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TOPICAL REVIEW

Potential mediators linking gut bacteria to metabolic health: a critical view

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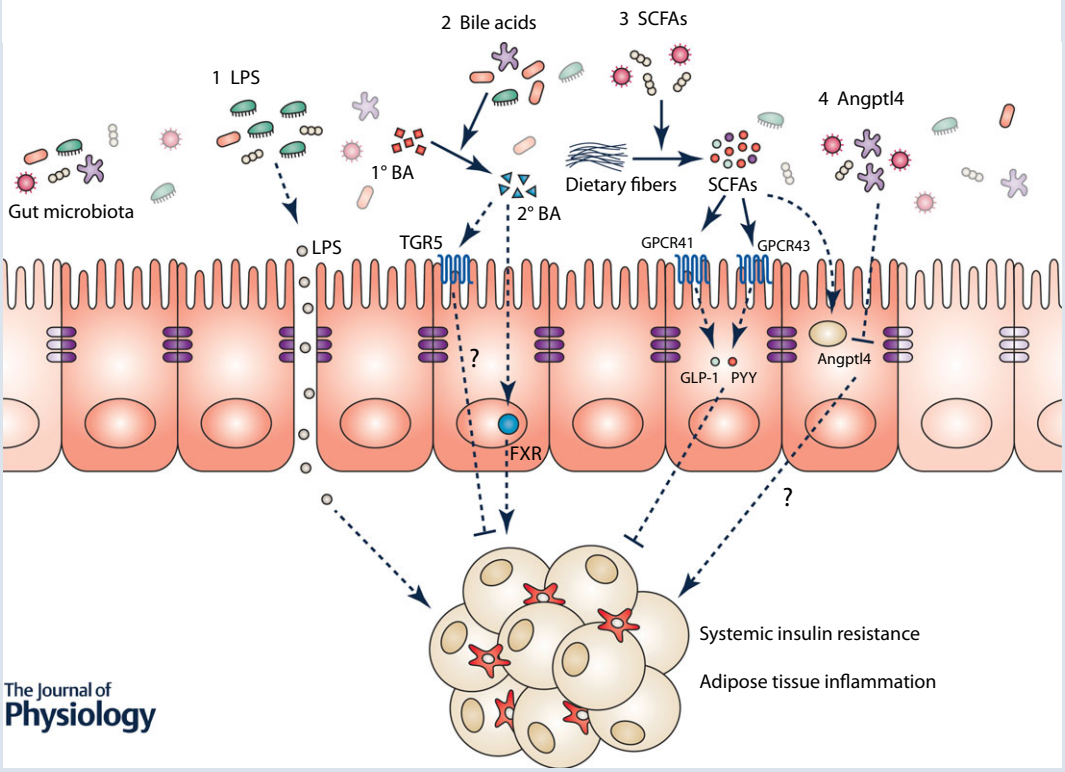

Abstract Growing evidence suggests that the bacteria present in our gut may play a role in mediating the effect of genetics and lifestyle on obesity and metabolic diseases. Most of the current literature on gut bacteria consists of cross-sectional and correlative studies, rendering it difficult to make any causal inferences as to the influence of gut bacteria on obesity and related metabolic disorders. Interventions with germ-free animals, treatment with antibiotic agents, and

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microbial transfer experiments have provided some evidence that disturbances in gut bacteria may causally contribute to obesity-related insulin resistance and adipose tissue inflammation. Several potential mediators have been hypothesized to link the activity and composition of gut bacteria to insulin resistance and adipose tissue function, including lipopolysaccharide, angiopoietin-like protein 4, bile acids and short-chain fatty acids. In this review we critically evaluate the current evidence related to the direct role of gut bacteria in obesity-related metabolic perturbations, with a focus on insulin resistance and adipose tissue inflammation. It is concluded that the knowledge base in support of a role for the gut microbiota in metabolic regulation and in particular insulin resistance and adipose tissue inflammation needs to be strengthened.

