

Supplementary Materials

Figure S1.

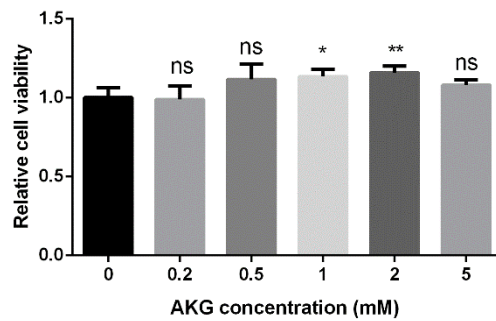


Figure S1. Relative cell viabilities of C2C12 myoblasts cultured in normal growth medium with different concentrations of AKG. $n = 5$ for each group. * $p < 0.05$, ** $p < 0.01$.

Figure S2.

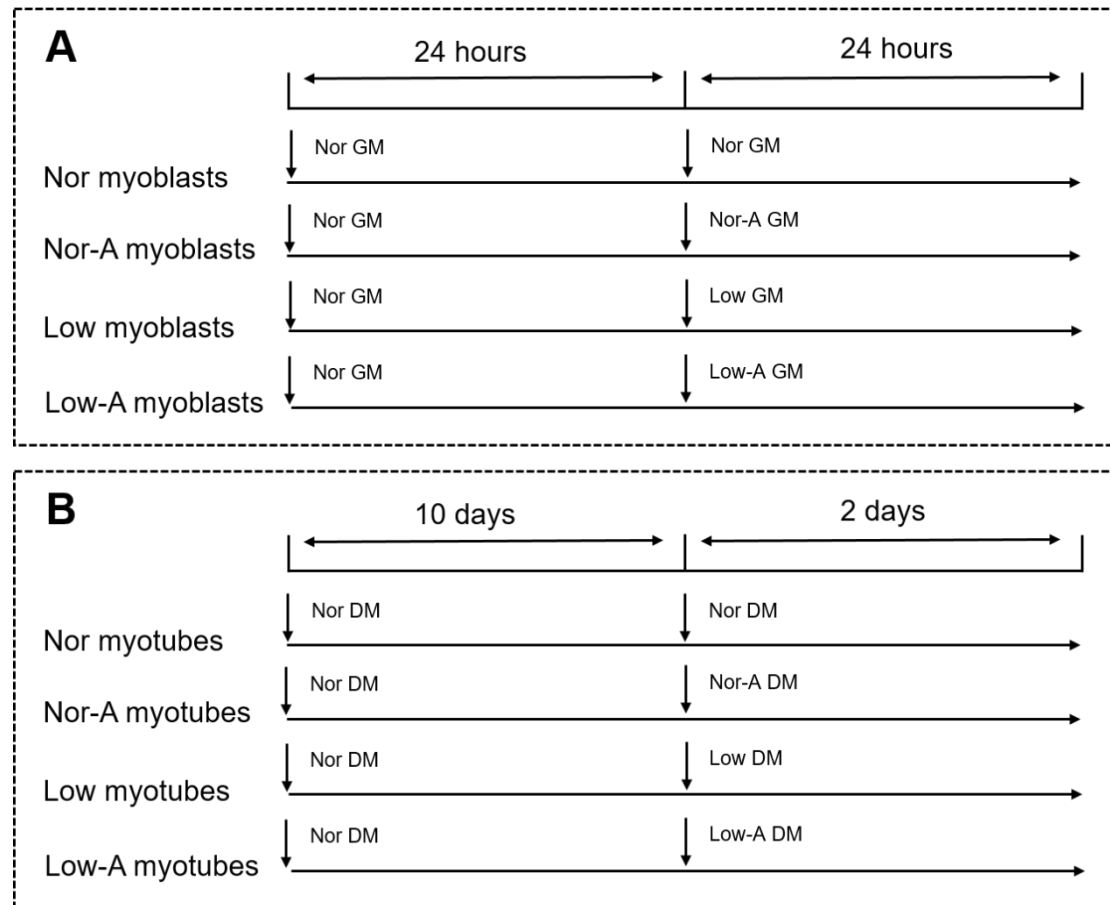


Figure S2. Schematic representation of the experimental design. (A) C2C12 cells were firstly cultured in normal growth medium (Nor GM) to reach 50% confluence for 24 hours and then cultured in four different media for another 24 hours. Nor-A GM: normal growth medium supplemented with AKG; Low GM: low-glucose growth medium; Low GM: low-glucose growth medium supplemented with AKG. **(B)** C2C12 cells were firstly cultured in normal differentiation medium (Nor DM) to form myotubes for 10 days and then cultured in four different media for another 2 days. Nor-A GM: normal growth medium supplemented with AKG; Low GM: low-glucose growth medium; Low GM: low-glucose growth medium supplemented with AKG.

Figure S3.

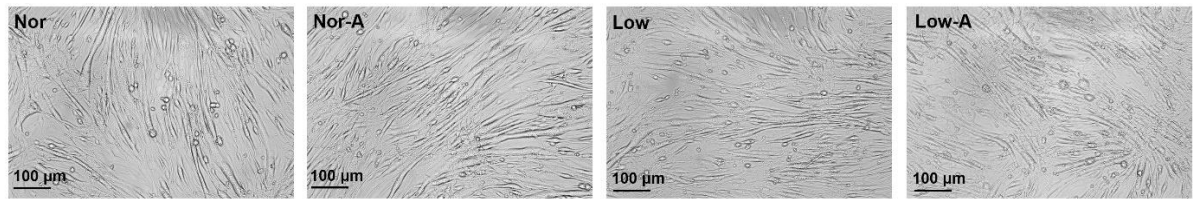


Figure S3. Morphologies of C2C12 myotubes cultured in normal DM and low-glucose DM with or without AKG supplementation.

Figure S4.

