

## Supplemental Material

# Change in Metabolomic Profile Associated with an Average Increase in Plain Water Intake of > +1 L/Day, Sustained Over 4 Weeks, in Healthy Young Men with Initial Total Water Intake Below 2 L/Day

Jodi D. Stookey<sup>a</sup> Bernhard Paulweber<sup>b</sup> Thomas K. Felder<sup>c</sup> Florian Lang<sup>d</sup>  
Dieter Häussinger<sup>e</sup> David W. Killilea<sup>f</sup> Frans A. Kuypers<sup>g</sup> Markus Ritter<sup>h,i,j,k</sup>

<sup>a</sup>Water and Hydration Translational Epidemiological Research, San Francisco, USA, <sup>b</sup>Department of Internal Medicine, St. Johanns University Hospital, Paracelsus Medical University, Salzburg, Austria, <sup>c</sup>Department of Laboratory Medicine, Paracelsus Medical University, Salzburg, Austria, <sup>d</sup>Department of Physiology, Eberhard Karls University, Tuebingen, Germany, <sup>e</sup>Clinic for Gastroenterology, Hepatology, and Infectious Diseases, Medical Faculty, Heinrich Heine University Düsseldorf, Düsseldorf, Germany, <sup>f</sup>MLK Core Manager, Research Resource Program, Office of Research, University of California, San Francisco, USA, <sup>g</sup>Division of Hematology, Department of Pediatrics, RBC Lab, University of California, San Francisco, USA, <sup>h</sup>Center of Physiology, Pathophysiology and Biophysics, Institute of Physiology and Pathophysiology, Salzburg, Austria, <sup>i</sup>Ludwig Boltzmann Institute for Arthritis and Rehabilitation, Paracelsus Medical University, Salzburg, Austria, <sup>j</sup>Gastein Research Institute, Paracelsus Medical University, Salzburg, Austria, <sup>k</sup>Kathmandu University School of Medical Sciences, Dhulikhel, Nepal

**Supplementary Table 1**

*Indices of fasting macronutrient and energy metabolism for Adapt Study participants who changed hydration classification.* Macronutrient concentrations were determined by Quest Diagnostics (San Jose, CA) as part of the serum comprehensive metabolic panel. Plasma insulin was determined at CHORI Oakland CA by ELISA using a commercially available kit (IBL International Corp.). The Homeostasis Model Assessment index of insulin resistance (HOMA-IR) was calculated using the formula described by Matthews et al. [174, 175]: HOMA-IR = (insulin (mIU/mL) x [glucose (mmol/L)/22.5]. The fasting recumbent resting metabolic rate and respiratory quotient were calculated from gas exchange rates which were recorded at 10 sec intervals during a 5-min measurement period (Quark RMR COSMED Chicago, USA) (see [96, 97]). \*Significantly different (p<0.05) from corresponding Week 1 value in fixed effect regression models.

<b>Fasting macronutrient and energy metabolism</b>			<b>Week 1</b>	<b>Weeks 3-6</b>	<b>Week 6</b>
			<b>Mean (SE)</b>	<b>Mean (SE)</b>	<b>Mean (SE)</b>
<b>Macronutrient indices</b>	Serum glucose	mg/dL	75.3 (2.3)	79.8 (1.1)*	81.8 (2.0)*
	Blood urea nitrogen (BUN)	mg/dL	14.0 (0.8)	13.9 (0.8)	13.3 (2.2)
	BUN: creatinine	ratio	18.3 (1.1)	17.0 (0.8)	16.0 (2.0)
	Serum total protein	g/dL	7.6 (0.1)	7.2 (0.1)*	7.2 (0.1)*
	Serum albumin	g/dL	4.9 (0.2)	4.7 (0.04)*	4.7 (0.1)*
<b>Plasma insulin</b>	Plasma insulin	uIU/mL	11.7 (1.9)	8.8 (0.6)	8.3 (1.6)
	HOMA-IR	ratio	2.2 (0.4)	1.7 (0.1)	1.7 (0.3)
<b>Recumbent indirect calorimetry</b>	Resting metabolic rate	kcal/d	2184.5 (173.8)	2215.6 (58.8)	2162.3 (127.0)
	Respiratory quotient	ratio	0.70 (0.05)	0.73 (0.02)	0.77 (0.03)*

