Measuring Vitamin D₃ Metabolic Status, Comparison between Vitamin D Deficient and Sufficient Individuals

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Table S1. MRM parameters for detection of target metabolites and ISs.

Analyte	Data acquisition segments	Precursor ion (*m/z*)	Fragmentor (V)	Adduct	Collision energy (eV)	Dwell time (ms)	Quantitation transition	RT (min)
1,24,25(OH)₃D₃	1 – 3.5 min	397.3	80	[M+H–2H₂O]⁺	30	500	397.3 → 107.1	2.8
24,25(OH)₂D₃		399.3	170	[M+H–H₂O]⁺	30		399.3 → 121.0	4.1
24,25(OH)₂D₃-d₆	3.5 – 7 min	405.5	170	[M+H–H₂O]⁺	20	130	405.3 → 121.0	
1,25(OH)₂D₃		399.3	140	[M+H–H₂O]⁺	30		399.3 → 227.1	5.2
1,25(OH)₂D₃-d₃		402.5	140	[M+H–H₂O]⁺	20		402.3 → 230.1	

25(OH)D₃	7 – 10 min	383.3	145	[M+H–H₂O]⁺	30	250	383.3 → 107.1	7.5
25(OH)D₃-d₃		386.3	145	[M+H–H₂O]⁺	30		386.3 → 107.1	
Vitamin D₃	10 – 14 min	385.3	85	[M+H]⁺	30	250	385.3 → 107.0	11.2
Vitamin D₃-d₃		388.3	120	[M+H]⁺	25		388.3 → 162.1	

Table S2. Calibration models for determination of target metabolites in plasma.

Analyte	LOD ($pg\ mL^{-1}$)	LOQ ($pg\ mL^{-1}$)	Calibration range	Equation	Coefficient of determination
$1,24,25(OH)_3D_3$	0.3	1.0	$1.0 - 1000\ pg\ mL^{-1}$	$y = 0.0043 \pm 0.0001\times + 0.1489 \pm 0.0248$	0.9964
$24,25(OH)_2D_3$	3.0	10.0	$0.01 - 20\ ng\ mL^{-1}$	$y = 0.2142 \pm 0.0031\times - 0.0635 \pm 0.0119$	0.9966
$1,25(OH)_2D_3$	1.2	4.0	$4.0 - 500\ pg\ mL^{-1}$	$y = 0.0026 \pm 0.0001\times - 0.1254 \pm 0.0166$	0.9947
$25(OH)D_3$	0.3	1.0	$0.001 - 250\ ng\ mL^{-1}$	$y = 0.1417 \pm 0.0011\times + 0.02269 \pm 0.0113$	0.9991
Vitamin D_3	1.5	5.0	$0.005 - 25\ ng\ mL^{-1}$	$y = 0.1121 \pm 0.0015\times + 0.0756 \pm 0.0154$	0.9973

Table S3. Spearman correlation results between metabolites and ratios involved in vitamin D_3 metabolism.

