Monitoring the response of the human urinary metabolome to brief maximal exercise by a combination of RP-UPLC-MS and ^{1}H NMR spectroscopy

Alexandros Pechlivanis[§,†,⊥] *Konstantinos G. Papaioannou,* [‡] *George Tsalis,* [‡] *Ploutarchos Saraslanidis,* [‡] *Vassilis Mougios*[‡,¶] *and Georgios A. Theodoridis*[†,*]

§ Biomolecular Medicine, Division of Computational and Systems Medicine, Department of Surgery and Cancer, Faculty of Medicine, Imperial College London, SW7 2AZ, London, U. K.

† School of Chemistry, Aristotle University of Thessaloniki, 54124 Greece

‡ School of Physical Education and Sport Science at Thessaloniki, Aristotle University of Thessaloniki, 54124 Greece

Table of Contents

Table S1. Changes in human urine metabolite levels, identified by 1H NMR spectroscopy, after maximal intermittent exercise. Data are mean ± sd, relative to the mean of pre-exercise.

Table S2. Summary of model characteristics from OPLS-DA multivariate statistical analyses of data obtained from all RP-UPLC-MS analyses and the 1H NMR analysis.

Figure S1. Scores plots after PCA of the data obtained by RP-UPLC-MS analysis, at ESI+ mode and ESI- mode

Figure S2. S-plot from the OPLS-DA model on the basis of RP-UPLC-MS ESI+ data, comparing the baseline samples with the samples obtained 1 h after exercise

Figure S3. Mass spectra of hypoxanthine

Figure S4. Scores plot from the OPLS-DA model on the basis of RP-UPLC-MS ESI- data, comparing, in pairwise fashion, the baseline samples

Figure S5. Median 1H NMR spectra of urine samples

Figure S6. Characteristic 1H NMR spectral regions from the urine samples of one individual, obtained at baseline, and 1, 1.5, as well as 2 h post-exercise.

Figure S7. Scores plot for the OPLS-DA model on the basis of 1H NMR data, comparing baseline samples with those at 1.5 h after exercise

Figure S8. Top panel: scores plot for the OPLS-DA model on the basis of 1H NMR data, comparing baseline samples with those at 2 h after exercise

Figure S9. Top panel: scores plot for the OPLS-DA model on the basis of 1H NMR data, comparing samples at 1 h with those at 2 h after exercise

Table S1. Changes in human urine metabolite levels, identified by 1H NMR spectroscopy, after maximal intermittent exercise. Data are mean ± sd, relative to the mean of pre-exercise.

Metabolite key	Metabolite	Pre-exercise	1 h post-exercise	1.5 h post-exercise	2 h post-exercise
M1	2-Hydroxyisovalerate	1.00 ± 0.24	1.48 ± 0.43	0.97 ± 0.22	1.00 ± 0.48
M2	2-Hydroxybutyrate	1.00 ± 0.24	2.29 ± 0.79	1.15 ± 0.34	1.00 ± 0.31
M3	2-Oxoisocaproate	1.00 ± 0.10	1.90 ± 0.49	1.16 ± 0.28	0.99 ± 0.39
M4	3-Methyl-2-oxovalerate	1.00 ± 0.21	2.23 ± 0.48	1.27 ± 0.39	0.99 ± 0.53
M5	Valine	1.00 ± 0.14	0.89 ± 0.09	0.91 ± 0.12	1.01 ± 0.28
M6	Isoleucine	1.00 ± 0.13	0.99 ± 0.09	0.88 ± 0.11	0.99 ± 0.31
M7	3-Hydroxyisobutyrate	1.00 ± 0.17	1.97 ± 0.57	1.21 ± 0.37	0.99 ± 0.37
M8	2-Oxoisovalerate	1.00 ± 0.11	1.11 ± 0.09	0.98 ± 0.14	0.93 ± 0.30
M9	3-Hydroxybutyrate	1.00 ± 0.21	1.43 ± 0.36	1.00 ± 0.13	0.98 ± 0.18
M10	Lactate	1.00 ± 0.16	191.32 ± 61.04	18.15 ± 25.17	3.15 ± 5.33
M11	*2*-Hydroxyisobutyrate	1.00 ± 0.34	2.61 ± 0.88	1.30 ± 0.40	1.04 ± 0.33
M12	Alanine	1.00 ± 0.16	1.46 ± 0.31	0.97 ± 0.19	0.83 ± 0.13
M13	Acetate	1.00 ± 0.20	1.81 ± 0.53	1.27 ± 0.30	1.04 ± 0.18
M14	Acetoacetate	1.00 ± 0.08	1.08 ± 0.12	1.02 ± 0.08	1.10 ± 0.17
M15	Oxaloacetate	1.00 ± 0.35	0.93 ± 0.20	0.94 ± 0.34	0.96 ± 0.36
M16	Pyruvate	1.00 ± 0.10	9.87 ± 3.90	1.52 ± 0.99	1.08 ± 0.27
M17	Succinate	1.00 ± 0.28	0.69 ± 0.15	0.92 ± 0.33	0.83 ± 0.18
M18	Citrate	1.00 ± 0.36	0.65 ± 0.20	1.10 ± 0.33	0.96 ± 0.40
M19	Dimethylamine	1.00 ± 0.25	0.89 ± 0.10	1.00 ± 0.18	0.85 ± 0.17
M20	Trimethylamine	1.00 ± 0.42	0.81 ± 0.27	0.94 ± 0.33	0.83 ± 0.28
M21	2-Oxoglutarate	1.00 ± 0.26	0.85 ± 0.14	0.89 ± 0.20	0.83 ± 0.17
M22	Creatinine	1.00 ± 0.22	0.89 ± 0.11	0.96 ± 0.17	0.80 ± 0.16
M23	Malonate	1.00 ± 0.62	1.04 ± 0.45	1.01 ± 0.47	1.22 ± 1.15
M24	Carnitine	1.00 ± 0.56	0.88 ± 0.42	1.09 ± 0.57	0.93 ± 0.50
M25	Trimethylamine *N*-oxide	1.00 ± 0.29	0.81 ± 0.37	0.90 ± 0.21	0.79 ± 0.24
M26	Taurine	1.00 ± 0.38	0.79 ± 0.28	1.01 ± 0.43	0.90 ± 0.42
M27	Glycine	1.00 ± 0.24	0.60 ± 0.11	0.78 ± 0.17	0.70 ± 0.20
M28	Allantoin	1.00 ± 0.33	0.88 ± 0.34	1.01 ± 0.35	1.02 ± 0.61
M31	Fumarate	1.00 ± 0.23	1.80 ± 0.45	1.18 ± 0.66	2.00 ± 1.50
M32	*trans*-Aconitate	1.00 ± 0.19	1.08 ± 0.30	1.17 ± 0.39	1.39 ± 0.66
M33	4-Aminohippurate	1.00 ± 0.41	0.65 ± 0.25	0.83 ± 0.34	0.71 ± 0.53
M34	Tyrosine	1.00 ± 0.22	0.78 ± 0.16	1.00 ± 0.43	0.92 ± 0.37
M35	Hippurate	1.00 ± 0.61	0.69 ± 0.33	0.65 ± 0.29	0.58 ± 0.24
M37	Hypoxanthine	1.00 ± 0.77	15.37 ± 4.85	17.39 ± 7.74	9.62 ± 5.01
M38	Inosine	1.00 ± 0.38	10.93 ± 10.30	5.91 ± 7.58	3.94 ± 4.15
M39	Formate	1.00 ± 0.33	0.50 ± 0.19	0.86 ± 0.38	1.22 ± 0.41

