Supplemental materials for

“Metabolic alterations in multiple sclerosis and the impact of vitamin D supplementation”

Supplementary Methods

Metabolomics Analysis

Samples were stored at –80°C until processed. Sample preparation was carried out as described previously at Metabolon, Inc [1]. Briefly, recovery standards were added prior to the first step in the extraction process for quality control purposes. To remove protein, dissociate small molecules bound to protein or trapped in the precipitated protein matrix, and to recover chemically diverse metabolites, proteins were precipitated with methanol under vigorous shaking for 2 min (Glen Mills Genogrinder 2000) followed by centrifugation. The resulting extract was divided into five fractions: one for analysis by ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS; positive ionization), one for analysis by UPLC-MS/MS (negative ionization), one for the UPLC-MS/MS polar platform (negative ionization), one for analysis by gas chromatography–mass spectrometry (GC-MS), and one sample was reserved for backup.

Three types of controls were analyzed in concert with the experimental samples: samples generated from a pool of extensively characterized human plasma or generated from a small portion of each experimental sample of interest served as technical replicate throughout the data set; extracted water samples served as process blanks; and a cocktail of standards spiked into every analyzed sample allowed instrument performance monitoring. Instrument variability was determined by calculating the median relative standard deviation (RSD) for the standards that were added to each sample prior to injection into the mass spectrometers (median RSD typically = 4-6%; $n \geq 30$ standards). Overall process variability was determined by calculating the median RSD for all endogenous metabolites (i.e., non-instrument standards) present in 100% of the

pooled human plasma or client matrix samples (median RSD = 10-14%; n = several hundred metabolites). Experimental samples and controls were randomized across the platform run.

Mass Spectrometry Analysis

Non-targeted MS analysis was performed at Metabolon, Inc. Extracts were subjected to either GC-MS or UPLC-MS/MS [1,2]. The chromatography was standardized and, once the method was validated no further changes were made. As part of Metabolon's general practice, all columns were purchased from a single manufacturer's lot at the outset of experiments. All solvents were similarly purchased in bulk from a single manufacturer's lot in sufficient quantity to complete all related experiments. For each sample, vacuum-dried samples were dissolved in injection solvent containing eight or more injection standards at fixed concentrations, depending on the platform. The internal standards were used both to assure injection and chromatographic consistency. Instruments were tuned and calibrated for mass resolution and mass accuracy daily.

The UPLC-MS/MS platform utilized a Waters Acquity UPLC with Waters UPLC BEH C18-2.1×100 mm, 1.7 μm columns and a Thermo Scientific Q-Exactive high resolution/accurate mass spectrometer interfaced with a heated electrospray ionization (HESI-II) source and Orbitrap mass analyzer operated at 35,000 mass resolution. The sample extract was dried then reconstituted in acidic or basic LC-compatible solvents, each of which contained 8 or more injection standards at fixed concentrations to ensure injection and chromatographic consistency. One aliquot was analyzed using acidic, positive ion-optimized conditions and the other using basic, negative ion-optimized conditions in two independent injections using separate dedicated columns. Extracts reconstituted in acidic conditions were gradient eluted using water and methanol containing 0.1% formic acid, while the basic extracts, which also used water/methanol, contained 6.5mM ammonium bicarbonate. A third aliquot was analyzed via negative ionization following elution

from a HILIC column using a gradient consisting of water and acetonitrile with 10mM Ammonium Formate. The MS analysis alternated between MS and data-dependent MS^2 scans using dynamic exclusion, and the scan range was from 80-1000 *m/z*.

The samples destined for analysis by GC-MS were dried under vacuum desiccation for a minimum of 18 h prior to being derivatized under dried nitrogen using bistrimethyl-silyltrifluoroacetamide. Derivatized samples were separated on a 5% diphenyl / 95% dimethyl polysiloxane fused silica column (20 m x 0.18 mm ID; 0.18 um film thickness) with helium as carrier gas and a temperature ramp from 60° to 340°C in a 17.5 min period. All samples were analyzed on a Thermo-Finnigan Trace DSQ fast-scanning single-quadrupole MS using electron impact ionization (EI) and operated at unit mass resolving power. The scan range was from 50–750 m/z.

