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Strategies to promote abundance of *Akkermansia muciniphila*, an emerging probiotics in the gut, evidence from dietary intervention studies

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Abstract

Akkermansia muciniphila is a mucin-degrading bacterium commonly found in human gut. *A. muciniphila* has been inversely associated with obesity, diabetes, inflammation, and metabolic disorders. Due to its highly promising probiotic activities against obesity and diabetes, *A. muciniphila* has drawn intensive interest for research and development in recent years. A number of human and animal studies have shown that the abundance of *A. muciniphila* in the gut can be enhanced through dietary interventions. The present review focuses on evidence-based dietary strategies of improving *A. muciniphila* abundance in the gut by critically appraising up-to-date available human and animal intervention studies on *A. muciniphila* growth and their impact on risk factors of obesity and diabetes. Their potential mechanisms in promoting *A. muciniphila* are also discussed along with the discussions of mechanism of action for *A. muciniphila* to exert probiotic functions.

Keywords

Probiotics; *Akkermansia muciniphila*; Dietary supplementation; Gut microbiota; Obesity; Diabetes

1. Introduction

Akkermansia muciniphila is a mucin-degrading bacterium belonging to the phylum *Verrucomicrobia* (Derrien, Collado, Ben-Amor, Salminen, & de Vos, 2008). *A. muciniphila* was first isolated and identified when using purified mucin as the only carbon source in the growing medium in a belief that specific gut microbes have ability to utilize the mucus glycans as carbon sources (Derrien, Vaughan, Plugge, & de Vos, 2004). *A. muciniphila* is commonly found in human gut, representing 3–5% of the microbial community in humans (Belzer & de Vos, 2012; Derrien et al., 2004). The bacterium is also found in a wide variety of other species partly due to its mucin-degrading capability which provides an ecological advantage, especially in a condition of lacking other dietary sources except mucin (as the only constant source of nutrients) (Lukovac et al., 2014). Since its discovery in 2004 by Derrien et al. (2004), *A. muciniphila* has quickly become a popular research topic due to its newly discovered probiotic properties (Derrien et al., 2008; Dingemanse et al., 2015; van Passel et al., 2011). The bacterium is more abundant in the gut of healthy subjects than in that of diabetic and obese patients (Karlsson et al., 2012; Santacruz et al., 2010; Tilg & Moschen, 2014) and patients with bowel diseases (Png et al., 2010) and metabolic disorders

(Brahe et al., 2015; Collado, Isolauri, Laitinen, & Salminen, 2010). Recent intervention studies also confirmed an inverse correlation of *A. muciniphila* abundance with body weight (Everard et al., 2013; Shin et al., 2014), inflammation (Hansen et al., 2014), metabolic syndrome (Roopchand et al., 2015), and both type 1 diabetes (Hansen et al., 2012a, 2012b) and type 2 diabetes (Hansen et al., 2012a, 2012b; Shin et al., 2014). Collectively, the increasing body of evidence from animal and human studies suggest that *A. muciniphila* is a highly promising probiotic, especially its potential for the prevention and treatment of diabetes, obesity, and their associated metabolic disorders, which is of great interest for future research and development.

The exact mechanisms by which *A. muciniphila* exerts the beneficial impact on health have not been fully elucidated. The positive modulation of mucus thickness and gut barrier integrity by *A. muciniphila* could be the key for its aforementioned probiotic activities. *A. muciniphila* supplementation was able to restore mucus thickness in obese and type 2 diabetic mice where gut mucus was disrupted by high fat diet treatment; the treatment also resulted in a significant reduction of serum lipopolysaccharides (LPS), a metabolic endotoxemia, and improved the metabolic profile (Everard et al., 2013). LPS is a major component of the outer membrane of gut gram-negative bacteria and its presence in circulation often indicates gut permeability, thus a disruption of intestinal mucus (Turner, 2009). Intestinal mucus is synthesized and secreted by the goblet cells consisting of two layers: an inner unstirred layer devoid of bacteria and a thicker multi-laminated outer layer with commensal bacteria (Johansson et al., 2008). Its major components, mucins which contain approximately 20% protein and 80% carbohydrates are a source of nutrients for *A. muciniphila* (Ambort et al., 2012). The intestinal adherent mucus gel layers in humans has a thickness from around 200 μM up to 800–900 μM depending partly on the sites (Derrien et al., 2010; Pullan et al., 1994). The mucus creates a protective barrier that prevents noxious agents, destructive hydrolases, and microorganisms from directly contacting the epithelial cell layer (Pullan et al., 1994; Turner, 2009). Mucin has a turnover rate of 6–12 h with the inner layer at a rate of about 1 h and its secretion is believed to be regulated by neural, hormonal, and paracrine effects (Ambort et al., 2012; Linden, Sutton, Karlsson, Korolik, & McGuckin, 2008; Plaisancié et al., 1998). Recent studies showed that *A. muciniphila*, despite its utilization of mucin as a source of nutrients, actually were positively associated with the mucus thickness and intestinal barrier integrity in humans and animals (Collado, Derrien, Isolauri, de Vos, & Salminen, 2007; Everard et al., 2013; Ganesh, 2013; Zhong, 2015). How *A. muciniphila* could promote mucus thickness is not known. One of the reasons could be *A. muciniphila* stimulates mucus turnover rate by making short-chain fatty acids from the degraded mucin, the preferable energy sources for the host epithelium which synthesize and secret mucin. Indeed, *A. muciniphila* supplementation increased the number of mucin-producing goblet cells in mice (Shin et al., 2014).

