Supplementary Data

Known Metabolite	Derivative	HMDB ID	Identification Ion	RT (min)	MSL (level)
4-Hydroxyproline	3TMS	HMDB00725	230	12.540	1
Alanine	2TMS	HMDB00161	190	8.777	1
Arginine	3TMS	HMDB00517	256	14.810	1
Asparagine	3TMS	HMDB00168	231	13.684	1
Aspartate	2TMS	HMDB00191	306	12.479	1
Cholesterol	1TMS	HMDB00067	458	22.263	1
Citrate	2TMS	HMDB00094	465	14.734	1
Dodecanoate	1TMS	HMDB00638	257	13.584	1
Erythronate	2TMS	HMDB00613	292	12.627	1
Fructose	1MEOX/5TMS	HMDB00660	319	15.030	1
Fumarate	2TMS	HMDB00134	245	11.051	1
Glutamate	3TMS	HMDB03339	246	13.279	1
Gluconic Acid	6TMS	HMDB00625	333	15.948	1
Glucose	1MEOX/5TMS	HMDB00122	319	15.168	1
Glycine	2TMS	HMDB00123	204	8.953	1
Glycerol	3TMS	HMDB00131	205	10.301	1
Glycerol-3-P	4TMS	HMDB00126	299	14.317	1
Hexadecanoic Acid Methyl Ester	1TMS	HMDB61859	270	15.600	1
Hexadecanoate	1TMS	HMDB00220	313	16.338	1
Isoleucine	1TMS	HMDB00172	188	9.530	1
Inositol	6TMS	HMDB02985	432	16.547	1
Lactate	2TMS	HMDB00190	191	8.101	1
Leucine	2TMS	HMDB00687	158	10.325	1
Lysine	4TMS	HMDB00182	230	15.483	1
Malate	3TMS	HMDB00744	335	12.199	1
Mannose	1MEOX/5TMS	HMDB00169	319	15.395	1
Octadecanoate	1TMS	HMDB00827	356	17.532	1
Phenylalanine	2TMS	HMDB00159	192	13.411	1
Pipecolate	2TMS	HMDB00070	156	10.904	1
Proline	2TMS	HMDB00162	186	13.056	1
Rhamnose	4TMS	HMDB00849	277	14.052	1
Serine	3TMS	HMDB00187	204	11.098	1
Sorbose	1MEOX/5TMS	HMDB01266	307	15.130	1
Succinate	2TMS	HMDB00254	247	10.731	1
Sucrose	8TMS	HMDB00258	451	19.512	1
Tartaric Acid	4TMS	HMDB00956	219	12.952	1
Threonine	3TMS	HMDB00167	218	11.323	1
Tyrosine	3TMS	HMDB00158	308	15.420	1
Urea	2TMS	HMDB00294	189	10.450	1
Uric Acid	4TMS	HMDB00289	456	16.621	1
Valine	2TMS	HMDB00883	144	9.822	1
Xylitol	5TMS	HMDB02917	307	13.979	1
Xylose	4TMS	HMDB00098	217	13.547	1

Table S1. Parameters of the 43 known metabolites within the initially investigated PBQC (verified within all samples).

Unknown Metabolite	Identification Ion	RT (min)	REST x-FC	*p*	EX10 x-FC	*p*	EX30 x-FC	*p*	RC60 x-FC	*p*
1	258	8.91	-1.21 ± 0.07		-1.07 ± 0.04		1.11 ± 0.07		-1.413 ± 0.07	*0.000*
2	233	9.98	1.16 ± 0.18		-1.29 ± 0.10		-1.40 ± 0.08	*0.020*	-1.426 ± 0.09	
3	246	12.15	1.41 ± 0.48		-1.19 ± 0.31		-1.72 ± 0.24		-2.293 ± 0.26	*0.023*
4	292	12.75	1.49 ± 0.44		1.04 ± 0.49		-1.59 ± 0.10	*0.017*	-2.077 ± 0.05	*0.014*
5	329	12.85	-1.21 ± 0.18		-1.85 ± 0.15	*0.001*	-3.03 ± 0.17	*0.001*	-4.206 ± 0.17	*0.000*
6	313	12.86	1.36 ± 0.17		-1.17 ± 0.10		-2.10 ± 0.14	*0.033*	-1.958 ± 0.14	*0.001*
7	292	13.08	-1.01 ± 0.19		-1.66 ± 0.24		-2.21 ± 0.22	*0.035*	-3.009 ± 0.25	*0.004*
8	326	13.73	-1.35 ± 0.15		1.58 ± 0.14	*0.009*	1.84 ± 0.07	*0.001*	1.007 ± 0.19	
9	369	14.87	1.36 ± 0.11		-1.58 ± 0.05	*0.000*	-2.72 ± 0.09	*0.001*	-1.524 ± 0.10	*0.004*
10	565	17.84	1.13 ± 0.11		-1.74 ± 0.14	*0.011*	-5.35 ± 0.18		-4.125 ± 0.09	*0.000*
11	329	18.32	1.24 ± 0.32		-2.57 ± 0.39	*0.020*	-2.50 ± 0.26		-9.844 ± 0.37	
12	363	18.38	-1.11 ± 0.37		1.07 ± 0.35		-3.20 ± 0.23	*0.007*	-3.253 ± 0.23	*0.020*

