Short-chain fatty acids in control of energy metabolism

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Short-chain fatty acids (SCFAs), especially acetate, propionate and butyrate, are the end products from the intestinal microbial fermentation of dietary fibers and resistant starch. It has been well documented that plasma and colonic SCFAs are associated with metabolic syndromes. Recently, the involvement of SCFAs in energy homeostasis regulation has been extensively studied. The importance of SCFAs on energy metabolism has highlighted the potential of modulating SCFAs as a nutritional target to prevent and counteract metabolism disorders and its associated diseases such as obesity and type 2 diabetes. Here, we summarize the current knowledge about the biological properties of SCFAs with their impact on the energy homeostasis.

Keywords

short-chain fatty acids, gut health, energy homeostasis, GPR41/43

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INTRODUCTION

The balance of energy storage and release, also known as energy homeostasis, is crucial for overall health and even survival (Woods 1998). It has been well characterized that obesity, the result of long-term positive energy balance, increases the risk for many diseases such as type 2 diabetes, heart disease, hypertension, stroke, liver disease, colon cancer, and osteoarthritis (Must, Spadano et al. 1999). Similarly, long-term negative energy balance also contributes to several body-disorders, including decline in metabolism, loss of bone mass, decreases in thyroid hormones, and reduction in physical performance (Pollock, Grogan et al. 2010). Therefore, the ability to adapt to variety of energy intakes and outputs is vital.

It has long been recognized that dietary fiber and resistant starch would benefit a range of health issues, such as lowering cholesterol level and controlling blood glucose level (Brown, Rosner et al. 1999, Causey, Feirtag et al. 2000, Boers, MacAulay et al. 2016). Recently, studies have revealed that these beneficial effects of high-fiber diets are, at least partly, attributed to its end-products of gut microbiota fermentation as SCFAs (Canfora, Jocken et al. 2015, De Vadder, Kovatcheva-Datchary et al. 2016). Dietary intake of fiber or resistant starch are linked to enhanced SCFAs production in gut as well as high-peripheral circulating SCFAs (Wong, de Souza et al. 2006, Tarini and Wolever 2010). Human study in obese women found three-month consumption of a mixture of inulin and oligofructose improved postprandial glucose response and enriched butyrate-producing bacteria in gut (Dewulf, Cani et al. 2013). Recent studies in

healthy individuals also found consumption of fiber-rich bread (e.g. barley kernel-based bread, wholegrain rye bread) resulted in improved markers of glucose metabolism with increased serum SCFAs concentrations (Lappi, Mykkanen et al. 2014, Nilsson, Johansson-Boll et al. 2015). Animal studies also show supplementation with inulin-type fructans (fructo-oligosaccharides [FOS]) improved HFD-induced diabetes in mice with improved glucose tolerance, and glucose-stimulated insulin secretion (GSIS) as well as decreased intestinal endotoxin levels and circulate pro-inflammatory cytokines (Cani, Neyrinck et al. 2007).

There has been increasing interest in the idea that a strong link exists between the SCFAs and energy homeostasis. Recent studies have provided a wealth of evidence for the coherent understanding of the multi-level network in which SCFAs exert their effects. The aim of the present mini-review is to summarize the current knowledge about the biological properties of SCFAs with their impact on the energy homeostasis.

EFFECTS OF COLONIC SCFA ABSORPTION IN HUMANS

In humans, acetate, propionate and butyrate are three major SCFAs produced by intestinal fermentation, which account for >95% of SCFA content in gut (Velazquez, Lederer et al. 1997). The production of SCFAs mainly occurs in the proximal colon (including ascending colon and transverse colon) due to the highest level of substrate for colonic fermentation existing there. The concentration of SCFAs can reach around 70 to 140 mM in the proximal colon (Topping and Clifton 2001). Interestingly, although proximal colon is the major place for SCFA production in

gut, distal colonic acetate infusion, but not proximal colonic acetate infusion in obese man, can increase fasting fat oxidation, with a pronounced increase in plasma PYY and postprandial insulin, suggesting the distal SCFAs levels may be important for exerting their functions (van der Beek, Canfora et al. 2016). After being produced in the gastrointestinal (GI) tract, SCFAs are absorbed rapidly and efficiently. It is estimated that less than 10% of SCFAs produced in the colon would be excreted in the feces (Boets, Deroover et al. 2015). At least four putative mechanisms have been described for the colonic absorption of SCFAs, including non-ionic diffusion (Charney, Micic et al. 1998), exchange with bicarbonate in a ratio of 1:1 (Harig, Soergel et al. 1991, Mascolo, Rajendran et al. 1991, Harig, Ng et al. 1996), co-transportation with cations *via* the hydrogen-coupled monocarboxylate transporters (MCT1, MCT2, and MCT4) (Nedjadi, Moran et al. 2014), as well as sodium-coupled monocarboxylate transporter 1 (SMCT1) (Teramae, Yoshikawa et al. 2010). These mechanisms permit SCFAs to regulate lumen pH, epithelial cells volume and cations (especially Na^+) absorption, and further influence the absorption of other nutrients. Indeed, *in vivo* studies have shown the estimated daily colonic SCFAs absorption in human is roughly equivalent to the sodium absorption (300 mmol), suggesting absorption of these two nutrients may be tightly integrated in the colon (Sellin 1999). Furthermore, study also demonstrated that reduced SCFA production (e.g antibiotic induced decrease in colonic microbiota) may results in diarrhea while administration of resistant starch can be the treatment of acute diarrhea by stimulate colonic SCFAs production as well as

improved sodium absorption (Binder 2010). Besides, butyrate is largely absorbed in the colon and plays as the major energy source for colonocytes (Roediger 1980, Wong, de Souza et al. 2006). Butyrate oxidation can make up around 70% and 60% of the oxygen consumption in human descending colon and ascending colon when physiological levels of glucose and butyrate were present (Roediger 1980).

