Supplementary information

HR-MAS NMR Metabolomics profile of Vero cells under the influence of virus infection and nsP2 inhibitor: A Chikungunya Case Study

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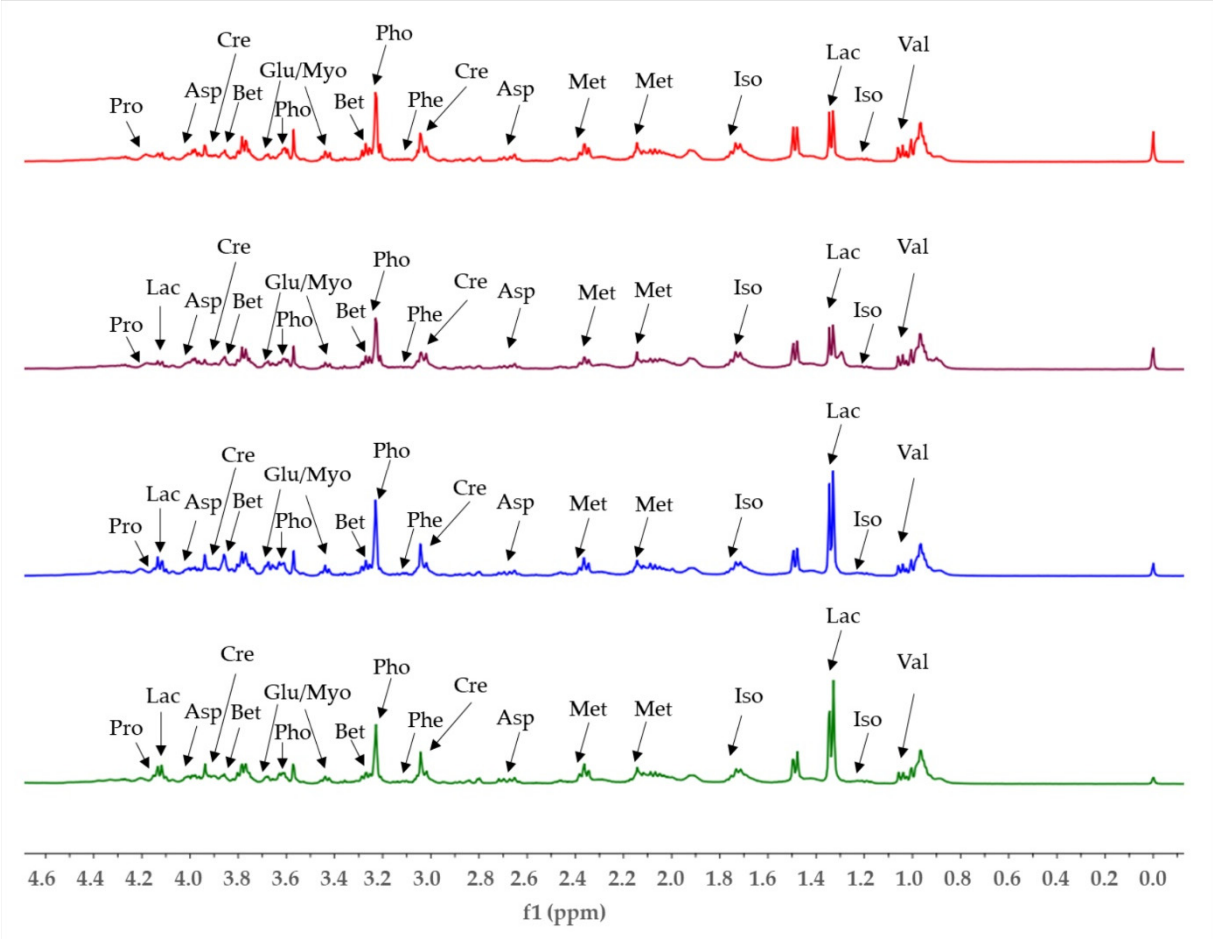