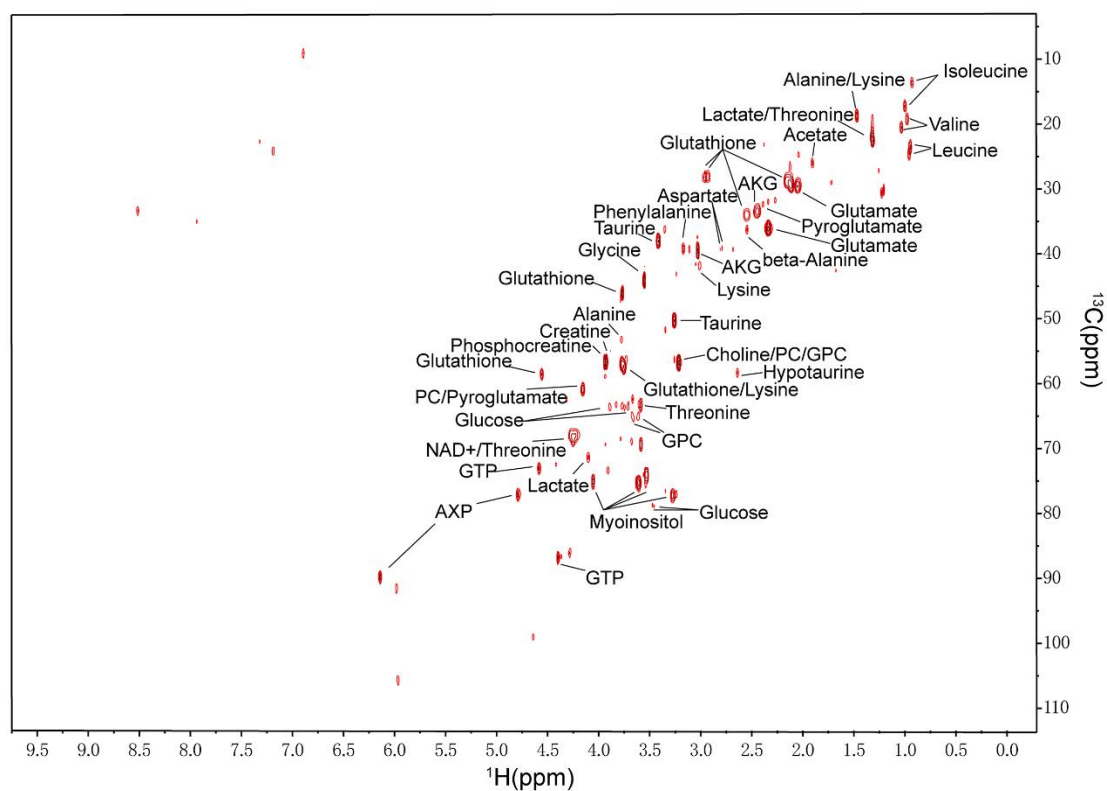


Figure S4. Representative 2D ^1H - ^{13}C HSQC spectrum of aqueous extracts derived from C2C12 myoblasts recorded on 850 MHz NMR spectrometer.

