Identifying the factors influencing outcome in probiotic studies in overweight and obese patients: host or microbiome?

We read with interest Rodriguez and colleagues' study, using microbiota transfer from obese stool donors into inulin-treated *hum-ob* mice, to define a gut microbiome signature predicting response to prebiotic.[1] However, the impact of other microbiome-based interventions (and particularly probiotics) on weight loss in humans is highly-variable between individuals.[2] We were interested as to whether baseline gut microbiota, or aspects of host physiology, may predict weight loss during probiotic studies.

In our recent double-blind study (ISRCTN12562026), overweight/obese adults were randomised to either 6 months of Lab4P probiotic (containing lactobacilli and bifidobacteria) or placebo.[3] A higher proportion of participants receiving probiotic lost weight compared with those receiving placebo, and the extent of weight loss in the probiotic arm was greater than

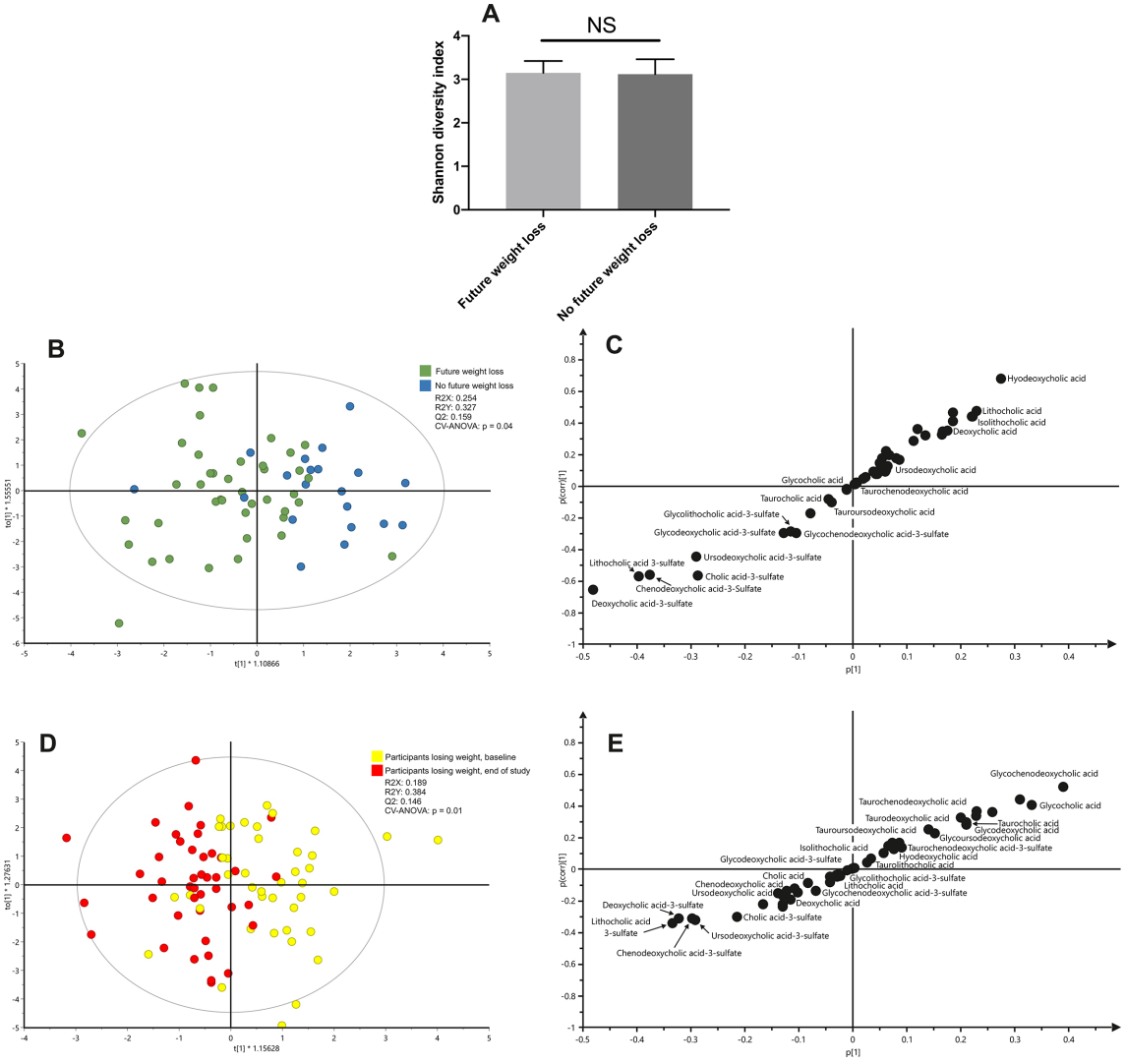

Figure 1 Effect of probiotics on stool microbiome and bile acid profiles in patients losing weight. (A) Shannon diversity index of baseline samples from participants losing weight (n=41) versus those not losing weight (n=19) during the study (as assessed from 16S rRNA gene profiling of stool samples; Student's t-test); (B) orthogonal partial least squares discriminant analysis (OPLS-DA) of stool bile acid profiles (assessed via LC-MS) of baseline samples from participants losing weight versus those not losing weight; (C) S-plot from OPLS-DA in 1B, with all assayed sulfated bile acids clustering on the left of the plot, consistent with their enrichment in baseline stool samples from those participants losing weight during the study; (D) OPLS-DA of stool bile acid profiles of baseline versus end of study samples from those participants losing weight during the study (n=41 in both groups); (E) S-plot from OPLS-DA in 1D, with most assayed sulfated bile acids clustering on the left of the plot, consistent with their enrichment in baseline (compared with end of study) stool samples from those participants losing weight during the study. CV-ANOVA, coefficient of variance analysis of variance; LC-MS, liquid chromatography-mass spectrometry.

the placebo group.[3] We subsequently performed metataxonome and metabonome analysis on stool samples from study participants (using 16S rRNA gene sequencing and liquid chromatography-mass spectrometry bile acid profiling, respectively, applying established protocols[4 5]).

