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Short chain fatty acids in human gut and metabolic health

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REVIEW ARTICLE

Abstract

Evidence is accumulating that short chain fatty acids (SCFA) play an important role in the maintenance of gut and metabolic health. The SCFA acetate, propionate and butyrate are produced from the microbial fermentation of indigestible carbohydrates and appear to be key mediators of the beneficial effects elicited by the gut microbiome. Microbial SCFA production is essential for gut integrity by regulating the luminal pH, mucus production, providing fuel for epithelial cells and effects on mucosal immune function. SCFA also directly modulate host metabolic health through a range of tissue-specific mechanisms related to appetite regulation, energy expenditure, glucose homeostasis and immunomodulation. Therefore, an increased microbial SCFA production can be considered as a health benefit, but data are mainly based on animal studies, whereas well-controlled human studies are limited. In this review an expert group by ILSI Europe's Prebiotics Task Force discussed the current scientific knowledge on SCFA to consider the relationship between SCFA and gut and metabolic health with a particular focus on human evidence. Overall, the available mechanistic data and limited human data on the metabolic consequences of elevated gut-derived SCFA production strongly suggest that increasing SCFA production could be a valuable strategy in the preventing gastro-intestinal dysfunction, obesity and type 2 diabetes mellitus. Nevertheless, there is an urgent need for well controlled longer term human SCFA intervention studies, including measurement of SCFA fluxes and kinetics, the heterogeneity in response based on metabolic phenotype, the type of dietary fibre and fermentation site in fibre intervention studies and the control for factors that could shape the microbiome like diet, physical activity and use of medication.

Keywords: prebiotics, SCFA, dietary fibre, gut health, metabolic health

1. Introduction

There is accumulating evidence that the gut microbiome is of importance in mucosal immunity, barrier integrity and gut health as well in the regulation of host metabolic health. The gut microbiota is able to ferment indigestible food components such as prebiotics and soluble dietary fibres. Prebiotic consumption can result in the microbial production of short-chain fatty acids (SCFA), including acetate, butyrate and propionate, as major end products. These metabolites emerge as one of the key mechanism by which prebiotics exert their beneficial health effects (Gibson *et al.*, 2017; Koh *et al.*, 2016). These include health effects that are attributed to increased microbial SCFA formation including an improved gut barrier function and reduced intestinal inflammation. In addition, the SCFA butyrate is an important fuel for the colonocytes and impairment in fuel supply may play a role in a reduced gastro-intestinal functioning. Additionally, SCFA may affect gastro-intestinal transit and motility. Besides effects on gastro-intestinal health, SCFA may play a role in the pathophysiology of obesity and related diseases such as type 2 diabetes mellitus (T2DM) by affecting body weight control through effects on energy intake and energy expenditure, as well as insulin sensitivity and systemic low-grade (Canfora *et al.*, 2015; Chambers *et al.*, 2018c; Rios-Covian *et al.*, 2016).

Increasing SCFA production and a more saccharolytic fermentation may in that context be considered as a health benefit, but data are mostly based on animal studies and human studies are limited. Additionally, the majority of mechanistic evidence from animal studies used different routes to administer SCFA (e.g. oral vs intragastric infusions vs colonic infusions vs gut microbial fermentation), which may not all directly translate to the human situation. Consequently, a thorough review of the available evidence is required, with a particular focus on human clinical interventions or on human *ex vivo* studies such as human gut microbiota colonic models.

Therefore, an expert group has been set up by ILSI Europe's Prebiotics Task Force, investigating and reviewing the current scientific knowledge on SCFA to consider the relationship between SCFA and human health as well as whether an increased gut-derived SCFA production can be regarded as a biomarker of gut and metabolic health. The manuscript and its conclusions have been refined in a workshop with scientific experts, industrial representatives and policy makers.

In the review, we present current scientific evidence on health effects of gut-derived SCFA with a focus on evidence from human studies and interventions and only from *in vitro* or animal research where almost no human research is available. We first provide an overview of intestinal SCFA production, metabolism as well as rate of absorption, which is followed by an overview of SCFA in relation to gastro-intestinal health. Subsequently, effects on metabolic health are addressed, including effects on energy balance, substrate metabolism and low-grade inflammation.

2. Method section

The search strategy conducted was developed through internal discussion within the research team, and deployed within the PubMed and/or SCOPUS bibliographic database. The search used a combination of terms targeting title headings, keywords or free-text words, with specific filters finally applied.

With respect to all topics on physiological effects the following search terms for SCFA was used: (short chain fatty acid*[TI] OR SCFA[TI] OR butyrate[TI] OR butanoate[TI] OR butyric acid[TI] OR butanoic acid[TI] OR acetate[TI] OR ethanoate[TI] OR acetic acid[TI] OR ethanoic acid[TI] OR propionate[TI] OR propanoate[TI] OR propanoic acid[TI] OR propionic acid[TI]).

This search string was combined with other specific search terms as detailed in the supplementary data. References cited in this review include primarily English language original research and some specific reviews by experts in the field.

The focus was on studies in humans, but important mechanistic insights from animal or '*in vitro*' studies were included. For the parts on body weight control and metabolic health a systematic narrative approach was used. No protocol for this review has been published. Detailed information on the applied search can be found in the supplementary data.

3. Short chain fatty acid metabolism

Human gut microbiota generate short chain fatty acids

The human large intestine is home to a large and diverse population of microbial species, with particularly large numbers of bacteria. The phylogenetic core, dynamics and stability of this ecosystem have been intensively studied by high throughput 16S ribosomal RNA gene sequencing-based technologies (see recent reviews (Almeida *et al.*, 2019; Falony *et al.*, 2016; Hornung *et al.*, 2018; Shetty *et al.*, 2017). Briefly, the dominant bacterial phyla are *Bacteroidetes* and *Firmicutes*, while minor phyla include *Actinobacteria*, *Proteobacteria* and *Verrucomicrobia*, and methanogens are dominant amongst the *Archaea*. Notably eight abundant genera, namely *Bacteroides, Eubacterium, Faecalibacterium, Alistipes, Ruminococcus, Clostridium, Roseburia* and *Blautia,* were identified in more than 1000 humans (Shetty *et al.*, 2017). These microbes utilise a diverse range of dietary substrates to produce an array of

metabolites, many of which are important for the health of the host. Since the majority of gut microbes are bacteria, here we focus mainly on bacterial metabolism of dietary substrates to SCFA. Such substrates include carbohydrates, prebiotics, plant material and proteins that are not digested in the small intestine, either because they are non-digestible or bypass the host's enzymatic/absorptive capacity (due to overload or disease).

Under the anaerobic conditions prevailing in the colon, the carbohydrate material will be preferentially fermented in the proximal colon, where they will be degraded into monosaccharides by microbial hydrolysis and subsequently fermented to phosphoenolpyruvate via the Embden-Meyerhof pathway. The phosphoenolpyruvate intermediate will then be used to produce the SCFA via different reactions.

Production of acetate may occur via acetyl-coenzyme A to acetyl-CoA and then to acetate by numerous anaerobic gut bacteria or via the Wood-Ljungdahl pathway. Acetate can be produced by most gut microbiota species and generally reaches the highest levels in the colon as compared to other SCFA as described below.

Propionate can be produced via the succinate, acrylate or propanediol pathways. The phylogenetic distribution of these three routes used for propionate formation within the human gut bacteria was studied using genomic and metagenomic analyses, and by designing primer sets for the detection of diagnostic genes for these pathways (Reichardt *et al.*, 2014). These researchers showed that the dominant route for propionate production from hexose sugars was determined to be the succinate pathway, as indicated by the widespread distribution of the *mmdA* gene that encodes methylmalonyl-CoA decarboxylase in *Bacteroidetes* and in many Negativicutes. The acrylate pathway, based on detection of the *lcdA* gene, encoding lactoyl-CoA dehydratase, was restricted to a few species within the *Lachnospiraceae* and *Negativicutes*. Support for the latter was obtained by stable isotope probing for lactate utilisation in *Coprococcus catus* (*Lachnospiraceae*). The propanediol pathway detected, using the *pduP* gene encoding propionaldehyde dehydrogenase, that processes deoxy sugars such as fucose and rhamnose was more prevalent within the *Lachnospiraceae* occurring in relatives of *Ruminococcus obeum* and in *Roseburia inulinivorans* (Reichardt *et al.*, 2014).

Butyrate is generally formed by condensation of two molecules of acetyl-CoA via either butyrate kinase or butyryl-CoA:acetate CoA-transferase. Study of the human gut microbiota via primers for the genes encoding these enzymes showed that in most cases butyryl-CoA:acetate CoA-transferase, rather than butyrate kinase, appears to perform the final step in butyrate synthesis and is possible only in the presence of acetate (Louis *et al.*, 2004). Butyrate production is widely distributed among phylogenetically diverse human colonic Gram-positive *Firmicutes*. Two abundant groups are related to *Eubacterium rectale/ Roseburia* spp. and to *Faecalibacterium prausnitzii* (Louis *et al.*, 2010). While most species have the capacity for either propionate or butyrate production from hexose sugars, some species of *Lachnospiraceae* (*C. catus* and *R. inulinivorans*) have been demonstrated to switch from butyrate to propionate production on different substrates (Reichardt *et al.*, 2014; Scott *et al.*, 2006). Besides these pathways for SCFA production, there will be numerous other routes to SCFA as some microbes grow on intermediate products of fermentation, such as hydrogen gas (H_2), lactate, succinate, formate and ethanol, and convert these to end products including SCFA. Other microbes, particularly Archaea species can metabolise carbon dioxide (CO_2) either yielding methane (CH_4) or acetate.

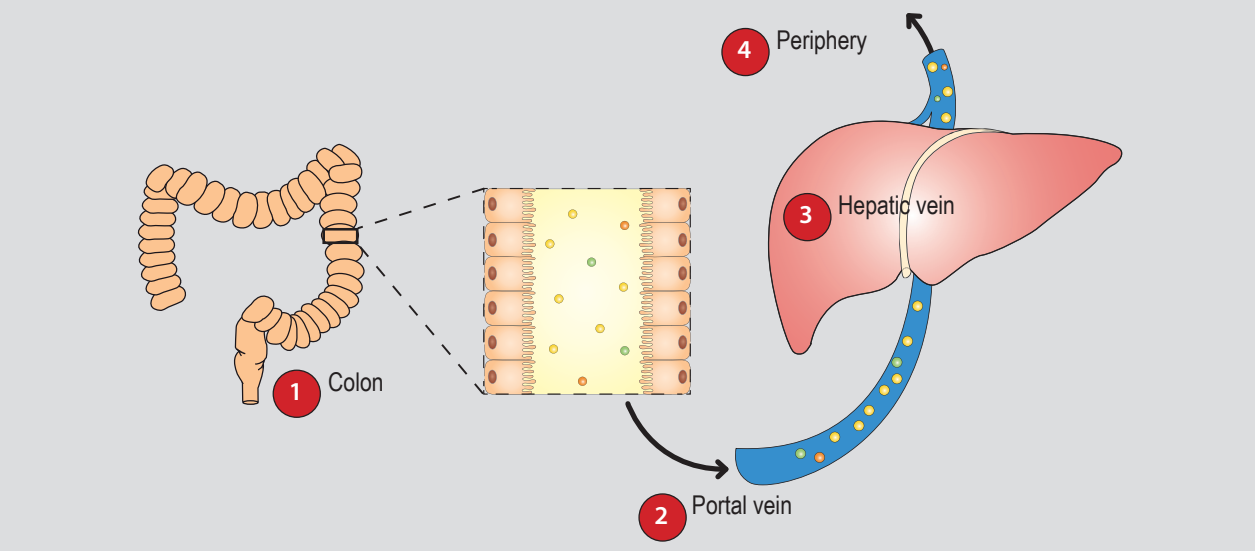
Short chain fatty acid concentrations in the colon

During production in the colon, SCFA are rapidly and almost completely absorbed in colonocytes, or through the portal vein into the liver, or into systemic circulation. Thus only a minor part (approximately 5-10%) is excreted in faeces. Consequently, faecal measurements are not considered as accurate representative for *in vivo* colonic production and should be interpreted with caution (Alles *et al.*, 1996; Boets *et al.*, 2015). Recently, it was shown that longer rectosigmoid transit time was associated with low concentrations of faecal SCFA (Muller *et al.*, 2020). The data suggest that faecal SCFA should be considered as most representative for the SCFA production and/or absorption in the very distal colon and not necessarily other or more proximal colonic sites. Nevertheless, there have been numerous studies that measure SCFA concentrations in faeces upon supplementation with different substrates such as dietary fibres, prebiotics, specific foods and diets, or in comparing certain health and lifestyle conditions. Occasionally, the concentrations of (certain) SCFA in faeces have been correlated with specific gut bacterial types, such as reduction of butyrate-producing bacteria abundance with decline in faecal butyrate on a low carbohydrate weight loss diet (Duncan *et al.*, 2007).

Gaining accurate knowledge on *in vivo* production and absorption kinetics of (individual) SCFA and their relative proportions is challenging due to the inaccessibility of the colon and the rapid absorption by the colonocytes. Human colonic *in vitro* gut models are widely used to estimate levels and ratios of SCFA production from different substrates, but they cannot account for *in vivo* absorption (Aguirre *et al.*, 2016; Grootaert *et al.*, 2009). In this review we focus on the available *in vivo* studies.

While there were several lines of evidence that microbial fermentation of polysaccharides and fibres in the human gut resulted in SCFA formation as detected in faeces, a landmark study confirmed this in human sudden death victims examined within 4 h of death (Cummings *et al.*, 1987). Analysis of the intestinal contents of the victims showed low SCFA concentrations (acetate, propionate, butyrate) in the terminal ileum (13±6 mmol/kg), which dramatically increased in the ascending colon (137-197 mmol/kg) and decreased towards the descending colon (86-97 mmol/kg) (Cummings *et al.*, 1987; MacFarlane *et al.*, 1992). A molar ratio of acetate:propionate:butyrate:valerate:caproate was estimated at 54:20:21:4:1 in these initial studies (Figure 1) (MacFarlane and Gibson, 1995; MacFarlane *et al.*, 1992). Higher SCFA concentrations in the proximal colon are the result of greater production in combination with relatively less absorption having occurred. As the luminal colonic contents move through the colon, SCFA absorption across the epithelium and bacterial utilisation reduce the measurable concentrations. The rate of transit through the colon affects the amount of absorption, and has been shown to correlate with detectable concentrations of SCFA in faeces (Lewis and Heaton, 1997). In contrast, end products of protein fermentation increase from the proximal to distal colon (MacFarlane *et al.*, 1992). Increased fermentation also reduces the pH, which in turn affects the microbial composition and SCFA production. Based on various studies, approximately 100-400 mmol SCFA can be theoretically produced within the human colon upon a daily ingestion of around 10 g of fibres (Cummings and MacFarlane, 1997; McBurney and Thompson, 1989).

A non-invasive methodology to measure SCFA production in the human colon is stable isotope technology, in which labelled carbohydrates are consumed and metabolites monitored in blood or expired air. One of the first studies was in premature infants to determine if lactose fermentation occurred in the colon (Kien *et al.*, 1992). A [1-^{13}C]-acetate orogastric infusion technique was used during constant orogastric feeding, which gave an estimated mean rate of entry of acetic acid of 64.3±38.6% (24%-136%) of the potential two carbon units from dietary lactose in the infants.



	Colon		Portal vein		Hepatic vein		Periphery	
	R	µmol/kg	R	µmol/l	R	µmol/l	R	µmol/l
Acetate	60	43,500-63,400	71	182-263	81	71-220	90	5-220
Propionate	20	14,200-26,700	21	18-30	12	0.8-7	5	3-8
Butyrate	20	14,700-24,400	8	15-30	7	0.5-12	5	7-10

Figure 1. Short chain fatty acid (SCFA) molar ratio and concentration. Data derived from victims who suddenly died and/or from patients undergoing major upper abdominal surgery. Data are expressed as µmol per kg of luminal content in the colon and µmol/l of blood in the portal and hepatic veins and in peripheral blood. Acetate is the most abundant SCFA in the large intestine, portal vein, hepatic vein and peripheral circulation and concentrations decrease, respectively. Note that if one approximates that 1 kg of luminal matter is equivalent to 1 l, the drop in concentration from the colon to the portal vein indicates that about 99.5% of each of the three SCFAs is used by the gut mucosa. The subsequent drop in concentrations between the portal and the hepatic veins further indicates that the remaining low level of propionate and butyrate are mainly extracted by the liver. Note this occurs to a lesser extent for acetate which remains in reasonable levels in the hepatic vein.

More recently a stable-isotope dilution method was used to quantify the *in vivo* colonic production of SCFA in healthy humans after consumption of 15 gram prebiotic inulin (Boets *et al.*, 2015). Primed continuous intravenous infusion with ^{13}C-SCFA were applied while breath and blood samples were collected; it was estimated that 137±75 mmol acetate, 11±9 mmol propionate, and 20±17 mmol butyrate were produced over a 12 h period. Comparison of this study outcome to *in vitro* human colonic gut models showed slightly different production ratios for the three SCFA. Although stable isotope dilution technology is the preferred way to estimate rates of SCFA appearance and disposal, the rapid turnover of the different SCFA may complicate interpretation of the data. Reappearance of label after metabolism inside peripheral cells may also lead to underestimation of the actual fluxes.

Breath gas (H_2 or CH_4) are used to estimate fermentation to SCFA, but these markers only give a global indication of fermentation. New approaches for sampling SCFA directly in the gut are being investigated such as the IntelliCap system (Maurer *et al.*, 2015), which would help alleviate the current issues with faecal samples only representing the balance between production, utilisation and absorption.

Regulation of short chain fatty acid production and cross-feeding

Besides production of butyrate, propionate and acetate, bacteria also produce intermediary SCFAs like formate, succinate and lactate in pure culture which are detected only in very small amounts in faeces, primarily due to bacterial cross-feeding (Figure 2). The diverse community structure in the large intestine means that bacteria do not act in isolation, and bacterial interactions, notably cross-feeding, i.e. the utilisation of substrates or metabolites released by other bacteria, are crucial in determining the levels of final SCFA in the colon. Moreover, bacterial cross-feeding plays a key role in maintaining the diversity and stability of this complex ecosystem. Bacteria can cross-feed on both SCFA products and/or smaller carbohydrate moieties released from the initial degradation of complex carbohydrates. For effective cross-feeding, bacteria have to be in close proximity. Hence, the spatial organisation and niche occupation of the bacterial community is important. Bacterial cross-feeding also drives biofilm formation where bacterial symbiosis is particularly important (MacFarlane and MacFarlane, 1997). Due to the limited amount of *in vivo* studies on cross-feeding, the phenomenon of cross-feeding is described below with examples of *in vitro* studies using gut models or specific co-cultures and supported when possible by the available *in vivo* studies.