Compound Identification, Quantification, and Data Curation

Metabolites were identified by automated comparison of the ion features in the experimental samples to a reference library of chemical standard entries that included retention time, molecular weight (m/z), preferred adducts, and in-source fragments as well as associated MS spectra and curated by visual inspection for quality control using software developed at Metabolon [3]. Identification of known chemical entities is based on comparison to metabolomic library entries of purified standards. Commercially available purified standard compounds have been acquired and registered into LIMS for distribution to both the UPLC-MS/MS and GC-MS platforms for determination of their detectable characteristics. Additional mass spectral entries have been created for structurally unnamed biochemicals, which have been identified by virtue of their recurrent nature (both chromatographic and mass spectral). These compounds have the potential to be identified by future acquisition of a matching purified standard or by classical

structural analysis. Peaks were quantified using area-under-the-curve. Raw area counts for each metabolite in each sample were normalized to correct for variation resulting from instrument inter-day tuning differences by the median value for each run-day, therefore, setting the medians to 1.0 for each run. This preserved variation between samples but allowed metabolites of widely different raw peak areas to be compared on a similar graphical scale. We also included blinded duplicates (n=6 sample pairs) to assess reliability of the measured metabolites. The median intra-class correlation coefficient for the detected metabolites was 0.77 (interquartile range: 0.53 to 0.91).

Supplementary References

1. Evans AM, DeHaven CD, Barrett T, Mitchell M, Milgram E. Integrated, nontargeted ultrahigh performance liquid chromatography/electrospray ionization tandem mass spectrometry platform for the identification and relative quantification of the small-molecule complement of biological systems. *Anal Chem*. 2009;81(16):6656-6667. doi:10.1021/ac901536h.
2. Sha W, da Costa K-A, Fischer LM, et al. Metabolomic profiling can predict which humans will develop liver dysfunction when deprived of dietary choline. *FASEB J*. 2010;24(8):2962-2975. doi:10.1096/fj.09-154054.
3. Dehaven CD, Evans AM, Dai H, Lawton KA. Organization of GC/MS and LC/MS metabolomics data into chemical libraries. *J Cheminform*. 2010;2(1):9. doi:10.1186/1758-2946-2-9.

Supplementary Table 1: Cross-sectional Cohort: Contents of Green and Brown Metabolite Modules significantly altered between MS patients and HC.

Module	Metabolite	MM* Score	Mean Standardized Metabolite Level** (95% CI)		
			HC	MS	P-value for difference
Green Module	gamma-glutamylleucine	0.91	-0.27 (-0.58, 0.04)	0.45 (0.14, 0.76)	0.002
	gamma-glutamylisoleucine*	0.88	-0.1 (-0.44, 0.24)	0.25 (-0.09, 0.59)	0.16
	gamma-glutamylvaline	0.85	-0.28 (-0.59, 0.03)	0.42 (0.11, 0.73)	0.003
	leucine	0.83	-0.35 (-0.68, -0.01)	0.44 (0.11, 0.78)	0.002
	valine	0.83	-0.34 (-0.67, -0.02)	0.45 (0.12, 0.77)	0.002
	isoleucine	0.80	-0.28 (-0.64, 0.07)	0.38 (0.02, 0.73)	0.01
	tyrosine	0.72	0.02 (-0.34, 0.38)	0.19 (-0.17, 0.55)	0.52
	gamma-glutamylmethionine	0.72	0.02 (-0.34, 0.38)	0.14 (-0.21, 0.5)	0.64
	gamma-glutamylphenylalanine	0.71	-0.1 (-0.43, 0.23)	0.25 (-0.08, 0.58)	0.15
	gamma-glutamyltyrosine	0.70	0.11 (-0.23, 0.46)	0.1 (-0.24, 0.44)	0.96
	2-aminoadipate	0.70	-0.35 (-0.67, -0.03)	0.46 (0.15, 0.78)	0.0009
	methionine	0.65	-0.1 (-0.47, 0.28)	0.22 (-0.16, 0.59)	0.25
	phenylalanine	0.63	-0.17 (-0.5, 0.17)	0.25 (-0.09, 0.58)	0.09
	2-methylbutyrylcarnitine (C5)	0.62	0 (-0.34, 0.33)	0.15 (-0.19, 0.48)	0.54
	indoleacetate	0.59	-0.05 (-0.41, 0.3)	0.17 (-0.18, 0.53)	0.39
	gamma-glutamyltryptophan	0.58	-0.09 (-0.42, 0.25)	0.14 (-0.19, 0.47)	0.35
	propionylcarnitine	0.54	-0.07 (-0.39, 0.26)	0.19 (-0.13, 0.52)	0.27
	N-acetylkynurenine (2)	0.53	-0.09 (-0.4, 0.22)	0.14 (-0.17, 0.46)	0.3
	isovalerylcarnitine	0.53	-0.07 (-0.41, 0.26)	0.25 (-0.09, 0.58)	0.19
	ornithine	0.52	-0.26 (-0.59, 0.07)	0.36 (0.03, 0.69)	0.01
	methyl indole-3-acetate	0.52	-0.08 (-0.45, 0.29)	0.16 (-0.21, 0.53)	0.38
	cysteine-glutathione disulfide	0.5	-0.13 (-0.5, 0.24)	0.18 (-0.18, 0.55)	0.24
	thymol sulfate	0.48	-0.27 (-0.63, 0.1)	0.21 (-0.16, 0.57)	0.08
	kynurenate	0.45	-0.29 (-0.66, 0.08)	0.27 (-0.1, 0.64)	0.04
	succinylcarnitine	0.44	-0.06 (-0.41, 0.28)	0.16 (-0.19, 0.51)	0.38
	cholate	0.43	-0.09 (-0.43, 0.26)	0.23 (-0.11, 0.57)	0.21

