

Supplementary Materials

Figure S1.

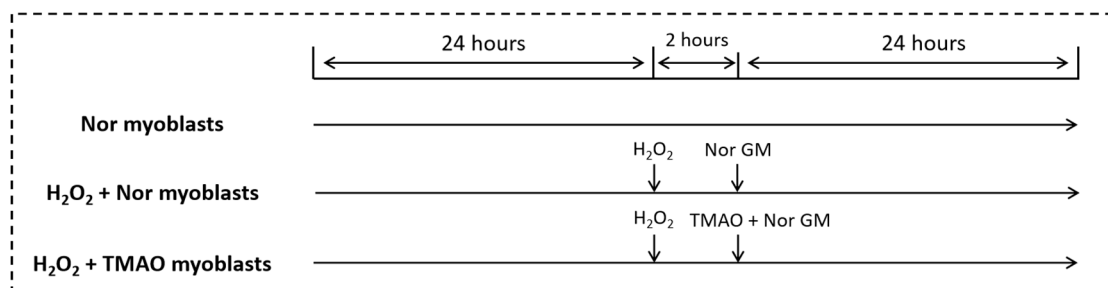


Figure S1. Schematic representation of the cell experimental design. C2C12 cells were firstly cultured in normal growth medium (Nor GM) to reach 50-60% confluence for 24 h. H₂O₂ was added to C2C12 myoblasts at a final concentration of 0.5 mM for 2 h, and then the medium was removed. Nor GM was added in the H₂O₂-treated (H₂O₂) group; TMAO was dissolved in normal growth medium to C2C12 myoblasts for 24 h in the TMAO-treated (TMAO) groups with three final concentrations of TMAO (1, 5 and 10 mM).

Figure S2.

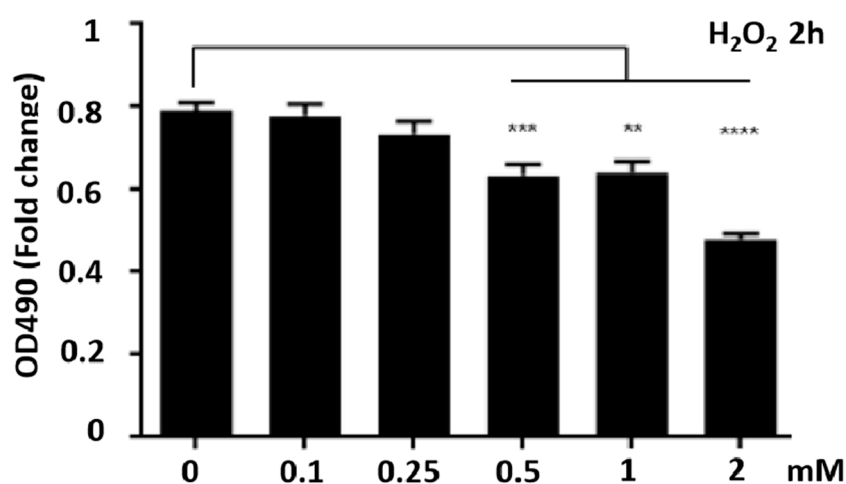


Figure S2. Cell viabilities of C2C12 myoblasts treated by H₂O₂ at various concentrations for 2h. Cell viabilities were assessed by measured OD_{490 nm} values with the MTS approach ($n = 6$). ** $p < 0.01$, *** $P < 0.001$, **** $p < 0.0001$

Figure S3.