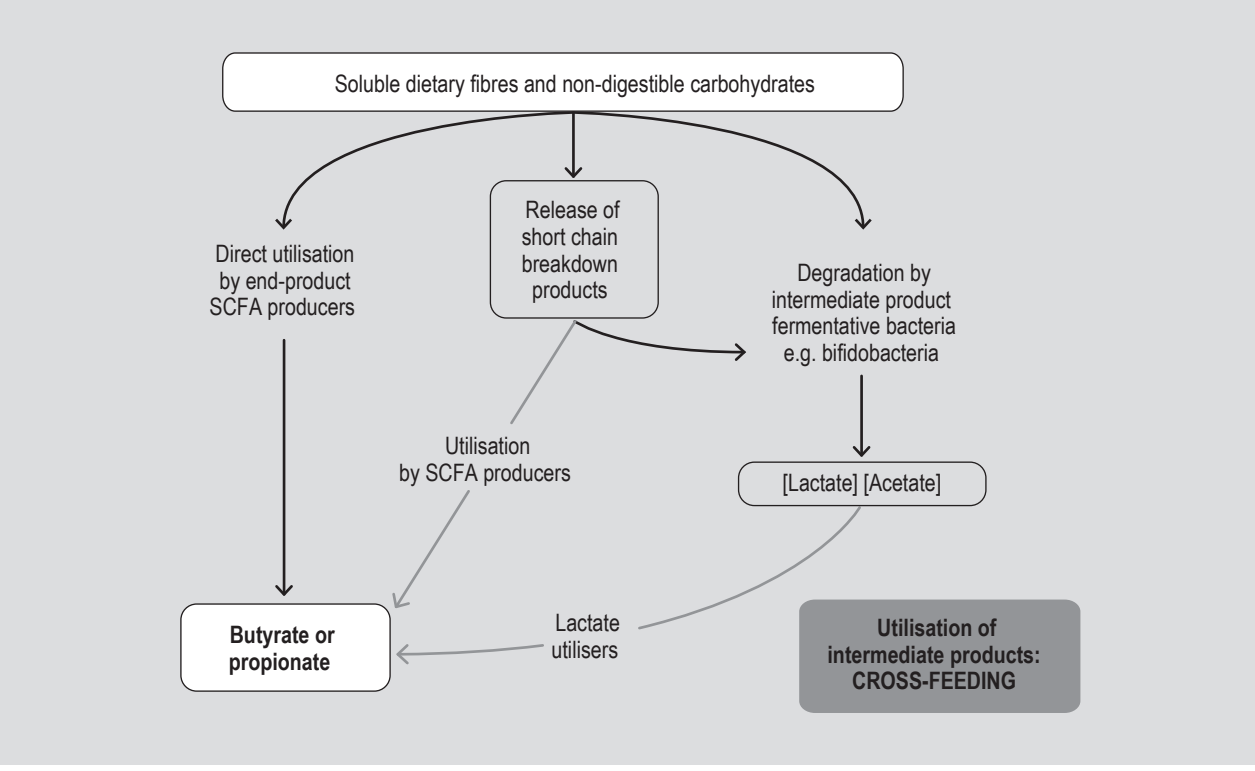


Figure 2. Bacterial cross-feeding. Schematic illustration of the direct fermentation of substrates (non-digestible carbohydrates, soluble fibres and specific prebiotics), or the cross-feeding on intermediate products, to produce the short chain fatty acids (SCFA) end-products propionate and butyrate.

Some of the early work establishing bacterial cross-feeding mechanisms was triggered by the 'prebiotic conundrum'. Specifically, the inulin-type-fructan prebiotics enhance the growth of bifidobacteria, yet also stimulate butyrate production, while bifidobacteria produce lactate, acetate, formate and ethanol, but never butyrate. Lactate is in fact only detected at low concentrations (<5 mM) in faeces from healthy humans. Several studies revealed that lactate-utilising, butyrate-producing bacteria, including *Eubacterium hallii* and *Anaerostipes* species, removed lactate from laboratory media in co-cultures with *Bifidobacterium adolescentis*, thereby producing butyrate (Duncan *et al.*, 2004). Isotope studies using ^{13}-C labelled lactate and [U-^{13}C]glucose confirmed the stimulatory effect of prebiotic fructans on the carbon flow from lactate or acetate into butyrate (Belenguer *et al.*, 2006; Morrison *et al.*, 2006). Detailed co-culture experiments of bifidobacteria strains with other human gut bacterial strains, which are able to use different carbohydrate growth substrates, demonstrated that two types of cross-feeding occur during prebiotic degradation: (1) conversion of the lactate and acetate produced by bifidobacteria during growth into butyrate; and (2) direct utilisation of released complex carbohydrate breakdown products by butyrate-producing bacteria (Belenguer *et al.*, 2006; Falony *et al.*, 2006; Morrison *et al.*, 2006).

The substrate complexity, including chain length, is important in determining which bacteria can utilise which complex carbohydrates for growth. Only a few gut bacteria can use long-chain or complex carbohydrates, with many more microbes benefiting from release of short-chain length intermediary products by these primary degraders. Therefore, bacterial scavenging and fermentation of released mono- and oligo-saccharide products is an effective cross-feeding strategy.

It is beyond the scope of this review to cover the cross-feeding identified for all these complex substrates but a few examples for prebiotics, starch and intestinal mucin are discussed below. The impact of a range of prebiotics, non-digestible oligosaccharides and soluble fibres on the human stool microbiota composition from clinical studies was recently reviewed, which largely supports the *in vitro* studies and identifies some further potential cross-feeding interactions (Swanson *et al.*, 2020).

The key starch utilising bacteria were identified by introducing radio-labelled starch into a colonic fermenter system and tracking the ^{13}-C incorporation into 16S rRNA (Kovatcheva-Datchary *et al.*, 2009). Several bacterial species namely *Ruminococcus bromii, Eubacterium rectale, B. adolescentis* and *Prevotella* species contributed to starch degradation, and *R. bromii* has been identified as a keystone starch degrader, releasing oligo- and mono-saccharides that can be used by other bacteria for growth by cross-feeding (Ze *et al.*, 2012). Human studies with starch supplementation supported the key role of *R. bromii* and *E. rectale* in starch degradation (Martínez *et al.*, 2010; Walker *et al.*, 2011). The main fermentation products of *R. bromii* are acetate and formate whilst *E. rectale* produces butyrate.

Within the inulin-type fructans, there are a range of degradation capabilities by members of the gut microbiota and even a few bifidobacterial species have the capacity to grow on long-chain inulin (Rossi *et al.*, 2005; Scott *et al.*, 2013). The different abilities of bifidobacteria to utilise different chain lengths of inulin means that within-species cross-feeding occurs. In both co-cultures and animal models introduction of (multiple) *Bifidobacterium* species led to induction of genes involved in carbohydrate metabolism and increased SCFA production (Turroni *et al.*, 2016; Turroni *et al.*, 2015). The release of acetate by bifidobacteria was shown to stimulate the growth of *F. prausnitzii* on oligofructose. However, this only happened when slow preferential degradation of oligofructose occurred by *B. adolescentis* internally, and not during rapid efficient degradation of all chain lengths by other bifidobacteria species (Moens *et al.*, 2016). Some butyrate producing bacteria such as *F. prausnitzii* are able to utilise oligofructose directly for growth (Scott *et al.*, 2013). Such *in vitro* mechanistic studies investigating inulin-type-fructans are supported by human studies following dietary consumption and subsequent analyses of the faecal microbiota by molecular technologies such as 16S rRNA sequencing or real-time PCR (Dewulf *et al.*, 2013; Healey *et al.*, 2018; Ramirez-Farias *et al.*, 2009).

The intestinal mucus layer is also a carbohydrate source utilised to generate SCFA, although few gut anaerobes can use mucin directly for growth. *Akkermansia muciniphila* specialises in the degradation of host mucus and resides within the mucin layer (Van Herreweghen *et al.*, 2017). Co-culture of *A. muciniphila* with butyrate-producing bacteria such as *E. hallii* showed that the release of oligo-saccharides and production of acetate by *A. muciniphila* stimulated *E. hallii* growth and led to the synthesis of propionate, butyrate, vitamin B12, and 1,2-propanediol production (Belzer *et al.*, 2017). Transcription of various *A. muciniphila* genes, including those involved in mucin degradation, were differentially regulated in co-culture with *A. caccae* (Chia *et al.*, 2018). These studies clearly showed that bi-directional syntrophy is important in the complex gastrointestinal ecosystem. *Bifidobacterium bifidum* is also able to utilise mucin as a growth substrate, releasing monosaccharides and lactate. In co-culture experiments the growth of *E. hallii* and other bifidobacteria species on mucin were enabled by *B. bifidum*, resulting in altered SCFA profiles, including the utilisation of lactate by the *E. hallii* (Bunesova *et al.*, 2018). The latter interaction may be particularly important in the early colonisation of the infant gut, and the removal of potentially damaging lactate.

In silico models are developing in complexity and are now proving useful in unravelling the bacterial interactions in the gut ecosystem. Bacterial cross-feeding on both SCFA and carbohydrate degradation products are essential parameters to include in such models to obtain realistic outputs (Hoek and Merks, 2017). *In silico* models are initially validated by comparison with results from *in vitro* models. Thus, the flow of ^{13}C labelled starch to propionate via succinate, with the remainder found in acetate and butyrate, could be accurately demonstrated. In addition, it was demonstrated that butyrate is mainly synthesised through cross-feeding on acetate (Binsl *et al.*, 2010). The key role of lactate-utilising bacteria in converting lactate and acetate into butyrate in the human colon was also confirmed *in silico*, with findings replicating those from the batch culture experiments (Muñoz-Tamayo *et al.*, 2011). Substrate preferences of bacteria have also been replicated using mathematical models, verifying the relative importance of cross-feeding on SCFA and carbohydrate degradation products (Van Wey *et al.*, 2016). Moving forward, such models need to consider not only the presence and activity of different bacterial species, but also the relative abundance and competitive abilities of bacteria within the complex community for the different substrates, and the spatial organisation of bacteria within the ecosystem (Henson and Phalak, 2017; Rosendale *et al.*, 2017). The more knowledge we have about bacterial interactions *in vitro*, the more accurate such *in silico* models can be in mimicking the gut ecosystem and thus the more knowledge we can gain about the impact of specific dietary substances on bacterial cross-feeding and metabolic outputs.

Apical and basolateral short chain fatty acid transport

Research on mechanisms involved in trans-epithelial transport of SCFA has been mostly carried out using isolated colonocytes. Both apical and basolateral transport have been studied. Apical transport has been proposed to follow separate routes. Depending on the pH prevailing in the intestinal milieu the SCFA can be protonated and diffuse across the membrane, but since the pKa of the different SCFA is around 4.8 and the luminal pH around 6, most of the SCFA are in the dissociated form and are transported into enterocytes by active transport systems (Sellin, 1999). Three types of transporters have been found: (1) SCFA-bicarbonate co-transport: the activity of this system has been demonstrated though the proteins responsible have not yet been identified (Harig *et al.*, 1996; Mascolo *et al.*, 1991; Vidyasagar *et al.*, 2005); (2) the monocarboxylate transporter (MCT)1-4, with most work done on MCT1 (*SLC16A1)* (Teramae *et al.*, 2010) which also transports lactate and pyruvate in a proton co-dependent way (Teramae *et al.*, 2010); and (3) the sodium dependent monocarboxylate transporter (SMCT, *SLC5A8).* SMCT1 has a preference for butyrate and transports propionate and acetate at a slower rate, although it is probably still the main transporter for them (Gupta *et al.*, 2006; Takebe *et al.*, 2005). Disruption of MCT1 in mice has been shown to be lethal. Experiments in the heterozygous mice showed a distinct intestinal phenotype pointing to an important role for this protein (Lengacher *et al.*, 2013). In view of the fact that MCT1 expression is ubiquitous we will have to wait until intestinal models will be generated to understand the specific role of MCT1.

Since the pH in the cytosol of the enterocytes is higher than in the intestinal lumen, all SCFA will be ionised and cannot diffuse back to the intestinal lumen or out of the basolateral side of the cells. Therefore, on the basolateral side an active SCFA transport is required. Both SCFA/HCO_3 antiport and SCFA/cation symport have been suggested to be involved. The affinity of the basolateral SCFA/HCO_3 antiporter for butyrate is much lower compared to the apical transporter (Km = 17.5 vs 1.5 mM) suggesting that two different proteins are involved (Halestrap and Meredith, 2004; Harig *et al.*, 1996). MCT4 and MCT5 have been found at the basolateral side of the membrane (Gill *et al.*, 2005) and could be involved in transport but the importance of their role is as yet unknown. Clearly more work has to be carried out before we really understand the molecular mechanisms of, in particular, basolateral SCFA transport.

Systemic concentrations of short chain fatty acids

Compared to colonic levels, the plasma concentrations of the three main SCFA are very low: 5-220; <8; <10 μM for acetate, propionate and butyrate, respectively (Bouter *et al.*, 2018; Kootte *et al.*, 2017; Neis *et al.*, 2018; Van der Beek *et al.*, 2015).

The acute SCFA profiles in the systemic circulation derived from the colonic fermentation of ^{13}C-barley meals (Verbeke *et al.*, 2010) or,^{13}C-inulin (Deroover *et al.*, 2017) were determined using a stable isotope approach, which demonstrated that SCFA profiles in blood could change following intake of different dietary fibres. Several studies noted that there were large inter- and intra-individual variations in plasma SCFA concentrations, which may be due to differences in diet, gut microbiota, SCFA absorption and metabolism (Deroover *et al.*, 2017; Peters *et al.*, 1992).

Although portal concentrations of SCFA could be two times higher compared to the periphery blood (Cummings *et al.*, 1987; Neis *et al.*, 2018), there is a massive concentration gradient between the intestinal lumen and the blood compartment. Apparently, intestinal uptake is slower than either colonocyte metabolism or removal from the blood by other organs. This suggests that the transporters at the apical side of the colonocytes are important rate controlling steps for SCFA metabolism by the host. Since the lumen-blood gradient is higher for propionate and butyrate compared to acetate this may be particularly true for these two SCFAs (Figure 1). As discussed in the section

on gluconeogenesis below, colonocytes actively metabolise butyrate, which may explain the low concentration of this SCFA in the blood.

4. Short chain fatty acids in gut health

Gut health is most often described by a combination of characteristics including: (1) an adequate production of gastrointestinal secretions leading to appropriate digestion and absorption of food, minerals and water; (2) a predictable and regular stool pattern and stool consistency; (3) a balanced intestinal microbiota; (4) an adequately functioning intestinal barrier; and (5) a status of well-being and absence of gastrointestinal diseases (Bischoff, 2011). In the following paragraphs, we provide an overview of how SCFA may impact the different aspects of gut health.

Short chain fatty acids, gut permeability and tight junctions

Interventions that modulate the human intestinal microbiota and thus likely SCFA production, yielded mixed results with respect to gut permeability (Reijnders *et al.*, 2016; West *et al.*, 2012). Most studies either did not measure SCFA or only measured faecal SCFA, which hampers drawing correlations between SCFA and intestinal permeability. In children with persistent diarrhoea, supplementation with green bananas (containing resistant starch) and pectin for 1 week significantly reduced intestinal permeability and stool output (Rabbani *et al.*, 2004). Small intestinal permeability was also improved in healthy subjects after consumption of inulin-enriched pasta for 8 weeks compared to control pasta (Russo *et al.*, 2012a). In contrast, 2 weeks of probiotics/FOS (1.5×10^{10} cfu probiotic mixture + 10 g fructo-oligosaccharides (FOS)) supplementation to healthy subjects did not affect intestinal permeability (Wilms *et al.*, 2016). Similarly, prebiotic supplementation (galacto-oligosaccharides (GOS), 5.5 g/d for 12 weeks) had no significant effect on intestinal permeability in 29 men with well-controlled type 2 diabetes (Pedersen *et al.*, 2016). Finally, no effect on intestinal permeability was observed after administration of prebiotics to critically ill patients (Jain *et al.*, 2004), burn patients (Olguin *et al.*, 2005) or preterm infants (Westerbeek *et al.*, 2011).

The connection between the epithelial cells is formed by tight junctions (TJ), adherent junctions and desmosomes, which consist of specific transmembrane proteins that are intracellularly connected. As the epithelial cells proliferate rapidly and renew quickly, tight junction proteins need to be strictly regulated to avoid detrimental effects on membrane integrity. The impact of SCFA, in particular of butyrate, on tight junctions has mainly been studied in *in vitro* studies and in various experimental animal models.

In monolayers of differentiated intestinal epithelial cells (cdx2-IEC), butyrate contributed to the intestinal barrier function by increasing the expression of the TJ protein claudin-1 (Wang *et al.*, 2012). Furthermore, butyrate mitigated the negative effects of lipopolysaccharides (LPS) on epithelial integrity concurrent with a selective upregulation of expression of TJ proteins in an *in vitro* model using porcine intestinal epithelial cell line (IPEC-J2) (Yan and Ajuwon, 2017). The effect of butyrate on tight junction protein complex integrity in colonic epithelial cells includes the activation of AMP-activated protein kinase (AMPK) activity (Peng *et al.*, 2009). Phosphorylation of AMPK was increased in cells after incubation with SCFA and inhibition of AMPK phosphorylation prevented the effects on permeability. In addition, butyrate also induced the redistribution of the TJ proteins occludin and zonula occludens-1 (ZO-1) in the cellular membrane (Elamin *et al.*, 2013; Miao *et al.*, 2016; Peng *et al.*, 2009). These protective effects seem largely concentration dependent, with higher doses causing epithelial barrier disruption. The protective effect of butyrate on tight junction protein expression may also depend on stimulation of interleukin (IL)10-RA (Zheng *et al.*, 2017). IL10-RA inhibits the claudin-2 TJ protein, known to increase intestinal permeability. Furthermore, butyrate may improve the barrier function by stabilising the transcription factor hypoxia-inducible factor (HIF). Intracellular oxidation of butyrate results in depletion of oxygen and stabilisation of HIF, which targets genes in the intestinal cells that are critical for microbial defence, barrier production and mucin production (Kelly *et al.*, 2015).

Animal studies mostly use interventions with prebiotics of fermentable fibre and presume that the effects on barrier integrity are mediated by an increase in SCFA production. In mice, navy and black beans supplementation altered the microbial community structure resulting in enhanced SCFA production. Multiple aspects of mucus and epithelial barrier integrity were enhanced (Monk *et al.*, 2017). Similarly, feeding fermentable dietary fibre to mice increased faecal SCFA and protected the colonic barrier by increased expression of TJ proteins (ZO-1 and ZO-2, occluding, junctional adhesion molecule A (JAMA) and claudin-7) (Hung and Suzuki, 2018). When genetically obese mice (*ob/ob*) were fed a prebiotic-enriched diet, intestinal permeability was reduced and tight-junction integrity (expression of ZO-1 and occludin) was increased. The effects were mediated by endogenous production of glucagon-like peptide 2 (GLP-2) as administration of a GLP-2 antagonist abolished and pharmacological GLP-2 treatment mimicked the prebiotic effect (Cani *et al.*, 2009b). Since SCFA concentrations were not measured, it is not clear whether the increased GLP-2 concentrations were mediated by prebiotic-induced SCFA production.