	pro-hydroxy-pro	0.38	-0.14 (-0.51, 0.23)	0.15 (-0.22, 0.53)	0.28
	N-acetylcarnosine	0.37	0.02 (-0.28, 0.32)	0.17 (-0.13, 0.48)	0.48
	adenosine 3',5'-cyclic monophosphate (cAMP)	0.36	-0.11 (-0.47, 0.26)	0.11 (-0.26, 0.47)	0.42
	N-6-trimethyllysine	0.33	-0.24 (-0.55, 0.07)	0.1 (-0.2, 0.41)	0.13
	uracil	0.31	-0.18 (-0.54, 0.18)	0.25 (-0.11, 0.61)	0.1
	chenodeoxycholate	0.26	0.09 (-0.24, 0.42)	0.06 (-0.27, 0.39)	0.91
	dimethylglycine	0.18	0.23 (-0.13, 0.59)	-0.18 (-0.54, 0.18)	0.13
	cystine	0.04	-0.12 (-0.48, 0.24)	0.08 (-0.27, 0.44)	0.44
	13-HODE + 9-HODE	-0.15	-0.06 (-0.42, 0.29)	-0.09 (-0.45, 0.26)	0.91
	myo-inositol	-0.25	0.1 (-0.28, 0.47)	-0.19 (-0.57, 0.19)	0.3
	2-isopropylmalate	-0.37	0.04 (-0.35, 0.43)	-0.06 (-0.45, 0.32)	0.72
Brown Module	3-hydroxypyridine sulfate	0.86	0.06 (-0.31, 0.44)	-0.07 (-0.45, 0.31)	0.62
	N-(2-furoyl)glycine	0.85	0.13 (-0.25, 0.51)	-0.14 (-0.52, 0.24)	0.34
	maleate	0.83	0.21 (-0.16, 0.59)	-0.22 (-0.59, 0.15)	0.12
	1-methylxanthine	0.82	0.26 (-0.1, 0.62)	-0.22 (-0.58, 0.14)	0.07
	1-methylurate	0.81	0.26 (-0.1, 0.63)	-0.2 (-0.56, 0.16)	0.09
	trigonelline (N'-methylnicotinate)	0.81	0.15 (-0.22, 0.53)	-0.19 (-0.57, 0.19)	0.22
	3-methyl catechol sulfate (1)	0.78	0.07 (-0.31, 0.44)	-0.06 (-0.43, 0.31)	0.64
	O-methylcatechol sulfate	0.75	0.01 (-0.37, 0.4)	-0.08 (-0.47, 0.31)	0.75
	catechol sulfate	0.75	0.08 (-0.31, 0.47)	-0.15 (-0.55, 0.24)	0.42
	caffeine	0.75	0.28 (-0.08, 0.65)	-0.33 (-0.7, 0.04)	0.02
	o-cresol sulfate	0.74	0.11 (-0.26, 0.48)	-0.09 (-0.46, 0.29)	0.47
	paraxanthine	0.74	0.27 (-0.08, 0.63)	-0.29 (-0.64, 0.06)	0.03
	1,3,7-trimethylurate	0.74	0.18 (-0.2, 0.56)	-0.21 (-0.59, 0.18)	0.17
	quinate	0.73	0.08 (-0.29, 0.46)	-0.05 (-0.42, 0.33)	0.63
	3-methyl catechol sulfate (2)	0.70	0.18 (-0.21, 0.56)	-0.18 (-0.56, 0.21)	0.21
	theophylline	0.66	0.21 (-0.16, 0.57)	-0.23 (-0.59, 0.13)	0.10
	ferulic acid 4-sulfate	0.65	0.23 (-0.16, 0.62)	-0.26 (-0.64, 0.13)	0.09
	5-acetylamino-6-amino-3-methyluracil	0.64	0.27 (-0.05, 0.6)	-0.14 (-0.47, 0.18)	0.09
	1,2,3-benzenetriol sulfate (2)	0.63	0.23 (-0.14, 0.59)	-0.32 (-0.69, 0.04)	0.04
	1,7-dimethylurate	0.63	0.25 (-0.11, 0.61)	-0.26 (-0.62, 0.1)	0.05

