined the tissue weight and results showed that inguinal and epididymal white adipose tissue weights were decreased (Figure S1C-D), liver and muscle weights were not affected (data not shown), and the unit length weights of jejunum and ileum were increased (Figure 1D-E). Histology analysis
showed villus length of small intestines was increased by propionate or inulin treatment which could attribute to the unit length weight increase (Figure S1E-F). Meanwhile, triglyceride level in jejunum and ileum as well as serum was significantly lower in propionate or inulin group (Figure 1F-H). These results showed dietary propionate and inulin decreased whole body fat mass and intestinal triglyceride content without affecting lipid absorption.

Figure 1. Effect of propionate and inulin on metabolic phenotype in mice fed a chow diet. (A) Fat mass of mice treated with propionate or inulin for 2 weeks. (B) Average daily food intake. (C) Fecal triglyceride content. (D-E) The unit length weight of jejunum (D) and ileum (E). (F) Triglyceride content in the jejunum. (G) Triglyceride content in the ileum. (H) Triglyceride content in serum. Data are mean ± SEM, n=6-8 per group. Ctrl, control; NaP, sodium propionate; TG, triglyceride. *P <0.05 versus control.

3.2. Propionate and Inulin Induce Intestinal Lipolysis Gene Expression

We next measured the expression of lipid metabolism key enzymes in tissues. Results showed ATGL and hormone sensitive lipase (HSL) which are key lipases that catalyze cytoplasmic lipolysis [43] and rate-limiting fatty acid oxidation enzyme carnitine palmitoyltransferase-1α (Cpt1α) expressions were not affected by propionate or inulin in liver and adipose tissues (Figure S2A-B), suggesting liver and adipose lipid metabolism might not be significantly affected by either propionate or inulin. However, CD36 mRNA expression was decreased in adipose tissues, which could indicate the uptake of lipid in adipose tissues was lower by propionate or inulin (Figure S2B). Notably, the expression of ATGL and HSL was significantly increased in the jejunum (Figure 2A) and ileum (Figure 2B) after propionate and inulin treatment. LAL expression was also elevated in the jejunum, which is responsible for breaking down triglyceride via lipophagy (Figure 2A-B). Intestinal triglyceride resynthesis enzymes monoacylglycerol acyltransferase 2 (MGAT2) and diacylglycerol acyltransferase 1 (DGAT1) expressions were not changed in response to propionate or inulin (Figure 2C-D), suggesting intestinal triglyceride resynthesis was not affected. Meanwhile, Cpt1α was elevated, suggesting an increase of fatty acid oxidation (Figure 2E-F). Together, these
results suggested propionate and inulin could increase intestinal lipolysis to reduce intestinal triglyceride content and whole body fat mass.

Figure 2. Effect of propionate and inulin on small intestinal lipid metabolism gene in mice. (A-B) Lipolysis key lipases (ATGL, HSL and LAL) in the jejunum (A) and ileum (B). (C-D) Triglyceride synthesis genes (MGAT2 and DGAT1) in the jejunum (C) and ileum (D). (E-F) The mRNA level of Cpt1α in the jejunum (E) and ileum (F). Data are mean ± SEM, n=6-8 per group. *P <0.05 versus control.

3.3. Propionate is Able to Directly Induce Lipolysis Gene Expression and Decreases Lipid Content in Caco-2 Cells

We next investigated the direct effects of propionate on intestinal lipid lipolysis using Caco-2 cells, which have a phenotype that resembles the enterocytes of small intestine [44]. Oil Red O staining of Caco-2 cells showed a decreased number of lipid droplets after 24 h incubation with 0.1 and 0.5 mmol/L propionate, while no change with acetate and butyrate incubation at the same dose (Figure 3A and Figure S3A). And Caco-2 cell vitality was not affected (Figure S3B). Triglyceride content in Caco-2 cells was also significantly decreased by 0.1 and 0.5 mmol/L propionate (Figure 3B). The expression of ATGL, HSL and LAL was significantly increased in Caco-2 cells incubated with 0.5 mmol/L propionate while no change in acetate or butyrate treatment (Figure 3C-E). DAGT1 expression was not changed by SCFAs (Figure S3C), suggested SCFAs also did not affect triglyceride synthesis in Caco-2 cell. These data indicated that propionate was able to directly promote intestinal lipolysis gene expression and decrease enterocytes triglyceride content.
Figure 3. Effect of SCFAs on lipid metabolism in Caco-2 cells. (A) The OD value of Caco-2 cells after incubated with 0.1 and 0.5 mmol/L SCFAs for 24 h by Oil Red O staining. (B) Intracellular triglyceride content of Caco-2 cells treated with 0.1 and 0.5 mmol/L SCFAs for 24 h. (C-E) The mRNA level of ATGL(C), HSL(D) and LAL (E) in Caco-2 cells by 24 h incubation with 0.1 and 0.5 mmol/L SCFAs. Data are means ± SEM, n=4 per group. Representative results of the three independent experiments are shown. Ctrl, control; NaA, sodium acetate; NaB, sodium butyrate; NaP, sodium propionate. *P <0.05 versus control.