In IL-10 knock-out mice which spontaneously develop chronic colitis, multi-fibre feeding also increased epithelial

expression and correct localisation of TJ proteins occludin and ZO-1 (Wang *et al.*, 2016). Also in mice with experimentally induced autoimmune hepatitis that were fed a high fibre diet, expression of occludin, ZO-1 and claudin-1 was significantly increased (Hu *et al.*, 2018). Administration of (partially hydrolysed) guar gum to dextran sodium sulphate (DSS)-treated mice resulted in doubling of the total faecal SCFA concentrations compared to controls concomitantly with 60-120% increases in expression of occludin and claudin-3, 4 and 7 (Hung and Suzuki, 2016). Furthermore, oral administration of butyrate (9 mM in drinking water) but not a mixture of SCFA (35 mM acetate, 15 mM propionate and 9 mM butyrate) prevented a reduction in small intestinal permeability induced by the chemotherapeutic drug 5-fluorouracil (Ferreira *et al.*, 2012).

In weaned piglets, administration of encapsulated sodium butyrate allowed halving the dose of antibiotics for preventing diarrhoea (Huang *et al.*, 2015). Compared to control animals, functional permeability measures (urinary lactulose mannitol ratios) and occludin protein expression were significantly increased. In an animal model of impaired intestinal barrier function, oral administration of butyrate not only improved intestinal histology, but also reduced increased gut permeability, resulting in lowered mortality (Han *et al.*, 2015).

In conclusion, although there is mechanistic support for effects of SCFA on TJ and barrier function, evidence in humans for effects of microbial modulation or SCFA on gut permeability is mixed.

Short chain fatty acids affect mucus production

Mucus is secreted by specialised Goblet cells of the intestinal mucosa. It forms an essential layer that varies in thickness and chemical composition along the gastrointestinal (GI) tract. Mucus is a biological lubricant, but also serves as a nutrient source for specialised gut microbes. Most importantly, mucus acts as a chemical-physical barrier that contributes to the protection against luminal agents such as chemicals, toxins, pathogens and allergens (for review see Pelaseyed *et al.*, 2014).

An increase in mucus production is often deduced from increased expression of the MUC2 gene that codes for mucin 2, the most prominent mucin secreted by intestinal epithelial cells. SCFA, in particular butyrate, stimulate MUC2 gene expression via selective acetylation/methylation of histones of the MUC2 gene (Burger-van Paassen *et al.*, 2009; Hatayama *et al.*, 2007) or through the synthesis of prostaglandin E1 as shown in *in vitro* co-culture model of functionally interacting intestinal epithelial and mesenchymal cells (Willemsen *et al.*, 2003). Administration of butyrate enemas to mice stimulated the expression of MUC1, 2, 3 and 4 in different ways along the colon. In the proximal colon, MUC2 was preferentially increased by butyrate although the number of MUC2 positive cells was not changed. Surprisingly, upregulation of MUC2 expression resulted in a decrease in thickness of the adherent mucus layer (Gaudier *et al.*, 2009). Nevertheless, MUC2 expression was not affected in rectal biopsies from healthy subjects and patients with ulcerative colitis (UC) in remission after daily administration of butyrate enemas (60 ml, 100 mM butyrate) for two weeks (Hamer *et al.*, 2010).

Other studies directly measured mucus production. *Ex vivo* experiments using colonic tissue biopsies from colitis and cancer patients showed that physiological concentrations of butyrate stimulate mucin synthesis as demonstrated by increased incorporation of [^{3}H]-labelled N-acetylglucosamine into mucin (Finnie *et al.*, 1995). Furthermore, in an isolated perfused rat colon loop model, luminal administration of acetate and butyrate, but not propionate, enhanced mucin secretion in a dose dependent manner. The increased secretion of mucin was accompanied by histological changes of goblet cells that reflected increased activation of mucin exocytosis (Barcelo *et al.*, 2000).

Increased mucin synthesis was also supported by oral administration of prebiotics that stimulate luminal SCFA production through microbial metabolism. In a humanised rodent model, oral administration of two prebiotic fibres increased mucin concentration in caecum and faeces. Interestingly, the elevated mucin production was associated with a lower abundance of mucin-degrading bacteria in the caecum, whereas mucin degradation seemed more prominent in the colon (Van den Abbeele *et al.*, 2011).

Besides affecting mucin production, butyrate may also affect its quality. Via posttranslational modification, carbohydrate side chains of varying length and sequence are added to the protein backbone of the mucins. In this way, mucins can be modified by sialylation, sulphation, acetylation, and fucosylation resulting in different proportions of neutral, acidic and sulphated mucins. Highly glycosylated structures of mucins give the mucus gel its physico-chemical properties (i.e. viscosity, hydrophobicity). Treatment of human intestinal goblet cells with butyrate mainly upregulated galectin-1 gene expression (Gaudier *et al.*, 2004). Galection-1 is a regulatory glycosylation enzyme with a protective role in experimental colitis. Moreover, the sugar chains are involved in the interactions between the luminal microbiota and the epithelial cells since bacteria use these sugars not only as growth substrates, but also as adhesion sites. *In vitro* studies using LS174T colonic epithelial cells indicated that butyrate also improved the adherence of beneficial microbes such as *Lactobacillus* and bifidobacteria, whereas it prevented the adherence and potential translocation of pathogenic *Escherichia coli* bacteria (Jung *et al.*, 2015).

Short chain fatty acids and immune reactivity

Short chain fatty acids affect mucosal immune function

SCFA may have a versatile role in supporting immune homeostasis (Davie, 2003; McKenzie *et al.*, 2017; McNabney and Henagan, 2017; Schilderink *et al.*, 2016). Human evidence for effects of SCFA on intestinal immune function or inflammation is limited. The anti-inflammatory effects of butyrate have mainly been investigated in patients with UC where the impact of topical butyrate application using enemas on intestinal inflammation was evaluated (for a review, see Hamer *et al.*, 2008). Such direct intestinal butyrate administration reduced the intestinal inflammatory status in colitis patients (Vernia *et al.*, 1995). Moreover, the clinical anti-inflammatory activity of butyrate in UC patients was demonstrated through oral intake of fermentable dietary fibres (60 g oat bran/d corresponding to 20 g of dietary fibre) (Hallert *et al.*, 2003). Although most studies contained relatively low numbers of participants and not all were placebo-controlled, there is a cautious optimism in favour of butyrate treatment. Nevertheless, butyrate treatment has not entered clinical application. A possible explanation is the difficulty to administer butyrate in the distal colon and the availability of effective alternative treatment options. Hence, both luminal administration and endogenous stimulation of butyrate production through microbial fermentation of dietary fibres seems to prevent pathological conditions of intestinal inflammation.

There is mechanistic evidence for an effect of SCFA on mucosal immune and inflammatory status, mainly from cell line and small animal models. SCFA, in particular butyrate, alter the secretion of pro-inflammatory mediators (e.g. interferon (IFN)-γ IL-1, IL-2, IL-6, IL-8, tumour necrosis factor (TNF)-α), as well as molecules involved in the homing of inflammatory cells and cell interactions (e.g. intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), T-cell receptors). Basically, these effects occur through inhibition of mitogen-activated protein kinases (MAPK) pathways and inhibition of nuclear factor κB (NF-κB) in intestinal epithelial cells (Inan *et al.*, 2000; Schwab *et al.*, 2007). Activation of the G-protein-coupled receptors (GPRs) GPR41 and GPR43 (also known as free fatty acid receptor (FFARs) FFAR3 and FFAR2, respectively) on the immune cell membranes by butyrate results in decreased cAMP levels and increased cytoplasmic calcium levels leading to activation of MAPK, which transfers a signal from the cell surface to the nucleus (Li *et al.*, 2017). The increased intracellular calcium concentrations also result in activation of the NLRP3 inflammasome and subsequent activation of caspase-1. Activated caspase-1 in turn converts pro-IL-18 into IL-18, which promotes epithelial repair (Macia *et al.*, 2015). Inhibition of NF-κB is mediated by activation of β-arrestin 2 and results in decreased transcription of genes encoding many pro-inflammatory genes. Furthermore, the production of reactive oxygen species (ROS) during oxidative stress and involved in inflammatory processes, can be mitigated by SCFA (e.g. butyrate) through stimulation of antioxidant glutathion (GSH) (Russo *et al.*, 2012b). At physiological dose, SCFA also inhibit pro-inflammatory cytokine release by intestinal epithelial cells induced by toll-like receptor (TLR) activators such as LPS (Iraporda *et al.*, 2015).

Furthermore, SCFA modulate the function of dendritic cells (DCs). DCs are unique, not only because they are able to access the luminal content, but also because they either induce immune responses or maintain tolerance, depending on their stage of maturation.

Whereas incubation of human monocyte-derived DCs with acetate did not elicit significant changes in the transcriptome, butyrate and propionate reduced the release of several pro-inflammatory chemokines and inhibited the expression of LPS-induced cytokines (Nastasi *et al.*, 2015). Incubation of DCs with butyrate in an early stage interfered with the maturation of the cells and kept them in an immature state. Major histocompatibility complex molecules, co-stimulatory molecules and classical DC-associated molecules were downregulated and the major T helper1-skewing factors IL-12 and IFN-γ were substantially suppressed whereas the anti-inflammatory cytokine IL-10 was increased (Liu *et al.*, 2012). Similarly, DCs exposed to butyrate expressed the immunosuppressive enzymes indoleamine 2,3-dioxygenase 1 (IDO1) and the vitamin A-converting enzyme aldehyde dehydrogenase 1A2 (Aldh1A2), promoted conversion of naive T-cells into immunosuppressive FoxP3+ T_{regs} and suppressed conversion of naive T-cells into pro-inflammatory IFN-γ-producing cells (Gurav *et al.*, 2015). Moreover, all SCFA restored colonic T_{reg} population and function in germfree and SPF-mice thus protecting against colitis in an activated T-cell transfer model (Arpaia *et al.*, 2013; Smith *et al.*, 2013).

The impact of butyrate on differentiation and phenotypical changes of DCs was further reflected in functional assays revealing increased phagocytic activity and reduced T-cell stimulatory capacity (Millard *et al.*, 2002). The induction of IL-10 producing Tr1 regulatory T cells by butyrate depended on the induction of retinoic acid producing enzymes (Kaisar *et al.*, 2017; Schilderink *et al.*, 2016).

Humoral immune responses are an important element of immune surveillance in the gut. Secretory IgA (SIgA) is the most prominent antibody produced in the intestinal mucosa that protects the intestines against bacterial and viral infections. Microbiota plays a role in supporting SIgA production. Acetate, but not butyrate, increased faecal IgA and IgA-positive B-cells in the lamina propria of wildtype mice but had no effect in GPR43 knockout (KO) mice,

indicating that the process was mediated through specific SCFA receptor interaction. Retinoic acid, which can be stimulated by butyrate (Schilderink *et al.*, 2016), played an important role in the increased production of IgA by B-cells (Wu *et al.*, 2017). The role of retinoic acid was confirmed *in vivo*. Here, dietary fibre intake stimulated SCFA production, which was linked to an increased expression of vitamin A converting enzymes, a higher production of luminal IgA and increased presence of regulatory T cells in the intestinal mucosa (Goverse *et al.*, 2017). Increasing colonic SCFA production by high-fibre feeding also increased IgA production and generation of T_{reg} cells in a mouse model of food allergy. The protective effect of SCFA on allergic sensitisation was further supported by the observation that mice lacking key SCFA receptors (GPR43 or GPR109A) exhibited aggravated allergic symptoms (Tan *et al.*, 2016).

SCFA and antimicrobial proteins

The two major families of mammalian anti-microbial proteins are defensins and cathelicidins. They have the capacity to kill and/or inactivate bacteria, fungi, and enveloped viruses *in vitro* and also contribute to host defence *in vivo* (Zasloff, 2006). LL-37 is the only cathelicidin host defence peptide in humans. In addition to its bactericidal activities against gram-positive and gram-negative bacteria, LL-37 can neutralise bacterial LPS and is chemotactic for human peripheral monocytes, neutrophils, and CD4+ T lymphocytes (Hase *et al.*, 2002). Butyrate is a potent inducer of cathelicidin expression when incubated *in vitro* with colonic carcinoma cell lines (Schauber *et al.*, 2006). Moreover, synthesis of LL-37 was enhanced with butyrate in the presence of exogenously added purified MUC2, indicating regulatory mechanisms between MUC2 and cathelicidins in the colonic mucosa (Cobo *et al.*, 2017).

Short chain fatty acids provide energy to the colonocytes

In contrast to small intestinal epithelial cells that primarily use glucose and glutamine as energy substrates (Mithieux, 2001), SCFA constitute the major energy source of colonocytes. Already in 1980, Roediger demonstrated that 70% of the total amount of oxygen consumed by human colonocytes *in vitro* was used for butyrate oxidation (Roediger, 1980). SCFA oxidation has been estimated to recover 600-750 kJ of energy per day from undigested carbohydrate, corresponding to approximately 8% of the daily recommended energy needs (Bergman, 1990; McNeil, 1984). After absorption into the cells, SCFA diffuse through the inner mitochondrial membrane and enter the citric acid cycle to generate ATP. Complete oxidation of one mole acetate, propionate and butyrate provides 10, 18 and 27 mole ATP, respectively (Clausen and Mortensen, 1995). Butyrate is oxidised at a faster rate compared to acetate and propionate, as demonstrated in *in vitro* experiments by measuring $^{14}CO_2$ production after incubation of human colonocytes with ^{14}C-labelled acetate, propionate and butyrate (Clausen and Mortensen, 1995). Half of the maximal oxidation rate was obtained for butyrate at a concentration of 82 μM whereas higher concentrations were required for acetate (565 μM) and propionate (369 μM).

Both *in vitro* and *in vivo* (animal and human) studies have indicated that butyrate oxidation is impaired in UC resulting in a state of energy deficiency ultimately leading to a reduction in sodium absorption and mucin secretion and a shorter life of the colonocytes (De Preter *et al.*, 2011). These results confirm that a certain level of butyrate oxidation is vital for a healthy colonic epithelium. Yet, it remains unclear whether levels above normal provide additional benefits.

Short chain fatty acids affect motility and transit

Experimental studies in rats have shown that SCFA affect colonic motility although results are contradictory and appear to depend on luminal SCFA concentrations. Infusion of physiological SCFA concentrations (100 mM) into the rat colon did not affect colon motility whereas high concentrations (500 mM) significantly reduced colon contractility (Cherbut *et al.*, 1998). Also the mechanism of action is not clear and may involve secretion of peptide YY (PYY) (Cherbut *et al.*, 1998), interaction with GPR41 and GPR43 (Dass *et al.*, 2007) or stimulation of the efferent vagal nerves by serotonin released from enterochromaffin cells (Fukumoto *et al.*, 2003). Furthermore, the effects of SCFA on motility might differ among species and there is not yet direct evidence that SCFA influence colon motility in humans (Cherbut, 2003; Jouet *et al.*, 2013).

Short chain fatty acids and mineral absorption

There is compelling data that prebiotics, such as inulin-type fructans and GOS lead to increased mineral absorption, in particular enhancing calcium absorption (Coxam, 2007). Moreover, effects have also been proposed for the absorption of other minerals such as magnesium and phosphorus. SCFA generated from the colonic fermentation of prebiotic fibres are the proposed main protagonist contributing to this effect, with several mechanisms of actions suggested.

Due to technical and logistic difficulties in analysing the effects of SCFA on mineral absorption *in vivo*, data from human studies is scarce. A trial with six healthy male volunteers investigated the effects of SCFA on calcium absorption in the distal colon with rectal infusions (Trinidad *et al.*, 1993). 30 mmol of calcium were infused together with different amounts of SCFA (0, 22.5 or 45 mmol, 3:1 mixture of acetate and propionate). Infusion of SCFA together with calcium led to dose-dependent increases in serum calcium concentrations. Follow-on studies demonstrated that propionate exhibited a greater effect

than acetate at higher doses of calcium (Trinidad *et al.*, 1993, 1997, 1999). Moreover, an increase in the absorption of the SCFA acetate and propionate at higher doses of calcium was demonstrated. Therefore, the hypothesis of a calcium-hydrogen exchange similar to that proposed by Lutz and Scharrer (1991) was supported. Comprehensive data is available from *in vitro* studies, which support the mechanism of action. The production of SCFA led to a decreased pH of the colonic contents and thereby may reduce the complexation of minerals like calcium (Weaver, 2015), resulting in more calcium being available for uptake in the colon. However, the decreased colonic pH might not be the only mechanism by which SCFA enhance calcium absorption. In a series of *in vitro* experiments, SCFA were added to Caco-2 human intestinal epithelial cells and resulted in an increase in mRNA expression of proteins involved in paracellular calcium uptake (Fukushima *et al.*, 2009, 2012). Transcription of transient receptor potential vanilloid type 6 (TRPV6/CaT1/ECaC2), which is located in small intestinal mucosal epithelial cells on the luminal side where it promotes transcellular calcium uptake, was elevated in Caco2-cells upon stimulation with sodium-butyrate and sodium-propionate (Fukushima *et al.*, 2009). Moreover, mRNA expression of calbindin-D9k, a calcium-binding protein that transports intracellular calcium to the basolateral membrane of mucosal epithelial cells, was upregulated (Fukushima *et al.*, 2012). Furthermore, in experiments with rat cecum and colon explants in an Ussing chamber it has been demonstrated that the presence of SCFA increased transepithelial calcium transport (Mineo *et al.*, 2001; Raschka and Daniel, 2005). Similar results were reported by Kashimura *et al.* (1996) who used hindgut segments from rats to analyse the stimulatory effects of SCFA on the absorption of calcium, magnesium and phosphorus. They also showed an increase in absorption of these minerals upon addition of acetic and butyric acid. These findings support the concept of additional stimulatory effects of SCFA on mineral, particularly calcium, uptake. Hence, stimulatory effects of SCFA on mineral absorption go beyond the effect of lowering pH as the sole underlying mechanism.

Three animal experiments attempting to directly determine the effect of SCFA on mineral absorption were identified. In a luminal perfusion experiment with rats, Lutz and Scharrer (1991) demonstrated in an *in vivo* setting that acetate and butyrate caused an increase in calcium absorption in the distal colon. In the proximal colon, this calcium absorption enhancing effect was not observed (Lutz and Scharrer, 1991). The authors hypothesised that a calcium-hydrogen exchanger located in the apical membrane of the epithelium might be involved in calcium absorption by the distal colon (Lutz and Scharrer, 1991). In accordance with the mechanistic *in vitro* and *ex vivo* studies described above, Kashimura *et al.* (1996) demonstrated that the intake of different substrates increased mineral absorption and retention in rats corresponding to the fermentability of these substrates. Furthermore, Fukushima *et al.* (2009) found changes in gene expression in rat colorectal mucosa cells in response to a diet containing FOS, which were in accordance to those observed *in vitro* in Caco-2 cells. Namely, TRPV6 and calbindin-D9k mRNA expression were increased after the FOS diet, indicating that SCFA are the mediators of this calcium absorption enhancing effect (Fukushima *et al.*, 2009).

