

Supplementary Information

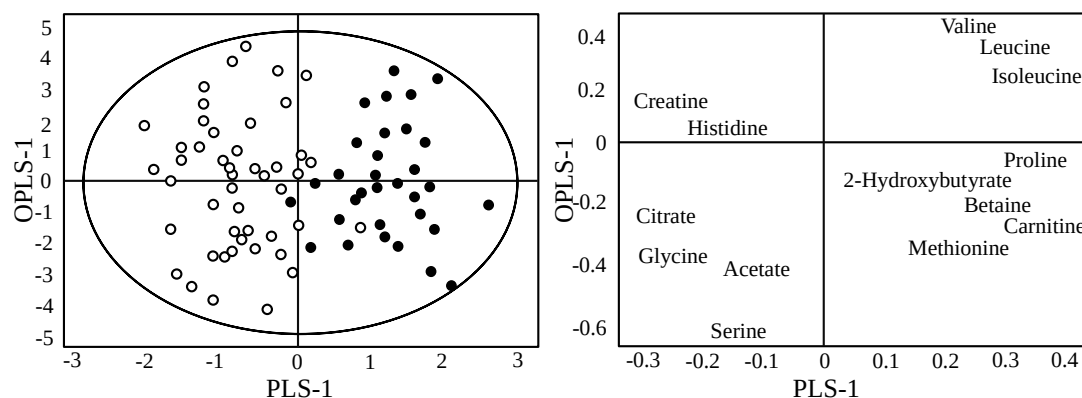
Serum Metabolomics of Activity Energy Expenditure and its Relation to Metabolic Syndrome and Obesity

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Supplementary Figure S1. Supervised OPLS-DA score scatter plot and loadings plot showing a close to complete separation between men (dots) and women (circles) based on the serum metabolome. Every dot/circle represents one participant. The score scatter plot (left) and loading plot (right) are superimposable and indicate which metabolites that associate with either gender. The model was based on two components, including one orthogonal component (Y-axis), with a R^2 of 0.73 and a Q^2 of 0.61. The distinct clustering of gender indicates that there are considerable differences in the overall metabolome of men and women. The ellipse represents the 95% confidence interval.

Y-variables	Unit	Range (mean)		R ² / Q ² values	
<i>Continuous Y-variables</i>		Women (n=47)	Men (n=35)	Women	Men
Age	years	30-59 (46)	33-60 (51)	0.57/0.16*	0.75/0.06
BMI value	Kg/m ²	16.8-30.3 (23.5)	20.0-33.4 (26.0)	0.25/0.10**	0.41/0.24
Body fat percentage	%	14.3-44.9 (30.3)	10.9-34.0 (21.7)	0.37/0.11****	0.49/0.30
Waist circumference	cm	64.8-98.7 (82.4)	74.2-155.3 (94.0)	0.34/0.22****	0.37/0.20
Hip circumference	cm	81.8-122.0 (99.9)	92.8-111.8 (99.0)	0.45/0.21	N/A
Waist to hip ratio		0.73-0.91 (0.82)	0.79-1.04 (0.91)	0.20/0.09****	0.40/0.23
AEE_{DLW}	Kcal	489-2974 (1200)	630-3129 (1437)	0.24/0.09	0.27/0.08
AEE/kg_{DLW}	Kcal/kg	8.3-49.8 (18.9)	9.0-37.1 (17.3)	N/A	N/A
Moderate physical activity	Hours/day	0.05-9.4 (1.6)	0-6.8 (1.8)	N/A	0.34/0.11
Vigorous physical activity	Hours/day	0-2.6 (0.5)	0-2.1 (0.6)	0.25/0.14	0.23/0.07
PAL_{DLW}	Kcal	1.6-3.6 (2.1)	1.5-3.0 (2.0)	N/A	N/A
<i>Qualitative Y-variables</i>					
BMI class		Normal weight= 77%	Normal weight= 31%	0.42/0.14****	0.57/0.33
		Overweight= 23%	Overweight= 69%		
MetS		0-3 (0)	0-3 (0)	0.19/0.12	0.31/0.12
		0= 60% 1= 34%	0= 54% 1= 17%		
		2= 4% 3= 2%	2= 17% 3= 11%		