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Abstract figure legend Hypothetical model of gut microbial participation in adipose tissue inflammation and insulin resistance. Several mediators are hypothesized to link changes in gut bacterial composition to adipose tissue inflammation and insulin resistance. (1) Disturbances in gut microbial composition may disrupt the gut barrier thereby causing leakage of lipopolysaccharides (LPS) into the bloodstream. Low levels of LPS in the bloodstream can cause adipose tissue inflammation and insulin resistance. (2) Alterations in gut microbiota and subsequent changes in the conversion of primary to secondary bile acids may alter farnesoid X receptor (FXR) signalling and impact adipose tissue inflammation and insulin resistance. Whether alterations in gut microbiota influence adipose tissue inflammation and insulin resistance via bile acid-mediated G protein-coupled bile acid receptor 1 (TGR5) signalling is completely unknown. (3) Microbial fermentation of dietary fibres generates short-chain fatty acids (SCFAs). By activating G protein-coupled receptors (GPCR) 41 and 43, SCFAs induce secretion of glucagon-like peptide-1 (GLP-1) and peptide YY (PYY), which may inhibit adipose tissue inflammation and insulin resistance. (4) Inhibition of angiopoietin-like protein 4 (ANGPTL4) by the gut microbiota may promote adiposity, but whether regulation of (intestinal) ANGPTL4 by the gut microbiota affects adipose tissue inflammation and insulin resistance is completely unknown. Dashed arrows indicate putative pathways for which more research is needed to investigate the direct impact of microbiota on adipose tissue inflammation and insulin resistance.

Abbreviations ANGPTL4, angiopoietin-like protein 4; FXR, farnesoid X receptor; GLP-1, glucagon-like peptide-1; GPCR, G protein-coupled receptor; LPS, lipopolysaccharide; PYY, peptide YY; SCFA, short-chain fatty acid; TGR5, G protein-coupled bile acid receptor 1.

Introduction

Obesity is associated with a variety of metabolic complications including dyslipidaemia and insulin resistance. Evidence is accumulating that the development of insulin resistance and other complications of obesity may be driven by a heightened inflammatory state of the adipose tissue. Elevated adipose tissue inflammation is characterized by the influx of various immune cells, including macrophages, and the upregulation of numerous inflammatory cytokines (Boutens & Stienstra, 2016).

Obesity and related metabolic diseases are believed to be the result of an interaction between genetics and lifestyle factors, such as diet and physical activity. In the last decade, there has been growing realization that part of the effect of genetics and lifestyle on obesity and metabolic diseases may be mediated by the microorganisms residing in our gastrointestinal tract, which are referred to as the gut microbiota (Janssen & Kersten, 2015; Wu *et al.* 2015). Indeed, changes in gut microbiota composition have been observed in people with obesity (Ley *et al.* 2006; Schwiertz *et al.* 2010), and obesity-related diseases such as type 2 diabetes (Qin *et al.* 2012), non-alcoholic fatty liver disease (Mouzaki *et al.* 2013; Jiang *et al.* 2015) and cardiovascular disease (Karlsson *et al.* 2012).

The gut microbiota predominantly consists of bacteria but also includes viruses, fungi, protozoa, and archaea. The composition of the gut microbiota varies greatly between individuals, reflecting the impact of the host genome and environmental factors, such as lifestyle, hygiene, the use of antibiotics and especially diet. In addition, the intestinal microbiota composition may be affected by specific disease states (Benson *et al.* 2010; Sommer & Bäckhed, 2013). Conversely, the intestinal microbiota may impact the host and contribute to certain diseases (Rabot *et al.* 2010; Ridaura *et al.* 2013; Gregory *et al.* 2015; Schaubeck *et al.* 2016). Most of the contemporary literature on the relation between the composition of the gut microbiota and obesity and related parameters consists of cross-sectional and correlative studies. Because of the complicated interaction between the host, environmental factors and the gut microbiota, no causal inferences can be drawn from these studies about the influence of gut bacteria on obesity

and obesity-related disturbances, which represents a major limitation. In this review we discuss the current evidence related to the direct role of gut bacteria in obesity-related metabolic perturbations, with a focus on insulin resistance and adipose tissue inflammation. In the first part, we describe the results of various interventions that addressed the causal role of gut bacteria in metabolic diseases. In the second part, we describe mechanistic studies on potential mediators that may link changes in gut bacteria to obesity-related insulin resistance and adipose tissue inflammation.

