

## Urinary vitamin C excretion as a biomarker of compliance in a fruit and vegetable intervention study

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Observational evidence consistently suggests fruit and vegetable (FV)-rich diets may protect against many non-communicable diseases<sup>(1)</sup>. Progression of research on the FV-disease risk relationship is hindered by inaccuracies involved in traditional methods of assessing dietary intake<sup>(2)</sup>. Accurate, reliable, independent, objective nutritional biomarkers are therefore crucial to modern epidemiological research. Plasma vitamin C concentration is widely used as an indicator of FV intake<sup>(2)</sup>, however little attention has focused on use of urinary vitamin C as a biomarker of FV intake. Thus, using data from a randomised FV study, we determined the use of 24-hour urinary vitamin C as a potential novel objective biomarker of compliance of participants in FV interventions.

Participants were aged 40–65 years and hypertensive (blood pressure of 140–179/90–109 mmHg). Following a one portion FV/day four-week run-in-period, participants were randomised to consume 1,3 or 6 portions FV/day for eight-weeks. Fasting plasma and 24-hour urine samples were collected at the start and end of the intervention. Vitamin C was measured using a fluorimetric method on an automated Cobas Fara centrifugal analyser.

A total of 117 subjects completed the 12-week study. Across the intervention groups plasma vitamin C increased, but plateaued between 3 and 6 portions/d, and the test for linear trend across the groups did not reach statistical significance ( $p = 0.06$ ). In contrast, for urinary vitamin C, regardless of whether values were standardised for creatinine, or volume, or not standardised, the difference between the three groups was statistically significant, and increased as FV intake increased (comparison between 1, 3 and 6 portions/day groups,  $p$ -value for linear trend all  $<0.001$ ).

			1 portions/d	3 portions/d	6 portions/d	$p$ -value
Fruit and vegetable intake (portions/d) <sup>1</sup>	Baseline	No. of subjects	27	33	34	–
		Mean	0.9	1.1	1.1	0.25
	Change at 8 wk	SD	0.4	0.5	0.5	
		Mean	0.2	2.2	4.5	$<0.001$
		95% CI	0.0, 0.3	1.8,2.5	4.2,4.7	
Plasma vitamin C ( $\mu\text{mol/l}$ ) <sup>2</sup>	Baseline	No. of subjects	29	38	38	–
		Mean	23.7	25.7	27.9	0.62
	Change at 8 wk	IQR	15.5,34.3	20.9,36.7	19.2,45.0	
		Mean	1.09	1.51	1.52	0.06
		95% CI	0.90,1.32	1.20,1.91	1.26,1.83	
Urinary vitamin C status ( $\mu\text{mol/l}$ ) <sup>2</sup>	Baseline	No. of subjects	21	29	20	–
		Mean	4.54	8.05	4.98	0.58
	Change at 8 wk	IQR	0.40,27.15	2.44,22.45	0.40,134.18	
		Mean	0.80	1.89	12.02	$<0.001$
		95% CI	0.34, 1.84	0.74,4.86	3.48,41.56	

<sup>1</sup> All baseline values are mean (SD), and all changes mean (95% CI). Changes were calculated as week 8 – baseline. <sup>2</sup>Variables were logarithmically transformed. All baseline values are geometric mean (IQR), and all change values are geometric mean (95% CIs) of the ratio of the week 8 to baseline value.

Urinary vitamin C (24 h) increased linearly with increasing FV consumption, in contrast to plateauing plasma vitamin C concentration. This suggests 24-hour urinary vitamin C is a potential objective biomarker of compliance in FV intervention studies.

1. Dehghan M, Akhtar-Danesh N, McMillan CR, Thabane L (2007) *Nutr J* 6, 41.
2. Jenab M, Slimani N, Bictash M, Ferrari P, Bingham SA (2009) *Hum Genet* 125, 507–525.