Table S2. Summary of model characteristics from OPLS-DA multivariate statistical analyses of data obtained from all RP-UPLC-MS analyses and the 1H NMR analysis.

Comparisons	Analysis	Components (predictive + orthogonal)	R^2X	R^2Y	Q^2Y	CV-ANOVA, *P* value
Pre vs 1 h	LC +	1 + 2	0.307	0.946	0.869	9.35×10^{-024}
Pre vs 1 h	LC -	1 + 3	0.339	0.951	0.861	7.50×10^{-021}
Pre vs 1.5 h	LC +	1 + 3	0.39	0.923	0.746	7.92×10^{-014}
Pre vs 1.5 h	LC -	1 + 3	0.333	0.911	0.622	4.11×10^{-009}
Pre vs 2 h	LC +	1 + 3	0.557	0.920	0.715	1.69×10^{-012}
Pre vs 2 h	LC -	1 + 3	0.431	0.897	0.658	3.22×10^{-010}
1 h vs 1.5 h	LC +	1 + 4	0.441	0.944	0.733	3.25×10^{-012}
1 h vs 1.5 h	LC -	1 + 3	0.268	0.904	0.680	2.09×10^{-011}
1 h vs 2 h	LC +	1 + 4	0.594	0.977	0.880	$2.47 \times 10e^{-021}$
1 h vs 2 h	LC -	1 + 3	0.405	0.938	0.793	1.64×10^{-016}
Pre vs 1 h	^{1}H NMR	1 + 1	0.373	0.764	0.595	3.23×10^{-011}
Pre vs 1.5 h	^{1}H NMR	1 + 3	0.414	0.949	0.520	1.11×10^{-006}
Pre vs 2 h	^{1}H NMR	1 + 4	0.506	0.979	0.523	7.58×10^{-006}
1 h vs 1.5 h	^{1}H NMR	1 + 1	0.36	0.643	0.422	9.67×10^{-007}
1 h vs 2 h	^{1}H NMR	1 + 1	0.455	0.727	0.624	3.54×10^{-012}

Pre: pre-exercise. 1 h, 1.5 h, 2 h: 1, 1.5, 2 h post-exercise. LC +, LC - : RP-UPLC-MS in ESI+ and ESI- modes.

