Circulatory and Urinary B-Vitamin Responses to Multivitamin Supplement Ingestion Differ Between Older and Younger Adults

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## **Supplemental Tables**

**Table S1:** Urine output and creatinine concentration of the study participants at fasting and 1-4 h following multivitamin and mineral supplement ingestion.

TT *		Old		Younger		P value		
Urine	M	F	M	F	Age	Sex	Age x sex	
Volume fasting (g)	41.1 ± 2.3	45.1 ± 4.3	$46.9 \pm 5.6$	32.1 ± 5.7	0.456	0.267	0.058	
Volume 1-4 h (g)	$408.7 \pm 88$	$489.9 \pm 113.7$	$469 \pm 147.2$	$508.9 \pm 83.4$	0.735	0.606	0.860	
Creatinine fasting (mmol/L)	$11.7 \pm 0.9$	$8.4 \pm 1.5$	$18.9 \pm 3.6$	$17.5 \pm 3.7$	0.005*	0.401	0.734	
Creatinine 1-4 h (mmol/L)	$7.5 \pm 1.2$	$5 \pm 1.7$	$8.7 \pm 2.2$	$3.2 \pm 0.5$	0.813	0.012*	0.328	

M: male; F: female. \*, significant main age or sex effects, P < 0.05.

Values are presented as mean ± SEM (n=20 in each age group with 10 males and 10 females each.)

Statistical analysis was performed using general linear univariate model with age and sex as fixed factors;  $\alpha$  *P* < 0.05.

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Table S2. Baseline B-vitamin and vitamer concentration in circulation and urine of the study participants measured at fasting.

	Older adults		Younger adults		Effect		
Circulation	M (n=10)	F (n=10)	M (n=10)	F (n=10)	Age	Sex	Age x sex
Vitamin B <sub>12</sub> 1	351.9 ± 31.9a	$535.4 \pm 64.7^{b}$	$569.6 \pm 29.7^{b}$	$473.2 \pm 53.4$	0.109	0.363	0.005 <sup>+</sup>
Folate <sup>1</sup>	$16.5 \pm 3.2$	$15.9 \pm 1.4$	$10.1 \pm 1.1$	$8.9 \pm 1.1$	$0.001^{*}$	0.689	0.878
Pyridoxine	$0.2 \pm 0.1$	$0.2 \pm 0.0$	$0.3 \pm 0.2$	$0.1 \pm 0.0$	0.506	0.333	0.352
Pyridoxal	$9.2 \pm 3.2$	$4.6 \pm 1.5$	$10.1 \pm 1.5$	$11.6 \pm 2.9$	0.108	0.520	0.217
Pyridoxamine	$1.0 \pm 0.2$	$0.9 \pm 0.3$	$1.2 \pm 0.2$	$0.6 \pm 0.2$	0.988	0.116	0.247
PLP	$2.1 \pm 1.6$	$1.0 \pm 0.3$	$1.1 \pm 0.5$	$2.7 \pm 1.9$	0.822	0.864	0.298
4-pyridoxic acid	$20.7 \pm 5.6$	$21.9 \pm 2.2$	$16.6 \pm 2.3$	$17.1 \pm 3.2$	0.222	0.819	0.916
Thiamine	$1.3 \pm 0.4$	$2 \pm 0.3$	$0.5 \pm 0.1$	$0.6 \pm 0.2$	$0.001^{*}$	0.161	0.276
Pantothenic acid	$113.3 \pm 4.1^{a}$	$169.5 \pm 22.1$ <sup>b</sup>	$124.3 \pm 13.1$	$106.6 \pm 12.3^{a}$	0.080	0.189	$0.014^{+}$
Riboflavin	$18.1 \pm 2.3$	$17.9 \pm 2.5$	$10.1 \pm 1.1$	$20.1 \pm 5.1$	0.352	0.127	0.108
FMN	$4.4 \pm 0.9$	$6.9 \pm 1.2$	$7.7 \pm 1.6$	$8.6 \pm 1.5$	0.064	0.186	0.520
Nicotinic acid	$1.7 \pm 0.4$	$2.1 \pm 0.4$	$2.5 \pm 1.2$	$2.3 \pm 0.4$	0.512	0.896	0.667
Nicotinamide	$331.1 \pm 63.7$	$503.6 \pm 68.4$	$641.3 \pm 266.7$	$724.2 \pm 74.5$	0.078	0.388	0.761
Nicotinuric acid	$582.4 \pm 145.6$	$196.7 \pm 48.2^{a}$	$358.6 \pm 90.1$	$778.4 \pm 262.5$ <sup>b</sup>	0.267	0.915	$0.016^{+}$
Urine							
Folic acid	$0.4 \pm 0.1$	$0.9 \pm 0.4$	$0.3\pm0.1$	$0.8 \pm 0.4$	0.715	0.093	0.984
Pyridoxine	$0.1\pm0.0$	$0.1 \pm 0.0$	$0.1 \pm 0.0$	$0.1 \pm 0.0$	0.116	0.395	0.501
Pyridoxal	$250.8 \pm 45.3$	$332.7 \pm 39.5$	$244.8 \pm 50.7$	$343.5 \pm 71.0$	0.964	0.097	0.875
Pyridoxamine	$4.5 \pm 0.3$	$4.9 \pm 0.5$	$3.4 \pm 0.3$	$5.1 \pm 0.4$	0.341	0.089	0.280
PLP	$32.2 \pm 15.4$	$125.9 \pm 60.2$	$13.4 \pm 6.7$	$423.2 \pm 281.5$	0.234	0.01*	0.091
4-pyridoxic acid	$2316.4 \pm 834.0$	$1938.8 \pm 174.6$	$1451.7 \pm 149.2$	$2195.6 \pm 546.3$	0.556	0.722	0.280
Thiamine	$40.5 \pm 7.9$	$46.8 \pm 8.0$	$40.5 \pm 6.1$	$34.0 \pm 6.9$	0.383	0.994	0.385
Pantothenic acid	$1890.9 \pm 85.5$	$3363.5 \pm 454.4$	$1697.2 \pm 391.0$	$2433.0 \pm 500.7$	0.161	0.008*	0.355
Riboflavin	$323.1 \pm 56.7$	$352.4 \pm 63.2$	$149.9 \pm 27.8$	$227.5 \pm 74.8$	$0.015^{*}$	0.365	0.681
FMN	$241.8 \pm 32.1$	$288.1 \pm 49.5$	$187.9 \pm 16.9$	$248.0 \pm 41.2$	0.212	0.159	0.853
Nicotinic acid	$65.4 \pm 5.7$	$100.0 \pm 18.2$	$34.6 \pm 3.8$	$59.6 \pm 10.4$	$0.003^{*}$	$0.01^{*}$	0.664
Nicotinamide	$772.5 \pm 30.6$	$731.4 \pm 81.7$	$611.6 \pm 45.0^{a}$	$834.9 \pm 71.7^{b}$	0.64	0.143	0.036 <sup>†</sup>
Nicotinuric acid	$995.9 \pm 254.6$	$414.4 \pm 107.9^{a}$	$336.5 \pm 96.8^{a}$	$1540.2 \pm 478.8$ <sup>b</sup>	0.412	0.275	0.003+
Biotin	$12.3 \pm 0.6$	$19.5 \pm 2.2$	$13.6 \pm 1.9$	$22.4 \pm 7.2$	0.589	$0.047^{*}$	0.851