Research has shown that the abundance of *A. muciniphila* was 3300-fold lower in leptin-deficient obese (ob/ob) mice than in their lean littermates (Everard et al., 2013). The bacterium has also been negatively associated with energy extraction from diet in cold condition and caloric restriction (Chevalier et al., 2015; Dao et al., 2016). The beneficial functions of *A. muciniphila* and its potential mechanisms have recently discussed by Derrien, Belzer, and de Vos (2016). Here we thoroughly examined all up-to-date available

intervention studies on *A. muciniphila* in attempting to identify evidence and practical dietary strategies to promote the growth of *A. muciniphila*, an emerging probiotic in the gut.

2. Supplementation with viable *A. muciniphila*: effective and consistent from animal studies

Despite the factor that *A. muciniphila* has been discovered for more than 10 years, there was no human study available on direct dietary supplementation with *A. muciniphila.* Nevertheless, three mice intervention studies have been reported. All of them showed a significant increase of *A. muciniphila* in the gut and/or feces of the recipient mice (two different mice models) (Everard et al., 2013; Li, Lin, Vanhoutte, Woo, & Xu, 2016; Shin et al., 2014). A Western high-fat diet on Apoe−/−mice for 8 weeks reduced the abundance of *A. muciniphila* from around 7.0×10^9/g feces to 4.6×10^9/g feces, an over 100-fold decrease. While, supplementation with *A. muciniphila* (5×10^9 cfu for 8 weeks) on the Western diet was able to restore the abundance of *A. muciniphila* back to 8.0×10^9/g feces (Li et al., 2016). Interestingly, supplementation of *A. muciniphila* did not significantly change in the composition of gut microbiota (except the bacterium itself). The *A. muciniphila* supplementation reduced the size of atherosclerotic plaques and ameliorated both aortic and systemic inflammation in Western diet-fed Apoe−/−mice (Li et al., 2016). The other two studies used diet-induced obese (Power et al.) mice. Oral treatment of *A. muciniphila* (2×10^8 cfu for 4 weeks) increased *A. muciniphila* abundance in the fecal content of the high-fat-fed DIO mice (from about 10^8/g feces in the high-fat only-fed group to 10^{10}/g feces in the treatment group) (Everard et al., 2013). Again, the high-fat diet significantly changed the gut microbiota whereas *A. muciniphila* treatment did not modify this profile (Everard et al., 2013). Another study also found that oral supplementation of *A. muciniphila* (4.0×10^8 cfu/d for 6 weeks) was able to restore its abundance in high-fat diet-fed DIO Mice, which was significantly reduced after 4 weeks of HFD (Shin et al., 2014). Collectively, all three studies showed that Western high-fat diet in as short as 4 weeks were able to significantly reduce the abundance of *A. muciniphila* and supplementation of viable *A. muciniphila* is able to restore its abundance back.

Dietary supplementation of *A. muciniphila* did not change microbiota profile (Everard et al., 2013; Li et al., 2016), suggesting a minimum interaction with other gut bacteria. However, other studies found that abundance of *A. muciniphila* was positively associated with the richness of *Gordonibacter*, *Ruminococcaceae*, and *Methanobrevibacter smithii* (Arumugam et al., 2011; Dao et al., 2016). No further report on their interactions or investigation on possible cross-feeding are available. Interestingly, supplementation of *A. muciniphila* did not increase its abundance in DIO mice on normal control diet (both groups at around 10^{10}/g feces) (Everard et al., 2013), indicating that there could be a up limit for the growth of *A. muciniphila* in the gut and *A. muciniphila* supplementation may not be deliverable for healthy subjects.