Supplementary Table S1. All analyzed variables with their respective range of variability and average values as well as the values of explained variance (R²) and predictive ability (Q²). Supervised multivariate statistical models were created using partial least square regression (PLS) for the continuous Y-variables and discriminant analysis (DA) were applied for qualitative Y-variables, using PLS-DA when comparing more groups than two (i.e. MetS₀₋₃) and orthogonal PLS-DA when comparing two groups (i.e. BMI class or MetS_{any} vs MetS_{zero}). N/A indicates when no statistical model

could be created. * $p < 0.01$, ** $p < 0.001$ and *** $p < 0.0001$. Abbreviations are as follows in alphabetical order: AEE_{DLW}, activity energy expenditure; BMI, body mass index; MetS, metabolic syndrome; PAL_{DLW}, physical activity level.

Serum metabolite	MEN			WOMEN			ALL PARTICIPANTS		
	Average	SD	Median	Average	SD	Median	Average	SD	Median
2-Aminobutyrate	9.1	4.1	9.1	7.8	3.1	7.7	8.4	3.6	8.9
2-Hydroxybutyrate	16.5	7.6	17.3	13.9	5.3	13.1	15.0	6.5	14.8
<i>2-Hydroxyisovalerate</i>	<i>3.3</i>	<i>1.8</i>	<i>3.5</i>	<i>3.3</i>	<i>1.4</i>	<i>3.5</i>	<i>3.3</i>	<i>1.6</i>	<i>3.5</i>
3-Hydroxybutyrate	28.9	42.8	15.5	22.1	18.0	14	25.0	31.1	15
Acetate	16.4	4.7	15.7	17.1	5.1	16.1	16.8	4.9	16.1
Acetoacetate	12.5	12.7	9.3	9.5	4.9	9	10.8	9.1	9.3
<i>Acetone</i>	<i>4.2</i>	<i>3.4</i>	<i>3.2</i>	<i>3.9</i>	<i>2.6</i>	<i>3</i>	<i>4.0</i>	<i>2.9</i>	<i>3.15</i>
Alanine	162.2	29.5	152.9	147.2	31.0	141.5	153.6	31.1	147.2
Arginine	42.1	15.5	49.1	42.9	12.0	49.1	42.5	13.5	49.1
Betaine	19.9	5.8	19.3	15.7	4.4	15.1	17.5	5.4	16.9
<i>Butyrate</i>	<i>2.8</i>	<i>1.6</i>	<i>2.7</i>	<i>2.8</i>	<i>1.3</i>	<i>2.7</i>	<i>2.8</i>	<i>1.4</i>	<i>2.7</i>
Carnitine	18.5	4.6	19.1	13.8	3.1	14	15.8	4.5	15.65
<i>Choline</i>	<i>4.4</i>	<i>1.8</i>	<i>4.6</i>	<i>4.2</i>	<i>0.8</i>	<i>4</i>	<i>4.3</i>	<i>1.3</i>	<i>4.25</i>
Citrate	54.9	13.5	56.2	61.4	11.6	60.7	58.6	12.8	58.9
Creatine	9.8	4.4	8.4	13.1	5.8	12.1	11.7	5.5	11.4
<i>Creatine phosphate</i>	<i>5.1</i>	<i>2.2</i>	<i>4.9</i>	<i>4.6</i>	<i>1.9</i>	<i>4.9</i>	<i>4.8</i>	<i>2.1</i>	<i>4.9</i>
Creatinine	21.0	5.9	21.1	17.9	5.7	17	19.2	6.0	19.05
<i>Dimethyl sulfone</i>	<i>5.3</i>	<i>11.5</i>	<i>3.2</i>	<i>7.5</i>	<i>24.0</i>	<i>3.8</i>	<i>6.6</i>	<i>19.6</i>	<i>3.45</i>
<i>Dimethylamine</i>	<i>2.0</i>	<i>2.2</i>	<i>1.3</i>	<i>1.8</i>	<i>1.8</i>	<i>1.3</i>	<i>1.9</i>	<i>2.0</i>	<i>1.3</i>
<i>Formate</i>	<i>3.3</i>	<i>3.7</i>	<i>0</i>	<i>3.6</i>	<i>4.2</i>	<i>0</i>	<i>3.4</i>	<i>4.0</i>	<i>0</i>
Glucose	1986.1	230.9	1982.5	1839.8	219.5	1846.4	1902.2	234.6	1907.9
Glutamine	211.8	29.1	212.4	201.3	26.9	203.8	205.8	28.2	211.6
Glycerol	889.8	251.8	942.9	880.9	316.5	841.8	884.7	289.0	911.4
Glycine	96.1	20.7	93	120.3	44.3	109.8	110.0	37.9	98.65
Histidine	26.5	8.1	28.5	28.8	4.7	28.1	27.9	6.4	28.3
Isoleucine	27.3	4.1	29.1	21.4	3.3	20.8	23.9	4.7	23.2

