Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity

Amandine Everard\textsuperscript{a}, Clara Belzer\textsuperscript{b}, Lucie Geurts\textsuperscript{c}, Janneke P. Ouwerkerk\textsuperscript{d}, Céline Druart\textsuperscript{a}, Laure B. Bindels\textsuperscript{a}, Yves Guiot\textsuperscript{c}, Muriel Derrien\textsuperscript{b}, Giulio G. Muccioli\textsuperscript{d}, Nathalie M. Delzenne\textsuperscript{a}, Willem M. de Vos\textsuperscript{b,e}, and Patrice D. Cani\textsuperscript{a,1}

\textsuperscript{a}Metabolism and Nutrition Research Group, Walloon Excellence in Life sciences and BioTechnology (WELBIO), Louvain Drug Research Institute, Université catholique de Louvain, B-1200 Brussels, Belgium; \textsuperscript{b}Laboratory of Microbiology, Wageningen University, 6703 HB, Wageningen, The Netherlands; \textsuperscript{c}Department of Pathology, Cliniques Universitaires Saint-Luc, Université catholique de Louvain, B-1200 Brussels, Belgium; \textsuperscript{d}Bioanalysis and Pharmacology of Bioactive Lipids Research Group, Louvain Drug Research Institute, Université catholique de Louvain, B-1200 Brussels, Belgium; and \textsuperscript{e}Departments of Bacteriology and Immunology and Veterinary Biosciences, University of Helsinki, 00014 Helsingin yliopisto, Helsinki, Finland

Obesity and type 2 diabetes are characterized by altered gut microbiota, inflammation, and gut barrier disruption. Microbial composition and the mechanisms of interaction with the host that affect gut barrier function during obesity and type 2 diabetes have not been elucidated. We recently isolated Akkermansia muciniphila, which is a mucin-degrading bacterium that resides in the mucus layer. The presence of this bacterium inversely correlates with body weight in rodents and humans. However, the precise physiological roles played by this bacterium during obesity and metabolic disorders are unknown. This study demonstrated that the abundance of A. muciniphila decreased in obese and type 2 diabetic mice. We also observed that prebiotic feeding normalized A. muciniphila abundance, which correlated with an improved metabolic profile. In addition, we demonstrated that A. muciniphila treatment reversed high-fat diet-induced metabolic disorders, including fat-mass gain, metabolic endotoxemia, adipose tissue inflammation, and insulin resistance. A. muciniphila administration increased the intestinal levels of endocannabinoids that control inflammation, the gut barrier, and gut peptide secretion. Finally, we demonstrated that all these effects required viable A. muciniphila because treatment with heat-killed cells did not improve the metabolic profile or the mucus layer thickness. In summary, this study provides substantial insight into the intricate mechanisms of bacterial (i.e., A. muciniphila) regulation of the cross-talk between the host and gut microbiota. These results also provide a rationale for the development of a treatment that uses this human mucus colonizer for the prevention or treatment of obesity and its associated metabolic disorders.

Gut microbiota were once characterized as bystanders in the intestinal tract, but their active role in intestinal physiology is now widely investigated. In particular, the mutualism that exists between gut microbiota and the host has received much attention. Obesity and type 2 diabetes are characterized by altered gut microbiota (1), inflammation (2), and gut barrier disruption (3–5). We recently demonstrated an association of obesity and type 2 diabetes with increased gut permeability, which induced metabolic endotoxemia and metabolic inflammation (3–5). Unequivocal evidence demonstrates that gut microbiota influence whole-body metabolism (1, 6) by affecting the energy balance (6), gut permeability (4, 5), serum lipopolysaccharides (i.e., metabolic endotoxemia (7)), and metabolic inflammation (3–5, 7) that are associated with obesity and associated disorders. However, the microbial composition and the exact mechanisms of interaction between these two partners that affect host–gut barrier function and metabolism remain unclear.

The intestinal epithelium is the interface for the interaction between gut microbiota and host tissues (8). This barrier is enhanced by the presence of a mucus layer and immune factors that are produced by the host (9). Antimicrobial peptides for innate immunity are produced by Paneth cells (e.g., α-defensins, lysozyme C, phospholipases, and C-type lectin, primarily generating islet-derived 3-gamma, RegIIIγ or enterocytes (RegIIIγ) (10–12). Adaptive immune system effectors that are secreted into the intestinal lumen, such as IgA, may also restrict bacterial penetration into the host mucosa and mucosal tissue (13). These immune factors allow the host to control its interactions with gut microbiota and shape its microbial community (14).

The endocannabinoid system has also been implicated in the control of the gut barrier and inflammation (5, 14). One lipid in this system, 2-arachidonoylglycerol (2-AG), reduces metabolic endotoxemia and systemic inflammation (15). Another acylglycerol, 2-palmitoylglycerol (2-PG), potentiates the antiinflammatory effects of 2-AG (16). Importantly, 2-oleoylglycerol (2-OG) stimulates the release of gut peptides, such as glucagon-like peptide-1 (GLP-1) and glucagon-like peptide-2 (GLP-2), from intestinal L-cells (17). These peptides are implicated in the control of glucose homeostasis and gut barrier function, respectively (4).

Recently, Akkermansia muciniphila has been identified as a mucin-degrading bacteria that resides in the mucus layer (18), and it is the dominant human bacterium that abundantly colonizes this nutrient-rich environment (18). A. muciniphila may represent 3–5% of the microbial community (18, 19) in healthy subjects, and its abundance inversely correlates with body weight (20–23) and type 1 diabetes (24) in mice and humans, although a recent metagenomic study found that some of the genes belonging to A. muciniphila were enriched in type 2 diabetic subjects (25).