Figure S1. HR-MAS ^{1}H-NMR T_2-edited (CPMG) spectra (400 MHz) of all four conditions demonstrating the most differential metabolites among them. Vero E6 control sample spectrum is represented in green (CC), cells treated with WDL (CW) are in blue, cells infected and treated with the molecule (CWV) are represented in purple, and CHIKV-infected cells are represented in red (CV). Identified metabolites are Val (Valine); Lac (Lactate); Iso (Isoleucine); Pro (Proline); Met (Methionine); Asp (Aspartate); Cre (Creatine); Phe (Phenylalanine); Pho (Phosphocholine); Glu (Glucose); Bet (Betaine); Myo (Myo-inositol).

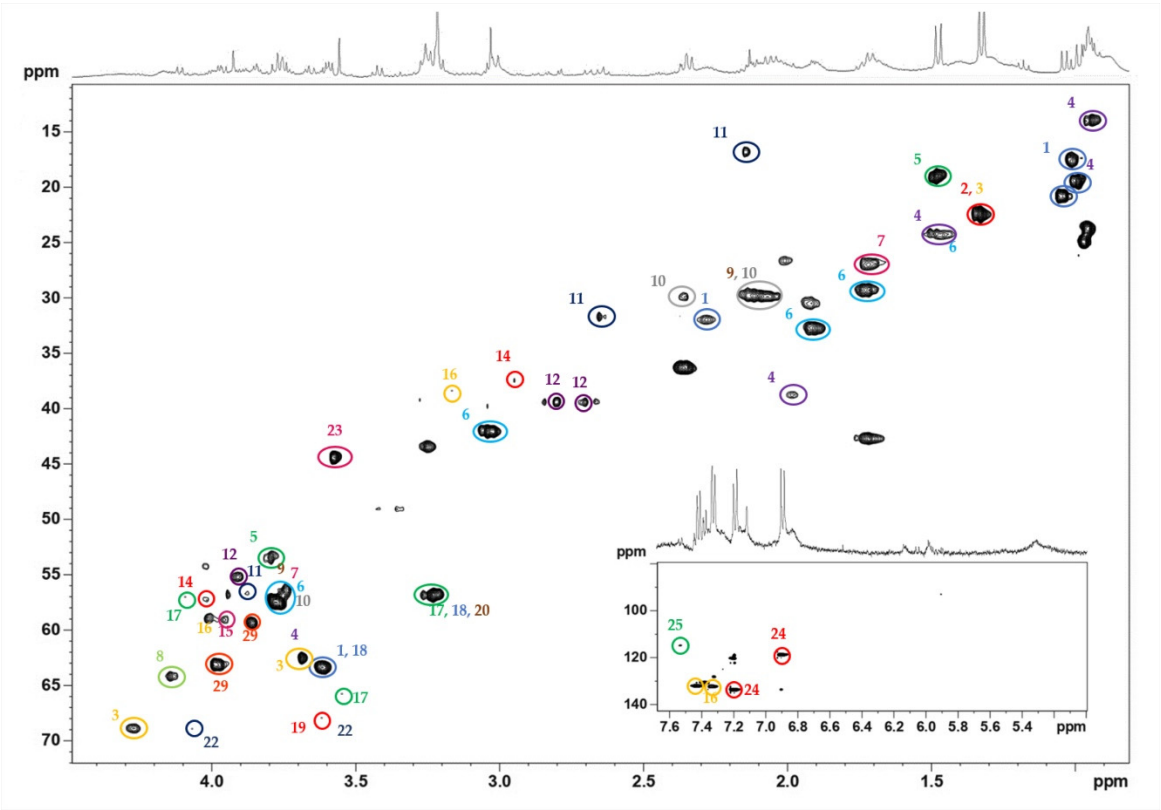


Figure S2. HR-MAS 2D-HSQC spectrum of Vero E6 cells samples acquired on a Bruker Avance III 400 MHz. The image of the spectrum comprises the region between 0 and 4.50 ppm, amplified 20 times, and the small image on the right corner shows the 5.20 to 7.60 ppm region for 1H chemical shifts and from 10 to 70 ppm for 13C. Identified metabolites are numbered according to Figure S1 and Table S1.

