

Blood Metabolite Profiling of Antarctic Expedition Members: An ^1H NMR Spectroscopy-Based Study

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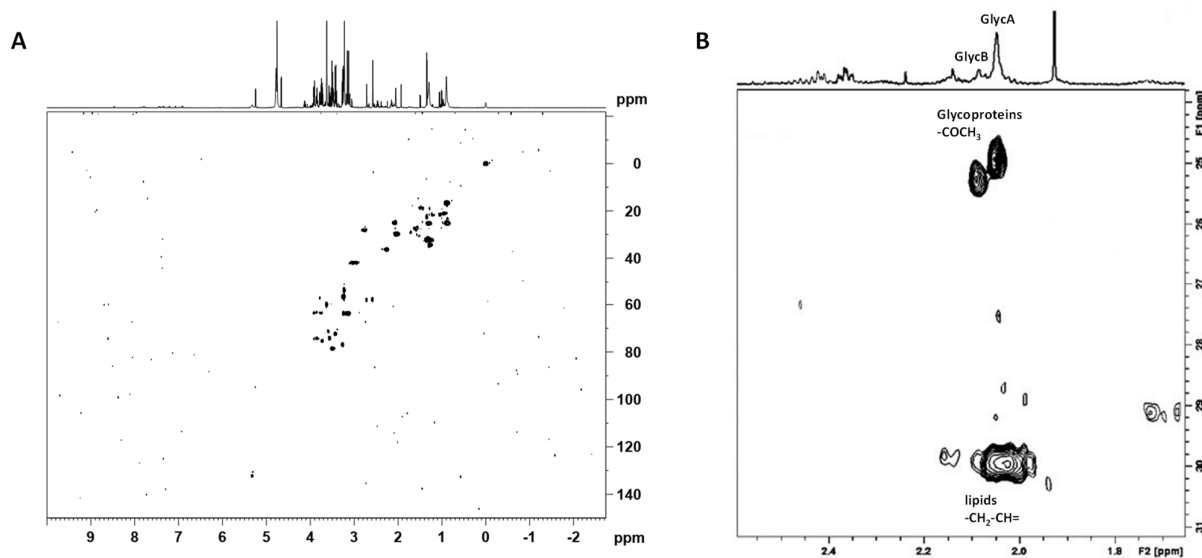


Figure S1. A) 600MHz ^1H - ^{13}C HSQC spectrum of human serum and B) relative expansion in the region 2.5-1.5 ppm (for ^1H) and 31-20 ppm (for ^{13}C); -COCH₃ acetyl groups of glycoproteins, GlycA and GlycB [19].

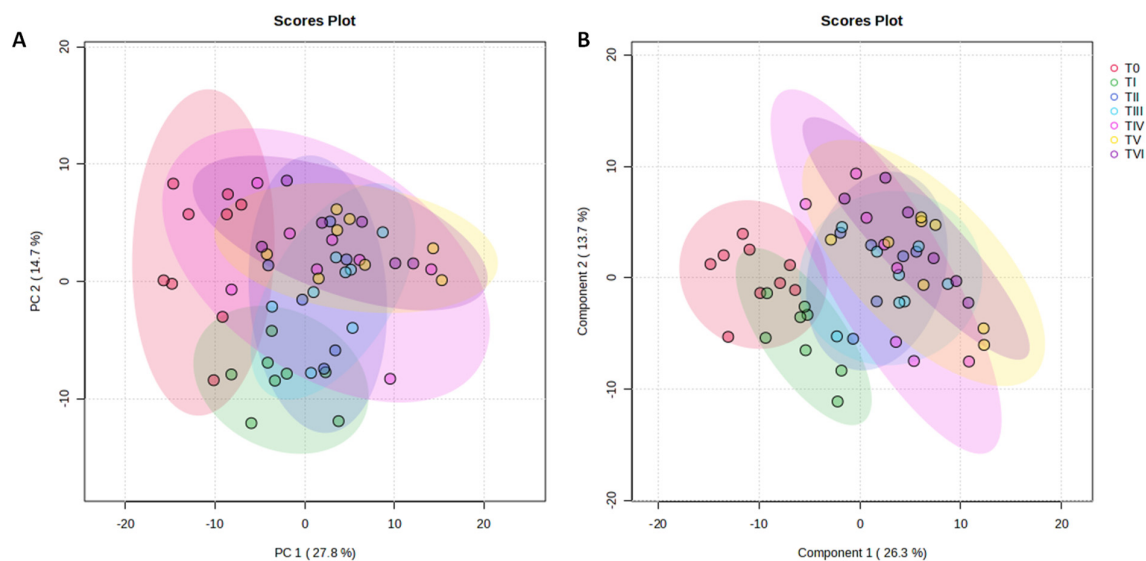


Figure S1. A) PCA and **(B)** PLS-DA score plots obtained from the explorative (PCA model) and supervised (PLS-DA model) analyses performed on the whole spectral data. From statistical analyses, relatively higher levels of glucose, glutamate and N-acetyl glycoproteins (GlycA) and relative low content of lipids and glutamine were found in samples collected at time “T0”, with respect to the other serum samples (PCA = principal component analysis; PLS-DA = partial least squares discriminant analysis initial sampling session (T0) and subsequent samplings after 7 (TII), 14 (TIII), 90 (TIV), 180 (TV), and 270 (TVI) days from the arrival.