We recently discovered that the administration of prebiotics (oligofructose) to genetically obese mice increased the abundance of A. muciniphila by ~100-fold (23). However, the direct implications of A. muciniphila for obesity and type 2 diabetes have not been determined, and the precise physiological roles it plays during these processes are not known.

Our previous results and the close proximity of this bacterium to the human intestinal epithelium support the hypothesis that A. muciniphila plays a crucial role in the mutualism between the gut microbiota and host that controls gut barrier function and other physiological and homeostatic functions during obesity and type 2 diabetes. We administered alive or heat-killed A. muciniphila to mice that were fed a high-fat diet and investigated the gut barrier, glucose homeostasis, and adipose tissue metabolism to test this hypothesis.


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1To whom correspondence should be addressed. E-mail: patrice.cani@uclouvain.be.

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Results

A. muciniphila Abundance Decreased in Obese and Type 2 Diabetic Mice. We observed that the abundance of A. muciniphila was 3,300-fold lower in leptin-deficient obese mice than in their lean littermates (Fig. 1A). We also observed a 100-fold decrease of this bacterium in high-fat-(HF)-fed mice (Fig. 1B).

Prebiotic Treatment Restored Basal Levels of A. muciniphila and Improved Metabolic Endotoxemia and Related Disorders That Are Associated with HF-Diет-induced Obesity. Prebiotics (oligofructose) completely restored A. muciniphila counts in both models (Fig. 1B and C), therefore supporting the data obtained in our previous study performed in ob/ob mice (23). Administration of prebiotics in HF-fed mice abolished metabolic endotoxemia (Fig. 1D) and normalized the CD11c subpopulation of macrophages in adipose tissue, which is the primary population of increased adipose tissue macrophages in obesity (2) (Fig. 1E). Administration of prebiotics also reduced the total fat mass, the mass of the different fat pads (i.e., s.c., mesenteric, and epididymal), and the body weight (Fig. 1F and Fig. S1 A–C). These results were significant and inversely correlated with A. muciniphila abundance (Fig. 1G and Fig. S1 D and E). However, the role of the lack of A. muciniphila in the molecular mechanisms that underlie the onset of these disorders has not been demonstrated, and whether an increased abundance of A. muciniphila reverses these disorders must be investigated. A. muciniphila was orally administered to control or HF-fed mice for 4 wk to address these questions.

HF Diet Altered the Gut Microbiota Composition, Whereas A. muciniphila Did Not Significantly Induce Changes. A. muciniphila treatment was associated with an increase in A. muciniphila abundance in the cecal content of mice (Fig. S2A). We also demonstrated that an HF diet significantly changes the gut microbiota using a phylogenetic microarray (Mouse Intestinal Tract Chip, MITChip) (10, 23), as shown by principal component analyses (Fig. 2A), dendrogram clustering, and representational difference analysis (Fig. S2 B and C), whereas A. muciniphila treatment did not modify this profile (Fig. 2A and Fig. S2 B and C).

A. muciniphila Improved Metabolic Disorders in Diet-induced Obese Mice. A. muciniphila treatment normalized diet-induced metabolic endotoxemia, adiposity, and the adipose tissue marker CD11c (Fig. 2B–D and Fig. S3A). Similarly, A. muciniphila treatment reduced body weight and improved body composition (i.e., fat mass/lean mass ratio) (Fig. S3 B and C) without changes in food intake (Fig. S3D). We demonstrated that A. muciniphila treatment completely reversed diet-induced fasting hyperglycemia (Fig. 2E) via a mechanism that was associated with a 40% reduction in hepatic glucose-6-phosphatase expression (Fig. 2F), thereby suggesting a reduction in gluconeogenesis. Notably, the insulin resistance index was similarly reduced after A. muciniphila treatment (Fig. S3E). These results suggest a key role for A. muciniphila in gut barrier function, metabolic inflammation, and fat storage. Therefore, we hypothesized that A. muciniphila would impact adipose tissue metabolism. We demonstrated that A. muciniphila treatment under an HF diet increased the mRNA expression of markers of adipocyte differentiation (e.g., CCAAT/enhancer-binding protein-α, encoded by Cebpα) and lipid oxidation (e.g., carnitine palmitoyltransferase-1, encoded by Cpt1; acyl-CoA oxidase, encoded by Acox1; peroxisome proliferator-activated receptor γ coactivator, encoded by Pgc1α; and peroxisome proliferator-activated receptor alpha, encoded by Ppara) without affecting lipogenesis markers (e.g., acetyl-CoA carboxylase, encoded by Accl) and fatty acid synthase, encoded by Fasn) (Fig. 2G). These data further confirm our hypothesis that A. muciniphila controls fat storage, adipose tissue metabolism, and glucose homeostasis.