In summary (Figure 3), there is consistent mechanistic evidence that SCFA, in particular butyrate, are important regulators of intestinal barrier function through mechanisms that include stimulation of mucin synthesis and quality, the synthesis of antimicrobial peptides as well as reducing epithelial permeability targeting tight junction complex integrity. Furthermore, SCFA may regulate mucosal immunity, luminal administration and endogenous stimulation of butyrate production seem to benefit pathological conditions of intestinal inflammation. Nevertheless, human intervention studies are limited and report mixed results of SCFA on gut permeability as well as mucosal immunity and inflammation. Finally, a consistent effect of SCFA on colonic calcium absorption has been demonstrated *in vitro*, *ex vivo* and confirmed by rectal infusion experiments in rats and humans.

5. Short chain fatty acids in metabolic health

Body weight control

This part of the review aims to provide an integrated view on the impact of SCFA on energy homeostasis by collating and examining the recent evidence around (1) the effect of increasing the provision of SCFA on body weight, energy intake and energy expenditure, and (2) the effect of SCFA receptors, through rodent GPR41 and GPR43 knockout studies, on body weight, energy intake and energy expenditure (Figure 4).

Body weight: selected studies

In total, 19 studies reporting a body weight outcome that met inclusion criteria were identified. Fourteen studies reported the effect of increasing the provision of SCFA on body weight. Of these, 10 studies were conducted in mice (8 in C57BL/6 mice, 1 in E3LCETP mice and 1 in LDLr KO mice) and 3 in rats (2 in non-described rats and 1 in OLETF/LETO rats) and only a single study was conducted in humans (overweight/obese adults). Within the rodent studies, the SCFA investigated were acetate in 6 studies, butyrate in 10 studies, propionate in 4 studies and a mixture of acetate, butyrate and propionate in 1 study. Acetate was administered orally via diet in 3 studies, oral injection in 1 study, intraperitoneal injection in 1 study and intragastric infusion in 1 study. Butyrate was administered orally via diet

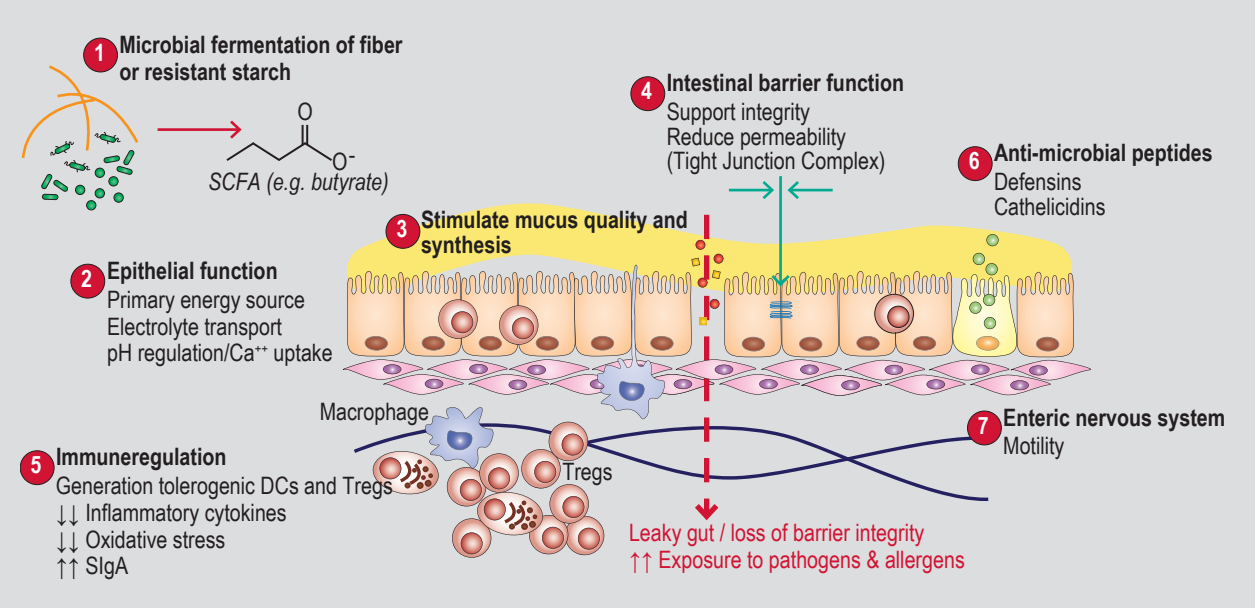


Figure 3. Effects of short chain fatty acids (SCFA) on intestinal mucosal homeostasis. Bacteria inhabiting the gastrointestinal tract can ferment fibres and oligosaccharides to produce SCFAs, e.g. acetate, propionate and butyrate (1). These metabolites play an important role in the microbiome-host interface, and maintain intestinal homeostasis through supporting epithelial cell function (2), strengthening intestinal barrier integrity (3, 4) and affecting immunoregulatory processes, e.g. the generation of tolerogenic DC and T cells and anti-inflammatory processes (5). SCFA also support the innate immune defence against pathogenic bacteria through the stimulation of antimicrobial molecules (6). Moreover, they can act on the enteric nervous system (7) affecting gut motility and the gut-brain axis, the latter substantiating their potential beneficial role in certain neurological disorders.

in 8 studies, oral gavage in 1 study and intragastric gavage in 1 study. Propionate was administered orally via diet in all 4 studies. The mixture of SCFA was administered orally via diet. In the human study, the SCFA investigated was propionate, which was administered orally via diet. Five studies reporting the effect of SCFA receptor knockout on body weight were identified, of which 2 studies investigated GPRR41-KO and 3 study investigated GPR43-KO.

Short chain fatty acids and body weight

The investigation into the effect of SFCA supplementation and body weight in humans is at present limited with only one study reporting body weight change (Table 1, human), with the majority of the current literature focussing mainly on fibre supplementation and endogenous SCFA production rather than supplementation. Chambers *et al.* (2015) demonstrated that propionate supplementation to human subjects protected against weight gain as part of a habitual diet. A 10 g a day supplementation of an inulin-propionate ester, delivering additional 2.36 g propionate into the colon, for 24 weeks resulted in a significantly reduced weight gain compared to control. Only 4% of the participants in the inulin-propionate ester group gained greater or equal to 3% body weight, compared with 25% in the control group, with an additional 17% in the control group gaining at least 5% body weight. Currently, the evidence that SCFA supplementation in humans influences body weight is limited to this single study investigating propionate supplementation, and thus generalising the result in the context of human body weight effect is not currently possible.

Thirteen studies were identified (Table 1, rodents), which have recorded the impact of SCFA administration on body weight in rodents, and 12 of the 13 showed a beneficial effect of SCFA administration on weight loss, prevention of weight gain or attenuation of weight gain. Ten studies investigated the effects of butyrate administration on body weight in rodents, of which all reported improvements in body weight compared to control. Li *et al.* (2017) showed butyrate, incorporated into a high fat diet (HFD) for 9 weeks reduced diet induced obesity in mice by 27% compared with no butyrate in a HFD control group. Similar results were observed by Gao *et al.* (2009), who showed that butyrate supplementation within a HFD significantly reduced HFD-induced body weight gain over a 16 week period, compared to control. Following an additional 5 weeks of butyrate supplementation, a 10.2% reduction in body weight was observed. A further study by Arnoldussen *et al.* (2017), showed that 2 months of butyrate supplementation on a HFD in mid-adult mice tended to reduce body weight by 16%, but this was not significantly different. However, in late-adult mice (>10 months old) there was a 23% reduction in body weight ($P<0.001$) in diet induced obese mice fed a HFD at 12 months, highlighting the longer-term effect of

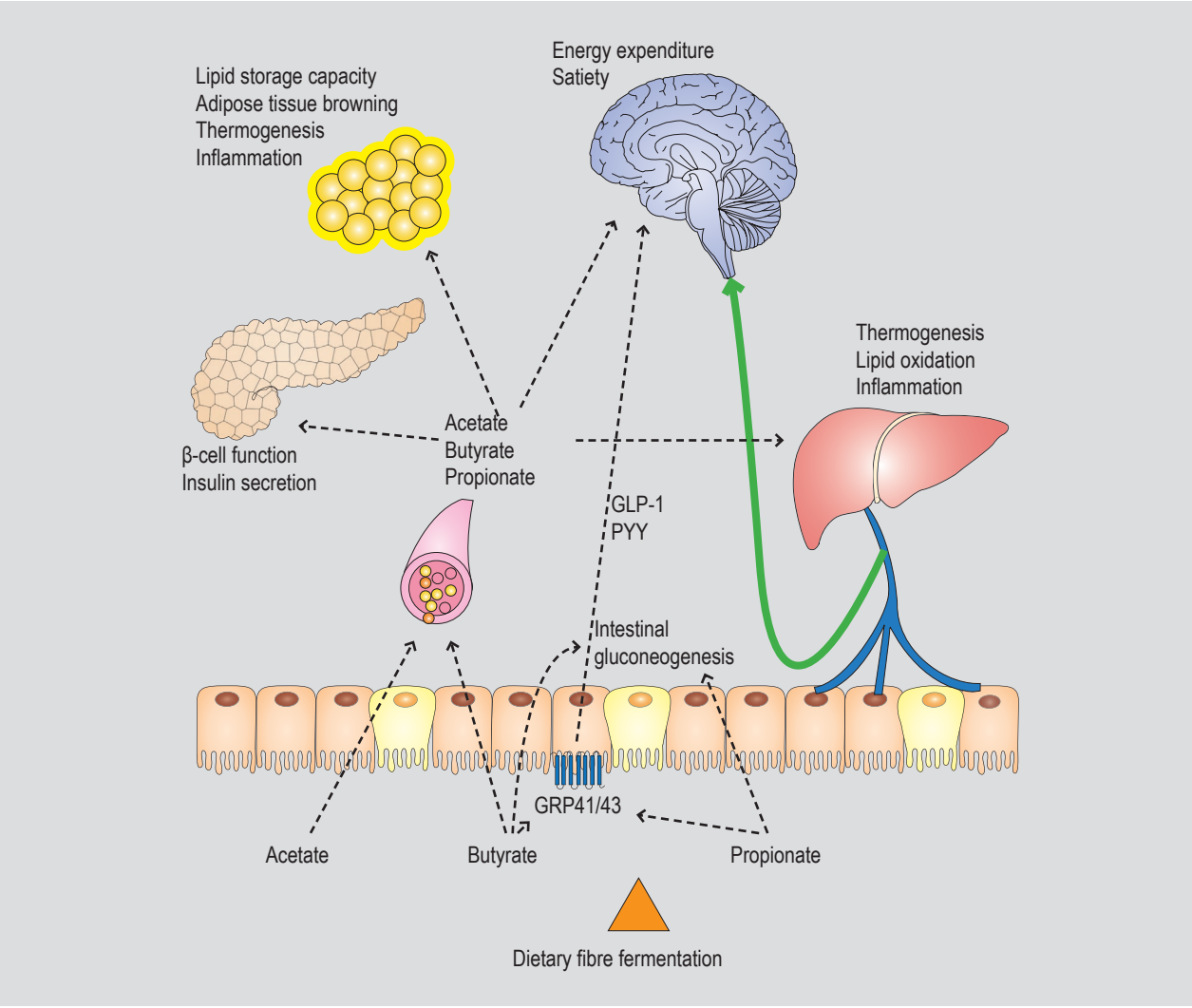


Figure 4. Short chain fatty acids (SCFA) and metabolic health. The best studied mechanism include the capability of mainly propionate and butyrate bind to G-protein-coupled receptors (GPR41 and GPR43) in the colon leading to the production of the gut hormones peptide YY (PYY) and glucagon-like peptide 1 (GLP-1), thereby affecting satiety and glucose homeostasis. Propionate and butyrate activate intestinal gluconeogenesis by several complementary mechanisms. Released glucose initiates a neural signal conveyed by portal nerves (in green) to the brain, resulting in metabolic benefits (satiety, insulin sensitivity). SCFA can also reach the circulation and can, thereby, directly affect peripheral tissue substrate metabolism and function. In the adipose tissue, acetate and propionate might attenuate intracellular lipolysis and affect the lipid buffering capacity. Acetate, and especially propionate and butyrate might reduce the secretion of proinflammatory cytokines and chemokines, thereby possibly reducing local macrophage infiltration. Mainly acetate and butyrate may increase thermogenesis and browning in adipose tissue. Acetate and butyrate increase liver lipid oxidation and decrease inflammation. Propionate may improve β-cell function and SCFA may affect glucose-stimulated insulin secretion.

butyrate on attenuating weight gain on HFD. Additional animal studies have also reported an attenuation of body weight gain with butyrate administration (De Vadder *et al.*, 2014; Den Besten *et al.*, 2015; Dutzan *et al.*, 2017; Guo *et al.*, 2016; Henagan *et al.*, 2015; Hong *et al.*, 2016; Kang *et al.*, 2017; Lim *et al.*, 2012; Lu *et al.*, 2016; Zhou *et al.*, 2017).

The role of acetate supplementation in body weight regulation appears to be less clear with both positive and negative effects. Acetate supplementation has been shown to attenuate HFD associated weight gain (Den Besten *et al.*, 2015; Guo *et al.*, 2016; Kimura *et al.*, 2013; Lim *et al.*, 2012; Lu *et al.*, 2016; Yamashita *et al.*, 2007). In the studies by Guo *et al.* (2016) and Lu *et al.* (2016) it was shown that dietary supplementation of acetate, propionate, butyrate or a mixture of the three SCFA significantly suppressed HFD induce weight gain, with acetate having the greatest overall effect (≈72%). However, an investigation by Perry *et al.* (2016) reported that chronic intragastric infusion of acetate for 10 days promoted an increase in body weight compared

Table 1. Tabulated summary of the effects of increasing the provision of short chain fatty acids (SCFA) on body weight in humans and rodents.[1]

Reference	Sample characteristics	Outcome
Humans		
Chambers *et al.*, 2015	19 Male, 30 Female (n=49), age 40-65 y, BMI 25-40 kg/m^2 SCFA: propionate Dose: 2.6 g (equivalent in colon) Administered via: oral; habitual diet or habitual diet + propionate Duration: 24 w	Significant reduction in BW gain (~5%) (P=0.033), and prevention of increase in BW (~3%) (P=0.036), with habitual diet + propionate compared to habitual diet.
Rodents		
Arnoldussen *et al.*, 2017	LDLr KO male mice (n=30), age 3 or 6 m SCFA: butyrate Dose: 5% w/w Administered via: Oral; HFD or HFD + butyrate Duration: Age 3 m mice = 4 m on HFD + 2 m HFD + butyrate; age 6 m mice = 6 m on HFD + 2 m HFD + butyrate	In baseline age 3 m mice, no-significant effect of HFD + butyrate compared to HFD (P>0.05). In baseline age 6 m mice, significant reduction in BW (23%) with HFD + butyrate compared to HFD (P<0.001).
Den Besten *et al.*, 2015	C57Bl/6J male mice (n=6-8), age 2 m SCFA: acetate, butyrate, and propionate Dose: 5% w/w Administered via: oral; HFD or HFD + acetate, butyrate or propionate Duration: 12 wk	Significant reduction in BW gain (~ -8 g) with HFD + either SCFA compared to HFD (P<0.05).
De Vadder *et al.*, 2014	Sprague-Dawley male rats (n=6), age 6-8 wk, BW 275-300 g SCFA: butyrate and propionate Dose: 5% w/w Administered via: oral; standard diet or standard diet + butyrate or propionate Duration: 10-14 d	Significant prevention of BW gain (~ -5 g) with standard diet + butyrate or propionate compared to standard diet (P<0.05).
Gao *et al.*, 2009	Dietary-obese C57BL/6J male mice (n=10), age 4 wk SCFA: butyrate Dose: 5% w/w Administered via: oral; HFD or HFD + butyrate Duration: 16 and 21 wk	At 16 wk, significant reduction in BW gain (~ -17 g) with HFD + butyrate compared to HFD (P<0.05). At 21 wk, further significant reduction in BW gain (10.2%) with HFD + butyrate compared to HFD (P<0.05).
Hong *et al.*, 2016	C57BL/6 male mice (n=8) on HFD diet SCFA: butyrate Dose: 80 mg Administered via: oral; HFD or HFD + butyrate gavage dosing Duration: 5 doses in 10 d	Significant reduction in BW gain (~ -3 g) with HFD + butyrate gavage dosing compared to HFD (P<0.05).
Henagan *et al.*, 2015	C57BL/6J male mice (n=10 per group), age 5 wk SCFA: butyrate Dose: 5% w/w Administered via: oral; LFD, HFD or HFD + butyrate Duration: 10 wk	Significant prevention of BW gain (~ -8 g) with HFD + butyrate compared to HFD.[2]
Li *et al.*, 2018	E3L.CETP male mice (n=8-10), age 10-12 wk SCFA: butyrate Dose: 5% w/w Administered via: oral; HFD or HFD + butyrate Duration: 9 wk	Significant prevention of BW gain (~27% / ~ -10 g) with HFD + butyrate compared to HFD (P<0.001).
Lin *et al.*, 2012	C57BL/6N male mice (n=8), age 3 m SCFA: acetate, butyrate, and propionate Dose: acetate (3.74%), butyrate (5%), propionate (4.3%) Administered via: oral; HFD or HFD + acetate, butyrate or propionate Duration: 4 wk	Significant suppression of BW gain (~40%) with HFD + acetate compared to HFD (P<0.001). Significant prevention of BW gain (~40%) with HFD + butyrate and propionate compared to HFD (P<0.0001).

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Table 1. Continued.

Reference	Sample characteristics	Outcome
Lu *et al.*, 2016	C57BL/6J male mice (n=15), age 3-4 wk SCFA: acetate, butyrate, propionate and their mixture Dose: ND Administered via: oral; HFD or HFD + acetate, butyrate, propionate or mixture Duration: 16 wk	Significant suppression of BW gain with HFD + acetate, butyrate, propionate or mixture compared to HFD (P<0.05). Acetate had greatest effect on BW gain suppression (~72.31%). Magnitude of the other SCFAs on body weight not reported.
Perry *et al.*, 2016	Rats (n=16) SCFA: acetate Dose: 20 µmol/kg/min for 60 mins Administered via: gastric; intragastric infusion Duration: 10 d	Significant increase in BW (8%) with acetate intragastric infusion compared to no acetate intragastric infusion (P<0.0001).
Sahuri-Arisoylu *et al.*, 2016	Male C57BL/6 mice (n=8 per group); NFD and HFD groups SCFA: acetate Dose: 4.41 mM/200 µl; 3 injections per wk. Administered via: intraperitoneal injection; 4-(2-hydroxyethyl)-1-piperazine-ethanesulfonic acid or nanoparticle-derived acetate Duration: 6 wk	No significant difference in BW in both NFD and HFD groups (P>0.05).
Yamashita *et al.*, 2007	OLETF rats (n=4), age 4 wks; LETO rats (n=3-4), age 4 wks SCFA: acetate Dose: 5.2 mg/kg Administered via: oral; injection Duration: 5 d/wk for 6 m	At 30 wks, significant reduction in BW gain (~ -114 g) with oral acetate injection in OLETF rats compared to LETO rats (P<0.01).
Zhou *et al.*, 2017	C57BL/6J male mice (n=15) SCFA: butyrate Dose: 200 mg/kg BW Administered via: Gastric; HFD or HFD + intragastric gavage of butyrate Duration: 8 wk	Significant attenuation of HFD-induced BW gain (~10%) with HFD + iIntragastric gavage of butyrate compared to HFD (P<0.05).