ROLES OF SCFAs IN GUT-BRAIN AXIS

After produced in the gut, SCFAs can reach the systemic circulation *via* superior mesenteric vein (SMV) or inferior mesenteric vein (IMV). Acetate is the major SCFA found in the peripheral circulation. In human, the concentration of acetate can reach several hundred μM in blood while the concentration of propionate and butyrate are negligible (few μM) (Wolever, Josse et al. 1997). Furthermore, free SCFAs can cross the blood-brain barrier *via* monocarboxylate transporters (Vijay and Morris 2014), which makes SCFAs can function as signaling molecules for transmitting the state of the intestines to the brain. Quantitative analysis of cerebrospinal fluid (CSF) by high-resolution nuclear magnetic resonance spectroscopy (HR-NMR) has revealed the existence of approximately 30 – 40 μM SCFAs in CSF (Nagashima, Morio et al. 2010). Indeed, acetate also serves as an important energy source for astrocytes in brain (Muir, Berl et al. 1986, Waniewski and Martin 1998). Positron emission tomography–computed tomography (PET-CT) also confirmed that i.v. administration or colonic infusions of acetate led to acetate uptake by the brain (Frost, Sleeth et al. 2014). Recent study further pointed

out that gut microbiota-derived SCFAs can influence blood-brain barrier permeability (Braniste, Al-Asmakh et al. 2014). Furthermore, SCFAs were proved to be an appetite-regulating agent in brain. Both i.p. injection (500 mg / kg body weight, in C57BL/6 mice) and i.c.v injection (2.5 μM acetate, in male Wistar rats) led to reduction in acute food intake at 1 and 2 h post injection (Frost, Sleeth et al. 2014). The underlying mechanism seems to involve acetate induced neuronal activation in the arcuate nucleus (ARC) in hypothalamus, where increased anorexingenic neuropeptides melanocortin precursor pro-opiomelanocortin (POMC) and decreased orexigenic neuropeptides agouti-related peptide (AgRP) expression were observed with reduced hypothalamic AMPK activity and consequent increased ACC activity (Frost, Sleeth et al. 2014). Using a functional magnetic resonance imaging (fMRI), it was revealed that increasing colonic propionate production by consumption of inulin-propionate ester significantly lowered blood oxygen level-dependent (BOLD) signal during food picture evaluation in the caudate and nucleus accumbens in healthy non-obese men, suggesting colonic propionate may attenuate reward-based eating behavior *via* striatal pathways in human (Byrne, Chambers et al. 2016). Besides to their appetite suppressing properties in CNS, SCFAs also mediate autonomic nervous system (ANS) activities. Propionate (i.p. injection, 1g/ kg body weight, in mice) was found to directly strengthen sympathetic nervous system (SNS) activation as well as downstream physiological effects such as increase in heart rate, oxygen consumption and energy expenditure (Kimura, Inoue et al. 2011). Besides, propionate also can induce action potentials and firing

frequency in sympathetic neurons, suggesting that propionate directly increases sympathetic activity at the sympathetic ganglion level (Kimura, Inoue et al. 2011). Recently, it was reported that HFD-induced acetate production in gut can increase acetate levels in brain and lead to activation of the parasympathetic nervous system, which further increase ghrelin secretion, glucose-stimulated insulin secretion (GSIS), and consequent hyperphagia, hypertriglyceridaemia, lipid accumulation in liver and skeletal muscle (Perry, Peng et al. 2016). This latest evidence further highlighted the complexity of regulatory effects of SCFAs on energy metabolism since it hinted HFD-induced increase in plasma SCFAs (especially acetate) will deteriorate obesity related metabolic syndrome, which seems to be opposite to the beneficial effects of consumption of dietary fibers.

The intestine can also relay signals of nutritional and energy status from gut to brain *via* the secretion of gut-derived hormones (Maric, Gazibara et al. 2014). Recently with the discovery of regulatory effects of SCFAs on a range of gut-derived hormones, SCFAs were recognized as important regulator of food intake. Both *in vitro* and *in vivo* studies have revealed that SCFAs stimulate the secretion of GLP-1 and PYY in L-cells (Tolhurst, Heffron et al. 2012, Chambers, Viardot et al. 2015, Psichas, Sleeth et al. 2015). Additionally, increased circulating concentrations of GLP-1 and PYY after SCFAs administration also support the effects of SCFAs on these gut-derived hormones secretion (Tolhurst, Heffron et al. 2012, Psichas, Sleeth et al. 2015). Human studies also highlighted the effect of intestinal microbes produced SCFAs on

secretion of gut hormones. Healthy individuals consuming additional inulin demonstrated increased plasma GLP-1 rapidly (0.5 h post-administration) and reduced ghrelin level after a followed test meal (after 4 h) (Tarini and Wolever 2010). Consuming brown beans rich in prebiotic non-digestible oligosaccharides at evening meals was also found to increase plasma levels of SCFAs (such as propionate and isobutyrate), PYY and GLP-2 but decrease level of ghrelin after a standard breakfast (Nilsson, Johansson et al. 2013). Consistent with these observed short-term effects, a number of human studies also revealed long-term supplementation of prebiotic fibers (e.g. oligo fructose and cereal β-glucans) or direct delivery of propionate to the human colon may up-regulate PYY and GLP-1 secretion and down-regulate ghrelin secretion with increase in SCFAs (Parnell and Reimer 2009, Verhoef, Meyer et al. 2011, Chambers, Viardot et al. 2015). Hence, regulation of gut-derived hormones, especially appetite regulating hormones such as PYY, GLP-1 and ghrelin, may also contribute to the overall beneficial effects of SCFAs on suppressing appetite and lowering energy intake.

ROLE OF SCFAs IN PANCREATIC ISLETS

Besides the indirect effect of SCFAs on insulin secretion *via* parasympathetic nervous system mentioned above, evidence also support the existence of direct effect of SCFAs on pancreatic beta cells. However, it seems different conclusions were reached from a range of studies (Shah, Wongsurawat et al. 1977, Patel and Singh 1979, Tiengo, Valerio et al. 1981, Ximenes, Hirata et al. 2007, McNelis, Lee et al. 2015, Priyadarshini and Layden 2015, Priyadarshini, Villa et al.

