European Review for Medical and Pharmacological Sciences 2019; 23: 8075-8083

Akkermansia muciniphila: key player in metabolic and gastrointestinal disorders

I.G. MACCHIONE[1,2], L.R. LOPETUSO[1,2], G. IANIRO[1,2], M. NAPOLI[1,2], G. GIBIINO[1,2], G. RIZZATTI[1,2], V. PETITO[1], A. GASBARRINI[1,2], F. SCALDAFERRI[1,2]

[1]Istituto di Patologia Speciale Medica, Università Cattolica del Sacro Cuore, Rome, Italy
[2]UOC Medicina Interna e Gastroenterologia, Area Medicina Interna, Gastroenterologia ed Oncologia Medica, Dipartimento di Scienze Gastroenterologiche, Endocrino-Metaboliche e Nefro-Urologiche, Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome, Italy

Abstract. – OBJECTIVE: Gut microbiota has a key role in host metabolic regulation and immune response, and its dysbiosis represents one of the main causes of gastrointestinal diseases. In this scenario, *Akkermansia muciniphila* is a crucial player in keeping the integrity of the gastrointestinal tract.

MATERIALS AND METHODS: This review focuses on the correlation between gut microbiota and intestinal homeostasis, primarily exploring *A. muciniphila* and its involvement in the development of metabolic disorders and gastrointestinal diseases.

RESULTS: *Akkermansia muciniphila* belongs to the Verrucomicrobia phylum, and it colonizes the mucus layer in the gastrointestinal tract, representing 1 to 4% of the fecal microbiota. It stimulates mucosal microbial networks, and it improves intestinal barrier function, providing crucial host immunological responses. Several studies have demonstrated the possible involvement of *A. muciniphila* in the development of intestinal and metabolic disorders. Indeed, adipose and glucose metabolisms are influenced by *A. muciniphila*, and its levels inversely correlate to inflammatory conditions, such as inflammatory bowel disease, obesity, and diabetes. Conversely, its therapeutic administration decreases their development.

CONCLUSIONS: *A. muciniphila* exerts a key role in the maintenance of intestinal health and in host metabolic modulation. Future studies could open new horizons towards its potential therapeutic applications in gastrointestinal and extra-intestinal diseases.

Key Words:
Akkermansia muciniphila, Gut microbiota, Gastrointestinal diseases, Metabolic diseases, Obesity, IBD, Colitis.

Introduction

The collection of bacteria, *Archaea* and *Eukarya* colonising the GI tract, usually known as the "gut microbiota", has been estimated to exceed 10^{14} microorganisms, which encompasses ~10 times more bacterial cells than the number of human cells and over 100 times the amount of genomic content (microbiome) as the human genome[1,2].

Latest evidence supports the role of gut microbiota in several host functions, and this dysbiotic condition has been linked to cardiovascular disease, neurodegenerative conditions, psychiatric disorders, and autoimmune conditions[3-6].

Early studies[7,8] sought to identify the normal set of microbes that colonizes healthy people, by culture and characterization of physiological properties. The introduction of strictly anaerobic techniques in the 1970s allowed the recovery of more than 300 bacterial species.

Then, above all, the introduction of 16SrRNA profiling studies provided invaluable insights into the taxonomic composition of the gut microbiota, which in turn has facilitated the inference of broad evolutionary patterns. However, species diversity may only present the top of the iceberg of microbial complexity in the gut[9-12]. Recent studies applying methods that go beyond the typical 16SrRNA profiling approach have provided compelling evidence that the bacterial species of the gut microbiota are composed of a multitude of strains, which are likely to influence gut microbiota functions.

Despite variability in species composition, the mammalian gut microbiota is dominated by relatively few bacterial phyla, including *Firmicutes*, *Bacteroidetes*, *Proteobacteria*, *Actinobacteria*, and *Verrucomicrobia*. Each phylum is generally classified in a hierarchical manner into sub-phyla, classes, orders, families, genera, and finally species and strains. Some of the earliest efforts to

Corresponding Author: Franco Scaldaferri, MD; e-mail: franco.scaldaferri@policlinicogemelli.it

sequence 16SrRNA genes directly from samples showed that 85-95% of bacterial abundance corresponding to known species could be attributed to three bacterial groups related to *Bacteroides*, *Clostridium cluster XIVa*, and *Clostridium cluster IV*[13,14].

Among these, *Verrucomicrobia* belongs to the *Planctomycetes-Verrucomicrobia-Chlamydiae* bacterial superphylum[15]. The first microbe of this phylum, *Akkermansia muciniphila*, was originally isolated from a fecal sample from a healthy female in a specific medium that contained purified mucins, sole carbon source, suggesting specific metabolic properties located in the interface between the luminal bacteria and the host[16]. Since then, many functions were hypothesized to support the role of *Verrucomicrobia* in the modulation of metabolism in the human host. In this review, we will summarize the current evidence on *Verrucomicrobia*, gut pathophysiology, and metabolic disorders.

The Verrucomicrobia Phylum

Verrucomicrobia are Gram-negative bacteria belonging to the *Planctomycetes*, *Verrucomicrobia*, *Chlamydiae* (PVC) superphylum, that is a group of six bacterial phyla: *Planctomycetes*, *Verrucomicrobia*, *Chlamydiae*, *Lentisphaerae*, *Poribacteria*, and *OP3*[17,18]. The bacteria from this superphylum were previously suggested to have a compartmentalized cell plan with a cytoplasmic membrane as the outermost membrane, and an intracytoplasmic membrane containing a condensed nucleoid and ribosomes[19]. However, more recently it has been suggested that these bacteria have an outer and an inner membrane (IM) with possible invaginations of the IM inside the cytoplasm, representing a variation and not an exception of the Gram-negative cell plan[20].