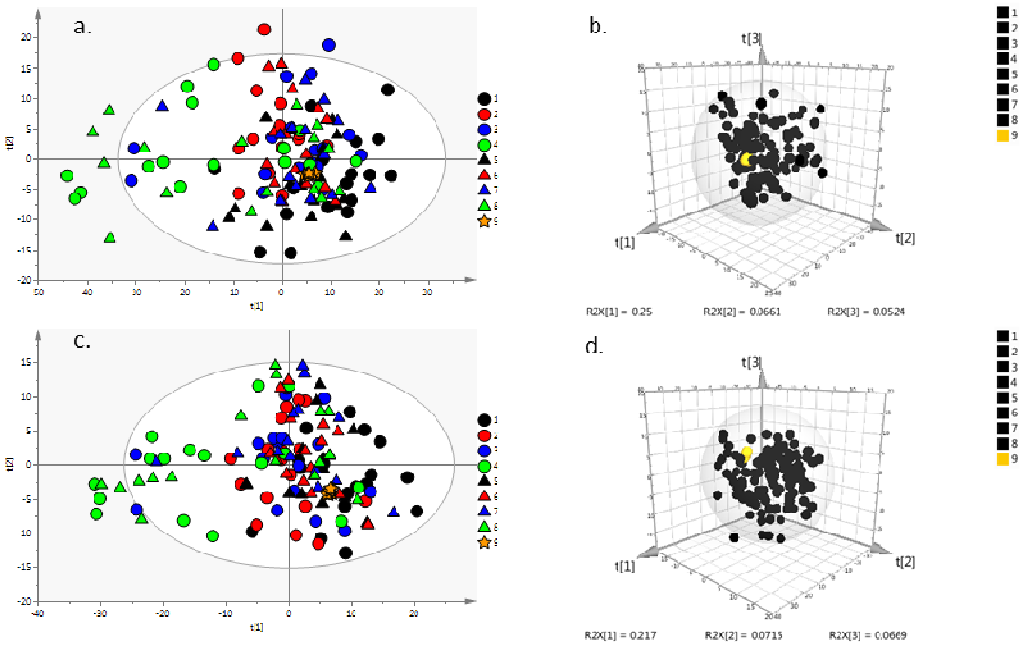

Figure S1. Scores plots after PCA of the data obtained by RP-UPLC-MS analysis, at ESI+ mode (a and b) and ESI- mode (c and d), of urine samples from 17 men, taken at baseline and up to 2 h after three 80-m sprint runs, performed twice on separate days (test-retest). a, c, two-dimensional scores plots (PC1 vs PC2): ● test, baseline; ● test, 1 h post-exercise; ● test, 1.5 h post-exercise; ● test, 2 h post-exercise; ▲ retest, baseline; ▲ retest, 1 h post-exercise; ▲ retest, 1.5 h post-exercise; ▲ retest, 2 h post-exercise; ★ QC samples. b, d, three-dimensional scores plots (PC1 vs PC2 vs PC3): ● all experimental samples; ■ QC samples.

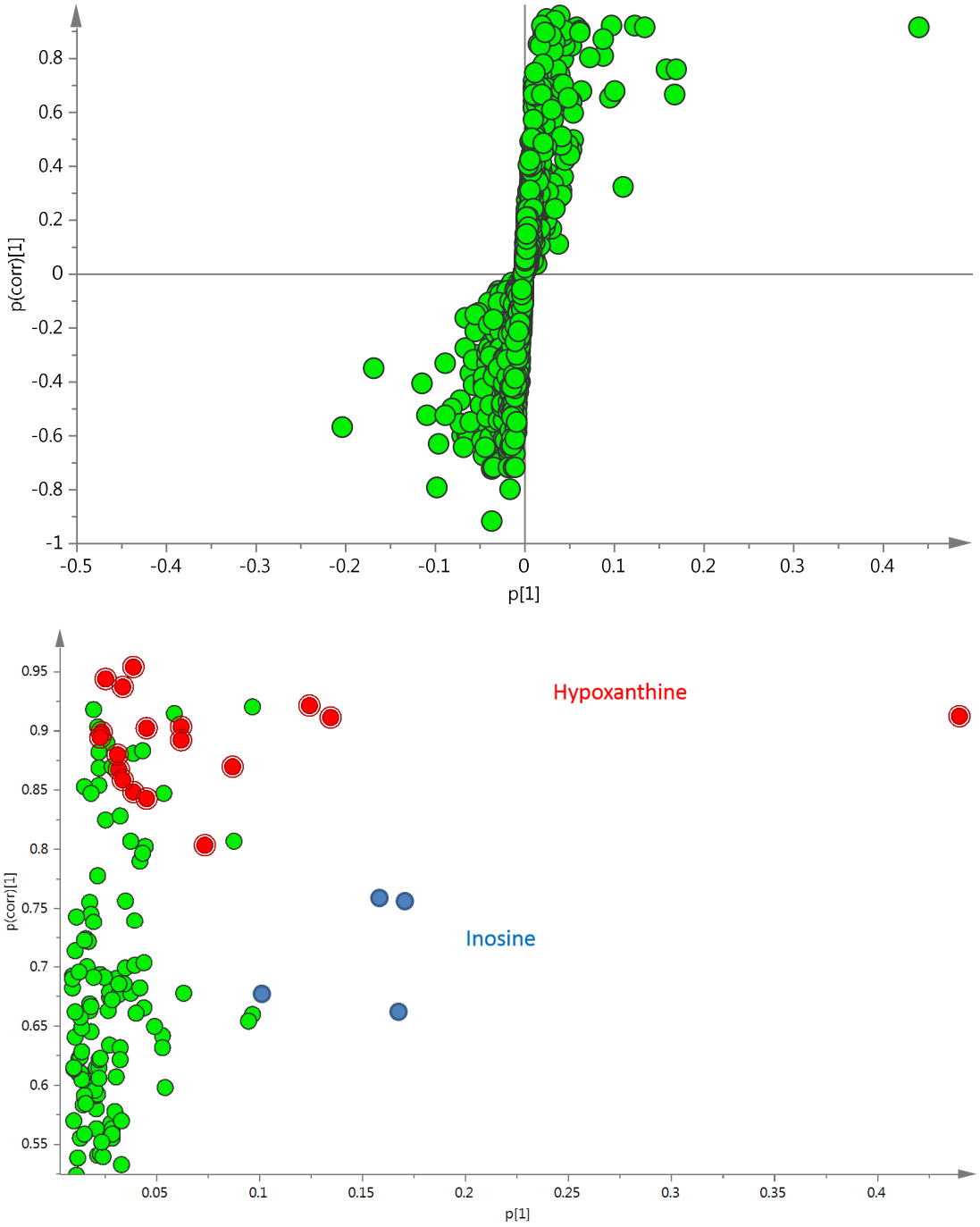

Figure S2. S-plot from the OPLS-DA model on the basis of RP-UPLC-MS ESI+ data, comparing the baseline samples with the samples obtained 1 h after exercise. In the expanded view (lower panel), we highlight the features that are assigned to hypoxanthine and inosine and that are increased post-exercise.