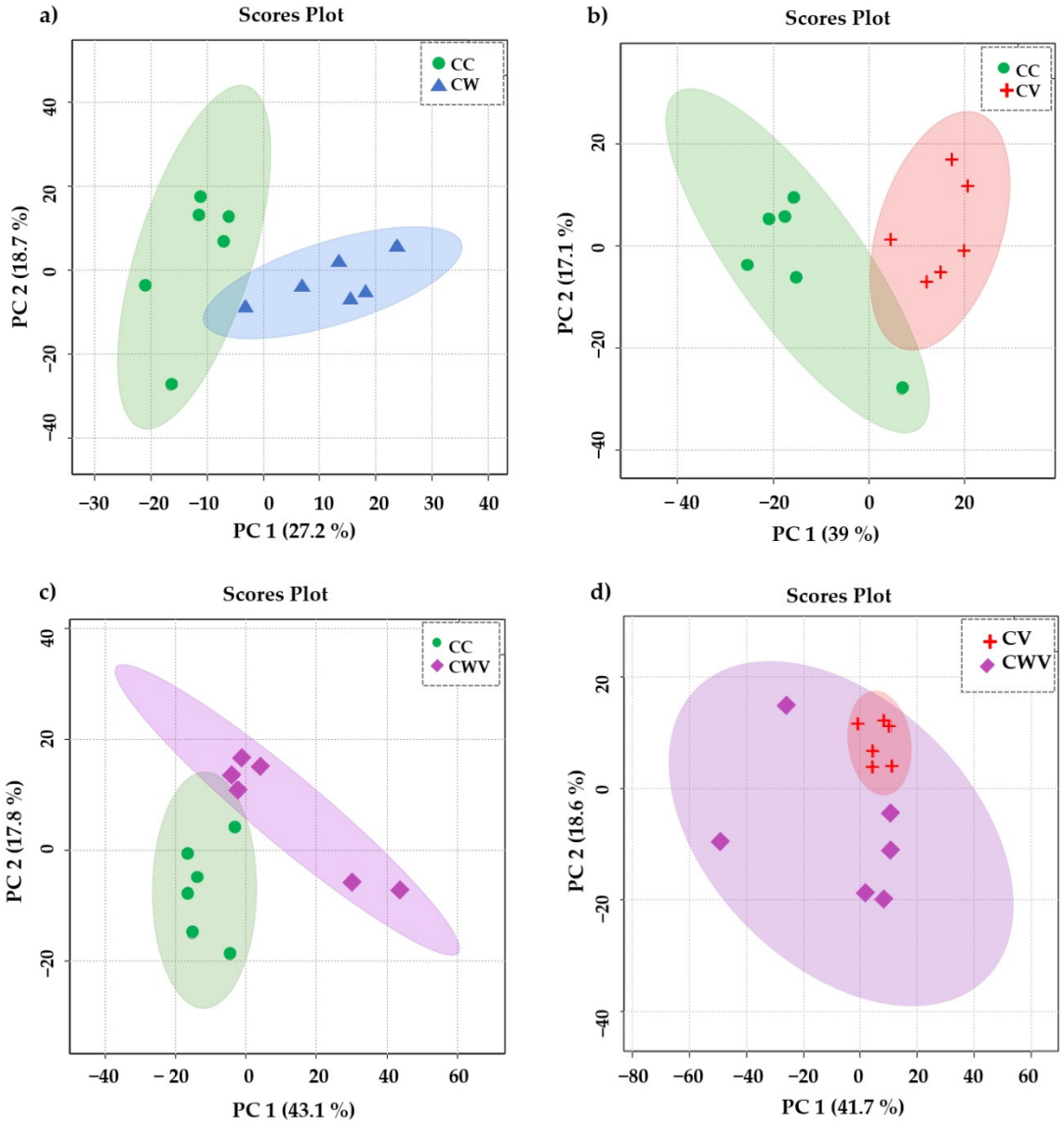


Figure S3. PCA scores plot in 2D of the HR-MAS 1H-NMR data showing the intrinsic groupings of the conditions compared to the healthy cell control (CC), shown in green, and no outliers. (**a**) The cells treated with WDL (CW) in blue, (**b**) the cells infected with CHIKV in red (CV), and (**c**) the CHIKV-infected cells treated with WDL in purple (CWV). The explained variance with the first two principal components is shown in the y and x axes.