Verrucomicrobia phylum is involved in soil-based environments[21], in aquatic environments[22,23] and, not secondarily, is associated with eukaryotic species[24-27].

Akkermansia muciniphila belongs to this phylum, a bacterium that shares only very little similarity with *Verrucomicrobia* genomes, indicating how this phylum includes a vast bio-diversity of species[28].

A. muciniphila colonizes the mucus layer of the human gastrointestinal (GI) tract (12) and constitutes 1 to 4% of the fecal microbiota[29]. It is considered to be an anaerobic bacterium, although a more recent study[30] showed that it has a good survival when exposed to atmospheric oxygen, so that it can be defined as an aerotolerant anaerobic bacterium.

A. muciniphila encodes for 567 secreted proteins, such as sugar hydrolase, sialidase, and sulfatase, which are involved in mucin utilization. It is thus considered a mucin-degrading bacterium[31]. In fact, intestinal mucins, the highly glycosylated proteins of the epithelial mucus layer, represent the main source of carbon and nitrogen for this species[16] that can be found in high numbers in mucosal biopsy specimens of the human colon[32]. Moreover, *A. muciniphila* has been found to have a role not only in mucins biodegradation, but also in stimulating mucin production[33,34]. Because of its influence on mucin metabolism and consequently on the thickness of the mucus layer, a crucial role of *A. muciniphila* in gut barrier function is easy to be hypothesized. Indeed, it has been demonstrated that *A. muciniphila* is depleted in patients affected by inflammatory bowel disease (IBD), in which a reduction in barrier functions has been largely proved[35,36]. *A. muciniphila* reduction has been shown in ulcerative colitis (UC) and Crohn's disease (CD), both in clinically active disease and during remission[35,36]. A study of 2011 also demonstrated an inverse correlation between *A. muciniphila* levels and the severity of acute appendicitis[37].

Moreover, Reunanen et al[30] examined the effects of *A. muciniphila* on *in vitro* colonic cells lines Caco2 and HT-29, highlighting the capacity of this bacteria to adhere to the epithelium and to strengthen the intestinal barrier. The same study showed that *A. muciniphila* can induce a weak pro-inflammatory activity stimulating enterocytes production of interleukin 8 (IL-8) at cell concentrations 100-fold higher than those of *E. coli*. Thus, this bacterium does not activate a strong inflammatory cascade in the epithelium but seems to be able to keep the mucosa-associated immune system alerted at an appropriate level. These results are in line with other *in vivo* studies that link *A. muciniphila* to a non-inflamed mucosa[35,36] and suggest important host-bacteria interactions involved in the maintenance of host immune response.

Moreover, *A. muciniphila* has a crucial role in metabolic homeostasis. Studies on animals[38-40] showed a lower abundance of *A. muciniphila* in mice fed with high-fat diet (HFD), obese mice, and with type 2 diabetes-like symptoms. These results were confirmed by clinical trials that reported a negative correlation between *A.*

muciniphila and dietary fat intake, serum total cholesterol, and LDL[41]. A possible role of *A. muciniphila* in preventing metabolic disorders was supposed by a study[42] on HFD fed mice, in which a daily administration of 10^8 CFU of this strain for four weeks improved metabolic profile, prevented weight gain, and reduced LPS endotoxemia related to metabolic disorders. Overall, the effects of *A. muciniphila* on metabolism represent a field of great interest that may lead to possible future therapeutic applications in such disorders.

Gut Microbiota and Obesity

Obesity is a major risk factor for the development of common diseases, such as type 2 diabetes, metabolic syndrome, and cardiovascular disease, which are leading causes of morbidity and mortality in developed nations with an estimated cost exceeding hundreds of billion euro in the last years and an estimated 250,000 deaths per year[43].

Both environmental and genetic factors contribute to obesity and T2D development. Many researches emphasize the role of the gut microbiota as critical metabolism modifier and recent studies[44-46] showed its role in the development of obesity and progression to T2D. On one side, obesity determines a significant reduction of microbial variety and T2D causes dysbiosis with a reduction in butyrate-producing bacteria levels on the other. In this connection, it was observed that *Roseburia* and *Faecalibacterium prausnitzii* were less abundant in the feces of T2D patients compared to controls[47,48].

Human gut microbiota exerts some key-functions, such as digestion of otherwise indigestible substrates, production of specific vitamins impossible to synthesize *via* human enzymatic reactions, modification of host metabolism through the production of certain metabolites as short-chain fatty acids (SCFA), or *via* translocation of endotoxins[49].

One of them, known as the gram-negative bacterial wall compound lipopolysaccharide (LPS), is an innate immune regulator capable of activating macrophages in both adipose tissue and peripheral blood[50]. Some investigations confirm that the translocation of specific intestinal bacteria plays an important role in metabolism and development of obesity and insulin resistance. Ley et al[51] reported a higher ratio of *Firmicutes*/*Bacteroidetes* and a lower number of *Bacteroidetes* in 12 obese adult men and women randomly assigned to either a low-fat or low-carbohydrate diet for one year, when compared to lean, normal-weight individuals at baseline. However, the ratio returned to normal in those individuals who had successful and sustained weight loss.

Jumpertz et al[52] reported differences in the fecal microbiota in lean and obese individuals during diets, that varied in caloric content (2400 kcal/day *vs.* 3400 kcal/day), showing that an altered nutrient loading induced rapid changes in the gut bacterial community.