2015, Tang, Ahmed et al. 2015). Shah J.H. *et al.,* firstly reported acetate infusion (0.4 µM/min) in rat significantly potentiated GSIS, hinting the positive regulatory effect of SCFAs on insulin release (Shah, Wongsurawat et al. 1977), however, two independent studies using isolated islets of rat demonstrated the opposite effects of acetate on pancreatic islets (Patel and Singh 1979, Tiengo, Valerio et al. 1981). Recently, with the discovery of opposite effects of two SCFA sensing GPCRs on GSIS upon SCFA activation (GPR41 was found to inhibit glucose-dependent insulin secretion while GPR43 was reported to potentiate insulin secretion) (McNelis, Lee et al. 2015, Priyadarshini and Layden 2015, Priyadarshini, Villa et al. 2015), it seems the balance between GPR43 and GPR41 expression and activation can fine-tune the net effects of SCFAs on insulin secretion, which may explain the discrepancy between studies. However, more studies are needed to clarify how SCFAs influence insulin secretion in islets. Recently, loss of GPR43 (SCFA sensing GPCR) was found to cause impaired islet mass and beta cell survival in mice (Villa, Priyadarshini et al. 2016), suggesting circulating SCFAs may regulate beta cell mass and functions by direct mechanisms.

ROLE OF SCFAs IN LIVER

Liver is an essential metabolic organ, where propionate and butyrate is mainly absorbed (Wong, de Souza et al. 2006). Recently, a human study to measure SCFAs exchange across the gut and liver during major upper abdominal surgery showed very little butyrate and propionate produced in the GI tract could escape the splanchnic area because of the highly efficient hepatic

uptake (Bloemen, Venema et al. 2009).

Imbalance between lipid formation and breakdown, glucose production and catabolism, and cholesterol synthesis and secretion may contribute to aberrant liver energy metabolism (Shen and Shi 2015). SCFAs metabolism in liver can directly affect energy status as sources of energy. For example, propionate can be largely converted to glucose (gluconeogenesis) in liver *via* entering tricarboxylic acid (TCA) cycle (Demigne, Yacoub et al. 1986, Bloemen, Olde Damink et al. 2010, den Besten, Lange et al. 2013). Acetate also can be changed into substrates for the synthesis of cholesterol, long-chain fatty acids (LCFAs) (den Besten, Lange et al. 2013). Besides, variety of research also demonstrated that SCFAs is beneficial for hepatic metabolism. A number of animal studies have revealed the dietary SCFAs induce a switch from hepatic lipogenesis to hepatic beta-oxidation, thus decreasing hepatic steatosis, increasing energy expenditure and providing a protective mechanism against high fat diet (HFD) challenge (Yamashita, Maruta et al. 2009, den Besten, Bleeker et al. 2015). Activation of UCP2-AMPK-ACC pathway seems to be required for this SCFAs mediated beneficial effects on liver metabolism (den Besten, Bleeker et al. 2015). SCFAs also demonstrated the effects on glucose metabolism in liver. Rat fed with dietary containing acetate (0.2% w/w) showed significant higher glycogen, citrate, and lower xylulose-5-phosphate concentrations in liver, suggesting the occurrence of acetate-activated gluconeogenesis and -inactivated glycolysis *via* inactivation of fructose-2,6-bisphosphate synthesis (Fushimi, Tayama et al. 2001). SCFAs were also reported to suppress the cholesterol

synthesis in liver. Diet supplement with SCFAs for 14 days significantly decreased liver cholesterol synthesis as well as plasma cholesterol levels compared to the prebiotic fiber-free diet groups (Hara, Haga et al. 1999). Recently, using liposome encapsulated acetate (LITA), which only delivered acetate peripherally, decreased lipid accumulation, suppressed lipogenesis, and increased mitochondrial functions were also observed in liver of HFD mice, suggesting this anti-lipogenic action of SCFAs in liver should be independent of central action (Sahuri-Arisoylu, Brody et al. 2016).

ROLE OF SCFAs IN ADIPOSE TISSUE

Adipose tissue also has a major influence on energy homeostasis (Rosen and Spiegelman 2006). SCFAs have been demonstrated to promote adipogenesis in 3T3-L1 preadipocytes with down-regulation of the lipid accumulation and up-regulation of PPARγ, AP2 and leptin expression (Hong, Nishimura et al. 2005). These effects seem to happen at the late stage of adipogenesis since exposing 3T3-L1 preadipocytes to acetate or propionate failed to induce any significant changes in nuclear receptors expression, leptin secretion and intracellular triglyceride accumulation at the early stage of differentiation (Frost, Cai et al. 2014). Recently, the pro-adipogenic potential of acetate in immortalized brown adipocytes were also reported (Hu, Kyrou et al. 2016). Interestingly it seems that in rodent adipocytes models, acetate exerts its pro-adipogenic effects at markedly lower concentrations in white adipocytes 3T3-L1 (10^{-7} M) than immortalized brown adipocytes (10^{-3} -10^{-2} M). Considering the fact that circulating acetate

concentration reflects the nutrient status, this might indicate that acetate may act on white and brown adipocytes differently depending on the nutrient status.

Interestingly, the pro-adipogenic effects of SCFAs were not only found in pre-adipocytes from rodent cell models. It has also been demonstrated that SCFAs enhance adipocyte differentiation in porcine adipose tissue (Li, Yao et al. 2014), suggesting pro-adipogenic effect of SCFAs might widely exist in many species. However, acetate treatment failed to alter the process of adipogenesis (using aP2 expression as indicator) using preadipocytes isolated from human omental adipose tissue (OAT) (Dewulf, Ge et al. 2013). Therefore, more evidence is needed to elucidate the effects of SCFAs on adipogenesis in humans.