We observed no difference in stool microbiota alpha-diversity at baseline between participants losing weight during the study versus those who did not (figure 1A); furthermore, no differences were observed in microbiota composition between groups at any taxonomic level. Conversely, on stool bile acid profiling, a valid supervised multivariate model could be constructed separating baseline samples from participants losing weight during the study versus those who did not (p=0.04; figure 1B). Discriminatory feature identification was performed via S-plot, with baseline samples from participants losing weight demonstrating enrichment in all identified sulfated bile acids compared with those not losing weight (figure 1C). A similar multivariate analysis compared stool bile acid profiles from baseline versus end of study from all participants losing weight during the study; a further valid supervised multivariate model could be made (p=0.01; figure 1D), with most sulfated bile acids enriched in pre-intervention samples (figure 1E). Of participants taking probiotics, a much higher proportion lost weight during the study duration than did not (n=27/34 *vs* *n*=7/34, respectively). A trend was observed towards enrichment of stool sulfated bile acids in baseline stool samples from participants in the probiotic arm subsequently losing weight versus those who did not; however, this did not reach statistical significance (p=0.11), perhaps reflecting the relatively small number of participants not losing weight in the probiotic arm.

Bile acid sulfation is performed in the liver by host enzymes (as a means of bile acid detoxification and elimination), while microbial sulfatases cleave −3-sulfate groups from bile acids intracolonically.[6 7] Previous rodent work has identified that probiotic use is associated with suppressed activity of the farnesoid X receptor-fibroblast growth factor 15 (FXR-FGF15) axis;[8] furthermore, FXR-null mice have higher expression of the gene for the sulfation enzyme Sult2a1 and increased faecal excretion of lithocholic acid-3-sulfate,[9] providing a potential link between sulfated bile acids and a pathway with pleiotropic effects on host metabolism and weight.

We are uncertain as to whether sulfated faecal bile acids directly influence body weight, if they are a marker of mechanisms that do, or are a proxy of diet. We also cannot state if this association between enriched faecal sulfated bile acids and future weight loss may suggest excessive host sulfation or reduced gut microbiota desulfation functionality in certain overweight/obese patients; however, the latter explanation seems less likely given the lack of baseline gut microbiota differences between groups.

While a gut *microbial* signature appears to underpin future weight loss in response to prebiotics, it may be that *host* metabolic functions predict such changes for weight loss in probiotic studies. Obesity is a complex, multifactorial disease, with potentially variable dominant mechanisms in different individuals; it is feasible that any benefit of microbial interventions may be mediated via their impact on aberrant host physiology, rather than purely amelioration of gut microbiome perturbation.

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