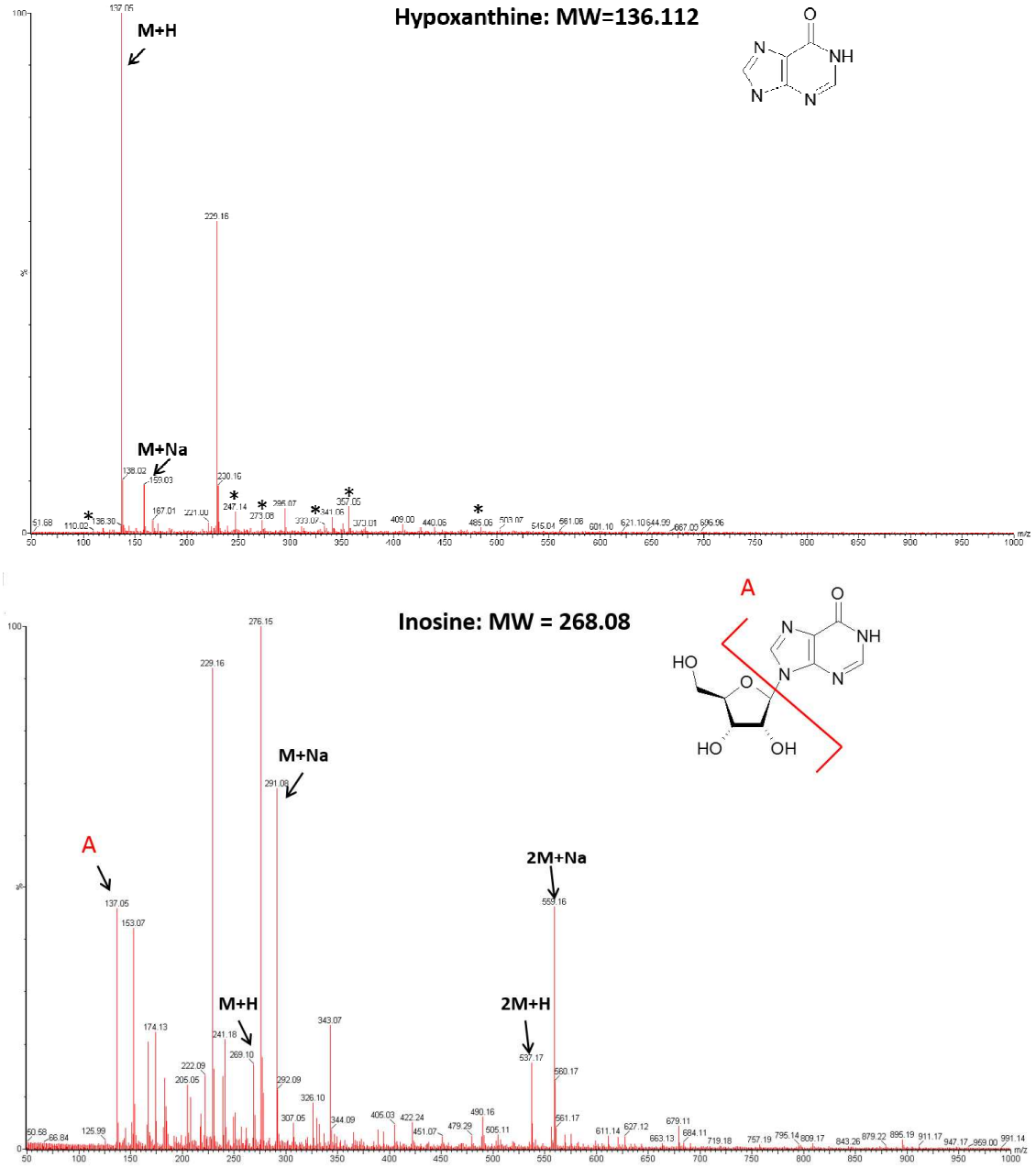

Figure S3. Mass spectra of hypoxanthine and inosine with the identified fragments and adducts that were found significant in the OPLS-DA models. Asterisks denote unidentified features.