3.4. Propionate Induces Intestinal Lipolysis through phosphorylation of AMPK

Since SCFAs are endogenous ligands for FFARs, we first tested whether propionate increased intestinal lipolysis through FFARs in vivo. Results showed neither FFAR2 or FFAR3 was affected by propionate or inulin treatment in the jejunum (Figure S4A-B). Instead, immunoblot analyses showed that phosphorylation of AMPK (p-AMPK, Thr172) in the jejunum and ileum was significantly elevated (Figure 4A-B). In vitro studies also showed propionate directly increased p-AMPK dose-dependently in Caco-2 cells (Figure 4C). To investigate role of AMPK signaling in propionate regulation of intestinal lipolysis, we used a specific inhibitor of p-AMPK (compound C, Cc). Caco-2 cells treated with 10 μmol/L Cc was able to inhibit p-AMPK protein expression (Figure S5B), meanwhile significantly increased triglyceride content (Figure S5A) and decreased ATGL, HSL and LAL mRNA expression after 24 h incubation (Figure 4E-G). More importantly, when elevation of p-AMPK by propionate was blocked by Cc (Figure S5B), the reduction of cell triglyceride content by propionate treatment was blocked simultaneously in Caco-2 cells (Figure 4D) as well as the increase of ATGL, HSL and LAL expression (Figure 4E-G). These data suggested that AMPK signaling had a direct role in propionate regulation on intestinal lipolysis.
Figure 4. Propionate regulates lipolysis dependent on p-AMPK in Caco-2 cells. (A-B) Protein expression of p-AMPK and AMPK in the jejunum (A) and ileum (B) of mice treated with propionate or inulin. (C) Protein expression of p-AMPK and AMPK in Caco-2 cells treated with 0.1 and 0.5 mmol/L propionate for 24 h. (D) Intracellular triglyceride content of Caco-2 cells when stimulated with compound C (Cc, 10 μmol/L) in the presence of propionate for 24 h. (E-G) The mRNA expression of ATGL (E), HSL (F) and LAL (G) in Caco-2 cells when stimulated with compound C (Cc, 10 μmol/L) in the presence of propionate for 24 h. Data are means ± SEM, n=4 per group. *P <0.05 versus control.

3.5. Propionate Upregulates LSD1 Downstream of p-AMPK to Induce Intestinal Lipolysis

Previous study showed AMPK could regulate LSD1 in Caco-2 cells, and LSD1 has important role in lipid metabolism [39]. We measured the protein expression of LSD1 in the jejunum and ileum. Results showed that propionate and inulin significantly increased LSD1 expression in both jejunum and ileum (Figure 5A-B). In vitro studies also showed propionate directly increased LSD1 dose-dependently in Caco-2 cells (Figure 5C). Inhibition of LSD1 by its inhibitor GSK2879552 (5 μmol/L) (Figure S5C) significantly increased triglyceride level in Caco-2 cells (Figure 5D) and decreased ATGL, HSL (P=0.062) and LAL expression (Figure 5E-G). More importantly, when elevation of LSD1 by propionate was blocked by GSK2879552 treatment (Figure S5C), the decrease of triglyceride level by propionate was completely blocked (Figure 5D), as well as the increase in ATGL, HSL and LAL expression (Figure 5E-G). Notably, inhibition of p-AMPK expression decreased LSD1 protein level and when treated with propionate, inhibition of p-AMPK blocked propionate-induced increase of LSD1 in Caco-2 cell (Figure 5H). However, inhibition of LSD1 protein expression by GSK2879552 did not affect either p-AMPK or propionate-induced increase of p-AMPK in Caco-2 cell (Figure 5I). Taken together, these data suggested that propionate upregulated LSD1 downstream of p-AMPK to induce intestinal lipolysis.
Figure 5. LSD1 regulates propionate-induced lipolysis in Caco-2 cells. (A-B) Protein expression of LSD1 in the jejunum (A) and ileum (B) of mice treated with propionate or inulin. (C) Protein expression of LSD1 in Caco-2 cells treated with 0.1 and 0.5 mmol/L propionate for 24 h. (D) Intracellular triglyceride content of Caco-2 cells stimulated with or without GSK2879552 (GSK552, 5μmol/L) in the presence of propionate for 24 h. (E-G) The mRNA expression of ATGL (E), HSL (F) and LAL (G) of Caco-2 cells treated with or without GSK552 in the presence of propionate for 24 h. (H) Protein expression of LSD1 in Caco-2 cells treated with or without Cc in the presence of propionate for 24 h. (I) Protein expression of p-AMPK in Caco-2 cells stimulated with or without GSK552 in the presence of propionate for 24 h. Data are means ± SEM, n=4 per group. *P < 0.05 versus control.