A high-fat diet also has an important role in the change of gut microbiota; indeed, Western diet induces an important intestinal dysbiosis, especially the proliferation of gram-negative bacteria, such as *Enterobacteriaceae.* This condition promotes local inflammation, and it increases intestinal permeability[53].

Examination of gut microbiota has been extended to children, but the results are heterogeneous. However, it is now quite definite that it is involved in triggering an innate immune response in early life, resulting in the maturation of gut-associated lymphoid tissue and developing adaptive immunity[54].

Some studies reported an increased level of *Firmicutes* and an increased *Firmicutes*/*Bacteroidetes* ratio in obese individuals[55], while others showed increased levels of *Bacteroidetes* and an increased *Bacteroidetes*/*Firmicutes* ratio[56].

A report from Egypt further demonstrated different results[57], suggesting that the composition of gut microbiota may depend on the environment, particularly in children.

Obese phenotype seems to be transmissible, as shown further[58]. It was observed that in the murine models, the germ-free mice remain significantly leaner than the conventional raised mice, despite a significantly higher food intake[59].

Vice versa, colonization of GF mice with caecum-derived microbiota of conventional mice ("conventionalization") demonstrated a significant increase in total body fat content without changes in the caloric intake[60]. This finding was later reproduced using inoculation in GF mice with feces of obese human subjects showing identical effects[61].

Akkermansia Muciniphila and Obesity: Evidence from Mouse Models

Some of the previous studies[62] have found a link between caloric intake and the abundance of *Akkermansia muciniphila*.

Administration of prebiotics in high fat diet-fed mice abolished the metabolic endotoxemia that

characterizes the impaired metabolic state present in obese subjects, reduced the total fat mass and the body weight. Of note, these results were significantly and inversely correlated with *A. muciniphila* abundance[63].

Schneeberger et al[64] reported that the levels of *A. muciniphila* in mice are inversely associated with inflammatory markers, lipid synthesis, and several plasma markers of insulin resistance, cardiovascular risk, and adiposity. The greatest impact on body weight gain and adiposity in mice occurs after 6 consecutive weeks of HFD administration.

There was a significative inverse correlation between *A. muciniphila* levels and inflammatory markers, all circulating parameters (i.e., insulin, glucose, triglycerides, and leptin) and positive correlation with 118 out of 13 genes involved in fatty oxidation, synthesis, and browning, while *Bifidobacterium spp.* abundance was significantly and negatively correlated with 3 inflammatory markers and leptin, and positively correlated with only 2 genes involved in fatty oxidation.

Therefore, these data indicate that HFD affects specific gut bacteria, and highlight that the abundance of *A. muciniphila* progressively declines with prolonged dietary treatment in mice, and that this effect is exacerbated upon HFD. It is also demonstrated that this bacterium declines before the onset of metabolic alterations, thereby suggesting a causative implication in the disease progression[64].

Moreover, the gut concentration of *A. muciniphila* is increased and decreased respectively in mice fed with fish oil-enriched diet and lard-enriched diet, and this effect is associated with better control of the gut barrier function and lower adipose tissue inflammation, a phenomenon that can be transferred to germ-free recipient mice[65].

Likewise, other authors[33-42] demonstrated that gut barrier dysfunction, the weight and fat gain in mice fed with HFD can be reduced with simultaneous administration of *A. muciniphila*.

Finally, there is also a link between age and *A. muciniphila* in mice, since the intestinal levels of this bacterium are lower in older mice. HFD heavily influence adipose tissue profile and intestinal microbiota in a way that mimicked aging[64].

A. Muciniphila and Obesity: Evidence from Human Studies

Emerging studies evaluated the relationship between the gut abundance of *Akkermansia muciniphila* and body weight in human. There is evidence of inverse correlation between these two variables[66]. Specifically, it was observed a reduction in numbers of *Bifidobacterium* and *A. muciniphila* and an increase in numbers of *Staphylococcus*, *Enterobacteriaceae*, and *Escherichia Coli* in overweight pregnant compared with normal-weight pregnant women.

Their gut microbiota composition was analysed by quantitative real-time PCR. In the whole population, the increased total bacteria and *Staphylococcus* numbers were correlated to increased plasma cholesterol levels, where instead the increased *Bacteroides* numbers were linked to increased HDL-cholesterol and folic acid levels, and reduced TAG levels[67].

Karlsson et al[68] evaluated the gut microbiota of twenty overweight or obese children and twenty normal weight control children of 4-5 years of age. Interestingly, the levels of *A. muciniphila* were significantly reduced in obese/overweight children, whereas the concentration of the gram-negative *Enterobacteriaceae* family was significantly higher in the same group. The concentration of *Bifidobacterium* was inversely correlated to alanine aminotransferase (ALT) in obese/overweight children.

Since diabetes and overweight have been related to increased gut permeability and low-grade inflammation, LPS-induced endotoxemia has been suggested as one of the causative agents of metabolic disorders related to obesity. The *in vitro* observation that *A. muciniphila* fortifies epithelial barrier function could provide a working hypothesis to rationalize the *in vivo* findings connecting decreased fecal *A. muciniphila* levels with diabetes and obesity. This could reveal one possible mechanism behind the protective effect of the bacterium against high-fat-diet-induced LPS endotoxemia in obese mice[30].

At this time, no study relationship between *A. muciniphila* and the markers of hypothalamic control of food intake has been evaluated.

In accordance with all this evidence, we can suppose that in humans *A. muciniphila* influences the response to caloric restriction diet in terms of improvement of inflammatory markers, insulin resistance, and glycemia.

