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Propionate promotes intestinal lipolysis and metabolic benefits via AMPK/LSD1 pathway in mice

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Abstract

Dietary fibers and their microbial fermentation products short-chain fatty acids promote metabolic benefits, but the underlying mechanisms are still unclear. Recent studies indicate that intestinal lipid handling is under regulatory control and has broad influence on whole body energy homeostasis. Here we reported that dietary inulin and propionate significantly decreased whole body fat mass without affecting food intake in mice fed with chow diet. Meanwhile, triglyceride (TG) content was decreased and lipolysis gene expression, such as adipose triglyceride lipase (*Atgl*), hormone-sensitive lipase (*Hsl*) and lysosomal acid lipase (*Lal*) was elevated in the jejunum and ileum of inulin- and propionate-treated mice. *In vitro* studies on Caco-2 cells showed propionate directly induced enterocyte *Atgl*, *Hsl* and *Lal* gene expression and decreased TG content, via activation of phosphorylation of AMP-activated protein kinase (p-AMPK) and lysine-specific demethylase 1 (LSD1). Moreover, inulin and propionate could increase intestinal lipolysis under high-fat diet (HFD)-fed condition which contributed to the prevention of HFD-induced obesity. Our study suggests that dietary fiber inulin and its microbial fermentation product propionate can regulate metabolic homeostasis through regulating intestinal lipid handling, which may provide a novel therapeutic target for both prevention and treatment of obesity.

Introduction

Metabolic disorders such as obesity and type 2 diabetes have become major threats to human health worldwide (Franks *et al.* 2016). It is well recognized that dietary fiber intervention is one of the most efficient strategies for improving metabolic health (Parnell *et al.* 2009, Dewulf *et al.* 2013, Kovatcheva-Datchary *et al.* 2015, Zhao *et al.* 2018). However, the mechanisms for dietary fibers on metabolic regulation still need further investigation.

These beneficial effects of dietary fibers are, at least partially, attributed to their fermentation end products by gut microbiota, short-chain fatty acids (SCFAs) (Koh *et al.* 2016, Makki *et al.* 2018). 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 (Schwiertz *et al.* 2010,

Rahat-Rozenbloom *et al.* 2014). However, emerging evidence shows propionate has beneficial effects on metabolic regulation (Lin *et al.* 2012, Chambers *et al.* 2015, Den Besten *et al.* 2015a, Lu *et al.* 2016, Chambers *et al.* 2018). 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 (Ridaura *et al.* 2013). Moreover, recent studies in obese humans suggested long-term propionate administration prevented body weight gain and reduced lipid accumulation (Chambers *et al.* 2015, Chambers *et al.* 2018). Dietary propionate supplementation also protected against high-fat diet (HFD)-induced obesity and insulin resistance in mice (Den Besten *et al.* 2015a). These beneficial effects could attribute to appetite reduction and increased resting energy expenditure via promoting lipid oxidation by propionate (Chambers *et al.* 2015, Den Besten *et al.* 2015a). Therefore, conflicting results suggest the mechanisms of propionate in metabolic regulation need further investigation.

Recent evidence suggests propionate can regulate metabolic homeostasis in gut. The study by Vadder *et al.* showed propionate activated intestinal gluconeogenesis to improve whole body glucose metabolism (De Vadder *et al.* 2014). Meanwhile, lipid handling by the gut is being recognized as under regulatory control and has broad ramifications for whole body energy homeostasis (Abumrad *et al.* 2012). Recent studies showed that lipid storage might be present within enterocytes, primarily as triglyceride (TG). Intestinal triglyceride goes through resynthesis, cytoplasmic storage and mobilization as well as secretion in chylomicron particles (Xiao *et al.* 2018). Regulation of intestinal lipid metabolism could affect enterocyte lipid content and whole body fat accumulation (Cao *et al.* 2004, Uchida *et al.* 2013, Nelson *et al.* 2014, Luo *et al.* 2018). Catabolism of lipid in the intestine includes two aspects, cytoplasmic lipolysis and autophagic lipolysis which is termed lipophagy (Singh *et al.* 2012, Young *et al.* 2013). 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 (Obrowsky *et al.* 2013). Lysosomal acid lipase (*Lal*) is the enzyme that breaks down triglyceride via lipophagy (Ward *et al.* 2016). LAL deficiency in mice also resulted in massive accumulation of triglyceride and cholesteryl ester in small intestine (Du *et al.* 2001). Whether SCFAs, especially propionate, have an effect on intestinal lipid metabolism is worth studying.