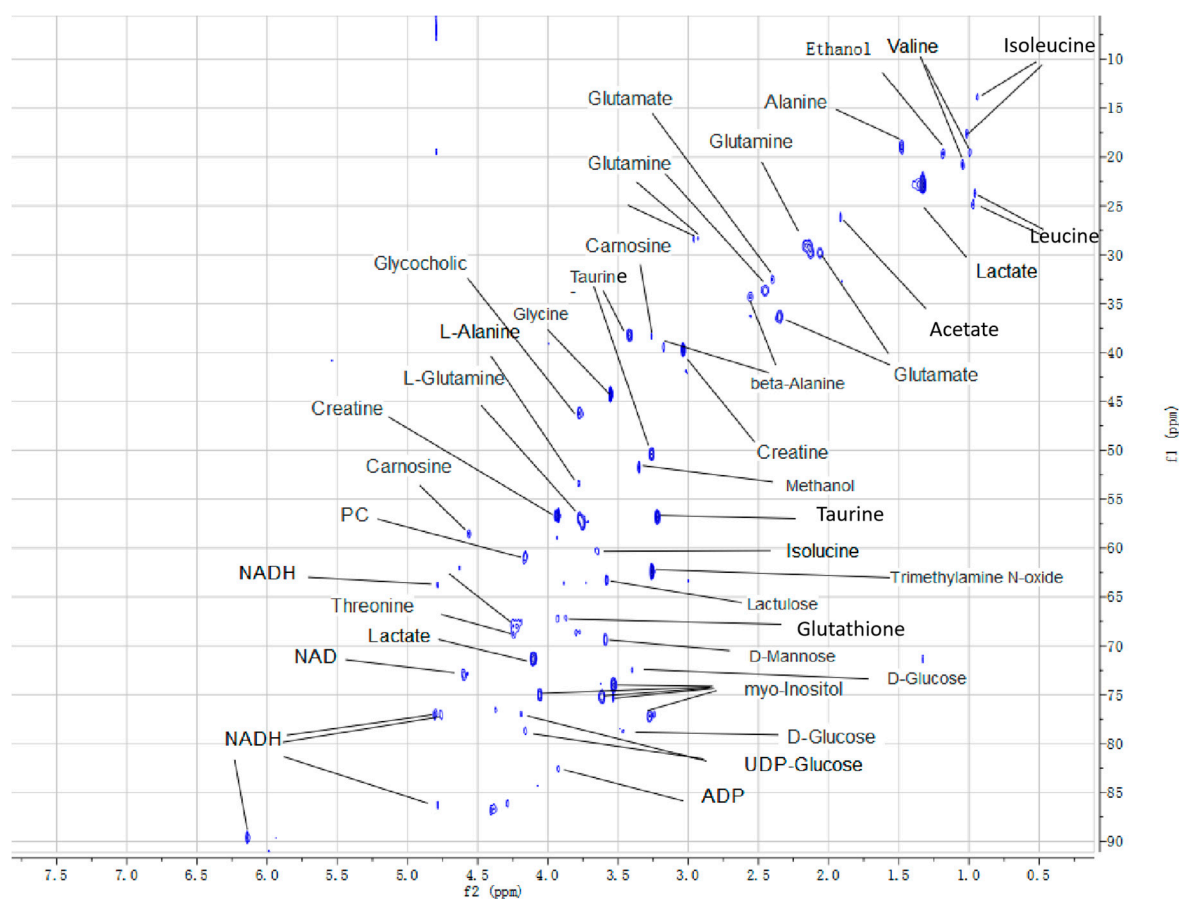
