LETTERS
https://doi.org/10.1038/s41591-019-0495-2
nature medicine

Supplementation with *Akkermansia muciniphila* in overweight and obese human volunteers: a proof-of-concept exploratory study

Clara Depommier[1,9], Amandine Everard[1,9], Céline Druart[1], Hubert Plovier[1], Matthias Van Hul[1], Sara Vieira-Silva[2,3], Gwen Falony[2,3], Jeroen Raes[2,3], Dominique Maiter[4,5], Nathalie M. Delzenne[6], Marie de Barsy[4,5,10], Audrey Loumaye[4,5,10], Michel P. Hermans[4,5,10], Jean-Paul Thissen[4,5,10], Willem M. de Vos[7,8,10] and Patrice D. Cani[1*]

Metabolic syndrome is characterized by a constellation of comorbidities that predispose individuals to an increased risk of developing cardiovascular pathologies as well as type 2 diabetes mellitus[1]. The gut microbiota is a new key contributor involved in the onset of obesity-related disorders[2]. In humans, studies have provided evidence for a negative correlation between *Akkermansia muciniphila* abundance and overweight, obesity, untreated type 2 diabetes mellitus or hypertension[3–8]. Since the administration of *A. muciniphila* has never been investigated in humans, we conducted a randomized, double-blind, placebo-controlled pilot study in overweight/obese insulin-resistant volunteers; 40 were enrolled and 32 completed the trial. The primary end points were safety, tolerability and metabolic parameters (that is, insulin resistance, circulating lipids, visceral adiposity and body mass). Secondary outcomes were gut barrier function (that is, plasma lipopolysaccharides) and gut microbiota composition. In this single-center study, we demonstrated that daily oral supplementation of 10^{10} *A. muciniphila* bacteria either live or pasteurized for three months was safe and well tolerated. Compared to placebo, pasteurized *A. muciniphila* improved insulin sensitivity ($+28.62 \pm 7.02\%$, $P = 0.002$), and reduced insulinemia ($-34.08 \pm 7.12\%$, $P = 0.006$) and plasma total cholesterol ($-8.68 \pm 2.38\%$, $P = 0.02$). Pasteurized *A. muciniphila* supplementation slightly decreased body weight (-2.27 ± 0.92 kg, $P = 0.091$) compared to the placebo group, and fat mass (-1.37 ± 0.82 kg, $P = 0.092$) and hip circumference (-2.63 ± 1.14 cm, $P = 0.091$) compared to baseline. After three months of supplementation, *A. muciniphila* reduced the levels of the relevant blood markers for liver dysfunction and inflammation while the overall gut microbiome structure was unaffected. In conclusion, this proof-of-concept study (clinical trial no. NCT02637115) shows that the intervention was safe and well tolerated and that supplementation with *A. muciniphila* improves several metabolic parameters.

To overcome the worldwide evolution of cardiometabolic diseases, research has increasingly focused its attention on interventions that target the gut microbiota[2]. Among commensal bacteria residing in the intestine, *Akkermansia muciniphila* has attracted growing interest for its health-promoting effects[9]. In rodents, treatment with *A. muciniphila* reduces obesity and related disorders, such as glucose intolerance, insulin resistance, steatosis and gut permeability[10–12]. Recently, in rodents, we serendipitously discovered that pasteurization of *A. muciniphila* enhances its beneficial properties on adiposity, insulin resistance and glucose tolerance[11]. However, translational evaluation of *A. muciniphila* for human investigation was hampered by the need for animal-derived compounds in the growth medium used to culture this bacterium. We circumvented this major issue by developing a synthetic medium compatible with human administration[11].

The main objectives of this exploratory study were (1) to evaluate the feasibility, safety and tolerance of *A. muciniphila* supplementation, and (2) to explore for the first time the metabolic effects of *A. muciniphila* supplementation in humans. The study was designed as an exploratory and proof-of-concept study for a first supplementation in humans. The primary outcomes were safety, tolerability (that is, hepatic and renal function, inflammation) and metabolic parameters (that is, insulin resistance, circulating lipids, visceral adiposity and body mass index (BMI)). The secondary outcomes were gut barrier function (that is, plasma lipopolysaccharides (LPS)/metabolic endotoxemia), gut microbiota composition and metabolites. In 2017, the first reported preliminary human data from this study obtained using 5 volunteers per group suggested that treatment with either placebo, two doses of live *A. muciniphila* (low dose of 10^9 bacteria per day or high dose of 10^{10} bacteria per day) or pasteurized *A. muciniphila* (10^{10} bacteria per day) was safe in individuals with excess body weight; no changes in safety parameters or reported adverse events were observed after 15 d of daily administration[11].

In this study, we further extended this randomized, double-blind, placebo-controlled proof-of-concept and feasibility study using daily oral administration of *A. muciniphila* for 3 months,

[1]Metabolism and Nutrition Research Group, Louvain Drug Research Institute, WELBIO, Walloon Excellence in Life Sciences and BIOtechnology, UCLouvain, Université catholique de Louvain, Brussels, Belgium. [2]Laboratory of Molecular Bacteriology—Department of Microbiology and Immunology, KU Leuven, Leuven, Belgium. [3]Center for Microbiology, VIB, Leuven, Belgium. [4]Pôle EDIN, Institut de Recherches Expérimentales et Cliniques, UCLouvain, Université catholique de Louvain, Louvain-la-Neuve, Belgium. [5]Division of Endocrinology and Nutrition, Cliniques universitaires St-Luc, Brussels, Belgium. [6]Metabolism and Nutrition Research Group, Louvain Drug Research Institute, UCLouvain, Université catholique de Louvain, Brussels, Belgium. [7]Laboratory of Microbiology, Wageningen University, Wageningen, the Netherlands. [8]Human Microbiome Research Program, Faculty of Medicine, University of Helsinki, Helsinki, Finland. [9]These authors contributed equally: Clara Depommier, Amandine Everard. [10]These authors jointly supervised this work: Marie de Barsy, Audrey Loumaye, Michel P. Hermans, Jean-Paul Thissen, Willem M. de Vos. *e-mail: Patrice.cani@uclouvain.be

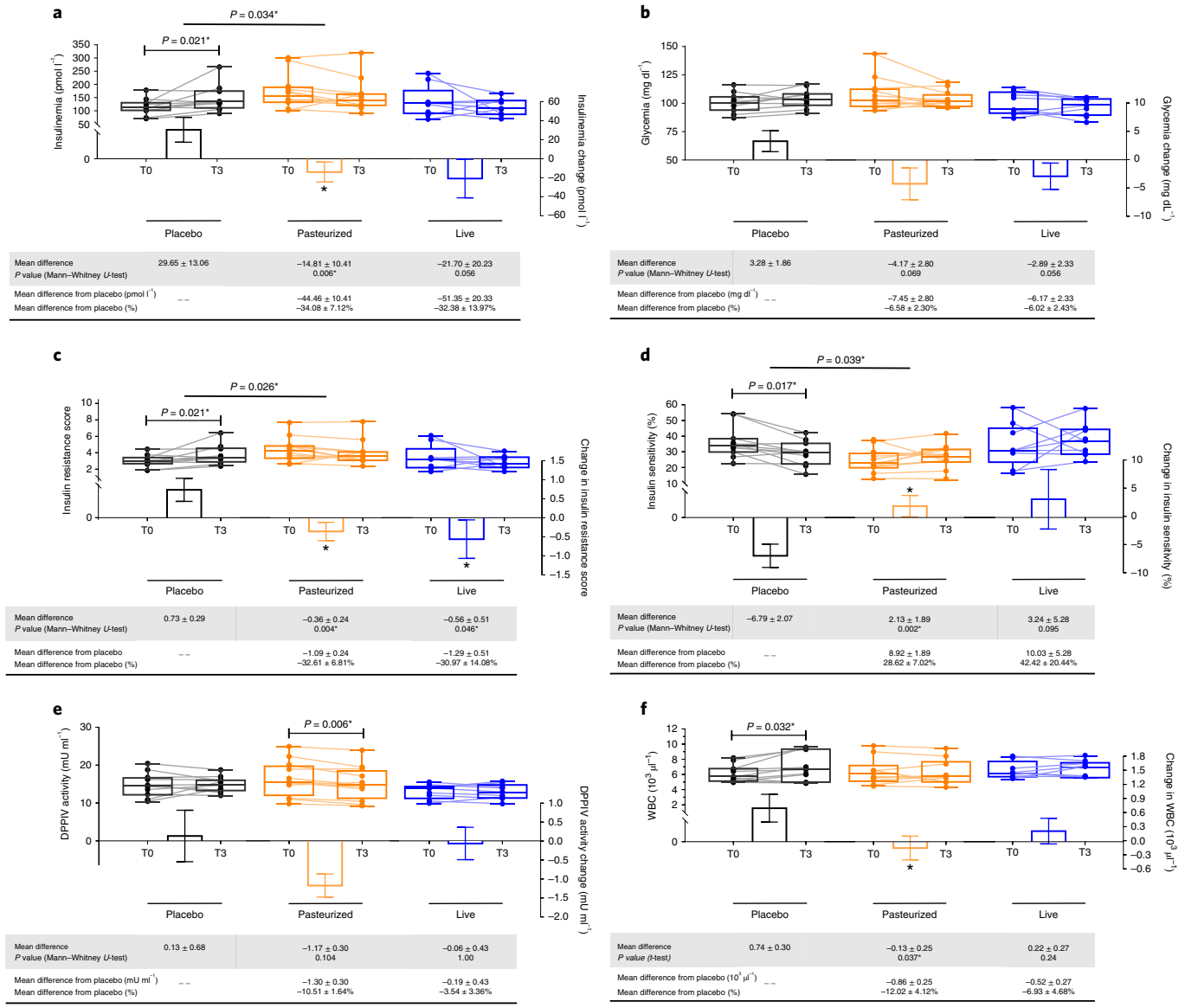


Fig. 1 | Changes in parameters related to glucose metabolism and WBC. a, Insulinemia. **b**, Glycemia. **c**, Insulin resistance score. **d**, Insulin sensitivity. **e**, DDPIV activity. **f**, White blood cell count. Differential values (mean difference and mean difference from placebo) are expressed as the mean ± s.e.m., either as raw data or as percentages. The bars represent the mean change from baseline value per group, with their s.e.m. Mann–Whitney *U*-tests or unpaired *t*-tests were performed to compare the differential values of both treated groups versus the placebo group (intergroup changes), according to the distribution. The respective *P* values are indicated in the table below each plot; when the test is significant, the bars are marked with an asterisk. The lines represent the raw values before and after three months of supplementation. The distribution of values within each group for each timing is illustrated by a box-and-whisker plot. In the box plots, the line in the middle of the box is plotted at the median, and the inferior and superior limits of the box correspond to the 25th and the 75th percentiles, respectively. The whiskers correspond to the minimum and maximum values. Matched-pairs Wilcoxon signed-rank tests or paired *t*-tests were performed to verify changes from baseline (intragroup changes), according to the distribution. When the difference is significant, a capped line is marked above the group concerned with the corresponding *P* value. Changes between 0 and 3 months across the 3 groups were analyzed with Kruskal–Wallis or one-way ANOVA tests according to the distribution; group-wise comparisons were performed using Bonferroni and Tukey's corrections for multiple testing, respectively. When the difference is significant, a line is marked above the concerned groups with the corresponding *P* value. Placebo group, $n = 11$; pasteurized bacteria group, $n = 12$; live bacteria group, $n = 9$ for all parameters, except for WBC: placebo, $n = 11$; pasteurized bacteria group, $n = 11$; live bacteria group, $n = 8$. All tests were two-tailed. $^*P < 0.05$.

either live or pasteurized and compared their effects at the highest dose tested, that is, at 10^{10} bacteria per day, in individuals exhibiting excess body weight (overweight or obese), insulin resistance and metabolic syndrome.