[1] BW = body weight; BMI = body mass index; Exp = experiment; GF = germ free; HFD = high fat diet; KO = knockout; LFD = low fat diet; ND = no data; NFD = no fat diet; w/w = weight by weight.
[2] *P*-value not reported.

to control treated rodents. Similarly, Sahuri-Arisoylu *et al.* (2016) found that intraperitoneal injection of nanoparticle-derived acetate for 6 weeks increased body weight when on a normal chow diet, although such difference was not observed in mice fed a high fat diet.

Although it is the least studied out of the three SCFA in rodents, propionate has shown to directly attenuate high fat diet weight gain in all 4 studies identified with a 10-14 day up to 12 week duration (De Vadder *et al.*, 2014; Den Besten *et al.*, 2015; Guo *et al.*, 2016; Lim *et al.*, 2012; Lu *et al.*, 2016). Two separate studies have looked at the combination of all three SCFA on body weight, demonstrating that there was a significant reduction in body weight gain (Den Besten *et al.*, 2015; Guo *et al.*, 2016; Lu *et al.*, 2016). In general, rodent studies highlight that oral SCFA supplementation attenuates weight gain associated with a high fat diet. However, there is currently a lack of evidence to suggest that SCFA supplementation results in direct weight loss or prevents weight gain when added to normal chow.

Mechanistically, five studies were identified (Table 2) that investigated the impact of either GPR43 or GPR41 KO on body weight in rodents, 4 of which have found body weight difference between GPR-KO mice and there control. The initial work of Samuel *et al.* (2008) reported the effect of GPR41-KO on weight gain in germ free (GF) raised mice and those colonised with the saccharolytic bacterium, *Bacteroides thetaiotamicron* and the methanogenic archaeon, *Methanobrevibacter smithii* (Bt/Ms). GPR41-KO had no effect on weight gain compared to wild types under GF raised conditions, whilst weight gain was significantly lower in GPR-KO mice compared to wild types in the Bt/Ms colonised mice, suggesting that GPR41 signalling contributes to body weight gain. This observation is not supported by others who found that GPR41-KO in mice

Table 2. Tabulated summary of the effects of short chain fatty acid (SCFA) receptor knockout on body weight.[1]

Reference	SCFA receptor	Sample characteristics	Duration	Outcome
Bellahcene *et al.*, 2013	GPR41	GPR41-KO vs wildtype C57BL/6 mice (n=9). HFD	40 wk	No significant difference in BW (P>0.05).
Bjursell *et al.*, 2011	GPR43	Male GPR43-KO (n=9) vs wildtype C57BL/6 mice (n=7). Normal chow and HFD groups	35 wk	Significantly lower BW in GPR43-KO mice compared to wildtype C57BL/6 mice, when on HFD.[2]
Kimura *et al.*, 2013	GPR43	Male GPR43-KO vs wildtype 129/Sv mice (n=7). Normal chow and HFD groups	14-16 wk	Significant increase in BW on normal chow (~8 g) and HFD (~20 g) in GPR43-KO mice compared to wildtype 129/Sv mice (P<0.005).
Lin *et al.*, 2012	GPR41	Male GPR41-KO mice. Exp 1 (n=8-13), exp 2 (n=34-41). Butyrate (5%), propionate (4.3%) or acetate (3.74%) in HFD	Exp 1: 1 wk, exp 2: 8 d	Exp 1: no significant difference (P>0.05). Acetate result not reported. Exp 2: significant suppression of BW gain (~ -2 g) in HFD + butyrate and HFD + propionate (P<0.001). Acetate not reported.
Samuel *et al.*, 2008	GPR41	Wildtype vs GPR41-KO mice with and without a model fermentative microbial community (n=4-9 per group)	4 wk	No significant difference in BW between GF wildtype and GPR41-KO mice (P>0.05). Significant reduction in BW gain in GPR41-KO mice compared to wildtype mice with model fermentative microbial community (P<0.05).

[1] BW = body weight; Exp = experiment; GF = germ free; HFD = high fat diet; KO = knockout.
[2] Magnitude or *P*-value not reported.

(Bellahcene *et al.*, 2013; Lim *et al.*, 2012) had no impact on body weight compared to wild-type mice on standard chow diet or HFD. Furthermore, initial work by Bjursell *et al.* (2011) identified that GPR43-KO mice had lower body weight gain compared to wild type controls after 35 weeks of a HFD. No differences were observed on the normal chow diet. Conversely, Kimura *et al.* (2013) demonstrated that body weight of both normal chow and high fat diet fed GPR43-KO mice were significantly higher than that of wild-type mice. In summary, the available studies do not report a consistent impact of either GPR43 or GPR41 signalling on weight gain in rodent KO models.

In summary, the current literature appears to support the preventative effect of butyrate and propionate against HFD-induced weight gain in rodents, although there is conflicting data regarding studies investigating acetate supplementation. The vast majority of this evidence is derived from rodent studies (evidence from a single human study) and further inclusion of a body weight outcome is warranted in human studies to complement work investigating energy intake and/or energy expenditure.

Short chain fatty acids and energy intake

In total, 16 studies reporting an energy intake outcome were identified, which met inclusion criteria. 12 studies reporting the effect of increasing the provision of SCFA on energy intake were identified, of which 7 studies were conducted in mice, 1 study in Sprague and Dawley rats and – importantly – 4 studies were conducted in overweight/obese adult humans. Within the rodent studies, the SCFA investigated were acetate in 4 studies, butyrate in 6 studies and propionate in 3 studies. Acetate was administered orally via diet in 2 studies, and intraperitoneal injection in 2 studies. Butyrate was administered orally via diet in 5 studies and either intragastric gavage or intravenous injection in 1 study. Propionate was administered orally via diet in all 3 studies. Within human studies, the SCFA investigated was propionate in all 4 studies, which was administered orally via diet. Four studies reporting the effect of SCFA receptor knockout on energy expenditure were identified. Of which 2 studies investigated GPR43 and 2 studies investigated GPR41-KO.

Four studies have investigated the impact of SCFA on energy intake in human volunteers, all of which investigate the effect of propionate supplementation on energy intake (Table 3, humans). Darzi *et al.* (2012) incorporated propionate into sourdough bread and found no difference in energy intake at an *ad libitum* test meal or over the 24 h period following consumption. It is likely that the orally supplemented propionate would have been absorbed in the upper gastrointestinal tract, meaning little would reach the colon and the major site of SCFA production. The three other studies have employed methods where propionate is directly delivered to the lower gastrointestinal tract. An example of this being the inulin-SCFA ester, a unique delivery system, whereby SCFA are conjugated by an ester linkage to inulin, a carrier molecule. The ester

Table 3. Tabulated summary of the effects of increasing the provision of short chain fatty acids (SCFA) on energy intake in humans and rodents.[1]

Reference	Sample characteristics	Outcome
Humans		
Byrne *et al.*, 2016	Male (n=20), age: 26-61 y, BMI 25.2±0.5 kg/m^2 SCFA: propionate Dose: 2.6 g (equivalent in colon) Administered via: oral; taken at breakfast: propionate or inulin control Duration: 1 d	Significant reduction in EI with propionate compared to Inulin control (P=0.03).
Chambers *et al.*, 2015	19 Male, 30 Female (n=49), age 40-65 y, BMI 25-40 kg/m^2 SCFA: propionate Dose: 2.6 g (equivalent in colon) Administered via: oral; habitual diet or habitual diet + propionate Duration: 24 wk	Acute phase, significant reduction in EI with habitual diet + propionate compared to habitual diet (P<0.01). No significant difference at 24 wks (P>0.05).
Darzi, Frost and Robertson, 2012	11 Male, 9 Female (n=20), age 25.1±4.6 y, BMI 23.1±2.4 kg/m^2 SCFA: propionate Dose: 6 mmol Administered via: oral; taken at breakfast: bread + propionate or bread Duration: 1 d	No significant difference in EI (P>0.05).
Polyviou *et al.*, 2016	Overweight healthy males (n=9), age 38±9 y, BMI 29.8±1.5 kg/m^2 SCFA: propionate Dose: 10g/d Administered via: oral; propionate or inulin control Duration: 7 d	Significant reduction in EI during *ad libitum* test meal compared to inulin (P=0.025).
Rodents		
Den Besten *et al.*, 2015	C57Bl/6J male mice (n=6-8), age 2 m SCFA: acetate, butyrate, and propionate Dose: 5% w/w Administered via: oral; HFD or HFD + acetate, butyrate or propionate Duration: 12 wk	No significant difference in EI between HFD or HFD + either SCFA (P>0.05).
De Vadder *et al.*, 2014	Sprague-Dawley male rats (n=6), age 6-8 wk SCFA: butyrate and propionate Dose: 5% w/w Administered via: oral; standard diet or standard diet + butyrate or propionate Duration: 10-14 d	No significant difference in food intake between HFD or HFD + either SCFA (P>0.05).
Frost *et al.*, 2014	C57BL/6 male mice (n=7-22), age 6-8 wk SCFA: acetate Dose: 500 mg/kg (intraperitoneal injection); 2.5 µmol (intracerebroventricular administration) Administered via: injection; intraperitoneal injection or intraperitoneal injection + acetate or intracerebroventricular administration or intracerebroventricular administration + acetate Duration: 4 h	Significant suppression in food intake at 1-2 h and reduction in cumulative food intake 2-4 h with intracerebroventricular administration + acetate compared to intracerebroventricular administration.
Gao *et al.*, 2009	Dietary-obese C57BL/6J male mice (n=10), age 4 wk SCFA: butyrate Dose: 5% w/w Administered via: oral; HFD or HFD + butyrate Duration: 16 and 21 wk	No significant difference in food intake between HFD or HFD + butyrate (P>0.05).

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Table 3. Continued.

Reference	Sample characteristics	Outcome
Henagan *et al.*, 2015	C57BL/6J male mice (n=10 per group), age 5 wk SCFA: butyrate Dose: 5% w/w Administered via: oral; LFD, HFD or HFD + butyrate Duration: 10 wk	No significant difference in food intake with HFD + butyrate compared to HFD (P>0.05).
Li *et al.*, 2018	E3L.CETP male mice (n=8-10), age 10-12 wk SCFA: butyrate Dose: 6 M in 0.15 ml (intra-gastric gavage); 15 mM or 150 mM in 0.1 ml (intravenous injection); 5% w/w (diet) Administered via: intragastric gavage or intravenous injection or HFD or HFD + butyrate Duration: 1 h and 9 wk	After 1 h, significant reduction in food intake with intragastric gavage + butyrate compared to HFD (P<0.05). No significant difference with intravenous injection or HFD + butyrate compared to HFD (P>0.05). After 9 wk, significant reduction in food intake with intragastric gavage + butyrate (~ -22%) compared to HFD (P<0.01). No significant difference with intravenous injection or HFD + butyrate compared to HFD (P>0.05).
Lin *et al.*, 2012	C57BL/6N male mice (n=8), age 3 m SCFA: acetate, butyrate, and propionate Dose: acetate (3.74%), butyrate (5%), propionate (4.3%) Administered via: oral; HFD or HFD + acetate, butyrate or propionate Duration: 4 wk	Significant reduction in 9-day cumulative food intake (~ -22%) with HFD + acetate compared to HFD (P<0.05). No significant with HFD + butyrate or propionate compared to HFD (P>0.05).
Sahuri-Arisoylu *et al.*, 2016	Male C57BL/6 mice (n=8 per group); NFD and HFD groups SCFA: acetate Dose: 4.41 mM/200 µl, 3 injections per wk Administered via: intraperitoneal injection; 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid or nanoparticle-derived acetate Duration: 6 wk	No significant difference in EI in both NFD and HFD (P>0.05).

[1] BMI = body mass index; EI = Energy intake; HFD = high fat diet; LFD = low fat diet; NFD = no fat diet; w/w = weight by weight.

linkage can only be broken down via bacterial fermentation meaning a known amount of SCFA is delivered directly to the colon and released upon microbiota fermentation. A number of feeding studies have been carried out in humans using the inulin-propionate ester added to a standard food product using a dose of 10 g, which results in a 2.5-fold increase in daily propionate production based on UK non-starch polysaccharide (NSP) intake. It has been shown that an acute increase in colonic propionate production using inulin-propionate ester reduces *ad libitum* energy (Chambers *et al.*, 2015). Similarly, *ad libitum* energy intake was reduced following a 7-day supplementation (Polyviou *et al.*, 2016). The effect of increased colonic propionate on reward-based eating behaviour was further investigated by assessing brain anticipatory reward responses during a food picture evaluation task following the consumption of inulin-propionate ester by Byrne *et al.* (2016). Elevated colonic propionate reduced activation in brain regions associated with reward-processing during a food picture evaluation task and reduced the appeal of high-energy foods and *ad libitum* energy intake. While there are a number of studies investigating the effect of propionate on food intake in humans, there are no known studies that have investigated the effects of acetate or butyrate.

Five studies have reported the effects of incorporating SCFA into the diet of rodents (Table 3, rodents), with four observing no effects on energy intake (De Vadder *et al.*, 2014; Den Besten *et al.*, 2015; Gao *et al.*, 2009; Henagan *et al.*, 2015). However, Lim *et al.* (2012) reported a significant reduction in cumulative energy intake following acetate supplementation to a high fat diet. Two further studies in mice have used different routes of administration, and suggest SCFA supplementation can suppress energy intake. Li *et al.* (2017) reported a reduction in energy intake following both the acute and chronic intragastric administration of butyrate. Interestingly, this acute reduction in energy intake was not present following the intravenous administration of butyrate. This disparity in results may be due to the fact that intragastric

administration allows butyrate to reach its natural site of production and therefore interact with intestinal receptors and/or metabolism, which is not achieved with peripheral administration. Frost *et al.* (2014) investigated the effect of acetate on energy intake. While colonic delivery of encapsulated acetate did not change food intake, intraperitoneal and central brain administration of acetate in mice was found to acutely (4 h) reduce food intake. Longer-term intraperitoneal administration of acetate (6 weeks), however, found no difference in mice on no fat and high fat diets (Sahuri-Arisoylu *et al.*, 2016). Acetate, the SCFA that circulates at the highest concentrations, has been detected at concentrations of 30-40 μM in cerebrospinal fluid (Nagashima and Morio, 2010) and can be used as an energy source by the brain (Bhatt *et al.*, 2013; Jiang *et al.*, 2013). Frost *et al.* (2014) also confirmed that colonic acetate crosses the blood brain barrier, absorbed by the brain and reduces food intake. Furthermore, acetate-induced neuronal activation in the hypothalamus following intraperitoneal administration was observed by Frost *et al.* (2014), suggesting that acetate plays a direct role in central appetite regulation.

The available rodent and human studies therefore suggest that oral SCFA supplementation does not modulate energy intake, whilst administering SCFA to the lower gut may reduce energy intake. The SCFA receptors GPR43 and GPR41 are co-expressed in glucagon-like peptide 1 (GLP-1) and PYY expressing cells (Karaki *et al.*, 2006), which led to the suggestion that SCFA might reduce food intake via stimulating the release of these hormones. The distal ileum and colon contain a high number of enteroendocrine L-cells, which release the anorectic hormones GLP-1 and PYY. Several studies in *in vitro* models of enteroendocrine cell lines have probed this effect of SCFA in gut hormone release, (Psichas *et al.*, 2014; Tolhurst *et al.*). These studies suggest that SCFA can stimulate anorectic gut hormone release via GPR43. It has also been suggested that high levels of SCFA in the lower gut could modulate energy intake via gut-brain neural circuits. For example, De Vadder *et al.* (2014) reported that elevated colonic propionate production could induce vagal signalling in the gut or portal vein via GPR41. Similarly, Li *et al.* (2017) found that the decrease in food intake following intragastric administration of butyrate in mice, was blocked after vagotomy. As previously mentioned, there is also evidence to suggest that large increases in circulating acetate may having a direct effect on the brain leading to a suppression in appetite (Frost *et al.*, 2014). In regard to identified studies in this area, four studies (Table 4) investigated the impact of either GPR43- or GPR41-KO on energy intake while two studies assessed the role of GPR43 in energy intake in GPR43-KO mice. Bjursell *et al.* (2011) reported a significant increase in energy intake relative to body weight, in GPR43-KO mice fed a high fat diet for 38 weeks. However, there was no difference in food intake in mice fed normal chow. Similarly, Kimura *et al.* (2013) reported a significant increase in food intake in GPR43-KO mice fed a high fat diet, but not when fed normal chow. The other two studies investigated the role of GPR41 in food intake in GPR41-KO mice. Lin *et al.* reported a significant increase in food intake in GPR41 KO mice fed a high fat diet (Lin *et al.*, 2012). However, Bellahcene *et al.* (2013) reported no significant effect of genotype on food intake in both low fat and high fat diet mice. In summary,

Table 4. Tabulated summary of effects of short chain fatty acid (SCFA) receptor knockout on energy intake.

Reference	SCFA receptor	Sample characteristics	Duration	Outcome
Bellahcene *et al.*, 2013	GPR41	GPR41-KO vs wildtype C57BL/6 mice (n=9). LFD and HFD	40 wk	No significant difference in food intake (P>0.5).
Bjursell *et al.*, 2011	GPR43	Male GPR43-KO (n=9) vs wildtype C57BL/6 mice (n=7). Normal chow and HFD groups	14-15 wk, 37-35 wk	Significant increase in absolute food intake with GPR43-KO mice compared to wildtype C57BL/6 mice, when on normal chow (P<0.05). Significant increase in relative food intake at 38 wks, and absolute food intake at 15 and 38 wks with GPR43-KO mice compared to wildtype C57BL/6 mice, when on HFD (P<0.05).
Kimura *et al.*, 2013	GPR43	Male GPR43-KO vs wildtype 129/Sv mice (n=7). Normal chow and HFD groups	14-16 wk	Significant increase in food intake with GPR43-KO mice compared to wildtype 129/Sv mice, when on HFD (P<0.05). No significant difference in food intake on LFD (P>0.05).
Lin *et al.*, 2012	GPR41	Male GPR41-KO mice vs wildtype C57BL/6 mice (n=34-41). Butyrate (5%), propionate (4.3%) or acetate (3.74%) in HFD	8 d	Significant increase in food intake (~ -9%) with GPR41-KO mice compared to wildtype C57BL/6 mice (P<0.05).