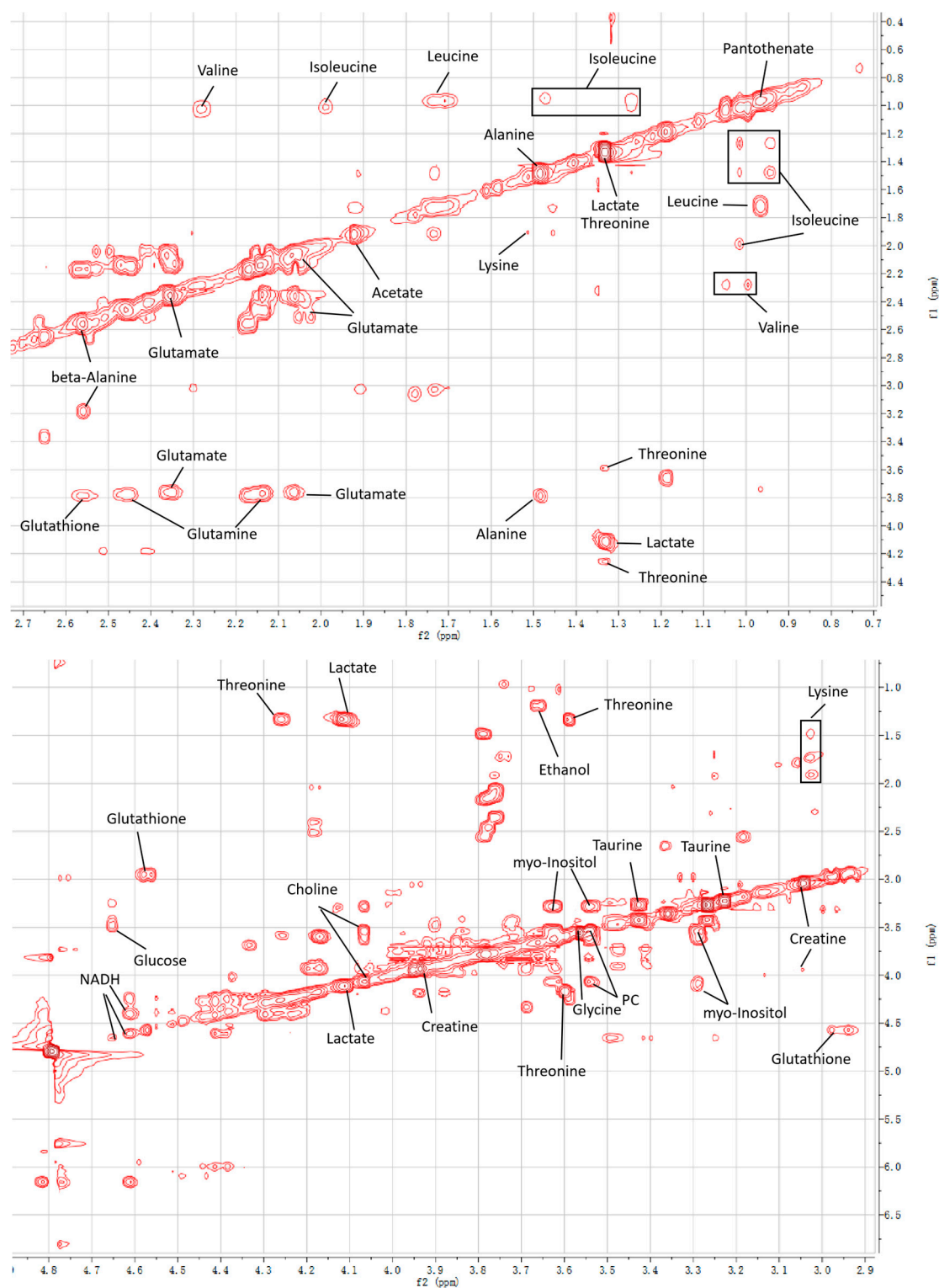