Individuals were enrolled and underwent randomization to receive either a placebo, live *A. muciniphila* (10^{10} bacteria per day) or pasteurized *A. muciniphila* (10^{10} bacteria per day) as a supplement for 3 months, with the specific advice to keep to their normal dietary intake and physical activity during the study period (see flow chart in Extended Data Fig. 1). Although participants were randomized, we found that before starting supplementation (that is, at T0), participants receiving pasteurized cells exhibited significantly higher levels of insulin and lower insulin sensitivity than those in the placebo group (Supplementary Table 1). In the interest of safety, an early visit was scheduled 15 d after the start of supplementation. We found that both safety and tolerability were

similar between the two groups receiving the different forms of *A. muciniphila* compared to the placebo group (Supplementary Tables 2 and 3), except for a higher white blood cell count (WBC) in the placebo and treated groups (Supplementary Table 2). We further followed safety and tolerability parameters until three months after the start of supplementation and did not observe any adverse events (Supplementary Tables 4 and 5). In addition, compliance was higher than 99% in all groups (Supplementary Table 5).

After 3 months, the placebo group exhibited a significant increase of fasting plasma insulin ($P < 0.05$, T3 versus T0; Fig. 1a), contrary to participants receiving both forms of *A. muciniphila* where reduced plasma insulin levels (approximately 30%) were observed compared to the placebo group (Fig. 1a). This effect was significant between the pasteurized *A. muciniphila* and placebo groups (Fig. 1a). Fasting glycemia was not affected (Fig. 1b); however, participants were not highly hyperglycemic at baseline (Supplementary Table 1).

We also measured insulin sensitivity and resistance (homeostatic model assessment (HOMA) method) and found that insulin sensitivity was significantly reduced at T3 in the placebo group (Fig. 1c,d). Both forms of *A. muciniphila* improved this parameter. Indeed, pasteurized *A. muciniphila* markedly and significantly improved the insulin sensitivity index by about 30% compared to the placebo group (Fig. 1d) and live *A. muciniphila* significantly improved the insulin resistance score (Fig. 1c). Hemoglobin A1c (HbA1c) was not modified by supplementation with *A. muciniphila* (Supplementary Table 4); however, this may be explained by the fact that participants did not have diabetes and had normal HbA1c at baseline (Supplementary Table 1).

Besides its impact on incretins and glucose metabolism, the activity of the enzyme dipeptidyl peptidase 4 (DPP4) is thought to be involved in modulating inflammation. Indeed, several studies have shown a lower inflammatory tone when DPP4 inhibitors were used, thereby suggesting that this enzyme may contribute to improving glucose metabolism and lowering cardiometabolic risk by other mechanisms than modulating incretin levels[13–15]. In this study, we found that pasteurized *A. muciniphila* significantly lowered DPP4 activity at the end of the 3-month period compared to baseline (Fig. 1e). This parameter remained stable in both the placebo and live *A. muciniphila* groups. Consistent with the hypothesis that this enzyme may contribute to improving glucose metabolism and lowering cardiometabolic risk by other mechanisms than modulating incretins levels, we did not find any significant changes in plasma glucagon-like peptide-1 (GLP-1) levels (Extended Data Fig. 2).

WBC counts are elevated in obesity[16] and numerous very large cohort studies and meta-analyses have clearly linked elevated WBC counts with glucose intolerance or the risk of developing type 2 diabetes[17]. More recently, WBC counts were suggested as predictors for incident type 2 diabetes mellitus (T2DM) in obese individuals[18–20]. Therefore, in accordance with these observations, we measured WBC counts in the study groups. Interestingly, we found that WBC remained significantly increased compared to baseline and week 2 in the placebo group (Supplementary Table 2 and Fig. 1f), whereas pasteurized *A. muciniphila* supplementation completely abolished this effect, resulting in significantly lower WBC counts in the pasteurized *A. muciniphila* group compared to the placebo group (Fig. 1f). The magnitude of the differences between T0 and T3 or the placebo group (that is, 866 cells μl^{-1}) is highly significant, since a difference between 300 and 1,000 cells μl^{-1} is clinically relevant[17–20].

Although C-reactive protein was not significantly changed (Supplementary Table 4), we measured other markers associated with cardiometabolic risk. We found lower soluble CD40 ligand levels in the pasteurized versus the placebo group, but this effect did not reach significance ($P = 0.059$; Extended Data Fig. 2a). The chemokine growth-regulated oncogene/CXCL1 was decreased in the pasteurized *A. muciniphila* group at T3 versus T0 and versus the placebo group ($P = 0.055$), whereas monocyte chemoattractant protein 1 (MCP1) decreased by 21% versus placebo but did not reach significance (Extended Data Fig. 2b,c).

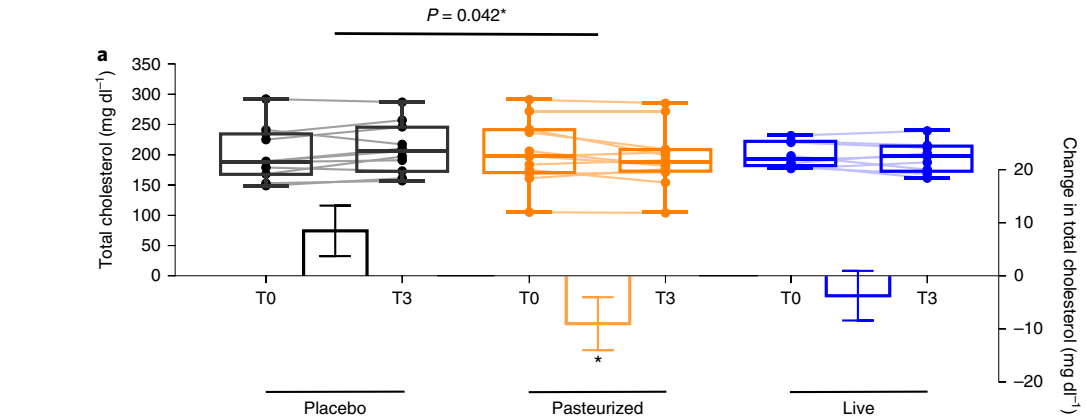
Recent studies showed that *A. muciniphila* gavage reduces plasma cholesterol in rodents[11,21] and can also prevent the development of atherosclerosis[22]. We found that administration of pasteurized *A. muciniphila* significantly decreased total cholesterol by 8.68% compared to placebo (Fig. 2a), whereas low-density lipoprotein (LDL) cholesterol was −7.53% lower and triglycerides were −15.71% lower but did not reach significance (Fig. 2b,c). Interestingly, the magnitude of the effects on lipids observed was equivalent to that induced by dietary supplementation with phytosterols according to a recent meta-analysis[23].

Numerous large cohort studies have linked raised activity of hepatic enzymes such as γ-glutamyltransferase (GGT), aspartate aminotransferase (AST) and alanine transaminase (ALT) to adverse changes in glucose and lipid metabolism, to the extent that those enzymes are considered inflammatory markers and risk factors for the development of insulin resistance and incident T2DM[24–27]. In rodents, several studies[12,28–30] have reported that supplementation with *A. muciniphila* reduces GGT, AST and ALT levels as well as hepatic steatosis. Strikingly, pasteurized *A. muciniphila* significantly reduced both GGT and AST levels after 3 months compared to baseline (Fig. 3b,c), but not ALT (Fig. 3a). Particularly, GGT levels were markedly and significantly decreased by about 24% in the pasteurized *A. muciniphila* group compared to the T3 levels observed in the placebo group ($P = 0.009$). None of these parameters were affected by supplementation with live *A. muciniphila* (Fig. 3a–c).

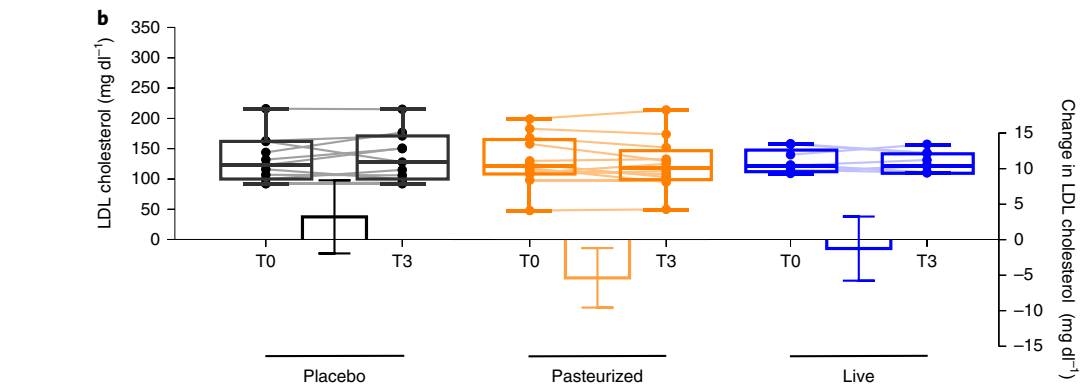
To further explore the potential mechanisms underlying the reduction of GGT and AST, we focused on plasma LPS. Indeed, numerous data obtained in humans suggest that translocation of endotoxins contributes to liver injury[31–33] as well as insulin resistance[32,34]. Moreover, we and others have shown that *A. muciniphila* reinforces gut barrier function and eventually reduces plasma

Fig. 2 | Changes in parameters related to lipid metabolism. a, Total cholesterol. **b**, LDL cholesterol. **c**, Triglycerides. The differential values (mean difference and mean difference from placebo) are expressed as the mean ± s.e.m., either as raw data or as percentages. The bars represent the mean change from baseline value per group, with their s.e.m. Mann–Whitney *U*-tests or unpaired *t*-tests were performed to compare the differential values of both treated groups versus the placebo group (intergroup changes), according to the distribution. The respective *P* values are indicated in the table below each plot; when the test is significant, the bars are marked with an asterisk. The lines represent the raw values before and three months after receiving treatment. The distribution of values within each group for each timing is illustrated by a box-and-whisker plot. In the box plots, the line in the middle of the box is plotted at the median, and the inferior and superior limits of the box correspond to the 25th and the 75th percentiles, respectively. The whiskers correspond to the minimum and maximum values. Matched-pairs Wilcoxon signed-ranks tests or paired *t*-tests were performed to verify changes from baseline (intragroup changes), according to the distribution; when drawn, the capped line above the group concerned shows the corresponding *P* value. When the difference is significant, a capped line is marked above the group concerned. Changes between 0 and 3 months across the 3 groups were analyzed with a Kruskal–Wallis or one-way ANOVA test according to the distribution; group-wise comparisons were performed using Bonferroni and Tukey's corrections for multiple testing, respectively. When the difference is significant, a line is marked above the groups concerned with the corresponding *P* value. Placebo group, $n = 11$; pasteurized bacteria group, $n = 12$; live bacteria group, $n = 9$ for all parameters. All tests were two-tailed. $^*P < 0.05$.