	levulinate (4-oxovalerate)	0.63	-0.04 (-0.41, 0.34)	0 (-0.37, 0.38)	0.89
	methylsuccinate	0.60	0.01 (-0.36, 0.38)	-0.04 (-0.41, 0.34)	0.86
	1,3-dimethylurate	0.59	0.18 (-0.18, 0.53)	-0.1 (-0.46, 0.25)	0.28
	3-methoxycatechol sulfate (1)	0.54	0.12 (-0.24, 0.47)	-0.25 (-0.6, 0.11)	0.16
	5-acetylamino-6-formylamino-3-methyluracil	0.46	0.04 (-0.32, 0.4)	-0.1 (-0.46, 0.26)	0.59
	cis-urocanate	0.33	0.25 (-0.11, 0.62)	-0.18 (-0.55, 0.18)	0.11
	picolinate	0.28	-0.15 (-0.53, 0.22)	0.2 (-0.17, 0.58)	0.19
	N-acetylisoleucine	0.28	-0.1 (-0.45, 0.25)	0.22 (-0.13, 0.57)	0.21
	betaine	0.23	0.09 (-0.26, 0.44)	0.04 (-0.31, 0.39)	0.84
	glycoursodeoxycholate	0.22	0.02 (-0.35, 0.39)	0.07 (-0.3, 0.44)	0.85
	N2,N5-diacetylornithine	0.2	-0.17 (-0.55, 0.2)	0.12 (-0.26, 0.49)	0.30
	glutamine	0.15	0.1 (-0.27, 0.48)	0.01 (-0.36, 0.38)	0.73
	pyridoxate	0.10	0 (-0.39, 0.39)	0.08 (-0.31, 0.47)	0.78
	N-acetylleucine	0.08	-0.21 (-0.56, 0.14)	0.32 (-0.03, 0.67)	0.04
	gamma-glutamylglutamine	0.08	0.03 (-0.34, 0.4)	0.05 (-0.32, 0.42)	0.94
	EDTA	0.06	0.07 (-0.29, 0.43)	0.02 (-0.34, 0.38)	0.86
	lysine	0.04	-0.19 (-0.57, 0.19)	0.23 (-0.15, 0.61)	0.13
	tyramine O-sulfate	-0.06	0.19 (-0.18, 0.57)	-0.14 (-0.52, 0.23)	0.22
	cysteine s-sulfate	-0.25	0.14 (-0.24, 0.51)	-0.1 (-0.47, 0.28)	0.40
	4-hydroxychlorothalonil	-0.26	-0.07 (-0.44, 0.31)	0.04 (-0.34, 0.41)	0.71
	glutamate	-0.41	-0.03 (-0.41, 0.35)	0.16 (-0.22, 0.54)	0.48

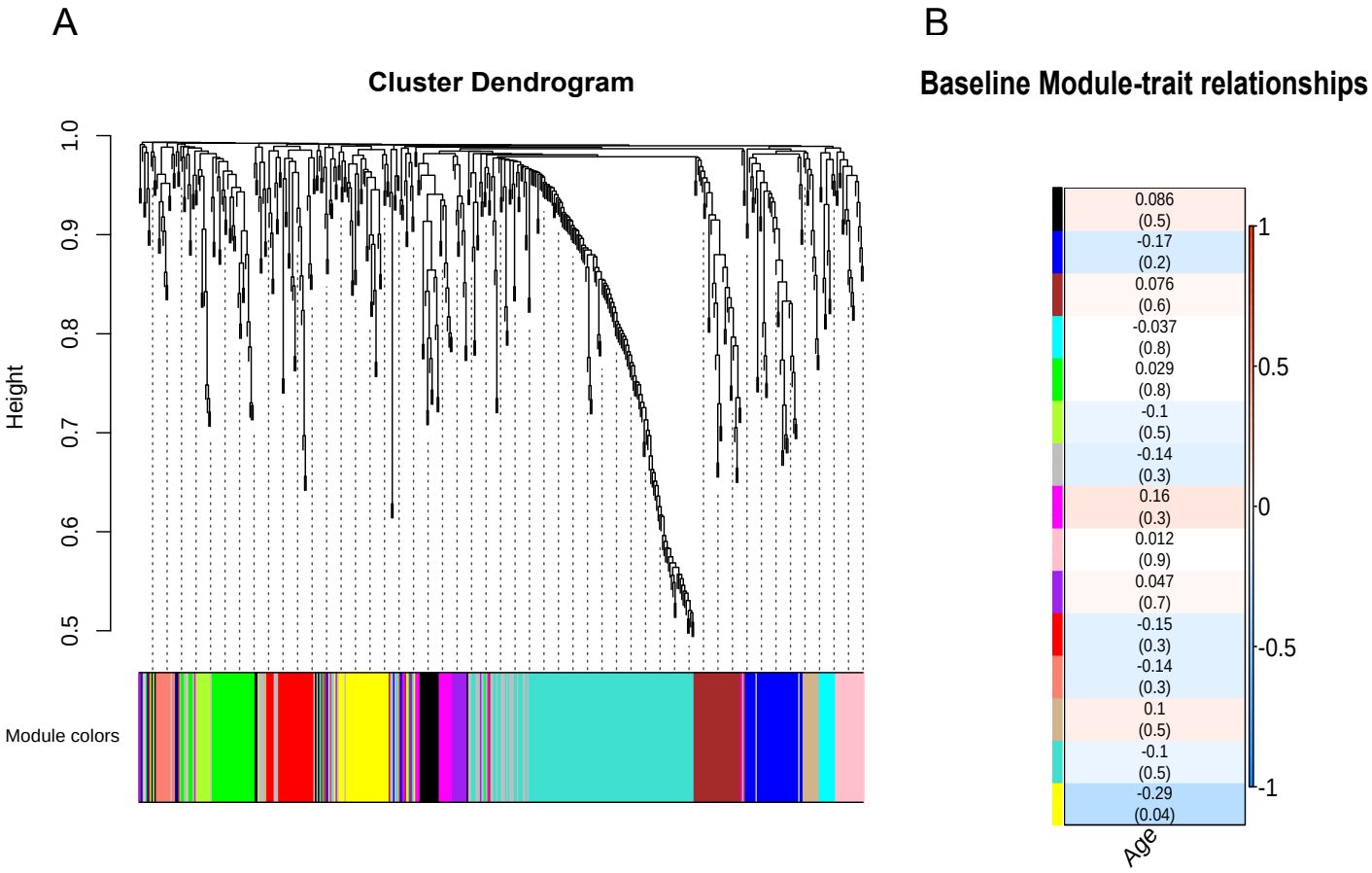
Supplementary Table 2: Vitamin D Cohort Novel Pathway Analyses: Contents of Metabolite Modules significantly* altered by Vitamin D Supplementation differently between MS and HC