Lactate	705.3	147.8	697.5	630.8	139.8	583.1	662.6	147.1	644.55
Leucine	53.1	7.8	53.9	43.3	5.9	42.3	47.5	8.3	45.7
Lysine	56.1	10.5	55.6	50.5	13.5	47.7	52.9	12.5	51
Methionine	11.1	3.3	11.3	9.7	2.3	9.8	10.3	2.8	10.45
Methyl succinate	6.7	3.2	7	6.2	2.0	5.9	6.4	2.6	6.25
Myo-inositol	17.1	9.8	16.1	16.7	7.9	14.3	16.9	8.7	15.25
<i>O-Phosphocholine</i>	<i>1.7</i>	<i>0.8</i>	<i>2</i>	<i>1.6</i>	<i>0.6</i>	<i>2</i>	<i>1.6</i>	<i>0.7</i>	<i>2</i>
Ornithine	26.2	7.2	26.8	22.6	6.8	21.8	24.2	7.2	23.9
Phenylalanine	25.1	4.9	24.2	24.4	3.9	24.2	24.7	4.4	24.2
Proline	86.1	26.2	82.6	64.6	16.2	61.7	73.8	23.5	70.65
Pyruvate	18.0	6.3	17.6	18.8	5.9	18.1	18.4	6.0	17.85
Serine	50.4	12.8	51.4	53.7	12.6	53.3	52.3	12.7	51.9
Taurine	48.8	25.7	52.4	53.5	18.6	55.7	51.5	21.9	54.65
Threonine	54.8	21.8	56.8	57.4	20.9	55.3	56.3	21.2	55.7
Tyrosine	30.0	6.1	29.2	29.3	5.6	29	29.6	5.8	29.1
Urea	273.3	225.1	273.6	229.5	186.1	260.3	248.2	203.5	262.05
Valine	103.6	14.7	104.1	86.7	13.1	84.7	93.9	16.0	93.2

Supplementary Table S2. All serum metabolites detected with qualitative profiling of ¹H NMR spectra. Metabolites with mean and/or average values below 5uL were excluded in all analysis because their low signal/noise ratio, as indicated in italics. Average and median values as well as standard deviation (SD) are shown for all participants and for men and women separately.

	Body fat percentage	BMI class	MetS
Lower	3-Hydroxybutyrate	Creatinine	Creatinine
	Acetoacetate	Serine	Glutamine
	Serine	3-Hydroxybutyrate	Glycine
	Glycine	Acetoacetate	Serine
	Glutamine		Ornithine
	Urea		Lactate
			3-Hydroxybutyrate
			Carnitine
Higher	Tyrosine	Carnitine	Creatine
	Carnitine	Tyrosine	Myo-inositol
	Pyruvate	Phenylalanine	Urea
	Arginine	Glucose	Methyl succinate
	Phenylalanine	Proline	
	Methyl succinate	Arginine	
	Glucose	Pyruvate	
	Creatine		
	Proline		
	Isoleucine		
	Body fat percentage	Waist circumference	Waist:hip ratio
Lower	Serine	Glycine	Serine
	Glycine	Serine	Glycine
		Urea	Urea
Higher	Pyruvate	Pyruvate	Pyruvate
	Carnitine	Carnitine	Carnitine
	Creatine	Tyrosine	Arginine
	Arginine	Arginine	Tyrosine
	Proline	Methyl succinate	Creatine
	Tyrosine	Creatine	Proline
	Phenylalanine	Valine	Isoleucine
	Methyl succinate	Isoleucine	Valine