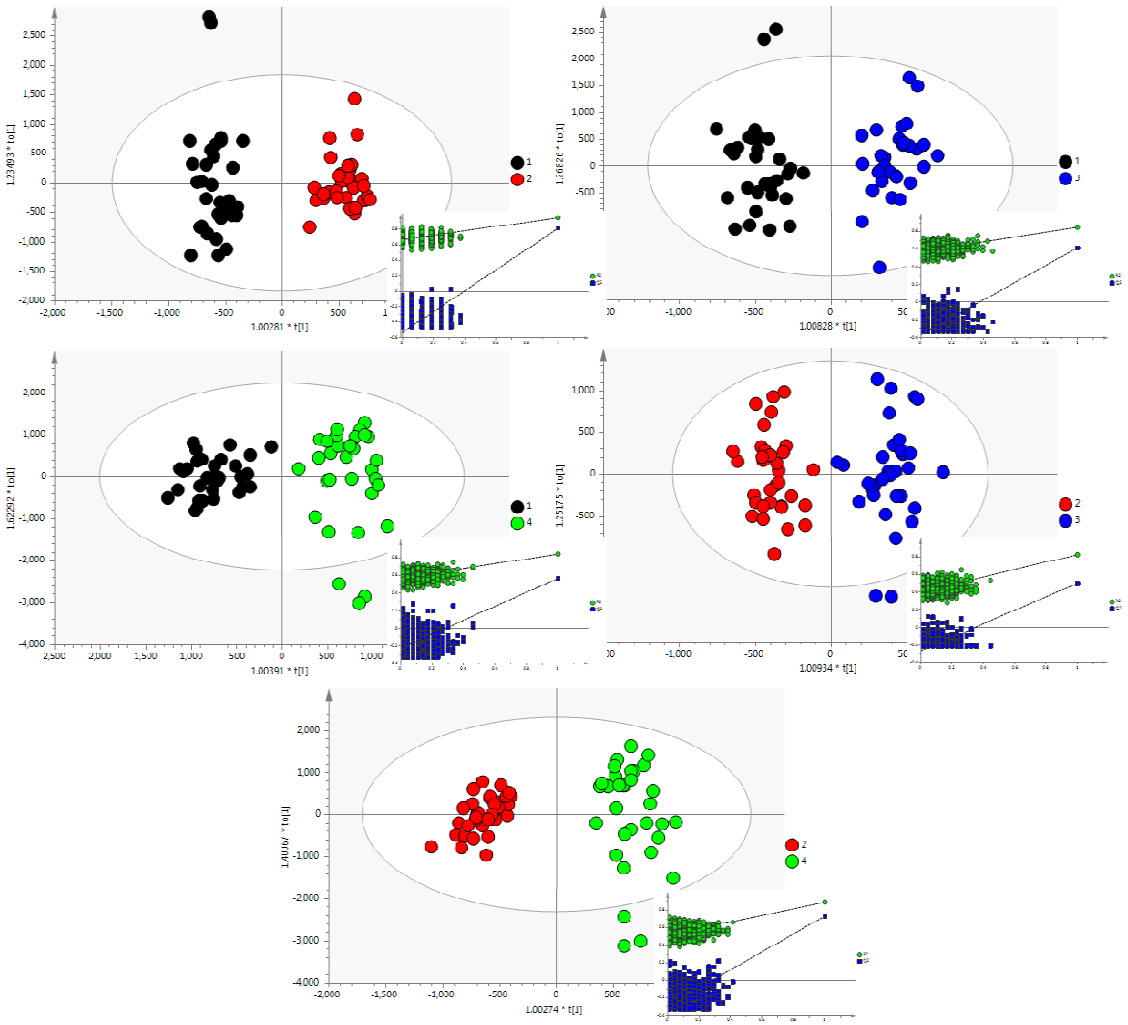

Figure S4. Scores plot from the OPLS-DA model on the basis of RP-UPLC-MS ESI- data, comparing, in pairwise fashion, the baseline samples (●), the samples obtained 1 h after exercise (●), the samples obtained 1.5 h after exercise(●), and the samples obtained 2 h after exercise (●). Inserts depict the permutation plots for each OPLS-DA model. No valid model could be constructed for the comparison of 1.5 and 2 h.