Figure S5

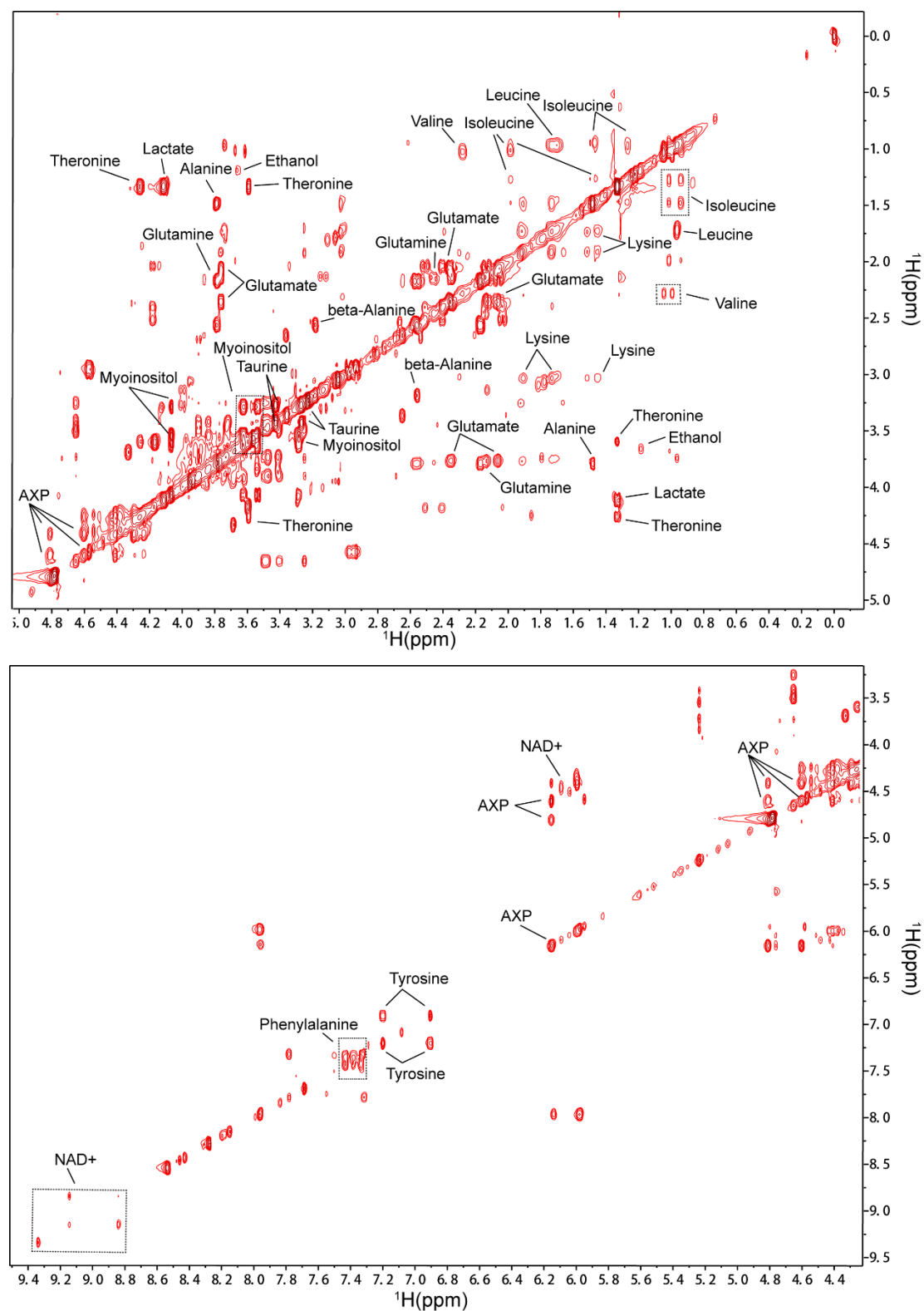


Figure S5. Representative 2D ^1H - ^1H TOCSY spectrum of aqueous extracts derived from C2C12 myoblasts recorded on 850 MHz NMR spectrometer.

Figure S6

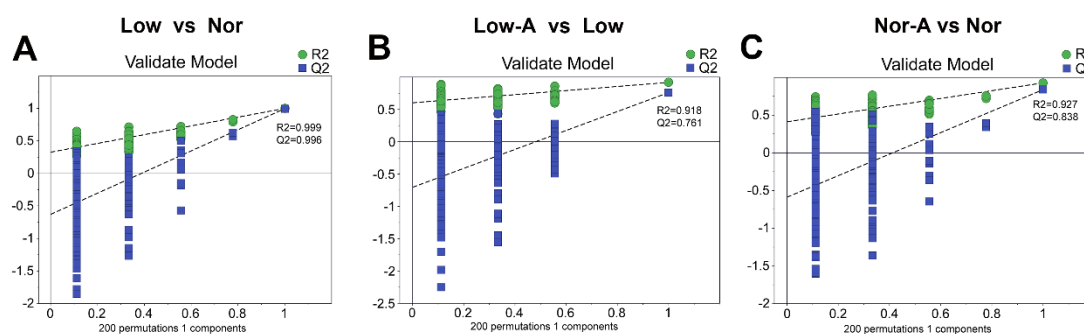


Figure S6. Cross-validation plots of OPLS-DA models of Low vs Nor, Low-A vs Low, Nor-A vs Nor.