[1] HFD = high fat diet; LFD = low fat diet; KO = knockout.

the majority of current studies suggest that GPR43 and GPR41 have stimulatory effects on energy intake, with as 3 of the 4 identified studies showing KO of these receptors increases energy intake in mice fed a high fat diet.

In summary, studies observing the impact of oral SCFA administration have repeatedly failed to demonstrate an impact on energy intake, which may be related to the absorption of the exogenous SCFA in the upper GI tract. Investigations that have targeted delivery of SCFA to the lower GI tract have shown reductions in energy intake, which may be related to anorectic gut hormone release and/or direct neural gut-brain signalling via GPR43 and GPR41 receptors.

Short chain fatty acids and energy expenditure

In total, 14 studies assessing energy expenditure outcome were identified which met inclusion criteria. Ten studies studying the effect of provision of SCFA on energy expenditure were identified. Of which, 6 studies were conducted in mice (5 in C57BL/6 mice and 1 in E3LCETP mice), 1 study in OLETF rats, and 3 studies were conducted in adult humans (2 in overweight/obese and 1 in body mass index (BMI) 18-35 kg/m^2). Within the rodent studies, the SCFA investigated were acetate in 4 studies, butyrate in 3 studies and propionate in 2 studies. Acetate was administered orally via diet in 3 studies and by intraperitoneal injection in 1 study. Butyrate was administered orally via diet in all 3 studies. Propionate was administered orally via diet in 1 study and by intraperitoneal injection in 1 study. Within human studies, the SCFA's investigated were acetate in 1 study, propionate in 1 study and mixtures of acetate, propionate and butyrate in 1 study. Acetate was administered in distal and proximal colon in 1 study. SCFA mixtures were administered in distal colon in 1 study. Propionate was administered orally via diet in 1 study.

Four studies reporting the effect SCFA receptor knockout on energy expenditure were identified. Of which 2 studies investigated GPR43 knockout and 2 studies investigated GPR41 knockout mice. Data from the rodent studies are summarised in Table 5 (rodents). Three studies have quantified the impact of SCFA on energy expenditure in human volunteers (Table 5, humans). These studies have only recorded the acute impact over 2-3 h and the potential chronic effects of SCFA on energy expenditure remain untested. Van der Beek *et al.* (2016) first investigated the impact of infusing sodium acetate to either the proximal or distal colon in overweight and obese humans. The authors report no significant effect of colonic acetate infusion in either the overnight fasted state or after volunteers ingested an oral glucose load. The same authors, however, report positive effects of infusing a SCFA mixture, high in either acetate or propionate, to the distal colon. These effects in overweight and obese males were only observed in the overnight fasted state and not following ingestion of a glucose load. The impact of oral SCFA supplementation on energy expenditure in humans has only been assessed by Chambers *et al.* (2018b). The investigation reported that oral supplementation with sodium propionate increased energy expenditure in overnight fasted volunteers. In summary, the available data suggests that SCFA supplementation in humans acutely increases energy expenditure; however, future work is needed to clarify if this effect is only observed in fasted conditions or other physiological states. Similarly, to the rodent data, the available studies have not compared the SCFA intervention vs an energy-matched control. Consequently, the increase in energy expenditure above basal fasting level reported by Canfora *et al.* (2017a) and Chambers *et al.* (2018b) maybe a response to the energy content of the SCFA, rather than any unique stimulatory effect of the SCFA on energy metabolism.

Hattori *et al.* (2010) reported that oral acetate supplementation in mice acutely stimulates energy expenditure compared to a water control, whilst Kimura *et al.* (2011) described that intraperitoneal injection of propionate increased oxygen consumption compared to phosphate buffered saline. These studies would indicate that SCFA supplementation acutely increases energy expenditure in rodents, however, in both studies the control intervention was not energy matched. As gut-derived SCFA play important roles as substrates in host carbohydrate and lipid metabolism (Den Besten *et al.*, 2013), within these studies it is presently unclear if the observed increase in energy expenditure in response to acute SCFA provision, is simply a result of metabolic handling stimulated by the energy content of the SCFA rather than a unique stimulatory effect of the SCFA themselves.

The impact of chronic SCFA administration on energy expenditure in rodents has been reported in five studies. An investigation by Li *et al.* (2017) observed that incorporating sodium butyrate to a HFD had no effect on energy expenditure after 1 week in mice, despite an elevation in whole-body lipid oxidation rates. Conversely, Gao *et al.* (2009) demonstrated that supplementing a high fat diet with sodium butyrate enhanced energy expenditure recorded after 1 and 10 week. This stimulatory effect on energy expenditure was only observed during the 12 h night cycle, with no differences observed during the 12 h light phase. Yamashita *et al.* (2009) reported a similar outcome in rats orally administered acetate over a 20-week period, as acetate only increased energy expenditure in the 12 h 'active' night period and not during the 12 h 'resting' phase. However, the observation that SCFAs only increase energy expenditure during the night period is not supported by more investigations by Den Besten *et al.* (2015) and Sahuri-Arisoylu *et al.* (2016). Den Besten *et al.* (2015) reported that incorporating either sodium acetate, sodium propionate or sodium butyrate into a high fat diet enhanced energy

Table 5. Tabulated summary of the effects of increasing the provision of short chain fatty acids (SCFA) on energy expenditure in humans and rodents.[1]

Reference	Sample characteristics		Outcome
Humans			
Canfora *et al.*, 2017	Overweight/obese males (n=12)		Significant increase in EEx with high acetate and high propionate compared to placebo in overnight fasted state (P<0.05). No significant difference in EEx in postprandial state (P>0.05).
	SCFA:	acetate, butyrate and propionate	
	Dose:	Placebo (40 mM sodium chloride); high acetate (24 mM sodium acetate, 8 mM sodium propionate, 8 mM sodium butyrate); high propionate (18 mM sodium acetate, 14 mM sodium propionate, 8 mM sodium butyrate); high butyrate (18 mM sodium acetate, 8 mM sodium propionate, 14 mM sodium butyrate)	
	Administered via:	distal colon; placebo or high acetate or high propionate or high butyrate	
	Duration:	EEx measured after infusions for 2 h following overnight fast and 2 h after 75 g oral glucose load	
Chambers *et al.*, 2018	Males and females (n=18), BMI 18-35 kg/m^2		Significant increase in EEx with propionate compared to control.
	SCFA:	propionate	
	Dose:	control (4,164 mg sodium chloride); propionate (6,845 mg sodium propionate)	
	Administered via:	oral; capsules ingested over 2 h in overnight fasted state: control or propionate	
	Duration:	EEx measured for 3 h	
Van der Beek *et al.*, 2016	Overweight/obese males (n=6)		No significant increase in EEx in either proximal or distal colon or fasted or postprandial states (P>0.05).
	SCFA:	acetate	
	Dose:	100 and 180 mmol/l	
	Administered via:	proximal and distal colon; placebo (sodium chloride) or acetate	
	Duration:	EEx measured after infusions for 2 h following overnight fast and 2 h after 75 g oral glucose load	
Rodents			
Den Besten *et al.*, 2015	C57Bl/6J male mice (n=6-8), age 2 m		Significant enhancement of EEx overall, and in both 'day' (06:30 to 18:00) and 'night' (18:00 to 06:30) periods with HFD + SCFA compared to HFD (P<0.05).
	SCFA:	acetate, butyrate, and propionate	
	Dose:	5% w/w	
	Administered via:	oral; HFD or HFD + acetate, butyrate or propionate	
	Duration:	12 wk	
Gao *et al.*, 2009	Dietary-obese C57BL/6J male mice (n=10), age 4 wk		Significant increase in EEx after both 1 and 10 wk of supplementation in 12 h 'night' phase (P <0.05) in HFD + butyrate compared to HFD. No significant difference in 12 h 'day' phase (P>0.05).
	SCFA:	butyrate	
	Dose:	5% w/w	
	Administered via:	oral; HFD or HFD + butyrate	
	Duration:	EEx measured after 1 wk and 10 wk.	
Hattori *et al.*, 2010	C57BL/6 male mice (n=9), age 9 wk		Significant increase in EEx with acetate compared to water (P<0.05).
	SCFA:	acetate	
	Dose:	1.5% in 10 ml/kg water	
	Administered via:	oral; acetate or water	
	Duration:	3 h post administration	
Kimura *et al.*, 2011	C57BL/6 male mice (n=8), age 4-16 wk		Significant increase in oxygen consumption with propionate compared to PBS (P<0.05).
	SCFA:	propionate	
	Dose:	1 g/kg	
	Administered via:	intraperitoneal injection; PBS or propionate	
	Duration:	40 min post administration	

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Table 5. Continued.

Reference	Sample characteristics		Outcome
Li *et al.*, 2018	E3L.CETP male mice (n=8-10), age 10-12 wk		No significant effect in EEx overall or during either day or night periods between HFD or HFD + butyrate (P>0.05).
	SCFA:	butyrate	
	Dose:	5% w/w	
	Administered via:	oral; HFD or HFD + butyrate	
	Duration:	EEx measured for 3 d following 1 wk intervention	
Sahuri-Arisoylu *et al.*, 2016	Male C57BL/6 mice (n=8 per group), NFD and HFD groups		Significant increase in EEx during both 'light' (07:00 to 17:00) and 'dark' (17:00 to 07:00) periods P<0.05), when on HFD with acetate compared to control. Significant increase in EEx during 'dark' period, when on NFD (P<0.05).
	SCFA:	acetate	
	Dose:	4.41 mM/200 µl; 3 injections per wk	
	Administered via:	intraperitoneal injection; 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid or nanoparticle-derived acetate	
	Duration:	EEx (heat dissipation) measured after 6 wk	
Yamashita *et al.* 2009	OLETF male rats (n=3-4), age 4 wk		Significant increase in oxygen consumption at 20 wk in 'active' period (19:30-07:30) (P<0.05) with normal chow + acetate compared to normal chow + distilled water. No significant differences in 'resting' period (07:30-19:30) (P>0.05).
	SCFA:	acetate	
	Dose:	5.2 mg/kg in 5 ml/kg distilled water)	
	Administered via:	oral; normal chow + distilled water or normal chow + acetate	
	Duration:	oxygen consumption measured at 20 wk	

[1] BMI = body mass index; EEx = Energy expenditure; HFD = high fat diet; KO = knockout; NFD = no fat diet; PBS = phosphate buffered saline.

expenditure after 10 weeks in mice during both 12 h day and night periods. In addition, Sahuri-Arisoylu *et al.* (2016) found that Intraperitoneal injection of nanoparticle-derived acetate for 6 weeks increases energy expenditure on a high fat diet during both 12 h day and night periods. In summary, the majority of studies have reported that chronic administration of SCFA promotes energy expenditure in rodents, but within the current literature discrepancy exists as to whether these effects are observed during the active/night and/or the resting/light periods.

Mechanistically, the positive effect of SCFA on energy expenditure could be due to stimulation of GPR43 and GPR41. Four studies were identified (Table 6) which have investigated the impact of either GPR43 or GPR41 knockout on energy expenditure in rodents. The initial work of Bjursell *et al.* (2011) identified that GPR43-KO promoted energy expenditure in mice. No differences were reported between GPR43-KO and wild-type mice fed a normal fat chow diet, however in mice fed HFD, GPR43-KO was associated with higher energy expenditure when mice were studied at 34-35 weeks. Three subsequent studies reported opposite effects and demonstrated that mice lacking either GPR43 or GPR41 have lower energy expenditure compared to wild-type controls. Kimura *et al.* (2011) reported that oxygen consumption was significantly lower at 14-16 weeks in GPR41-KO mice fed a normal chow diet. However, this effect was not observed when mice had been starved for 48 h. Later work by the same research group (Kimura *et al.*, 2013) described that GPR43-KO lowers energy expenditure in 16-week old mice fed a high fat diet. Furthermore, Bellahcene *et al.* (2013) reported that male GPR41-KO mice fed a high fat diet had lower energy expenditure at 15, 27 and 40 weeks of age compared to wild-type controls. This study suggests gender differences in GPR41-KO mice, as these differences were not recorded in female mice. In summary, the majority of current studies suggest that GPR43 and GPR41 have stimulatory effects on energy expenditure, as KO of these receptors lowers measured rates of energy expenditure in rodents. These effects have predominantly been tested in male mice. Interestingly, the only study that investigated gender differences suggested that GPR41-KO only influences energy expenditure in males. Future work is needed to clarify the possible differential effects of GPR43 and GPR41 KO on males and females.

It has consistently been reported that the increase in energy expenditure stimulated by SCFA is associated with a promotion in whole-body lipid oxidation (Den Besten *et al.*, 2015; Gao *et al.*, 2009; Hattori *et al.*, 2010; Li *et al.*, 2017). All three human studies also support the observations in rodent models that administration of SCFA stimulates whole-body lipid oxidation (Canfora *et al.*, 2017b; Chambers *et al.*, 2018b; Van der Beek *et al.*, 2016). The increase in energy expenditure and lipid oxidation by SCFA administration has been postulated to be due to stimulation of sympathetic nervous system (SNS) activity, via GPR41 expressed at the

Table 6. Tabulated summary of effects of short chain fatty acid (SCFA) receptor knockout on energy expenditure.[1]

Reference	SCFA receptor	Sample characteristics	Duration	Outcome
Bellahcene *et al.*, 2013	GPR41	GPR41-KO vs wildtype C57BL/6 mice (n=9). HFD	EEx measured at 15, 27 and 40 wk	Significant reduction in 24 h EEx in male GPR41-KO mice at 15, 27 and 40 wk compared to wildtype C57BL/6 mice (P<0.05). No significant difference in female mice (P>0.05).
Bjursell *et al.*, 2011	GPR43	Male GPR43-KO (n=9) vs wildtype C57BL/6 mice (n=7). Normal chow and HFD groups	EEx measured at 11-12 and 34-35 wk	Significant increase in EEx at 34-35 wk with GPR43-KO mice at 15, 27 and 40 wk compared to wildtype C57BL/6 mice, when on HFD (P<0.05). No significant difference in EEx on normal chow or at 11-12 wk on HFD (P>0.05).
Kimura *et al.*, 2011	GPR41	Male GPR41-KO vs wildtype C57BL/6 mice (n=5-7).	oxygen consumption measured at 14-16 wk	Significant reduction in oxygen consumption during feeding in GPR41-KO mice compared to wildtype C57BL/6 mice (P<0.05).
Kimura *et al.*, 2013	GPR43	Male GPR43-KO vs wildtype 129/Sv mice (n=7). Normal chow and HFD groups	EEx measured at 16 wk	Significant reduction in 24 h EEx in GPR43-KO mice compared to wildtype 129/Sv mice (P<0.05).

[1] EEx = energy expenditure; HFD = high fat diet; KO = knockout.

level of the sympathetic ganglion (Kimura *et al.*, 2011), an increase in brown adipose tissue (BAT) activity (Li *et al.*, 2017; Sahuri-Arisoylu *et al.*, 2016) and via suppression of PPARγ in peripheral tissues, which upregulates lipid oxidation (Den Besten *et al.*, 2015).

Conclusion body weight control

Based on the balance of the available data we can conclude that: (1) SCFA supplementation appears to prevent weight gain associated with HFD in rodents, while human studies are limited. (2) SCFA supplementation is not associated with reductions in energy intake. The route of administration of exogenous SCFA may impact on the observed appetite-suppressive effect. Oral delivery of SCFA has consistently been shown not to modulate energy intake, whilst methods that target delivery of SCFA to the lower gut have been shown to reduce energy intake. (3) SCFA administration has been shown to stimulate energy expenditure by promoting lipid oxidation. At present, the vast majority of the available evidence has been obtained from animal models. Direct translation into humans may be limited, thus future translational work should support the development of novel interventions to increase SCFA production to improve long-term energy homeostasis.

Short chain fatty acids and glucose homeostasis, including insulin secretion and insulin sensitivity

Disturbances in substrate metabolism and inter-organ crosstalk between gut-adipose tissue, liver and skeletal muscle may play an important role in the aetiology of ectopic fat accumulation and insulin resistance. In this section, a systematic review, using defined search strings is done on human studies relating SCFA administration or dietary fibres-related SCFA production to glycaemic control, insulin sensitivity and insulin secretion. Second, we summarise studies on the effect of SCFA on factors that contribute to the progression of insulin resistance such as adipose tissue dysfunction, characterised by a reduced lipid storage capacity and low-grade inflammation, as well as impairments in substrate handling and oxidation in metabolically active tissues (DeFronzo *et al.*, 1981). The aforementioned effects of SCFA on gut barrier function and gut integrity can also contribute, to the development of insulin resistance and impairments in glucose homeostasis (Cani *et al.*, 2007; Henao-Mejia *et al.*, 2012; Tremaroli and Bäckhed, 2012). In this section the focus is on the effects of SCFA on adipose tissue, liver and skeletal muscle and β-cell function and metabolism as well as on low-grade inflammation (summarised in Figure 4).