Module	Metabolite		MM* Score	Individual Metabolite Model Results for Δ (95% CI) in metabolite levels associated with Vitamin D Supplementation		
				HC	MS	P-value for difference
Green Module	gamma-glutamylleucine		0.91	-0.16 (-0.34, 0.02)	0.2 (-0.01, 0.4)	0.010247938
	gamma-glutamylhistidine		0.90	-0.29 (-0.47, -0.1)	0.23 (0.08, 0.38)	1.86E-05
	gamma-glutamylisoleucine*		0.88	-0.28 (-0.45, -0.11)	0.18 (-0.06, 0.42)	0.002416426
	gamma-glutamylvaline		0.84	-0.21 (-0.39, -0.03)	0.27 (0.05, 0.48)	0.00072747
	methionine sulfoxide		0.83	-0.13 (-0.33, 0.07)	0.03 (-0.09, 0.16)	0.178303581
	glutamate		0.82	-0.05 (-0.23, 0.13)	0.1 (-0.05, 0.25)	0.204901512
	gamma-glutamyl-epsilon-lysine		0.78	0.04 (-0.11, 0.2)	0.13 (-0.16, 0.43)	0.58977446
	gamma-glutamylglycine		0.75	-0.19 (-0.41, 0.03)	0.29 (0.08, 0.49)	0.001458049
	cysteine sulfinic acid		0.72	0.08 (-0.28, 0.44)	0.14 (-0.17, 0.46)	0.792893356
	gamma-glutamylglutamate		0.71	-0.25 (-0.42, -0.08)	0.06 (-0.13, 0.25)	0.017134018
	5-oxoproline		0.70	0.05 (-0.1, 0.19)	0.01 (-0.19, 0.21)	0.767730818
	gamma-glutamylphenylalanine		0.69	-0.24 (-0.46, -0.01)	0.12 (-0.13, 0.37)	0.039607557
	isovalerate		0.64	-0.27 (-0.52, -0.03)	-0.01 (-0.17, 0.15)	0.075576675
	erythronate*		0.60	-0.41 (-0.76, -0.07)	-0.05 (-0.38, 0.28)	0.121932346
	gamma-glutamylalanine		0.59	0.02 (-0.28, 0.32)	0.38 (0.05, 0.7)	0.105205662
	4-guanidinobutanoate		0.57	-0.07 (-0.35, 0.22)	0.02 (-0.13, 0.17)	0.587389934
	dimethylarginine (SDMA + ADMA)		0.55	-0.17 (-0.56, 0.22)	0.01 (-0.44, 0.47)	0.553796002
	12-HEPE		0.55	-0.18 (-0.39, 0.02)	0.04 (-0.16, 0.25)	0.11693568
	4-imidazoleacetate		0.54	-0.08 (-0.3, 0.14)	-0.15 (-0.34, 0.04)	0.628742806
	trans-urocanate		0.53	-0.19 (-0.39, 0.01)	-0.37 (-0.74, 0.01)	0.42117302
	quinolinate		0.43	-0.18 (-0.57, 0.22)	-0.07 (-0.55, 0.4)	0.72425762
	gamma-glutamylmethionine		0.41	-0.04 (-0.44, 0.36)	0.31 (-0.19, 0.82)	0.280334599
	sebacate (decanedioate)		0.40	0.03 (-0.25, 0.32)	-0.03 (-0.28, 0.21)	0.722695154
	isovalerylcarnitine		0.34	-0.04 (-0.39, 0.32)	0.18 (-0.52, 0.88)	0.584324772
	myo-inositol		0.33	-0.04 (-0.39, 0.31)	-0.07 (-0.4, 0.27)	0.908200159