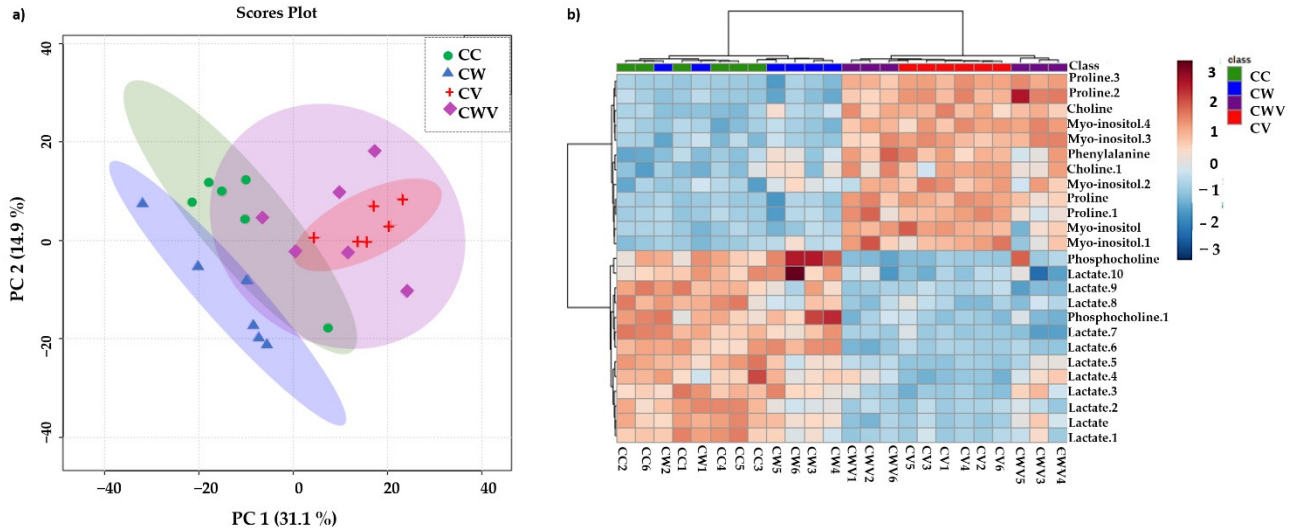


Figure S4. (**a**) PCA scores plot in 2D of the HR-MAS ^{1}H-NMR data showing the intrinsic groupings of all the conditions: the healthy cell control (CC), shown in green, the cells treated with WDL (CW) in blue, the cells infected with CHIKV in red (CV), and (**c**) the CHIKV-infected cells treated with WDL in purple (CWV). The explained variance with the first two principal components is shown in the y and x axes. (**b**) Hierarchical clustering through heat maps analysis using Euclidean distance. The lines represent the discriminant variables from the PLS-DA analysis for all conditions CC × CV × CW × CWV. The columns represent the samples, and the colour bars on the right represent the relative concentrations of the discriminant metabolites (red: higher relative concentration; blue: lower relative concentration).