	AEE _{DLW}	Moderate physical activity	Vigorous physical activity
Lower		Glycine	Taurine Methionine
Higher	Lactate	Taurine	Leucine
	Serine	Carnitine	Glycine
	Phenylalanine	Pyruvate	Creatinine
	Glucose	Lactate	Isoleucine
	Creatinine	Phenylalanine	Valine
	Leucine	Tyrosine	Betaine
	Carnitine	2-Hydroxybutyrate	Serine
	Lysine	Glycerol	Lysine
	Valine	Histidine	Acetate
	Ornithine	Threonine	Arginine
	2-Hydroxybutyrate		
	Histidine		

Supplementary Table S3. Metabolic profiles for men showing all analyzed variables with the most influential (VIP>1) metabolites listed. Negative (lower) and positive correlations (higher) between metabolites and each variable are indicated. No multivariate models could be created for hip circumference, AEE/kg or PAL.

	BMI value	BMI class	MetS	Body fat percentage
Lower	Serine	Serine	Serine	Glycerol
	Methyl succinate	Glycine	Myo-inositol	Serine
	Myo-inositol	Methyl succinate	Creatinine	Myo-inositol
	3-Hydroxybutyrate	Creatinine	Arginine	Creatinine
	2-Hydroxybutyrate	Citrate	Betaine	3-Hydroxybutyrate
	Citrate	2-Aminobutyrate	Acetoacetate	Citrate
		2-Hydroxybutyrate		
		3-Hydroxybutyrate		
Higher	Pyruvate	Glucose	Histidine	Lysine
	Alanine	Carnitine	Lysine	Glycine
	Glucose	Ornithine		Carnitine
	Ornithine	Pyruvate		Creatine
	Creatine	Alanine		
	Carnitine			
	Hip circumference	Waist circumference	Waist:hip ratio	
Lower	Serine	Serine	Serine	
	Myo-inositol	Myo-inositol	Creatinine	
	3-Hydroxybutyrate	3-Hydroxybutyrate	Myo-inositol	
		2-Hydroxybutyrate	Methyl succinate	
		Methyl succinate	Phenylalanine	
			2-Hydroxybutyrate	
			Glutamine	
			Citrate	
			2-Aminobutyrate	
			Leucine	
Higher	Lysine	Glucose	Urea	
	Creatine	Pyruvate		
	Histidine	Carnitine		
	Phenylalanine	Alanine		

Pyruvate
Carnitine
Glucose
Valine
Lactate
Glutamine
Alanine

	AEE	Vigorous physical activity
Lower	Ornithine	Urea 3-Hydroxybutyrate Acetoacetate
Higher	Methyl succinate	Glutamine
	Lysine	Creatine
	Histidine	Phenylalanine
	Creatinine	Carnitine
	Carnitine	Lactate
	Acetoacetate	Threonine
	2-Aminobutyrate	Glucose Myo-inositol Ornithine Alanine

Supplementary Table S4. Metabolic profiles for women showing all analyzed variables with the most influential (VIP>1) metabolites listed. Negative (lower) and positive correlations (higher) between metabolites and each variable are indicated. No multivariate models could be created for moderate physical activity, AEE/kg or PAL.