One important mechanism for SCFA-mediated regulation is via free fatty acid receptors, FFAR2 and FFAR3 (preferentially activated by propionate) (Samuel *et al.* 2008, Kimura *et al.* 2013). However, evidence suggested FFAR-independent mechanisms could also be involved for propionate, as *Ffar3*-deficient mice were still sensitive to SCFA metabolic regulation (Lin *et al.* 2012, Christiansen *et al.* 2018). 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 (Tang *et al.* 2011, Eamin *et al.* 2013, Den Besten *et al.* 2015a). AMPK is a vital regulator of lipid metabolism in multiple organs (Daval *et al.* 2005, Ahmadian *et al.* 2011, Mottillo *et al.* 2016). Meanwhile, propionate also acts as a modulator of epigenome by altering histone acetylation (Johnstone 2002). Lysine-specific demethylase 1 (LSD1), an epigenetic regulator by altering histone methylation, particularly plays a key role in lipid metabolism in adipose tissue (Duteil *et al.* 2014, 2017). Recent study demonstrated that intestinal AMPK deficiency in mice and Caco-2 cell regulated LSD1 activity (Sun *et al.* 2017), indicating an intrinsic link between AMPK and LSD1 in intestines.

In this study, we showed that inulin and propionate decreased whole body fat mass in chow diet -fed mice without affecting food intake. Meanwhile, propionate and inulin induced intestinal lipolysis and decreased intracellular TG content. Propionate directly increased lipolysis genes expression such as *Atgl*, hormone-sensitive lipase (*Hsl*) and *Lal* in Caco-2 cells via activation of phosphorylation of AMPK and LSD1. These beneficial effects of propionate and inulin on intestinal lipolysis were also present in HFD-fed mice to contribute to the prevention of diet-induced obesity (DIO). Thus, we report a mechanism linking microbial fermentation metabolite 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.

Materials and methods

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). 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 SCFA treatment studies, 8-week-old male mice were fed on chow diet and were dividedly gavaged with sodium acetate (S2889, Sigma), sodium propionate (P1880, Sigma), sodium butyrate (303410, Sigma) or inulin (BENEO-Orafti, Belgium) at 2000 mg/kg body weight in saline solution every day for 2 weeks. For DIO studies, 8-week-old male mice were pre-treated with HFD (D12451, 45% fat, Research Diets) for 3 weeks and then were dividedly gavaged with propionate or inulin at 2000 mg/kg body weight every day with HFD feeding 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 experiments, all mice were killed after 6 h fasting and tissue samples and plasma were collected for further analysis.

Tissue, fecal and serum lipid analysis

Lipids were extracted from tissues following the method of Folch *et al.* by a minor modification (Folch-Pi *et al.* 1957). Briefly, tissues were homogenized with chloroform:methanol (2:1) to a final volume 20 times of the tissue sample. After dispersion, the whole mixture was agitated for a minimum of overnight at room temperature. Then, the solvent was added with 0.2 volume of 0.9% NaCl. After vortex and spin at $2000\,\text{g}$ for 10 min, the bottom chloroform phase containing lipids was evaporated under a nitrogen stream. Finally, the dry content was dissolved in butanol:Triton X-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.* (Kraus *et al.* 2015). Triglyceride contents in the tissues, feces and blood were assayed using a triglyceride assay kit (Nanjing Jiancheng, A110-2).

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% CO_2 at 37°C. For SCFA 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 collected for further analysis.

RNA extraction and quantitative RT-PCR

Total RNA was extracted from tissues and cells using TRIzol reagent (Invitrogen), according to the manufacturer's instructions. Total RNA (1 μg) was reverse transcribed with Revert-Aid first strand cDNA synthesis kit (Thermo Scientific; #K1622) for quantitative RT-PCR with Power SYBR Green PCR master mix (Applied Biosystems, 1708040) and Q6 real-time PCR system (Applied Biosystems). The relative abundance of mRNA was standardized with Tata-binding protein (TBP) or β-Actin as the invariant control. Primer sequences are given in Supplementary Table 1 (see section on supplementary data given at the end of this article).

Western blotting

The tissues and cells were homogenized in the lysis buffer (RIPA, BioTeke) containing 1 mmol/L protease inhibitor PMSF (P7626, Sigma) and centrifuged at $12,000\,\text{g}$ 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. Antibodies, anti-AMPK (#2532, 1/1000), anti-phospho-AMPK (#2535, 1/1000), anti-LSD1 (#2184, 1/1000) were purchased from Cell Signaling Technology; 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).

Oil Red O staining

Fresh tissue samples were isolated and fixed with 3.7% formaldehyde for 48 h at 4°C. The samples were then incubated in 30% sucrose for 12 h and embedded in Tissue-Tek O.C.T. Compound. Serial sections (8 μM) were made and stained with 0.3% oil Red O (Sigma, O0625) for 10 min. Caco-2 cells were washed with pre-cold PBS three times and fixed with 3.7% formaldehyde for 30 min. 0.3% oil Red O in isopropanol was then added directly to the

fixed cells and incubated for 1h. The red lipid droplets were visualized by microscopy (NIS-Elements; Nikon) and dissolved in isopropanol to quantify by a microplate reader (Thermo Fisher Scientific).

Statistical analysis

The data were processed with SPSS software version 19.0 (SPSS Inc.). 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 ± s.e.m.