**Supplementary Table 2**

*Fold change of known compounds in first-morning urine, post-bolus urine and post-bolus serum between Week 1 and Week 6 in participants who changed hydration classification. Compounds in blue decreased 2-fold or more for 3 or more study participants. Compounds in red increased 2-fold or more for 3 or more study participants.*

<b>Supplementary Table 2</b>					
mz/rt	Name of known compound	KEGG id	Log fold change		
			First-morning urine	Post-bolus urine	Post-bolus serum
129/1078536	cholesterol	C00187	-0.63	3.77	-4.16
319/659798	glucose	C00221	-1.37	1.82	-4.09
191/217657	lactic acid	C01432	-1.33	2.34	-2.98
142/364523	proline	C00148	-0.29	2.62	-2.91
237/1066526	tocopherol alpha-	C00376	-1.12	2.22	-2.91
144/313502	valine	C00183	-0.97	1.16	-2.56
156/600000	glutamine	C00064	-1.56	2.00	-2.52
202/780482	tryptophan	C00078	-2.02	1.08	-2.46
311/706508	palmitoleic acid	C08362	-1.75	0.78	-2.27
131/258161	2-hydroxybutanoic acid	C05984	-0.92	2.18	-2.12
158/346101	leucine	C00123	-1.68	0.70	-2.03
191/278829	3-hydroxybutyric acid	C01089	-1.43	1.36	-2.00
206/725465	2-hydroxyhippuric acid	C07588	-0.51	-1.84	-1.98
345/632897	quinic acid	C00296	-0.88	-2.60	-1.98
156/663483	lysine	C00047	-1.40	0.54	-1.93
116/240378	alanine	C00041	-0.02	1.75	-1.87
339/781527	oleic acid	C00712	0.19	1.74	-1.85
144/849710	oleamide NIST	C19670	-0.69	-1.24	-1.78
246/529100	glutamic acid	C00025	-0.04	1.67	-1.69
218/455754	aminomalonate	C00872	-0.89	0.50	-1.68
174/594051	ornithine	C00077	0.10	1.66	-1.65
150/777414	linoleic acid	C01595	-0.66	1.99	-1.64
230/897184	inosine	C00294	-1.22	0.49	-1.57
218/537804	phenylalanine	C00079	-0.92	0.81	-1.55
179/673176	34-dihydroxyhydrocinnamic acid	C10447	-2.49	-1.42	-1.53
218/409568	threonine	C00188	-0.62	1.35	-1.52
264/646701	adenine	C00147	-1.30	0.03	-1.47
158/359251	isoleucine	C00407	-0.94	0.79	-1.38
128/637588	methionine sulfoxide	C02989	-1.63	-0.52	-1.36
305/730022	myo-inositol	C00137	-1.25	-0.74	-1.29
202/764586	indole-3-lactate	C02043	-1.21	-0.26	-1.20
189/323728	urea	C00086	0.50	0.56	-1.13
257/651275	N-carbamylglutamate	C05829	-0.89	-0.92	-0.96
204/946601	maltose	C00208	-1.23	-0.28	-0.95
176/483560	methionine	C00073	-0.20	0.46	-0.73
218/671252	tyrosine	C00082	-1.32	-0.29	-0.67
204/611100	shikimic acid	C00493	-1.32	-1.00	-0.65
223/1026121	tocopherol gamma-	C02483	-1.27	-2.46	-0.62
248/435564	beta-alanine	C00099	-0.21	-1.00	-0.54

Cont'd.