Figure S7

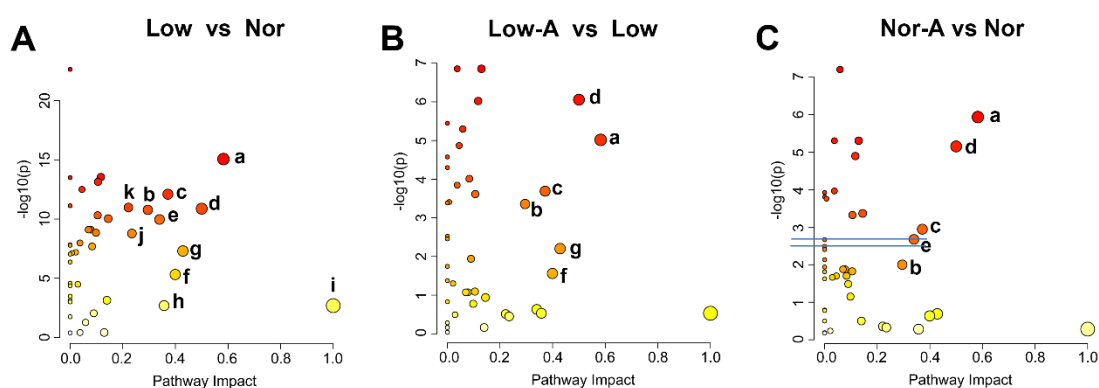


Figure S7. Significantly altered metabolic pathways of Low vs Nor, Low-A vs Low, Nor-A vs Nor. Characters in the panels represent significantly altered metabolic pathways: a. Alanine, aspartate and glutamate metabolism; b. Glycine, serine and threonine metabolism; c. Glutathione metabolism; d. D-Glutamine and D-glutamate metabolism; e. Starch and sucrose metabolism; f. beta-Alanine metabolism; g. Taurine and hypotaurine metabolism; h. Phenylalanine metabolism; i. Phenylalanine, tyrosine and tryptophan biosynthesis; j. Nicotinate and nicotinamide metabolism; k. Histidine metabolism.

Table S1. Resonance assignments of aqueous extracts derived from C2C12 myoblasts.

NO.	Metabolites	δ ^1H (ppm) and multiplicity	Moieties
1	Leucine	0.96 (d), 0.97 (d), 1.69 (m), 1.70 (m), 1.73 (m), 3.73 (m)	α -CH ₃ , α -CH ₃ , γ -CH, β -CH ₂ , α -CH
2	Isoleucine	0.94 (t), 1.01(d), 1.21 (m), 1.42 (m), 2.00 (m), 3.67 (d)	δ -CH ₃ , γ -CH ₃ , half γ -CH ₂ , half γ -C H ₂ , β -CH, α -CH
3	Valine	0.99 (d), 1.05 (d), 2.26 (m), 3.60 (d)	γ -CH ₃ , γ -CH ₃ , β -CH, α -CH
4	Ethanol	1.17 (t), 3.65 (q)	δ -CH ₃ , CH ₂
5	Threonine	1.30 (d), 3.58 (d), 4.24 (m)	γ -CH ₂ , β -CH
6	Lactate	1.33 (d), 4.11 (q)	β -CH ₃ , α -CH
7	Alanine	1.47 (d), 3.78 (q)	β -CH ₃ , α -CH
8	Acetate	1.91 (s)	CH ₃
9	Methionine	1.98 (m), 2.13 (s), 2.17 (m), 2.66 (dd), 3.78 (m)	δ -CH ₃ , γ -CH ₂ , β -CH ₂
10	Pyroglutamate	2.05 (m), 2.39 (d), 2.51 (m), 4.18 (dd)	β -CH, γ -CH ₂ , β -CH, α -CH