These studies also suggest a causal role of intestinal barrier function and LPS in mediating *A. muciniphila* activities (Everard et al., 2013; Li et al., 2016). Bacterial-derived LPS play an essential role in the inflammatory process of inflammatory bowel diseases (IBD) and

other metabolic disorders (Cani et al., 2008; de La Serre et al., 2010; Yu, Flynn, Turner, & Buret, 2005). An increase of serum LPS often suggests an intestinal barrier dysfunction or a leaking gut barrier (Turner, 2009). Thickness of the mucin layer is an important measure of intestinal permeability (Atuma, Strugala, Allen, & Holm, 2001). Research showed that a high-fat-diet (60% fat for 4 weeks) resulted in a 46% thinner mucus layer in DIO mice (Everard et al., 2013). Despite that *A. muciniphila* has been known as a mucin-degrading bacterium (Collado et al., 2007), the bacterium actually increased the thickness of the mucin layer (Li et al., 2016), resulting in a decrease in intestinal permeability and subsequently reduced gut derived LPS penetrating into circulation in Western diet-fed Apoe−/−mice (Li et al., 2016). More interestingly, chronic infusion of LPS was able to complete abolish the benefits of *A. muciniphila* supplementation (the amelioration of inflammation and atherosclerosis), strongly suggesting a causal role of LPS in mediating *A. muciniphila* activities (Li et al., 2016) which could be the result of the reduced intestinal permeability upon *A. muciniphila* supplementation. The LPS-reducing ability (by up to 50% reduction in serum) of *A. muciniphila* supplementation was also confirmed in DIO mice model (Everard et al., 2013; Shin et al., 2014), which could be due to its ability to restore the thickness of the mucin layer and gut barrier function once were damaged by high-fat diet. Although *A. muciniphila* supplementation showed no significant effects on body weight, lipid and glucose metabolism on Apoe−/−mice (Li et al., 2016), it indeed reduced body weight gain, hyperglycemia, insulin resistance on high-fat diet treated DIO mice (Everard et al., 2013; Shin et al., 2014). It is important to note that oral treatment with a lower dose (4.0×10^6 cfu) of viable *A. muciniphila* did not ameliorate the impaired glucose tolerance in high-fat-fed DIO mice, suggesting that there is a dose response limit for *A. muciniphila* exert its beneficial effects (Shin et al., 2014).

3. Supplement with other selected probiotics promoted *A. muciniphila* growth in the gut

There was an animal study found that oral administration of a mixture of *Lactobacillus rhamnosus* LMG S-28148 and *Bifidobacterium animalis* subsp. *lactis* LMG P-28149 for 14 weeks (5 days/week, 5×10^8 CFU of each strain in PBS) increased *A. muciniphila* abundance in the fecal content of the high-fat-fed DIO mice by approximately 100 fold (from $10^{6.5}$/g feces on high-fat-fed group to $10^{8.5}$/g feces in high-fat plus probiotics group) (Alard et al., 2016). In this study, a significant inverse correlation was detected between the body weight gain and the abundance of *A. muciniphila* ($P < 0.001$) which is consistent to other intervention studies (Everard et al., 2013; Shin et al., 2014). The effectiveness of this mix probiotics on *A. muciniphila* abundance is surprisingly comparable to that of the direct *A. muciniphila* supplementation on the same mice model (Everard et al., 2013; Shin et al., 2014). It is likely the strain of *B. animalis* subsp. *lactis* LMG P-28149 not *L. rhamnosus* LMG S-28148 exert such an *A. muciniphila*-promoting effect since the authors further conducted a 7-week feeding study on individual strains (10^9 CFU each) with the same protocol and found that the benefits of the mix probiotics was attributed to *B. animalis* not *L. rhamnosus* (Alard et al., 2016), though the mechanisms are unclear. Interestingly, previous studies showed that *A. muciniphila* supplementation did not change the gut microbiota profile in DIO mice and Apoe−/−mice (Li et al., 2016), suggesting that *A. muciniphila* does

not directly interact with other gut bacteria. The authors also used a dynamic *in vitro* gut model showing that the mix probiotics induced a significant time-dependent increase of total SCFAs in the simulated ascending colon as well as a shift from acetate to butyrate and propionate. These observations suggest that an increase of SCFAs may be a promoting effect for *A. muciniphila* since SCFAs promote mucin growth and *A. muciniphila* is a mucin-degrading bacterium.

4. Supplementation of prebiotics, fructo-oligosaccharides promoted *A. muciniphila* abundance

Although no human study has been reported, three animal studies consistently showed that oral administration of fructo-oligosaccharides (oligofructose or FOS), a common prebiotic, promoted the growth of *A. muciniphila* in the gut of DIO and *ob/ob* mice and Sprague–Dawley rats models (Everard et al., 2011, 2013; Reid, Eller, Nettleton, & Reimer, 2015). A high-fat diet (60% fat for 8 weeks) on DIO mice led to a 100-fold decrease of *A. muciniphila* in feces (from 10^9/g feces on a standard diet to 10^7/g feces on high-fat diet), however, prebiotics supplementation (FOS, 0.3 g/d with the high-fat diet for 8 weeks) completely restored its concentration back to the level comparable to the mice fed with a standard diet (10^9/g feces) (Everard et al., 2013). The prebiotic effect of FOS on *A. muciniphila* was even more significant on *ob/ob* mice, prebiotic supplementation (FOS, 0.3 g/d with a standard diet for 5 weeks) increased the bacterium abundance by approximately 1000-fold (10^7/g feces in the control group versus 10^{10}/g feces in the prebiotic group) (Everard et al., 2013). Another study found that 5 weeks of oral FOS administration at the same dose increased the abundance of *A. muciniphila* in *ob/ob* mice by over 80 folds, along with the higher abundance of *Bifidobacterium* spp. and the *E. rectale/C. coccoides* group (Everard et al., 2011). In a recent study on newborn male Sprague–Dawley rats, FOS supplementation (10 % in diet for 16 weeks) increased *A. muciniphila* abundance by 2–3 folds without affecting food intake and body weight but showing a trend of increasing glucose metabolism (Reid et al., 2015).