3.6. Propionate and Inulin Protect Against HFD-Induced Obesity
Finally, we confirmed whether the beneficial effects of propionate and inulin still existed in HFD fed mice. During 2-weeks HFD feeding, propionate and inulin completely prevented HFD-induced body weight gain (Figure 6A), accompanied by decreased fat mass (Figure 6B). White adipose tissue weights were decreased consistent with lower fat mass (Figure S6A-B). Also, no change of food intake and fecal triglyceride level was observed which indicated propionate and inulin did not affect fat absorption by gastrointestinal tract on HFD (Figure 6C-D). Consistent with the results in chow diet fed mice, jejunal and ileal as well as serum triglyceride levels were significantly decreased by propionate or inulin treatment on HFD (Figure 6E-G). The expression of ATGL, HSL and Cpt1α in the jejunum and ileum was increased in propionate or inulin treatment (Figure 6H-K), whereas MGAT2 and DGAT1 were not affected (Figure S6C-D). In addition, protein expression of p-AMPK and LSD1 was significantly increased in the jejunum and ileum of HFD fed-mice treated with propionate or inulin (Figure 7L-O). All together, these findings indicated that propionate and inulin inhibited HFD-induced obesity by promoting intestinal lipolysis.

**Figure 6.** Effect of propionate and inulin on metabolic phenotype in HFD-fed mice. (A) Body weight gain of HFD-fed mice treated with propionate or inulin for 2 weeks, and cumulative weight gain is expressed as a percentage of initial body weight. (B) Fat mass of HFD-fed mice treated with propionate or inulin. (C) Average daily food intake. (D) Fecal triglyceride content. (E-F) Triglyceride content in the jejunum (E) and ileum (F) of HFD-fed mice. (G) Triglyceride content in serum. (H-I) The mRNA expression of ATGL, HSL and LAL in the jejunum (H) and ileum (I) of HFD-fed mice. (J-K) The mRNA expression of Cpt1α in the jejunum (J) and ileum (K) of HFD-fed mice. (L-M) Protein expression of p-AMPK and AMPK in the jejunum (L) and ileum (M) of HFD-fed mice. (N-O) Protein expression of LSD1 in the jejunum (N) and ileum (O) of HFD-fed mice. Data are means ± SEM, n=5-7 per group. *P <0.05 versus control.

4. Discussion
Here we examined the metabolic effects of propionate and inulin. We found that propionate produced from microbial fermentation of dietary fibers decreases whole body fat content without affecting lipid absorption. Especially, propionate has a direct influence on inducing intestinal lipolysis via AMPK and LSD1 that could play a key role to improve host metabolic physiology such as adiposity [45].

The beneficial effects of dietary fibers and SCFAs on metabolic control, such as resistance to diet-induced obesity, have been described independently [11,12,14,16,46]. The beneficial effect of propionate has been suggested to be caused by increasing fatty acid oxidation in liver, therefore increased energy expenditure [10,12]. Other study has also showed that propionate promoted lipid oxidation in the adipose tissue [11]. Previous study showed the majority (>90%) of propionate was absorbed by the gastrointestinal tract [47]. Here, our data showed dietary propionate significantly decreased whole body fat mass without affecting food intake (Figure 1A-B and Figure 6B-C), meanwhile, lipid metabolism was not significantly affected in either liver or adipose tissue (Figure S2A-B). Instead, propionate directly induced intestinal lipolysis and decreased enterocytes lipid storage (Figure 2A-B). Recent studies suggest small intestine is an important organ in regulating whole body lipid metabolism. Dietary lipids go through enterocytes intracellular transport and metabolism, storage to finally transport to tissues via lymphatics and the circulation. We thus propose that propionate increases enterocytes lipolysis to reduce lipid storage, which then decreases lipid transport to tissues, since data showed serum triglyceride was lower and and CD36 expression in adipose tissue was downregulated by propionate treatment (Figure 1H and Figure S2B). Further studies such as on genetically modified animal models need to be done to verify effects of intestinal regulation on whole body energy homeostasis by propionate, this would give us a clue to a new perspective.