Variable	AEE _{DLW}		AEE/kg _{DLW}		PAL _{DLW}	
	High	Low	High	Low	High	Low
	Average (SD)	Average (SD)	Average (SD)	Average (SD)	Average (SD)	Average (SD)
MetS risk factors	1.0 (0.0)**	1.6 (0.8)			1.1 (0.26)*	1.6 (0.85)
BMI			23.1 (1.4)**	25.8 (3.0)	23.4 (1.6)**	25.9 (3.2)
Body fat percentage			29.4 (4.2)**	36.2 (5.2)	30.3 (4.5)**	36.4 (5.5)
Waist circumference			82.9 (4.9)**	90.1 (6.4)		
Hip circumference			99.9 (3.4)**	105.9 (6.5)	100.8 (3.9)*	105.8 (6.7)

Variable	Moderate physical activity		Vigorous physical activity	
	High	Low	High	Low
	Average (SD)	Average (SD)	Average (SD)	Average (SD)
BMI			20.5 (2.0)**	22.8 (1.6)
Body fat percentage			22.5 (5.6)*	27.9 (2.6)
Hip circumference	97.7 (3.8)**	91.3 (5.0)	91.4 (5.4)*	97.6 (3.4)

Supplementary Table S5. Body measures and MetS risk factors that were different between high and low groups of AEE_{DLW}, AEE/kg_{DLW}, PAL_{DLW} as well as moderate and vigorous physical activity in women. Statistical significance is indicated for the respective high compared to the low groups, *p<0.05, **p<0.01.

Pathway	N(Compounds)	Hits	Raw p	-log(p)	Holm p	FDR	Impact
Sphingolipid metabolism	25	1	3.93E-04	7.84E+00	1.96E-02	1.19E-02	0
Methane metabolism	34	2	4.75E-04	7.65E+00	2.33E-02	1.19E-02	0.02
Cyanoamino acid metabolism	16	3	1.27E-03	6.67E+00	6.07E-02	2.11E-02	0
Glycine, serine and threonine metabolism	48	6	1.82E-03	6.31E+00	8.55E-02	2.27E-02	0.44
Sulfur metabolism	18	2	3.13E-03	5.77E+00	1.44E-01	3.13E-02	0.03
Cysteine and methionine metabolism	56	3	4.68E-03	5.36E+00	2.11E-01	3.90E-02	0.07
Primary bile acid biosynthesis	47	2	3.40E-02	3.38E+00	1.00E+00	2.43E-01	0.02
Porphyrin and chlorophyll metabolism	104	2	5.07E-02	2.98E+00	1.00E+00	3.17E-01	0
Aminoacyl-tRNA biosynthesis	75	13	9.41E-02	2.36E+00	1.00E+00	4.43E-01	0.11
Glycerolipid metabolism	32	1	1.11E-01	2.20E+00	1.00E+00	4.43E-01	0.19
Thiamine metabolism	24	2	1.14E-01	2.17E+00	1.00E+00	4.43E-01	0
Glutathione metabolism	38	2	1.17E-01	2.14E+00	1.00E+00	4.43E-01	0
Purine metabolism	92	3	1.19E-01	2.13E+00	1.00E+00	4.43E-01	0.01
Citrate cycle (TCA cycle)	20	2	1.33E-01	2.02E+00	1.00E+00	4.43E-01	0.15
Glyoxylate and dicarboxylate metabolism	50	2	1.33E-01	2.02E+00	1.00E+00	4.43E-01	0