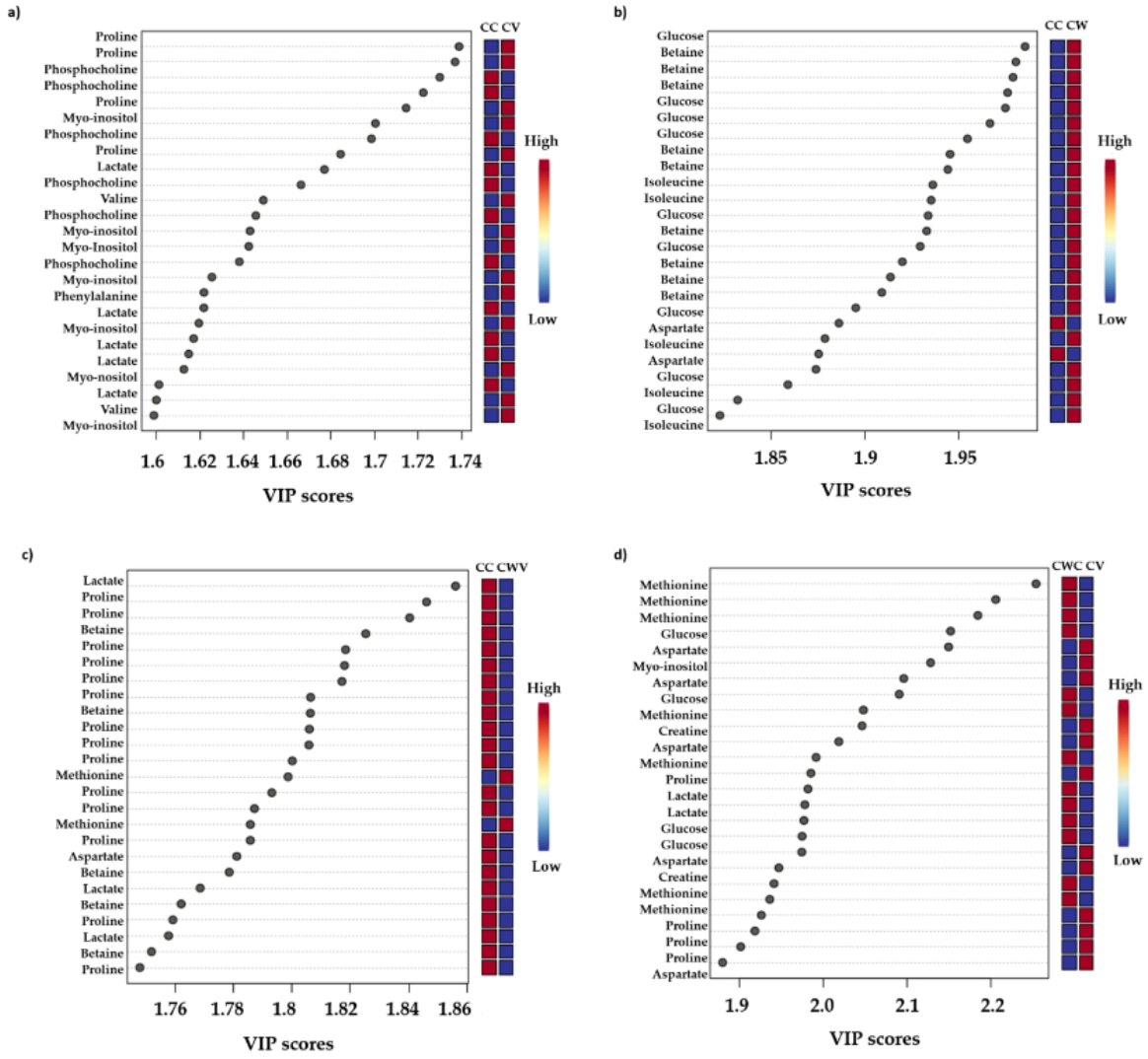


Figure S5. VIP plots of the most discriminant variables from the PLS-DA models, with scores > 1.5. (**a**) CC × CV; (**b**) CC × CW; (**c**) CC × CWV; (**d**) CV × CWV .

Table S1. Cell toxicity of WDL in Vero E6 cell lineage.

Concentration	Cell Viability (%)*
400 µM	45.5 ± 17.0
300 µM	59.0 ± 13.0
200 µM	68.0 ± 8.0
100 µM	74.7 ± 14.0
50 µM	93.6 ± 10.1

* Values with 95% confidence intervals (CI).