It appeared that prebiotic effect on body weight was inconsistent in different animal models (Everard et al., 2011, 2013; Reid et al., 2015). However, in mice studies, FOS supplementation significantly reduced the total fat mass accompanied by a significant reduction in serum LPS level (by over 50%) and a significant improvement in glycemic control (Everard et al., 2011, 2013). Unfortunately, so far only FOS has been evaluated for such an effect and it is not known whether other prebiotics such as inulin, galacto-oligosaccharides (GOS), lactulose, etc., which all possess similar *A. muciniphila*-promoting activities. It is also unclear how FOS could promote the growth of *A. muciniphila in vivo* and the potential mechanisms have not been discussed by the previous authors. There are also currently no published *in vitro* culture data on *A. muciniphila* utilization of prebiotics including FOS. *A. muciniphila* was unable to utilize specific carbon sources such as glucose, cellobiose, lactose, galactose, xylose, fucose, rhamnose, maltose upon *in vitro* incubation (Derrien et al., 2004). However, based on its genome data, *A. muciniphila* appears capable of metabolizing a variety of carbon sources previously found non-utilizable including galactose, cellobiose, melibiose and fructose (Derrien et al., 2004; van Passel et al., 2011).

Our preliminary *in vitro* culture experiments showed that, among 16 different carbon sources including prebiotics and dietary fibers (FOS, xylooligosaccharide, inulin, galacto-oligosaccharides, isomalto oligosaccharides Karaya gum, potato starch unmodified, methylcellulose fiber, D(+)-raffinose pentahydrate, tragacanth gum, grapefruit pectin, Acacia senegal tummy fiber, beta glucan, psyllium husk, oligo-chitosan, and galactomune), adding FOS into the media significantly promoted *A. muciniphila* growth but other fibers/prebiotics did not exhibited such a strong growth-promoting activity, suggesting FOS may be a preferable nutrient for *A. muciniphila*.

5. FODMAP in diet promoted *A. muciniphila*: two human studies suggest A positive association

'FODMAP' refers to fermentable Oligo-, Di- and Mono-saccharides and Polyols—which includes fructose, lactose, oligosaccharides, polyols, and sugar alcohols (polyols, such as sorbitol, mannitol, xylitol and maltitol) which share some distinct functional properties: poor absorption in the small intestine due to lack of hydrolases (fructans, galactans) or limitation of transport across the epithelium (fructose) and rapid fermentation due to their short-chain nature (lactose and oligosaccharides) as compared with dietary fibers (Gibson & Shepherd, 2005, 2010). The ingestion of FODMAPs could increase rapidly-fermentable carbohydrates and subsequently result in functional gut symptoms (Gibson & Shepherd, 2010). For this reason, low FODMAP diets have been widely applied to treat irritable bowel syndrome (IBS) (Halmos, Power, Shepherd, Gibson, & Muir, 2014). However, there currently no clearly defined cut-off values differentiating high or low FODMAP diets. FODMAPs are usually calculated based on individual foods with fructose and fructans being the most widespread in the diet (Gibson & Shepherd, 2010). Recent human studies showed that FODMAP content in diet might significantly affect *A. muciniphila* abundance (Halmos et al., 2015, 2016). A cross over study on 7 patients with Crohn's disease revealed that the patients receiving 21 days of low (containing 3.05% FODMAP) or typical (''Australian" containing 23.7% FODMAP) had *A. muciniphila* abundance of 3.75 or 5.08 (Log_{10} copies of 16S rRNA gene/g feces), respectively, FODMAP diets with 21-day washout in between (Halmos et al., 2016). It should be noted that the low FODMAP diet also significantly lowered the relative abundance of butyrate-producing *Clostridium cluster XIVa*, however, SCFA, pH and total bacterial abundance remained unaltered (Gibson & Shepherd, 2005).