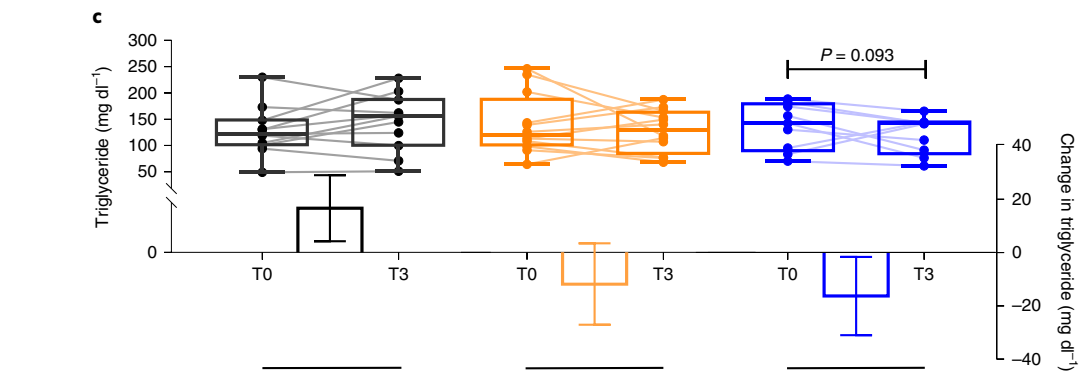
LPS[10,11,22,29]. Therefore, we measured plasma LPS before and after *A. muciniphila* supplementation. Pasteurized *A. muciniphila* significantly decreases LPS compared to baseline, but also compared to the placebo group at T3 (Fig. 3d). Thus, we speculated that such significant findings could be involved in the favorable metabolic changes observed, such as improved glucose metabolism and hepatic inflammatory markers and decreased WBC. It is worth nothing that pasteurized *A. muciniphila* supplementation decreases



	Placebo	Pasteurized	Live
Mean difference	8.55 ± 4.77	−8.92 ± 5.02	−3.67 ± 4.70
P (*t*-test)		0.02*	0.088
Mean difference from placebo (mg dl^{-1})	– –	−17.46 ± 5.02	−12.21 ± 4.70
Mean difference from placebo (%)		−8.68 ± 2.38%	−6.70 ± 2.47%



	Placebo	Pasteurized	Live
Mean difference	4.64 ± 5.77	−5.33 ± 4.19	−1.22 ± 4.52
P (Mann–Whitney *U*-test)		0.24	0.46
Mean difference from placebo (mg dl^{-1})	– –	−9.97 ± 4.19	−5.86 ± 4.52
Mean difference from placebo (%)		−7.53 ± 3.12%	−4.35 ± 3.48%



	Placebo	Pasteurized	Live
Mean difference	16.64 ± 12.37	−12.5 ± 15.29	−16.33 ± 14.68
P (Mann–Whitney *U*-test)		0.19	0.080
Mean difference from placebo (mg dl^{-1})	– –	−29.14 ± 15.29	−32.97 ± 14.68
Mean difference from placebo (%)		−15.71 ± 10.66%	−20.05 ± 13.20%

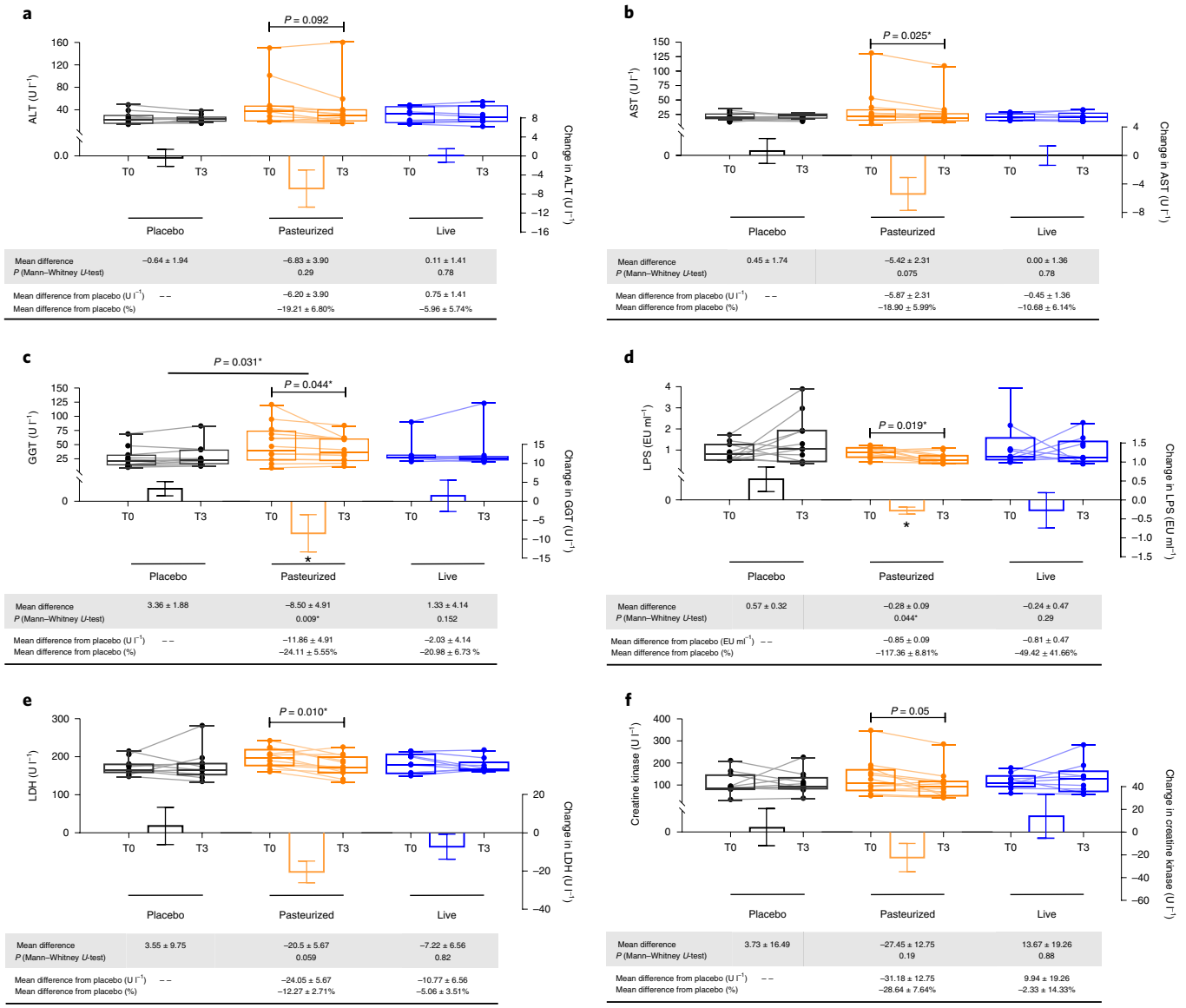


Fig. 3 | Changes in hepatic and general enzymes. a, ALT activity. **b**, AST activity. **c**, γ-Glutamyltransferase activity. **d**, LPS activity. **e**, LDH activity. **f**, Creatine kinase activity. Differential values (mean difference and mean difference from placebo) are expressed as the mean ± s.e.m., either as raw data or as percentages. The bars represent the mean change from baseline value per group, with their s.e.m. Mann–Whitney *U*-tests were performed to compare the differential values of both treated groups versus the placebo group (intergroup changes), according to the distribution. The respective *P* values are indicated in the table below each plot; when the test is significant, the bars are marked with an asterisk. The lines represent the raw values before and after three months of supplementation. The distribution of values within each group for each timing is illustrated by a box-and-whisker plot. In the box plots, the line in the middle of the box is plotted at the median, and the inferior and superior limits of the box correspond to the 25th and the 75th percentiles, respectively. The whiskers correspond to the minimum and maximum values. Matched-pairs Wilcoxon signed-rank tests were performed to verify changes from baseline (intragroup changes), according to the distribution. When the difference is significant, a capped line is marked above the group concerned with the corresponding *P* value. Kruskal–Wallis analyses were used to compare changes between 0 and 3 months across the 3 groups according to the distribution. All group-wise comparisons were performed using Bonferroni's correction for multiple testing. When the difference is significant, a line is marked above the groups concerned with the corresponding *P* value. Placebo group, $n = 11$; pasteurized bacteria group, $n = 12$; live bacteria group, $n = 9$ for all parameters except for creatine kinase: placebo group, $n = 11$; pasteurized bacteria group, $n = 11$; live bacteria group, $n = 8$. All tests were two-tailed. *$P < 0.05$.

serum lactate dehydrogenase (LDH) and creatine kinase levels at T3 versus T0, two enzymes considered valid markers of whole-body tissue damage and muscle-specific injury, respectively (Fig. 3e,f).

Since gut microbiota have been linked with metabolism and cardiometabolic risk factors[2,35,36], and *A. muciniphila* is linked with improved metabolic parameters[36,37] we measured the levels of *A. muciniphila* at baseline and after supplementation (Extended Data Fig. 3a). First, we found that the abundance of *A. muciniphila* was similar between groups at baseline, whereas supplementation significantly increased by 1.7–2.6 log the quantity of *A. muciniphila* recovered in the feces of the pasteurized and live *A. muciniphila* groups, respectively (Extended Data Fig. 3a). Interestingly, baseline characterization of the fecal microbiome performed on the 3 groups showed that there was no significant difference between groups at baseline (permutational multivariate analysis of variance (MANOVA), $R^2 = 0.066$, $P = 0.51$; Extended Data Fig. 3b).