Table S2. Chemical shifts, spectral peaks multiplicities and coupling constants of some of the most important metabolites.

Metabolite	Nº	^{1}H Chemical shifts (ppm), spectral peaks multiplicities and coupling constant	^{13}C Chemical shifts (ppm), spectral peaks multiplicities and coupling constant
Valine	1	0.97 d (*J* = 8.0 Hz); 1.05 d (*J* = 7.0 Hz); 2.28 m; 3.61 d (*J* = 4.0 Hz)	17.1, 19.3, 20.5, 31.6, 63.2
Lactate	2	1.33 d (*J* = 7.0 Hz); 4.12 q (*J* = 7.0 Hz)	22.4
Threonine	3	1.33 d (J = 6.7 Hz); 3.60 (J = 6.7 Hz); 4.26 m	22.6, 63.2, 68.8
Isoleucine	4	0.93 t (*J* = 8.0 Hz); 0.99 d (*J* = 9.0 Hz); 1.24 m; 1.46 m; 1.97 m; 3.67 d (*J* = 4.0 Hz)	14.1, 17.1, 24.1, 38.6, 62.5
Alanine	5	1.48 d (*J* = 7.3 Hz); 3.79 q (*J* = 7.3 Hz)	18.8, 53.1
Lysine	6	1.43 m; 1.73 m; 1.91 m; 3.03 t; 3.75 t (*J* = 6.1 Hz)	24.1, 29.2, 32.6, 42.1, 57.4
Arginine	7	1.68 m (*J* = 7.0 Hz); 1.90 m (*J* = 7.3 Hz); 3.25 t (*J* = 6.9 Hz); 3.75 t (*J* = 6.1 Hz)	26.4, 30.4, 43.3, 57.2
Proline	8	2.10 m; 2.36 m 3.58m; 4.14 dd (*J* = 8.6, 6.4 Hz)	31.1, 64.2
Glutamate	9	2.12 m; 2.37 m; 3.77 dd (*J* = 7.2, 4.7 Hz)	30.2, 31.6, 56.5
Glutamine	10	2.14 m; 2.45 m; 3.77 t (*J* = 6.2 Hz)	29.4, 33.8, 57.2
Methionine	11	2.14 m; 2.17 s; 2.65 t (*J* = 7.6 Hz); 3.85 dd (*J*=7.1, 5.4 Hz)	16.6, 32.3, 31.5, 56.6
Aspartate	12	2.68 dd (*J* = 17.5, 8.9 Hz); 2.82 dd (*J* = 17.5, 3.7 Hz); 3.92 dd (*J* = 8.8, 3.8 Hz)	39.3, 55.1
Hypotaurine	13	2.55 t (*J* = 6.8 Hz); 3.23 t (*J* = 6.8 Hz)	-
Asparagine	14	2.88 m; 2.94 m; 3.99 dd (*J* = 7.7, 4.3 Hz)	37.4, 37.6, 54.1
Creatine	15	3.04 s; 3.93 s	59.1
Phenylalanine	16	3.11 dd (*J* = 14.6, 8.0 Hz); 3.28 dd (*J* = 14.6, 5.5 Hz); 4.00 dd (*J*	39.1, 58.9, 132.2, 131.7, 131.4

		= 7.9, 5.3 Hz); 7.33 m; 7.39 m; 7.42 m	
Choline	17	3.21 s; 3.51 dd (*J* = 5.8, 4.2 Hz); 4.06 dd	56.5, 70.2, 58.1
Phosphocholine	18	3.23 s; 3.62 m; 4.22 m	56.7, 64.1
Glucose	19	3.31 m; 3.44 m; 3.54 m	74.1
Betaine	20	3.26 s; 3.89 s	56.8
Trimethylamine-N-oxide	21	3.27 s	-
Myo-inositol	22	3.27 t (*J* = 11.8 Hz); 3.52 dd (*J* = 9.9, 2.9); 3.61 t (*J* = 9.7 Hz); 4.05 t (*J* = 2.9 Hz)	77.2, 73.8, 75, 74.9
Glycine	23	3.57 s	44.3
Tyrosine	24	6.89 m; 7.19 m	118.8, 133.6
Tryptophan	25	7.53 m; 7.72 m	115.1, 121.3
UDP-N-acetylglucosamine **UDP-glucose** **UDP-glucuronate**	26	5.97 d; 7.95 d	144.2
Fumarate	27	6.53 s	-
ATP	28	6.15 d; 8.26 s; 8.56 s	-
Serine	29	3.86 dd; 3.97 m	59.2, 63.3