Possible interventions to study the role of gut bacteria in metabolic diseases

To investigate whether the gut bacteria contribute to insulin resistance and adipose tissue inflammation, interventions with germ-free animals, treatment with antibiotic agents, and bacterial transfer experiments are conducted. Germ-free animals are maintained free from any microorganisms throughout life and are therefore useful to elucidate the role of the gut microbiota in metabolic disorders. Compared to conventionalized mice, germ-free mice fed a high-fat diet were found to have an improved glucose tolerance, improved insulin sensitivity, and reduced adipose tissue inflammation (Bäckhed *et al.* 2004; Rabot *et al.* 2010; Caesar *et al.* 2012). However, it is unclear whether the elevated insulin resistance and adipose tissue inflammation in conventionalized mice as compared to germ-free mice are directly mediated by the gut microbiota, or indirectly via a higher body weight gain. To circumvent the effects of body weight and adiposity on adipose tissue inflammation, Caesar *et al.* (2015) compared germ-free and body weight-matched conventionally raised mice. Interestingly, adipose tissue inflammation was improved in germ-free mice as compared with conventionally raised mice. While this study thus suggests that the gut microbiota promote adipose tissue inflammation, it is important to realize that interventions with germ-free mice have certain limitations. Indeed, germ-free mice have large defects in the development of the immune system and antibody production, show morphological defects in the intestine, and may suffer from a vitamin deficiency, which may significantly affect the experimental outcome (Smith *et al.* 2007; Yi & Li, 2012).

To overcome these limitations, administration of antibiotics and microbial transfer are a popular alternative to modulate gut bacterial composition. Antibiotics suppress the gut bacteria (Cani *et al.* 2008; Carvalho *et al.* 2012). Similar to the observations in germ-free mice, treatment with antibiotics improved the glucose tolerance and reduced the infiltration of macrophages in adipose tissue in mice (Cani *et al.* 2008; Membrez *et al.* 2008; Carvalho *et al.* 2012). In contrast, in humans, administration of a cocktail of antibiotics for 4 days had no effect on glucose tolerance (Mikkelsen *et al.* 2015), while treatment with vancomycin for 1 week decreased the peripheral insulin sensitivity (Vrieze *et al.* 2014). One disadvantage of antibiotics is that they do not suppress all gut bacteria, which might result in the overgrowth of non-targeted bacteria (Walsh *et al.* 2014; Morgun *et al.* 2015), or outgrowth of intestinal fungi (Mulligan *et al.* 1982; Dollive *et al.* 2013). Furthermore, it is important to note that antibiotics possess direct anti-inflammatory properties and may cause mitochondrial dysfunction, independent of their bactericidal or bacteriostatic effects (Tauber & Nau, 2008; Steel *et al.* 2012; Wang *et al.* 2015).

Microbial transfer can be achieved by oral gavage or intrarectal administration of bacteria, or by co-housing animals. Transfer of microbiota from obesity-prone – but not from obesity-resistant – mice to germ-free mice increased weight gain, increased homeostasis model assessment of insulin resistance (HOMA-IR), and stimulated the infiltration of macrophages in adipose tissue (Duca *et al.* 2014). Another study showed that mice receiving microbiota from obese mice at weaning had an improved glucose tolerance as compared with mice receiving microbiota from lean mice, but not when the microbial transfer was performed at 8 weeks of age (Ellekilde *et al.* 2014). The role of the gut microbiota in human obesity and insulin resistance has been investigated by transplanting faecal microbiota from female adult twins discordant in obesity to germ-free mice. While an increase in obesity and adiposity was observed in mice receiving the gut microbiota from the obese twin as compared with mice receiving the gut microbiota from the lean twin, only a trend towards impaired glucose tolerance ($P = 0.06$) was observed (Ridaura *et al.* 2013). Interestingly, the transplantation of gut microbiota from lean individuals to patients with metabolic syndrome improved insulin sensitivity (Vrieze *et al.* 2012). Of importance, the effectiveness of bacterial transfer depends on whether bacteria are able to colonize in the recipient's intestinal microbial niche.

Microbial transfer can also be achieved by co-housing, which is exclusively applied in animals. Importantly, cohousing of mice with different genotypes will equalize intestinal bacterial composition in the co-housed animals. Therefore, only if the gut microbiota have profound effects on the phenotype might differences between the two co-housed groups yield significant results (Laukens *et al.* 2016).