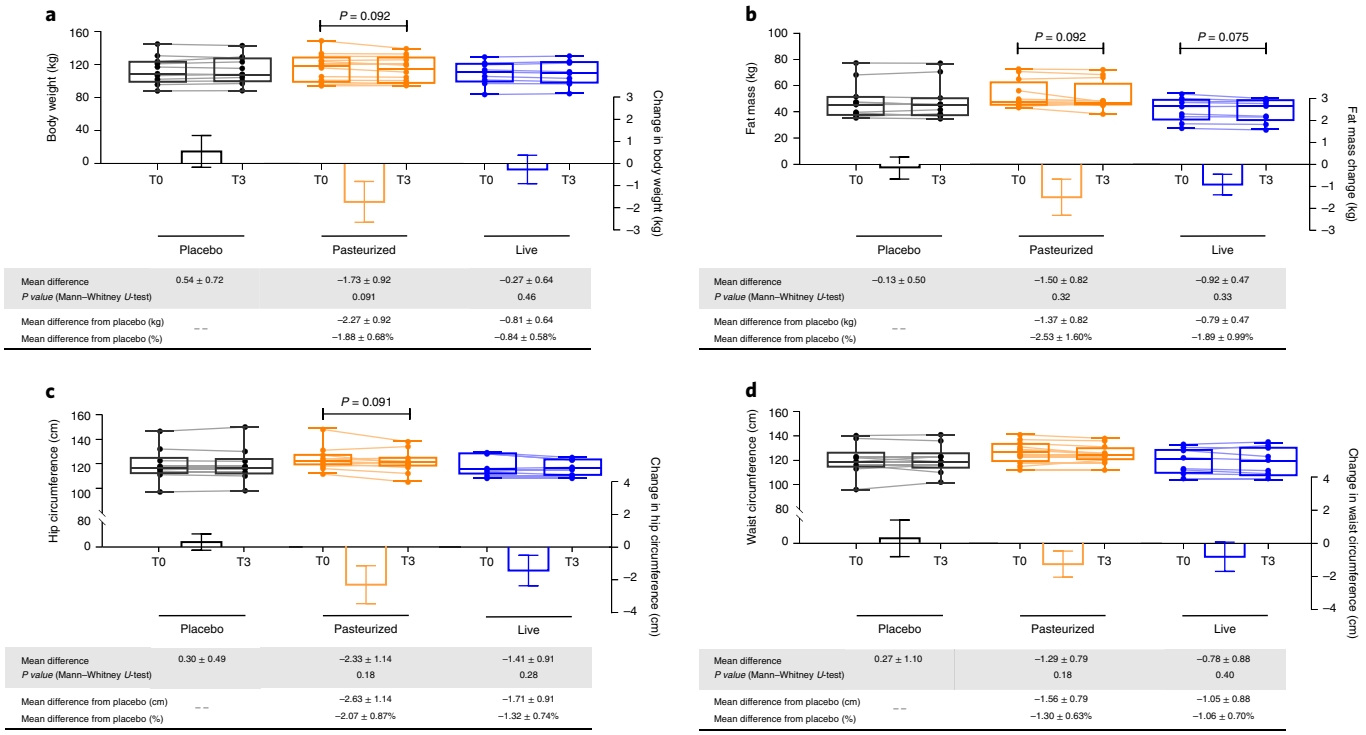


Fig. 4 | Changes in anthropometric parameters. a, Body weight. **b**, Fat mass. **c**, Hip circumference. **d**, Waist circumference. Differential values (mean difference and mean difference from placebo) are expressed as the mean ± s.e.m., either as raw data or as percentages. The bars represent the mean change from baseline value per group, with their s.e.m. Mann–Whitney *U*-tests were performed to compare the differential values of both treated groups versus the placebo group (intergroup changes) according to the distribution. The respective *P* values are indicated in the table below each plot. The lines represent the raw values before and after three months of supplementation. The distribution of values within each group for each timing is illustrated by a box-and-whisker plot. In the box plots, the line in the middle of the box is plotted at the median, and the inferior and superior limits of the box correspond to the 25th and the 75th percentiles, respectively. The whiskers correspond to the minimum and maximum values. Matched-pairs Wilcoxon signed-rank tests were performed to verify changes from baseline (intragroup changes) according to the distribution and, when drawn, the capped line above the group concerned shows the corresponding *P* value. Kruskal–Wallis analysis was used to compare changes between 0 and 3 months across the 3 groups according to the distribution. All group-wise comparisons were performed using Bonferroni's correction for multiple testing. Placebo group, $n=11$; pasteurized bacteria group, $n=12$; live bacteria group, $n=9$ for all parameters except for hip and waist: placebo group, $n=10$; pasteurized bacteria group, $n=12$; live bacteria group, $n=9$. All tests were two-tailed.

Moreover, at the end of the intervention, the difference in gut microbiome composition between the 3 groups was slightly higher than at baseline while still non-significant (permutational MANOVA, $R^2=0.075$, $P=0.18$; Extended Data Fig. 3b). We evaluated the alteration in microbiota composition from baseline to end point (pairing per individual) and found that none of the treatments induced significant community-wide compositional change, although treatment with live bacteria had a slightly higher impact (partial distance-based redundancy analysis (dbRDA), adjusted $R^2=0.03$, $P=0.095$), than pasteurized (partial dbRDA, adjusted $R^2=0.02$, $P=0.14$) and placebo (partial dbRDA, adjusted $R^2=0.01$, $P=0.66$). Therefore, these results demonstrate that supplementation with either pasteurized or live *A. muciniphila* did not affect the overall structure of the gut microbiome. This finding is in line with previous data obtained in rodents, which showed that the gut microbiome of mice supplemented with live *A. muciniphila* was not significantly modified[10].

We also observed that the administration of pasteurized *A. muciniphila* slightly decreased body weight by approximately −2.27 kg ($P=0.09$), fat mass by approximately −1.37 kg ($P=0.09$) and hip circumference by −2.63 cm ($P=0.09$) (Fig. 4a–c) compared to the placebo group. Waist circumference was decreased by approximately 1.56 cm, but this change did not reach statistical significance (Fig. 4d). These differences are all of clinical relevance in the context of metabolic disorders and we may not rule out that improvement of different metabolic parameters is associated with the impact of supplementation on body weight, fat mass and hip circumference.

Our study has several limitations. Although most of the primary outcomes were reached, we did not find significant changes in visceral adiposity and BMI. However, we did not use specific and accurate methods such as dual-energy X-ray absorptiometry to precisely estimate the quantity of visceral versus subcutaneous fat. Also, this pilot and exploratory study enrolled a small number of individuals; thus, the study was not powered to deliver definitive conclusions on the end points related to metabolic parameters. Also, physical activity level and precise calories intake were not determined using dedicated measures. However, all the groups were investigated blindly; therefore, we may argue that any confounding factors were probably equally distributed between the different groups. Finally, we observed comparable apparent worsening of the phenotype of the placebo group over time as noted in other studies[38–40].

In conclusion, this proof-of-concept prospective study shows the feasibility of culturing and administering *A. muciniphila* to humans. Our data unequivocally show that administration of a daily dose as high as of 10^{10} cells of *A. muciniphila* is safe in the longer term (that is, 3 months).

This study provides a promising start for the development of future clinical interventions with appropriate design to confirm and extend our findings, which show the safety and impact of oral

supplementation with *A. muciniphila* in overweight or obese insulin-resistant individuals.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, statements of code and data availability and associated accession codes are available at https://doi.org/10.1038/s41591-019-0495-2.

Received: 2 October 2018; Accepted: 22 May 2019;
Published online: 1 July 2019

References

1. O'Neill, S. & O'Driscoll, L. Metabolic syndrome: a closer look at the growing epidemic and its associated pathologies. *Obes. Rev.* **16**, 1–12 (2015).
2. Cani, P. D. et al. Microbial regulation of organismal energy homeostasis. *Nat. Metab.* **1**, 34–46 (2019).
3. Brahe, L. K. et al. Specific gut microbiota features and metabolic markers in postmenopausal women with obesity. *Nutr. Diabetes* **5**, e159 (2015).
4. Zhang, X. et al. Human gut microbiota changes reveal the progression of glucose intolerance. *PLoS ONE* **8**, e71108 (2013).
5. Yassour, M. et al. Sub-clinical detection of gut microbial biomarkers of obesity and type 2 diabetes. *Genome Med.* **8**, 17 (2016).
6. Li, J. et al. Gut microbiota dysbiosis contributes to the development of hypertension. *Microbiome* **5**, 14 (2017).
7. Liu, R. et al. Gut microbiome and serum metabolome alterations in obesity and after weight-loss intervention. *Nat. Med.* **23**, 859–868 (2017).
8. Dao, M. C. et al. *Akkermansia muciniphila* and improved metabolic health during a dietary intervention in obesity: relationship with gut microbiome richness and ecology. *Gut* **65**, 426–436 (2016).
9. Cani, P. D. & de Vos, W. M. Next-generation beneficial microbes: the case of *Akkermansia muciniphila*. *Front. Microbiol.* **8**, 1765 (2017).
10. Everard, A. et al. Cross-talk between *Akkermansia muciniphila* and intestinal epithelium controls diet-induced obesity. *Proc. Natl Acad. Sci. USA* **110**, 9066–9071 (2013).
11. Plovier, H. et al. A purified membrane protein from *Akkermansia muciniphila* or the pasteurized bacterium improves metabolism in obese and diabetic mice. *Nat. Med.* **23**, 107–113 (2017).
12. Everard, A. et al. Intestinal epithelial *N*-acylphosphatidylethanolamine phospholipase D links dietary fat to metabolic adaptations in obesity and steatosis. *Nat. Commun.* **10**, 457 (2019).
13. Atkin, S. L. et al. Effect of dipeptidyl peptidase-4 inhibitors on circulating tumor necrosis factor-α concentrations: a systematic review and meta-analysis of controlled trials. *J. Diabetes Complicat.* **31**, 1458–1464 (2017).
14. Akoumianakis, I. & Antoniades, C. Dipeptidyl peptidase IV inhibitors as novel regulators of vascular disease. *Vascul. Pharmacol.* **96–98**, 1–4 (2017).
15. Olivares, M. et al. The potential role of the dipeptidyl peptidase-4-like activity from the gut microbiota on the host health. *Front. Microbiol.* **9**, 1900 (2018).
16. Veronelli, A. et al. White blood cells in obesity and diabetes: effects of weight loss and normalization of glucose metabolism. *Diabetes Care* **27**, 2501–2502 (2004).
17. Ohshita, K. et al. Elevated white blood cell count in subjects with impaired glucose tolerance. *Diabetes Care* **27**, 491–496 (2004).
18. Gu, Y. et al. White blood cells count as an indicator to identify whether obesity leads to increased risk of type 2 diabetes. *Diabetes Res. Clin. Pract.* **141**, 140–147 (2018).
19. Zhang, H. et al. White blood cell subtypes and risk of type 2 diabetes. *J. Diabetes Complicat.* **31**, 31–37 (2017).
20. Twig, G. et al. White blood cells count and incidence of type 2 diabetes in young men. *Diabetes Care* **36**, 276–282 (2013).
21. Shen, J. et al. Low-density lipoprotein receptor signaling mediates the triglyceride-lowering action of *Akkermansia muciniphila* in genetic-induced hyperlipidemia. *Arterioscler. Thromb. Vasc. Biol.* **36**, 1448–1456 (2016).
22. Li, J., Lin, S., Vanhoutte, P. M., Woo, C. W. & Xu, A. *Akkermansia muciniphila* protects against atherosclerosis by preventing metabolic endotoxemia-induced inflammation in *Apoe*$^{-/-}$ mice. *Circulation* **133**, 2434–2446 (2016).
23. Ras, R. T. et al. Consumption of plant sterol-enriched foods and effects on plasma plant sterol concentrations—a meta-analysis of randomized controlled studies. *Atherosclerosis* **230**, 336–346 (2013).
24. Wannamethee, S. G., Shaper, A. G., Lennon, L. & Whincup, P. H. Hepatic enzymes, the metabolic syndrome, and the risk of type 2 diabetes in older men. *Diabetes Care* **28**, 2913–2918 (2005).
25. Rantala, A. O. et al. Gamma-glutamyl transpeptidase and the metabolic syndrome. *J. Intern. Med.* **248**, 230–238 (2000).
26. Lim, J. S., Lee, D. H., Park, J. Y., Jin, S. H. & Jacobs, D. R. Jr. A strong interaction between serum γ-glutamyltransferase and obesity on the risk of prevalent type 2 diabetes: results from the Third National Health and Nutrition Examination Survey. *Clin. Chem.* **53**, 1092–1098 (2007).
27. Fraser, A. et al. Alanine aminotransferase, γ-glutamyltransferase, and incident diabetes: the British Women's Heart and Health Study and meta-analysis. *Diabetes Care* **32**, 741–750 (2009).
28. Hänninen, A. et al. *Akkermansia muciniphila* induces gut microbiota remodelling and controls islet autoimmunity in NOD mice. *Gut* **67**, 1445–1453 (2018).
29. Grander, C. et al. Recovery of ethanol-induced *Akkermansia muciniphila* depletion ameliorates alcoholic liver disease. *Gut* **67**, 891–901 (2018).
30. Wu, W. et al. Protective effect of *Akkermansia muciniphila* against immune-mediated liver injury in a mouse model. *Front. Microbiol.* **8**, 1804 (2017).
31. Harte, A. L. et al. Elevated endotoxin levels in non-alcoholic fatty liver disease. *J. Inflamm. (Lond.)* **7**, 15 (2010).
32. Cani, P. D. et al. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* **56**, 1761–1772 (2007).
33. Miele, L. et al. Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. *Hepatology* **49**, 1877–1887 (2009).
34. Lassenius, M. I. et al. Bacterial endotoxin activity in human serum is associated with dyslipidemia, insulin resistance, obesity, and chronic inflammation. *Diabetes Care* **34**, 1809–1815 (2011).
35. Allin, K. H. et al. Aberrant intestinal microbiota in individuals with prediabetes. *Diabetologia* **61**, 810–820 (2018).
36. Wu, H. et al. Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug. *Nat. Med.* **23**, 850–858 (2017).
37. Dao, M. C. et al. *Akkermansia muciniphila* and improved metabolic health during a dietary intervention in obesity: relationship with gut microbiome richness and ecology. *Gut* **65**, 426–436 (2016).
38. Scherbaum, W. A. et al. Efficacy and tolerability of vildagliptin in drug-naïve patients with type 2 diabetes and mild hyperglycaemia. *Diabetes Obes. Metab.* **10**, 675–682 (2008).
39. Rosenstock, J., Rigby, S. P., Ford, D. M., Tao, B. & Chou, H. S. The glucose and lipid effects of colesevelam as monotherapy in drug-naïve type 2 diabetes. *Horm. Metab. Res.* **46**, 348–353 (2014).
40. Kim, S. G. et al. Efficacy and safety of lobeglitazone monotherapy in patients with type 2 diabetes mellitus over 24-weeks: a multicenter, randomized, double-blind, parallel-group, placebo controlled trial. *PLoS ONE* **9**, e92843 (2014).