In addition, i.p administration of acetate at mM level was found to induce the increase in browning of white adipose tissue (WAT) both in HFD- and NFD-fed mice. Since stimulating the browning of WAT has been proven to demonstrate anti-obesity and anti-diabetic effects in rodent models, therefore, SCFAs may also help to improve metabolic health *via* increasing energy expenditure by WAT browning (Sahuri-Arisoylu, Brody et al. 2016).

SCFAs also mediate various aspects of glucose and lipid metabolism in adipose tissue. SCFAs inhibit lipolysis in white adipocytes *in vitro* (Hong, Nishimura et al. 2005) and reduce free fatty acid (FFA) levels in circulation in mice *in vivo* (Hong, Nishimura et al. 2005), which correlates with the decreased ATGL expression in SAT (Sahuri-Arisoylu, Brody et al. 2016) and decreased phosphorylation of HSL at Ser563 (Aberdein, Schweizer et al. 2014). Notably, unlike

plasma FFA lowering agent nicotinic acid, acetate decreases plasma FFA levels without causing undesirable side effects such as flushing, indicating SCFAs such as acetate might be a potential treatment of dyslipidemia than nicotinic acid treatment (Hong, Nishimura et al. 2005). Similar to the effects in liver, SCFAs were also found to increase lipid oxidation in adipose tissue *via* PPAR dependent UCP2-AMPK-ACC pathway activation (den Besten, Bleeker et al. 2015). *In vivo* studies also highlighted the roles of SCFAs in BAT activity. Acetate injection (5.2 mg / kg body weight) decreased lipid accumulation in BAT and increased transcripts of lipolytic genes (such as LCACD, 3KACT, and PPARδ) (Yamashita, Maruta et al. 2009), while butyrate administration (5g/kg body weight) was found to increase adaptive thermogenesis and thermogenesis-related genes (PGC-1α and UCP1) expression in BAT of HFD- mice (Gao, Yin et al. 2009).

Besides, SCFA also affect the cytokines secretion in adipose tissue. *Ex vivo* study using human omental adipose tissue (OAT) and subcutaneous adipose tissue (SAT) has revealed the propionic acid incubation significantly stimulated leptin secretion, suggesting SCFAs may also modulate appetite *via* directly regulating adipokines expression in adipose tissue (Al-Lahham, Roelofsen et al. 2010)

ROLE OF SCFAs IN SKELETAL MUSCLE

SCFAs were also found to mediate glucose metabolism and fatty acid utilization in skeletal muscle. Evidence suggest that AMPK (an important regulator of skeletal muscle metabolism) activation may be the key signal for SCFAs exerting their effects in muscle. Injection of acetate

(10.5 mg/ kg body weight) led to the increase in AMP/ATP ratio and subsequent AMPK activation in muscles in both normal and Otsuka Long-Evans Tokushima Fatty (OLETF) rat rapidly (2-3 mins post-injection) (Yamashita, Maruta et al. 2009). In addition, six months of acetate injections (5.2 mg / kg body weight) led to the increase in the transcription of myoglobin and Glut4 in abdominal muscle of OLETF rats (Yamashita, Maruta et al. 2009). Besides, animal study also demonstrated dietary acetate supplementation (0.2% w/w in diet) induced glycogen storage and decrease glycolysis in gastrocnemius, suggesting SCFAs may enhance glycogen repletion in skeletal muscle (Fushimi, Tayama et al. 2001).

Furthermore, butyrate administration also increased the transformation of skeletal muscle fiber from type I (glycolytic type) into type II (oxidative type) in the vastus lateralis, gastrocnemius, and soleus of HFD-mice *via* activation of AMPK and p38. Consistently, PGC-1α, the main regulator of this transformation was also increased by butyrate treatment (Gao, Yin et al. 2009).

SCFA RECEPTORS

Since the discovery of SCFAs sensing receptors (GPR41 and GPR43) in the early 21st century (Brown, Goldsworthy et al. 2003), SCFAs have been regarded as the signalling molecules involved in various cellular processes. GPR41 and GPR43 have been demonstrated to be indispensable for a range of SCFA-mediated effects. Although numerous studies have suggested the beneficial effects of SCFA administration on appetite regulation and energy

homeostasis, the exact roles of these SCFA sensing GPCRs on energy homeostasis remain unclear. Knockout mice studies provided inconsistent and even contradictory results about the effects of loss of GPR41 and/or GPR43 on energy metabolism (Ge, Li et al. 2008, Bjursell, Admyre et al. 2011, Tolhurst, Heffron et al. 2012, Kimura, Ozawa et al. 2013, McNelis, Lee et al. 2015, Priyadarshini, Villa et al. 2015). SCFAs may exert their functions on metabolism *via* both GPCR-dependent and GPCR-independent mechanisms. Therefore, high selective agonists and antagonists for SCFAs sensing receptors as well as tissue-specific GPR41 and/or GPR43 knock-out mice are needed in future studies to elucidate the involvement of these receptors in SCFA-mediated effects. Notably, the crosstalk between GPR41 and GPR43 expression may also make this task more complex since loss of GPR41 caused the decrease of GPR43 (Zaibi, Stocker et al. 2010). Recently, a study using dual knock out of GPR41 and GPR43 in mice showed increased insulin secretion and improved glucose tolerance in type 2 diabetes after the loss of both GPR41 and GPR43 in the animals (Tang, Ahmed et al. 2015). However, evidence is still not enough to explain the discrepancy between the observed functions of SCFAs and their receptors on metabolism.