A. muciniphila Treatment Exerted Minor Effects on Antibacterial Peptide Content in the Ileum and IgA Levels in the Feces. Recent data suggest that the intestinal mucosa contributes to the maintenance of the gut barrier by secreting antimicrobial peptides for innate immunity that are produced by Paneth cells (e.g., α-defensins, lysozyme C, phospholipases, and C-type lectin, primarily the RegIIIy) or enterocytes (RegIIIy) (10, 12). We measured the expression of Paneth and epithelial cell antibacterial markers in the ileum to elucidate the impact of the HF diet and A. muciniphila treatment on gut barrier function. A. muciniphila increased the
A. muciniphila 

sterile anaerobic PBS for 4 wk and fed a control (CT) or HF diet (HF) (CT in red cecal contents of control mice treated with a daily oral gavage containing using the MITChip phylogenetic modifying gut microbiota composition. (5). We demonstrated an association of decreased monoacylglycerol lipase expression with obesity, adipose tissue macrophage in 

endotoxemia and systemic inflammation (5). We also demonstrated previously that the pharmaco-

expression of Reg3γ (RegIIIγ) under the control diet, but this effect was not observed in HF-fed mice (Fig. S4A). Pla2g2a and Defa expression were similar between groups, but Lys1 expression tended to be lower after bacterial administration (Fig. S4 B–D). We also measured IgA in fecal samples as an adaptive immune system factor (13). Fecal IgA levels were not affected by the treatments (Fig. S4E), which suggests that A. muciniphila controls gut barrier function by other mechanisms of epithelial signaling (26).

A. muciniphila Increased Endocannabinoid (Acylglycerols) Content in the Ileum. We previously observed a link between gut microbiota and intestinal endocannabinoid system tone (5). We demonstrated an association of decreased monoacylglycerol lipase expression with improved gut barrier function and decreased metabolic inflammation (5). We also demonstrated previously that the pharmacological inhibition of monoacylglycerol lipase reduced metabolic endotoxemia and systemic inflammation (15), which suggests a direct link between acylglycerols and gut barrier function. Therefore, we measured intestinal acylglycerol levels and demonstrated that A. muciniphila treatment increased the levels of 2-OG, 2-AG, and 2-PG (Fig. 3A). These results support a direct link between A. muciniphila administration and intestinal levels of acylglycerols that are involved in glucose and intestinal homeostasis.

A. muciniphila Counteracted Diet-Induced Colon Mucosal Barrier Dysfunction During Obesity. Recent evidence suggests that interactions between the gut microbiota and mucus layer are dynamic systems that affect mucus barrier biology (9, 27). Therefore, we investigated the impact of A. muciniphila treatment on the thickness of the inner mucus layer. We demonstrated a 46% thinner mucus layer in HF-fed mice, and A. muciniphila treatment counteracted this decrease (Fig. 3 B and C).

Viable but Not Heat-Killed A. muciniphila Counteracted Diet-Induced Metabolic and Mucosal Barrier Dysfunction During Obesity. To further demonstrate whether A. muciniphila has to be alive to exert its metabolic effects, we have compared the impact of viable A. muciniphila administration with that of heat-killed A. muciniphila. We found that viable A. muciniphila counteracted diet-induced metabolic endotoxemia, fat mass development, and altered adipose tissue metabolism (Fig. 4 A, B, and D and Fig. S5A) to a similar extent as observed in the first set of experiments (Fig. 2 B, C, and G and Fig. S3A). Importantly, these effects were not observed after administration of heat-killed A. muciniphila (Fig. 4 A, B, and D and Fig. S5A). In addition, we found that viable A. muciniphila significantly reduced plasma glucose levels after an oral glucose tolerance test (Fig. 4C), whereas heat-killed A. muciniphila exhibited glucose intolerance similar to that of HF-fed mice (Fig. 4C). Finally, we confirmed that viable A. muciniphila...
restored mucus layer thickness upon HF-diet, whereas we found that heat-killed A. muciniphila did not improve mucus layer thickness compared with HF (Fig. 4 E and F). It is worth noting that we found 100-fold more viable A. muciniphila recovered from the cecal and colonic content of A. muciniphila-treated mice compared with the HF and heat-killed bacteria groups (HF-Akk: 9.5 ± 1.02 log10 cells/mg of content; HF and HF-K-Akk: 6.8 ± 0.51 log10 cells/mg of content; P = 0.0059), thereby evidencing the viability of A. muciniphila after oral administration.

This study confirms that that HF diet-induced obesity is associated with changes in gut microbiota composition (7) (28) (Fig. 2A and Fig. S2 B and C). However, antimicrobial peptides in the ileum were not affected by the treatments. In contrast, Reg3g expression in colon epithelial cells was significantly reduced, by ~50%, in HF and heat-killed A. muciniphila treated mice, whereas viable A. muciniphila treatment completely blunted this effect and increased Reg3g expression upon HF diet (Fig. S5B).

Discussion

This study demonstrated a dramatic decrease in A. muciniphila in genetically and diet-induced obese mice. We demonstrated that prebiotic (oligofructose) treatment restored A. muciniphila abundance and improved gut barrier and metabolic parameters. However, the mechanisms that were responsible for the bloom in A. muciniphila caused by prebiotic administration are not clear. A. muciniphila does not grow on oligofructose-enriched media (in vitro), which suggests that complex cross-feeding interactions contributed to this effect. However, it has been previously shown in rats that oligofructose feeding increases the number of goblet cells and mucus layer thickness (29). Thus, whether oligofructose feeding increases A. muciniphila by providing the main source of energy for this bacterium and thereby favoring its growth or whether the increase of A. muciniphila increases mucus production and degradation (i.e., turnover) remain to be demonstrated. Oligofructose changes more than 100 different taxa in mice (23). Therefore, we cannot exclude that oligofructose induces specific changes in the gut bacteria and cross-feeding promoting the growth of A. muciniphila. In the present study, we investigated the direct impact of A. muciniphila. We reversed the pathological phenotype by restoring the physiological abundance of this strain in obese and diabetic mice. These results demonstrated the key role of A. muciniphila in the pathophysiology of obesity, type 2 diabetes, and metabolic inflammation. These experiments clearly demonstrate that viable A. muciniphila controls gut barrier function, fat mass storage, and glucose homeostasis in obese and type 2 diabetic mice via several mechanisms. These results provide proof of concept in this context. The major weaknesses in investigations of the role of gut microbiota in the etiology of obesity and type 2 diabetes is the reliance on conclusions that are based on correlative data between bacteria (or one genus) and physiological parameters, because most of the gut bacteria have been identified at the phylogenetic level (i.e., through metagenomic approaches) but have never been cultured.