11	Glutamate	2.08 (m), 2.12 (m), 2.34 (m), 2.37 (m), 3.75 (m)	Half β -CH ₂ , half β -CH ₂ , half γ -CH ₂ , half γ -CH ₂ , α -CH
12	Lysine	1.43 (m), 1.49 (m), 1.70 (m), 1.91 (m), 3.02 (t), 3.75 (t)	half γ -CH ₂ , half γ -CH ₂ , δ -CH ₂ , β -CH ₂ , ϵ -CH ₂ , α -CH
13	Glutamine	2.13 (m), 2.45 (m), 3.77 (t)	γ -CH ₂ , β -CH ₂ , α -CH
14	Glutathione	2.15 (m), 2.55 (m), 2.96 (m), 3.77 (m), 4.56 (m)	β -CH ₂ , γ -CH ₂ , CH ₂ -SH, α -CH & CH ₂ -NH, CH-NH
15	α -Ketoglutarate (AKG)	2.45 (t), 3.01 (t)	CH ₂ , CH ₂
16	beta-Alanine	2.54 (t), 3.17 (t)	CH ₂ , CH ₂
17	Aspartate	2.68 (dd); 2.81 (dd); 3.90 (dd)	β -CH ₂ ; α -CH
18	Creatine	3.04 (s), 3.93 (s)	N-CH ₃ , CH ₂
19	Phosphocreatine	3.05 (s), 4.05 (s)	N-CH ₃ , CH ₂
20	Tyrosine	3.05 (dd), 3.19 (dd), 6.92 (d), 7.19 (d)	half β -CH ₂ , half β -CH ₂ , β -CH, α -CH
21	Phenylalanine	3.12 (dd), 3.30 (dd), 3.99 (dd), 7.33 (d), 7.37 (t), 7.43 (t)	α -CH, half β -CH ₂ , half β -CH ₂ , α -CH, β -CH, γ -CH
22	Choline	3.21 (s), 3.51 (dd), 4.04 (t)	N-(CH ₃) ₃ , α -CH ₂ , CH ₂ OH
23	Phosphocholine (PC)	3.22 (s), 3.60 (t), 4.18 (m)	N-(CH ₃) ₃ , N-CH ₂ , CH ₂ OH
24	sn-Glycero-3-phosphocholine (GPC)	3.23 (s), 3.60 (dd), 3.68 (dd), 3.87 (m), 3.94 (m), 4.33 (m)	N-(CH ₃) ₃ , half $^1\text{CH}_2$, $^2\text{CH}_2$, half $^2\text{CH}_2$, half $^3\text{CH}_2$, half $^3\text{CH}_2$, $^1\text{CH}_2$
25	Taurine	3.24 (t), 3.41 (t)	$^1\text{CH}_2$, $^2\text{CH}_2$
26	Myo-inositol	3.28 (t), 3.53 (dd), 3.63 (t), 4.07 (t) β ((3.24 (dd), 3.48 (t), 3.90 (dd)),	^2CH , $^4,6\text{CH}$, $^1,3\text{CH}$, ^5CH
27	Glucose	α (3.54 (dd), 3.71 (t), 3.72 (dd), 3.83 (m))	β (H ₂ , H ₃ , H ₅), α (H ₂ , H ₃ , H ₆)