Akkermansia Muciniphila and Intestinal Barrier Modulation

Mucus is an essential part of maintaining the health of the gastrointestinal tract and its functional integrity. It represents both an efficient barrier that protects the epithelial surface, preventing

particles or extraneous agent penetration, and an excellent lubricant for metabolic processes[69]. The mucus layer offers substrates for bacterial growth, adhesion, and protection.

The resident microbiota also represents an essential resource to ensure an effective gut barrier; it provides to obtain nutrients, it processes necessary molecules for mucosal integrity, and it competes with pathogens to gain space[70]. Conversely, gut microbiota composition has been shown to influence mucus barrier properties. Higher levels of specific bacterial species are able in the inflammatory conditions to increase the permeability of the mucus layer and thus decrease the barrier function[71]. Therefore, its presence influences intestinal colonization by attracting bacteria that have the ability to survive and proliferate within the mucus layer[29].

Akkermansia muciniphila is known to colonize the mucosal layer of the human intestine where it triggers both hosts metabolic and immune responses. It was found being particularly effective in increasing mucus thickness and increasing gut barrier function. Its metabolic and mucolytic activity make *A. muciniphila* a key species in the mucus layer, stimulating beneficial mucosal microbial networks. Specifically, the production of short-chain fatty acids is beneficial to the host and microbiota members[72]. It was demonstrated that *A. muciniphila* colonizes the intestinal tract in early life and develops within a year to a level close to that observed in healthy adults[29]. In the colon of a healthy human, *A. muciniphila* is present in high levels with an abundance of approximately 3%[37]. Its specialization in mucin degradation makes it a key organism at the mucosal interface between the lumen and host cells[73]. Thanks to *A. muciniphila*, intestinal mucin is degraded into mainly propionic and acetic acid that become the substrate for *F. prausnitzii*, one of the main producers of butyrate in the intestine. This leads to the inhibition of inflammation in the gastrointestinal tract and to the prevention of the increased intestinal permeability[74].

A. muciniphila also has an active role in stimulation and regulation of host immunity, improving intestinal barrier function, and probably providing competitive exclusion at the host-microbe interface[28,75]. It is positively correlated with a lean phenotype, reduced body weight gain, increate metabolic responses, and with the restoration of gut barrier function by modulation of mucus layer thickness[76]. Notably, low levels of *A. muciniphila* have been observed in patients with inflammatory bowel diseases (mainly in ulcerative colitis) and metabolic disorders, which suggest that it may have potential anti-inflammatory properties[73]. Specifically, recent trials demonstrated that *A. muciniphila* increased the endogenous production of specific bioactive lipids, especially Glucagon-like peptide 2 (GLP-2), involved in gut barrier regulation[77].

Next research challenge will be to analyze the molecular mechanism of actions involved in the metabolic and immunological *A. muciniphila* properties in order to find potential therapeutic applications.

Akkermansia Muciniphila and Gastrointestinal Diseases

The relation between mucin and bacteria varies depending on the microbiota, and several studies[16] have reported a potential involvement of mucin-degrading bacteria in the pathogenesis of intestinal diseases. The levels of *Akkermansia muciniphila* have been shown to be inversely correlated with several disorders, such as inflammatory bowel diseases (IBD), obesity, diabetes, and appendicitis, but few information exists about its immunological mechanisms of action[76].

In IBD, the abundance of *A. muciniphila* was found to be decreased[75]. Indeed, several studies[76] have demonstrated its depletion in the fecal microbiota of ulcerative colitis patients, and low levels of *A. muciniphila* have also been found in biopsies of intestinal mucosa from IBD-patients in comparison to healthy controls.

Also, individuals with acute appendicitis were found to have low levels of *A. muciniphila*, and the amount of *A. muciniphila* was inversely related to the severity of appendicitis[37].

Because of its ability to degrade mucins, it was supposed that *A. muciniphila* could contribute to intestinal inflammation because low levels of mucin allow the pathogen to access the mucosa. To support this, trials with SIHUMI mice reported that the intestinal inflammation induced by *Salmonella typhimurium* was exacerbated by *A. muciniphila*, suggesting that this was based on its ability to interfere with host mucus formation and production[77].

However, several trials have demonstrated that *A. muciniphila* controls fat storage, adipose tissue metabolism, and glucose homeostasis[42]. It is widely considered as a novel potential candidate to improve metabolic disorders associated with obesity, diabetes, liver diseases, and cardiometabolic disorders. Indeed, its administration has been shown

to reduce the development of such diseases[78]. This outline, therefore, the key role of *A. muciniphila* in GI and extra GI diseases and how important it is to keep investigating its mechanisms in order to find many other potential therapeutic approaches.

Therapeutic Role of Akkermansia Muciniphila for the Treatment of Metabolic Disorders

The examination of *Akkermansia muciniphila* beneficial properties in metabolic regulation and immune response led to better investigate its potential therapeutic effects in the treatment of certain diseases and metabolic disorders.

Notably, *A. muciniphila* seems to have an active role in amine butyrate production and propionate extracellular pool output. It is also crucial in hydrogen sulfide production[79], that may have anti-inflammatory, muscular relaxing, and antioxidant properties[80].

Prebiotic feeding strongly increases the presence of *A. muciniphila* and it improves metabolic disorders. All trials in which animals were treated with *A. muciniphila* showed that it reduces body weight and fat-mass gain, hepatic steatosis, inflammation, cholesterol levels, and atherosclerosis; improves insulin sensitivity and restores gut barrier function by influencing different factors, like mucus-layer thickness, tight-junction proteins, antimicrobial peptides, and immunity[81].