The interventions mentioned above are commonly used to investigate the role of the gut bacteria in metabolic health. However, these interventions cannot discriminate between pathogenic and beneficial bacteria. Bacteria also confer health benefits related to, for example, vitamin synthesis, tissue homeostasis and immune function (Smith *et al.* 2007; Sommer & Bäckhed, 2013). As a result,

the loss of beneficial bacteria can have detrimental effects on the host. A more targeted approach to modulate the intestinal bacteria, for example by administrating specific bacterial species, is expected to give more specific insight into which and how bacteria can affect the health of the host. For instance, administration of *Escherichia coli* to germ-free mice has been shown to impair glucose tolerance (Caesar *et al.* 2012), whereas administration of *Akkermansia muciniphila* to conventional mice was shown to improve glucose tolerance (Everard *et al.* 2013). To successfully administer bacterial species, bacteria need to remain viable during storage, survive the passage through the gastrointestinal tract, and be able to colonize the intestine. Although the number of bacteria that can be cultured has gone up significantly, still many gastrointestinal bacteria cannot be cultured (Li *et al.* 2014; Rajilić-Stojanović & de Vos, 2014). Hence, due to this limitation, the more targeted approach is only applicable to a selection of bacterial strains.

Overall, current evidence lends some credence to the notion that changes in the gut bacterial composition contribute to insulin resistance and adipose tissue inflammation. However, additional human and animal studies are needed to bolster the causal link between the gut bacteria and obesity-related metabolic parameters.

Potential mediators

Several potential mediators have been hypothesized to link the activity and composition of the gut microbiota to insulin resistance and adipose tissue function, including lipopolysaccharide (LPS), angiopoietin-like protein 4 (Angptl4), bile acids and short-chain fatty acids (SCFAs).

Lipopolysaccharides. LPS or so-called endotoxin is a major component of the gram-negative bacterial cell wall. The first indications that LPS may be involved in obesity and related metabolic disorders were reported by Cani *et al.* (2007). Continuous subcutaneous infusion of LPS for 4 weeks induced weight gain, insulin resistance, and adipose tissue inflammation to a similar extent as high-fat feeding. Importantly, high-fat feeding was found to elevate plasma LPS levels, which was associated with an increased gut permeability and a decreased expression of the tight junction proteins zonula occludens-1 and occludin. Treatment of mice fed a high-fat diet with antibiotics did not increase the gut permeability and plasma LPS levels, suggesting a role for the gut bacteria in this process. In addition to reduced LPS levels, antibiotic-treated mice also displayed less macrophage infiltration in adipose tissue and improved glucose and insulin tolerance (Cani *et al.* 2008). That the suppression of the gut bacteria improves insulin resistance and adipose tissue inflammation concurrent with a reduction in plasma LPS levels has also been found in other studies that used mice fed a high-fat diet (Membrez *et al.* 2008; Carvalho *et al.* 2012), *ob/ob* mice (Cani *et al.* 2008), or germ-free mice (Caesar *et al.* 2012). Interestingly, supplementing *A. muciniphila* during high-fat feeding reduced fat mass gain and improved glucose tolerance in association with reduced serum LPS levels. These findings were not observed with heat-killed *A. muciniphila*, suggesting that live *A. muciniphila* has a profound role in gut barrier function (Everard *et al.* 2013).

It is important to note that not all mouse studies have provided supportive evidence for a role of LPS in insulin resistance (Lichtenstein *et al.* 2010; Caesar et al., 2012, 2015; Laugerette *et al.* 2012). Whereas administration of the gram-negative bacterium *E. coli* W3110 to germ-free mice increased plasma LPS, administration of the isogenic mutant MLK1067 did not. However, both strains increased adipose tissue weight and impaired glucose tolerance, suggesting that gut-derived LPS is not essential for adiposity and glucose and insulin tolerance in mice (Caesar *et al.* 2012). In another study by the same authors, obesity, adipose tissue inflammation and insulin resistance in mice fed a lard-based high-fat diet were not accompanied by a significant elevation in plasma LPS levels as compared with mice fed a fish oil-based high-fat diet (Caesar *et al.* 2015). However, lard-feeding significantly increased toll-like receptor 4 activation (Caesar *et al.* 2015), which might reflect an increased sensitivity of the mice to LPS, a notion that was also raised in another study (Huang *et al.* 2007).

Whereas the original publication by Cani *et al.* (2008) in mice reported a stimulatory effect of LPS on insulin resistance, subsequent studies in rats have failed to find an effect of LPS administration on insulin resistance (Liu *et al.* 2010; Dudele *et al.* 2015). In humans, LPS injection in healthy subjects was shown to induce insulin resistance and adipose tissue inflammation (Dandona *et al.* 2010; Mehta *et al.* 2010). To investigate whether the changes in gut bacterial composition associated with overfeeding and obesity can elevate plasma LPS levels in humans, healthy humans were placed on high-fat/hypercaloric diets in two independent studies. Laugerette *et al.* (2014) reported that although overfeeding for 8 weeks caused marked weight gain, it did not influence the fasting plasma LPS levels. By contrast, Pendyala *et al.* (2012) observed that 4 weeks of Western diet raised the plasma LPS levels. Interestingly, the increased LPS levels were not accompanied by higher serum pro-inflammatory cytokine levels.