The same research group also conducted a second dietary FOD-MAP intervention on different subjects in a cross-over design: 27 IBS and 6 healthy subjects whom were randomly allocated one of two 21-day provided diets, differing only in FODMAP content (low 3.05 g/day vs Australian 23.7 g/day), and then crossed over to the other diet with ≥21-day washout period (Halmos et al., 2015). Australian diet (high FODMAP) had the relative abundance of *A. muciniphila* at 0.1% versus 0.02% (low FODMAP diet) of total bacteria, their absolute abundance were 5.46 and 4.29 Log_{10} copies of 16S rRNA gene/g feces, respectively (Halmos et al., 2015). The high FODMAP diet also significantly increased the abundance of butyrate-producing *Clostridium cluster XIVa* (Halmos et al., 2015). However, since the authors pooled the analysis together for all subjects (Halmos et al., 2015), it is not known whether FODMAP diets affected the IBS patients and healthy subjects differently

since this study was not aimed to compare fecal microbiota and biochemical indices in healthy subjects with those who have IBS. Contrast to the same intervention on Crohn patients, for IBS patients and health subjects, the low FODMAP diet was associated with higher fecal pH, greater microbial diversity and reduced total bacterial abundance (by 47%) compared with the Australian diet. Nevertheless, fecal SCFA concentration was unaffected (Halmos et al., 2015). It also should be noted that the same research group found that in the same cross-over study protocol on 30 patients with IBS, a diet low in FODMAP effectively reduced functional gastrointestinal symptoms (Halmos et al., 2014). Collectively, the two human studies found that a positive association of FODMAP in diets with *A. muciniphila* abundance in different patients/health subjects. FODMAP diets contained 1.57 and 5.49 Oligosaccharides, respectively (Halmos et al., 2015, 2016), however, it is unclear whether the change of *A. muciniphila* induced by low versus high FODMAP diets are attributable to their difference in oligosaccharide content. Also, unfortunately, there is no human study available investigating the role of FODMAP diet on *A. muciniphila* abundance in obese/diabetic subjects who are associated with low *A. muciniphila* abundance (Everard et al., 2013; Shin et al., 2014).

6. Evidence from dietary polyphenol: inconsistent results from human and animal studies

Dietary polyphenols are natural antioxidants and many of them such as phenolic acids, flavones, and anthocyanins possess strong antimicrobial activity (Daglia, 2012; Parkar, Stevenson, & Skinner, 2008). Both antioxidant and antimicrobial activities of dietary polyphenols could potentially reshape the gut microbiota ecology because, on the one hand, many gut bacteria such as *A. muciniphila* are obligate anaerobes, which are extremely vulnerable under the attacks of free oxygen radicals (Daglia, 2012); therefore, dietary antioxidants once ingested may help protect those obligate anaerobes and modify gut microbiota by scavenging oxygen radicals (Roopchand et al., 2015). On the other hand, certain dietary polyphenols have antimicrobial activity against specific bacteria while other bacteria could be promoted. For instance, a recent study showed that a black tea or a red wine grape extract (RWGE), both containing complex dietary polyphenol mixtures, significantly promoted growth of *A. muciniphila* in an *in vitro* gut microbial ecosystem, namely simulator of the intestinal microbial ecosystem (SHIME) (Kemperman et al., 2013).

There are five dietary intervention studies available including a human trial investigating possible effect of dietary polyphenols on the growth of *A. muciniphila* in the gut. The results however, were inconsistent (as shown in Table 1): two showed that oral intake of dietary polyphenols promoted *A. muciniphila* abundance, the other three including the human study showed no effects. Specifically, dietary supplementation of cranberry extract and Concord grape polyphenols increased *A. muciniphila* abundance in feces from 2% to over 30% (OTU sequences) and from 6.2% to 49.1% on the high-fat fed DIO mice, respectively (Anhê et al., 2015; Roopchand et al., 2015). Whereas the intake of pomegranate extract, green tea extract, and whole California table grape showed no effect on *A. muciniphila* abundance of healthy humans or DIO mice (Axling et al., 2012; Baldwin et al., 2016; Li et al., 2015). The inconsistent results suggest that the *A. muciniphila*-promoting effects of dietary polyphenols

are highly depended on their chemical nature and sources. It is unclear whether the significant activity of cranberry extract and Concord grape polyphenols can be translated to humans and more importantly what specific polyphenols are responsible for such activities. It should be noted that although green tea powder showed no effect on *A. muciniphila* abundance, a reduction in body weight gain and other metabolic benefits were observed in high-fat fed obese mice (Axling et al., 2012). Also, in the human study, although pomegranate extract did not change *A. muciniphila* abundance in the feces, the bacterium was 33-fold and 47-fold higher in stool samples of the subjects who are able to produce pomegranate metabolite urolithin as compared to non-producers at baseline and after 4 weeks dietary treatment, respectively. This suggested that *A. muciniphila* might play an important role in the breakdown of phenolic compounds in the intestine and specific group of humans may benefit more from polyphenols for promoting *A. muciniphila* (Li et al., 2015). In summary, since dietary polyphenols are so diverse and abundant, a validated *in vitro* SHIME model would be very helpful as a quick tool to screen and detect which polyphenols are effective in promoting *A. muciniphila* growth, then animal and human studies could be followed up for verifying their activities.