Supplementary Table 2 Continued

mz/rt	Name of known compound	KEGG id	Log fold change		
			First-morning urine	Post-bolus urine	Post-bolus serum
233/463180	malic acid	C00711	-1.01	-0.38	-0.50
156/485935	oxoproline	C01879	-1.14	-0.63	-0.47
217/633603	15-anhydroglucitol	C07326	-1.18	-0.63	-0.46
117/674647	pentadecanoic acid	C16537	-2.00	-0.30	-0.34
217/861508	uridine	C00299	-1.45	-1.32	-0.28
307/641863	fructose	C02336	-0.53	-2.54	-0.26
248/368707	glycine	C00037	-0.40	-0.47	-0.25
202/684929	indole-3-acetate	C00954	-0.63	-0.88	-0.17
357/590747	glycerol-alpha-phosphate	C03189	-1.30	-1.13	-0.14
205/344428	glycerol	C00116	-1.36	-0.35	-0.09
245/390775	fumaric acid	C00122	-1.39	-0.67	-0.02
129/901749	1-monopalmitin	C01885	-1.00	-1.06	0.01
189/377495	glyceric acid	C00258	-1.70	-0.86	0.02
217/541086	lyxose	C00476	-1.34	-2.32	0.04
157/621404	citrulline	C00327	-1.30	-2.08	0.05
232/480387	aspartic acid	C00049	-0.01	-0.03	0.06
154/663739	histidine	C00135	-0.71	-0.70	0.07
268/416586	4-methylcatechol	C06730	-0.57	-1.58	0.11
104/434422	hydrocinnamic acid	C05629	-1.06	-0.77	0.12
299/603912	phosphoethanolamine	C00346	-1.43	-1.24	0.17
265/619128	hypoxanthine	C00262	-1.85	-1.20	0.21
233/325027	4-hydroxybutyric acid	C00989	-1.46	-1.15	0.23
314/345365	phosphate	C00009	0.59	-1.19	0.25
292/534291	tartaric acid	C00898	-1.00	-3.05	0.29
218/395020	serine	C00065	-1.59	-1.04	0.30
151/218927	phenol	C00146	-1.49	-1.77	0.31
191/935640	lactose	C00243	-1.16	-2.48	0.34
325/926133	xanthosine	C01762	-1.13	-1.22	0.36
140/484934	trans-4-hydroxyproline	C01157	-1.57	-1.18	0.37
100/464991	parabanic acid NIST	C00802	-1.39	-1.82	0.45
218/804619	cystine	C01420	-0.93	0.91	0.45
247/506306	2-hydroxyglutaric acid	C02630	-1.45	-2.71	0.46
160/537635	ibuprofen	C01588	0.34	0.38	0.46
117/920648	behenic acid	C08281	-1.14	-2.23	0.47
361/1123027	1-kestose	C03661	-0.81	-2.92	0.50
188/553078	asparagine	C00152	1.12	0.59	0.51
223/537925	4-hydroxybenzoate	C00156	-1.39	-1.69	0.54
217/828606	glucoheptulose	C02076	-1.87	-1.76	0.54
353/701688	xanthine	C00385	-1.36	-1.63	0.58
247/370608	succinic acid	C00042	-1.04	-1.83	0.67

Cont'd.

Supplementary Table 2 Continued

mz/rt	Name of known compound	KEGG id	Log fold change		
			First-morning urine	Post-bolus urine	Post-bolus serum
130/285825	2-aminobutyric acid	C02721	-0.81	-1.30	0.68
179/339214	benzoic acid	C00180	-1.85	-1.58	0.70
292/497572	threonic acid	C01620	-1.52	-1.47	0.71
190/260513	oxalic acid	C00209	-1.14	-0.25	0.74
220/645815	gluconic acid lactone	C00198	-1.58	-2.45	0.74
441/730691	uric acid	C00366	-1.28	-1.67	0.76
217/553078	ribose	C00121	-1.74	-2.01	0.78
239/411619	resorcinol	C01751	-0.69	-1.16	0.83
261/421596	glutaric acid	C00489	-0.93	-1.75	0.84
155/523205	pimelic acid	C02656	-1.36	-2.13	0.85
160/943858	cellobiose	C01971	-0.56	-1.66	0.91
217/567437	xylitol	C00379	-1.46	-3.21	0.94
309/673225	4-pyridoxic acid	C00847	-0.58	-2.54	1.05
319/722897	N-acetylmannosamine	C00645	-2.55	-3.35	1.08
165/280360	p-cresol	C01468	0.48	-0.94	1.15
103/544100	xylose	C00181	-1.09	-3.26	1.21
254/376695	catechol	C00090	-0.80	-2.59	1.24
111/474435	adipic acid	C06104	-1.76	-2.09	1.24
291/690887	pantothenic acid	C12276	-0.89	-2.23	1.25
201/343457	caprylic acid	C06423	-0.94	-1.64	1.28
307/563801	pentitol	D00061	-1.82	-1.92	1.29
177/227636	glycolic acid	C00160	-1.87	-2.54	1.32
290/626989	28-dihydroxyquinoline	C06342	-1.67	-3.14	1.37
204/1000661	galactinol	C01235	-1.78	-3.01	1.38
117/856421	arachidic acid	C06425	-0.65	-1.54	1.38
247/456203	citramalic acid	C00815	-1.39	-2.69	1.40
273/617342	citric acid	C00158	-0.58	-2.74	1.43
319/663215	mannitol	C00392	-1.75	-2.21	1.45
248/452655	3-aminoisobutyric acid	C05145	-0.49	-0.26	1.47
307/631835	tagatose	C00795	-2.69	-2.65	1.51
319/746341	n-acetyl-d-hexosamine	C03878	-1.92	-3.22	1.52
179/542795	4-hydroxyphenylacetic acid	C00642	-1.22	-2.89	1.54
217/704741	hexitol	C00392	-1.73	-3.55	1.57
239/521803	124-benzenetriol	C02814	-0.45	-1.39	1.60
217/667922	sorbitol	C00794	-2.35	-2.82	1.61
292/599680	ribonic acid	C01685	-1.76	-2.67	1.65
217/575497	ribitol	C00474	-1.88	-3.53	1.67
333/689527	mannonic acid NIST	C00514	-0.33	-2.69	1.67

