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**Effects of Sleep Restriction on the Human Plasma Metabolome**

Running title: Plasma Metabolome Changes and Sleep Loss

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Table 1. Sleep, diet, physical activity, and weight maintenance during each condition<sup>a</sup>

	8.5-h TIB <sup>e</sup>	5.5-h TIB <sup>e</sup>
<b>8-night average sleep data<sup>b</sup></b>		
Going-to-bed time (h:min)	23:54 (0:20)	1:03 (0:02) **
Out-of-bed time (h:min)	8:26 (0:20)	6:32 (0:02) **
Total sleep time (h:min/day)	6:56 (0:34)	4:44 (0:17) **
Sleep onset latency (min) <sup>d</sup>	0:18 (0:20)	0:09 (0:11) **
Sleep efficiency (%)	81 (7)	86 (5) **
<b>7-day average food intake<sup>b</sup></b>		
Energy consumption (kcal/kg)	32 (2)	32 (3)
Carbohydrate (% of energy)	50 (2)	50 (2)
Protein (% of energy)	16 (1)	16 (1)
Fat (% of energy)	34 (1)	34 (1)
<b>7-day average physical activity<sup>b</sup></b>		
Total activity count (thousands/day)	70.3 (39.1)	71.0 (45.2)
<b>Body weight maintenance<sup>c</sup></b>		
Pre-treatment body weight (kg)	73.5 (12.7)	73.1 (12.4)
Post-treatment body weight (kg)	73.4 (12.6)	73.0 (12.7)
7-day change in body weight (kg)	-0.1 (0.5)	0.0 (0.6)
Body weight coefficient of variability (%)	0.5 (0.2)	0.4 (0.1)

<sup>a</sup> Data are mean (SD). <sup>b</sup> Measures of sleep, food intake, and physical activity were compared using repeated-measures analysis of variance with order of treatment and gender as between-subject factors. <sup>c</sup> Weight maintenance was assessed using paired t-tests. <sup>d</sup> Square root transformed data used for comparison; <sup>e</sup> TIB: time-in-bed (h/day). \*\* P<0.01.

Table 2. Summary of detected biochemical changes following experimental sleep restriction.

Super pathway	Sub-pathway	Biochemical name	TIB 5.5h / 8.5h ratio	p value	q value
Amino acid	Glycine, serine & threonine metabolism	N-acetylthreonine	1.30	<0.001	0.186
		betaine	1.04	0.051	0.808
	Histidine metabolism	histidine	1.06	0.041	0.808
	Lysine metabolism	glutaroyl carnitine	1.12	0.028	0.808
	Phenylalanine & tyrosine metabolism	phenyllactate	1.35	0.010	0.478
	Tryptophan metabolism	5-hydroxytryptophan (serotonin)	1.08	0.073	0.808
		C-glycosyltryptophan	1.07	0.036	0.808
	Valine, leucine & isoleucine metabolism	isoleucine	1.10	0.003	0.302
	Cysteine, methionine, SAM & taurine metabolism	N-formylmethionine	1.17	0.099	0.808
		N-acetylmethionine	1.24	0.055	0.808
	Creatine metabolism	creatine	1.13	0.086	0.808
Peptide	gamma-glutamyl	gamma-glutamylglutamine	1.16	0.087	0.808
Carbo-hydrate	Fructose, mannose, galactose, starch & sucrose	mannose	0.80	0.039	0.808
		glucose	0.89	0.086	0.808
	Glycolysis, gluconeogenesis, & pyruvate metabolism	1,6-anhydroglucose	1.51	0.001	0.186
		gluconate	0.66	0.086	0.808
		xylonate	0.74	0.088	0.808
Energy	Krebs cycle	malate	1.27	0.075	0.808
Lipid	Medium-chain fatty acid	caproate (6:0)	1.11	0.076	0.808
	Carnitine metabolism	2-decenoyl carnitine	1.29	0.095	0.808
	Bile acid metabolism	deoxycholate	1.38	0.059	0.808
		glycocholate sulfate	1.23	0.008	0.465
	Lysolipid	2-myristoylglycerophosphocholine	1.77	0.066	0.808
	Sterol/steroid	cholesterol	1.11	0.030	0.808
		beta-sitosterol	0.73	0.030	0.808
		7-alpha-hydroxy-3-oxo-4-cholestenoate	1.15	0.030	0.808
Nucleotide	Pyrimidine metabolism, uracil containing	pseudouridine	1.06	0.080	0.808
Cofactors & vitamins	Pantothenate & CoA metabolism	pantothenate	1.12	0.023	0.808
	Tocopherol metabolism	gamma-CEHC	1.32	0.038	0.808
Xenobiotics	Benzoate metabolism	benzoate	1.10	0.009	0.465
	Chemical	2-hydroxyisobutyrate	0.87	0.058	0.808
	Food component/plant	piperine	0.64	0.006	0.465
	Xanthine metabolism	theophylline	0.83	0.090	0.808

Sleep-loss-related significant and trending elevations in plasma metabolites are highlighted respectively in red and orange, whereas significant and trending declines are highlighted respectively in green and light green.