7. Metformin consistently increased *A. muciniphila* abundance

Metformin has been used as a first-line drug for treatment of type 2 diabetes (Nauck et al., 2009). Despite its nature as the most popular anti-diabetic drug, the therapeutic mechanisms are not fully understood (Zhou et al., 2001). Interestingly, recent human and animal studies revealed that metformin was able to modulate the gut microbiota and this effect was associated with its anti-inflammatory and anti-obesity as well as its therapeutic efficacy on glucose metabolism (Lee & Ko, 2014; Napolitano et al., 2014; Shin et al., 2014; Zhou et al., 2016). Although no human trails available on *A. muciniphila*, all animal studies consistently showed that metformin significant promoted *A. muciniphila* abundance (as shown in Table 2).

These studies showed that high-fat diet significantly reduced *A. muciniphila* in C57BL/6 J mice. Oral treatment of metformin (100–300 mg/bw) either in drinking water or oral gavage was able to restore its abundance after 4–10 weeks of intervention. Interestingly, metformin treatment had no effect on abundance of control diet fed mice (Shin et al., 2014). The glycemic control and other metabolic disorders associated with diet-induced obesity were significantly improved after metformin treatment. However, it is not known whether the benefits of metformin are mediated through the stimulation of *A. muciniphila.* One of the most significant findings in these studies is that the pretreatment of a combination of antibiotics (carbenicillin, metronidazole, neomycin and vancomycin) on HFD-fed mice before metformin treatment abolished the metformin activity (Shin et al., 2014), which strongly suggesting that the gut bacteria (i.e. *A muciniphila*) play an important role mediating metformin activity. Further, exogenous LPS administration (subcutaneous injection, 50 μg/kg/day for 5 days) nearly completely blocked all these beneficial effects of metformin on glucose metabolism, insulin signaling and redox status in the mice (Zhou et al., 2016), indicating that LPS also plays an essential role in mediating metformin activity. Previous studies also consistently showed that *A. muciniphila* administration significantly reduced serum LPS levels (Everard et al., 2013; Li et al., 2016). There are currently no

reports showing that metformin could directly stimulate mucin production. However, metformin treatment the number of mucin-producing goblet cells increased upon which also increased *A. muciniphila* abundance in mice (Shin et al., 2014). It is likely that metformin promotes *A. muciniphila* resulting in increase of goblet cells because *A. muciniphila* supplementation without metformin also stimulated production of goblet cells in mice (Shin et al., 2014). These findings raise a hypothesis that anti-diabetic activity of metformin is mediated by modulation of gut microbiota, especially the increase of *A muciniphila*, resulting in a reduction in serum LPS levels which in turn reduces inflammation and metabolic disorders. The first evidence that metformin modulated the human gut microbiome profile in diabetes patients was reported by Napolitano et al. (2014), however, *A. muciniphila* was not investigated in this study. Human studies are urgently needed to confirm whether metformin is able to stimulate the abundance of *A. muciniphila* in obese humans and its role in mediating metformin activity.

8. Rhubarb extract promoted *A. muciniphila* abundance

Rhubarb (Da Huang) is a well-known Chinese herbal medicine used as a laxative for treatment of constipation, jaundice, gastrointestinal hemorrhage, and ulcers (Huang, Lu, Shen, Chung, & Ong, 2007; Matsuda et al., 2001). Rhubarb mainly contains anthraquinone derivatives which have been reported with anticancer and hepatoprotective activities (Huang et al., 2007; Zhao, Wang, Zhou, Shan, & Xiao, 2009). A recent paper however showed that Rhubarb extract modified gut microbiota of a standard diet (AIN93M)-fed DIO mice (Neyrinck et al., 2016). Supplementation of Rhubarb extract (0.3% in a standard AIN93M diet for 17 days) increased the relative abundance of *A. muciniphila* to 38.9% of fecal total bacteria (measured by pyrosequencing of 16sRNA gene) in DIO mice (12-wk-old) mice (as compared to 9.4% for mice on the standard diet only). The increase was very remarkable considering the treatment only lasted for 17 days. Coincidentally, the relative abundance of *Firmicutes* was reduced to 24.1% (from 48.7% in control mice) (Neyrinck et al., 2016). Rhubarb extract also improved intestinal homeostasis and alcohol-induced oxidative stress and inflammation in the liver (Neyrinck et al., 2016). It remains to be determined that how Rhubarb extract could induce such a drastic change on gut microbiota especially the increase of *A. muciniphila*. It should be pointed out that the fiber content in the rhubarb extract is very low (in case of this study, its concertation in the diet is 0.08%), making it unlikely to be effective (Neyrinck et al., 2016). Similarly, the polyphenols in rhubarb extract may not contribute to the microbiota-modifying effect due to its low concentration. Therefore, it is likely the anthraquinone derivatives in Rhubarb extract are responsible for modulating mice gut microbiota and increasing *A. muciniphila* abundance. Further studies using purified Rhubarb anthraquinones are necessary to confirm whether anthraquinones are promising dietary components for modulating gut microbiota and promoting *A. muciniphila*.