A. muciniphila especially acts with a specific protein called Amuc_1100, which is involved in immunomodulatory aspects[81]. Recently, Ottman et al[76] have reported that the administration of the purified recombinant Amuc_1100 protein improved glucose tolerance and decreased body weight and fat mass gain in mice fed on high-fat diet in comparison to untreated mice.

In this scenario, the essential role of *A. muciniphila* in intestinal health maintenance, especially in immunomodulation of metabolic disorders is clear. Consequently, future research will need to enhance the key-role of this microorganism and its proteins in metabolic regulation and host immune modulation, in order to improve its therapeutic application in gastrointestinal diseases.

Conclusions

Gut microbiota is an active element to ensure mucosal integrity, and its balance is necessary for intestinal health maintenance. Every component has a specific role in metabolic function, and immune modulation and dysbiosis condition is related to the development of gastrointestinal impairment. In this background, a crucial role is played by *Akkermansia muciniphila*, a mucin-degrading bacterium, which revealed important metabolic and immunological properties. Several studies have demonstrated how it is centrally involved in controlling of fat storage and glucose homeostasis; its abundance is inversely related to inflammatory conditions, such as dyslipidaemia, obesity, inflammatory bowel disease (IBD), and type 2 diabetes.

In accordance with these observations, other detailed studies would be needed to deepen *A. muciniphila* activity, in order to improve its use in the therapeutic application for metabolic and gastrointestinal disorders.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Neish AS. Microbes in gastrointestinal health and disease. Gastroenterol 2009; 136: 65-80.
2) Gill SR, Pop M, DeBoy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, Gordon JI, Relman DA. Fraser-Ligget CM, Nelson KE. Metagenomic analysis of the human distal gut microbiome. Science 2006; 312: 1355-1359.
3) Ahmadmehrabi S, Tang WHW. Gut microbiome and its role in cardiovascular diseases. Curr Opin Cardiol 2017; 32: 761-766.
4) Quigley EMM. Microbiota-brain-gut axis and neurodegenerative diseases. Curr Neurol Neurosci Rep 2017; 17: 94.
5) Rios AC, Maurya PK, Pedrini M, Zeni-Graiff M, Asevedo E, Mansur RB, Wieck A, Grassi-Oliveira R, Mclntyre RS, Hayashi MAF, Brietzke E. Microbiota abnormalities and the therapeutic potential of probiotics in the treatment of mood disorders. Rev Neurosci 2017; 28: 739-749.
6) Maeda Y, Takeda K. Role of gut microbiota in rheumatoid arthritis. J Clin Med 2017; 6: 60.
7) Rajilić-Stojanović M, de Vos WM. The first 1000 cultured species of the human gastrointestinal microbiota. FEMS Microbiol Rev 2014; 38: 996-1047.
8) Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The human microbiome project. Nature 2007; 449: 804-810.
9) Faith JJ, Guruge JL, Charbonneau M, Subramanian S, Seedorf H, Goodman AL, Clemente JC, Knight R,

Heath AC, Leibel RL, Rosenbaum M, Gordon JI. The long-term stability of the human gut microbiota. Science 2013; 341: 1237439.

10) Schloissnig S, Arumugam M, Sunagawa S, Mitreva M, Tap J, Zhu A, Waller A, Mende DR, Kultima JR, Martin J, Kota K, Sunyaev R, Weinstock GM, Bork P. Genomic variation landscape of the human gut microbiome. Nature 2013; 493: 45-50.

11) Greenblum S, Carr R, Borenstein E. Extensive strain-level copy-number variation across human gut microbiome species. Cell 2015; 160: 583-594.

12) Zhu A, Sunagawa S, Mende DR, Bork P. Inter-individual differences in the gene content of human gut bacterial species. Genome Biol 2015; 16: 82.

13) Suau A, Bonnet R, Sutren M, Godon JJ, Gibson GR, Collins MD, Doré J. Direct analysis of genes encoding 16S rRNA from complex communities reveals many novel molecular species within the human gut. Appl Environ Microbiol 1999; 65: 4799-4807.

14) Hold GL, Pryde SE, Russell VJ, Furrie E, Flint HJ. Assessment of microbial diversity in human colonic samples by 16S rDNA sequence analysis. FEMS Microbiol Ecol 2002; 39: 33-39.

15) van Niftrik L, Devos DP. Editorial: Planctomycetes-Verrucomicrobia-Chlamydiae Bacterial Superphylum: new model organisms for evolutionary cell biology. Front Microbiol 2017; 8: 1458.

16) Derrien M, Vaughan EE, Plugge CM, de Vos WM. Akkermansia municiphila gen. nov., sp. nov., a human intestinal mucin-degrading bacterium. Int J Syst Evol Microbiol 2004; 54 (Pt 5): 1469-1476.

17) Wagner M, Horn M. The Planctomycetes, Verrucomicrobia, Chlamydiae and sister phyla comprise a superphylum with biotechnological and medical relevance. Curr Opin Biotechnol 2006; 17: 241-249.

18) Gupta RS, Bhandari V, Naushad HS. Molecular signatures for the pvc clade (Planctomycetes, Verrucomicrobia, Chlamydiae, and Lentisphaerae) of bacteria provide insights into their evolutionary relationships. Front Microbiol 2012; 3: 327.

19) Lee KC, Webb RI, Janssen PH, Sangwan P, Romeo T, Staley JT, Fuerst JA. Phylum Verrucomicrobia representatives share a compartmentalized cell plan with members of bacterial phylum Planctomycetes. BMC Microbiol 2009; 9: 5.