Several reports have demonstrated the importance of selected bacteria [i.e., Lactobacillus spp (30, 31), Bifidobacterium spp (32, 33), and Bacteroides uniformis CECTT 7771 (34)] on fat mass development during diet-induced obesity, but the aims of these studies were different from that of the present study. These studies investigated the impact of supplementation with one specific probiotic strain or strains that were isolated from healthy infants on physiological parameters. Here we investigated the strain that is affected during obesity and type 2 diabetes in humans and rodents (18, 23). Probiotics have far fewer opportunities for direct contact with the mucosa, but A. muciniphila may induces differential host responses because of more intensive contact with the host mucosa (26). To further confirm this hypothesis, we have treated HF-fed mice with a probiotic (i.e., Lactobacillus plantarum WCFS1). We found that L. plantarum administration did not change fat mass development, adipose tissue metabolism, mucus layer thickness, colon Reg3g mRNA, and metabolic endotoxemia (Fig. S6 A–E). Therefore, these data suggest that A. muciniphila induces specific host responses compared with other putative beneficial microbes.

A. muciniphila is a Gram-negative bacteria (i.e., it contains LPS) that constitutes 3–5% of the gut microbial community. However, our study clearly demonstrated the lack of a direct relationship between the abundance of Gram-negative bacteria within the gut and metabolic endotoxemia (i.e., that is caused by serum LPS) because gut colonization by A. muciniphila decreased metabolic endotoxemia arising on an HF diet. One explanation for this
counterintuitive result may be that *A. muciniphila* regulates gut barrier function at different levels. Previous data suggest that gut microbiota contribute to gut barrier alterations during obesity and metabolic endotoxemia (4). However, the different mechanisms of interaction between bacteria and the host that affect gut barrier function during obesity and type 2 diabetes have not been elucidated. This study identified an association of obesity with a decrease in mucus thickness, which supports an additional mechanism of increased gut permeability (i.e., metabolic endotoxemia) that is characteristic of obesity and associated disorders. Furthermore, we demonstrated that *A. muciniphila* restored this mucus layer, which suggests that this mechanism contributes to the reduction in metabolic endotoxemia that was observed during *A. muciniphila* treatment. Moreover, we found that viable *A. muciniphila* induces these effects, whereas heat-killed *A. muciniphila* did not protect the mice from diet-induced obesity and associated disorders.

These results suggest that the presence of viable *A. muciniphila* within the mucus layer is a crucial mechanism in the control of host mucus turnover (19), which improves gut barrier function. However, we cannot exclude additional mechanisms that have been implicated in the regulation of gut barrier. For example, we previously demonstrated that gut microbiota control gut peptides (e.g., GLP-2) that regulate epithelial cell proliferation and gut barrier function (4). Prebiotics stimulate GLP-1 and GLP-2 secretion by acting on the enteroncephaline L-cells that are primarily in the ileum and colon (6). The abundance of *A. muciniphila* is associated with higher L-cell activity (i.e., GLP-1 and GLP-2 secretion) (4, 23), but the mechanisms underlying this relationship are not known. Here, we demonstrated that *A. muciniphila* administration significantly increased intestinal levels of 2-OG, which stimulates glucagon-like peptide secretion from intestinal L-cells (17). Altogether our data suggest that this could be a key mechanism by which *A. muciniphila* controls gut barrier function, metabolic endotoxemia, and metabolism. We also demonstrated that *A. muciniphila* administration increased 2-AG intestinal levels. We recently demonstrated that an increase in 2-AG endogenous levels induced by selective monoacylglycerol lipase inhibitor protects against trinitrobenzene sulfonic acid-induced colitis in mice (15) and reduces metabolic endotoxemia as well as the level of circulating inflammatory cytokines and peripheral and brain inflammation. Therefore, the increased 2-AG levels that were observed after *A. muciniphila* treatment may have also contributed to the reduced inflammation. However, whether the induction of these endocannabinoids after *A. muciniphila* treatment constitutes the molecular event that links these metabolic features warrants further investigation.

Specifically, we demonstrated that the restoration of the physiological abundance of *A. muciniphila* reduced diet-induced body weight gain, fat mass development, and fasting hyperglycemia without affecting food intake. This variation in energy storage is explained by the normalization of adipose tissue adipogenesis (i.e., differentiation and lipogenesis) and fatty acid oxidation. We have previously demonstrated that higher circulating LPS levels inhibit adipose tissue differentiation and lipogenesis, thereby contributing to altered adipose tissue metabolism characterizing obesity (5). Thus, we postulate that *A. muciniphila* restores gut barrier function and thereby contributes to normalize metabolic endotoxemia and adipose tissue metabolism. We found that *A. muciniphila* improved glucose tolerance and decreased endogenous hepatic glucose production. These findings are not in agreement with the apparent but low association of *A. muciniphila* genes with type 2 diabetes-associated metagenome-wide associated studies (25). Nevertheless, the data by Qin et al. remain to be confirmed because this related to only 337 of the 2,176 *A. muciniphila* genes (35) and may be confounded by dietary or pharmaceutical treatments specifically favoring its growth in the human intestine.