Acknowledgements

We thank A. Barrois for the excellent technical assistance. We are very grateful to M. Buysschaert for his continuous support and helpful criticism during the preparation of this project. We also thank the volunteers who participated in this study. P.D.C. is a senior research associate at Fonds de la Recherche Scientifique (FRS-FNRS). A.E. is a research associated at FRS-FNRS. C. Druart was supported by a FIRST Spin-Off grant from the Walloon Region (no. 1410053). Research in the Wageningen laboratory of W.M.d.V. was partially supported by a European Research Council (ERC) Advanced Grant no. 250172 (Microbes Inside), the SIAM Gravity Grant no. 024.002.002 and the Spinoza Award of the Netherlands Organization for Scientific Research. P.D.C. is the recipient of grants from the FNRS (nos. J.0084.15 and 3.4579.11) and Projet de Recherche (no. T.0138.14) and FRFS-WELBIO grants (no. WELBIO-CR-2017C-02). This work was supported by the Funds Baillet Latour (Grant for Medical Research 2015), a prize of the Banque Transatlantique Belgium and a FIRST Spin-Off grant from the Walloon Region (no. 1410053). P.D.C. is a recipient of the PoC ERC grant 2016 (no. Microbes4U_713547) and ERC Starting Grant 2013 (no. 336452-ENIGMO). P.D.C. and J.R. are recipients of a grant from the FNRS and FWO (EOS program no. 30770923).

Author contributions

P.D.C. conceived the project. J.-P.T., M.P.H., A.L., D.M., A.E., C. Depommier, C. Druart, H.P., M.V.H., W.M.d.V. and P.D.C. designed the clinical study. P.D.C. supervised the clinical part of the study and W.M.d.V. contributed to the microbial culturing of *A. muciniphila*. P.D.C., A.E., C. Depommier, C. Druart, M.d.B., J.-P.T., A.L., D.M. and M.P.H. performed the clinical part of the study. N.M.D. contributed to interpretation of the results. S.V.-S., G.F. and J.R. performed the fecal microbiome sequencing and analysis. P.D.C., A.E. and C. Depommier performed the experiments and interpreted all the results. P.D.C., A.E. and C. Depommier generated the figures and tables. P.D.C. and C. Depommier wrote the manuscript. All authors discussed the results and approved the manuscript.

Competing interests

A.E., C. Druart, H.P., P.D.C. and W.M.d.V. are inventors of patent applications (nos. PCT/EP2013/073972, PCT/EP2016/071327 and PCT/EP2016/060033 filed with the European Patent Office, Australia, Brazil, Canada, China, the Eurasian Patent Organization, Israel, India, Hong Kong, Japan, South Korea, Mexico, New Zealand and the United States) dealing with the use of *A. muciniphila* and its components in the context of obesity and related disorders. P.D.C. and W.M.d.V. are cofounders of A-Mansia Biotech S.A.

Additional information

Extended data is available for this paper at https://doi.org/10.1038/s41591-019-0495-2.

Supplementary information is available for this paper at https://doi.org/10.1038/s41591-019-0495-2.

Reprints and permissions information is available at www.nature.com/reprints.

Correspondence and requests for materials should be addressed to P.D.C.

Peer Review Information: Joao Monteiro was the primary editor on this article and managed its editorial process and peer review in collaboration with the rest of the editorial team.

Methods

Participants and study design. This study was designed as a randomized, placebo-controlled, parallel-group pilot study. Between December 2015 and December 2017, 32 overweight/obese individuals (BMI > 25 kg m^{-2}) aged between 18 and 70 years volunteered to participate and were enrolled in the study. Eligible participants had been diagnosed with metabolic syndrome according to the National Cholesterol Education Program Adult Treatment Panel III definition, that is, at least three of the five following criteria: fasting glycemia > 100 mg dl^{-1}; blood pressure ≥ 130/85 mmHg or antihypertensive treatment; fasting triglyceridemia ≥ 150 mg dl^{-1}; high-density lipoprotein (HDL) cholesterol < 40 mg dl^{-1} for men, < 50 mg dl^{-1} for women; and/or waist circumference > 102 cm for men, >88 cm for women and whose insulin sensitivity was <75% (refs.[41,42]), evaluated using HOMA-modeling of insulin sensitivity (HOMA Calculator, University of Oxford). Written informed consent was obtained from each participant and the study protocol was approved by the Commission d'Ethique Biomédicale Hospitalo-facultaire of the Université catholique de Louvain. The study was registered at https://clinicaltrials.gov as trial no. NCT02637115.

Participants were recruited at the Cliniques universitaires Saint-Luc in Brussels. A total of 160 participants aged 18–70 years were screened. Forty-five overweight or obese individuals with insulin resistance and metabolic syndrome were eligible for inclusion. In this group, five declined to participate. Therefore, 40 individuals were enrolled and received either a placebo, live *A. muciniphila* (10^{10} bacteria per day) or pasteurized *A. muciniphila* (10^{10} bacteria per day) as a supplement for 3 months, with the specific advice to keep to their normal dietary intake and physical activity during the study period (see flow chart in Extended Data Fig. 1). To prevent any viability or shelf life issues, the *A. muciniphila* bacteria were delivered to participants frozen in glycerol. The placebo contained the same amount of glycerol. The viable count of the *A. muciniphila* bacteria delivered to participants did not change during the entire intervention (data not shown).

Of the 40 participants, 7 had to be excluded before study completion: 1 in the placebo group; 1 in the pasteurized bacteria group; and 5 in the live bacteria group. Three early terminations were due to personal reasons (that is, mainly because of the difficulty to attend the nine scheduled visits at the hospital) and four were due to untimely use of antibiotics during the study. One additional participant in the placebo group was excluded from the analysis because of protocol violation. This resulted in a total of 32 participants: a placebo group of 11 participants; a pasteurized bacteria group of 12 participants; and a live bacteria group of 9 participants. These 32 participants completed the 3-month supplementation. Participants were allocated to one of the treatment arms following a randomized block design with a block size of eight. The Microsoft Excel randomization function was used to generate the allocation sequence. Participants and physicians were both blinded to treatment allocation. Apart from the placebo group (administered an equivalent volume of sterile PBS containing glycerol), participants were assigned to ingest either 10^{10} cells of live *A. muciniphila* or 10^{10} cells of pasteurized *A. muciniphila* in PBS containing glycerol daily for 3 months. Packages containing either glycerol (placebo) or glycerol and *A. muciniphila* (pasteurized and live bacteria groups) were given to participants every 2 weeks during follow-up visits, with the instructions to take one dose every morning on an empty stomach. Participants were instructed to keep the packages in the freezer compartment of a home refrigerator until a dose was needed. A temperature sensor was also provided to all participants to monitor the temperature during transport and home storage (at −20 °C). Anaerobic fermentation, concentration and packaging of the bacteria and placebo were performed according to the hazard analysis and critical control points quality system using the medium level food grade as described previously[11]. Pasteurization consisted of heat treatment at 70 °C for 30 min of fresh *A. muciniphila*.