20) Devos DP. PVC bacteria: variation of, but not exception to, the Gram-negative cell plan. Trends Microbiol 2014; 22: 14-20.

21) Sangwan P, Kovac S, Davis KER, Sait M, Janssen PH. Detection and cultivation of soil verrucomicrobia. Appl Environ Microbiol 2005; 71: 8402-8410.

22) Martiny AC, Albrechtsen HJ, Arvin E, Molin S. Identification of bacteria in biofilm and bulk water samples from a nonchlorinated model drinking water distribution system: detection of a large nitrite-oxidizing population associated with Nitrospira spp. Appl Environ Microbiol 2005; 71: 8611-8617.

23) Haukka K, Kolmonen E, Hyder R, Hietala J, Vakkilainen K, Kairesalo T, Haario H., Sivonen K. Effect of nutrient loading on bacterioplankton community composition in lake mesocosms. Microb Ecol 2006; 51: 137-146.

24) Petroni G, Spring S, Schleifer K-H, Verni F, Rosati G. Defensive extrusive ectosymbionts of Euplotidium (Ciliophora) that contain microtubule-like structures are bacteria related to Verrucomicrobia. Proc Natl Acad Sci U S A 2000; 97: 1813-1817.

25) Vandekerckhove TTM, Coomans A, Cornelis K, Baert P, Gillis M. Use of the Verrucomicrobia-specific probe EUB338-III and fluorescent in situ hybridization for detection of "Candidatus xiphinematobacter" cells in nematode hosts. Appl Environ Microbiol 2002; 68: 3121-3125.

26) Shinzato N, Muramatsu M, Matsui T, Watanabe Y. Molecular phylogenetic diversity of the bacterial community in the gut of the termite Coptotermes formosanus. Biosci Biotechnol Biochem 2005; 69: 1145-1155.

27) Wang M, Ahrné S, Jeppsson B, Molin G. Comparison of bacterial diversity along the human intestinal tract by direct cloning and sequencing of 16S rRNA genes. FEMS Microbiol Ecol 2005; 54: 219-231.

28) de Vos WM. Microbe profile: Akkermansia muciniphila: a conserved intestinal symbiont that acts as the gatekeeper of our mucosa. Microbiol 2017; 163: 646-648.

29) Collado MC, Derrien M, Isolauri E, de Vos WM, Salminen S. Intestinal integrity and Akkermansia muciniphila, a mucin-degrading member of the intestinal microbiota present in infants, adults, and the elderly. Appl Environ Microbiol 2007; 73: 7767-7770.

30) Reunanen J, Kainulainen V, Huuskonen L, Ottman N, Belzer C, Huhtinen H, de Vos W, Satokari R. Akkermansia muciniphila adheres to enterocytes and strengthens the integrity of the epithelial cell layer. Appl Environ Microbiol 2015; 81: 3655-3662.

31) van Passel MWJ, Kant R, Zoetendal EG, Plugge CM, Derrien M, Malfatti SA, Chain PSG, Woyke T, Palva A, de Vos WM, Smidt H. The genome of Akkermansia muciniphila, a dedicated intestinal mucin degrader, and its use in exploring intestinal metagenomes. PLoS One 2011; 6: e16876.

32) Lyra A, Forssten S, Rolny P, Wettergren Y, Lahtinen SJ, Salli K, Cedgård L, Odin E, Gustavsson B, Ouwehand A. Comparison of bacterial quantities in left and right colon biopsies and faeces. World J Gastroenterol 2012; 18: 4404-4411.

33) Shin NR, Lee JC, Lee HY, Kim MS, Whon TW, Lee MS, Bae JW. An increase in the Akkermansia spp. population induced by metformin treatment improves glucose homeostasis in diet-induced obese mice. Gut 2014; 63: 727-735.

34) Lee H, Ko G. Effect of metformin on metabolic improvement and gut microbiota. Appl Environ Microbiol 2014; 80: 5935-5943.

35) Png CW, Lindén SK, Gilshenan KS, Zoetendal EG, McSweeney CS, Sly LI, McGuckin MA, Florin TH.

Mucolytic bacteria with increased prevalence in IBD mucosa augment in vitro utilization of mucin by other bacteria. Am J Gastroenterol 2010; 105: 2420-2428.

36) Rajilić-StojanoviĐ M, Shanahan F, Guarner F, de Vos WM. Phylogenetic analysis of dysbiosis in ulcerative colitis during remission. Inflamm Bowel Dis 2013; 19: 481-488.

37) Swidsinski A, Dörffel Y, Loening-Baucke V, Theissig F, Rückert JC, Ismail M, Rau WA, Gaschler D, Weizenegger M, Kühn S, Schilling J, Dörffel WV. Acute appendicitis is characterised by local invasion with Fusobacterium nucleatum/necrophorum. Gut 2011; 60: 34-40.

38) Kiilerich P, Myrmel LS, Fjære E, Hao Q, Hugenholtz F, Sonne SB, Derrien M, Pedersen LM, Petersen RK, Mortensen A, Licht TR, Rømer MU, Vogel UB, Waagbø LJ, Giallourou N, Feng Q, Xiao L, Liu C, Liasert B, Kleerebezem M, Wang J, Madsen L, Kristiansen K. Effect of a long-term high-protein diet on survival, obesity development, and gut microbiota in mice. Am J Physiol Endocrinol Metab 2016; 310: 886-899.