Dynamic insulin resistance assessments and the present results suggest improved insulin sensitivity. However, we cannot exclude the possibility that the improvements in glucose and lipid metabolism occurred via an LPS-dependent mechanism, as demonstrated previously (5, 7). We confirmed (7, 36) that an HF diet profoundly affected the gut microbiota composition, whereas *A. muciniphila* administration did not significantly affect this profile. Therefore, it is tempting to extrapolate our findings as a single-or species-dependent modulation of the gut microbiota. Moreover, because heat-killing of *A. muciniphila* completely abolished the metabolic effects it is unlikely that specific *A. muciniphila*-derived cell-envelope components may directly contribute to the phenotype observed with viable *A. muciniphila*. It is worth noting that this observation also minimizes the possibility that the host response was caused by a substance in the culture media. However, although not directly fitting with the aim of the present study, follow-up studies of the gut microbiome after viable *A. muciniphila* administration may identify the components that contribute to disease or the host physiological response (37).

Finally, we demonstrated that *A. muciniphila* regulates intestinal antimicrobial peptides in the colon (e.g., RegIIIγ). *A. muciniphila* exerted minor effects on antimicrobial peptide production in the ileum. RegIIIγ exerts direct bacieroidal activity against Gram-positive bacteria in the intestine. Therefore, *A. muciniphila* may manipulate host immunity to favor its own survival through an increase in RegIIIγ expression, which reduces the competition for resources and induces long-term tolerance for the development in the mucus layer. Here, we clearly found that viable *A. muciniphila* significantly increased RegIIIγ, whereas heat-killed *A. muciniphila*. Moreover, because heat-killing of *A. muciniphila* completely abolished the metabolic effects it is unlikely that specific *A. muciniphila*-derived cell-envelope components may directly contribute to the phenotype observed with viable *A. muciniphila*. It is worth noting that this observation also minimizes the possibility that the host response was caused by a substance in the culture media. However, although not directly fitting with the aim of the present study, follow-up studies of the gut microbiome after viable *A. muciniphila* administration may identify the components that contribute to disease or the host physiological response (37).

Materials and Methods

**Mice.** Male C57BL/6 mice were used in the four series of experiments. Cecal contents from genetic (ob/ob) and HF-fed obese and type 2 diabetic mice were harvested, immersed in liquid nitrogen, and stored at −80 °C for further *A. muciniphila* analysis. A subset of 10-wk-old C57BL/6J was fed a control diet (CT) or an HF diet (60% fat). The mice were treated with *A. muciniphila*.
by oral gavage at a dose 2.10^8 cfu/0.2 mL suspended in sterile anaerobic PBS (CT-Akk and HF-Akk), or heat-killed *A. muciniphila* (Muc^−^) (ATCC BAA-835) was grown anaerobically in a mucin-based basal medium as described previously (18). The cultures were washed and concentrated in anaerobic PBS that included 25% (vol/vol) glycerol to an end concentration of 1.10^10 cfu/mL under strict anaerobic conditions. Body composition was assessed using a 7.5MHz time-domain NMR. Blood, adipose depots, liver, cerebrospinal fluid, and intestinal segments (ileum, cecum, and colon) were collected at death and analyzed. A complete description of the mouse experiments and bacteria preparation is provided in *SI Material and Methods.*

**Gut Microbiota Analysis.** Gut microbiota analyses were performed using real-time quantitative PCR (qPCR) analysis and the MITChip, which is a phylogenetic microarray consisting of 3,580 different oligonucleotide probes that target two hypervariable regions of the 16S rRNA gene (the V1 and V6 regions). Analyses of the MITChip were performed as described previously (23, 40) and in *SI Material and Methods.*

**Gene Expression Analysis.** The expression of metabolic genes of interest and RNA expression profiles were analyzed using real-time qPCR analysis as described in *SI Material and Methods.*

**Measurement of Endocannabinoid Intestinal Levels.** Intestinal endocannabinoids were measured using an LTOQ Orbitrap mass spectrometer as described in *SI Material and Methods.*

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**Biochemical Analysis.** Plasma insulin and fecal IgA were analyzed using ELISA as described in *SI Material and Methods.* The thickness of the mucus layer was measured in proximal colon segments that were fixed in Carney's solution and in S-μm paraffin sections stained with alcian blue as described in *SI Material and Methods.* LPS concentrations in portal vein blood were measured using Endosafe-Multi-Cardiature System based on the limulus amebocyte lysate kinetic chromogenic methodology as described in *SI Material and Methods.*

**Statistical Analysis.** Data are expressed as means ± SEM. Differences between two groups were assessed using the unpaired two-tailed Student t test. Data sets that involved more than two groups were assessed using ANOVA followed by Newman-Keuls post hoc tests. Correlations were analyzed using Pearson's correlation. In the figures, data with different superscript letters are significantly different at *P* < 0.05, according to post hoc ANOVA statistical analyses. Data were analyzed using GraphPad Prism version 5.00 for Windows (GraphPad Software). The results were considered statistically significant when *P* < 0.05.

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Supporting Information

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SI Materials and Methods

Mice. **Experiment 1: Ob/Ob experiments.** For ob/ob vs. lean study, 6-wk-old ob/ob (n = 5 per group) mice (C57BL/6 background, Jackson Laboratories) were housed in a controlled environment (12-h daylight cycle, lights off at 0600 hours) in groups of two or three mice per cage, with free access to food and water. The mice were fed a control diet (A040) for 16 wk. Cecal content was harvested immersed in liquid nitrogen and stored at −80 °C for further **Akermannia muciniphila** analysis.