Figure S3. Representative 2D ^1H - ^{13}C HSQC spectrum of aqueous extracts derived from C2C12 myoblasts recorded on 850 MHz NMR spectrometer.

Figure S4.



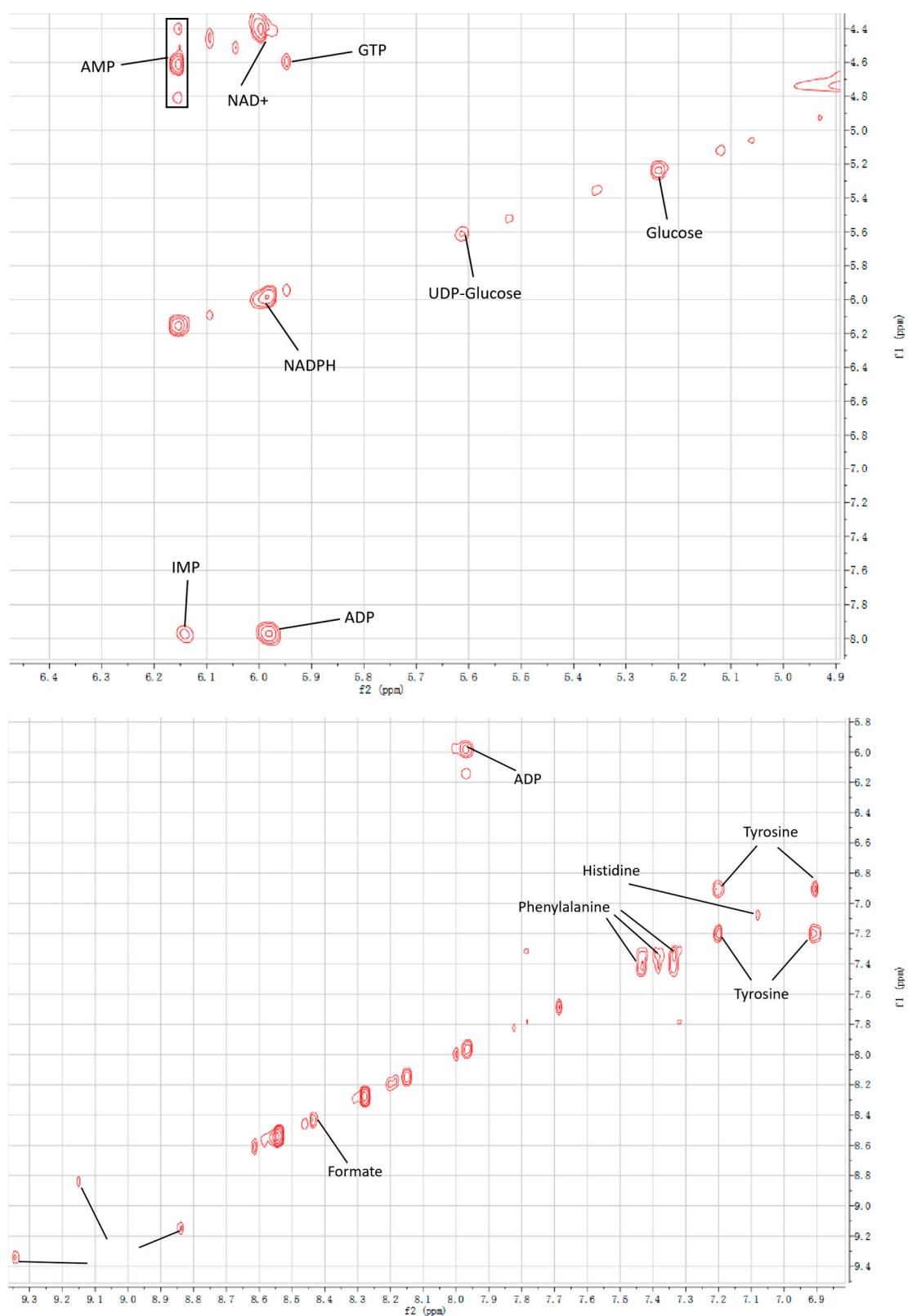


Figure S4. Representative 2D ^1H - ^1H TOCSY spectrum of aqueous extracts derived from C2C12 myoblasts recorded on 850 MHz NMR spectrometer.

Figure S5.