Cont'd.

Supplementary Table 2 Continued

mz/rt	Name of known compound	KEGG id	Log fold change		
			First-morning urine	Post-bolus urine	Post-bolus serum
117/547906	lauric acid	C02679	-1.07	-1.43	1.70
271/912704	sucrose	C00089	-0.51	-2.69	1.73
117/584895	fucose	C02095	-0.69	-3.25	1.74
220/500158	cysteine	C00097	-2.40	-2.79	1.75
189/590775	xylonic acid isomer	C02341	-1.03	-3.62	1.91
204/805227	glycerol-3-galactoside	C05401	-2.27	-4.53	1.92
292/489385	isothreonic acid	C00639	-1.32	-3.23	1.96
333/693148	gluconic acid	C00800	-1.54	-3.39	1.98
247/521554	3-hydroxy-3-methylglutaric acid	C03761	-1.13	-2.88	1.99
123/497561	5-hydroxymethyl-2-furoic acid NIST	C20448	-1.35	-3.57	2.03
290/777606	5-hydroxy-3-indoleacetic acid	C05635	-1.31	-3.28	2.12
217/570685	arabitol	C01904	-1.64	-3.42	2.13
217/573587	lyxitol	C00532	-1.65	-3.34	2.13
217/467595	threitol	C16884	-1.75	-4.02	2.13
158/548028	N-acetylaspartic acid	C01042	-1.12	-3.04	2.15
245/617338	isocitric acid	C00451	-1.29	-3.84	2.21
164/527648	3-hydroxyphenylacetic acid	C05593	0.18	-1.95	2.27
229/586815	aconitic acid	C00417	-1.74	-3.79	2.32
204/929908	lactulose	C07064	-1.63	-3.96	2.42
292/690882	galactonic acid	C00880	-0.80	-3.63	2.49
217/813899	pseudo uridine	C02067	-1.55	-3.41	2.55
206/637795	hippuric acid	C01586	-0.80	-6.32	2.65
241/385735	uracil	C00106	-1.60	-3.51	2.75
333/699211	saccharic acid	C00818	-1.17	-3.94	2.99
217/585473	UDP-glucuronic acid	C00167	-1.36	-3.22	3.84

**Table 3**

*Metabolic pathways potentially impacted by 4-week induced change in hydration.* Change in hydration was induced by instructing the study participants to sustain +>1 L/d increased drinking water over 4 weeks. Post-bolus urine and serum were collected 60-90 min after consuming a 750 mL bolus of drinking water after overnight food and water restriction. Total: Total number of compounds on a given metabolic pathway. Expected: Expected number of compounds by chance. Hits: number of compounds observed to change by 2-fold or more. Raw p: Unadjusted p-value for the hypergeometric test. FDR: False detection rate. Impact: The impact factor is a measure of the centrality of a compound in a metabolic pathway. Red and blue font highlight significant p-values <0.05, FDR <0.05, or impact factor >0.20. Red font represents significantly increased pathways. Blue font represents significantly decreased pathways. Urine results are based on analyses that normalized urine feature abundance relative to creatinine. <sup>c</sup> Serum results based on analyses that normalized serum feature abundance relative to creatinine. <sup>p</sup> Serum results based on analyses that normalized serum feature abundance relative to the pooled Week 1 sample.