Arginine and proline metabolism	77	8	1.46E-01	1.92E+00	1.00E+00	4.57E-01	0.43
Nitrogen metabolism	39	6	2.00E-01	1.61E+00	1.00E+00	5.09E-01	0
Galactose metabolism	41		2.03E-01	1.59E+00	1.00E+00	5.09E-01	0
Lysine degradation	47	3	2.54E-01	1.37E+00	1.00E+00	5.09E-01	0.15
Vitamin B6 metabolism	32	1	2.71E-01	1.31E+00	1.00E+00	5.09E-01	0.02
Pentose and glucuronate interconversions	53	1	2.71E-01	1.31E+00	1.00E+00	5.09E-01	0
Nicotinate and nicotinamide metabolism	44	1	2.71E-01	1.31E+00	1.00E+00	5.09E-01	0
Terpenoid backbone biosynthesis	33	1	2.71E-01	1.31E+00	1.00E+00	5.09E-01	0
Inositol phosphate metabolism	39	1	2.82E-01	1.27E+00	1.00E+00	5.09E-01	0.14
Taurine and hypotaurine metabolism	20	3	2.82E-01	1.27E+00	1.00E+00	5.09E-01	0.35
Pyrimidine metabolism	60	2	2.84E-01	1.26E+00	1.00E+00	5.09E-01	0
Tyrosine metabolism	76	3	3.03E-01	1.19E+00	1.00E+00	5.09E-01	0.05
Synthesis and degradation of ketone bodies	6	2	3.04E-01	1.19E+00	1.00E+00	5.09E-01	0.7
Ascorbate and aldarate metabolism	45	2	3.05E-01	1.19E+00	1.00E+00	5.09E-01	0.02
Pentose phosphate pathway	32	2	3.15E-01	1.15E+00	1.00E+00	5.09E-01	0
Butanoate metabolism	40	3	3.20E-01	1.14E+00	1.00E+00	5.09E-01	0.13

Alanine, aspartate and glutamate metabolism	24	2	3.26E-01	1.12E+00	1.00E+00	5.09E-01	0.21
Starch and sucrose metabolism	50	1	3.54E-01	1.04E+00	1.00E+00	5.21E-01	0.02
Amino sugar and nucleotide sugar metabolism	88	1	3.54E-01	1.04E+00	1.00E+00	5.21E-01	0
D-Glutamine and D-glutamate metabolism	11	1	3.66E-01	1.01E+00	1.00E+00	5.23E-01	0.03
Pantothenate and CoA biosynthesis	27	2	4.25E-01	8.55E-01	1.00E+00	5.66E-01	0
Valine, leucine and isoleucine degradation	40	3	4.27E-01	8.50E-01	1.00E+00	5.66E-01	0.02
Valine, leucine and isoleucine biosynthesis	27	4	4.30E-01	8.43E-01	1.00E+00	5.66E-01	0.05
Phenylalanine metabolism	45	3	4.52E-01	7.93E-01	1.00E+00	5.80E-01	0.12
Phenylalanine, tyrosine and tryptophan biosynthesis	27	2	5.34E-01	6.27E-01	1.00E+00	6.62E-01	0.01
Propanoate metabolism	35	4	5.43E-01	6.11E-01	1.00E+00	6.62E-01	0.03
Ubiquinone and other terpenoid-quinone biosynthesis	36	1	5.87E-01	5.32E-01	1.00E+00	6.90E-01	0
Glycolysis or gluconeogenesis	31	4	5.93E-01	5.22E-01	1.00E+00	6.90E-01	0.1
Pyruvate metabolism	32	3	6.64E-01	4.09E-01	1.00E+00	7.42E-01	0.42
D-Arginine and D-ornithine metabolism	8	2	6.68E-01	4.04E-01	1.00E+00	7.42E-01	0
Histidine metabolism	44	1	7.47E-01	2.92E-01	1.00E+00	7.94E-01	0.14

beta-Alanine metabolism	28	1	7.47E-01	2.92E-01	1.00E+00	7.94E-01	0
Selenoamino acid metabolism	22	1	7.84E-01	2.43E-01	1.00E+00	8.17E-01	0
Lysine biosynthesis	32	1	8.79E-01	1.29E-01	1.00E+00	8.79E-01	0.1
Biotin metabolism	11	1	8.79E-01	1.29E-01	1.00E+00	8.79E-01	0

Supplementary Table S6. Pathway analysis for women comparing MetS_{any} and MetS_{zero} groups. Pathways listed by p-value (Raw p). N(compounds) refers to the number of compounds included in each pathway (library), whereas hits refers to the number of metabolites included in the pathway analysis. Holm adjusted p-values were used for interpretation with significance indicated by p<0.05. False discovery rate (FDR) is also included.