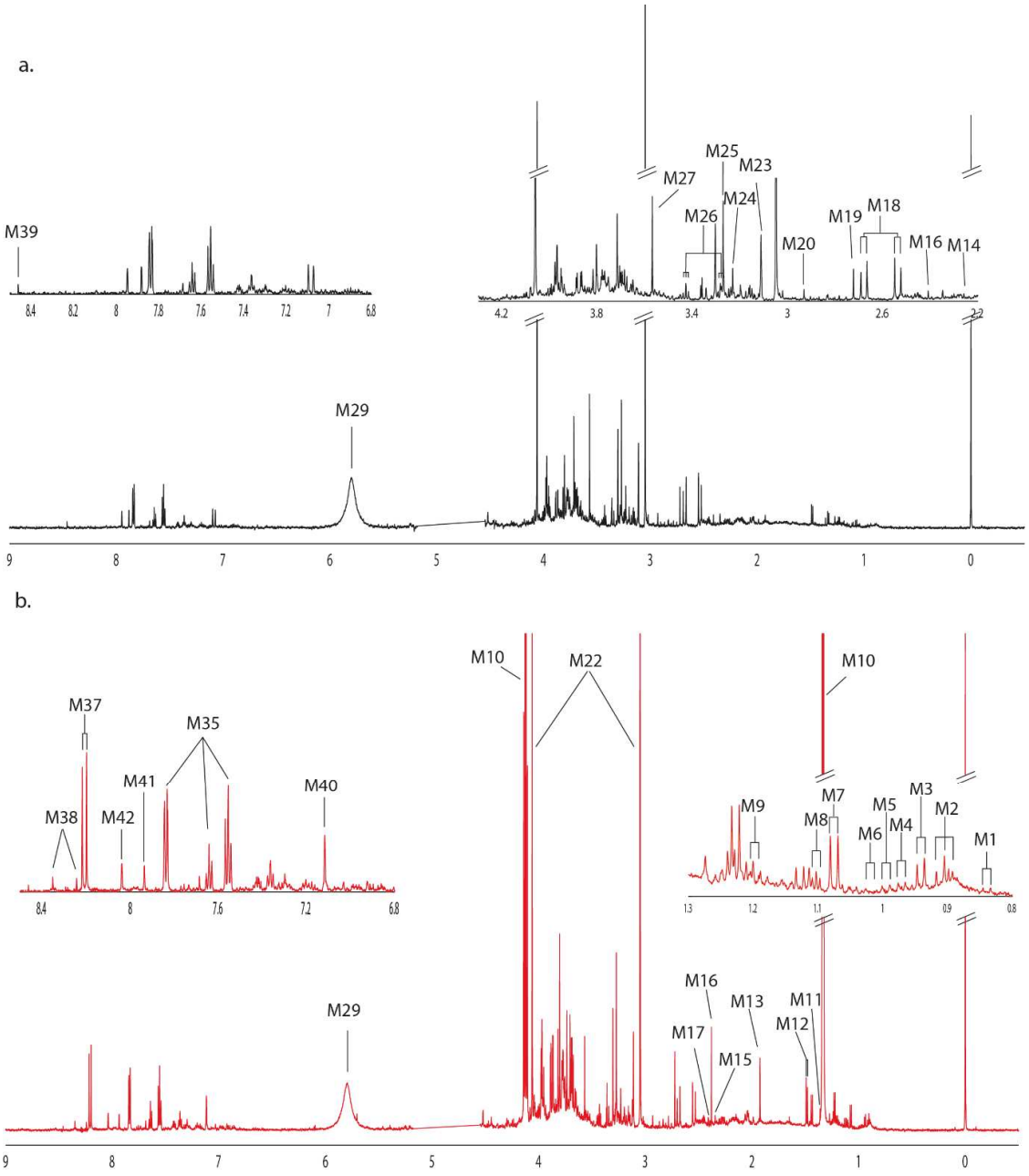

Figure S5. Median 1H NMR spectra of urine samples before (a, black), and one hour after exercise (b, red). For metabolite notation, see Table 1.

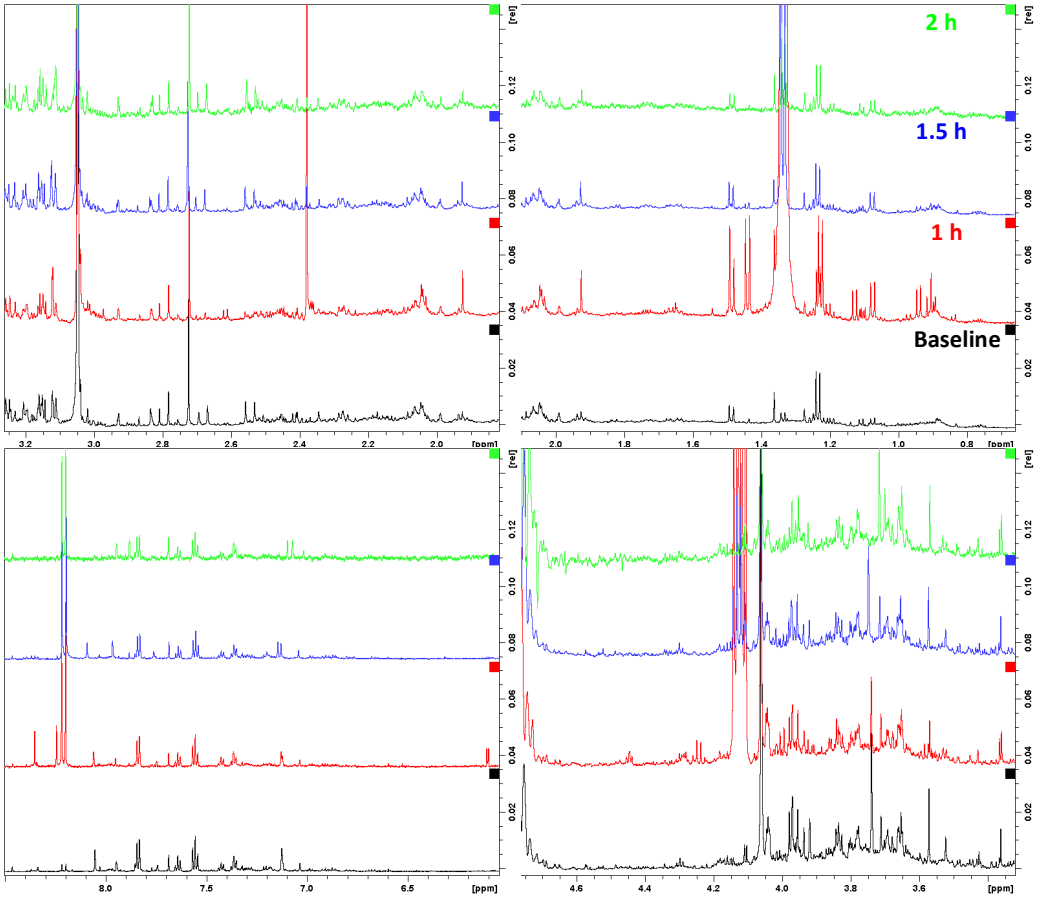

Figure S6. Characteristic 1H NMR spectral regions from the urine samples of one individual, obtained at baseline (black) and 1 (red), 1.5 (blue), as well as 2 h post-exercise (green).