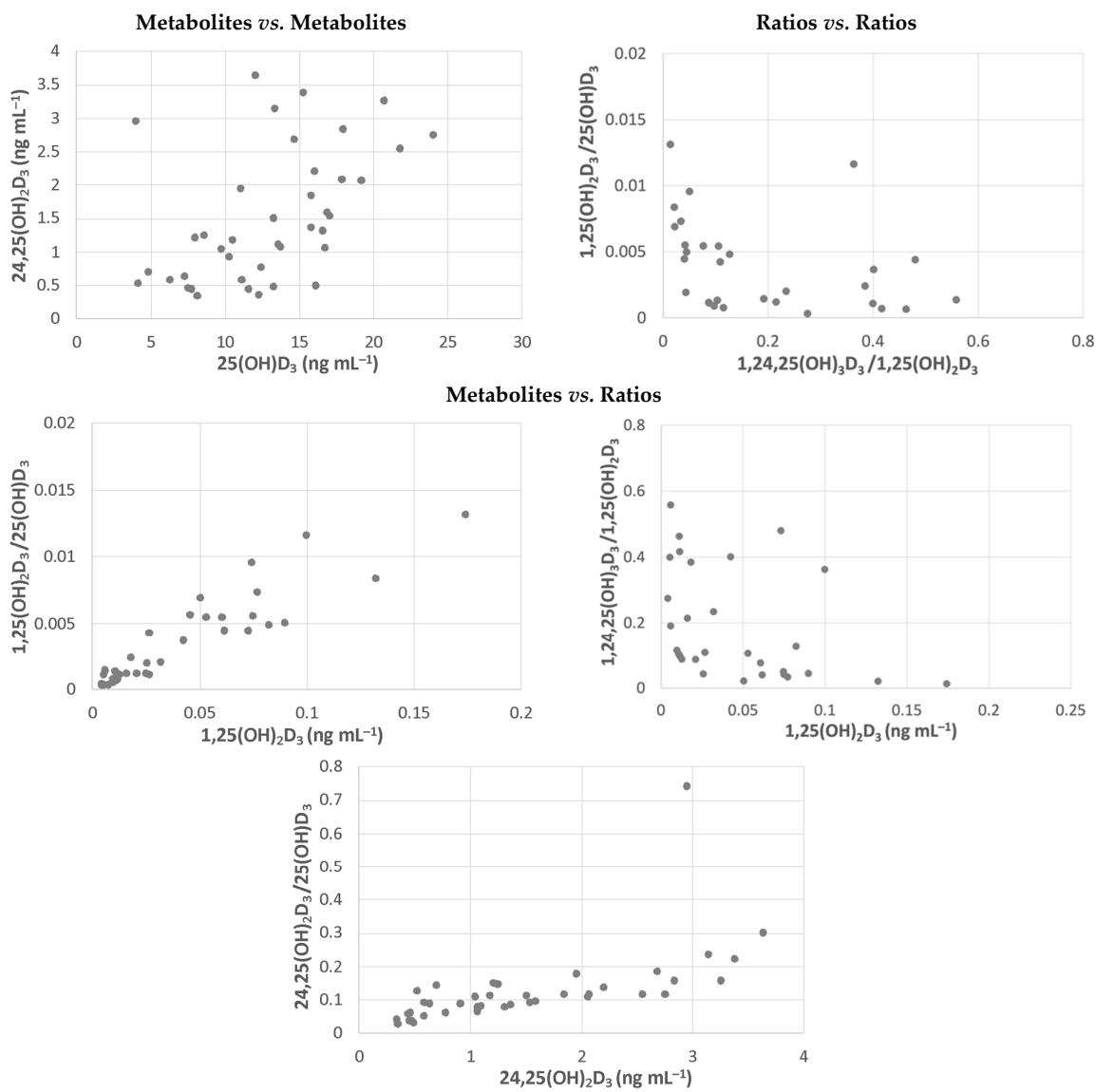|			*p-value*	Correlation coefficient, rho (ρ)
Metabolites *vs.* Metabolites	$25(OH)D_3$	$24,25(OH)_2D_3$	0.0004	0.54
Metabolites *vs.* Ratios	$1,25(OH)_2D_3$	$1,25(OH)_2D_3/25(OH)D_3$	0.0310	0.89
		$1,24,25(OH)_3D_3/1,25(OH)_2D_3$	<0.0001	−0.63
	$24,25(OH)_2D_3$	$24,25(OH)_2D_3/25(OH)D_3$	<0.0001	0.82
Ratios *vs.* Ratios	$1,24,25(OH)_3D_3/1,25(OH)_2D_3$	$1,25(OH)_2D_3/25(OH)D_3$	<0.0001	−0.70

Figure S1. Plots of significant correlations between metabolites and ratios involved in vitamin D₃ metabolism.