39) Nobel YR, Cox LM, Kirigin FF, Bokulich NA, Yamanishi S, Teitler I, Chung J, Sohn J, Barber CM, Goldfarb DS, Raju K, Abubucker S, Zhou Y, Ruiz VE, Li H, Mitreva M, Alekseyenko AV, Weinstock GM, Sodergren E, Blaser MJ. Metabolic and metagenomic outcomes from early-life pulsed antibiotic treatment. Nat Commun 2015; 6: 7486.

40) Cox LM, Yamanishi S, Sohn J, Alekseyenko A V, Leung JM, Cho I, Kim SG, Li H, Gao Z, Mahana D, Zárate Rodriguez JG, Rogers AB, Robin N, Loke P, Blaser MJ. Altering the intestinal microbiota during a critical developmental window has lasting metabolic consequences. Cell 2014; 158: 705-721.

41) Brahe LK, Le Chatelier E, Prifti E, Pons N, Kennedy S, Hansen T, Pedersen O, Astrup A, Ehrlich SD, Larsen LH. Specific gut microbiota features and metabolic markers in postmenopausal women with obesity. Nutr Diabetes 2015; 5: e159.

42) Everard A, Belzer C, Geurts L, Ouwerkerk JP, Druart C, Bindels LB, Guiot Y, Derrien M, Muccioli GG, Delzenne NM, de Vos WM, Cani PD. Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. Proc Natl Acad Sci U S A 2013; 110: 9066-9071.

43) Cecchini M, Sassi F, Lauer JA, Lee YY, Guajardo-Barron V, Chisholm D. Tackling of unhealthy diets, physical inactivity, and obesity: health effects and cost-effectiveness. Lancet 2010; 376: 1775-1784.

44) Wu GD, Chen J, Hoffmann C, Bittinger K, Chen YY, Keilbaugh SA, Bewtra M, Knights D, Walters WA, Knight R, Sinha R, Gilroy E, Gupta K, Baldassano R, Nessel L, Li H, Bushman FD, Lewis JD. Linking long-term dietary patterns with gut microbial enterotypes. Science 2011; 334: 105-108.

45) David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA, Biddinger SB, Dutton RJ, Turnbaugh PJ. Diet rapidly and reproducibly alters the human gut microbiome. Nature 2014; 505: 559-563.

46) Goodrich JK, Waters JL, Poole AC, Sutter JL, Koren O, Blekhman R, Beaumont M, Van Treuren W, Knight R, Bell JT, Spector TD, Clark AG, Ley RE. Human genetics shape the gut microbiome. Cell 2014; 159: 789-799.

47) Montandon SA, Jornayvaz FR. Effects of antidiabetic drugs on gut microbiota composition. Genes Basel 2017; 8: 250.

48) Aydin O, Nieuwdorp M, Gerdes V. The gut microbiome as a target for the treatment of type 2 diabetes. Curr Diab Rep 2018; 18: 55.

49) Gao Z, Yin J, Zhang J, Ward RE, Martin RJ, Lefevre M, Cefalu WT, Ye J. Butyrate improves insulin sensitivity and increases energy expenditure in mice. Diabetes 2009; 58: 1509-1517.

50) Cani PD, Neyrinck AM, Fava F, Knauf C, Burcelin RG, Tuohy KM, Gibson GR, Delzenne NM. Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. Diabetologia 2007; 50: 2374-2383.

51) Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. Nature 2006; 444: 1022-1023.

52) Jumpertz R, Le DS, Turnbaugh PJ, Trinidad C, Bogardus C, Gordon JI, Krakoff J. Energy-balance studies reveal associations between gut microbes, caloric load, and nutrient absorption in humans. Am J Clin Nutr 2011; 94: 58-65.

53) Bibbò S, Ianiro G, Giorgio V, Scaldaferri F, Masucci L, Gasbarrini A, Cammarota G. The role of diet on gut microbiota composition. Eur Rev Med Pharmacol Sci 2016; 20: 4742-4749.

54) Mangiola F, Nicoletti A, Gasbarrini A, Ponziani FR. Gut microbiota and aging. Eur Rev Med Pharmacol Sci 2018; 22: 7404-7413.

55) Xu P, Li M, Zhang J, Zhang T. Correlation of intestinal microbiota with overweight and obesity in Kazakh school children. BMC Microbiol 2012; 12: 283.

56) Zhu L, Baker SS, Gill C, Liu W, Alkhouri R, Baker RD, Gill SR. Characterization of gut microbiomes in nonalcoholic steatohepatitis (NASH) patients: a connection between endogenous alcohol and NASH. Hepatology 2013; 57: 601-609.

57) Ismail NA, Ragab SH, ElBaky AA, Shoeib ARS, Alhosary Y, Fekry D. Frequency of Firmicutes and Bacteroidetes in gut microbiota in obese and normal weight Egyptian children and adults. Arch Med Sci 2011; 7: 501-507.

58) Amar J, Serino M, Lange C, Chabo C, Iacovoni J, Mondot S, Lepage P, Klopp C, Mariette J, Bouchez O, Perez L, Courtney M, Marren M, Klopp P, Lantieri O, Doré J, Charles M, Balkau B, Burcelin R; D.E.S.I.R. Study Group. Involvement of tissue bacteria in the onset of diabetes in humans: evidence for a concept. Diabetologia 2011; 54: 3055-3061.

59) Backhed F, Ding H, Wang T, Hooper L V., Koh GY, Nagy A, Semenkovich CF, Gordon GI. The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci U S A 2004; 101: 15718-15723.

60) Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 2006; 444: 1027–1031.