Results

Dietary propionate and inulin reduce whole body fat mass and intestinal triglyceride content in chow diet-fed mice

We first examined the effects of dietary SCFAs and inulin, which is a fiber source that results in a rapid, extensive generation of SCFAs after fermentation, on body weight and fat mass in chow diet-fed mice. Our results showed that body weight gain was not affected after 2-week gavage feeding with 2000 mg/kg of SCFAs (Xiong *et al.* 2004) or inulin comparing to control group (Fig. 1A and Supplementary Fig. 1A), however, whole body fat mass was decreased by propionate and inulin (10.3–7.2% and 10.3–8.7%, respectively) (Fig. 1B). Importantly, there was no change of food intake (Fig. 1C) or fecal triglyceride level (Fig. 1D) under propionate or inulin treatment,

suggesting dietary lipid absorption might not be affected. Then we examined the tissue weight and results showed that inguinal and epididymal white adipose tissue weights were decreased (Supplementary Fig. 1C and D), liver and muscle weights were not affected (data not shown), and the unit length weights of jejunum and ileum were increased (Fig. 1E and F). Histology analysis showed villus length of small intestines was increased by propionate and inulin which could attribute to the unit length weight increase (Supplementary Fig. 1E and F). Meanwhile, results showed triglyceride levels in jejunum and ileum as well as serum and adipose tissue were significantly lower in propionate and inulin groups (Fig. 1G, H and I and Supplementary Fig. 1I and J); however, triglyceride level in liver was unaffected (Supplementary Fig. 1G and H). These results showed dietary propionate and inulin decreased whole body fat mass and intestinal triglyceride content without affecting food intake.

Propionate and inulin induce intestinal lipolysis gene expression

We next measured the expression of lipid metabolism key enzymes in tissues. Results showed *Atgl* and *Hsl*, which are key lipases that catalyze cytoplasmic lipolysis (Holm 2003), and rate-limiting fatty acid oxidation enzyme *Cpt1a* expression were not affected by propionate or inulin in liver and adipose tissue (Supplementary Fig. 2A and B). Meanwhile, cluster of differentiation 36 (*Cd36*) and fatty acid transporter member 4 (*Fatp4*) mRNA expressions were not affected in liver (Supplementary Fig. 2A),
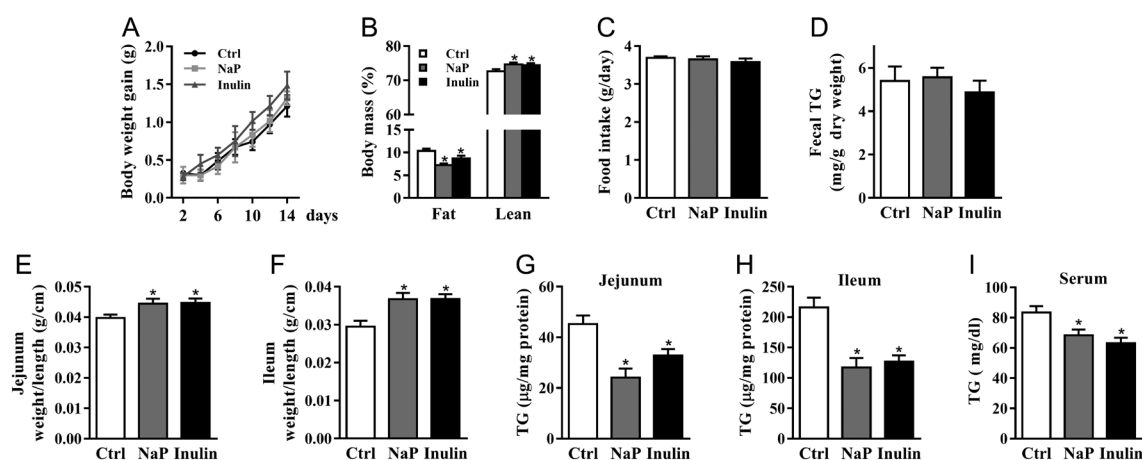

Figure 1
Effect of propionate and inulin on metabolic phenotype in mice fed a chow diet. (A, B and C) Body weight gain (A), fat mass (B) and food intake (C) of mice treated with propionate or inulin for 2 weeks. (D) Fecal triglyceride content. (E and F) The unit length weight of jejunum (E) and ileum (F). (G and H) Triglyceride content in the jejunum (G) and ileum (H). (I) Triglyceride content in serum. Data are mean ± s.e.m. and statistical analyses were performed using two-tailed Student's *t* test, *n* = 8 per group. Ctrl, control; NaP, sodium propionate. *$P < 0.05$ versus control.

together with unaffected TG content (Supplementary Fig. 1G and H), suggesting that liver lipid metabolism was not significantly regulated by propionate or inulin. Although *Fatp4* was unaffected, CD36 mRNA expression was decreased in adipose tissue (Supplementary Fig. 2B), which could indicate the lipid uptake in adipose tissue was lower by propionate or inulin to decrease the TG content (Supplementary Fig. 1I and J) since lipolysis and fatty acid oxidation were not affected.