**Experiment 2: Ob-prebiotic study.** Six-week-old ob/ob (n = 10 per group) mice (C57BL/6 background, Jackson Laboratories) were housed in a controlled environment (12-h daylight cycle, lights off at 0600 hours) in groups of two mice/cage, with free access to food and water. The mice were fed a control diet (A040) or a control diet supplemented with prebiotics (oligofructose) (10 g/100 g of diet) (Orafti) for 5 wk as previously described (1). This set of mice has been previously characterized by Everard et al. (1).

**Experiment 3: High-fat diet prebiotics experiments.** A set of 10-wk-old C57BL/6J mice (40 mice, n = 10 per group) (Charles River Laboratories) were housed in groups of five mice per cage, with free access to food and water. The mice were fed a control diet (A040) or a control diet supplemented with prebiotics (oligofructose) (Orafti) (0.3 g per mouse per day) added in tap water, or fed a high-fat (HF) diet [60% fat and 20% carbohydrates (kcal/100 g), D12492; Research Diet] or an HF diet supplemented with oligofructose (0.3 g per mouse per day) added in tap water. Treatment continued for 8 wk.

**Experiment 4: HF diet A. muciniphila treatment.** A set of 10-wk-old C57BL/6J mice (40 mice, n = 10 per group) (Charles River Laboratories) were housed in groups of 2 mice per cage (filter-top cages), with free access to food and water. The mice were fed a control diet (A040) or a control diet supplemented with prebiotics (oligofructose) (Orafti) (0.3 g per mouse per day) added in tap water, or fed a high-fat (HF) diet [60% fat and 20% carbohydrates (kcal/100 g), D12492; Research Diet] or an HF diet supplemented with oligofructose (0.3 g per mouse per day) added in tap water. Treatment continued for 4 wk. Control and HF groups were treated daily with an oral gavage of an equivalent volume of sterile anaerobic PBS containing a similar end concentration of glycerol (2.5%) (reduced with one drop of 100 mM titanium citrate) as the treatment groups for 4 wk. The viability of **A. muciniphila** was confirmed by serially diluting the cecal and fecal content immediately postmortem in anaerobe basal mucin-based medium (2) and confirmed with **A. muciniphila**-specific PCR primers (detailed below).

Food and water intake were recorded once per week. Pellets and spillage were weighed separately. Values for the weekly assessment were calculated on the basis of two mice per cage and five cages per group (n = 10 mice per group); the data were reported as cumulative food intake per mouse.

Body composition was assessed by using 7.5 MHz time domain-NMR (LF50 minispec; Bruker).

All mouse experiments were approved by and performed in accordance with the guidelines of the local ethics committee. Housing conditions were specified by the Belgian Law of April 6, 2010, regarding the protection of laboratory animals (agreement number LA1230314).

**Tissue Sampling.** The animals were anesthetized with isoflurane (Forene; Abbott) before exsanguination and tissue sampling, then mice were killed by cervical dislocation. Adipose depots (epi- didymal, s.c., and mesenteric) and liver were precisely dissected and weighed; the addition of the three adipose tissues corresponds to the adiposity index. The intestinal segments (ileum, cecum, and colon), cecal content, and adipose tissues were immersed in liquid nitrogen and stored at −80 °C for further analysis.

Mucus Layer Thickness. Proximal colon segments were immediately removed and fixed in Carnoy’s solution (ethanol 6: acid acetic 3: chloroform 1, vol/vol) for 2 h at 4 °C. They were then immersed in ethanol 100% for 24 h. Paraffin sections of 5 μm were stained with alcian blue. A minimum of 20 different measurements were made perpendicular to the inner mucus layer per field. Five to nineteen randomly selected fields were analyzed for each colon for a total of 4,549 measurements by using an image analyzer (Motic-image Plus 2.0ML; Motic).

**RNA Preparation and Real-Time Quantitative PCR Analysis.** Total RNA was prepared from tissues using TriPure reagent (Roche). Quantification and integrity analysis of total RNA was performed by running 1 μL of each sample on an Agilent 2100 Bioanalyzer (Agilent RNA 6000 Nano Kit). cDNA was prepared by reverse transcription of 1 μg total RNA using a Reverse Transcription System Kit (Promega). Real-time PCRs were performed with the StepOnePlus real-time PCR system and software (Applied Biosystems) using Mesa Fast qPCR (Eurogentec) for detection according to the manufacturer’s instructions. RPL19 RNA was chosen as the housekeeping gene. All samples were run in groups of 2 mice per cage (filter-top cages), and mice had free access to food and water. The mice were fed the same control diet or an HF diet as described above. **A. muciniphila** was daily administered by oral gavage at the dose 2.10^6 cfu/0.2 mL suspended in sterile anaerobic PBS. **A. muciniphila** was heat-killed by autoclaving (15 min, 121 °C, 225 kPa). A viability check by culturing on mucin-containing medium confirmed the absence of any viable cells. **Lactobacillus plantarum** WCF51 was grown anaerobically in MRS medium (Difco Lactobacilli MRS broth; BD), washed, concentrated, and manipulated an identical way as the **A. muciniphila** preparation. The two control groups (CT and HF) were treated daily with an oral gavage of an equivalent volume of sterile anaerobic PBS containing a similar end concentration of glycerol (2.5%) (reduced with one drop of 100 mM titanium citrate) as the treatment groups for 4 wk. The viability of **A. muciniphila** was confirmed by serially diluting the cecal and fecal content immediately postmortem in anaerobe basal mucin-based medium (2) and confirmed with **A. muciniphila**-specific PCR primers (detailed below).