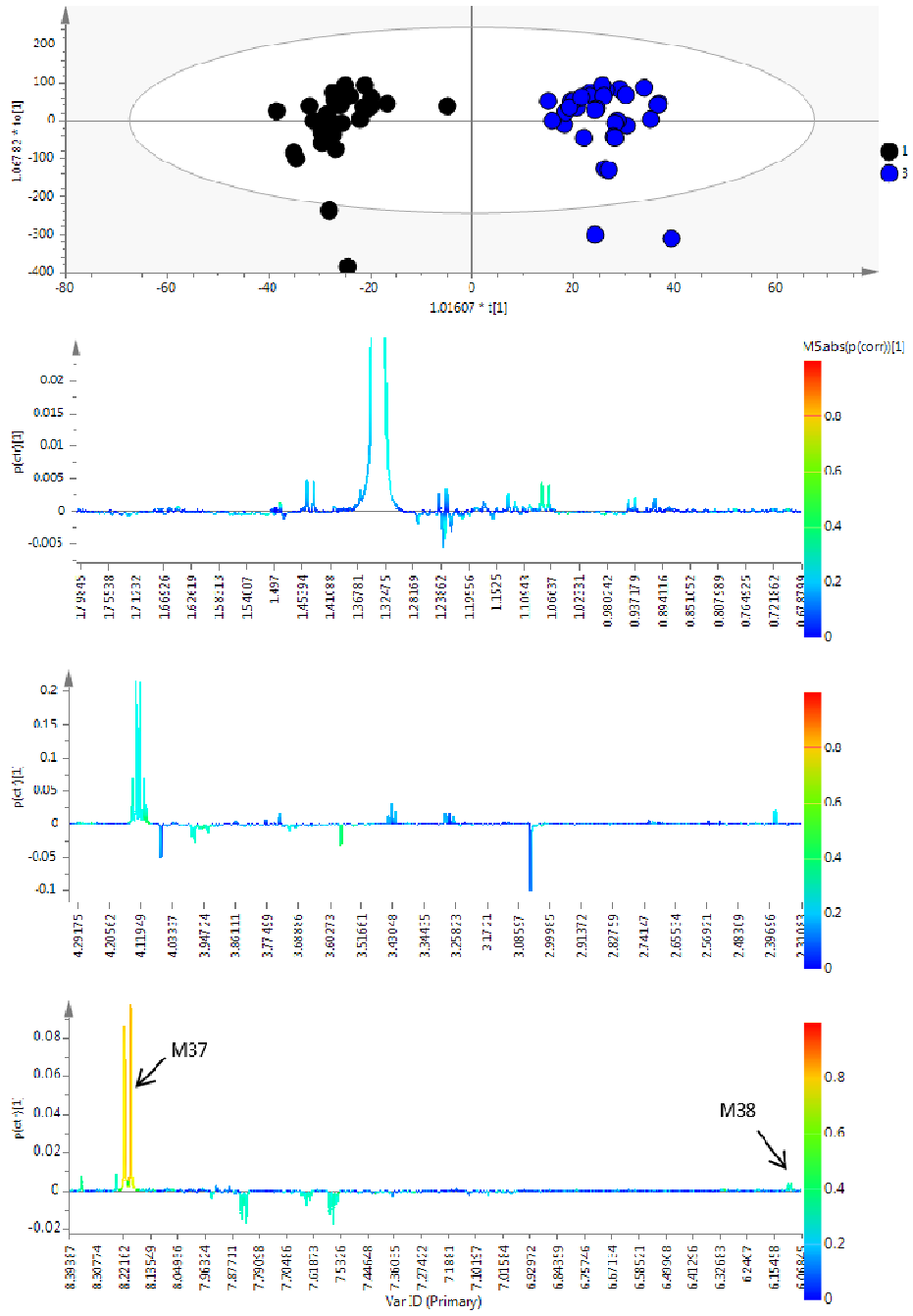

Figure S7. Top panel: scores plot for the OPLS-DA model on the basis of ^{1}H NMR data, comparing baseline samples (●) with those at 1.5 h after exercise (●). Lower panels: loadings plot. Numbering corresponds to metabolites in Table 1.