Notably, the expression of *Atgl* and *Hsl* was significantly increased in the jejunum (Fig. 2A) and ileum (Fig. 2B) after propionate and inulin treatment. Autophagic lipolysis enzyme *Lal* expression was also elevated in the jejunum (Fig. 2A). *Cd36* and *Fatp4* expression was not affected (Supplementary Fig. 2C and D), suggesting enterocyte lipid uptake was not significantly regulated. Intestinal triglyceride resynthesis enzymes monoacylglycerol acyltransferase 2 (*Mgat2*) and diacylglycerol acyltransferase 1 (*Dgat1*) expression were not changed in response to propionate or inulin (Fig. 2C and D), suggesting intestinal triglyceride resynthesis was not affected. Meanwhile, *Cpt1a* was elevated, suggesting an increase of fatty acid oxidation (Fig. 2E and F). Together, these results suggested propionate and inulin could increase intestinal lipolysis to reduce intestinal triglyceride content which contributed to the decreased whole body fat mass.

Propionate directly induces lipolysis gene expression and decreases lipid content in Caco-2 cells

We next investigated the direct effects of propionate on intestinal lipolysis using Caco-2 cells, which have a phenotype that resembles the enterocytes of small intestines (Borchardt 2011). 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 or butyrate incubation at the same dose (Fig. 3A and Supplementary Fig. 3A). Caco-2 cell vitality was not affected (Supplementary Fig. 3B). Triglyceride content in Caco-2 cells was also significantly decreased by 0.1 and 0.5 mmol/L propionate (Fig. 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 (Fig. 3C, D and E). Meanwhile, *Cd36* was not affected (Supplementary Fig. 3C), suggesting lipid uptake might not be a contributing factor to decreased lipid content under propionate treatment. *Dagt1* expression was not changed by SCFAs (Supplementary Fig. 3D), suggesting SCFAs also did not affect triglyceride synthesis in Caco-2 cells. These data indicated that propionate was able to directly promote intestinal lipolysis gene expression and decrease enterocytes triglyceride content.

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* nor *Ffar3* was affected by propionate or inulin treatment in the jejunum (Supplementary Fig. 4A and B). Instead, immunoblot analyses showed that phosphorylation of AMPK (p-AMPK, Thr172) in the jejunum and ileum was significantly elevated (Fig. 4A and B). *In vitro* studies also showed propionate directly increased p-AMPK dose-dependently in Caco-2 cells (Fig. 4C). To investigate the role of AMPK signaling in propionate regulation of intestinal lipolysis, we used a specific inhibitor of p-AMPK (compound C, Cc). 10 μmol/L Cc treatment to Caco-2 cells was able to inhibit p-AMPK protein expression (Supplementary Fig. 5B), meanwhile, significantly
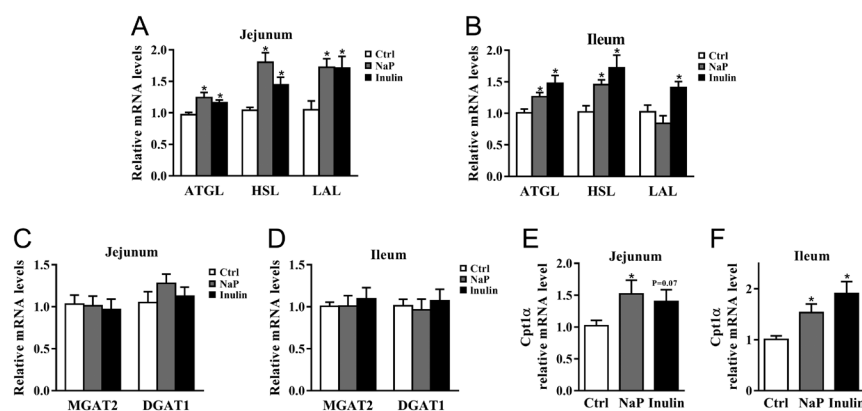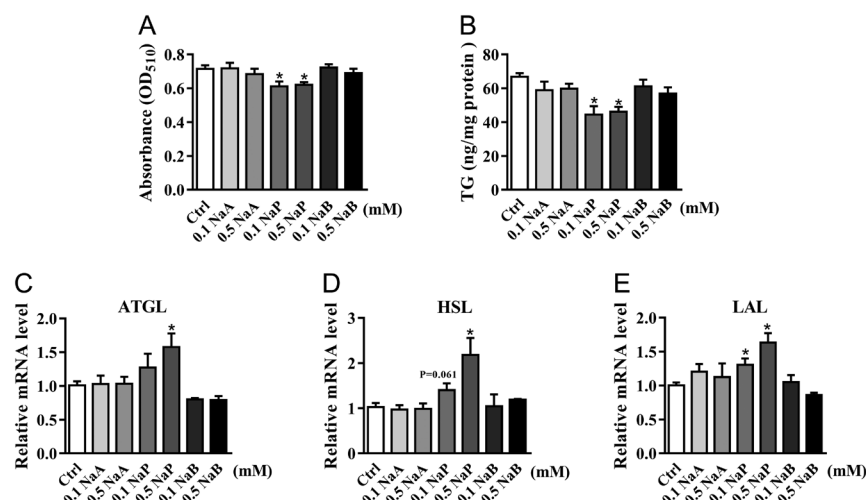
Figure 2