28	Glycine	3.57 (s)	α -CH ₂
29	UDP-glucose	5.62 (dd), 6.0 (m)	CH, 2CH
30	GTP	5.92 (d), 8.1 (s)	CH, CH
31	NAD ⁺	6.03 (d), 6.08 (s), 8.16 (s), 8.20 (m), 8.41 (s), 8.82 (d), 9.13 (d), 9.32 (s)	NH ₂ , NH ₂ (CO), d-CH, β -CH, ² CH, γ -CH, α -CH
32	AXP	6.14 (d), 8.27 (s), 8.58 (s)	NH ₂ , δ -CH, 2CH
33	Histidine	7.06 (s), 7.85 (s)	⁵ CH, ² CH
34	Formate	8.46 (s)	CH

^a Multiplicity: s, singlet; d, double; t, triplet; q, quartet; m, multiple; dd, double of double.

Table S2. Comparisons of metabolite levels between the Nor, Nor-A, Low and Low-A groups of C2C12 myoblasts based on relative NMR integrals with Student's t-test analyse.

Metabolites	Mean $\pm$ Standard error			
	Nor	Nor-A	Low	Low-A
Leucine	1.235 $\pm$ 0.0172	1.218 $\pm$ 0.009	1.391 $\pm$ 0.038 **	1.393 $\pm$ 0.023
Isoleucine	0.495 $\pm$ 0.013	0.492 $\pm$ 0.007	0.591 $\pm$ 0.020 **	0.586 $\pm$ 0.010
Valine	0.562 $\pm$ 0.013	0.560 $\pm$ 0.007	0.653 $\pm$ 0.018 ***	0.648 $\pm$ 0.009
Ethanol	0.092 $\pm$ 0.012	0.077 $\pm$ 0.008	0.107 $\pm$ 0.007	0.145 $\pm$ 0.012 #
Alanine	2.199 $\pm$ 0.090	1.919 $\pm$ 0.038 *	2.280 $\pm$ 0.032	2.257 $\pm$ 0.042
Acetate	0.243 $\pm$ 0.006	0.222 $\pm$ 0.006 *	0.343 $\pm$ 0.011 ****	0.320 $\pm$ 0.020
Glutathione	0.770 $\pm$ 0.013	0.686 $\pm$ 0.018 **	0.722 $\pm$ 0.008 **	0.705 $\pm$ 0.007
Glutamate	2.528 $\pm$ 0.068	2.453 $\pm$ 0.095	6.558 $\pm$ 0.028 ****	6.663 $\pm$ 0.068
AKG	0.151 $\pm$ 0.010	0.363 $\pm$ 0.020 ****	0.123 $\pm$ 0.009	0.242 $\pm$ 0.015 #####
Pyroglutamate	0.629 $\pm$ 0.017	0.789 $\pm$ 0.021 ****	0.312 $\pm$ 0.018 ****	0.303 $\pm$ 0.013
Glutamine	0.121 $\pm$ 0.009	0.176 $\pm$ 0.008 ***	1.653 $\pm$ 0.017 ****	1.528 $\pm$ 0.022 ###
Methionine	0.529 $\pm$ 0.007	0.500 $\pm$ 0.008 *	0.667 $\pm$ 0.005 ****	0.680 $\pm$ 0.005
Aspartate	0.115 $\pm$ 0.006	0.118 $\pm$ 0.005	0.574 $\pm$ 0.006 ****	0.565 $\pm$ 0.014