Table S3. Discriminant metabolites from the univariate analysis of CC × CV analysis (*T-test*, Fisher's LSD post-hoc test, $p < 0.05$).

Metabolite	**t.stat**	**p.value**	**-log10(p)**	**FDR**
Proline	-19.18	3.21E-09	8.49	1.88E-06
Proline	-18.47	4.64E-09	8.33	1.88E-06
Phosphocholine	16.29	1.57E-08	7.80	4.25E-06
Phosphocholine	14.61	4.48E-08	7.34	9.07E-06
Proline	-13.30	1.09E-07	6.95	1.77E-05
Myo-inositol	-11.62	3.93E-07	6.40	5.25E-05

Phosphocholine	11.44	4.54E-07	6.34	5.25E-05
Proline	-10.28	1.22E-06	5.91	0.00012
Lactate	9.79	1.91E-06	5.71	0.00017
Phosphocholine	9.18	3.43E-06	5.46	0.00027
Valine	-8.39	7.73E-06	5.11	0.00056
Phosphocholine	8.25	8.95E-06	5.04	0.00059
Myo-inositol	-8.15	1.00E-05	5.00	0.00059
Myo-inositol	8.12	1.02E-05	4.99	0.00059
Phosphocholine	7.96	1.21E-05	4.91	0.00065
Myo-inositol	-7.55	1.94E-05	4.71	0.00098
Phenylalanine	-7.43	2.21E-05	4.65	0.00099
Lactate	7.43	2.22E-05	4.65	0.00099
Myo-inositol	-7.36	2.39E-05	4.62	0.00102
Lactate	7.30	2.59E-05	4.58	0.00105
Lactate	7.23	2.80E-05	4.55	0.00108
Myo-inositol	-7.17	3.01E-05	4.52	0.00110
Lactate	6.87	4.30E-05	4.36	0.00145
Valine	-6.84	4.46E-05	4.35	0.00145
Myo-inositol	-6.82	4.61E-05	4.33	0.00145

Table S4. Discriminant metabolites from the univariate analysis of CC × CW analysis (*T-test*, Fisher's LSD post-hoc test, $p < 0.05$).

Metabolite	**t.stat**	**p.value**	**-log10(p)**	**FDR**
Glucose	-13.91	7.16E-8	7.14	2.95E-5
Betaine	-13.26	1.13E-7	6.94	2.95E-5
Betaine	-13.07	1.29E-7	6.88	2.95E-5
Betaine	-12.74	1.65E-7	6.78	2.95E-5
Glucose	-12.61	1.82E-7	6.73	2.95E-5
Glucose	-11.80	3.41E-7	6.46	4.60E-5
Glucose	-10.84	7.49E-7	6.12	8.67E-5
Betaine	-10.24	1.27E-6	5.89	1.22E-4
Betaine	-10.16	1.36E-6	5.86	1.22E-4
Isoleucine	-9.72	2.05E-6	5.68	1.50E-4