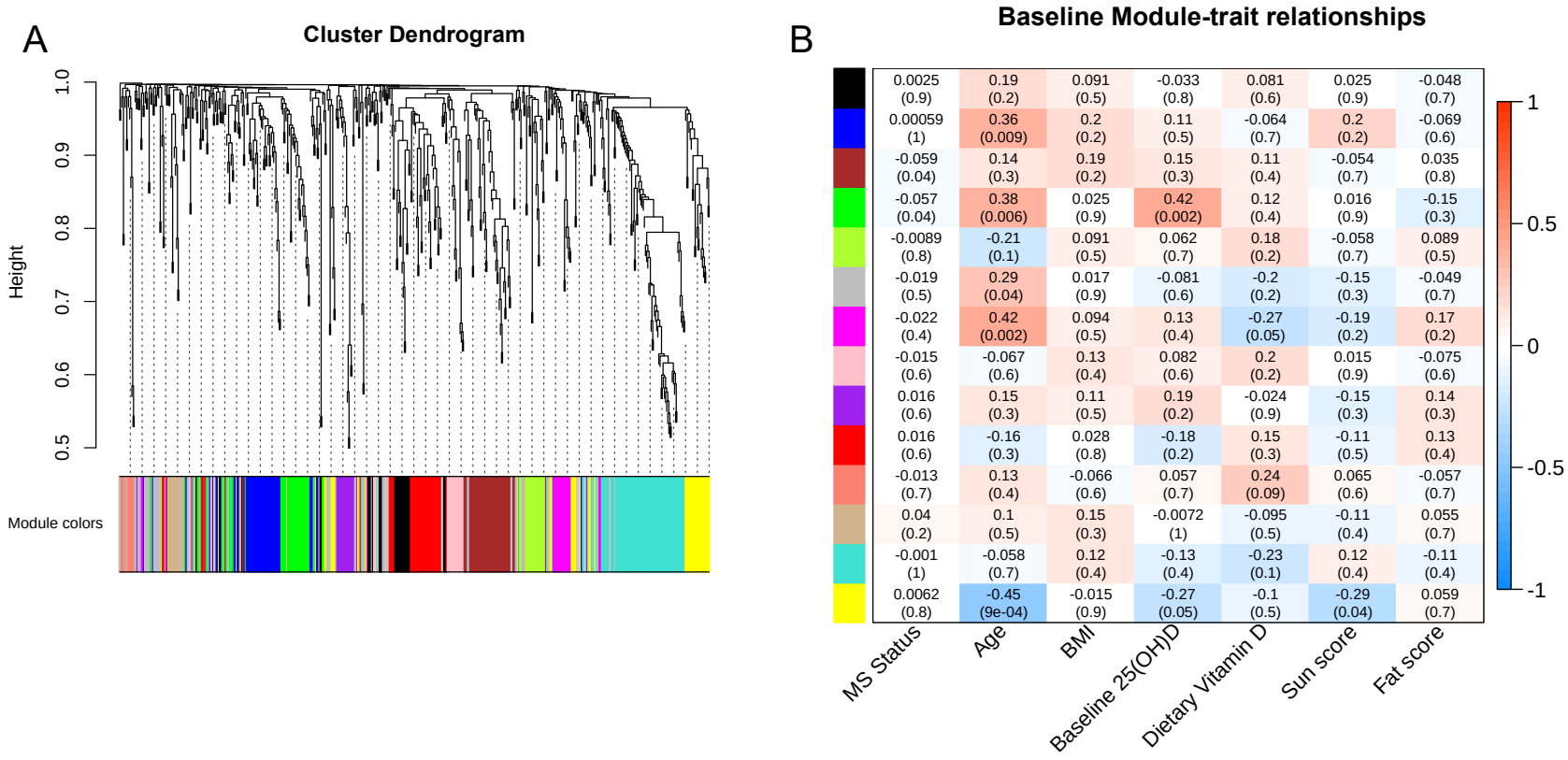
	glycerate		0.33	-0.1 (-0.52, 0.32)	-0.02 (-0.33, 0.29)	0.760240443
	tartronate (hydroxymalonate)		0.28	-0.01 (-0.46, 0.44)	0.16 (-0.3, 0.63)	0.581660833
	dimethyl sulfone		0.28	-0.71 (-0.99, -0.43)	-0.02 (-0.46, 0.43)	0.010846854
	pyroglutamine*		0.28	0.08 (-0.2, 0.36)	0.07 (-0.14, 0.28)	0.9454857
	succinylcarnitine		0.27	0 (-0.57, 0.56)	0 (-0.57, 0.58)	0.991806909
	N6-succinyladenosine		0.24	0.03 (-0.22, 0.29)	-0.12 (-0.51, 0.26)	0.509346717
	3-aminoisobutyrate		0.23	-0.22 (-0.65, 0.21)	-0.09 (-0.45, 0.26)	0.650430431
	gamma-glutamylglutamine		0.18	0.11 (-0.12, 0.34)	0.43 (0.01, 0.84)	0.188964807
	succinate		0.18	-0.13 (-0.44, 0.17)	0.07 (-0.46, 0.59)	0.514998803
	glutarylcarnitine (C5)		0.17	-0.09 (-0.71, 0.53)	-0.03 (-0.41, 0.36)	0.865047843
	pseudouridine		0.17	-0.27 (-0.72, 0.18)	0.07 (-0.24, 0.38)	0.206526567
	propionylcarnitine		0.15	-0.22 (-0.74, 0.31)	0.23 (-0.31, 0.77)	0.258968687
	glycolithocholate sulfate*		0.07	-0.26 (-0.69, 0.17)	-0.24 (-0.75, 0.28)	0.94700208
	glycerophosphorylcholine (GPC)		-0.01	-0.2 (-0.69, 0.3)	0.08 (-0.29, 0.45)	0.387529808
	1-methylnicotinamide		-0.03	0.03 (-0.39, 0.46)	-0.26 (-0.67, 0.14)	0.330574033
	serotonin		-0.33	-0.19 (-0.4, 0.01)	-0.01 (-0.45, 0.43)	0.460071345