Table 3. Summary of identified metabolites related to amino acid and peptide metabolism

Super pathway	Sub-pathway	Biochemical name	TIB 5.5h / 8.5h ratio	p value	q value
Amino acid	Glycine, serine & threonine metabolism	Glycine	1.02	0.863	0.897
		dimethylglycine	0.93	0.459	0.824
		N-acetylglycine	0.96	0.376	0.817
		beta-hydroxypyruvate	0.92	0.671	0.878
		Serine	1.00	0.956	0.913
		N-acetylserine	0.91	0.627	0.855
		Threonine	0.98	0.669	0.878
		N-acetylthreonine	1.30	<0.001	0.186
		Betaine	1.04	0.051	0.808
	Histidine metabolism	Histidine	1.06	0.041	0.808
		trans-urocanate	1.17	0.187	0.808
		3-methylhistidine	1.07	0.769	0.881
	Lysine metabolism	glutarate (pentanedioate)	1.16	0.877	0.897
		Lysine	1.19	0.279	0.817
		Pipecolate	1.25	0.306	0.817
		glutaroyl carnitine	1.12	0.028	0.808
	Phenylalanine & tyrosine metabolism	phenyllactate	1.35	0.010	0.478
		phenylalanine	1.06	0.242	0.808
		phenylacetate	0.94	0.626	0.855
		p-cresol sulfate	1.13	0.182	0.808
		Tyrosine	1.02	0.660	0.878
		3-(4-hydroxyphenyl)lactate	1.07	0.169	0.808
		3-methoxytyrosine	1.17	0.169	0.808
		phenylacetylglutamine	1.02	0.787	0.881
		3-phenylpropionate (hydrocinnamate)	0.81	0.281	0.817
		phenol sulfate	0.84	0.150	0.808
	Tryptophan metabolism	Kynurenine	1.07	0.368	0.817
		Tryptophan	1.05	0.188	0.808
		indolelactate	0.93	0.328	0.817
		indoleacetate	1.04	0.763	0.881
		tryptophan betaine	1.06	0.968	0.913
		serotonin (5HT)	1.08	0.073	0.808
		C-glycosyltryptophan	1.07	0.036	0.808
		3-indoxyl sulfate	1.06	0.395	0.817
		indolepropionate	1.03	0.932	0.911
	Valine, leucine & isoleucine metabolism	3-methyl-2-oxobutyrates	0.96	0.537	0.848
		3-methyl-2-oxovalerate	0.98	0.705	0.878
		levulinate (4-oxovalerate)	0.86	0.134	0.808
		beta-hydroxyisovalerate	1.02	0.514	0.836
		alpha-hydroxyisocaproate	1.08	0.688	0.878
		Isoleucine	1.10	0.003	0.302
		Leucine	1.08	0.103	0.808

		Valine	1.05	0.202	0.808
		3-hydroxyisobutyrate	0.95	0.330	0.817
		4-methyl-2-oxopentanoate	0.95	0.583	0.855
		3-hydroxy-2-ethylpropionate	1.12	0.572	0.855
		alpha-hydroxyisovalerate	0.97	0.577	0.855
		isobutyrylcarnitine	1.07	0.255	0.817
		2-methylbutyrylcarnitine	1.05	0.782	0.881
		isovalerylcarnitine	1.16	0.625	0.855
		tiglyl carnitine	1.14	0.407	0.822
		methylglutaryl carnitine	1.00	0.834	0.897
	Cysteine, methionine, SAM, & taurine metabolism	Cysteine	1.08	0.729	0.881
		S-methylcysteine	1.04	0.843	0.897
		N-formylmethionine	1.17	0.099	0.808
		Taurine	1.11	0.370	0.817
		Methionine	1.04	0.411	0.822
		N-acetylmethionine	1.24	0.055	0.808
		2-hydroxybutyrate	0.92	0.215	0.808
	Creatine metabolism	Creatine	1.13	0.086	0.808
		Creatinine	0.98	0.463	0.824
Peptide	gamma-glutamyl	gamma-glutamylvaline	1.02	0.538	0.848
		gamma-glutamylleucine	1.06	0.354	0.817
		gamma-glutamylisoleucine	1.11	0.168	0.808
		gamma-glutamylmethionine	1.13	0.297	0.817
		gamma-glutamylglutamate	0.99	0.486	0.833
		gamma-glutamylglutamine	1.16	0.087	0.808
		gamma-glutamylphenylalanine	1.00	0.885	0.897
		gamma-glutamyltyrosine	1.04	0.508	0.836
		gamma-glutamylthreonine	1.12	0.203	0.808
		gamma-glutamylalanine	1.13	0.239	0.808

Sleep-loss-related significant and trending elevations in plasma metabolites are highlighted respectively in red and orange.