CONCLUSIONS

The SCFAs produced by anaerobic microbial fermentation in the digestive tract or feces were widely identified in almost all mammalian species. This fermentation process is believed to be important for herbivorous species, which consume large amount of indigestible carbohydrates

such as cellulose, hemicellulose, pectin and oligosaccharides as food. Current estimates are that SCFAs contribute ~ 70 % to the caloric requirements of ruminants, such as sheep and cattle (Bergman, 1990). Besides, studies in ruminants also demonstrated the regulatory effects of SCFAs on the energy metabolism. One striking example is that intravascularly administration of SCFAs (propionate and butyrate) can stimulate insulin secretion in ruminant animals even stronger than glucose, which was more potential in other non-ruminant animals and humans. Although SCFAs is not counted as major energy source for humans, however, current evidence also strongly suggest the SCFAs production significantly influence the host energy metabolism. This may support the hypothesis that although humans and other and non-ruminant animals have evolutionarily adapted to an era where glucose and fats are more readily available, the ability of sensing nutrient status and regulating energy metabolism *via* SCFAs is still being kept.

Although no final conclusion has yet been reached on how SCFAs affect the energy homeostasis, however, a number of studies have indicated the alterations in SCFAs levels can be associated with changes of energy metabolism. Furthermore, most recent research indicated the addition of fermentable carbohydrates such as dietary fiber or resistant starch to the diets provide protective mechanisms against HFD challenge including reducing appetites, increasing energy expenditure, increasing insulin secretion and sensitives. These findings provide evidence for the potential application of modulating SCFAs and gut microbiota as a possible therapeutic method to prevent and counteract metabolism disorders and associated diseases such as obesity and type

2 diabetes. Indeed, a better understanding towards the roles of SCFAs in metabolism may provide useful information for keeping energy homeostasis in humans.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

Aberdein, N., M. Schweizer and D. Ball (2014). Sodium acetate decreases phosphorylation of hormone sensitive lipase in isoproterenol-stimulated 3T3-L1 mature adipocytes. *Adipocyte* **3**(2): 121-125.

Al-Lahham, S. H., H. Roelofsen, M. Priebe, D. Weening, M. Dijkstra, A. Hoek, F. Rezaee, K. Venema and R. J. Vonk (2010). Regulation of adipokine production in human adipose tissue by propionic acid. *Eur J Clin Invest* **40**(5): 401-407.

Bergman, E. N. (1990). Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. *Physiol Rev* **70**(2): 567-590.

Binder, H. J. (2010). Role of colonic short-chain fatty acid transport in diarrhea. *Annu Rev Physiol* **72**: 297-313.

Bjursell, M., T. Admyre, M. Goransson, A. E. Marley, D. M. Smith, J. Oscarsson and Y. M. Bohlooly (2011). Improved glucose control and reduced body fat mass in free fatty acid receptor 2-deficient mice fed a high-fat diet. *Am J Physiol Endocrinol Metab* **300**(1): E211-220.

Bloemen, J. G., S. W. Olde Damink, K. Venema, W. A. Buurman, R. Jalan and C. H. Dejong (2010). Short chain fatty acids exchange: Is the cirrhotic, dysfunctional liver still able to clear them? *Clin Nutr* **29**(3): 365-369.

Bloemen, J. G., K. Venema, M. C. van de Poll, S. W. Olde Damink, W. A. Buurman and C. H. Dejong (2009). Short chain fatty acids exchange across the gut and liver in humans measured at

surgery. *Clin Nutr* **28**(6): 657-661.

Boers, H. M., K. MacAulay, P. Murray, J. Seijen Ten Hoorn, A. R. Hoogenraad, H. P. Peters, M. A. Vente-Spreeuwenberg and D. J. Mela (2016). Efficacy of different fibres and flour mixes in South-Asian flatbreads for reducing post-prandial glucose responses in healthy adults. *Eur J Nutr*. 1-12.

Boets, E., L. Deroover, E. Houben, K. Vermeulen, S. V. Gomand, J. A. Delcour and K. Verbeke (2015). "Quantification of in vivo colonic short chain fatty acid production from inulin." *Nutrients* **7**(11): 8916-8929.

Braniste, V., M. Al-Asmakh, C. Kowal, F. Anuar, A. Abbaspour, M. Toth, A. Korecka, N. Bakocevic, L. G. Ng, P. Kundu, B. Gulyas, C. Halldin, K. Hultenby, H. Nilsson, H. Hebert, B. T. Volpe, B. Diamond and S. Pettersson (2014). The gut microbiota influences blood-brain barrier permeability in mice. *Sci Transl Med* **6**(263): 263ra158.

Brown, A. J., S. M. Goldsworthy, A. A. Barnes, M. M. Eilert, L. Tcheang, D. Daniels, A. I. Muir, M. J. Wigglesworth, I. Kinghorn, N. J. Fraser, N. B. Pike, J. C. Strum, K. M. Steplewski, P. R. Murdock, J. C. Holder, F. H. Marshall, P. G. Szekeres, S. Wilson, D. M. Ignar, S. M. Foord, A. Wise and S. J. Dowell (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.

Brown, L., B. Rosner, W. W. Willett and F. M. Sacks (1999). Cholesterol-lowering effects of

dietary fiber: a meta-analysis. *Am J Clin Nutr* **69**(1): 30-42.

Byrne, C. S., E. S. Chambers, H. Alhabeeb, N. Chhina, D. J. Morrison, T. Preston, C. Tedford, J. Fitzpatrick, C. Irani, A. Busza, I. Garcia-Perez, S. Fountana, E. Holmes, A. P. Goldstone and G. S. Frost (2016). Increased colonic propionate reduces anticipatory reward responses in the human striatum to high-energy foods. *Am J Clin Nutr* **104**(1): 5-14.

Canfora, E. E., J. W. Jocken and E. E. Blaak (2015). Short-chain fatty acids in control of body weight and insulin sensitivity. *Nat Rev Endocrinol* **11**(10): 577-591.

Cani, P. D., A. M. Neyrinck, F. Fava, C. Knauf, R. G. Burcelin, K. M. Tuohy, G. R. Gibson and N. M. Delzenne (2007). Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. *Diabetologia* **50**(11): 2374-2383.

Causey, J. L., J. M. Feirtag, D. D. Gallaher, B. C. Tungland and J. L. Slavin (2000). Effects of dietary inulin on serum lipids, blood glucose and the gastrointestinal environment in hypercholesterolemic men. *Nutrition Research* **20**(2): 191-201.

