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A metabolomics approach to discover and validate dietary biomarkers of green beans intake

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After decades of nutrition research, it is now well recognised that diet has a major influence on health and disease outcomes. The consumption of legumes has been associated with reduced risk of developing several noncommunicable diseases[1]. Accurate determination of intake of these foods is challenging with traditional methods of dietary assessment as these are susceptible to errors such as recall bias and difficulty estimating portion sizes[2]. Dietary biomarkers have emerged as a potential objective method of intake assessment, with a number of candidate biomarkers suggested for specific foods[3]. Currently, there are no validated biomarkers for green beans. Therefore, the objective of the current work is to discover and validate dietary biomarkers of green beans intake. A randomised cross-over acute intervention study was performed with 27 healthy participants that consumed a large portion of green beans, mixed berries, and mixed vegetables on 3 separate occasions over 3 weeks. Following an overnight fast, participants provided a first void urine sample and collected samples after 2-, 4-, 6-, and 24-hours post-consumption of the foods. Urinary metabolites were measured by liquid chromatography – mass spectrometry (LC-MS) in both positive and negative ionisation modes, and multivariate statistical analysis was performed to determine the features that changed post-consumption of green beans. Samples were further analysed by tandem mass spectrometry to obtain fragmentation patterns for metabolite identification. The putative biomarkers were assessed for a dose-response relationship in a follow-up study where 33 participants consumed 3 portion sizes of green beans.

Partial least squares discriminant analysis models demonstrated that the urinary metabolome was distinctly different following consumption of green beans (0 h v 4 h, Positive Mode ($R^2X = 0.51$, $Q^2=0.35$); Negative Mode ($R^2X = 0.28$, $Q^2=0.43$)). Variable importance in projection lists generated from the models and a repeated measures ANOVA identified the most discriminant features between pre- and post-consumption of green beans ($p < 0.05$). Examination of the features revealed that a total of 16 positive and 62 negative features displayed a differential time course after green beans intake compared to the control food. Putative identifications from spectral databases and the literature were assigned to metabolites, including trigonelline with mass to charge ratio (m/z) 138.055 and retention time (RT) 0.706, eucomic acid (m/z 239.0563, RT 10.196), 5-hydroxyanthranillic acid (m/z 154.0498, RT 4.697), hydroxyabscisic acid glucuronide (m/z 455.154, RT 12.573), and 3-hydroxysuberic acid (m/z 189.0767, RT 11.314). These metabolites, among others, displayed a dose-response relationship with increased urinary concentrations following increased green beans consumption. Confirmation of the metabolite identifications with authentic standards is on-going. Moreover, data from the dose-response study indicates that certain features can distinguish between portions of green beans intake. These metabolites will be combined into biomarker panels and assessed for their ability to predict intake.

References

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