Effect of propionate and inulin on small intestinal lipid metabolism gene in mice. (A and B) Relative mRNA expression of *Atgl*, *Hsl* and *Lal* in the jejunum (A) and ileum (B). (C and D) Relative mRNA expression of *Mgat2* and *Dgat1* in the jejunum (C) and ileum (D). (E and F) Relative mRNA expression of Cpt1α in the jejunum (E) and ileum (F). Data are mean ± s.e.m. and statistical analyses were performed using two-tailed Student's *t* test, *n* = 8 per group. *P < 0.05 versus control.
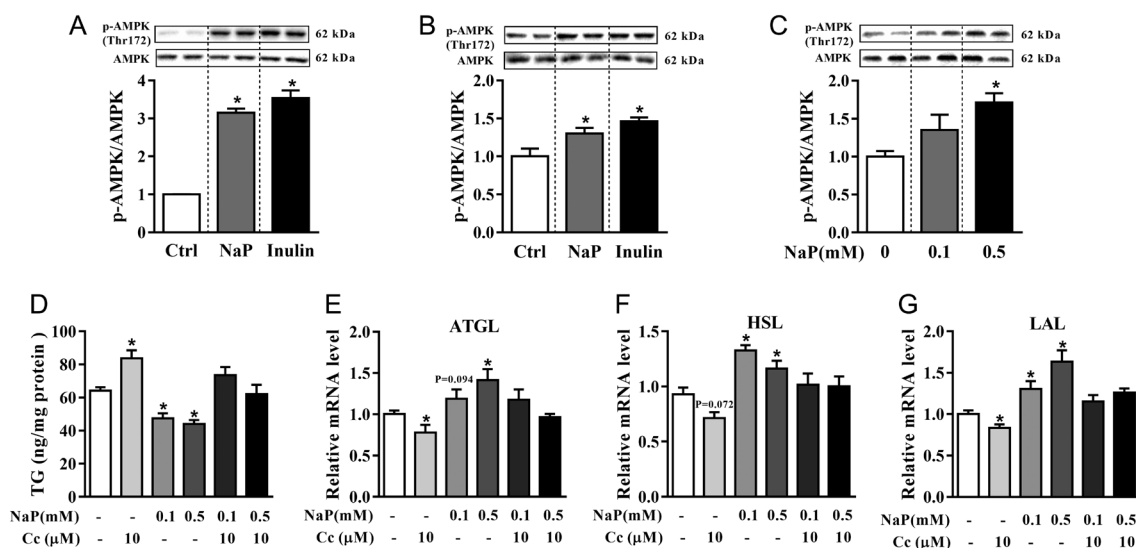
Figure 3

Effect of SCFAs on lipid metabolism in Caco-2 cells. (A) Triglyceride content was assessed in Caco-2 cells treated with 0.1 and 0.5 mmol/L SCFAs for 24 h by Oil Red O staining. (B) Triglyceride content of Caco-2 cells treated with 0.1 and 0.5 mmol/L SCFAs for 24 h. (C, D and E) Relative mRNA expression 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 ± S.E.M. and statistical analyses were performed using one-way ANOVA, $n = 6$ per group. Ctrl, control; NaA, sodium acetate; NaB, sodium butyrate; NaP, sodium propionate. *$P < 0.05$ indicates a significant difference.

increased triglyceride content (Supplementary Fig. 5A) and decreased *Atgl*, *Hsl* and *Lal* mRNA expression after 24-h incubation (Fig. 4E, F and G). More importantly, when elevation of p-AMPK by propionate was blocked by Cc (Supplementary Fig. 5B), the reduction of triglyceride content by propionate treatment was blocked simultaneously in Caco-2 cells (Fig. 4D) as well as the increase of ATGL, HSL and LAL expression (Fig. 4E, F and G). These data suggested that AMPK signaling had a direct role in propionate regulation on intestinal lipolysis and lipid content.

Propionate upregulates LSD1 downstream of p-AMPK to induce intestinal lipolysis

Previous studies showed AMPK could regulate LSD1 in Caco-2 cells, and LSD1 has important role in lipid metabolism regulation (Sun *et al.* 2017). We measured the protein expression of LSD1 in the jejunum and ileum and results showed that propionate and inulin significantly increased LSD1 expression in both jejunum and ileum (Fig. 5A and B). *In vitro* studies also showed propionate directly increased LSD1 dose-dependently

Figure 4

Propionate regulates lipolysis dependent on p-AMPK in Caco-2 cells. (A and B) Protein expression of p-AMPK and AMPK in the jejunum (A) and ileum (B) of mice treated with propionate or inulin, $n = 6$ mice per group. (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, $n = 4$ per group. (D) Triglyceride content of Caco-2 cells when stimulated with compound C (Cc, 10 μmol/L) in the presence of propionate for 24 h, $n = 6$ per group. (E, F and G) Relative 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, $n = 6$ per group. Data are means ± S.E.M. and and statistical analyses were performed using one-way ANOVA. *$P < 0.05$ indicates a significant difference.