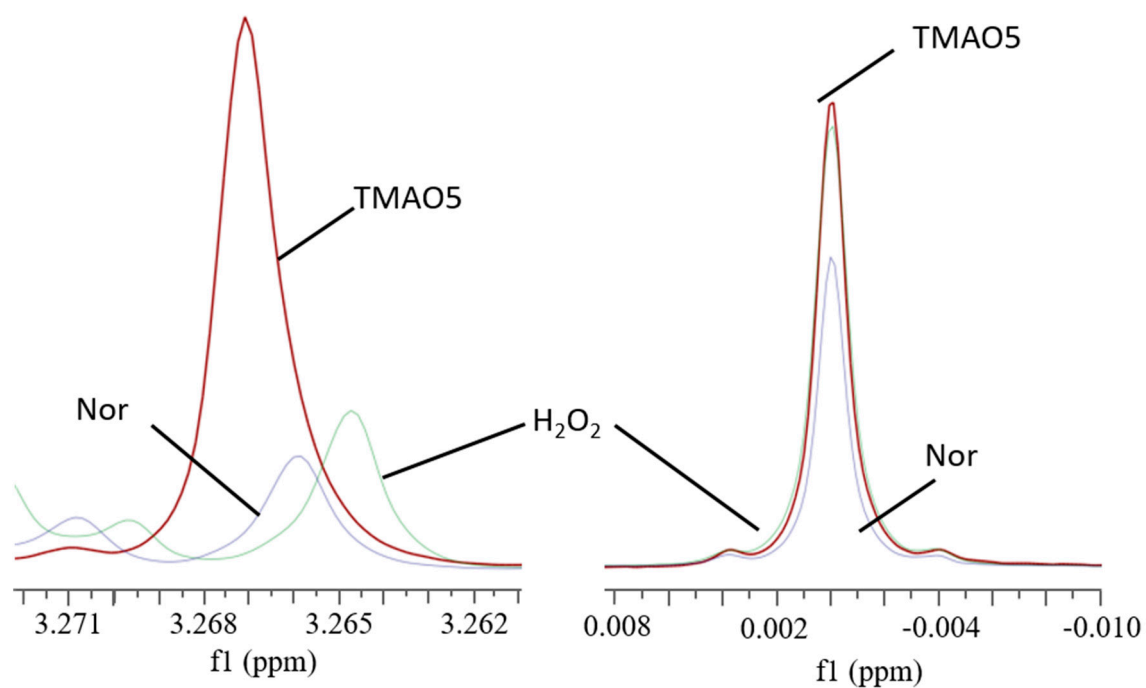


Figure S5. Local amplified regions of TMAO peaks in a typical 850 MHz ^1H NMR spectrum recorded on aqueous extracts derived from three groups of C2C12 myoblasts. The TSP peak was used as a chemical shift reference (δ 0.00). Blue line: spectral region from the Nor group. Green line: spectral region from the H_2O_2 group. Brown line: spectral region from the TMAO5 group.

Table S1. Identified metabolites in ¹H NMR spectra of aqueous extracts derived from C2C12 myoblasts