Table 4. Summary of identified metabolites related to lipid metabolism

Super pathway	Sub-pathway	Biochemical name	TIB 5.5h / 8.5h ratio	p value	q value
Lipid	Medium-chain fatty acid	caproate (6:0)	1.11	0.076	0.808
		heptanoate (7:0)	1.07	0.221	0.808
		caprylate (8:0)	1.06	0.358	0.817
		pelargonate (9:0)	1.05	0.325	0.817
		caprate (10:0)	1.04	0.421	0.822
		undecanoate (11:0)	1.13	0.137	0.808
		10-undecenoate (11:1n1)	1.18	0.271	0.817
		laurate (12:0)	1.07	0.239	0.808
		5-dodecenoate (12:1n7)	1.15	0.515	0.836
	Carnitine metabolism	Deoxycarnitine	1.02	0.911	0.907
		Carnitine	0.99	0.584	0.855
		3-dehydrocarnitine	1.08	0.261	0.817
		Acetylcarnitine	1.09	0.426	0.822
		hexanoylcarnitine	1.22	0.624	0.855
		octanoylcarnitine	1.38	0.301	0.817
		decanoylcarnitine	1.38	0.376	0.817
		2-decenoyl carnitine	1.29	0.095	0.808
		cis-4-decenoyl carnitine	1.17	0.499	0.836
		Laurylcarnitine	1.36	0.155	0.808
		palmitoylcarnitine	1.15	0.382	0.817
		stearoylcarnitine	1.18	0.426	0.822
		Oleoylcarnitine	1.24	0.197	0.808
	Bile acid metabolism	Cholate	2.41	0.355	0.817
		Glycocholate	1.15	0.796	0.881
		Deoxycholate	1.38	0.059	0.808
		glycodeoxycholate	1.03	0.776	0.881
		glycochenodeoxycholate	1.18	0.606	0.855
		glycolithocholate sulfate	0.92	0.890	0.897
		taurolithocholate 3-sulfate	0.90	0.996	0.913
		glycochenolate sulfate	1.23	0.008	0.465
		Taurochenolate sulfate	1.22	0.602	0.855
		glycoursodeoxycholate	0.92	0.510	0.836
	Lysolipid	1-palmitoylglycerophosphoethanolamine	1.17	0.101	0.808
		2-palmitoylglycerophosphoethanolamine	1.20	0.295	0.817
		1-stearoylglycerophosphoethanolamine	1.18	0.107	0.808
		1-oleoylglycerophosphoethanolamine	1.01	0.479	0.833
		2-oleoylglycerophosphoethanolamine	0.93	0.397	0.817
		1-linoleoylglycerophosphoethanolamine	1.21	0.709	0.878
		2-linoleoylglycerophosphoethanolamine	1.15	0.242	0.808
		1-arachidonoylglycerophosphoethanolamine	0.93	0.609	0.855