9. Caloric restriction (CR): inconsistent results from human and animal studies

Caloric restriction (CR) has been known for increasing longevity in mammals and reducing risk of age-associated diseases including cancer, atherosclerosis, and diabetes (Cohen et al.,

2004; Colman et al., 2009; Mattison et al., 2012), though the exact mechanisms are not known. A recent animal study showed that neonatal CD1 mice in undernutrition condition (timed separation of pups from dams: 12 h of separation per day for 11 days) resulted in a major phylum-level shifts in the distal intestinal microbiota, *A. muciniphila* being increased most significantly (by 50-fold) (Preidis et al., 2015). However, a more recent human study showed an opposite effect that CR actually reduced *A. muciniphila* abundance on obese or overweight subjects. The study on 49 human subjects (11 overweight and 38 obese) found that *A. muciniphila* at baseline was inversely related to fasting glucose, waist-to-hip ratio and subcutaneous adipocyte diameter (Dao et al., 2016). However, CR (CR diet with fibers and protein for 6 weeks) resulted in a decrease in *A. muciniphila* abundance in the Akk HI group (*A. muciniphila* abundance above median) and no change in the Akk LOW group though CR significantly improved insulin sensitivity and other clinical parameters in all groups (Dao et al., 2016). Nevertheless, the authors showed that subjects with higher baseline *A. muciniphila* exhibited more significant improvement in clinical parameters after CR, suggesting an interaction between CR and *A. muciniphila* (Dao et al., 2016). There are only two studies available up-to-date and the information is very limited about the impact of CR on gut microbiota and *A. muciniphila*, and their interactions warrants further investigations.

10. Selective antibiotic treatment remarkably promoted *A. muciniphila* abundance in humans and mice

Antibiotic treatment often result in significant change in the bacterial diversity of the gut (Hooper & Gordon, 2001; Manichanh et al., 2010; Pérez-Cobas et al., 2013). Recently, two studies (one on mice and one on humans) showed that antibiotic treatment was able to promote *A. muciniphila* as the most abundant bacterium in the gut (Dubourg et al., 2013; Hansen et al., 2012a, 2012b). Oral treatment of vancomycin on non-obese diabetic (NOD) mice (either from birth until weaning (day 28) or from 8 weeks of age until onset of diabetes) significantly reduced the abundance of once dominated *Firmicutes* and *Bacteroidetes*, promoting *Verrucomicrobia* (all reads within this phylum belonged to *A. muciniphila*) to be the most abundant phylum (>80%) in both groups of vancomycin-treated mice (Hansen et al., 2012a, 2012b). NOD mice spontaneously started to develop insulitis at 3–5 weeks of age (Tisch et al., 1993). Interestingly, vancomycin-treated NOD mice also had lower fasting glucose and cumulative diabetes incidence (73–75%) as compared to untreated NOD mice (93%) (Hansen et al., 2012a, 2012b), suggesting *A. muciniphila* promoted by vancomycin treatment may be involved. In an another study, *Verrucomicrobia* phylotype (*A. muciniphila*) was found unexpectedly in >40% of the total gut microbiota (in feces) from two patients who received a broad-spectrum antibiotic treatment (proportions of 44.9% for patient A with *Coxiella burnetii* vascular infection, receiving combination of doxycycline, hydroxychloroquine, piperacillin/tazobactam and teicoplanin and 84.6% for patients B admitted to the Intensive Care Unit, receiving10-day course of imipenem) (Dubourg et al., 2013). Interestingly, neither patient presented significant gastrointestinal disorders despite such a significant change in gut microbiota (Dubourg et al., 2013). An *in vitro* antibiotic susceptibility test showed that *A. muciniphila* was susceptible to imipenem, piperacillin/tazobactam, and doxycycline but was resistant to vancomycin, metronidazole, and penicillin

G (Dubourg et al., 2013). It is surprising that *A. muciniphila* is susceptible to imipenem but *A. muciniphila* represent over 80% of total microbiota in the stool sample of patient B who received 10-day course of imipenem. Nevertheless, both the studies demonstrated that antibiotic treatment can drastically change gut microbiota, especially *A. muciniphila*.

