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## Temporal microbial volatility remains unchanged with dietary fibre intervention and does not distinguish between responders and non-responders to fibre's blood pressure-lowering effects

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Increased temporal variability in the gut microbiome is associated with intestinal conditions such as ulcerative colitis and Crohn's disease, leading to the recently established concept of microbial volatility (1). Increased physiological stress has been shown to increase microbial volatility indicating that microbial volatility is susceptible to external interventions<sup>(1)</sup>. Dietary fibre positively affects the gut microbiome, but it is unclear if it impacts microbial volatility. The gut microbiota influences hypertension, and high-fibre intake reduces blood pressure  $(BP)^{(2)}$ . However, not all individuals exhibit a response to these fibre-based dietary changes, and the reasons for this variability remain unclear. Similarly, it is unknown whether the degree of stability of the gut microbiota consortium could be a determining factor in individual responsiveness to dietary interventions. Here, we aimed to identify: i) whether gut microbiome volatility differs when dietary fibre vs placebo interventions, and ii) whether microbiome volatility discriminates between BP responders and nonresponders to a high fibre intervention. Twenty treatment-naive participants with hypertension received either placebo or 40g per day of prebiotic acetylated and butyrylated high amylose maize starch (HAMSAB) supplementation for 3 weeks in a phase II randomised cross-over double-blind placebo-controlled trial<sup>(3)</sup>. Blood pressure was monitored at baseline and each endpoint by 24-hour ambulatory BP monitoring, with those experiencing a reduction between timepoints of  $\ge 2$  mmHg classified as responders. Baseline stool samples were collected, and the V4 region of the 16S gene was sequenced. Taxonomy was assigned by reference to the SILVA database. Microbial volatility between timepoints (e.g., pre- and post-intervention) was calculated as the Euclidian distance of centred log-ratio transformed genera counts (Aitchison distance). No difference was observed in microbial volatility between individuals when they received the dietary fibre intervention or the placebo ( $21.5 \pm 5.5$  vs  $20.5 \pm 7.7$ , p = 0.51). There was no significant difference between microbial volatility on the dietary intervention between responders and non-responders ( $21.8 \pm 4.9$  vs  $20.9 \pm 7.2$ , p = 0.84). There was no association between the change in BP during intervention and microbial volatility during intervention ( $r^2 = -0.09$ , p = 0.72). These data suggest that temporal volatility of the gut microbiota does not change with fibre intake or contribute to the BP response to dietary fibre intervention trials in people with hypertension.

## References

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