Chambers, E. S., A. Viardot, A. Psichas, D. J. Morrison, K. G. Murphy, S. E. Zac-Varghese, K. MacDougall, T. Preston, C. Tedford, G. S. Finlayson, J. E. Blundell, J. D. Bell, E. L. Thomas, S. Mt-Isa, D. Ashby, G. R. Gibson, S. Kolida, W. S. Dhillo, S. R. Bloom, W. Morley, S. Clegg and G. Frost (2015). Effects of targeted delivery of propionate to the human colon on appetite regulation, body weight maintenance and adiposity in overweight adults. *Gut* **64**(11): 1744-1754.

Charney, A. N., L. Micic and R. W. Egnor (1998). Nonionic diffusion of short-chain fatty acids across rat colon. *Am J Physiol* **274**(3 Pt 1): G518-524.

De Vadder, F., P. Kovatcheva-Datchary, C. Zitoun, A. Duchampt, F. Backhed and G. Mithieux (2016). Microbiota-produced succinate improves glucose homeostasis via intestinal gluconeogenesis. *Cell Metab* **24**(1): 151-157.

Demigne, C., C. Yacoub, C. Remesy and P. Fafournoux (1986). Propionate and butyrate metabolism in rat or sheep hepatocytes. *Biochim Biophys Acta* **875**(3): 535-542.

den Besten, G., A. Bleeker, A. Gerding, K. van Eunen, R. Havinga, T. H. van Dijk, M. H. Oosterveer, J. W. Jonker, A. K. Groen, D. J. Reijngoud and B. M. Bakker (2015). Short-Chain fatty acids protect against high-fat diet-induced obesity via a PPAR gamma-dependent switch fom lipogenesis to fat oxidation. *Diabetes* **64**(7): 2398-2408.

den Besten, G., K. Lange, R. Havinga, T. H. van Dijk, A. Gerding, K. van Eunen, M. Muller, A. K. Groen, G. J. Hooiveld, B. M. Bakker and D. J. Reijngoud (2013). Gut-derived short-chain fatty acids are vividly assimilated into host carbohydrates and lipids. *Am J Physiol Gastrointest Liver Physiol* **305**(12): G900-910.

Dewulf, E. M., P. D. Cani, S. P. Claus, S. Fuentes, P. G. Puylaert, A. M. Neyrinck, L. B. Bindels, W. M. de Vos, G. R. Gibson, J. P. Thissen and N. M. Delzenne (2013). Insight into the prebiotic concept: lessons from an exploratory, double blind intervention study with inulin-type fructans in obese women. *Gut* **62**(8): 1112-1121.

Dewulf, E. M., Q. Ge, L. B. Bindels, F. M. Sohet, P. D. Cani, S. M. Brichard and N. M. Delzenne (2013). Evaluation of the relationship between GPR43 and adiposity in human. *Nutr Metab (Lond)* **10**(1): 11.

Frost, G., Z. Cai, M. Raven, D. T. Otway, R. Mushtaq and J. D. Johnston (2014). Effect of short chain fatty acids on the expression of free fatty acid receptor 2 (Ffar2), Ffar3 and early-stage adipogenesis. *Nutr Diabetes* **4**: e128.

Frost, G., M. L. Sleeth, M. Sahuri-Arisoylu, B. Lizarbe, S. Cerdan, L. Brody, J. Anastasovska, S. Ghourab, M. Hankir, S. Zhang, D. Carling, J. R. Swann, G. Gibson, A. Viardot, D. Morrison, E. Louise Thomas and J. D. Bell (2014). The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nat Commun* **5**: 3611.

Fushimi, T., K. Tayama, M. Fukaya, K. Kitakoshi, N. Nakai, Y. Tsukamoto and Y. Sato (2001). Acetic acid feeding enhances glycogen repletion in liver and skeletal muscle of rats. *The Journal of Nutrition* **131**(7): 1973-1977.

Gao, Z., J. Yin, J. Zhang, R. E. Ward, R. J. Martin, M. Lefevre, W. T. Cefalu and J. Ye (2009). Butyrate improves insulin sensitivity and increases energy expenditure in mice. *Diabetes* **58**(7): 1509-1517.

Ge, H., X. Li, J. Weiszmann, P. Wang, H. Baribault, J. L. Chen, H. Tian and Y. Li (2008). Activation of G protein-coupled receptor 43 in adipocytes leads to inhibition of lipolysis and suppression of plasma free fatty acids. *Endocrinology* **149**(9): 4519-4526.

Hara, H., S. Haga, Y. Aoyama and S. Kiriyama (1999). Short-chain fatty acids suppress cholesterol synthesis in rat liver and intestine. *J Nutr* **129**(5): 942-948.

Harig, J. M., E. K. Ng, P. K. Dudeja, T. A. Brasitus and K. Ramaswamy (1996). Transport of n-butyrate into human colonic luminal membrane vesicles. *Am J Physiol* **271**(3 Pt 1): G415-422.

Harig, J. M., K. H. Soergel, J. A. Barry and K. Ramaswamy (1991). Transport of propionate by human ileal brush-border membrane vesicles. *Am J Physiol* **260**(5 Pt 1): G776-782.

Hong, Y. H., Y. Nishimura, D. Hishikawa, H. Tsuzuki, H. Miyahara, C. Gotoh, K. C. Choi, D. D. Feng, C. Chen, H. G. Lee, K. Katoh, S. G. Roh and S. Sasaki (2005). Acetate and propionate short chain fatty acids stimulate adipogenesis via GPCR43. *Endocrinology* **146**(12): 5092-5099.

Hu, J., I. Kyrou, B. K. Tan, G. K. Dimitriadis, M. Ramanjaneya, G. Tripathi, V. Patel, S. James, M. Kawan, J. Chen and H. S. Randeva (2016). Short-chain fatty acid acetate stimulates adipogenesis and mitochondrial biogenesis via GPR43 in brown adipocytes. *Endocrinology* **157**(5): 1881-1894.