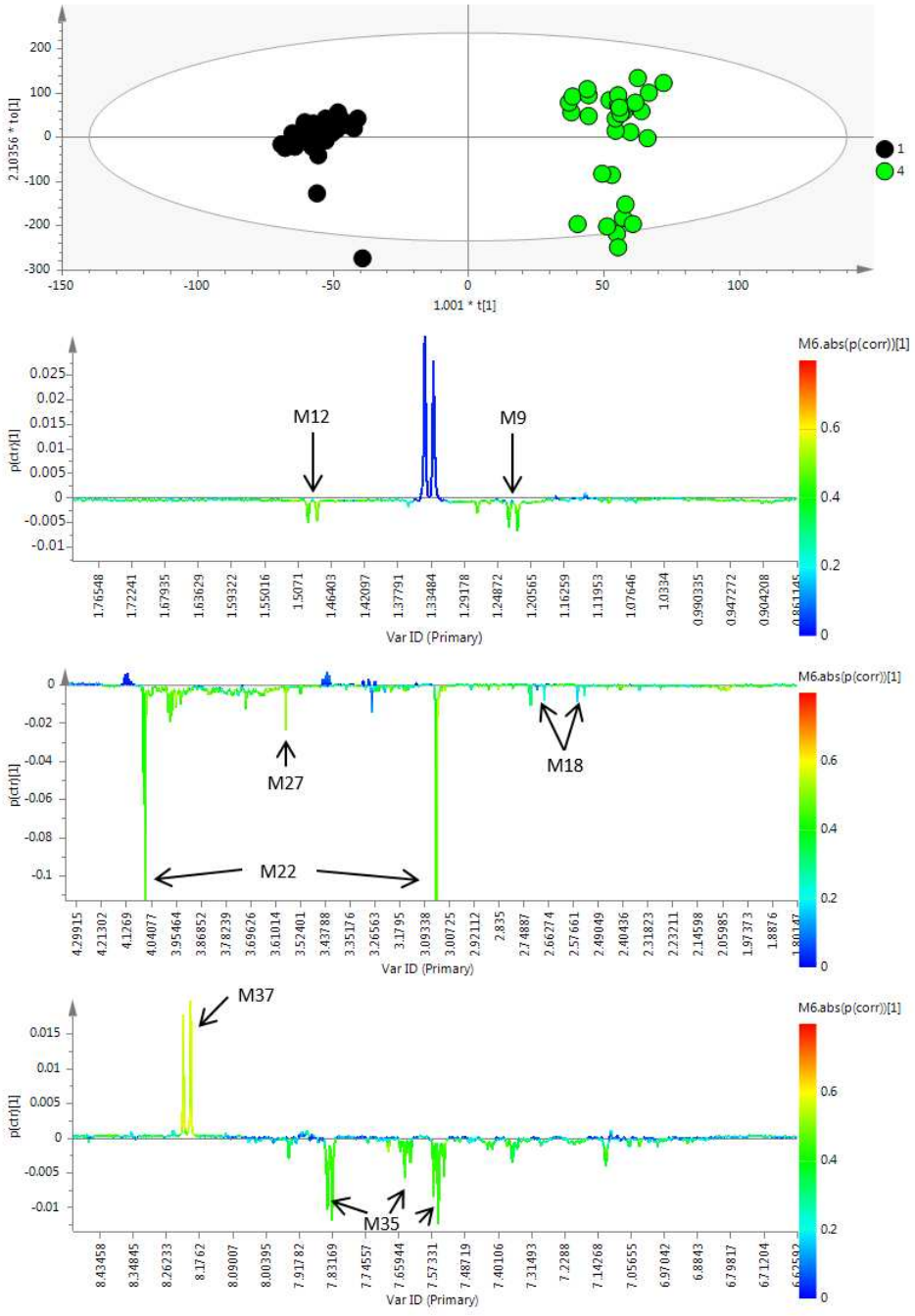

Figure S8. Top panel: scores plot for the OPLS-DA model on the basis of 1H NMR data, comparing baseline samples (●) with those at 2 h after exercise (●). Lower panels: loadings plot. Numbering corresponds to metabolites in Table 1.

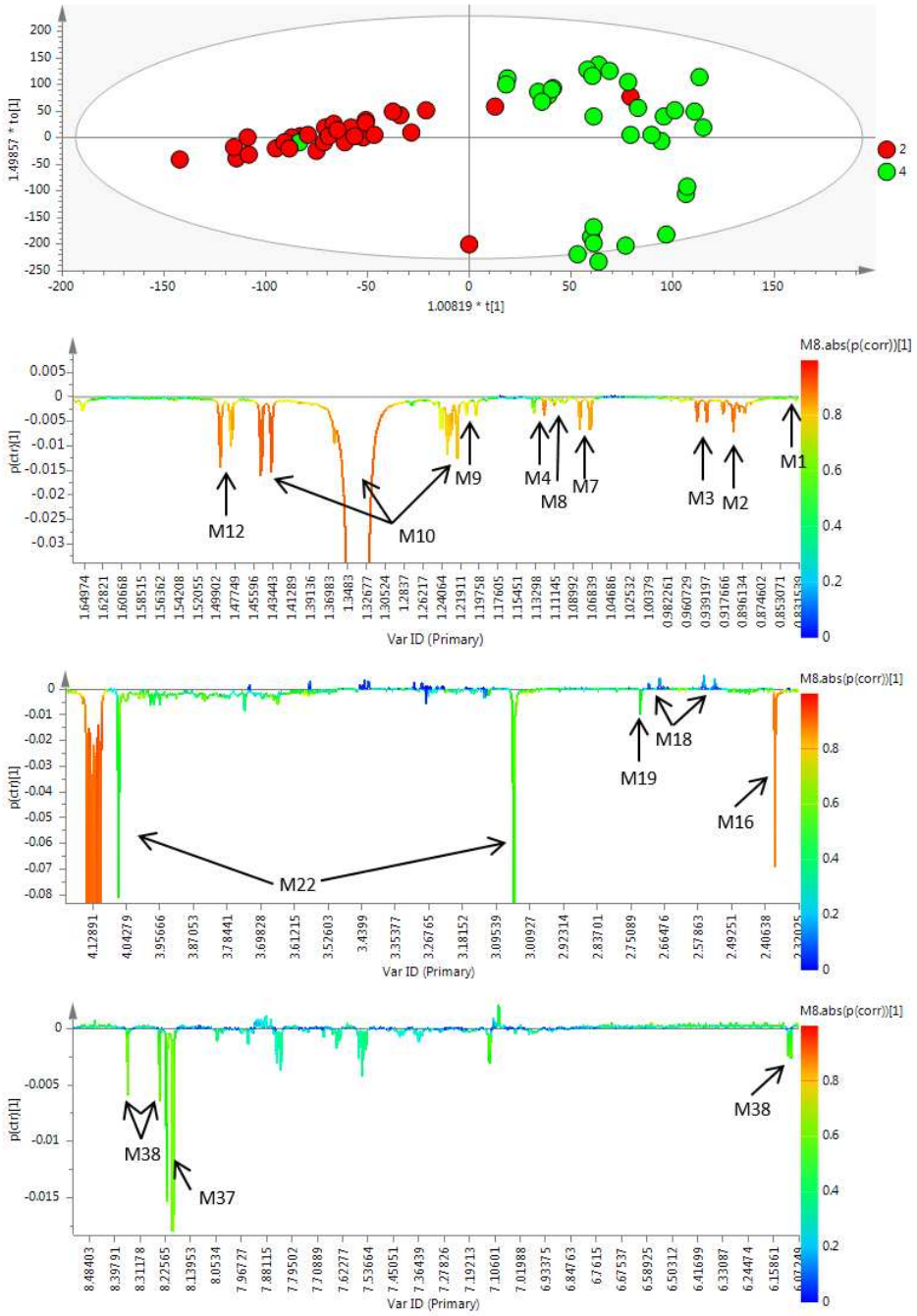

Figure S9. Top panel: scores plot for the OPLS-DA model on the basis of 1H NMR data, comparing samples at 1 h (●) with those at 2 h after exercise (●). Lower panels: loadings plot. Numbering corresponds to metabolites in Table 1.