Values are means  $\pm$  SEMs of concentrations in circulation (nmol/L) or urine (nmol/mmol creatinine), except otherwise stated. Circulatory ¹vitamin B₁₂ (pg/mL) and folate (mg/mL) were measured in serum, the rest in plasma samples folic acid and biotin were only detected in urine. Data were compared using general linear multivariate analysis of variance with age and sex as fixed factors. Sidak post-hoc test was applied for pairwise comparisons if significant age x sex interaction was present. Significant age x sex interaction (p < 0.05) is indicated by "†", main effects are indicated by "\*". Different superscript letters within a row indicate significantly different values between groups (p < 0.05). M, males; F, females.

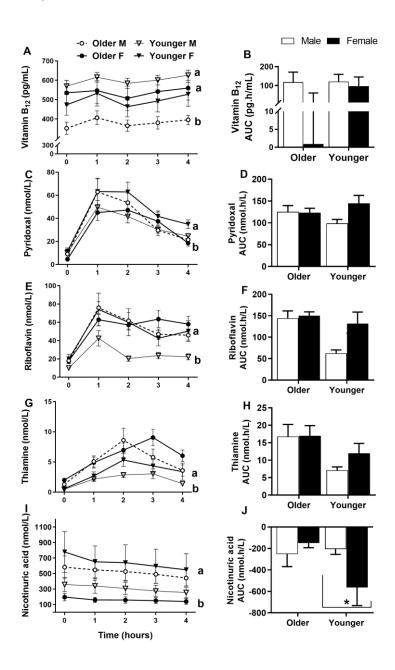
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Supplemental Figure 1.



**Figure S1.** Postprandial circulating B-vitamin concentrations with sex-specific effects in the older and younger adults following a multivitamin and mineral supplement ingestion. The panel on the left represents the time response concentration curves at each study time points including at fasting (Time=0 hour) and the right panel are the incremental area under the curves (iAUC) for the respective vitamins, (AB) serum vitamin  $B_{12}$ , (CD) pyridoxal, (EF) riboflavin, (GH) thiamine and (IJ) nicotinuric acid. Different alphabetical letters indicate a statistically significant difference (P = 0.05) between the groups indicated and asterisks (\*) indicate the difference in the 4-hour iAUC between age or sex groups.

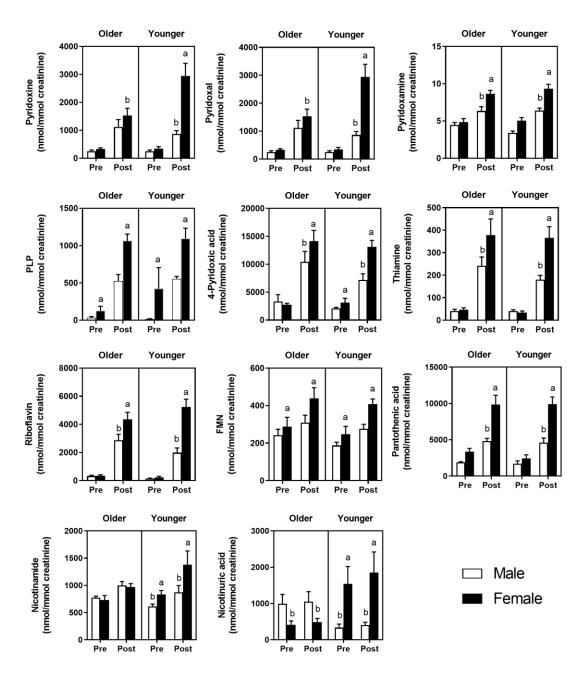
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Supplemental Figure 2.



**Figure S2.** Sex - specific effects on the urinary concentrations of B-vitamins and vitamers before (pre) and after (post) multivitamin supplement ingestion. Different alphabetical letters indicate significant differences with interactions (time x age x gender, P < 0.05) between or within age groups as indicated, whereas similar letters indicate a main sex effect, P=0.05) on vitamin concentration.