Simpson's paradox in proof-of-concept studies

To the Editor — We read with interest the article by Depommier et al. reporting improved insulin sensitivity, reduced cholesterol levels and decreased white blood cell counts in humans with metabolic syndrome after supplementation with *Akkermansia muciniphila*[1]. Although the authors carried out elaborate laboratory experiments to test their concepts, we feel that this study lacks in the fundamental information for a phase 1 human trial report. The predictor—*A. muciniphila* treatment—and the outcomes—obesity, metabolic syndrome and diabetes—are affected by lifestyle factors, glucose levels and diet, forming a complex confounding relationship. Thus, these factors must be considered even in a phase 1 trial, in which the main purpose is safety assessment. Without this consideration (or adjustment), the metabolic improvements reported by Depommier and colleagues may be an example of Simpson's paradox (which posits that when confounding factor(s) are not adjusted for, they can generate opposing and seemingly paradoxical results). If the observed results are a product of Simpson's paradox, stratifying the results according to the levels of diet and exercise will cause the observed beneficial effects to disappear. Randomization alone does not balance all the confounding factors, unless the sample size is sufficiently large[2].

A diet rich in polyphenols, polydextrose, butyrate and inulin is reported to increase *A. muciniphila* abundance[3]. Butyrate and other short-chain fatty acids from high-fiber diets play an important role in gut mucosa health[4], improve insulin resistance[4] and gut permeability[5] and affect the mucin layer, which is the substrate for *A. muciniphila*[6]. Thus, it is highly likely that diet will affect the abundance of *A. muciniphila* and reduce metabolic inflammation as well[5]. Therefore, phase 1 trials such as this one must follow the standard human phase 1 trial reporting format to show that confounding factors would not have biased their results, as was recently reported by Bajaj et al.[7]. Without elucidating the balanced confounding factors at baseline, the report may contain potentially biased results and trigger the lay media to produce flash headlines that may mislead the public.

Notably, the health benefits of the *A. muciniphila* membrane protein Amuc_1100 were more pronounced than those of live *A. muciniphila*[1]. Hence, supplementation with Amuc_1100 rather than live bacteria will be more appropriate in future research. Additionally, we suggest that phase 2 and 3 trials should have a cross-over design to mitigate any bias stemming from the imbalance in confounding factors in the parallel group design. We sincerely hope that Amuc_1100 can curtail the obesity pandemic worldwide. The investigators should realize that confounding must be balanced at baseline; otherwise the results will be biased. Also, blinding will not balance the confounding. ❐

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Competing interests

The authors declare no competing interests.

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