Taken together, the current literature on LPS as a potential causal link between disturbances in the gut microbiota and obesity-related insulin resistance and adipose tissue inflammation is inconsistent. One possible explanation for the inconsistent results might be the difficulty of accurately measuring LPS in blood. Most studies have used the FDA-approved Limulus amoebocyte lysate (LAL) assay, which measures LPS activity via an

enzymatic reaction. One drawback of the LAL assay is that several factors in plasma – such as bile salts and lipoproteins – can interact and thereby inactivate LPS, rendering LPS undetectable. In addition, anticoagulants, including EDTA and heparin, might interfere with the LAL assay (Hurley, 1995; Boutagy *et al.* 2016). Another drawback is the risk of contaminations, which may give rise to false-positive results.

Angiopoietin-like protein 4. Angiopoietin-like protein 4 (ANGPTL4) is a ubiquitously expressed glycoprotein that plays an important role in lipid metabolism by inhibiting the activity of the enzyme lipoprotein lipase (Dijk & Kersten, 2014). Lipoprotein lipase catalyses the hydrolysis of circulating triglycerides along the capillary lumen of muscle and fat tissue. Bäckhed *et al.* (2004, 2007) first identified ANGPTL4 as a potential link between the gut microbiota and fat storage. Whereas germ-free mice were protected against diet-induced obesity, germ-free mice lacking the *Angptl4* gene were not. Conventionalization of germ-free mice resulted in the suppression of *Angptl4* expression in the intestines but not in adipose tissue. Consequently, it was suggested that downregulation of intestinal *Angptl4* expression by gut microbiota may promote adipose tissue lipoprotein lipase activity and thereby peripheral fat storage (Bäckhed *et al.* 2004, 2007; El Aidy *et al.* 2013). In contrast, although Fleissner *et al.* similarly observed increased intestinal *Angptl4* expression in germ-free mice, conventional mice were leaner than their germ-free counterparts (Fleissner *et al.* 2010).

Currently, it is still unclear to what extent ANGPTL4 produced in the intestine has an endocrine function and is able to lower LPL activity in adipose tissue. Indeed, there is growing evidence suggesting that ANGPTL4 acts more locally instead of systemically (Dijk & Kersten, 2014). In the intestine, ANGPTL4 may primarily target pancreatic lipase and thereby reduce fat absorption (Mattijssen *et al.* 2014). Consequently, the inhibition of intestinal *Angptl4* upon conventionalization may promotes fat storage via elevated pancreatic lipase activity. In terms of glucose metabolism, while some studies have found an effect of ANGPTL4 overexpression on glucose metabolism (Xu *et al.* 2005; Lichtenstein *et al.* 2007), it remains to be determined whether ANGPTL4 plays an important regulatory role in insulin sensitivity and glucose homeostasis.

In apparent contrast to the finding of reduced intestinal *Angptl4* expression upon conventionalization, specific bacterial species and short-chain fatty acids potently induce ANGPTL4 in colonic cell lines (Are *et al.* 2008; Aronsson *et al.* 2010; Alex *et al.* 2013; Korecka *et al.* 2013). To further investigate the role of intestinal ANGPTL4 as a potential link between the gut microbiota and metabolic health, future studies using intestinal-specific *Angptl4* knockout mice should be performed.

Bile acids. Alterations in the gut bacterial composition have profound consequences for bile acid metabolism. For instance, it is known that the conversion of primary bile acids to secondary bile acids is carried out by the gut bacteria. Besides having an important role in the emulsification and absorption of dietary lipids, bile acids also serve as important signalling molecules that act through the farnesoid X receptor (FXR) and G protein-coupled bile acid receptor 1 (TGR5). By activating FXR and TGR5, bile acids can influence a variety of biological processes including bile acid metabolism, intestinal hormone secretion, inflammation, and lipid, glucose and energy metabolism. Accordingly, disturbances in the gut bacteria are suggested to affect metabolic parameters and pathways via changes in bile acid metabolism (Fiorucci & Distrutti, 2015).