Lysine	0.238 $\pm$ 0.007	0.264 $\pm$ 0.006 *	0.310 $\pm$ 0.011 ****	0.279 $\pm$ 0.009 #
Creatine	1.181 $\pm$ 0.118	1.105 $\pm$ 0.090	1.788 $\pm$ 0.035 ***	1.799 $\pm$ 0.033
Phosphocreatine	1.917 $\pm$ 0.065	1.663 $\pm$ 0.050 **	1.155 $\pm$ 0.035 ****	1.261 $\pm$ 0.041 (0.0673)
beta-Alanine	0.578 $\pm$ 0.007	0.554 $\pm$ 0.015	0.553 $\pm$ 0.006 *	0.585 $\pm$ 0.007 ##
Choline	0.171 $\pm$ 0.016	0.162 $\pm$ 0.005	0.156 $\pm$ 0.009	0.131 $\pm$ 0.008
PC	3.075 $\pm$ 0.077	2.633 $\pm$ 0.063 ****	4.029 $\pm$ 0.032 ****	4.419 $\pm$ 0.064 #####
GPC	1.218 $\pm$ 0.031	1.197 $\pm$ 0.036	0.622 $\pm$ 0.014 ****	0.597 $\pm$ 0.008
Taurine	3.442 $\pm$ 0.055	3.357 $\pm$ 0.034	3.997 $\pm$ 0.019 ****	4.092 $\pm$ 0.023 ##
Myoinositol	1.438 $\pm$ 0.026	1.210 $\pm$ 0.022 ****	1.414 $\pm$ 0.006	1.314 $\pm$ 0.010 ####
Glucose	0.204 $\pm$ 0.018	0.297 $\pm$ 0.025 **	0.018 $\pm$ 0.001 ****	0.018 $\pm$ 0.002
Glycine	3.460 $\pm$ 0.043	3.217 $\pm$ 0.058 **	2.914 $\pm$ 0.018 ****	3.086 $\pm$ 0.015 #####
Lactate	1.664 $\pm$ 0.056	2.067 $\pm$ 0.085 **	0.239 $\pm$ 0.020 ****	0.191 $\pm$ 0.015
Threonine	1.391 $\pm$ 0.049	1.215 $\pm$ 0.028 **	1.081 $\pm$ 0.04 **	1.165 $\pm$ 0.027
GTP	0.104 $\pm$ 0.003	0.095 $\pm$ 0.002 *	0.094 $\pm$ 0.001 **	0.101 $\pm$ 0.002 #
UDP-Glucose	0.033 $\pm$ 0.002	0.027 $\pm$ 0.002 *	0.010 $\pm$ 0.001 ****	0.007 $\pm$ 0.001
Tyrosine	0.151 $\pm$ 0.003	0.148 $\pm$ 0.001	0.168 $\pm$ 0.003 ***	0.170 $\pm$ 0.002
Phenylalanine	0.253 $\pm$ 0.007	0.256 $\pm$ 0.002	0.279 $\pm$ 0.006 *	0.292 $\pm$ 0.006
Histidine	0.020 $\pm$ 0.001	0.022 $\pm$ 0.001	0.025 $\pm$ 0.001 **	0.023 $\pm$ 0.000
NAD+	0.038 $\pm$ 0.002	0.036 $\pm$ 0.002	0.046 $\pm$ 0.001 ***	0.048 $\pm$ 0.001
Formate	0.036 $\pm$ 0.002	0.035 $\pm$ 0.002	0.046 $\pm$ 0.003 **	0.043 $\pm$ 0.002
AXP	0.647 $\pm$ 0.013	0.584 $\pm$ 0.014 **	0.753 $\pm$ 0.004 ****	0.756 $\pm$ 0.010