Supplementary Table 3							
Pathway	Specimen	Total	Expected	Hits	Raw p	FDR	Impact
Alanine, aspartate & glutamate metabolism	First-morning urine	28	1.63	4	0.075	0.595	0.20
	Post-bolus urine	28	0.34	3	0.004	0.059	0.31
	Post-bolus serum <sup>c</sup>	28	0.60	3	0.020	0.281	0.31
	Post-bolus serum <sup>p</sup>	28	0.78	3	0.040	0.375	0.31
Aminoacyl-tRNA biosynthesis	First-morning urine	48	2.79	7	0.018	0.386	0.17
	Post-bolus urine	48	0.50	7	<0.001	<0.001	0
	Post-bolus serum <sup>c</sup>	48	1.02	11	<0.001	<0.001	0
	Post-bolus serum <sup>p</sup>	48	1.33	13	<0.001	0.001	0
Arachidonic acid metabolism	First-morning urine	36	2.07	2	0.623	1.000	0.31
	Post-bolus serum <sup>c</sup>	36	0.77	1	0.543	1.000	0.31
	Post-bolus serum <sup>p</sup>	36	0.99	1	0.641	1.000	0.31
Arginine & proline metabolism	Post-bolus urine	38	0.47	3	0.006	0.073	0.27
	Post-bolus serum <sup>c</sup>	38	0.81	3	0.045	0.388	0.27
	Post-bolus serum <sup>p</sup>	38	1.05	3	0.086	0.573	0.27
Arginine biosynthesis	First-morning urine	14	0.81	3	0.043	0.595	0.23
	Post-bolus urine	14	0.14	3	<0.001	0.013	0.18
	Post-bolus serum <sup>c</sup>	14	0.30	4	<0.001	0.004	0.18
	Post-bolus serum <sup>p</sup>	14	0.39	4	<0.001	0.012	0.18
Ascorbate & aldarate metabolism	First-morning urine	8	0.46	4	0.001	0.052	0.50
	Post-bolus urine	8	0.45	3	0.008	0.338	0.50
	Post-bolus serum <sup>c</sup>	8	0.26	3	0.002	0.095	0.50
	Post-bolus serum <sup>p</sup>	8	0.13	3	<0.001	0.017	0.50
Beta-Alanine metabolism	Post-bolus urine	21	1.19	2	0.337	1.000	0.40
	Post-bolus serum <sup>p</sup>	21	0.58	1	0.448	1.000	0.40
Biosynthesis of unsaturated fatty acids	Post-bolus urine	36	0.44	3	0.009	0.104	0
	Post-bolus serum <sup>c</sup>	36	0.77	3	0.039	0.388	0
Butanoate (butyrate) metabolism	Post-bolus urine	15	0.18	2	0.014	0.127	0
	Post-bolus serum <sup>c</sup>	15	0.32	2	0.039	0.388	0
D-Glutamine & D-glutamate metabolism	Post-bolus urine	6	0.07	2	0.002	0.035	0.50
	Post-bolus serum <sup>c</sup>	6	0.13	2	0.006	0.105	0.50
	Post-bolus serum <sup>p</sup>	6	0.17	2	0.011	0.147	0.50
Galactose metabolism	First-morning urine	27	1.56	6	0.004	0.100	0.18
	Post-bolus urine	27	1.53	5	0.016	0.441	0.22
	Post-bolus serum <sup>c</sup>	27	0.89	4	0.010	0.292	0.11
Glutathione metabolism	Post-bolus urine	28	0.34	2	0.045	0.374	0.02
	Post-bolus serum <sup>p</sup>	28	0.78	3	0.040	0.375	0.03