in Caco-2 cells (Fig. 5C). Inhibition of LSD1 by its inhibitor GSK2879552 (5 µmol/L) (Supplementary Fig. 5C) significantly increased triglyceride level in Caco-2 cells (Fig. 5D) and decreased *Atgl*, *Hsl* and *Lal* expression (Fig. 5E, F and G). More importantly, when elevation of LSD1 by propionate was blocked by GSK2879552 treatment (Supplementary Fig. 5C), the decrease of triglyceride level by propionate was completely blocked (Fig. 5D), as well as the increase in *Atgl*, *Hsl* and *Lal* expression (Fig. 5E, F and 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 (Fig. 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 (Fig. 5I). Taken together, these data suggested that propionate upregulated LSD1 downstream of p-AMPK to induce intestinal lipolysis.

Propionate and inulin induce intestinal lipolysis and protect against HFD-induced obesity

Finally, we examined whether the beneficial effects of propionate and inulin still existed in HFD-fed mice. Mice were pre-treated with HFD for 3 weeks and gavaged with propionate, inulin and saline with HFD feeding; results showed that propionate and inulin completely prevented HFD-induced body weight gain (Fig. 6A), due to decreased fat mass by 6.6 and 8.2% (Fig. 6B). White adipose tissue weights were decreased consistent with lower fat mass (Supplementary Fig. 6A and 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 (Fig. 6C and D). Liver and muscle weights were not affected (data unshown). TG content in liver was not affected (Supplementary Fig. 6E). Notably, intestines weights were not significantly changed by propionate or inulin in HFD-fed mice (Supplementary Fig. 6C and 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 (Fig. 6E, F and G). The expression of *Atgl*, *Hsl* and *Cpt1a* in the jejunum and ileum was increased in propionate or inulin treatment (Fig. 6H, I, J and K), whereas *Mgat2* and *Dgat1* were not affected (Supplementary Fig. 6F and G). 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 (Fig. 6L, M, N and O). All together, these findings indicated that propionate and inulin promoted intestinal lipolysis and inhibited HFD-induced obesity.
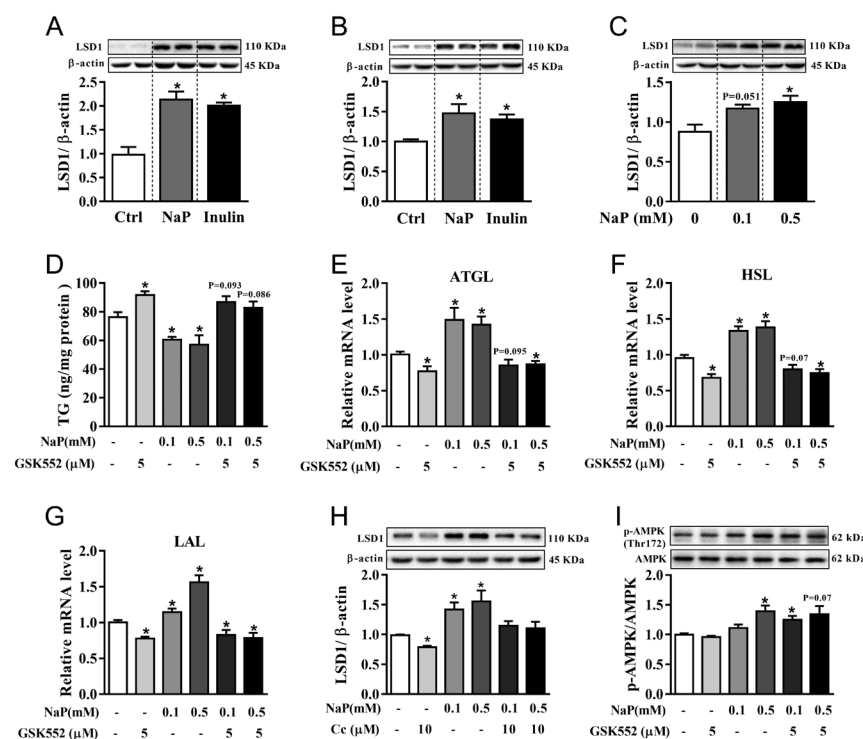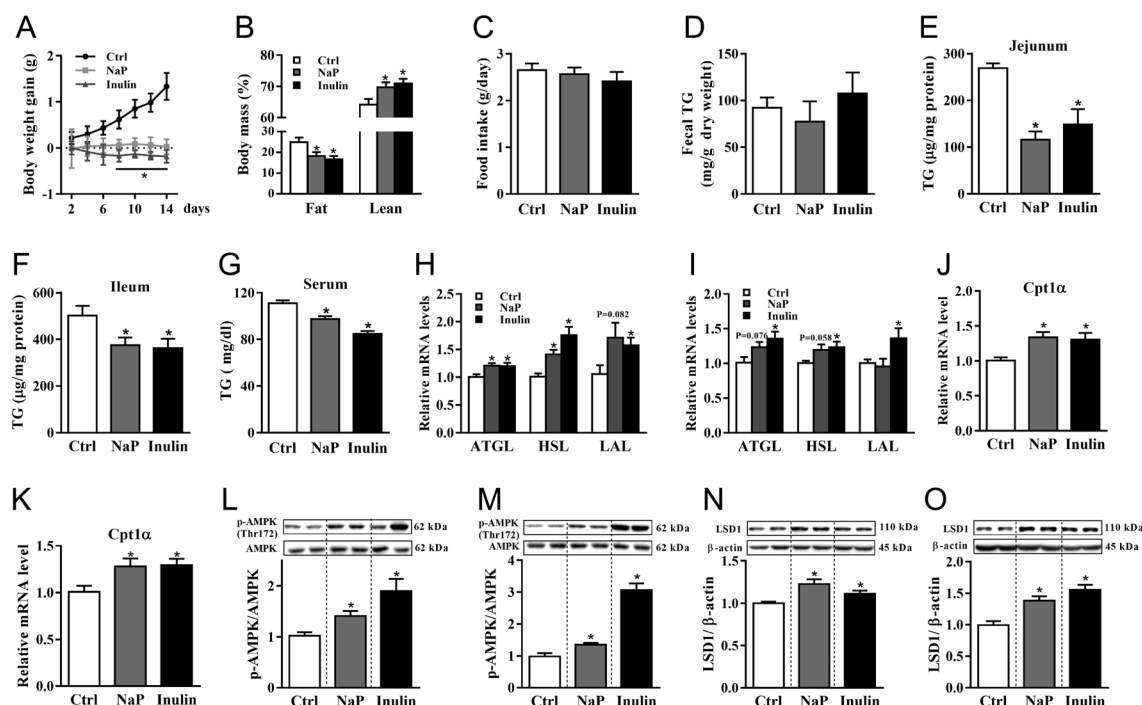