Exclusion criteria were: presence of acute or chronic progressive or chronic unstabilized diseases; alcohol consumption (>2 glasses per day); previous bariatric surgery; any surgery in the 3 months before the study or planned for 6 months after enrolling; pregnancy or pregnancy planned in the 6 months after enrolling; regular physical activity (>30 min of sports activities 3 times a week); consumption of dietary supplements (omega-3 fatty acids, probiotics, prebiotics, plant stanols/sterols) in the month before the study; inflammatory bowel disease or irritable bowel syndrome; diabetic gastrointestinal autonomic neuropathy (such as gastroparesis or reduced gastrointestinal motility); consumption of more than 30 g of dietary per day; consumption of vegetarian or unusual diets; lactose intolerance or milk protein allergy; gluten intolerance; current treatment with medications influencing the parameters of interest (glucose-lowering drugs such as metformin, DPP4 inhibitors, GLP-1 receptor agonists, acarbose, sulfonylureas, glinides, thiazolidinediones, sodium-glucose cotransporter-2 inhibitors, insulin, lactulose, consumption of antibiotics in the 2 months before or during the study, glucocorticoids, immunosuppressive agents, statins, fibrates, orlistat, cholestyramine or ezetimibe); and baseline HbA1c > 7.5%.

At baseline and at the end of the intervention, anthropometric measurements were assessed including body weight (kg) and BMI (kg m^{-2}). Waist and hip circumference (cm) were measured using a flexible tape. Fat mass (kg) was assessed using electric bioimpedance analysis (Body Composition Analyzer, type BC-418 MA; TANITA). Blood samples were collected at baseline and at the end of the intervention, after an overnight fast (8 h minimum). Based on the analytes of interest, different tubes were used: sodium fluoride-coated tubes for fasting glycemia and insulinemia; lithium-heparin-coated tubes for enzymatic activities; and LPS-free heparin sulfate-coated tubes for LPS measurement (BD Vacutainer glass sodium heparin tubes, catalog no. 368480). One set of tubes was sent directly to the hospital laboratory for the following blood analyses: fasting glycemia; insulinemia; HbA1c (%); total cholesterol; LDL cholesterol (calculated); HDL cholesterol; triglycerides; GGT; ALT; AST; LDH; creatine kinase; and WBC. The other tubes were brought to the research laboratory and kept on ice. Plasma was immediately isolated from whole blood by centrifugation at 4200*g* for 10 min at 4 °C and stored at −80 °C for further analyses.

For safety purposes, participants were asked to come back to the study hospital 2 weeks after the beginning of the intervention for blood sampling and clinical examination, allowing comparison of clinical parameters with baseline values. Blood sample analysis included C-reactive protein, urea, creatinine, glomerular filtration rate, AST, ALT, GGT, LDH, creatinine kinase, various coagulation parameters and hematologic profiling. Forty participants were included in this analysis at 2 weeks. The same measurements were performed at 3 months for the 32 participants who completed the intervention.

Compliance and presence of undesired side effects were monitored every 2 weeks during follow-up visits when participants were asked to fill in a questionnaire. We listed the adverse events most likely to occur during the study in the questionnaire. We also invited participants to point out any other adverse event that either newly emerged or worsened. The list of side effects included nausea, flatulence, bloating, cramps, borborygmus and gastric reflux. If adverse event(s) occurred, participants had to specify the number of days during which the effect(s) occurred. Each adverse event was calculated as the percentage of occurrence on the total number of days of intervention. Compliance was also assessed according to participants' daily records and the number of returned packages. Compliance was calculated as the percentage of the number of days where packages were actually ingested against the total number of days of the intervention. Participants were instructed to maintain their usual diets, levels of physical activity, current treatments and lifestyles throughout the intervention period. Quality control tests were also applied during the protocol; each participant received a 2-week supply of bacteria (14 bags + 1 bag in case of difficulties in attending the hospital and having to interrupt supplementation). Thus, participants came to the clinic every 2 weeks to receive a new supply containing 2 weeks of bacteria or placebo (15 bags, 14 + 1). At the hospital, bags were stored and kept at −80 °C before being delivered to participants. During transport from the laboratory to home and then in a refrigerator at home, we provided each participant with a device (TempTale4; Sensitech) to monitor the temperature throughout the study, including at home, to detect any potential temperature deviation over the period of supplementation. In addition, we randomly tested the viability of *A. muciniphila* (for the live bacteria group) by culturing the contents of the bags maintained at −80 °C but also those maintained at −20 °C, thereby validating the viability of cells at −20 °C.

Biochemical analyses. Insulin sensitivity and resistance were both analyzed using HOMA. This test consists of taking three blood samples at 5 min intervals for each individual. Insulinemia and glycemia were determined for each sample and the mean values were then entered in the HOMA2 calculator (v.2.3.3, available from http://www.dtu.ox.ac.uk/homacalculator/) to estimate insulin sensitivity (%) and insulin resistance.[43,44]

Insulinemia was evaluated by immunoanalysis. Glycemia was assessed by enzymatic test (hexokinase) with ultraviolet detection (Cobas 8000; Roche Diagnostics). HbA1c (%) was determined by high-performance liquid chromatography (G8 HPLC Analyzer; Tosoh). C-reactive protein was assessed by immunoturbidimetry (Cobas 8000). Total cholesterol, HDL cholesterol, triglycerides and GGT were dosed by enzymatic colorimetric method (Cobas 8000). LDL cholesterol concentrations were estimated using the Friedewald formula. AST and ALT were assessed by enzymatic dosage (International Federation of Clinical Chemistry and Laboratory Medicine) without activation by pyridoxal phosphate (Cobas 8000). A kinetic enzymatic test was performed to evaluate urea; creatinine was assessed by kinetic staining test (Jaffe method) (Cobas 8000). The glomerular filtration rate was estimated according to the CKD-EPI equation. The parameters related to muscle function (creatinine kinase and LDH) were assessed by ultraviolet test (Cobas 8000). All these tests were performed at the hospital laboratory.

Blood LPS endotoxin activity was measured with the Endosafe nexgen-MCS (Charles River Laboratories) based on the limulus amebocyte lysate kinetic chromogenic method, which measures color intensity directly related to the endotoxin concentration in a sample. Plasma was diluted 1/50 to 1/100 with endotoxin-free buffer (Charles River Laboratories) to minimize interference in the reaction and heated for 15 min at 70 °C. Each sample was diluted with endotoxin-free limulus amebocyte lysate reagent water (Charles River Laboratories) and treated in duplicate. Two spikes for each sample were included in the determination. All samples were validated for recovery and coefficient of variation. The lower limit of detection was 0.005 EU ml^{-1}.

Growth-related oncogene, sCD14L and MCP1 were assessed in each blood sample in duplicate using a MILLIPLEX MAP Human Cytokine/Chemokine

Magnetic Bead Panel; Merck Millipore) and measured using Luminex technology (BioPlex; Bio-Rad Laboratories) according to the manufacturer's instructions. Active plasma GLP-1 levels were determined by sandwich ELISA (Merck Millipore).

DPP4 activity was assessed by quantifying the production of p-nitroanilide (pNA) from glycine-proline-pNA (Sigma-Aldrich) using a standard curve of free pNA. For this, plasma samples were incubated for 30 min with glycine-proline-pNA at 37 °C and enzymatic activity was measured by kinetic analysis (380 nm) (SpectraMax M2; Molecular Devices).

Fecal microbiome analysis. *A. muciniphila* was quantified with quantitative PCR as described in Everard et al.[10] 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 taxonomic composition of fecal microbiota was determined by DNA extraction of fecal samples stored frozen (−80 °C) and library preparation for dual-index 16S ribosomal RNA gene sequencing as described in Vandeputte et al.[45]. Demultiplexing of the sequencing data was performed using LotuS55 v.1.565, followed by quality control and sequence variant matrix building using the DADA2 (ref. [46]) pipeline v.1.6.0 with taxonomic annotation by RDP classifier[47] v.2.12 using default parameters. Statistical analyses of microbiota composition were performed in R using the packages vegan (version 2.5-3)[48] and CoDaSeq (version 0.99-3)[49]. As recommended for microbiota composition data analysis, the abundance matrix was centered log-ratio-transformed (CoDaSeq:codaseq.clr) using the minimum proportional abundance detected for each taxon for the imputation of zeros. Samples with >10,000 reads ($n=63$ samples and genera with relative abundance >0.001 ($n=99$)) were included in the data analysis. Differences in microbiota profiles between treatment arms at baseline and at end point were evaluated by permutational MANOVA. Microbiota alteration from baseline to end point was evaluated per treatment arm and pairing by participant by performing a distance-based redundancy analysis (partial dbRDA, centered log-ratio-transformed matrix, Euclidean distance) by using time point as an explanatory variable while partialling out intraindividual similarity.

Fecal microbiota dissimilarity between samples was represented by genus-level principal coordinates analysis with Aitchison distance (Euclidean distance with centered log-ratio-transformed matrix) using the phyloseq (version 1.26.0), vegan (version 2.5-3) and ggplot2 R packages (version 3.1.0). Confidence ellipses for each of the six sample groups (corresponding to the three different treatment arms at baseline or at end point) were drawn at the 0.80 confidence level assuming a Student's *t*-distribution. The intervention effect is symbolized by colored arrows, with direction and length corresponding to the shift in group centroid from baseline to end point for each treatment arm. The arrows lengths were multiplied by five for visual clarity and the three arrows were re-centered to the centroid of all baseline samples (three arms confounded).

Statistical analysis. The normal distribution of continuous variables, expressed as raw data or as the difference between the two main time points (T0 and T3 months), was tested using the Shapiro–Wilk test. The appearance of box plots and Quantile–Quantile plots was also taken into account. All the following statistical tests were chosen in accordance with normality tests. For all parameters, and within each group, the intervention effect was calculated by subtracting the value obtained at T0 from the value obtained at T3 months for each participant. We named the differential value obtained 'mean difference'. The 'mean difference from placebo' was then calculated by subtracting the mean difference calculated for the placebo group from the mean difference calculated for the active group. The 'mean difference from placebo' was expressed as the raw data and as a percentage. Unpaired *t*-tests or non-parametric Mann–Whitney *U*-tests were used to assess the significance of differences between the mean differences of the two treated groups versus the mean differences of the placebo group. According to the distribution, either paired *t*-tests or non-parametric, two-tailed, matched-pairs Wilcoxon signed-rank tests were performed to identify the differences between T0 and T3 within each group. One-way analysis of variance (ANOVA) or Kruskal–Wallis tests were used to compare baseline parameters and the differential values across the three groups, according to the distribution; *P* values were adjusted using Bonferroni's correction. For baseline characteristics, the mean and s.d. were used to present the raw data of the normal variables, while the median and interquartile range were used to report non-normal variables. The data of the safety tables were expressed as the mean and s.d. Data presented in the figures were expressed as the mean and s.e.m. of the mean. Statistical analyses were conducted using SPSS v.23.0 (IBM Corporation). All tests were two-tailed and significance was set at $P<0.05$. Graphics were drawn with the Prism software v.7.0 (GraphPad Software).