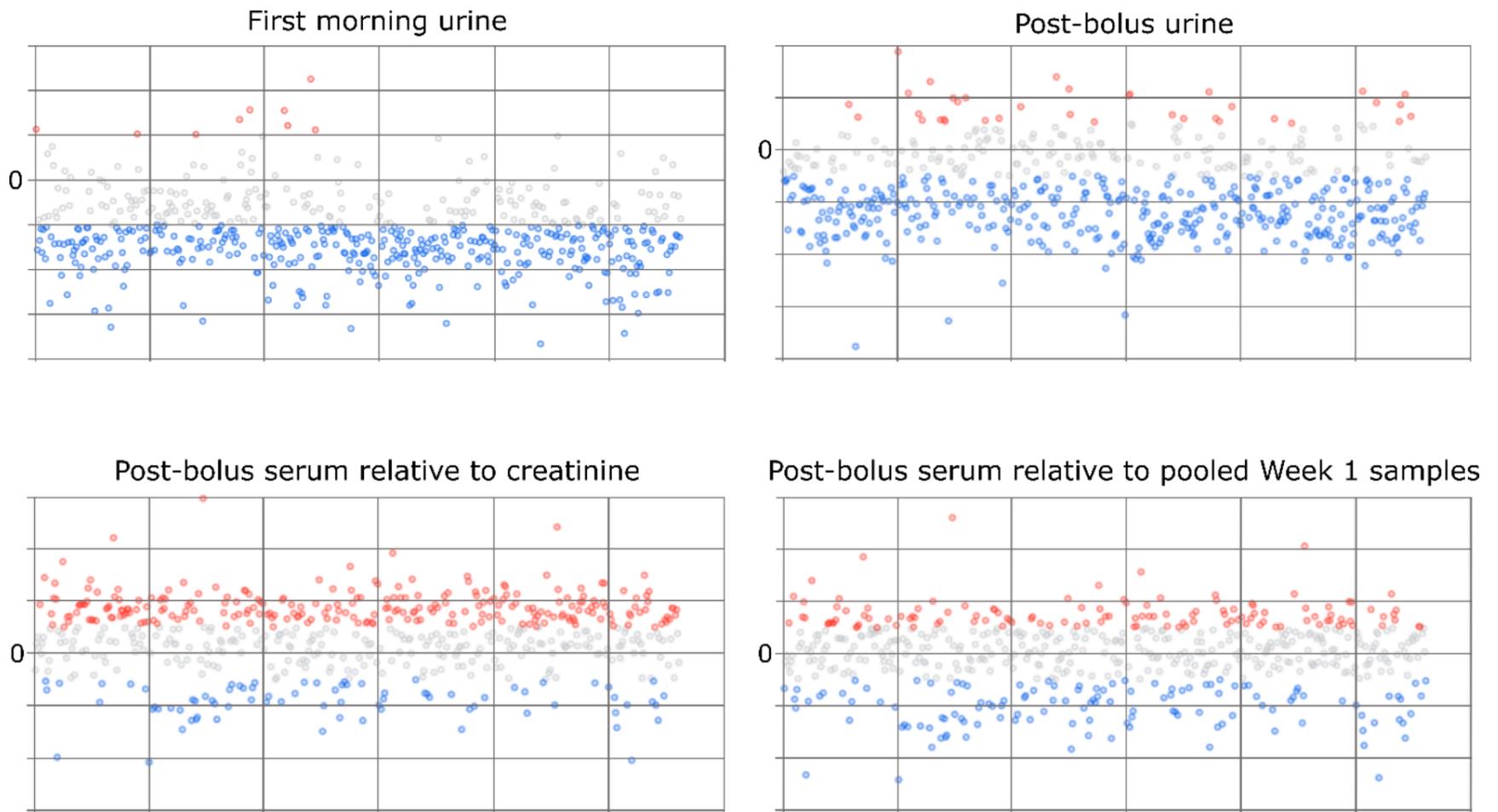
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Supplementary Table 3 Continued

