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Letter

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

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In our recent double-blind study (ISRCTN12562026), overweight/ obese adults were randomised to either six months of Lab4P probiotic (containing lactobacilli and bifidobacteria) or placebo [3]. A higher proportion of participants receiving probiotic lost weight compared to those receiving placebo, and the extent of weight loss in the probiotic arm was greater than the placebo group [3]. We subsequently performed metataxonomes and metabonomes analysis on stool samples from study participants (using 16S rRNA gene sequencing and liquid chromatography-mass spectrometry (LC-MS) 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 vs 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 vs 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 to those not losing weight (**Figure 1C**). A similar multivariate analysis compared stool bile acid profiles from baseline vs 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 vs those who did not; however,

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