61) Ridaura VK, Faith JJ, Rey FE, Cheng J, Duncan AE, Kau AL, Griffin NW, Lombard V, Henrissat B, Bain JR, Muehlbauer MJ, Ilkayeva O, Semenkovich CF, Funai K, Hayashi DK, Ly le BJ, Martini MC, Ursell LK, Clemente JC, Van Treuren W, Walters WA, Knight R, Newgard CB, Heath AC, Gordon J. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science 2013; 341: 1241214.

62) Remely M, Hippe B, Geretschlaeger I, Stegmayer S, Hoefinger I, Haslberger A. Increased gut microbiota diversity and abundance of Faecalibacterium prausnitzii and Akkermansia after fasting: a pilot study. Wien Klin Wochenschr 2015; 127: 394-398.

63) Everard A, Matamoros S, Geurts L, Delzenne NM, Cani PD. Saccharomyces boulardii administration changes gut microbiota and reduces hepatic steatosis, low-grade inflammation, and fat mass in obese and type 2 diabetic db/db mice. MBio 2014; 5: e01011-01014.

64) Schneeberger M, Everard A, Gómez-Valadés AG, Matamoros S, Ramírez S, Delzenne NM, Ramon G, Claret M, Cani PD. Akkermansia muciniphila inversely correlates with the onset of inflammation, altered adipose tissue metabolism and metabolic disorders during obesity in mice. Sci Rep 2015; 5: 16643.

65) Caesar R, Tremaroli V, Kovatcheva-Datchary P, Cani PD, Bäckhed F. Crosstalk between gut microbiota and dietary lipids aggravates WAT inflammation through TLR signaling. Cell Metab 2015; 22: 658-668

66) Everard A, Lazarevic V, Derrien M, Girard M, Muccioli GM, Neyrinck AM, Possemiers S, Van Holle A, François P, de Vos WM, Delzenne NM, Schrenzel J, Cani PD. Responses of gut microbiota and glucose and lipid metabolism to prebiotics in genetic obese and diet-induced leptin-resistant mice. Diabetes 2011; 60: 2775-2786.

67) Santacruz A, Collado MC, García-Valdés L, Segura MT, Marítn-Lagos JA, Anjos T, Martí-Romero M, Lopez RM, Florido J, Campoy C, SAnz Y. Gut microbiota composition is associated with body weight, weight gain and biochemical parameters in pregnant women. Br J Nutr 2010; 104: 83-92.

68) Karlsson CLJ, Önnerfält J, Xu J, Molin G, Ahrné S, Thorngren-Jerneck K. The microbiota of the gut in preschool children with normal and excessive body weight. Obesity 2012; 20: 2257-2261.

69) Cone RA. Barrier properties of mucus. Adv Drug Deliv Rev 2009; 61: 75-85.

70) Lopetuso LR, Scaldaferri F, Bruno G, Petito V, Franceschi F, Gasbarrini A. The therapeutic managment of gut barrier leaking: the emerging role for mucosal barrier protectors. Eur Rev Med Pharmacol Sci 2015; 19: 1068-1076.

71) Ottman N, Davids M, Suarez-Diez M, Boeren S, Schaap PJ, Martins dos Santos VAP, Smidt H, Belzer C, de Vos WM. Genomescale model and omics analysis of metabolic capacities of Akkermansia muciniphila reveal a preferential mucin-degrading lifestyle. Appl Environ Microbiol 2017; 83: e01014-01017

72) Ottman N, Geerlings SY, Aalvink S, de Vos WM, Belzer C. Action and function of Akkermansia muciniphila in microbiome ecology, health and disease. Best Pract Res Clin Gastroenterol 2017; 31: 637-642.

73) Derrien M, Belzer C, de Vos WM. Akkermansia muciniphila and its role in regulating host functions. Microb Pathog 2017; 106: 171-181.

74) Szachta P, Bartnicka A, Galecka M. Microbiota – a key to healing the gastrointestinal tract?. Pomeranian J life Sci 2016; 62: 21-24.

75) Geerlings S, Kostopoulos I, de Vos W, Belzer C. Akkermansia muciniphila in the human gastrointestinal tract: when, where, and how? Microorganisms 2018; 6. pii: E75.

76) Ottman N, Reunanen J, Meijerink M, Pietila TE, Kainulainen V, Klievink J, Huuskon Aalvink S, Skurnik M, Boeren S, Satokari R, Mercenier A, Palvi A, Smidt H, de Vos WM, Belzer C. Pili-like proteins of Akkermansia muciniphila modulate host immune responses and gut barrier function. PLoS One 2017; 12: e0173004.

77) Ganesh BP, Klopfleisch R, Loh G, Blaut M. Commensal Akkermansia muciniphila exacerbates gut inflammation in Salmonella typhimurium-infected gnotobiotic mice. PLoS One 2013; 8: e74963.

78) Cani PD, de Vos WM. Next-generation beneficial microbes: the case of Akkermansia muciniphila. Front Microbiol 2017; 8: 1765.

79) Rosario D, Benfeitas R, Bidkhori G, Zhang C, Uhlen M, Shoaie S, Mardinoglu A. Understanding the representative gut microbiota dysbiosis in metformin-treated Type 2 diabetes patients using genome-scale metabolic modeling. Front Physiol 2018; 9: 775.

80) Al Khodor S, Reichert B, Shatat IF. The microbiome and blood pressure: can microbes regulate our blood pressure?. Front Pediatr 2017; 5: 138.

81) Cani PD. Human gut microbiome: hopes, threats and promises. Gut 2018; 67: 1716-1725.