Pathway	Specimen	Total	Expected	Hits	Raw p	FDR	Impact
Glycerolipid metabolism	First-morning urine	16	0.93	3	0.061	0.595	0.54
	Post-bolus urine	16	0.91	1	0.609	1.000	0.21
Glycine, serine & threonine metabolism	First-morning urine	33	1.92	3	0.299	1.000	0.24
	Post-bolus urine	33	1.87	2	0.569	1.000	0.22
Glyoxylate & dicarboxylate metabolism	First-morning urine	32	1.86	5	0.034	0.575	0.22
Linoleic acid metabolism	Post-bolus urine	5	0.06	1	0.060	0.387	1.00
	Post-bolus serum <sup>c</sup>	5	0.10	1	0.991	0.613	1.00
	Post-bolus serum <sup>p</sup>	5	0.14	1	0.131	0.615	1.00
Nitrogen metabolism	Post-bolus urine	6	0.07	2	0.002	0.035	0
	Post-bolus serum <sup>c</sup>	6	0.13	2	0.006	0.105	0
	Post-bolus serum <sup>p</sup>	6	0.17	2	0.011	0.147	0
Pantothenate & CoA biosynthesis	Post-bolus serum <sup>p</sup>	19	0.30	2	0.036	1.000	0
Pentose & glucuronate interconversions	First-morning urine	18	1.05	5	0.003	0.100	0.45
	Post-bolus urine	18	1.02	5	0.003	0.211	0.45
	Post-bolus serum <sup>c</sup>	18	0.59	4	0.002	0.095	0.28
	Post-bolus serum <sup>p</sup>	18	0.29	3	0.003	0.108	0.20
Phenylalanine, tyrosine & tryptophan biosynthesis	First-morning urine	4	0.23	1	0.213	1.000	0.50
	Post-bolus serum <sup>c</sup>	4	0.09	1	0.083	0.578	0.50
	Post-bolus serum <sup>p</sup>	4	0.11	2	0.004	0.09	1.00
Purine metabolism	Post-bolus serum <sup>c</sup>	65	1.38	4	0.046	0.388	0.00
Retinol metabolism	First-morning urine	17	0.99	1	0.640	1.000	0.24
	Post-bolus urine	17	0.21	1	0.190	0.998	0.24
	Post-bolus serum <sup>c</sup>	17	0.36	1	0.308	1.000	0.24
Valine, leucine & isoleucine metabolism	Post-bolus serum <sup>p</sup>	17	0.47	1	0.472	0.382	0.24
	Post-bolus urine	8	0.10	3	<0.001	0.004	0
	Post-bolus serum <sup>c</sup>	8	0.17	4	<0.001	<0.001	0