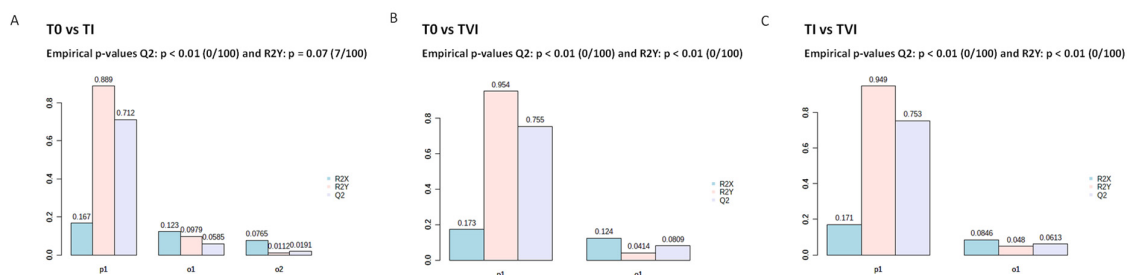


Figure S2. The overview of the OPLS-DA model performance for the selected groups and the permutation test. R²X and R²Y refer to the goodness and represent the model interpretation rate. Q² refers to the predicted variation (quality of prediction). R²Y and Q² closer to 1 indicate the more stable and reliable model.

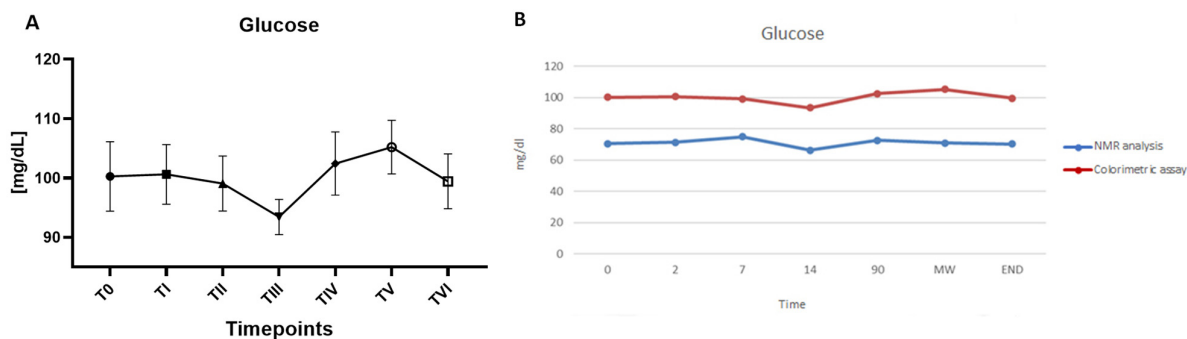


Figure S3. A) Colorimetric assay for glucose circulating levels of subjects in correspondence of the different timepoints. Values are represented as mean \pm SEM. Error bar indicates the interindividual fluctuation in the measured concentrations; **B)** Bruker ERETIC measurements used to directly measure the concentration of blood glucose. A good correlation has been obtained considering the concentration (mg/dL) calculated from the NMR spectra (^1H NMR signal at 5.25 ppm) and the colorimetric assay.

Table S1. Representative ^1H and ^{13}C NMR assignments of metabolites identified in human serum samples at 600 MHz (bs: broad signal; d: doublet; s: singlet; q: quartet; m: multiplet).