Food and water intake were recorded once per week. Pellets and spillage were weighed separately. Values for the weekly assessment were calculated on the basis of two mice per cage and five cages per group (n = 10 mice per group); the data were reported as cumulative food intake per mouse.

Body composition was assessed by using 7.5 MHz time domain-NMR (LF50 minispec; Bruker).

All mouse experiments were approved by and performed in accordance with the guidelines of the local ethics committee. Housing conditions were specified by the Belgian Law of April 6, 2010, regarding the protection of laboratory animals (agreement number LA1230314).

**Tissue Sampling.** The animals were anesthetized with isoflurane (Forene; Abbott) before exsanguination and tissue sampling, then mice were killed by cervical dislocation. Adipose depots (epi- didymal, s.c., and mesenteric) and liver were precisely dissected and weighed; the addition of the three adipose tissues corresponds to the adiposity index. The intestinal segments (ileum, cecum, and colon), cecal content, and adipose tissues were immersed in liquid nitrogen and stored at −80 °C for further analysis.

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duplicate in a single 96-well reaction plate, and data were analyzed according to the 2-\Delta\DeltaCT method. The identity and purity of the amplified product was checked through analysis of the melting curve carried out at the end of amplification. Primer sequences for the targeted mouse genes are presented in Table S1.

**Insulin Resistance Index.** Insulin resistance index was determined by multiplying the area under the curve (0 min and 15 min) of both blood glucose and plasma insulin obtained after an oral glucose load (2 g of glucose per kg of body weight) performed after 4 wk (*A. muciniphila* study) of treatment. Food was removed 2 h after the onset of the daylight cycle, and mice were treated after a 6-h fasting period as previously described (1).

**Biochemical Analyses.** Portal vein blood LPS concentration was measured using an Endosafe-Multi-Cartridge System (Charles River Laboratories) based on the Limulus amebocyte lysate (LAL) kinetic chromogenic methodology that measures color intensity directly related to the endotoxin concentration in a sample. Serum were diluted 1/10 with endotoxin-free buffer to minimize interference in the reaction (inhibition or enhancement) and heated 15 min at 70 °C. Each sample was diluted 1/70 or 1/100 with endotoxin-free LAL reagent water (Charles River Laboratories) and treated in duplicate, and two spikes for each sample were included in the determination. All samples have been validated for the recovery and the coefficient variation. The lower limit of detection was 0.005 EU/mL. Plasma insulin concentration was determined in 25 μL of plasma using an ELISA kit (Mercodia) according to the manufacturer’s instructions.

**DNA Isolation from Mouse Cecal Samples.** The cecal content of mice collected postmortem was stored at −80 °C. Metagenomic DNA was extracted from the cecal content using a QiAamp-DNA stool minikit (Qiagen) according to the manufacturer’s instructions.

**Measurement of Endocannabinoids Intestinal Levels.** Ileal tissues were homogenized in CHCl3 (10 mL), and a deuterated standard (200 pmol) were added. The extraction and the calibration curves were generated as previously described (3), and the data were normalized by tissue sample weight.

**qPCR: Primers and Conditions.** The primers and probes used to detect *A. muciniphila* were based on 16S rRNA gene sequences: forward *A. muciniphila*, CAGCACGTGAAGTGCCCCAC, reverse *A. muciniphila*, CCTTGCGGTTGGCTTCAGAT. Detection was achieved with StepOnePlus real-time PCR system and software (Applied Biosystems) using Mesa Fast qPCR (Eurogentec) according to the manufacturer’s instructions. Each assay was performed in duplicate in the same run. The cycle threshold of each sample was then compared with a standard curve (performed in triplicate) made by diluting genomic DNA (fivefold serial dilution) (DSMZ). The data are expressed as log of bacteria per g of cecal content.

**Mouse Intestinal Tract Chip: PCR Primers and Conditions.** The Mouse Intestinal Tract Chip (MITChip) is a phylogenetic microarray consisting of 3,580 different oligonucleotides specific for the mouse intestinal microbiota. Both the design and analysis of the MITChip were performed as previously described (1, 4).

**Statistical Analysis.** Data are expressed as means ± SEM. Differences between two groups were assessed using the unpaired two-tailed Student’s t test. Data sets involving more than two groups were assessed using ANOVA followed by Newman-Keuls post hoc tests after normalization by log transformation. Correlations were analyzed using Pearson’s correlation. Data with different superscript letters are significantly different (*P* < 0.05, according to post hoc ANOVA statistical analysis). Data were analyzed using GraphPad Prism version 5.00 for windows (GraphPad Software). Results were considered statistically significant when *P* < 0.05.

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**Figure S1.** Prebiotic-treated mice on an HF diet exhibited a decrease in s.c., mesenteric, and epididymal fat mass and in body weight. (A) s.c., mesenteric, and epididymal fat depot weights (g per 100 g body weight) measured in control diet-fed mice (CT), control diet-fed mice treated with prebiotics (CT-Pre), HF diet-fed mice (HF), and HF diet-fed mice treated with prebiotics (HF-Pre) (*n* = 10). (B) Final fat mass expressed in percentage of final body weight and measured by time-domain NMR (*n* = 10). (C) Final body weight (*n* = 10). (D) Pearson’s correlation between adipose tissue *CD11c* mRNA levels and *A. muciniphila* abundance (log10 of bacteria per g of cecal content) measured in the cecal content; (Inset) Pearson’s correlation coefficient (r) and the corresponding *P* value. (E) Pearson’s correlation between adipose tissue mass gain and cecal content of *A. muciniphila* (log10 of bacteria per g of cecal content); (Inset) Pearson’s correlation coefficient (r) and the corresponding *P* value. Data are shown as means ± SEM. Data with different superscript letters are significantly different (*P* < 0.05) according to post hoc ANOVA one-way statistical analysis.