11. High fat diet and alcohol could reduce abundance of *A. muciniphila*

Previous studies have consistently shown that high-fat diet significantly reduced *A. muciniphila* abundance in different animal models (Anhê et al., 2015; Axling et al., 2012; Everard et al., 2013; Li et al., 2016; Roopchand et al., 2015; Zhou et al., 2016). A treatment of high-fat diet (60% fat) for as short as 8 weeks on DIO mice led to a 100-fold decrease of *A. muciniphila* (Everard et al., 2013). Alcohol intake could also negatively affect *A. muciniphila* (Neyrinck et al., 2016). Acute alcohol administration (30% w/v, 6 g/kg body weight) caused 100-fold decrease in the relative abundance of *A. muciniphila* in fecal bacterial content of DIO mice (reduced from 9.3% (absolute abundance: about 9.8 Log_{10} cell number/g feces) to 3.8% (absolute abundance: about 7.8 Log_{10} cell number/g feces), accompanied by increased inflammation and oxidative stress (Neyrinck et al., 2016). Interestingly, supplementation of ground dietary flaxseed (10% in an AIN-93G basal diet for 3 weeks) caused a 30-fold reduction in *A. muciniphila* abundance in fecal total bacteria of DIO male mice (Power et al., 2016), despite that flaxseed supplementation improved intestinal barrier integrity by promoting colon goblet cell density, mucus production, and cecal short chain fatty acid levels (Power et al., 2016).

12. Summary

We have carefully examined a total of 24 the up-to-date available dietary intervention studies in search for evidence and strategies to increase *A. muciniphila*, a beneficial member of gut microbiota in the gut. Available evidence from animal studies showed that viable *A. muciniphila* or prebiotics (FOS) was able to consistently promote *A. muciniphila* abundance in the gut, suggesting a great potential for future development of dietary intervention approaches using viable bacterium or FOS for increasing gut *A. muciniphila.* Supplementation of *B. animalis* could also increase *A. muciniphila* by producing SCFA and facilitating mucin growth to feed the bacterium. Metformin and antibiotics treatment (vancomycin) also significantly promote *A. muciniphila* abundance in the gut but these strategies are not suitable for general public. Rhubarb extract is promising but more research is needed to confirm its activity and another concern about Rhubarb is that it is not a typical dietary ingredient. Dietary polyphenols are inconsistent, cranberry extract and Concord grape polyphenols are active but green tea and whole grape showed no effect. The inconsistency may be related to their difference in polyphenol profile but to identify the active polyphenols is challenging due to their abundance and diversity in the extract. It should also be noted that to maintain *A. muciniphila* abundance in the gut one may want to avoid high-fat diet and heavy alcohol consumption, though the results were based on the measurement of relative abundance of gut microbials.

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Table 1

Effects of dietary polyphenols on *A. muciniphila* abundance.

Dietary polyphenols	Subjects/Animal models	Treatment	Effects on *A. muciniphila*	Other effects	References
Pomegranate extract	Healthy humans	1 g/d for 4 weeks	No effect	N/A	Li et al. (2015)
Cranberry extract	DIO mice	200 mg/kg bw with a high-fat/high sucrose diet for 8 weeks	Increase relative abundance (operational taxonomic unit sequences) from 2% to over 30% in feces	Prevent increase of LPS and intestinal inflammation, body weight gain, visceral obesity, insulin resistance induced by high-fat/high sucrose diet	Anhê et al. (2015)
Green tea powder	DIO mice	4% in a high-fat diet for 22 weeks	No effect	Reduced the body fat content and hepatic triacylglycerol and cholesterol accumulation	Axling et al. (2012)
Concord grape polyphenols	DIO mice	1% in a high-fat diet for 13 weeks	Increased the relative abundance of *A. muciniphila* in cecal sample: from 6.2 to 49.1% 16 S rRNA sequences; fecal sample: from 7.5 to 54.8% 16 S rRNA sequences	Reduced serum LPS by 81%, inflammation, body weight gain, adiposity, glucose intolerance, increased gut barrier function	Roopchand et al. (2015)
Whole grape	DIO mice	3–5% California table grape in high-fat diet for 11 weeks	No significant effect	Reduced adiposity and markers of hepatic lipogenesis and alters gut microbiota in butter fat-fed mice	Baldwin et al. (2016)

Table 2

Effects of metformin on *A. muciniphila* abundance.

Metformin	Subjects/Animal models	Treatment	Effects on *A. muciniphila*	Other effects	References
Oral gavage	DIO mice	300 mg/kg bw with a high-fat diet for 6 weeks	Increase relative abundance from 0.3% to 2.9% in feces (versus 2.3% in control diet fed mice)	Improved glycemic control, Increased the number of mucin-producing goblet cells, reduced serum LPS; however, pretreatment of antibiotics abolished metformin activity	Shin et al. (2014)
In drinking water	DIO mice	100 mg/kg/d with a high-fat diet for 4 weeks	Restored the reduced abundance in the feces of (no specific data on number/ percentage)	Improved insulin sensitivity & glucose control, reduced serum LPS; exogenous LPS administration abolished all metformin activity	Zhou et al. (2016)
Oral gavage	DIO mice	300 mg/kg bw with a high-fat diet for 10 weeks	Increase relative abundance from 0.7% to 12.4% in feces	Improved metabolic disorders, increased the relative abundance of the phylum *Bacteroidetes*	Lee and Ko (2014)