Reply to 'Simpson's paradox in proof-of-concept studies'

Cani and Depommier reply: We appreciate the correspondence from Janket et al.[1] and their support concerning our study[2] showing that three months of supplementation with 10 billion *Akkermansia muciniphila* bacteria, either live or pasteurized, was safe and well tolerated and improved several cardiovascular risk factors in subjects with metabolic syndrome.

We agree with most of the points they make about the general design of pilot studies and the necessity of carefully monitoring confounding factors to mitigate the potential effects of Simpson's paradox. In their comments, they raise the interesting point that subjects should be stratified according to diet and physical activity in order to avoid any additional confounding effects. We fully agree with this, as this is exactly what we did in our pilot study. All subjects were carefully selected based on numerous criteria, including physical activity and dietary habits as determined using deep anamnesis, which was among tests we used; these also included a validated dietary questionnaire and blood analysis.

The risk that there could have been differences in basal levels of *A. muciniphila* between groups was mitigated by the fact that the levels of *A. muciniphila* were similar between groups at baseline and were not affected by the placebo, whereas the treated groups showed an increase in *A. muciniphila* titer ranging between 1.75 and 2.61 log per gram of feces (i.e., a 50–400-fold increase). This finding supports the likelihood that potential confounding factor related to *A. muciniphila* levels were scrutinized thoroughly.

Nevertheless, as Janket et al.[1] note, we and other have shown in numerous animal studies that dietary enrichment with various nutrients (e.g., prebiotic inulin and/or oligofructose) or certain polyphenols is associated with a strong change in the overall gut microbiota community, including an increase in *A. muciniphila* abundance. However, these effects on *A. muciniphila* are poorly reproduced in humans. A recent systematic review concluded that although the overall microbiota is altered by various dietary interventions, more research is needed to support the assumption that *A. muciniphila* can be targeted with dietary intervention because the results from this approach have not been consistent between studies[3]. As a matter of fact, we demonstrated in two human studies that either supplementation with isolated inulin and/or oligofructose (16 g/day)[4] or increasing the consumption of selected vegetables to naturally boost the intake of prebiotic fibers (15 g/day) induces a marked shift in the gut microbiota composition (e.g., a consistent increase in *Bifidobacterium* spp. and *Faecalibacterium prausnitzii* and a decrease in other specific taxa)[4,5]. However, although both types of regimens are associated with a strong impact on gut microbiota composition, none of these dietary approached induced changes in *A. muciniphila* levels[4,5].

Because the intake of all the nutrients mentioned by Janket et al.[1] were listed among our exclusion criteria, these confounding factors were excluded in our study[2].

We believe that our study cannot be viewed as a phase 1 trial, as such studies are designed differently and are used to find the best compromise in regard to the safety, tolerability, and pharmacodynamics (PD) and pharmacokinetics (PK) of an absorbed compound, and also normally include dose ranging to determine at what dose adverse effects begin to appear. Although we designed our nutritional study with all best efforts to achieve high quality standards, such as are used in testing pharmaceutical compounds, our study obviously did not include any escalation of doses to determine the minimal toxic dose, and it would have been impossible to assess PK and PD with this sort of nutritional approach.

Janket et al.[1] also point out that in our previous study in mice[6], we found that "the health benefits of the *A. muciniphila* membrane protein Amuc_1100 were more pronounced than those of live *A. muciniphila*." They suggest that supplementation with Amuc_1100 rather than live bacteria will be more appropriate in future research. The protein indeed replicated part of the effects of the live bacterium; however, we wish to highlight that the effects of the protein matched those observed with the pasteurized bacteria, which had greater efficacy than the live bacteria. Thus, using pasteurized *A. muciniphila* probably has different effects than simply increasing the levels of endogenous live bacteria or supplementing with a high dose of live bacteria.

In conclusion, the fact that many potential confounding factors were indeed taken into account and monitored clearly imply that Simpson's paradox may not apply to our study. ❐

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Competing interests

P.D.C. is an inventor on patent applications (PCT/EP2013/073972; PCT/EP2016/071327, and PCT/EP2016/060033 filed with the European Patent Office (EP) and the patent offices of Australia (AU), Brazil (BR), Canada (CA), China (CN), Eurasian Patent Organization (EA), Israel (IL), India (IN), Hong Kong (HK), Japan (JP), South Korea (KR), Mexico (MX), New Zealand (NZ), and the United States (US)) dealing with the use of *A. muciniphila* and its components in the context of obesity and related disorders. P.D.C. is a co-founder of A-Mansia Biotech SA.