		2-arachidonoylglycerophosphoethanolamine	1.01	0.891	0.897
		1-myristoylglycerophosphocholine	1.25	0.390	0.817
		2-myristoylglycerophosphocholine	1.77	0.066	0.808
		1-pentadecanoylglycerophosphocholine	1.30	0.201	0.808
		1-palmitoylglycerophosphocholine	1.22	0.154	0.808
		2-palmitoylglycerophosphocholine	1.29	0.327	0.817
		1-palmitoleoylglycerophosphocholine	1.24	0.388	0.817
		2-palmitoleoylglycerophosphocholine	1.07	0.916	0.907
		1-heptadecanoylglycerophosphocholine	1.24	0.145	0.808
		1-stearoylglycerophosphocholine	1.22	0.237	0.808
		2-stearoylglycerophosphocholine	1.21	0.155	0.808
		1-oleoylglycerophosphocholine	1.18	0.209	0.808
		2-oleoylglycerophosphocholine	1.26	0.279	0.817
		1-linoleoylglycerophosphocholine	1.22	0.419	0.822
		2-linoleoylglycerophosphocholine	1.22	0.580	0.855
		1-eicosadienoylglycerophosphocholine	1.19	0.178	0.808
		1-eicosatrienoylglycerophosphocholine	1.30	0.734	0.881
		1-arachidonoylglycerophosphocholine	1.29	0.359	0.817
		2-arachidonoylglycerophosphocholine	1.16	0.789	0.881
		1-docosapentaenoylglycerophosphocholine	1.21	0.776	0.881
		1-docosahexaenoylglycerophosphocholine	1.24	0.456	0.824
		1-palmitoylglycerophosphoinositol	1.04	0.707	0.878
		1-stearoylglycerophosphoinositol	0.92	0.249	0.817
		1-arachidonoylglycerophosphoinositol	0.91	0.523	0.836
		1-palmitoylplasmenylethanolamine	0.98	0.357	0.817
	Sterol & Steroid	Lathosterol	0.92	0.351	0.817
		Cholesterol	1.11	0.030	0.808
		7-alpha-hydroxycholesterol	1.12	0.770	0.881
		7-beta-hydroxycholesterol	1.13	0.580	0.855
		dehydroisoandrosterone sulfate (DHEA-S)	1.10	0.267	0.817
		epiandrosterone sulfate	1.00	0.793	0.881
		androsterone sulfate	1.03	0.995	0.913
		Cortisol	1.07	0.525	0.836
		Cortisone	0.94	0.375	0.817
		beta-sitosterol	0.73	0.030	0.808
		7-alpha-hydroxy-3-oxo-4-cholestenoate (7-Hoca)	1.15	0.030	0.808
		4-androsten-3beta,17beta-diol disulfate 1	1.01	0.232	0.808
		4-androsten-3beta,17beta-diol disulfate 2	1.01	0.718	0.881
		5alpha-androstan-3beta,17beta-diol disulfate	0.96	0.734	0.881
		5alpha-pregnan-3beta,20alpha-diol disulfate	1.08	0.852	0.897
		pregnen-diol disulfate	1.10	0.481	0.833
		pregn steroid monosulfate	1.06	0.983	0.913
		andro steroid monosulfate 1	0.98	0.503	0.836



	andro steroid monosulfate 2	1.02	0.762	0.881
	21-hydroxypregnenolone disulfate	1.08	0.379	0.817
	pregnenolone sulfate	1.03	0.556	0.855
	pregnanediol-3-glucuronide	1.05	0.884	0.897

Sleep-loss-related significant and trending elevations in plasma metabolites are highlighted respectively in red and orange, whereas significant declines are highlighted in green.

Table 5. Summary of identified metabolites related to carbohydrate and energy metabolism

Super pathway	Sub-pathway	Biochemical name	TIB 5.5h / 8.5h ratio	p value	q value
Carbohydrate	Fructose, mannose, galactose, starch & sucrose metabolism	fructose	0.76	0.369	0.817
		mannitol	0.73	0.352	0.817
		mannose	0.80	0.039	0.808
		sorbitol	0.90	0.338	0.817
	Glycolysis, gluconeogenesis & pyruvate metabolism	1,5-anhydroglucitol (1,5-AG)	0.96	0.451	0.824
		glycerate	0.96	0.350	0.817
		glucose	0.89	0.086	0.808
		1,6-anhydroglucose	1.51	0.001	0.186
		pyruvate	0.90	0.364	0.817
		lactate	1.07	0.440	0.824
	Nucleotide sugars & pentose metabolism	arabitol	0.93	0.449	0.824
		ribitol	1.02	0.817	0.895
		threitol	0.91	0.387	0.817
		gluconate	0.66	0.086	0.808
		ribose	0.71	0.360	0.817
		ribulose	0.88	0.573	0.855
		xylitol	0.96	0.884	0.897
		arabinose	1.01	0.804	0.887
		xylose	0.80	0.244	0.808
		xylonate	0.74	0.088	0.808
		xylulose	0.84	0.488	0.833
Energy	Krebs cycle	citrate	1.07	0.525	0.836
		cis-aconitate	1.11	0.205	0.808
		alpha-ketoglutarate	0.83	0.625	0.855
		succinate	1.19	0.257	0.817
		succinylcarnitine	1.14	0.115	0.808
		fumarate	1.17	0.240	0.808
		malate	1.27	0.075	0.808

Sleep-loss-related significant and trending elevations in plasma metabolites are highlighted respectively in red and orange, whereas significant and trending declines are highlighted respectively in green and light green.

**Effects of Sleep Restriction on the Human Plasma Metabolome****Highlights:**

- We examine the effects of recurrent sleep restriction on the plasma metabolome of adults with familial risk of type 2 diabetes.
- When sleep was curtailed the most pronounced metabolic signature was an elevation in multiple plasma amino acids and related metabolites.
- Sleep restriction also induced elevations in several fatty acid, bile acid, steroid hormone, and tricarboxylic acid cycle intermediates.
- Circulating levels of glucose, some monosaccharides, gluconate, and five-carbon sugar alcohols tended to decline when sleep was reduced.