Experiments in germ-free and antibiotic-treated mice (Swann *et al.* 2011; Sayin *et al.* 2013), as well as in humans treated with antibiotics (Vrieze *et al.* 2014), have indicated that the gut bacteria play an important role in the conversion of primary into secondary bile acids. In addition, germ-free and antibiotic-treated mice have an increased bile acid pool, increased biliary bile acid secretion and intestinal reabsorption, and decreased faecal bile acid excretion, indicating that the gut microbiota have a major impact on bile acid homeostasis (Sayin *et al.* 2013; Out *et al.* 2015). In turn, bile acids may impact the gut bacteria via their bactericidal properties, illustrating the complex relationship between the gut bacteria and bile acids (Mikkelsen *et al.* 2016).

The nuclear bile acid receptor FXR is expressed at high levels in the liver and small intestine, which are both tissues characterized by high concentrations of bile acids. Intestinal FXR signalling has been shown to protect against the development of obesity and to improve insulin resistance (Li *et al.* 2013). The impact of the microbiota on FXR signalling has been studied using germ-free and conventionally raised wild-type and FXR knockout mice. While the effect of the gut microbiota on insulin tolerance was found to be dependent on FXR, the influence of the gut microbiota on glucose tolerance was not (Parséus *et al.* 2016). Adipose tissue inflammation was significantly increased upon conventionalization in wild-type mice, but not in FXR-deficient mice, suggesting that the microbiota promote adipose tissue inflammation in an FXR-dependent manner (Parséus *et al.* 2016). Expression of FXR is relatively low in adipose tissue suggesting that effects of bile acids on adipose tissue inflammation are likely to be mediated via intestinal FXR.

The membrane bile acid receptor TGR5 is also mainly expressed in the intestine and has been implicated in glucose metabolism. Specifically, activation of TGR5 by bile acids was shown to improve insulin sensitivity and glucose tolerance via enhanced glucagon-like peptide-1

(GLP-1) secretion, which was blunted in mice lacking TGR5 (Harach *et al.* 2012; Potthoff *et al.* 2013).

Different bile acids are known to have a different potency towards FXR and TGR5 (de Aguiar Vallim *et al.* 2013). Accordingly, it is difficult to predict how changes in the gut bacterial composition and hence faecal bile acid composition affect bile acid signalling and subsequently impact metabolic processes. Moreover, the bile acid composition is substantially different between mice and humans, including differences in the production of the various primary bile acids and their conjugation with glycine in humans *versus* taurine in mice (Chiang, 2013). For this reason, the results obtained in studies on mice cannot easily be extrapolated to humans.

Short-chain fatty acids. SCFAs are the main intestinal bacterial fermentation end products of indigestible dietary components, such as dietary fibres. It has been hypothesized that part of the beneficial health effects of dietary fibres is mediated by SCFAs (Jakobsdottir *et al.* 2013; den Besten *et al.* 2014; Chassaing *et al.* 2015). In line with this notion, supplementation of SCFAs to a high-fat diet protected against diet-induced obesity and improved insulin sensitivity (Gao *et al.* 2009; Lin *et al.* 2012; den Besten *et al.* 2015a). Although these data support a direct impact of SCFA on metabolic health, absorption of orally ingested SCFAs takes place already in the small intestine and not in the caecum or colon. Different hormones with various physiological functions are produced along the gastrointestinal tract (Murphy & Bloom, 2006) and as a result, SCFAs absorbed in the small intestine may give rise to different metabolic effects as compared to SCFA produced by bacterial fermentation and absorbed in the large intestine (den Besten *et al.* 2015b).

The effects of SCFAs on glucose homeostasis are thought to be mediated via the secretion of GLP-1 and peptide YY (PYY) from enteroendocrine L-cells by activation of the G protein-coupled receptors (GPCR) 41 and 43. *In vitro* primary colonic cells lacking either GPCR41 or -43 were shown to secrete less GLP-1 and PYY after SCFA stimulation (Tolhurst *et al.* 2012; Psichas *et al.* 2015). Targeted delivery of the SCFA propionate to the colon increased GLP-1 and PYY in the portal vein in wild-type mice but not in mice lacking GPCR43 (Psichas *et al.* 2015). Additionally, mice lacking GPCR41 and -43 had impaired glucose tolerance (Tolhurst *et al.* 2012; Kimura *et al.* 2013). In humans, rectal propionate infusions have been shown to increase serum glucose levels, consistent with the hypothesis of propionate being a substrate for gluconeogenesis (Wolever *et al.* 1991). By contrast, while targeted delivery of propionate via an inulin-propionate ester effectively increased propionate in the colon and increased the postprandial plasma PYY and GLP-1 concentrations, no acute effects on plasma glucose levels were found (Chambers *et al.* 2015). Interestingly, inulin-propionate supplementation for 24 weeks prevented a deterioration in the glycaemic response in overweight adults (Chambers *et al.* 2015). However, it is unclear whether these effects are directly mediated by propionate impacting gut hormones, or indirectly via the reduction in body weight gain and adiposity in the inulin-propionate group as compared to the control group.