Metabolite	δ_{H} ppm (multiplicity, assignment)/ δ_{C} ppm
Acetate	1.92 (s, CH_3)/25.98
Acetoacetate	2.24 (s, CH_3)
Alanine	1.47(d, CH_3)/18.4
Choline	3.20 (s, $\text{N}(\text{CH}_3)_3$)/56.71
Citrate	2.54(d, half CH_2); 2.68(d, half CH_2)
Creatine/phosphocreatine	3.04(s, CH_3)/42.19; 3.96(s, CH_2)
Formate	8.46 (s, CH)
Glycerol	3.58(m, CH_2), 65.4; 3.67 (m, CH_2), 65.4
Glycine	3.57 (s, CH_2)/43.2
Glucose	3.25 (m, CH)/76.96; 3.42(m, CH)/72.5; 3.48(m, CH)/78.6; 3.55(m, CH)/74.27; 3.72(m, CH)/63.3; 3.85(m, CH)/63.4; 3.91/63.53; (m, CH_2); 4.64(d, CH); 5.24(d, CH)/94.88
Glutamate	2.08(m, half CH_2)/29.84; 2.13(m, half CH_2)/29.86; 2.36 (m, CH_2)/36.4; 3.78 (m, CH)/57.21
Glutamine	2.15(m, half CH_2)/29.88; 2.42(m, half CH_2)/36.48
3-Hydroxybutyrate	1.20(d, CH_3)/21.61
Histidine	7.07 (s, CH); 7.79 (s, CH)
Isoleucine	0.94(t, CH_3)/13.69; 1.02 (d, CH_3)
Lactate	1.33(d, CH_3)/22.9; 4.12 (q, CH)/71.4
Leucine	0.98(t, CH_3)/24.8; 1.73 (m, CH, CH_2)/42.72
Lipids, LDL	0.87(bs, CH_3)/16.74; 1.29(bs, CH_{2n})/34.7; 1.57(m, $\text{CH}_2\text{CH}_2\text{COOC}$)/27.53; 2.01(m, $\text{CH}_2\text{CH=}$)/29.9; 5.32(m, CH=CH)/132.43
Lipids, VLDL	0.90(bs, CH_3)/25.33; 1.32(bs, CH_{2n})/32.35; 1.57(m, $\text{CH}_2\text{CH}_2\text{COOC}$)/27.53; 2.01(m, $\text{CH}_2\text{CH=}$)/29.9; 5.32(m, CH=CH)/132.43
N-acetyl glycoproteins	2.05-2.07 (bs, COCH_3)/29.92
Phenylalanine	7.34(d, CH ring); 7.39(m, CH ring); 7.43(m, CH ring)
Tyrosine	6.90 (d, CH ring); 7.20 (d, CH ring)
Unidentified	2.82 (s, CH_3)/32.53
Valine	1.00(d, CH_3)/19.7; 1.05 (d, CH_3)/20.7; 2.28 (m, CH)/31.9

Table S3. Results from Pathway Analysis with MetPA for serum samples T0 vs T1 (Total number of compounds involved in each pathway and metabolites matched from the uploaded data; p is the original p -value calculated from the enrichment analysis; the impact is the pathway impact value calculated from pathway topology analysis).

ID	Pathway name	Total	Hits	p -value	$-\log(p)$	Holm p	FDR	Impact
A	D-glutamine and D-glutamate metabolism	6	2	0.0001758	6.755	0.028128	0.0004688	0.50
B	Alanine, aspartate and glutamate metabolism	28	2	0.0001758	6.755	0.028128	0.0004688	0.3109
C	Arginine biosynthesis	14	2	0.0001758	6.755	0.028128	0.0004688	0.11675

Table S4. Results from Pathway Analysis with MetPA for serum samples T0 vs TVI (Total number of compounds involved in each pathway and metabolites matched from the uploaded data; p is the original p -value calculated from the enrichment analysis; the impact is the pathway impact value calculated from pathway topology analysis).

ID	Pathway name	Total	Hits	p -value	$-\log(p)$	Holm p	FDR	Impact
A	D-glutamine and D-glutamate metabolism	6	1	2.06E-04	76.851	2.06E-03	2.06E-04	0.5
B	Alanine, aspartate and glutamate metabolism	28	1	2.06E-04	76.851	2.06E-03	2.06E-04	0.19712
C	Arginine biosynthesis	14	1	2.06E-04	76.851	2.06E-03	2.06E-04	0.11675

Table S5. Results from Pathway Analysis with MetPA for serum samples T1 vs TVI (Total number of compounds involved in each pathway and metabolites matched from the uploaded data; p is the original p -value calculated from the enrichment analysis; the impact is the pathway impact value calculated from pathway topology analysis).

ID	Pathway name	Total	Hits	p -value	$-\log(p)$	Holm p	FDR	Impact
A	Phenylalanine, tyrosine, and tryptophan biosynthesis	4	2	0.0066599	61.765	0.053279	0.001776	1.0
B	Phenylalanine metabolism	10	2	0.0066599	61.765	0.053279	0.001776	0.35714

Table S6. List of collected samples at different test sampling time (days).

Blood test sampling time (days)							
Sample ID	T0	T1	TII	TIII	TIV	TV	TVI
A	0	2	7	14	90	MW	END
B	0	2	7	14	90	MW	END
C	0	2	7	14	90	MW	END
D	0	2	7	14	90	MW	END
F	0	2	7	14	90	MW	END
G	0	2	7	14	90	MW	END
H	0	2	7	14	90	MW	END
K	0	2	7	14	90	MW	END