	threonate		-0.39	-0.37 (-0.66, -0.08)	-0.41 (-0.67, -0.16)	0.831891351
	oxalate (ethanedioate)		-0.43	0.19 (-0.09, 0.47)	-0.34 (-0.72, 0.04)	0.021937068
Red Module	2-stearoyl-GPE (18:0)*		0.81	-0.18 (-0.64, 0.29)	0.07 (-0.22, 0.37)	0.363977446
	1-stearoyl-GPE (18:0)		0.80	-0.28 (-0.76, 0.2)	0.14 (-0.08, 0.37)	0.121392251
	1-arachidonoyl-GPC (20:4)*		0.77	-0.25 (-0.66, 0.16)	0.06 (-0.19, 0.3)	0.205762559
	1-palmitoyl-GPI (16:0)*		0.77	-0.14 (-0.6, 0.32)	0.41 (0.07, 0.75)	0.049859128
	arachidonate (20:4n6)		0.77	-0.24 (-0.62, 0.14)	-0.14 (-0.38, 0.1)	0.654269689
	1-arachidonoyl-GPE (20:4)*		0.77	-0.26 (-0.67, 0.14)	0.19 (-0.14, 0.52)	0.08190052
	1-arachidonoyl-GPI (20:4)*		0.74	0.05 (-0.3, 0.4)	0.3 (0.01, 0.59)	0.263794848
	dihomo-linolenate (20:3n3 or n6)		0.73	-0.18 (-0.71, 0.36)	0.21 (-0.11, 0.54)	0.218690332
	1-oleoyl-GPI (18:1)*		0.72	-0.53 (-1.12, 0.07)	0.36 (-0.01, 0.73)	0.011392628
	mead acid (20:3n9)		0.69	-0.21 (-0.78, 0.36)	0.2 (-0.04, 0.44)	0.184495237
	1-linoleoyl-GPC (18:2)		0.68	-0.6 (-1.17, -0.02)	0.08 (-0.27, 0.43)	0.038995492
	2-hydroxypalmitate		0.67	-0.18 (-0.59, 0.23)	-0.09 (-0.41, 0.24)	0.738487724
	1-palmitoyl-GPG (16:0)*		0.67	-0.07 (-0.55, 0.41)	0.02 (-0.45, 0.5)	0.780768458
	1-linoleoyl-GPI (18:2)*		0.65	0.23 (-0.25, 0.72)	0.62 (0.27, 0.97)	0.191589907