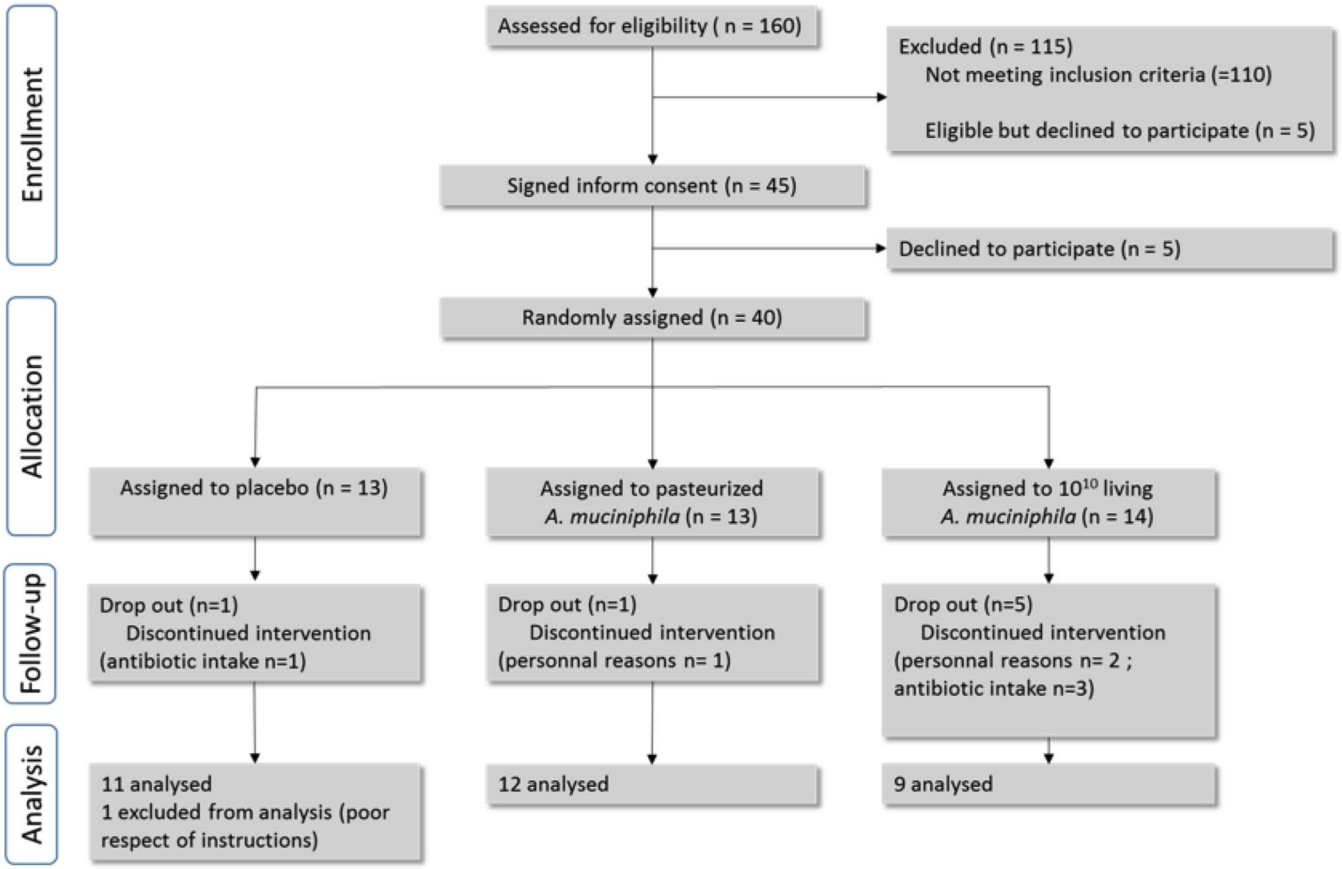
Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

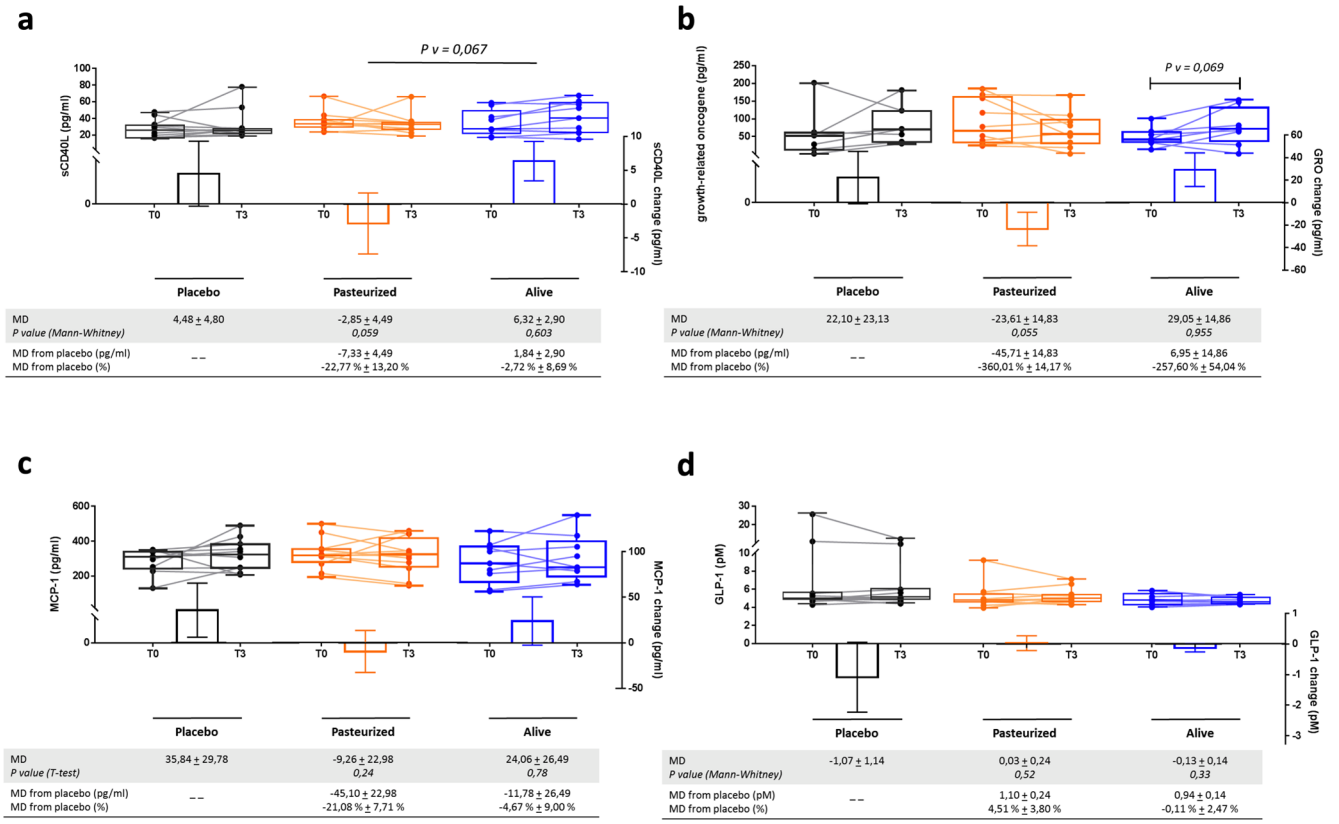
The data that support the findings of this study are available upon request. All figures are provided with individual values to have a direct access to the raw data. The 16S sequencing datasets generated during the current study are available from the European Genome-Phenome Archive (https://ega-archive.org/) under accession no. EGAS00001003585.

References

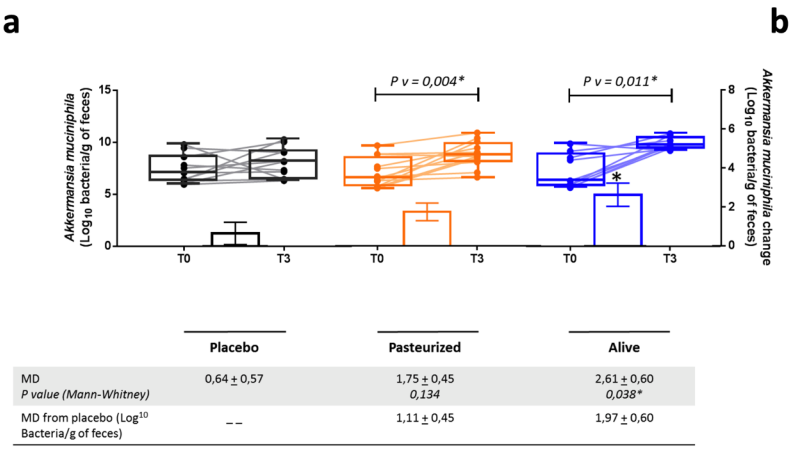
41. Werner, M., Tönjes, A., Stumvoll, M., Thiery, J. & Kratzsch, J. Assay-dependent variability of serum insulin levels during oral glucose tolerance test: influence on reference intervals for insulin and on cut-off values for insulin sensitivity indices. *Clin. Chem. Lab. Med.* **46**, 240–246 (2008).
42. Borza, D. et al. Influence of assay-dependent variability of serum insulin levels on insulin sensitivity indices. *Clin. Chem. Lab. Med.* **46**, 1655–1656 (2008).
43. Wallace, T. M., Levy, J. C. & Matthews, D. R. Use and abuse of HOMA modeling. *Diabetes Care* **27**, 1487–1495 (2004).
44. Dennis, J. M. et al. Precision medicine in type 2 diabetes: clinical markers of insulin resistance are associated with altered short- and long-term glycemic response to DPP-4 inhibitor therapy. *Diabetes Care* **41**, 705–712 (2018).
45. Vandeputte, D. et al. Quantitative microbiome profiling links gut community variation to microbial load. *Nature* **551**, 507–511 (2017).
46. Callahan, B. J. et al. DADA2: high-resolution sample inference from Illumina amplicon data. *Nat. Methods* **13**, 581–583 (2016).
47. Wang, Q., Garrity, G. M., Tiedje, J. M. & Cole, J. R. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Appl. Environ. Microbiol.* **73**, 5261–5267 (2007).
48. Oksanen, J. B. et al. vegan: community ecology package. R package version 2.5-3 (2018).
49. Gloor, G. B. & Reid, G. Compositional analysis: a valid approach to analyze microbiome high-throughput sequencing data. *Can. J. Microbiol.* **62**, 692–703 (2016).

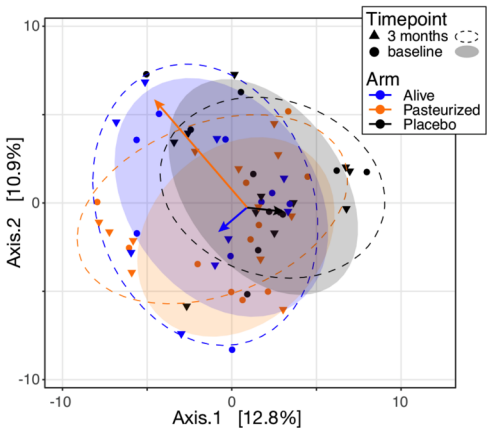


Extended Data Fig. 1 | Flow chart of the interventional study. Diagram of the participant selection procedure, which includes the following information: number of individuals enrolled at each step of the study progress; number of individuals included in the final analysis; details of the events that led to a reduction in group size.