NO.	Metabolites	δ ¹ H (ppm) and multiplicity	Moieties
1	Pantothenate	0.90(s), 0.94(s)	CH ₃ , CH ₃
2	Isoleucine	0.93(t), 1.00(d), 1.26(m), 1.42(m), 1.99(m), 3.66(d)	δ-CH ₃ , γ-CH ₃ , half γ-CH ₂ , half γ-CH ₂ , β-CH, α-CH
3	Leucine	0.94(d), 0.97(d), 1.71(m), 1.74(m), 3.73(m)	α-CH ₃ , α-CH ₃ , γ-CH, β-CH ₂ , α-CH
4	Valine	0.99(d), 1.05(d), 2.26(m), 3.60(d)	γ-CH ₃ , γ-CH ₃ , β-CH, α-CH
5	Ethanol	1.17 (t), 3.65 (q)	β-CH ₃ , CH ₂
6	Alanine	1.47(d), 3.78(q)	β-CH ₃ , α-CH
7	Putrescine	1.76 (m), 3.04 (m)	2CH ₂ , 2N-CH ₂
8	Acetate	1.91(s)	CH ₃
9	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
10	Glutamine	2.13(m), 2.42(m), 2.45(m), 3.77(t)	γ-CH ₂ , β-CH ₂ , α-CH
11	Methylamine (MA)	2.59 (s)	N-CH ₃
12	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
13	Dimethylamine (DMA)	2.72 (s)	CH ₃
14	Sarcosine	2.74 (s), 3.6 (s)	N-CH ₃ , α-CH ₂
15	Trimethylamine (TMA)	2.88 (s)	CH ₃
16	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
17	Lysine	1.43(m), 1.50(m), 1.73(m), 1.89(m), 1.92(m), 3.02(t), 3.75(t)	γ-CH ₂ , half γ-CH ₂ , δ-CH ₂ , β-CH ₂ , ε-CH ₂ , α-CH
18	Phosphocreatine (PCr)	3.05(s), 4.05(s)	N-CH ₃ , CH ₂
19	beta-Alanine	2.54(t), 3.17(t)	CH ₂ , CH ₂
20	Choline	3.21(s), 3.50(dd), 4.03(t)	N-(CH ₃) ₃ , N-CH ₂ , CH ₂ OH
21	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 CH ₂ , ² CH ₂ , half ¹ CH ₂ , half ³ CH ₂ , half ³ CH ₂ , ¹ CH ₂
22	Trimethylamine N-oxide (TMAO)	3.26(s)	CH ₃
23	Taurine	3.24(t), 3.41(t)	¹ CH ₂ , ² CH ₂
24	myo-Inositol	3.28(t), 3.53(dd), 3.63(t), 4.07(t)	² CH, ^{4,6} CH, ^{1,3} CH, ⁵ CH
25	Glycine	3.57(s)	α-CH ₂
26	Threonine	1.31(d), 3.59(d), 4.25(m)	γ-CH ₂ , β-CH
27	Creatine	3.04(s), 3.93(s)	N-CH ₃ , α-CH ₂
28	Lactate	1.33(d), 4.11(q)	β-CH ₃ , α-CH

29	O-Phosphocholine (PC)	3.20(s), 3.59(t), 4.18(m)	N-(CH ₃) ₃ , N-CH ₂ , CH ₂ OH
30	Glucose	β(3.24 (dd), 3.48 (t), 3.90 (dd)), 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)
31	UDP-Glucose (UDPG)	5.62 (dd), 6.0 (m)	CH, 2CH
32	UDP-N-Acetylglucosamine (UDP-GlcNAc)	2.07 (s), 5.5 (q), 6.0 (dd), 7.94 (d), 8.3 (d)	CH ₃ , CH, 2CH, CH, NH
33	NADH	4.37(m), 4.17(m), 4.69(t), 6.14(d), 8.45(s)	CH, CH, CH, NH ₂ , CH
34	Carnosine	2.68(m), 3.02(q), 7.06(s)	CH ₂ , CH
35	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
36	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
37	Pyridoxine	2.46(s), 4.81(s), 7.68(s)	CH ₃ , CH ₂ , CH
38	Histidine	7.06(s), 7.85(s)	⁵ CH, ² CH
39	GTP	5.92 (d), 8.1 (s)	CH, CH
40	AMP	6.14 (d), 8.27 (s), 8.58 (s)	NH ₂ , δ-CH, ² CH
41	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), δ-CH, β-CH, ² CH, γ-CH, α-CH
42	Formate	8.46(s)	CH
43	NADPH	4.20(m), 5.97(m), 8.23(s), 8.47(s)	CH, CH, CH, CH
44	ADP	6.13 (d), 8.27 (s), 8.58 (s)	NH ₂ , δ-CH, ² CH
45	IMP	4.37(m), 6.14(d), 8.22 (s), 8.58 (s)	CH, NH ₂ , CH, CH

Multiplicity: s, singlet; d, double; t, triplet; q, quartet; m, multiple; dd, double of double. Abbreviations: DMA, dimethylamine; TMA, trimethylamine; PCr, phosphocreatine; GPC, sn-Glycero-3-phosphocholine; TMAO, trimethylamine N-oxide; PC, O-phosphocholine; UDPG, UDP-glucose; UDP-GlcNAc, UDP-N-acetylglucosamine; GTP, guanosine triphosphate; AMP, adenosine monophosphate; NAD⁺, nicotinamide adenine dinucleotide; ADP, adenosine diphosphate; IMP, hypoxanthine nucleotide.