

## Urinary hippurate and proline betaine relative to fruit intake, blood pressure, and body mass index

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Recent application of metabolomics in small, well-controlled dietary intervention studies identified urinary excretion of hippurate (HP, gut-microbial metabolite of polyphenol metabolism) and proline betaine (PB) as markers of fruit intake.<sup>(1,2)</sup>

In a preliminary Metabolome-Wide Association Study using proton nuclear magnetic resonance (NMR) spectroscopy, we confirmed urinary excretion of HP and PB as consistent markers of total fruit intake across two free-living populations with repeated measurements. Here, we evaluated associations of urinary HP and PB and fruit intake with blood pressure (BP), and body mass index (BMI).

Cross-sectional data were used from 2,032 US and 449 UK participants ages 40 to 59 years of the INTERMAP (International Study of Macro- and Micronutrients and Blood Pressure) study. Fleshy fruit intakes, based on botanical classification and including 100 % fruit juices, were calculated from four in-depth 24-hr dietary recalls. Peak intensities of HP ( $\delta$ 7.84, doublet) and PB ( $\delta$ 3.11, singlet) were quantified from 24-hr urinary NMR spectra. Average metabolite excretions across quartiles of commonly consumed energy-adjusted (g/1000 kcal) fruit intakes and *P* for trend were calculated. Country-specific multivariable linear regression coefficients between fruits and metabolites were pooled, weighted by inverse of their variance.

Age-sex-sample adjusted Pearson correlations showed good reproducibility between visits for HP/PB in the US ( $r = 0.56/0.54$ ) and the UK ( $r = 0.59/0.74$ ). Correlations with HP/PB were 0.17/0.54 for total fruits, 0.21/0.20 for urinary potassium, and  $-0.23/-0.21$  for urinary sodium-to-potassium ratio. PB and HP were not intercorrelated ( $r = -0.03-0.12$ ). After adjustment for lifestyle factors, higher HP excretion by 2SD was inversely associated with systolic BP (Table), diastolic BP, and BMI. Significant associations with systolic BP prevailed with adjustment for BMI. Higher PB excretion was inversely associated with BMI, but not with BP. Total fruit intake was associated with a lower BMI and systolic BP; the latter attenuated with adjustment for BMI. Significant multivariable adjusted *P* for trends ( $P < 4 \times 10^{-6}$ ) across quartiles of intake were found between total fruit, apples and HP; similarly for total fruit and citrus fruit with PB. Correlations were 0.15 between apples and HP; 0.72 between citrus fruit and PB. Apples and citrus fruits were not related to BP or BMI.

In conclusion, higher urinary HP excretion was associated with lower systolic BP and BMI, higher urinary PB excretion with BMI. Associations of total fruit intake with systolic BP and BMI showed weaker, but comparable results.

	Systolic BP			Diastolic BP			BMI		
	Difference, mmHg	95 % CI		Difference, mmHg	95 % CI		Difference, mmHg	95 % CI	
PB	-0.86	-1.87	0.15	0.01	-0.70	0.71	-0.68	-1.09	-0.27
HP	-2.85	-3.86	-1.83	-1.13	-1.83	-0.42	-1.66	-2.07	-1.25
Fruit	-1.04	-2.11	0.04	-0.19	-0.94	0.55	-0.73	-1.17	-0.30
Citrus fruit	-0.29	-1.30	0.72	0.28	-0.42	0.98	-0.22	-0.64	0.19
Apples	-0.50	-1.51	0.52	-0.56	-1.26	0.14	-0.39	-0.79	0.02

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1. Heinzmann SS, Brown IJ, Chan Q *et al.* (2010) *AJCN* **92**,436–43.
2. Heinzmann SS, Merrifield CA, Rezzi S *et al.* (2012) *J Prot Res* **11**,643–55.