Figure 5

LSD1 regulates propionate-induced lipolysis in Caco-2 cells. (A and B) Protein expression of LSD1 in the jejunum (A) and ileum (B) of mice treated with propionate or inulin, *n* = 6 mice per group. (C) Protein expression of LSD1 in Caco-2 cells treated with 0.1 and 0.5 mmol/L propionate for 24 h, *n* = 4 per group. (D) Triglyceride content of Caco-2 cells stimulated with or without GSK2879552 (GSK552, 5 µmol/L) in the presence of propionate for 24 h, *n* = 6 per group. (E, F and G) Relative 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, *n* = 9 per group. (H) Protein expression of LSD1 in Caco-2 cells treated with or without Cc in the presence of propionate for 24 h, *n* = 4 per group. (I) Protein expression of p-AMPK in Caco-2 cells stimulated with or without GSK552 in the presence of propionate for 24 h, *n* = 4 per group. Data are means ± s.e.m. and statistical analyses were performed using one-way ANOVA. *$P < 0.05$ indicates a significant difference.

Figure 6

Effect of propionate and inulin on metabolic phenotype in HFD-fed mice. (A, B and C) Body weight gain (A), fat mass (B) and food intake (C) of HFD-fed mice treated with propionate or inulin for 2 weeks. (D) Fecal triglyceride content. (E and F) Triglyceride content in the jejunum (E) and ileum (F). (G) Triglyceride content in serum. (H and I) Relative mRNA expression of ATGL, HSL and LAL in the jejunum (H) and ileum (I). (J and K) Relative mRNA expression of Cpt1α in the jejunum (J) and ileum (K). (L and M) Protein expression of p-AMPK and AMPK in the jejunum (L) and ileum (M). (N and O) Protein expression of LSD1 in the jejunum (N) and ileum (O). $n = 6$ per group. Data are means ± S.E.M. and statistical analyses were performed using two-tailed Student's *t* test. *$P < 0.05$ versus control.

Discussion

Here we examined the metabolic effects of dietary fiber inulin and its SCFA fermentation products, and found that inulin and propionate decreased whole body fat content without affecting food intake in chow diet-fed mice. Especially, inulin and propionate induced intestinal lipolysis and decreased enterocyte TG content. *In vitro* studies on Caco-2 cells showed propionate directly increased lipolysis gene expression (such as *Atgl*, *Hsl* and *Lal*) to decrease TG content via activation of p-AMPK and LSD1. Moreover, inulin and propionate could also induce intestinal lipolysis in HFD-fed mice which could contribute to the prevention of DIO by inulin and propionate.