Kimura, I., D. Inoue, T. Maeda, T. Hara, A. Ichimura, S. Miyauchi, M. Kobayashi, A. Hirasawa and G. Tsujimoto (2011). Short-chain fatty acids and ketones directly regulate sympathetic nervous system via G protein-coupled receptor 41 (GPR41). *Proc Natl Acad Sci U S A* **108**(19): 8030-8035.

Kimura, I., K. Ozawa, D. Inoue, T. Imamura, K. Kimura, T. Maeda, K. Terasawa, D. Kashihara, K. Hirano, T. Tani, T. Takahashi, S. Miyauchi, G. Shioi, H. Inoue and G. Tsujimoto (2013). The

gut microbiota suppresses insulin-mediated fat accumulation via the short-chain fatty acid receptor GPR43. *Nat Commun* **4**: 1829.

Lappi, J., H. Mykkanen, K. E. Bach Knudsen, P. Kirjavainen, K. Katina, J. Pihlajamaki, K. Poutanen and M. Kolehmainen (2014). Postprandial glucose metabolism and SCFA after consuming wholegrain rye bread and wheat bread enriched with bioprocessed rye bran in individuals with mild gastrointestinal symptoms. *Nutr J* **13**: 104.

Li, G., W. Yao and H. Jiang (2014). Short-chain fatty acids enhance adipocyte differentiation in the stromal vascular fraction of porcine adipose tissue. *J Nutr* **144**(12): 1887-1895.

Maric, G., T. Gazibara, I. Zaletel, M. Labudovic Borovic, N. Tomanovic, M. Ciric and N. Puskas (2014). The role of gut hormones in appetite regulation (review). *Acta Physiol Hung* **101**(4): 395-407.

Mascolo, N., V. M. Rajendran and H. J. Binder (1991). Mechanism of short-chain fatty acid uptake by apical membrane vesicles of rat distal colon. *Gastroenterology* **101**(2): 331-338.

McNelis, J. C., Y. S. Lee, R. Mayoral, R. van der Kant, A. M. Johnson, J. Wollam and J. M. Olefsky (2015). GPR43 potentiates beta-cell function in obesity. *Diabetes* **64**(9): 3203-3217.

Muir, D., S. Berl and D. D. Clarke (1986). Acetate and fluoroacetate as possible markers for glial metabolism in vivo. *Brain Res* **380**(2): 336-340.

Must, A., J. Spadano, E. H. Coakley, A. E. Field, G. Colditz and W. H. Dietz (1999). The disease burden associated with overweight and obesity. *JAMA* **282**(16): 1523-1529.

Nagashima, H., Y. Morio, S. Meshitsuka, K. Yamane, Y. Nanjo and R. Teshima (2010). High-resolution nuclear magnetic resonance spectroscopic study of metabolites in the cerebrospinal fluid of patients with cervical myelopathy and lumbar radiculopathy. *Eur Spine J* **19**(8): 1363-1368.

Nedjadi, T., A. W. Moran, M. A. Al-Rammahi and S. P. Shirazi-Beechey (2014). Characterization of butyrate transport across the luminal membranes of equine large intestine. *Exp Physiol* **99**(10): 1335-1347.

Nilsson, A., E. Johansson, L. Ekstrom and I. Bjorck (2013). Effects of a brown beans evening meal on metabolic risk markers and appetite regulating hormones at a subsequent standardized breakfast: a randomized cross-over study. *PLoS One* **8**(4): e59985.

Nilsson, A. C., E. V. Johansson-Boll and I. M. Bjorck (2015). Increased gut hormones and insulin sensitivity index following a 3-d intervention with a barley kernel-based product: a randomised cross-over study in healthy middle-aged subjects. *Br J Nutr* **114**(6): 899-907.

Parnell, J. A. and R. A. Reimer (2009). Weight loss during oligofructose supplementation is associated with decreased ghrelin and increased peptide YY in overweight and obese adults. *Am J Clin Nutr* **89**(6): 1751-1759.

Patel, D. G. and S. P. Singh (1979). Effect of ethanol and its metabolites on glucose mediated insulin release from isolated islets of rats. *Metabolism* **28**(1): 85-89.

Perry, R. J., L. Peng, N. A. Barry, G. W. Cline, D. Zhang, R. L. Cardone, K. F. Petersen, R. G.

ACCEPTED MANUSCRIPT

Kibbey, A. L. Goodman and G. I. Shulman (2016). Acetate mediates a microbiome-brain-beta-cell axis to promote metabolic syndrome. *Nature* **534**(7606): 213-217.

Pollock, N., C. Grogan, M. Perry, C. Pedlar, K. Cooke, D. Morrissey and L. Dimitriou (2010). Bone-mineral density and other features of the female athlete triad in elite endurance runners: a longitudinal and cross-sectional observational study. *Int J Sport Nutr Exerc Metab* **20**(5): 418-426.

Priyadarshini, M. and B. T. Layden (2015). FFAR3 modulates insulin secretion and global gene expression in mouse islets. *Islets* **7**(2): e1045182.

Priyadarshini, M., S. R. Villa, M. Fuller, B. Wicksteed, C. R. Mackay, T. Alquier, V. Poitout, H. Mancebo, R. G. Mirmira, A. Gilchrist and B. T. Layden (2015). An acetate-specific GPCR, FFAR2, regulates insulin secretion. *Mol Endocrinol* **29**(7): 1055-1066.

Psichas, A., M. L. Sleeth, K. G. Murphy, L. Brooks, G. A. Bewick, A. C. Hanyaloglu, M. A. Ghatei, S. R. Bloom and G. Frost (2015). The short chain fatty acid propionate stimulates GLP-1 and PYY secretion via free fatty acid receptor 2 in rodents. *Int J Obes (Lond)* **39**(3): 424-429.