^a Relative levels of the metabolites were quantified based on the data normalized on the total area sum.

^b *p* values were calculated from independent samples t-test, statistical significances: * *p* < 0.05, ** *p* < 0.01, *** *p* < 0.001, **** *p* < 0.0001 compared to the Nor group, # *p* < 0.05, ## *p* < 0.01, #### *p* < 0.0001 compared to the Low group. Red/blue colors denote increased/decreased metabolites, respectively. *n* = 9 for each group.

Table S3. Relevant information of significantly altered metabolic pathways and the most influential metabolites.

NO.	Metabolic Pathway	Pathway impact	Match Status	Matched metabolites	p value		
					Low vs Nor	Low-A vs Low	Nor-A vs Nor
1	Alanine, aspartate and glutamate metabolism	0.583	5/28	Alanine, Aspartate, Glutamate, AKG, Glutamine,	8.664E-16	9.637E-6	1.157E-6
2	Glycine, serine and threonine metabolism	0.295	4/34	Choline, Threonine, Glycine, Creatine,	1.689E-11	4.382E-4	9.990E-3
3	Glutathione metabolism	0.371	4/28	Glycine, Glutathione, Glutamate, 5-Oxoproline	8.026E-13	2.044E-4	1.130E-3
4	D-Glutamine and D-glutamate metabolism	0.500	3/6	Glutamine, Glutamate, AKG	1.339E-11	8.777E-7	7.013E-6
5	Starch and sucrose metabolism	0.014	1/15	UDP-glucose	7.944E-10	8.445E-2	2.138E-3
6	beta-Alanine metabolism	0.399	3/21	Aspartate, beta-Alanine, Histidine	4.828E-6	2.754E-2	2.315E-1
7	Taurine and hypotaurine metabolism	0.429	1/8	Taurine	5.011E-8	6.268E-3	2.040E-1
8	Phenylalanine metabolism	0.357	2/12	Phenylalanine, Tyrosine	2.044E-3	2.943E-1	5.206E-1
9	Phenylalanine, tyrosine and tryptophan biosynthesis	1.000	2/4	Phenylalanine, Tyrosine	2.0443E-3	2.943E-1	5.206E-1
10	Nicotinate and nicotinamide metabolism	0.235	2/15	Aspartate, NAD ⁺	1.669E-9	3.553E-1	4.713E-1
11	Histidine metabolism	0.221	3/16	Histidine, Aspartate, Glutamate	1.056E-11	3.079E-1	4.414E-1

^a The metabolic pathway analysis was performed on the MetaboAnalyst 5.0 webserver (<https://www.metaboanalyst.ca>), using a combination of metabolite sets enrichment analysis ($p < 0.05$) and pathway topological analysis (pathway impact value > 0.2).