Table S2. Quantification ion, retention time (RT), x-FC (change in $HIE_{300\%}$ compared to the $HIE_{150\%}$ protocol) and p value for metabolites with unknown identities which showed significant differences between trials throughout the timeframe examined.

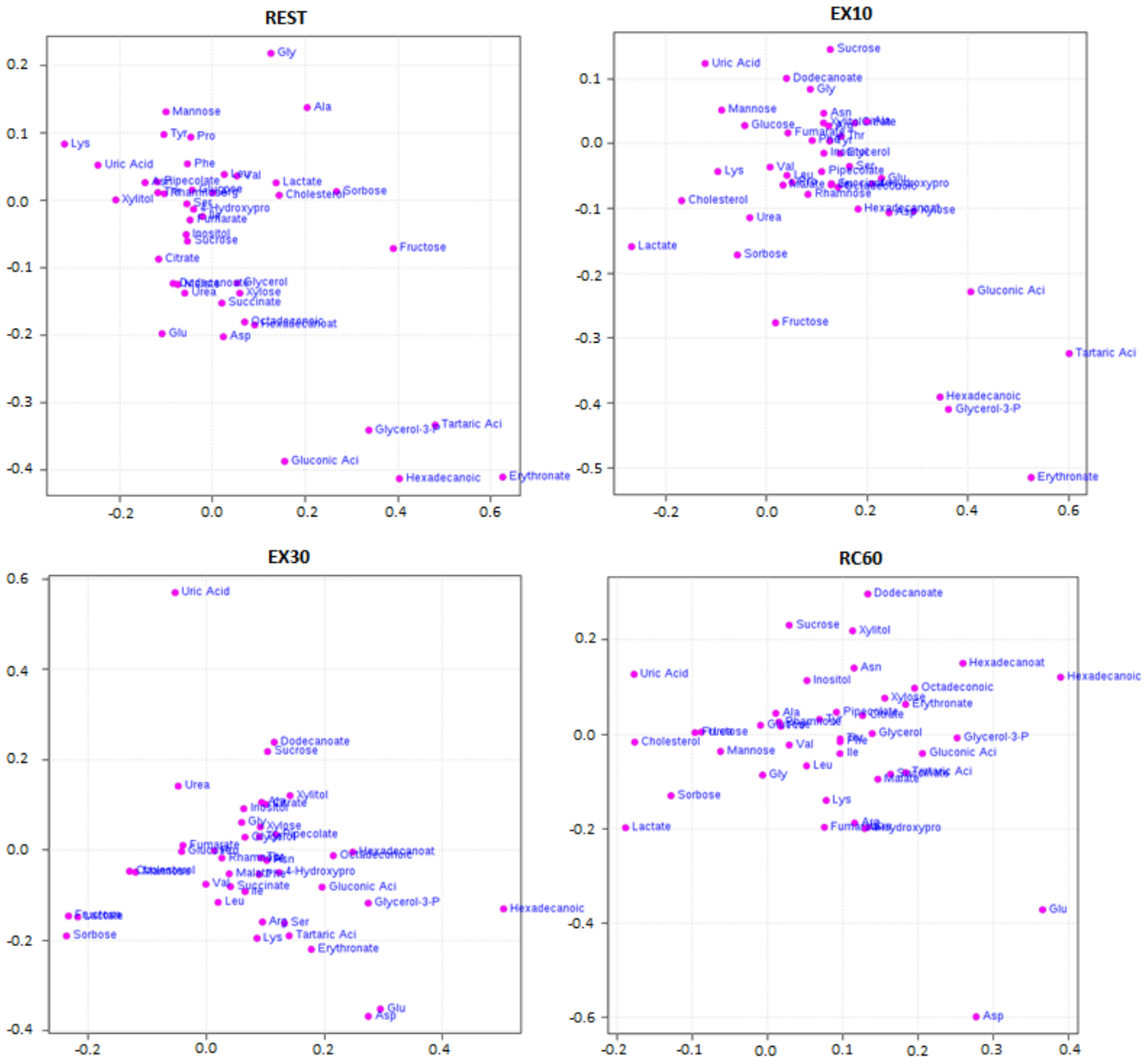

Fig. S1. 2D PLS-DS Loading Plots for PC1/PC2 from each time point of the low volume HIE protocols.