Extended Data Fig. 2 | Changes in inflammatory parameters and GLP-1. a, Soluble CD40 Ligand. **b**, Growth-related oncogene (CXCL1). **c**, MCP1. **d**, GLP-1. Differential values (mean difference and mean difference from placebo) are expressed as the mean + s.e.m., either as raw data or as percentages. The bars represent the mean change from baseline value per group, with their s.e.m. Mann–Whitney *U*-tests or unpaired *t*-tests were performed to compare the differential values of both treated groups versus the placebo group (intergroup changes), according to the distribution. The respective *P* values are shown in the table below each plot. The lines represent the raw values before and after 3 months of supplementation. The distribution of values within each group for each timing is illustrated by a box-and-whisker plot. In the box plots, the line in the middle of the box is plotted at the median, and the inferior and superior limits of the box correspond to the 25th and the 75th percentiles respectively. Matched-pairs Wilcoxon signed-rank tests or paired *t*-tests were performed to verify changes from baseline (intragroup changes), according to the distribution; when drawn, the capped line above the group concerned shows the corresponding *P* value. Changes between 0 and 3 months across the 3 groups were analyzed with Kruskal–Wallis or one-way ANOVA tests according to the distribution; group-wise comparisons were performed using Bonferroni's and Tukey's corrections for multiple testing, respectively. Placebo group, $n = 11$; pasteurized bacteria group, $n = 12$; live bacteria group, $n = 9$ for all parameters except for growth-related oncogene: placebo group, $n = 7$; pasteurized bacteria group, $n = 10$; live bacteria group, $n = 8$. All tests were two-tailed.





Extended Data Fig. 3 | Changes in fecal microbiome. a, *Akkermansia muciniphila* abundance in feces evaluated by quantitative PCR. Differential values (mean difference and mean difference from placebo) are expressed as the mean ± s.e.m. as raw data. The bars represent the mean change from baseline value per group, with their s.e.m. Mann–Whitney *U*-tests were performed to compare the differential values of both treated groups versus the placebo group (intergroup changes) according to the distribution. The respective *P* values are shown in the table below each plot. The lines represent the raw values before and after 3 months of supplementation. The distribution of values within each group for each timing is illustrated by a box-and-whisker plot. In the box plots, the line in the middle of the box is plotted at the median, and the inferior and superior limits of the box correspond to the 25th and the 75th percentiles, respectively. Matched-pairs Wilcoxon signed-rank tests were performed to verify changes from baseline (intragroup changes) according to the distribution. When the difference is significant, a capped line is marked above the group concerned with the corresponding *P* value. Kruskal–Wallis analyses were used to compare changes between 0 and 3 months across the 3 groups according to the distribution. Placebo group, $n = 11$; pasteurized bacteria group, $n = 12$; live bacteria, $n = 9$. All tests were two-tailed. $^*P < 0.05$. **b**, Visualization of participants' fecal microbiota composition at baseline and end point of the intervention. Fecal microbiota dissimilarity between samples is represented by principal coordinates analysis (genus-level Aitchison distance), with six sample groups corresponding to the three different treatment arms at baseline or at end point represented by confidence ellipses (80% confidence interval). Intervention effects are symbolized by the colored arrows, with direction and length corresponding to the shift in group centroid coordinates from baseline to end point for each treatment arm (rescaled ×4 and re-centered at the baseline global centroid). Placebo group, $n = 11$; pasteurized bacteria group, $n = 12$; live bacteria, $n = 9$.

nature research

Corresponding author(s): Patrice D. Cani

Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	Not applicable
Data analysis	- HOMA-IR to estimate insulin sensitivity (%) and insulin resistance was calculated by using the software HOMA calculator 2 (v2.3.3) available from http://www.dtu.ox.ac.uk/homacalculator/ - Statistical analyses were conducted using the SPSS software (version: 23.0 SPSS, INC). - Graphics were drawn using GraphPad Prism software (version 7.0, GraphPad Software, San Diego, CA, USA). - Demultiplexing of the sequencing data was performed using LotuS55 (version 1.565), followed by quality control and sequence variants matrix building using the DADA2 pipeline (version 1.6.0) with taxonomic annotation by RDP classifier (version 2.12) with default parameters. Statistical analyses of microbiota composition were performed in R using packages vegan and CoDaSeq. As recommended for microbiota compositional data analysis, the abundance matrix was centre log-ratio (clr) transformed (CoDaSeq:codaseq.clr). All these information are also mentioned in the methods section of the manuscript

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The data that support the findings of this study are available. All the figures are provided with individual values in order to have a direct access to the raw data. The 16S sequencing datasets generated during the current study are available in the EGA repository (European Genome-Phenome Archive, https://ega-archive.org/, accession number EGAS00001003585).

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	The sample size is described in the manuscript as well as the flow-chart (recruitment, selection, subjects included, drop outs). As sated in the manuscript, this study is a pilot proof-of-concept exploratory study, therefore, the study was not powered/designed at this stage to draw definitive conclusions of clinical outcomes. Rather the study was intended as an exploratory trial, the results of which will help design a properly powered study in the future, since several interesting results have been found and are statistically significant.
Data exclusions	As described in the methods, the exclusion criteria were pre-established and have led to the decision to exclude subjects from the dataset, and mentioned as drop-outs of the subjects during the study. Out of the 40 subjects, seven had to be excluded before completion of the study : 1 in the Placebo group, 1 in the Pasteurized group and five in the Alive group, with a total of 3 early termination due to personal reasons (i.e., mainly because of the difficulty to attend the nine scheduled visits at the hospital) and 4 due to untimely use of antibioitics during the study. One additional subject in the Placebo group was excluded from the analysis for protocol violation.
Replication	An experimental replication was not attempted for this study. This study reports the results of a pilot exploratory study. The method to produce Akkermansia has been published in Plovier et al Nature Medicine 2017 and is fully available. Although this is a pilot study a number of 9 to 12 subjects were enroled.
Randomization	Subjects were allocated to one of the treatment arms following a randomized block design with a block size of 8. The Microsoft Excel randomization function was used to generate the allocation sequence. Subjects and physicians were both blinded to the treatment allocation.
Blinding	All the subjects, the nursed and the physicians were blinded to the treatment allocation. The treatments were provided in a sachet with a numeric code and each subjects samples was also blinded by using a specific code which was not showing any link with the potential treatment.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☐	☒ Unique biological materials
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☐	☒ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Unique biological materials

Policy information about availability of materials

Obtaining unique materials	Unique Material consist in the bacterium Akkermansia muciniphila that have been provided to the volunteers. The strain of the bacteria is publicly available http://www.lgcstandards-atcc.org/Products/Collections/Microbiology_Collections/Bacteria.aspx strain code ATCC® BAA-835™ as well as the culture method used (https://www.nature.com/articles/nm.4236).

Human research participants

Policy information about studies involving human research participants

Population characteristics	A table described the overall characteristics of the volunteers is provided in the supplementary material. A description of the population is in the methods and described as follows: thirty-two overweight/obese subjects (BMI > 25 kg m-2) aged between 18 and 70 years were voluntary enrolled to participate. Eligible participants had been diagnosed with metabolic syndrome following the NCEP ATP III definition (at least three of the five following criteria: fasting glycemia > 100 mg/dl, blood pressure ≥ 130/85 mmHg or antihypertensive treatment, fasting triglyceridemia ≥ 150 mg/dl, HDL cholesterol <40 mg/dl for males, <50 mg/dl for females, and/or waist circumference >102 cm for males, >88 cm for females) and whose insulin sensitivity was <75% evaluated by HOMA-modelling of insulin sensitivity (HOMA Calculator the University of Oxford)
Recruitment	Participants were recruited at the Cliniques Universitaires Saint-Luc located in Brussels, Belgium, based on general advertizing (leaflets and website) and upon discussion with the physicians in charge of the study (3 differents), and blinded to the category of the treatment. Potential self-selection bias or other biases may have been present since all the subjects were interested by the potential effects linked to the bacteria tested (i.e., metabolic effects), however, because both the physicians and the volunteers were blinded to the category of treatments (i.e., placebo versus treatment) and the study was randomized, potential self-selection bias were likely not present and therefore may not impact the overall results.

Terms and Conditions

Springer Nature journal content, brought to you courtesy of Springer Nature Customer Service Center GmbH (“Springer Nature”).
Springer Nature supports a reasonable amount of sharing of research papers by authors, subscribers and authorised users (“Users”), for small-scale personal, non-commercial use provided that all copyright, trade and service marks and other proprietary notices are maintained. By accessing, sharing, receiving or otherwise using the Springer Nature journal content you agree to these terms of use (“Terms”). For these purposes, Springer Nature considers academic use (by researchers and students) to be non-commercial.
These Terms are supplementary and will apply in addition to any applicable website terms and conditions, a relevant site licence or a personal subscription. These Terms will prevail over any conflict or ambiguity with regards to the relevant terms, a site licence or a personal subscription (to the extent of the conflict or ambiguity only). For Creative Commons-licensed articles, the terms of the Creative Commons license used will apply.
We collect and use personal data to provide access to the Springer Nature journal content. We may also use these personal data internally within ResearchGate and Springer Nature and as agreed share it, in an anonymised way, for purposes of tracking, analysis and reporting. We will not otherwise disclose your personal data outside the ResearchGate or the Springer Nature group of companies unless we have your permission as detailed in the Privacy Policy.
While Users may use the Springer Nature journal content for small scale, personal non-commercial use, it is important to note that Users may not:

1. use such content for the purpose of providing other users with access on a regular or large scale basis or as a means to circumvent access control;
2. use such content where to do so would be considered a criminal or statutory offence in any jurisdiction, or gives rise to civil liability, or is otherwise unlawful;
3. falsely or misleadingly imply or suggest endorsement, approval , sponsorship, or association unless explicitly agreed to by Springer Nature in writing;
4. use bots or other automated methods to access the content or redirect messages
5. override any security feature or exclusionary protocol; or
6. share the content in order to create substitute for Springer Nature products or services or a systematic database of Springer Nature journal content.

In line with the restriction against commercial use, Springer Nature does not permit the creation of a product or service that creates revenue, royalties, rent or income from our content or its inclusion as part of a paid for service or for other commercial gain. Springer Nature journal content cannot be used for inter-library loans and librarians may not upload Springer Nature journal content on a large scale into their, or any other, institutional repository.
These terms of use are reviewed regularly and may be amended at any time. Springer Nature is not obligated to publish any information or content on this website and may remove it or features or functionality at our sole discretion, at any time with or without notice. Springer Nature may revoke this licence to you at any time and remove access to any copies of the Springer Nature journal content which have been saved.
To the fullest extent permitted by law, Springer Nature makes no warranties, representations or guarantees to Users, either express or implied with respect to the Springer nature journal content and all parties disclaim and waive any implied warranties or warranties imposed by law, including merchantability or fitness for any particular purpose.
Please note that these rights do not automatically extend to content, data or other material published by Springer Nature that may be licensed from third parties.
If you would like to use or distribute our Springer Nature journal content to a wider audience or on a regular basis or in any other manner not expressly permitted by these Terms, please contact Springer Nature at

onlineservice@springernature.com