Isoleucine	-9.67	2.15E-6	5.66	1.50E-4
Glucose	-9.59	2.32E-6	5.63	1.50E-4
Betaine	-9.55	2.40E-6	5.61	1.50E-4
Glucose	-9.38	2.83E-6	5.54	1.64E-4
Betaine	-8.95	4.32E-6	5.36	2.33E-4
Betaine	-8.70	5.60E-6	5.25	2.83E-4
Betaine	-8.52	6.71E-6	5.17	3.20E-4
Glucose	-8.04	1.11E-5	4.95	5.02E-4
Aspartate	7.77	1.50E-5	4.82	6.43E-4
Isoleucine	-7.56	1.91E-5	4.71	7.73E-4
Aspartate	7.47	2.11E-5	4.67	8.14E-4
Glucose	-7.43	2.21E-5	4.65	8.14E-4
Isoleucine	-7.07	3.39E-5	4.46	0.0011
Glucose	-6.52	6.71E-5	4.17	0.0022
Isoleucine	-6.34	8.36E-5	4.07	0.0026

Table S5. Discriminant metabolites from the univariate analysis of CC × CWV analysis (*T-test*, Fisher's LSD post-hoc test, $p < 0.05$).

Metabolite	**t.stat**	**p.value**	**-log10(p)**	**FDR**
Lactate	10.00	1.58E-6	5.79	9.43E-4
Proline	9.45	2.64E-6	5.57	9.43E-4
Proline	9.17	3.48E-6	5.45	9.43E-4
Betaine	8.53	6.66E-6	5.17	0.0010
Proline	8.27	8.73E-6	5.05	0.0010
Proline	8.26	8.86E-6	5.05	0.0010
Proline	8.23	9.16E-6	5.03	0.0010
Proline	7.87	1.35E-5	4.86	0.0010
Betaine	7.87	1.35E-5	4.86	0.0010
Proline	7.86	1.37E-5	4.86	0.0010
Proline	7.85	1.37E-5	4.86	0.0010
Proline	7.68	1.67E-5	4.77	0.0010
Methionine	-7.64	1.75E-5	4.75	0.0010
Proline	7.48	2.10E-5	4.67	0.0012
Proline	7.32	2.52E-5	4.59	0.0012
Methionine	-7.28	2.63E-5	4.57	0.0012
Proline	7.28	2.63E-5	4.57	0.0012
Aspartate	7.17	3.02E-5	4.51	0.0013
Betaine	7.10	3.26E-5	4.48	0.0013
Lactate	6.87	4.31E-5	4.36	0.0017
Betaine	6.73	5.13E-5	4.28	0.0019
Proline	6.67	5.53E-5	4.25	0.0020
Lactate	6.64	5.74E-5	4.24	0.0020
Betaine	6.52	6.65E-5	4.17	0.0022
Proline	6.45	7.34E-5	4.13	0.0023

Table S6. Discriminant metabolites from the univariate analysis of CV × CWV analysis (*T-test*, Fisher's LSD post-hoc test, $p < 0.05$).

Metabolite	t.stat	p.value	-log10(p)	FDR
Methionine	9.57	2.36E-6	5.62	0.001
Methionine	7.95	1.23E-5	4.90	0.004
Methionine	7.44	2.20E-5	4.65	0.005
Glucose	6.79	4.76E-5	4.32	0.008
Aspartate	-6.75	5.01E-5	4.29	0.008
Myo-inositol	-6.40	7.79E-5	4.10	0.010
Aspartate	-5.95	1.40E-4	3.85	0.010
Glucose	5.88	1.54E-4	3.81	0.015
Methionine	5.39	3.01E-4	3.52	0.024
Creatine	-5.38	3.08E-4	3.51	0.024
Aspartate	-5.11	4.53E-4	3.34	0.033
Methionine	4.87	6.43E-4	3.19	0.035
Proline	-4.82	6.93E-4	3.15	0.035
Lactate	4.80	7.22E-4	3.14	0.035
Lactate	4.77	7.56E-4	3.12	0.035
Glucose	4.76	7.65E-4	3.11	0.035
Glucose	4.74	7.85E-4	3.10	0.035
Aspartate	-4.74	7.89E-4	3.10	0.035
Creatine	-4.53	0.001	2.96	0.045
Methionine	4.49	0.001	2.94	0.046
Methionine	4.46	0.001	2.91	0.046
Proline	4.39	0.001	2.87	0.049