Dietary fibers have been linked to improve gut function [48]. Our results showed unit length weights of jejunum and ileum were elevated as well as villus length (Figure 1D-E and Figure S1E-F), indicating intestinal function improvement. And mRNA levels of y+LAT1 and b0,+AT which mediate uptake and release of cationic amino acid, and B0AT1 which mediates apical uptake of all neutral amino acids were markedly increased by propionate treatment in the jejunum (Figure S7A). When gavage-fed whily protein mixture, plasma arginine level was significantly increased after 30 min (Figure S7B). In addition, propionate did not affected the expression of genes involved in inflammatory response such as tumor necrosis factor-alpha (TNFα), nuclear factor kappa beta (NFκB) and interleukin-6 (IL-6) (Figure S7C), suggesting small intestinal inflammatory response was not activated. Therefore, it is likely for propionate to have a role in benefiting gut health by increasing intestinal lipolysis to provide energy for function improvements.

The metabolic beneficial effects of SCFAs have been described through activation of G-protein-coupled receptors, FFAR2 and FFAR3 [11,28,29,49,50]. Here, we first tested this pathway and neither FFAR2 or FFAR3 was affected by propionate in the intestine (Figure S4A-B). Our data suggest propionate and inulin directly induce enterocyte lipolysis via an increase in p-AMPK (Figure 4A-C). Similar study also reported that soluble dietary fiber could enhance the production of SCFAs which activates the intestinal epithelial AMPK activity [51]. Propionate-induced AMPK activity in HCT116 and SW480 cells was reported to be caused by the reduction of intracellular ATP level and AMP/ATP upregulation [32]. Meanwhile, there is also evidence showing SCFAs utilization as energy substrates could increase ATP which in turn drives the increase of cAMP [52].
SCFAs are also important modulators of the epigenome and regulate histone acetylation [53]. Here we found that propionate directly increases lipolysis gene expression in enterocytes through increase of LSD1, a histone demethylase (Figure 5A-C). Our data showed in enterocytes that treated with propionate, p-AMPK inhibitor completely blocked LSD1 increase and LSD1 inhibitor had no effect on p-AMPK (Figure 5H-I), indicating LSD1 is a downstream target of p-AMPK in enterocytes, as described earlier [39]. Studies from our lab as well as several independent groups showed LSD1 plays a key role in energy homeostasis [54]. For example, LSD1 significantly affects browning to regulate whole body lipid metabolism in adipose tissue [55]. However, the role of LSD1 in the intestine on metabolic homeostasis is unknown. Our data indicates inhibition of LSD1 expression as well as p-AMPK could decrease lipolysis in Caco-2 cells and increase triglyceride content (Figure 4D and 5D). Meanwhile, LSD1 expression in gut could be different between chow diet and HFD (Figure 5A-B and 7M-N). Therefore, we hypothesize that LSD1 could also regulate whole body energy metabolism in gut and the mechanisms worth further investigation.

In conclusion, we report a mechanism linking microbial fermentation of dietary fiber into SCFAs, especially propionate, and host energy homeostasis through intestinal lipid handling. We show that propionate has an important role in regulation of enterocyte lipolysis through activation of p-AMPK and LSD1. This further reveals the mechanism of propionate on host metabolism regulation. Since both the positive influence of dietary fiber on obesity [13] and intestinal lipid handling are known to be present in humans, these findings may provide novel perspectives in both treatment and prevention of metabolic diseases.

Supplementary Materials: The following are available online, Table S1: Primers used for quantitative real time-PCR, Figure S1: Effect of SCFAs on metabolic phenotype in chow diet fed mice, Figure S2: Effects of propionate and inulin on lipid metabolism gene in liver and white adipose tissue, Figure S3: Effects of SCFAs on lipid metabolism and cell viability in Caco-2 cells, Figure S4: Effect of propionate and inulin on FFARs in the jejunum, Figure S5: Effects of compound C and GSK2879552 on p-AMPK protein level in Caco-2 cells. Figure S6: Effect of propionate and inulin on metabolic phenotype in HFD fed mice. Figure S7: Effects of propionate and inulin on amino acid absorption and inflammatory cytokines in the jejunum.


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