Human data on effects of short chain fatty acids on glucose homeostasis and insulin sensitivity

Acute studies

Six intervention studies focused on the acute effects of SCFA administration on parameters of glucose homeostasis and insulin sensitivity (for more details see Table 7). Wolever *et al.* (1989) found no effects on blood glucose and insulin levels after rectal administration of acetate and propionate within 30 min. However, both mixtures decreased circulating fatty acid (FA) concentrations. In

Table 7. Short chain fatty acid (SCFA) intervention studies in humans and glucose homeostasis.[1]

Reference	Sample characteristics		Outcome
Bouter *et al.*, 2018	Participants:	9 healthy lean men as well as 10 men with metabolic syndrome	In the lean, but not in the metabolically compromised participants, an improvement in peripheral and hepatic insulin sensitivity.
	SCFA:	4 g sodium butyrate for 4 weeks	
	Design:	chronic; comparison of healthy men vs men with metabolic syndrome; parallel design	
Canfora *et al.*, 2017	Participants:	12 normoglycaemic overweight/obese (BMI; in kg/m^2, in a range 25-35) men	No changes in blood glucose, plasma insulin concentrations. Increase in PYY and decrease in free glycerol concentrations.
	SCFA:	rectal sodium-SCFA mixtures (180 mmol/l)	
	Design:	acute; placebo controlled (saline); four-way crossover	
Fernandes *et al.*, 2012	Participants:	9 healthy and 9 hyperinsulinemic adults	Acetate-induced FFA fall and rebound was greater in healthy than hyperinsulinemic participants (P<0.01). FFA fall and rebound was negatively associated with insulin resistance indices ((HOMA-IR with FFA fall and FFA rebound; insulinogenic index with FFA rebound).
	SCFA:	intravenous sodium acetate (140 mmol/l in 90 min)	
	Design:	acute; comparison of healthy vs hyperinsulinemic; parallel design	
Freeland *et al.*, 2010	Participants:	6 overweight/obese (BMI; in kg/m^2 >25), hyperinsulinemic women	No significant changes in glucose and insulin levels. Increase in PYY and decrease in TNF-α.
	SCFA:	sodium acetate, 60 mmol in 300 ml water; 200 mmol/l (rectally), intravenous 20 mmol in 100 ml water in 5 min	
	Design:	acute; placebo controlled (saline); four-way crossover	
Laurent *et al.*, 1995	Participants:	6 healthy adults	No changes in blood glucose, plasma insulin concentrations or hepatic glucose production. Decreased circulating FFA after all SCFA infusions.
	SCFA:	acetate (12 mmol/h), or propionate (4 mmol/h), or acetate + propionate (12 mmol/h + 4 mmol/h) for 3 h (gastric)	
	Design:	acute; placebo controlled (saline); four-way crossover	
Todesco *et al.*, 1991	Participants:	6 healthy adults	38% lowered 2 h glucose area under the curve values (P<0.05) and a lower glucose peak.
	SCFA:	oral sodium propionate (9.9 g per day) in white bread for 1 week	
	Design:	chronic; placebo controlled (standard white bread); two-way crossover	
Wolever *et al.*, 1991	Participants:	6 healthy adults	Acetate alone increased serum glucagon by ~26% (P<0.05) and decreased FFA levels by ~22% (P<0.05) but did not affect glucose or insulin levels. Propionate alone increased glucose by ~8% (P<0.05) and glucagon by ~13%, but no effects on insulin levels were observed.
	SCFA:	800 ml rectal infusions with 180 mmol acetate alone, 180 mmol propionate alone or a combination of both (180 mmol acetate + 60 mmol propionate)	
	Design:	acute; placebo controlled (saline); four-way crossover	
Venter *et al.*, 1990	Participants:	20 healthy female adults	Reduced fasting blood glucose and insulin response (P<0.05).
	SCFA:	oral sodium propionate (7.5 g capsules daily) for 7 weeks	
	Design:	chronic; placebo controlled (calcium phosphate); parallel design	
Wolever *et al.*, 1989	Participants:	6 healthy adults	No effects on circulating glucose or insulin. 40% decreased serum FFA after 180 mmol/l SCFA infusion (P<0.05).
	SCFA:	800 ml rectal infusions with 90 mmol acetate + 30 mmol propionate (90 mmol/l, isotonic) or 180 mmol acetate + 60 mmol propionate (180 mmol/l, hypertonic) within 30 min	
	Design:	acute; placebo controlled (saline); three-way crossover	

[1] BMI = body mass index; FFA = free fatty acid; PYY = protein YY; TNF-α = tumour necrosis factor-alpha.

a subsequent acute study by Wolever *et al.* (1991) rectal acetate infusions increased circulating glucagon and decreased circulating FA levels, but did not affect blood glucose or insulin levels. Rectal propionate infusions increased glucose and glucagon levels, but did not affect FA or insulin concentrations, suggesting a putative role of gut-derived propionate as a substrate for gluconeogenesis. Laurent *et al.* (1995) found no effects of 3 h gastric infusions of acetate and/or propionate on glucose metabolism, whilst all infusions reduced circulating FA concentrations. Also, Freeland and Wolever (2010) found no effects of acute intravenously and rectally infused sodium acetate on

glucose and insulin levels, whilst intravenous and rectal acetate infusions lowered plasma TNF-α and rectal acetate infusions increased PYY levels. Furthermore, Fernandes *et al.* (2012) found no differences in acetate clearance when acetate was infused intravenously in hyperinsulinaemic (HI) and healthy (NI) adults, but found a greater FA rebound in the NI group when compared to HI, which was correlated with IR indices, indicating a relation between the decreased lipolysis and improved insulin sensitivity. Canfora *et al.* (2017b) rectally infused mixtures of SCFA in overweight to obese men. The SCFA administration did not affect fasting or postprandial (after a 75 g glucose load) plasma glucose or insulin concentrations but increased fasting PYY and decreased fasting free glycerol concentrations.

Longer-term short chain fatty acid administration

We identified three human studies of longer-term (chronic) SCFA administration with outcomes on glucose homeostasis and insulin sensitivity (Table 8). In a study of Venter *et al.* (1990) propionate supplementation for 7 weeks resulted in decreased fasting glucose level and increased maximum insulin increments during an oral glucose tolerance test in healthy females. Todesco *et al.* (1991) showed that the

Table 8. Long-term dietary intervention studies with effects on glucose homeostasis and insulin sensitivity.[1]

Reference	Sample characteristics		Outcome
Robertson *et al.*, 2005	Participants: Nondigestible carbohydrate: Design:	10 healthy adults 30 g resistant starch (10 g three times) per day 4 wk placebo controlled (20 g digestible starch); two-way crossover	Improved whole-body insulin sensitivity (euglycemic-hyperinsulinaemic clamp) by ~13% (P<0.05).
Cani *et al.*, 2009	Participants: Nondigestible carbohydrate: Design:	10 healthy adults 16 g oligofructose (8 g twice) per day 2 wk placebo-controlled (16 g dextrin maltose); parallel design	2h postprandial glucose AUC was reduced by ~17% (P<0.05). Increased plasma GLP-1 and PYY levels and increased breath-hydrogen excretion.
Parnell *et al.*, 2009	Participants: Nondigestible carbohydrate: Design:	48 overweight/obese adults; BMI >25 kg/m^2 21 g oligofructose (7 g three times) per day 12 wk placebo-controlled (7.89 g isocaloric maltodextrin); parallel design	Absolute 6 h postprandial plasma glucose and insulin concentrations were reduced by 5% (P<0.01) and ~10% (P<0.01), respectively.
Dewulf *et al.*, 2013	Participants: Nondigestible carbohydrate: Design:	30 women with obesity; BMI >30 kg/m^2 16 g inulin/oligofructose mix (8 g twice) per day 3 mo placebo-controlled (16 g maltodextrin); parallel design	Reduced post-OGTT glucose response (7% (P<0.01). No effects on HOMA, fasting glucose and insulin and HbA1c.
Vulevic *et al.*, 2013	Participants: Nondigestible carbohydrate: Design:	45 overweight/obese adults, BMI >25 kg/m^2 5.5 g galactooligosaccharide mixture once a day 12 wk placebo-controlled (5.5 g maltodextrin); two-way crossover	Decreased fasting insulin (~14% (P<0.01), triglyceride and C-reactive protein plasma concentrations.
Dewulf *et al.,* 2013	Participants: Nondigestible carbohydrate: Design:	30 obese women, BMI >35 kg/m^2 Inulin/oligofructose mixture (16 g/day) 12 wk placebo-controlled (16 g maltodextrin); parallel design	Improved postprandial glucose response. However, no effects were found on HbA1c, HOMA and fasting insulin and glucose levels.
Canfora *et al.*, 2017	Participants: Nondigestible carbohydrate: Design:	44 prediabetic overweight/obese adults, BMI range 25-35 kg/m^2 15 g of galacto-oligosaccharide (3×5 g) per day 12 wk placebo-controlled (isocaloric maltodextrin); parallel design	No effects on glucose or insulin homeostasis. No increases in fasting SCFA plasma concentrations or faecal SCFA.
Zhao *et al.*, 2018	Participants: Nondigestible carbohydrate: Design:	43 adults with type 2 diabetes mellitus high-fibre diet; composed of whole grains, traditional Chinese medicinal foods, and prebiotics 12 weeks; isocaloric low fibre diet; parallel design	Complex fibre mixtures for 12 wk promoted SCFA producing bacteria and decreased haemoglobin A1c levels and fasting blood glucose and glucose tolerance by approximately 20% (P<0.05).

[1] BMI = body mass index; GLP-1 = glucagon-like peptide 1; OGTT = oral glucose tolerance test; PYY = peptide YY; AUC = area under the curve; SCFA = short chain fatty acids; HOMA = homeostatic model assessment.

daily propionate supplementation in bread for one week decreases glucose values, when compared to propionate-free bread. However, the glucose lowering effects were related to lower digestion of the bread-derived starch, since a higher faecal bulk was found in the propionate treated group. In a pilot study by Bouter *et al.* (2018), healthy lean men as well as men with metabolic syndrome were treated with 4g of sodium butyrate daily for 4 weeks. Interestingly, in the lean, but not in the metabolically compromised participants, an improvement in peripheral and hepatic insulin sensitivity (determined via hyperinsulinaemic-euglycaemic clamp) was observed. This pilot data suggests a disturbed SCFA handling/signalling in individuals with metabolic syndrome. However, the data should be confirmed in a larger cohort and underlying mechanisms need to be investigated.

Dietary fibre/prebiotic studies

Further indications for a beneficial effect of SCFA on insulin sensitivity and glucose homeostasis are derived from eight human dietary intervention studies using fermentable polysaccharides. Robertson *et al.* (2005) showed that 30 g per day resistant starch supplementation for 4 weeks enhanced whole-body and skeletal muscle insulin sensitivity in healthy subjects, which was accompanied by increased systemic acetate and propionate levels and an increase in adipose tissue and skeletal muscle acetate uptake. Cani *et al.* (2009a) showed that a 2-week treatment with oligofructose (16 g/day) decreased postprandial glucose levels, which was associated with plasma GLP-1 levels and an increased breath-hydrogen excretion (increased microbial fermentation) in healthy adults. In line, Parnell and Reimer (2009) found reduced postprandial glucose and insulin levels and increased PYY levels after oligofructose (21 g/day) intake for 12 weeks in overweight adults. Pouteau *et al.* (2010) investigated the effects of 5-week supplementation of the acetogenic fibres acacia gum and pectin (28 g/day) on glucose turnover and insulin sensitivity in 21 men with metabolic syndrome. Fasting glucose turnover improved compared to control. However, peripheral insulin sensitivity (measured via hyperinsulinaemic-euglycaemic clamp) did not change. In addition, Vulevic *et al.* (2013) found that a 12-week supplementation of a GOS mixture in overweight subjects beneficially altered faecal microbiota, fasting insulin and TAG concentrations. An elegant study of Dewulf *et al.* (2013) found an improved postprandial glucose response, and a shift towards a butyrate-producing gut microbiota composition after 3 months intake of an inulin/oligofructose mixture (16 g/day) in obese women. However, no effects were found on HbA1c, HOMA and fasting insulin and glucose levels. Canfora *et al.* (2017a) investigated the effect of the acetogenic fibre galactooligosaccharide (15 g/day) on insulin sensitivity in obese, pre-diabetic individuals. Besides 5-fold increases in *Bifidobacterium* spp., neither effects on SCFA plasma and faecal concentrations nor on peripheral and adipose tissue insulin sensitivity (as measured via hyperinsulinaemic-euglycaemic clamp technique) were found. A study by Zhao *et al.* (2018) showed that a diet rich in complex fibre mixtures for 12 weeks decreased haemoglobin A1c levels and fasting blood glucose and improved glucose tolerance by approximately 20% in T2DM individuals. A metagenomics analysis of the faecal microbiota revealed that the microbial pathways for acetate and butyrate production were significantly increased. There was also a trend for increased faecal acetate and butyrate concentrations, which coincided with increased circulating levels of fasting PYY and postprandial GLP-1. Interestingly, when a responder analyses was performed the microbiome of the positive responders showed a higher genetic microbial capacity to ferment fibres and to produce SCFA.

Together, these data provide direct and indirect evidence for a beneficial role of SCFA in glucose control and insulin sensitising. However, it has to be mentioned that recent well-controlled longer-term studies using fermentable dietary fibres failed to show beneficial effects on glucose homeostasis and insulin sensitivity. Interestingly, in these studies mainly individuals with a metabolically disturbed phenotype (metabolic syndrome, obesity or prediabetes) were included, which suggests a disturbed SCFA handling/signalling in these individuals. These controversial outcomes indicate the urgent need for well-controlled longer-term human SCFA intervention studies investigating effects on glucose homeostasis and insulin sensitivity in different metabolic phenotypes.

Short chain fatty acids affect adipose tissue metabolism

SCFA have been shown to affect adipose tissue metabolism. In particular, the systemically most abundant SCFA acetate might affect the adipocyte intracellular lipolysis. A decreased phosphorylation of hormone-sensitive lipase in a GPR-dependent manner might underlie this antilipolytic effect, as demonstrated by Aberdein *et al.* (2014) in 3T3-L1 rodent cell lines and by Jocken *et al.* (2017) in a human adipocyte model. Several *in vivo* studies also have shown that acetate can inhibit whole-body lipolysis in humans. For instance, Canfora *et al.* (2017b) demonstrated that rectal administrations of SCFA mixtures high in sodium acetate decreased circulating glycerol concentrations. Furthermore, Fernandes *et al.* (2012) showed that acute intravenously-administered acetate decreased plasma FFA levels in healthy and hyperinsulinaemic individuals with obesity. In contrast, butyrate seems to have pro-lipolytic properties as shown by Jocken *et al.* (2017) in a human adipocyte model and by Rumberger *et al.* (2014) and Jia *et al.* (2016) in rodent cell lines. The underlying mechanism might be an increase in histone hyperacetylation-associated β_3-adrenergic receptor activation and increased activity of the key lipolytic enzymes adipose triglyceride lipase (ATGL) and hormone-sensitive lipase (HSL). However, human *in vivo* data demonstrating

an increased lipolytic activity after butyrate administration are currently not available, which might be related to the low systemic abundance of butyrate.

Besides lipolysis, also adipogenesis may be affected by SCFA. Hong *et al.* (2005) demonstrated that treatment of 3T3-L1 preadipocytes with acetate and propionate for seven days increased adipogenesis. The increments in adipogenesis were related to an increased expression of GPR43 and peroxisome proliferator-activated receptor gamma 2 (PPARγ2), which is a transcription factor involved in early adipogenic differentiation. Of note, these the adipogenic effects of SCFA were completely abolished when GPR43 expression was blocked using siRNA, indicating that the GPR43 receptor is of major importance in the adipogenic potential of SCFA.

In summary, SCFA, in particular acetate, may affect adipose tissue function by increasing the lipid buffering capacity and preventing chronic low-grade inflammation, which may in the end positively affect insulin sensitivity. Potential mechanisms involved are the modulation of intracellular and extracellular lipolysis and PPARγ/GPR43-mediated adipogenesis.

Short chain fatty acids affect skeletal muscle metabolism

SCFA may improve skeletal muscle functioning by increasing the skeletal muscle lipid oxidative capacity as demonstrated in rodents. Yamashita *et al.* (2009) treated obese rats for a period of 6 months with acetate (5.2 mg/kg body weight) and found increased expression of genes involved in oxidative and glucose metabolism (i.e. myoglobin, glucose transporter type 4, pAMPK). Next, Fushimi *et al.* (2001) showed that six days dietary acetate supplementation in rats increased glycogen storage and decreased glycolysis in the gastrocnemius muscle. Gao *et al.* (2009) demonstrated that of a sodium butyrate enriched high-fat diet for 16 weeks enhanced the proportion of type 1 oxidative muscle fibres and expression of PPAR-δ resulting in enhanced mitochondrial fat oxidation in C57BL/6J mice. Together, these data indicate that SCFA may affect skeletal muscle glucose and oxidative metabolism and may increase muscle lipid turnover, thereby contributing to improved insulin sensitivity. Future investigation should focus on skeletal muscle SCFA uptake and their contribution to muscle oxidative metabolism in humans.

Short chain fatty acids affect liver metabolism

SCFA may affect hepatic glucose and lipid metabolism. Li *et al.* (2013) showed that bovine hepatocytes cultured with acetate in concentrations of 1.8 mmol/l to 7.2 mmol/l for 3 h increased the AMP/ATP ratio and AMPKα phosphorylation, which subsequently increased the expression of PPARα target genes involved in lipid oxidation. Moreover, oral and intravenous administration of acetate (Kondo *et al.*, 2009; Sakakibara *et al.*, 2006; Yamashita *et al.*, 2007) and all three SCFA (Den Besten *et al.*, 2015) in animal models of obesity and T2DM demonstrated a decreased liver lipid accumulation and improved glucose homeostasis. The related mechanisms include an increased hepatic AMPK activity, and increased expression of PPAR α target genes involved in, fat oxidation, thermogenesis, gluconeogenesis and lipogenesis. However, human studies focusing on SCFA effects on parameters of liver metabolism are currently lacking.

Short chain fatty acids affect β-cell function and insulin secretion

Rodent as well as human pancreatic β-cells express the SCFA receptors GPR41 and GPR43 (McNelis *et al.*, 2015; Priyadarshini *et al.*, 2015). Priyadarshini *et al.* (2015) demonstrated in obese and insulin resistant mice that SCFA have the capacity to increase glucose-stimulated insulin secretion via GPR43. McNelis *et al.* (2015) showed that depletion of GPR43 in mice with diet-induced obesity deteriorated β-cell function and increased β-cell mass. Moreover, Veprik *et al.* (2016) indicated that SCFA/GPR41 signalling is of particular importance in controlling pancreatic β-cell insulin secretion, since GPR41 knockout or overexpression in mice resulted in impairments in glucose control without effects on insulin sensitivity. By using a propionate-inulin-ester in a human *in vivo* study, Pingitore *et al.* (2017) demonstrated that propionate has beneficial effects on β-cell function and insulin secretion, which was independent of GLP-1. They also performed an *in vitro* follow-up experiment using human islets, which showed that propionate increased glucose-stimulated insulin release and maintained β-cell mass through inhibition of apoptosis. Thus, the SCFA/GPR axis is emerging as an important regulator of pancreatic insulin secretion and β-cell functioning.

Short chain fatty acids affect low-grade inflammation

Chronic low-grade (or systemic) inflammation results from the chronic activation of the immune system and the release of pro-inflammatory mediators such as TNF-α, IL like IL-6 and IL-1β and eicosanoids (McLoughlin *et al.*, 2017). These mediators are mainly produced in the cells of the immune system, but additionally, the adipose tissue serves as an endocrine organ secreting hormones (adipokines) involved in the regulation of energy metabolism (Roelofsen *et al.*, 2010). Dysregulation of adipokine production is regarded as one factor contributing to the chronic inflammatory state often accompanying obesity. Chronic low-grade inflammation is associated with the development of a variety of health impairments like diabetes, cardiovascular disease and cancer (McLoughlin *et al.*, 2017) and it has been shown that intake of prebiotics, i.e. fermentable dietary

fibre is inversely associated with inflammatory markers (McLoughlin *et al.*, 2017). This has been attributed to SCFA produced during their fermentation as mediators of the anti-inflammatory properties.