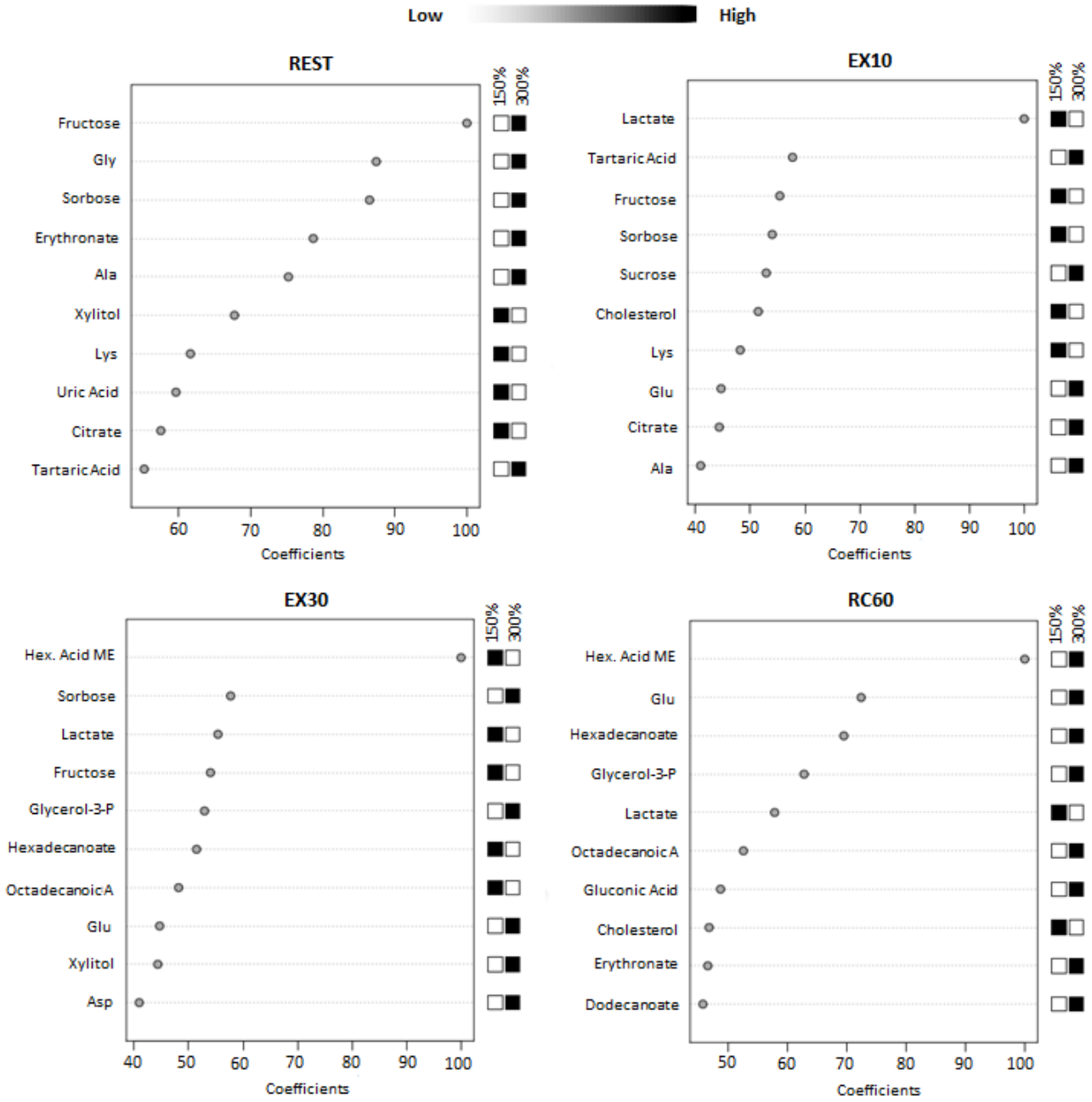

Fig. S2. Important features identified by overall coefficient scores of PLS-DA. Boxes ~~of~~ on the right of each time point~~s~~ coefficient graph indicate the relative concentration of the corresponding metabolite ~~in~~ for each exercise intensity level performed.