The direct effects of SCFAs on adipose tissue inflammation have been investigated in only a few studies. Since the effects of SCFAs on metabolic health frequently involve changes in obesity development (Canfora *et al.* 2015), which can be predicted to lead to changes in adipose tissue inflammation, the direct effects of SCFAs on adipose tissue inflammation are difficult to assess *in vivo*.

In vitro, SCFAs have been shown to exert anti-inflammatory effects by affecting cytokine production and chemotaxis (Cox, 2009; Maa *et al.* 2010; Liu *et al.* 2012). The effect of SCFAs on inflammation seems to be dependent on the concentration and the type of SCFA as well as on the type of immune cell (Vinolo *et al.* 2009; Bailón *et al.* 2010; Meijer *et al.* 2010). For example, Al-Lahham *et al.* (2012) showed that propionate reduced several inflammatory cytokines and chemokines in human omental adipose tissue explants, which could only be achieved using supraphysiological concentrations (3 mmol l^{-1}).

Taken together, there is some evidence that SCFAs affect insulin resistance and adipose tissue inflammation, but further research is necessary to better clarify the impact of intestinally derived SCFAs on insulin resistance and adipose tissue inflammation *in vivo*.

Conclusion

In conclusion, several potential mediators, including LPS, ANGPTL4, bile acids and SCFAs, have been proposed to link disturbances in the gut bacteria to obesity-related insulin resistance and adipose tissue inflammation. Currently, the literature on LPS as a potential causal link between the gut microbiota and obesity-related insulin resistance and adipose tissue inflammation is inconsistent, which may be related to the difficulty of accurately measuring LPS in blood. Bile acids are also suggested as possible mediators, but evidence from *in vivo* studies is limited. Since the gut bacteria impact bile acid composition and vice versa, it is difficult to investigate the role of bile acids as causal mediators of the gut bacteria. ANGPTL4 has been proposed as a link between the gut microbiota and obesity, but whether regulation of (intestinal) *Angptl4* by the gut microbiota also affects insulin resistance and adipose tissue inflammation is completely unknown. In contrast to LPS, ANGPTL4 and bile acids, SCFAs have been suggested to improve glucose tolerance and reduce adipose tissue inflammation, although further research is necessary to better clarify the

impact of intestinally derived SCFAs on insulin resistance and adipose tissue inflammation *in vivo*.

It should be noted that the observed effects of the gut microbiota and its potential mediators on insulin resistance and adipose inflammation are often confounded by changes in obesity development, which automatically impact insulin resistance and adipose tissue inflammation. Therefore, studies should be designed to better tease out the direct effect of the gut microbiota on insulin resistance and adipose tissue inflammation independent of obesity.

Studies on potential mediators linking disturbances in gut bacteria to insulin resistance and adipose tissue inflammation are mostly performed in mice. Although murine models provide a powerful tool to study host-gut microbe interactions, they do not always very well recapitulate the human situation, partly because the composition of the gut bacteria is quite dissimilar between humans and mice (Ley *et al.* 2005). Pre-clinical studies exploring the role of gut bacteria in obesity and metabolic regulation should therefore ideally be undertaken in a variety of mouse models on different diets. Moreover, as the gut bacterial composition can vary substantially in the same mouse strain depending on the animal facility and background diet, it is worthwhile to try to repeat existing studies in different animal facilities and using different diets. Finally, a more targeted approach involving modification of only one bacterial strain may provide more useful insight into the role of the gut bacteria in obesity and metabolic regulation, as compared to interventions in which nearly the entire bacterial population is modulated.

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Additional information

Competing interests

The authors report no conflict of interest.

Author contributions

All authors have approved the final version of the manuscript and agree to be accountable for all aspects of the work. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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