The beneficial effects of dietary fibers and SCFAs on metabolic control, such as resistance to DIO, have been described independently (Lin *et al.* 2012, De Vadder *et al.* 2014, Den Besten *et al.* 2015*a*,b, Lu *et al.* 2016). The effect of propionate has been suggested to be caused by increasing fatty acid oxidation in liver, therefore increased energy expenditure (Den Besten *et al.* 2015*a*, Chambers *et al.* 2018). Other study has also showed that

propionate promoted lipid oxidation in the adipose tissue (Lu *et al.* 2016). Previous studies showed the majority (>90%) of propionate was absorbed by the gastrointestinal tract (Bergman 1990). Here, our data showed dietary propionate significantly decreased whole body fat mass without affecting food intake (Fig. 1B and C and Fig. 6B and C); meanwhile, lipid metabolism was not significantly affected in either liver or adipose tissue (Supplementary Fig. 2A and B). Instead, propionate-induced intestinal lipolysis and decreased enterocytes lipid storage (Figs 2A and B, 3A and B). Recent studies suggest small intestine is an important organ in regulating whole body lipid metabolism. Dietary lipids go through enterocytes uptake 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 *Cd36* expression in adipose tissue was downregulated by propionate treatment (Fig. 1I and Supplementary Fig. 2B). In future studies, intestinal LSD1 and AMPK loss-of-function animal models could be applied to further

demonstrate the effects and mechanisms of propionate on intestinal lipid metabolism to regulate whole body energy homeostasis.

Dietary fibers have been linked to improve gut function (Kieffer *et al.* 2016). Our results showed unit length weights of jejunum and ileum were elevated as well as villus length (Fig. 1E and F and Supplementary Fig. 1E and F), indicating intestinal function improvement. mRNA levels of *Slc7a7* and *Slc7a9* which mediate uptake and release of cationic amino acids, and *Slc6a19* which mediates apical uptake of all neutral amino acids were markedly increased by propionate treatment in the jejunum (Supplementary Fig. 7A). To test intestinal absorption function, we gavage-fed mice with whey protein to both control and propionate-treated mice: results showed that the plasma arginine level was significantly increased after 30 min of treatment in the propionate group (Supplementary Fig. 7B). In addition, propionate did not affect 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) (Supplementary Fig. 7C), suggesting small intestinal inflammatory response was not activated. Therefore, it is likely for propionate to have a role in benefiting gut function by increasing intestinal lipolysis and providing more energy for function improvements since Cpt1α was also elevated (Fig. 2E and F).

The metabolic beneficial effects of SCFAs have been described through activation of G-protein-coupled receptors, *Ffar2* and *Ffar3* (Samuel *et al.* 2008, Tolhurst *et al.* 2012, Kimura *et al.* 2013, Psichas *et al.* 2015, Lu *et al.* 2016). Here, we first tested this pathway and neither *Ffar2* nor *Ffar3* was affected by propionate in the intestine (Supplementary Fig. 4A and B). Our data suggested propionate and inulin directly induced enterocyte lipolysis via an increase of p-AMPK (Fig. 4A, B and C). Similar studies also reported that soluble dietary fiber could enhance the production of SCFAs which activated the intestinal epithelial AMPK activity (Li *et al.* 2017). 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 (Tang *et al.* 2011). Meanwhile, there is also evidence showing SCFA utilization as energy substrates could increase ATP which in turn drives the increase of cAMP (Wang *et al.* 2012).

SCFAs are also important modulators of the epigenome and regulate histone acetylation (Fellows *et al.* 2018). Here we found that propionate increased lipolysis gene expression in enterocytes through elevation of LSD1, a histone demethylase (Fig. 5A, B and C). Our data showed in enterocytes that treated with propionate, p-AMPK inhibitor completely blocked LSD1 elevation and LSD1 inhibitor had no effect on p-AMPK (Fig. 5H and I), indicating LSD1 is a downstream target of p-AMPK in enterocytes, as described earlier (Sun *et al.* 2017). Studies from our lab as well as several independent groups showed LSD1 plays a key role in energy homeostasis (Nakao *et al.* 2019). For example, LSD1 significantly affects browning to regulate whole body lipid metabolism in adipose tissue (Duteil *et al.* 2016, Sambeat *et al.* 2016, Zeng *et al.* 2016). However, the role of LSD1 in the intestine on metabolic homeostasis is unknown. Our data indicate inhibition of LSD1 expression as well as p-AMPK could decrease lipolysis in Caco-2 cells and increase triglyceride content (Figs 4D and 5D). Meanwhile, LSD1 expression in gut could be different between chow diet and HFD (Figs 5A and B, 6N and O). Therefore, we hypothesize that LSD1 could also regulate whole body energy metabolism in gut and the mechanisms worth further investigation.

In summary, we report a link between dietary fiber and its SCFA microbial fermentation products, especially propionate, and host energy homeostasis through intestinal lipid handling. We show that propionate has a regulatory role in enterocyte lipolysis through activation of p-AMPK and LSD1. This further reveals the mechanisms of propionate on whole body metabolism regulation. Since both the positive influence of dietary fiber on obesity (Chambers *et al.* 2015) 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 such as obesity.

Author contribution statement

The authors' responsibilities were as follows: L Z and Y-L Y conducted the experimental design and had primary responsibility for the final content; D W, C-D L and M-L T performed the experiments and analyzed the data; D W and L Z wrote the manuscript and contributed to the discussion; C-Q T, G S and Q-Y J provided support on statistical analysis; and all authors read and approved the final manuscript.

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