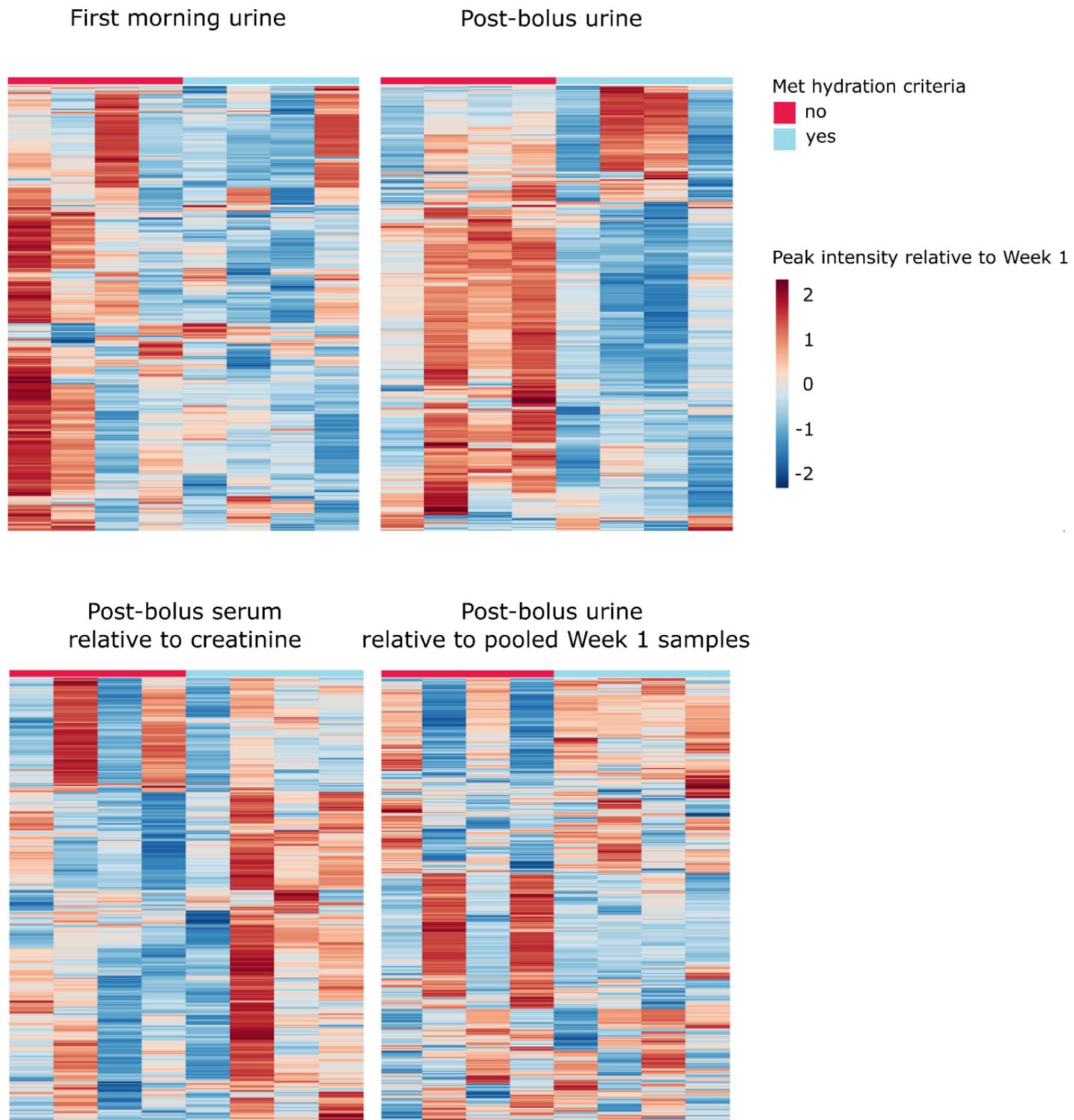
**Supplementary Figure 1**

*Fold-change of metabolomic features associated with 4 weeks of higher water intake.* Features identified in first-morning urine, post-bolus urine, and post-bolus serum were normalized relative to creatinine. Features identified in serum were also normalized relative to the pooled week 1 sample to reflect physiological conditions. Each figure describes the log-fold change (y-axis) between Week 1 and Week 6 of all features analyzed in the specimen (x-axis). Each dot represents one feature; Red color highlights features that increased 2-fold or more in specimen from three or more study participants. Blue color highlights features that decreased 2-fold or more in specimen from three or more study participants.



## Supplementary Figure 2

Metabolomic profiles at baseline and after 4 weeks of higher water intake for Adapt study participants who changed hydration classification. Specimen-specific heatmaps each summarize the metabolomic profiles of eight samples from four individuals measured at two time points. At Baseline (Week 1) all four participants did not meet one or more hydration criteria. In Week 6 all participants met all three hydration criteria (Serum osmolality <295 mOsm/kg H<sub>2</sub>O, Urine osmolality <800 mOsm/kg H<sub>2</sub>O, and Saliva osmolality <100 mOsm/kg H<sub>2</sub>O). Feature intensity (peak height) was normalized relative to creatinine for all urine samples. For serum samples, feature intensity was normalized relative to creatinine to account for hemodilution as well as relative to the pooled Week 1 sample to reflect physiological conditions. Blue color represents a decrease in peak intensity relative to Week 1.



**Supplementary Figure 3**

*Within- and between-week variation in metabolomic profile.* Features identified in first-morning urine, post-bolus urine, and post-bolus serum were normalized relative to creatinine. Features identified in serum were also normalized relative to the pooled week 1 sample to reflect physiological conditions. Pink represents data from the group of participants at baseline (Week 1). Blue represents data from the group in Week 6. Each dot represents a study participant. Orthogonal Partial Least Squares Discriminant Analysis (OPLS-DA) score plots describe the effect of time on the x-axis and within-week variation on the y-axis as an orthogonal T-score (OT score). Principal Components Analysis (PCA) score plots describe the unsupervised variation in the dataset. All score plots show 95% confidence regions for Week 1 and Week 6 data, respectively. Confidence regions that do not overlap are significantly different ( $p < 0.05$ ).