Fig. S2. Daily oral gavage with *A. muciniphila* restored *A. muciniphila* to basal levels in the cecal content without modifying gut microbiota composition. (A) *A. muciniphila* abundance (log_{10} of bacteria per g of cecal content) measured in the cecal content of mice treated with a daily oral gavage containing *A. muciniphila* (2.10^8 bacterial cells suspended in 200 μL of sterile anaerobic PBS) and fed a control (CT-Akk) or HF diet (HF-Akk), compared with mice fed a control (CT) or HF diet (HF) that were treated with a daily oral gavage containing an equivalent volume of sterile anaerobic PBS for 4 wk (n = 10). (B) Dendrogram clustering of the MITChip phylogenetic fingerprints of the gut microbiota. (C) Representational difference analysis (RDA) plot based on MITChip phylogenetic fingerprints of the gut microbiota; CT-Akk is noted as CTA, and HF-fed mice that received *A. muciniphila* are noted as HFA. Data are shown as means ± SEM. Data with different superscript letters are significantly different (P < 0.05) according to post hoc ANOVA one-way statistical analysis.
**Fig. S3.** *A. muciniphila* treatment reduced s.c., mesenteric, and epididymal fat mass, body weight, and insulin resistance in mice on an HF diet without affecting food intake. (A) s.c., mesenteric, and epididymal fat depot weights (g per 100 g body weight) measured in mice treated daily with an oral gavage of *A. muciniphila* and fed a control (CT-Akk) or HF diet (HF-Akk) or mice fed a control (CT) or HF diet and treated daily with an oral gavage of sterile anaerobic PBS (*n* = 10). (B) Final body weight (*n* = 10). (C) Final fat and lean mass expressed in percentage of final body weight and measured by time-domain NMR (*n* = 10). (D) Cumulative food intake (g) over the 4 wk of treatment. (E) Insulin resistance index was determined by multiplying the area under the curve (from 0 min to 15 min) of blood glucose and plasma insulin that were obtained after an oral glucose load (2 g glucose per kg of body weight) after 4 wk of treatment (*n* = 10). Data are shown as means ± SEM. Data with different superscript letters are significantly different (*P* < 0.05) according to post hoc ANOVA one-way statistical analysis.

**Fig. S4.** *A. muciniphila* treatment exerted minor effects on antibacterial peptide contents in the ileum and IgA levels in the feces. Antibacterial peptide mRNA expression: (A) regenerating islet-derived 3-γ (RegIIIγ, encoded by Reg3g), (B) phospholipase A2 group IIA (encoded by Pla2g2a), (C) α-defensins (encoded by Defa), and (D) lysozyme C (encoded by Lyz1) measured in the ileum of mice treated daily with an oral gavage of *A. muciniphila* (2.10^8 bacterial cells suspended in 200 μL of sterile anaerobic PBS) and fed a control (CT-Akk) or HF-diet (HF-Akk) or mice fed a control (CT) or HF diet and treated daily with an oral gavage of an equivalent volume of sterile anaerobic PBS for 4 wk (*n* = 10). (E) Fecal IgA levels (μg/g of feces). Data are shown as means ± SEM. Data with different superscript letters are significantly different (*P* < 0.05) according to post hoc ANOVA one-way statistical analysis.

**Fig. S5.** Heat-Killed *A. muciniphila* did not reduce s.c., mesenteric, and epididymal fat mass and did not increase colon antimicrobial peptides in mice on an HF diet. (A) s.c., mesenteric, and epididymal fat depot weights (g per 100 g body weight) measured in control mice fed a control (CT) or HF diet (HF) and treated with a daily oral gavage containing sterile anaerobic PBS and glycerol for 4 wk. Treated mice received an oral gavage of alive *A. muciniphila* (HF-Akk) or killed *A. muciniphila* (HF-K-Akk) (2.10^8 bacterial cells suspended in 200 μL of sterile anaerobic PBS) and fed a HF diet (*n* = 8). (B) mRNA expression of colon RegIIIγ (encoded by Reg3g) mRNA expression (*n* = 8–18); data represent the results from the two *A. muciniphila* studies. Data are shown as means ± SEM. Data with different superscript letters are significantly different (*P* < 0.05) according to post hoc ANOVA one-way statistical analysis.
Fig. S6. L. plantarum did not reduce fat mass and did not improve adipose tissue metabolism and gut barrier function in diet-induced obese mice. Control mice were fed a control (CT) or HF diet (HF) and treated with a daily oral gavage containing sterile anaerobic PBS and glycerol for 4 wk daily. Treated mice received an oral gavage of L. plantarum (HF-LP) (2.10^8 bacterial cells suspended in 200 μL of sterile anaerobic PBS) and fed a HF diet (n = 7–8). (A) Final fat mass measured by time-domain NMR (n = 7–8). s.c., mesenteric, and epididymal fat depot weights (g per 100 g body weight) (n = 7–8). (B) mRNA expression of markers of adipocyte differentiation (Cebpα, Lipogenesis) (n = 7–8). (C) Thickness of the mucus layer measured by histological analyses after alcian blue staining (n = 4–6). (D) Portal vein serum LPS levels (n = 6–7). (E) mRNA expression of colon RegIIIγ (encoded by Reg3g) (n = 8–18). Data are shown as means ± SEM. Data with different superscript letters are significantly different (P < 0.05) according to post hoc ANOVA one-way statistical analysis.

Table S1. Primer sequences

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