	2-palmitoleoyl-GPC (16:1)*		0.65	-0.11 (-0.48, 0.27)	-0.14 (-0.48, 0.2)	0.885208876
	1-oleoyl-GPG (18:1)*		0.64	-0.26 (-0.65, 0.14)	-0.18 (-0.64, 0.29)	0.792781799
	1-linoleoyl-GPE (18:2)*		0.64	-0.48 (-1.12, 0.16)	0.33 (-0.1, 0.76)	0.037115156
	2-hydroxystearate		0.63	-0.38 (-0.75, -0.01)	-0.1 (-0.45, 0.24)	0.286751628
	1-linolenoyl-GPC (18:3)*		0.61	-0.54 (-1.19, 0.12)	0.53 (0.13, 0.93)	0.005230549
	1-oleoyl-GPE (18:1)		0.59	-0.48 (-1.13, 0.18)	0.21 (-0.23, 0.65)	0.090222686
	2-oleoylglycerol (18:1)		0.56	-0.33 (-0.67, 0.01)	0.45 (0.19, 0.71)	0.000259177
	1-oleoyl-GPC (18:1)		0.56	-0.11 (-0.68, 0.46)	0.14 (-0.32, 0.61)	0.487920714
	1-palmitoyl-GPE (16:0)		0.54	-0.05 (-0.51, 0.41)	0.34 (0.01, 0.68)	0.180417484
	1-palmitoyl-GPC (16:0)		0.52	0.03 (-0.39, 0.45)	-0.07 (-0.54, 0.4)	0.746288782
	docosapentaenoate (n6 DPA; 22:5n6)		0.52	-0.01 (-0.33, 0.3)	0.04 (-0.24, 0.33)	0.788192746
	1-(1-enyl-stearoyl)-GPE (P-18:0)*		0.44	-0.43 (-0.76, -0.1)	0.11 (-0.24, 0.46)	0.024243823
	2-arachidonoylglycerol (20:4)		0.42	0.03 (-0.28, 0.35)	0.39 (0.02, 0.75)	0.148549114
	1-(1-enyl-palmitoyl)-GPE (P-16:0)*		0.39	-0.4 (-0.76, -0.04)	0 (-0.46, 0.46)	0.166697599
	1-(1-enyl-oleoyl)-GPE (P-18:1)*		0.35	-0.24 (-0.58, 0.11)	-0.04 (-0.42, 0.34)	0.441924002
	glycohyocholate		0.23	-0.49 (-1.09, 0.11)	-0.56 (-1.05, -0.08)	0.849117034
	malonate		0.22	-0.18 (-0.54, 0.19)	0 (-0.44, 0.43)	0.535559484
	citrate		0.19	-0.12 (-0.46, 0.23)	-0.16 (-0.59, 0.28)	0.882369555
	indolepropionate		0.17	0.11 (-0.2, 0.42)	0.11 (-0.34, 0.55)	0.989004134
	3-phenylpropionate (hydrocinnamate)		0.16	-0.34 (-0.6, -0.08)	-0.03 (-0.43, 0.37)	0.201562703
	methyl glucopyranoside (alpha + beta)		0.14	-0.05 (-0.48, 0.38)	-0.36 (-0.87, 0.15)	0.363473223
	N-acetylaspartate (NAA)		-0.10	0.12 (-0.15, 0.38)	-0.4 (-0.7, -0.11)	0.00745835
	N2,N2-dimethylguanosine		-0.11	0.07 (-0.32, 0.46)	-0.3 (-0.8, 0.19)	0.239328509
	N-acetylalanine		-0.11	0.23 (-0.54, 1.01)	0.1 (-0.25, 0.46)	0.764033046
	ursodeoxycholate		-0.20	0.07 (-0.45, 0.59)	0.07 (-0.29, 0.43)	0.990981611
	chenodeoxycholate		-0.21	0.02 (-0.34, 0.38)	0.09 (-0.37, 0.54)	0.834192158
	vanillylmandelate (VMA)		-0.22	0.12 (-0.3, 0.54)	-0.47 (-0.91, -0.03)	0.055654197

Supplementary Figure 1: *Cross-sectional Cohort:* Results of WGCNA characterizing metabolic modules



Supplementary Figure 2: *Vitamin D Supplementation Cohort:* Results of WGCNA characterizing metabolic modules.



Supplementary Figure Legends

Supplementary Figure 1: *Cross-sectional Cohort:* Results of WGCNA characterizing metabolic modules. Panel A depicts the clustering of metabolites into modules of highly correlated modules, represented by the various colors in the bar at the bottom of the figure. Panel B displays correlation of module eigen-metabolite values with age of the participants.

Supplementary Figure 2: *Vitamin D Supplementation Cohort:* Results of WGCNA characterizing metabolic modules. Panel A depicts the clustering of metabolites into modules of highly correlated metabolites represented by the color in the bar at the bottom of the panel. Panel B displays correlation of the module eigen-metabolite values at the baseline visit with clinical and laboratory parameters.