Roediger, W. E. (1980). Role of anaerobic bacteria in the metabolic welfare of the colonic mucosa in man. *Gut* **21**(9): 793-798.

Rosen, E. D. and B. M. Spiegelman (2006). Adipocytes as regulators of energy balance and glucose homeostasis. *Nature* **444**(7121): 847-853.

Sahuri-Arisoylu, M., L. P. Brody, J. R. Parkinson, H. Parkes, N. Navaratnam, A. D. Miller, E. L.

Thomas, G. Frost and J. D. Bell (2016). Reprogramming of hepatic fat accumulation and 'browning' of adipose tissue by the short-chain fatty acid acetate. *Int J Obes (Lond)*.

Sellin, J. H. (1999). SCFAs: The enigma of weak electrolyte transport in the colon. *News Physiol Sci* **14**: 58-64.

Shah, J. H., N. Wongsurawat and P. P. Aran (1977). Effect of ethanol on stimulus-induced insulin secretion and glucose tolerance. A study of mechanisms. *Diabetes* **26**(4): 271-277.

Shen, M. and H. Shi (2015). Sex hormones and their receptors regulate liver energy homeostasis. *Int J Endocrinol* **2015**: 294278.

Tang, C., K. Ahmed, A. Gille, S. Lu, H. J. Grone, S. Tunaru and S. Offermanns (2015). Loss of FFA2 and FFA3 increases insulin secretion and improves glucose tolerance in type 2 diabetes. *Nat Med* **21**(2): 173-177.

Tarini, J. and T. M. Wolever (2010). The fermentable fibre inulin increases postprandial serum short-chain fatty acids and reduces free-fatty acids and ghrelin in healthy subjects. *Appl Physiol Nutr Metab* **35**(1): 9-16.

Teramae, H., T. Yoshikawa, R. Inoue, K. Ushida, K. Takebe, J. Nio-Kobayashi and T. Iwanaga (2010). The cellular expression of SMCT2 and its comparison with other transporters for monocarboxylates in the mouse digestive tract. *Biomed Res* **31**(4): 239-249.

Tiengo, A., A. Valerio, M. Molinari, A. Meneghel and A. Lapolla (1981). Effect of ethanol, acetaldehyde, and acetate on insulin and glucagon secretion in the perfused rat pancreas.

Diabetes **30**(9): 705-709.

Tolhurst, G., H. Heffron, Y. S. Lam, H. E. Parker, A. M. Habib, E. Diakogiannaki, J. Cameron, J. Grosse, F. Reimann and F. M. Gribble (2012). Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein-coupled receptor FFAR2. *Diabetes* **61**(2): 364-371.

Topping, D. L. and P. M. Clifton (2001). Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. *Physiol Rev* **81**(3): 1031-1064.

van der Beek, C. M., E. E. Canfora, K. Lenaerts, F. J. Troost, S. W. Olde Damink, J. J. Holst, A. A. Masclee, C. H. Dejong and E. E. Blaak (2016). Distal, not proximal, colonic acetate infusions promote fat oxidation and improve metabolic markers in overweight/obese men. *Clin Sci (Lond)*.

Velazquez, O. C., H. M. Lederer and J. L. Rombeau (1997). Butyrate and the colonocyte. Production, absorption, metabolism, and therapeutic implications. *Adv Exp Med Biol* **427**: 123-134.

Verhoef, S. P., D. Meyer and K. R. Westerterp (2011). Effects of oligofructose on appetite profile, glucagon-like peptide 1 and peptide YY3-36 concentrations and energy intake. *Br J Nutr* **106**(11): 1757-1762.

Vijay, N. and M. E. Morris (2014). Role of monocarboxylate transporters in drug delivery to the brain. *Curr Pharm Des* **20**(10): 1487-1498.

Villa, S. R., M. Priyadarshini, M. H. Fuller, T. Bhardwaj, M. R. Brodsky, A. R. Angueira, R. E. Mosser, B. A. Carboneau, S. A. Tersey, H. Mancebo, A. Gilchrist, R. G. Mirmira, M. Gannon and

B. T. Layden (2016). Loss of Free Fatty Acid Receptor 2 leads to impaired islet mass and beta cell survival. *Sci Rep* **6**: 28159.

Waniewski, R. A. and D. L. Martin (1998). Preferential utilization of acetate by astrocytes is attributable to transport. *J Neurosci* **18**(14): 5225-5233.

Wolever, T. M., R. G. Josse, L. A. Leiter and J. L. Chiasson (1997). Time of day and glucose tolerance status affect serum short-chain fatty acid concentrations in humans. *Metabolism* **46**(7): 805-811.

Wong, J. M., R. de Souza, C. W. Kendall, A. Emam and D. J. Jenkins (2006). Colonic health: fermentation and short chain fatty acids. *J Clin Gastroenterol* **40**(3): 235-243.

Woods, S. C. (1998). Signals that regulate food intake and energy homeostasis. *Science* **280**(5368): 1378-1383.

Ximenes, H. M., A. E. Hirata, M. S. Rocha, R. Curi and A. R. Carpinelli (2007). Propionate inhibits glucose-induced insulin secretion in isolated rat pancreatic islets. *Cell Biochem Funct* **25**(2): 173-178.

Yamashita, H., H. Maruta, M. Jozuka, R. Kimura, H. Iwabuchi, M. Yamato, T. Saito, K. Fujisawa, Y. Takahashi, M. Kimoto, M. Hiemori and H. Tsuji (2009). Effects of acetate on lipid metabolism in muscles and adipose tissues of type 2 diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats. *Biosci Biotechnol Biochem* **73**(3): 570-576.

Zaibi, M. S., C. J. Stocker, J. O'Dowd, A. Davies, M. Bellahcene, M. A. Cawthorne, A. J. Brown,

D. M. Smith and J. R. Arch (2010). Roles of GPR41 and GPR43 in leptin secretory responses of murine adipocytes to short chain fatty acids. *FEBS Lett* **584**(11): 2381-2386.