Only a few human studies have been identified analysing the effect of SCFA on low-grade chronic or systemic inflammation. Not addressed here are the series of human studies examining SCFA topical therapy as a promising option in distinct forms of inflammatory bowel disease (Harig *et al.*, 1989; Scheppach, 1996). The effects of colonic administration of physiologically relevant SCFA mixtures was examined in a study in overweight and obese subjects. After colonic infusions of physiologically relevant SCFA mixtures, in concentrations and ratios reached after fibre intake, fasting circulating levels of the pro-inflammatory cytokine IL-1β decreased with a high acetate (60%) containing SCFA mixture compared to placebo, and was significantly lower compared to a high propionate (35%) SCFA mixture. Postprandial IL-1β levels did not differ between treatment groups. In addition, no significant differences between groups were reported in other proinflammatory cytokines such as TNF-α, IL-6 and IL-8 neither in the fasting nor in the postprandial period (Canfora *et al.*, 2017b). In the study by Van der Beek *et al.* (2016) a tendency for lower fasting plasma TNF-α concentrations was found after distal colonic acetate infusion with a 100 mmol/l yet not with a 180 mmol/l, as well as after proximal colonic acetate infusion Other inflammatory markers were not influenced. In two studies by Hamer *et al.* (2009; 2010) in patients with UC and healthy volunteers, butyrate (60 ml of a 100 mM solution) was administered via rectal enema once daily prior to sleeping. While a significant increase in mucosal IL-10/IL-12 was found in UC patients, no significant change of plasma C-reactive protein or other markers were found within the butyrate group. In females with high fasting plasma insulin levels, acetate was found to significantly decrease plasma TNF-α, while a suppressing effect was only confirmed after intravenous infusion of 20 mmol/l acetate (corresponding to an amount that would be produced in 1-4 h) yet not rectal acetate infusions of 60 mmol/l (Freeland and Wolever, 2010).

Different routes have been proposed by which microbiota-generated prebiotic fibre derived SCFA can modulate inflammatory processes. SCFA can bind to the G protein coupled receptors GPR41 and GPR43 and elicit intracellular signalling cascades like mitogen-activated protein kinases (MAPKs), protein kinase C (PKC) and transcription factors. Furthermore, SCFA act as histone deacetylase inhibitors, thereby altering the acetylation state of histones and other proteins and consequently leading to changes in the transcription of genes (Vinolo *et al.*, 2011). An influence on cyclooxygenase enzyme and thereby on eicosanoid production has also been proposed (Al-Lahham *et al.*, 2010a; Nurmi *et al.*, 2005).

Mechanistic insight into the direct effects of SCFA on inflammation comes mainly from *in vitro* and *ex vivo* experiments with isolated cells or tissues (Vinolo *et al.*, 2011). During inflammation, neutrophils are recruited into the inflamed tissues where they exert antibacterial actions by releasing ROS and granule enzymes and phagocytosis of bacteria (Aoyama *et al.*, 2010). However, excess pro-inflammatory mediators released by the neutrophils can also lead to damage of healthy host tissues if their apoptosis is delayed. In isolated human neutrophils, butyrate and propionate increased apoptosis via a caspase-dependent pathway (Aoyama *et al.*, 2010). In rat neutrophils, a decrease in ROS production was measured in response to butyrate treatment while propionate had no effect and acetate even increased ROS production (Vinolo *et al.*, 2009).

In a co-culture experiment with adipocytes and macrophages, Ohira *et al.* (2013) found significant dose-dependent decreases of the inflammatory markers TNF-α, monocyte chemoattractant protein 1 (MCP-1) and IL-6 after treatment with butyrate. In experiments using human omental adipose tissue (OAT) explants, Al-Lahham *et al.* (2012; 2010b) demonstrated that propionate inhibited secretion of inflammatory mediators like TNF-α and resistin. Since resistin is a marker for insulin resistance in humans and acts pro-inflammatory, its reduction is in agreement with the anti-inflammatory properties of SCFA.

The concentrations of the SCFA that were required to exert a specific action in the experiments reported above were often different. Furthermore, acetate, butyrate and propionate sometimes exhibit contrasting effects (e.g. Cavaglieri *et al.*, 2003). Thus, diverse statements can be found in the literature. However, it is possible that the amount of SCFA required to elicit an anti-inflammatory response indeed varies with different underlying causes of inflammation (Al-Lahham *et al.*, 2010a). Furthermore, the cell type used for the experiments might influence the outcome. Accordingly, Huuskonen *et al.* (2004) showed that butyrate has anti-inflammatory properties in primary, brain-derived microglial cells, but exerts pro-inflammatory effects in transformed, proliferating N9 microglial cells. According to the authors, this effect could be involved in the colon cancer protective role of butyrate. In T-cells, SCFA promoted differentiation depending on the cytokine milieu (Park *et al.*, 2015).

Confirmation of the anti-inflammatory properties that are often associated with SCFA comes from animal experiments. In ApoE knockout mice, feeding with butyrate reduced atherosclerotic lesions and lowered macrophage migration accompanied by a decrease in pro-inflammatory cytokines (Aguilar *et al.*, 2014). In mice treated intraperitoneally with

acetate, inflammatory processes after kidney injuries were decreased leading to attenuation of the detrimental effects of inflammation on renal function (Andrade-Oliveira *et al.*, 2015). Conversely, after systemic administration of supra-physiological doses of SCFA, renal tissue inflammation was increased due to dysregulation of T cell response (Park *et al.*, 2016). In another study with mice it was found that SCFA receptors GPR41 and GPR43 are required for an inflammatory response to bacterial infection and thus, a protective pro-inflammatory response (Kim *et al.*, 2013). In rodent models of colitis oral acetate administration was shown to be protective (Masui *et al.*, 2013).

Based on the totality of available data from *in vitro*/*ex vivo*, animal and limited human studies, there is plausible evidence supporting beneficial effects of SCFA generated from fermentation processes by the gut microbiota following prebiotic consumption on modulating and ameliorating low-grade inflammation.

Emerging concept in the gut-brain axis: gluconeogenesis in intestinal epithelium

Intestinal gluconeogenesis (IGN) is a function recently described in humans and all vertebrates studied up to now, that is able to influence energy homeostasis (for a review, Soty *et al.*, 2017). Basically, major metabolic benefits exerted by the SCFA butyrate and propionate relate to the activation of IGN in enterocytes. IGN releases glucose that is detected by the portal neural system, sending a signal to the brain. This initiates a set of brain-derived metabolic benefits in energy homeostasis, including decreased fat storage and body weight, improved insulin sensitivity and systemic glucose control (Soty *et al.*, 2017). Since butyrate and propionate bind to G protein-coupled receptor (GPR)41 and 43, which are both present in the enterocyte plasma membrane, an attractive hypothesis was that butyrate and propionate may modulate enterocyte metabolism via the activation of GPRs. However, this was not the case relating to IGN. Indeed, both SCFA activate IGN via complementary mechanisms that do not involve enterocyte GPR-41/43.

Instead, butyrate enters the enterocyte and is converted to butyryl-CoA, and subsequently, acetyl-CoA that is oxidised in the Krebs cycle and produces ATP. The ATP increase activates the production of cAMP via an ATP-induced activation of adenylate cyclase, independently of any hormonal stimulation, which finally activates the expression of the regulatory gluconeogenesis genes (glucose-6 phosphatase, and phosphoenolpyruvate carboxykinase-cytosolic form) (De Vadder *et al.*, 2014).

Propionate activates IGN via two distinct processes. Firstly, it binds to and activates neuronal GPR-41 receptors, especially those present in the gastrointestinal nerves surrounding the portal vein. This initiates a neural loop resulting in the release of vasoactive intestinal peptide (VIP) in the submucosal nervous plexus. VIP binds to its receptor VPAC1 in the enterocyte plasma membrane and activates adenylate cyclase, which activates the production of cAMP and the expression of IGN regulatory genes (De Vadder *et al.*, 2015). It is noteworthy that, owing to this neural-mediated relay, IGN gene expression is activated first in the distal gut but also along the whole intestine, even if the initial propionate signal is supposed to start from the colon where the bulk of microbiota resides (De Vadder *et al.*, 2014). Secondly, propionate serves as a glucose precursor for IGN, entering the enterocyte and the enterocyte metabolism via the formation of propionyl-CoA. The latter is converted to methylmalonyl-CoA by propionyl-CoA carboxylase, and the methylmalonyl-CoA is then converted to succinyl-CoA by methylmalonyl-CoA mutase (MUT). Succinyl-CoA enters the Krebs cycle to be finally converted to oxaloacetate and glucose via the IGN pathway (De Vadder *et al.*, 2014).

It is noteworthy that these studies have extended the notion of 'gut-brain' axis, classically ascribed until now to gastrointestinal hormones transported to the brain via the blood circulation. Indeed, fibre-derived signalling metabolites (propionate then glucose) are both sensed by the gastrointestinal nervous system locally, e.g. in the portal vein walls, and the signal regulating the hypothalamic homeostatic function is nervous in nature and peripheral in origin. Moreover, inactivating the gastrointestinal sensitive nerves by the neurotoxic agent capsaicin suppresses the metabolic benefits deriving from fibre, propionate or glucose (De Vadder *et al.*, 2014; Soty *et al.*, 2017). Similarly, the beneficial effects of the aforementioned nutrients do not take place in mice with deficient IGN (De Vadder *et al.*, 2014; Soty *et al.*, 2017).

Of note, the metabolic benefits conferred on the host by butyrate and propionate, *via* IGN, were deciphered in rat and mice studies. It is noteworthy that, while they are not expressed in the rat colon under normal feeding conditions, the regulatory genes of IGN are rapidly induced upon fibre feeding, via the action of propionate and butyrate (De Vadder *et al.*, 2014). Interestingly, IGN has since been demonstrated in humans under conditions known to be associated with dramatic metabolic benefits, such as after, gastric bypass surgery to treat obesity. The beneficial metabolic outcomes of the latter are very similar as those initiated by dietary fibre (Gutierrez-Repiso *et al.*, 2017; Hayes *et al.*, 2011; Mithieux, 2012). Moreover, it was reported that the outcomes of gastric bypass in obese humans were better when the starting IGN of the individual was high (Gutierrez-Repiso *et al.*, 2017). It was determined that around 6% of luminal propionate is converted to glucose from a tracer study (Boets *et al.*, 2017). Since SCFA concentrations may reach concentrations around hundreds mM in the colon lumen, this represents a huge

flux of propionate to the body glucose pool, while it cannot be estimated from such studies whether the conversion takes place in the intestine or the liver. Interestingly, the importance of normal metabolism by the intestinal mucosa of propionate deriving from gut microbial fermentation was emphasised in patients with MUT deficiency (methylmalonic aciduria), in which methylmalonyl-CoA accumulates and exerts deleterious effects. Indeed, the virtual elimination of gut microbial propionate production by antibiotic treatment decreased methylmalonate production, which substantially ameliorated health in these patients. Specifically, they retrieved appetite and increased food intake, while vomiting was virtually suppressed (Bain *et al.*, 1988).

Finally, these data suggest that IGN and portal to brain glucose signalling could be a key mediator of the metabolic benefits deriving from SCFA (Figure 4). Furthermore, they suggest that a normal propionate metabolism in the colonic mucosa is a requirement for health.

6. Discussion

There is mechanistic evidence that SCFA contribute to maintaining the intestinal barrier function and seem to benefit pathological conditions of intestinal inflammation, may prevent weight gain and may have positive effects on glucose homeostasis and insulin sensitivity. Nevertheless, human evidence is more limited and mixed. The question whether an increased microbial SCFA production can be seen as a health benefit as such is addressed in this review. The available evidence will be summarised below, followed by a conclusion under what conditions SCFA can be regarded as a health benefit, addressing the gaps in research and ending with perspectives for future research. This manuscript is the result of the work of an expert group of ILSI Europe's Prebiotics task force. The drafted manuscript, its discussion and conclusions have been refined based on a workshop with participation of scientific experts, industrial representatives as well as policy makers.

SCFA contribute to maintaining the intestinal barrier function through mechanisms that include stimulation of mucus synthesis and quality, and the synthesis of antimicrobial peptides as well as reducing epithelial permeability targeting tight junction complex integrity. Both luminal administration and endogenous stimulation of butyrate production through microbial fermentation of dietary fibres seem to benefit pathological conditions of intestinal inflammation. In human, interventions that modulate the microbiota and presumably also SCFA production yielded mixed results with respect to effects on gut permeability, as well as mucosal immunity and inflammation (Jain *et al.*, 2004; Olguin *et al.*, 2005; Pedersen *et al.*, 2016; Russo *et al.*, 2012a; Westerbeek *et al.*, 2011; Wilms *et al.*, 2016).

Based on the available data and our systemic review of literature, SCFA supplementation appears to prevent weight gain associated with a high HFD in rodents with limited human evidence. With respect to energy intake the balance of available data indicates that the route of SCFA administration may be of importance. Oral SCFA have consistently been shown not to reduce energy intake, which may be related to the absorption of SCFA in the upper GI tract. Targeted delivery to the lower gut may reduce energy intake, which may be related to anorectic gut hormone release and/or indirect neural gut-brain signalling via GPR41 and 43 receptors. There is also human evidence that SCFA administration may affect energy expenditure and fat oxidation. Here, oral propionate (Chambers *et al.*, 2018a) as well as distal colonic SCFA administration (Canfora *et al.*, 2017b) have been shown to increase energy expenditure and fat oxidation in acute settings during overnight fasted conditions.

Human data on effects of acute SCFA administration on metabolic health and glucose homeostasis show that SCFA affect whole lipolysis and hormones like PYY (Canfora *et al.*, 2017b; Van der Beek *et al.*, 2016). There are limited studies investigating the effect of longer term SCFA administration on glucose homeostasis and insulin sensitivity. Oral butyrate administration for 4 weeks improved hepatic and peripheral insulin sensitivity in lean but not in metabolically compromised individuals (Bouter *et al.*, 2018). Additionally, based on the totality of available data from *in vitro/ex vivo*, animal and limited human studies, there is mechanistic evidence supporting beneficial effects of SCFA generated from fermentation processes by the gut microbiota following prebiotic consumption on modulating and ameliorating low-grade inflammation.

Overall, human studies for health effects of SCFA are limited and *in vitro* and animal data do often not directly translate into the human condition. Furthermore, human studies are hampered by difficulties in measuring SCFA production, by differences in mode of administration and/or site of production, by variation in dietary fibres tested as well as diet composition and by variation in metabolic phenotype between individuals. Those factors will be addressed more extensively below.

In many studies, faecal SCFA are used as a biomarker of gut-derived SCFA production. However, concentrations of both faecal and circulating SCFA are the net result of bacterial production, bacterial cross-feeding on different SCFA, and colonocyte absorption. The body rapidly and almost completely absorbs gut microbially produced SCFA. Consequently, faecal measurements are not representative of *in vivo* colonic production, which is influenced by prebiotics or other colonic substrates, colonic pH and microbiota composition. Accurate knowledge of *in vivo* production and absorption kinetics of (individual) SCFA is challenging;

stable isotope technology is the superior non-invasive methodology to measure SCFA production in humans; however, this is not suitable for routine use. Nevertheless, this technology has been applied to prebiotic inulin which conclusively demonstrated increased SCFA production within the human colon (Boets *et al.*, 2015). Faecal SCFA concentrations are thus a balance of production, utilisation and absorption. Whilst the increases that are observed in human studies supplying more fermentable fibres to the complex microbial ecosystem indicates that faecal samples may be used as a proxy for increased SCFA production, care should be taken interpreting such data.

From an increasing number of studies it is evident that the mode of administration or site of fermentation is an important determinant of the metabolic response. Interestingly, as indicated above, acute human studies demonstrated that infusions of SCFA in the distal, but not proximal, colon modulated whole-body substrate metabolism, with an increased lipid oxidation and PYY concentration, and attenuated lipolysis (Van der Beek *et al.*, 2016). Therefore, an interesting future approach might be to increase the availability of dietary fibre in the more distal colonic site. Of note, an increased saccharolytic fermentation in the distal colon may also decrease microbial products derived from proteolytic fermentation (Hald *et al.*, 2016), such as the branched SCFA isobutyrate and isovalerate, ammonia and hydrogen sulphide. These products are far less studied, but high concentrations in the colon and systemic circulation of these products are related to adverse effect on gut and metabolic health (Blachier *et al.*, 2019; Canfora *et al.*, 2019; Da Silva *et al.*, 2018; Russell *et al.*, 2011). Additionally, targeted delivery of SCFA to the lower gut may impact energy intake, whilst oral administration has no such effect. Also, health effects may depend on the type of SCFA. Whilst gastro-intestinal health including mucosal immunity, immunity and integrity has been merely related to butyrate, in metabolic health, including body weight regulation and glucose homeostasis, also effects of propionate and acetate have been described in addition to those of butyrate.

Some of the conclusions with respect to SCFA come from prebiotic or dietary fibre studies in which (indicators of) SCFA production or microbial SCFA producers have been measured. The data from those studies are inconsistent which may relate to the characteristics of those fibres, including the site of fermentation, their fermentation type as well as the amount and type of SCFA produced. Notably, one study showed that fermentable oligosaccharides increased the abundance of bifidobacteria, but decreased the abundance of several other butyrate-producing bacteria, and even resulted in adverse effects on glucose homeostasis (Liu *et al.*, 2017). Also, other studies using one specific fibre did not show very pronounced metabolic effects as reviewed by Canfora *et al.* (2019). Although some studies using specific prebiotic supplements showed promising metabolic effects (Dehghan *et al.*, 2016; Dehghan *et al.*, 2013; Rao *et al.*, 2019), the message may be that it is important to keep the diversity of SCFA-producing bacteria high by providing different microbial substrates instead of stimulating one specific SCFA producing genus (Le Bourgot *et al.*, 2018; Rao *et al.*, 2019).

Besides that, SCFA intervention may not be similarly effective in all individuals. As indicated above, it has been shown that oral butyrate intervention for 4 wk improved insulin sensitivity in lean but not in obese individuals (Bouter *et al.*, 2018). Additionally, acetate infusion led to a greater FFA rebound in normal than in hyperinsulinemic individuals (Fernandes *et al.*, 2012). Thus, one size does not fit all and it may be the case that in metabolically compromised individuals, longer periods of intervention are required (>3 months) to induce physiologically relevant improvements.

7. Conclusions and perspectives

In conclusion, the available mechanistic data and limited human data on the metabolic consequences of elevated gut-derived SCFA production strongly suggest that increasing SCFA production could be a valuable strategy in the preventing gastro-intestinal dysfunction, obesity and type 2 diabetes mellitus. Nevertheless, there is an urgent need for well controlled longer term human SCFA intervention studies investigating effects on intestinal inflammation, body weight control, glucose homeostasis and insulin sensitivity as well as other parameters of metabolic health, taking the following factors into account:

More information on actual SCFA fluxes and kinetic studies on SCFA metabolism by means of stable isotope methodology, taken into account type of SCFA and site of production. Further, more information is needed on the transport of SCFA into the body as well as small and large intestinal SCFA handling.

There may be responders and non-responders to intervention, depending on initial microbial and/or metabolic profile. Non-responders to intervention may possibly need an intervention of longer duration.

In dietary fibre studies, the type of fibre(s) and site of fermentation, i.e. site of SCFA production may determine the outcome on human health and metabolism.

Controlling for factors that could shape the microbial composition and might directly affect host substrate and energy metabolism is also very important. Such factors include among other factors diet and physical activity, medications, as well as changes in gut transit time.

Combining *in vivo* gold-standard clinical techniques with advanced metabolomics, metatranscriptomics and metagenomics approaches will help to further determine the relationship between the microbiome and host gastrointestinal and metabolic health.

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