

Supplementary Materials

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

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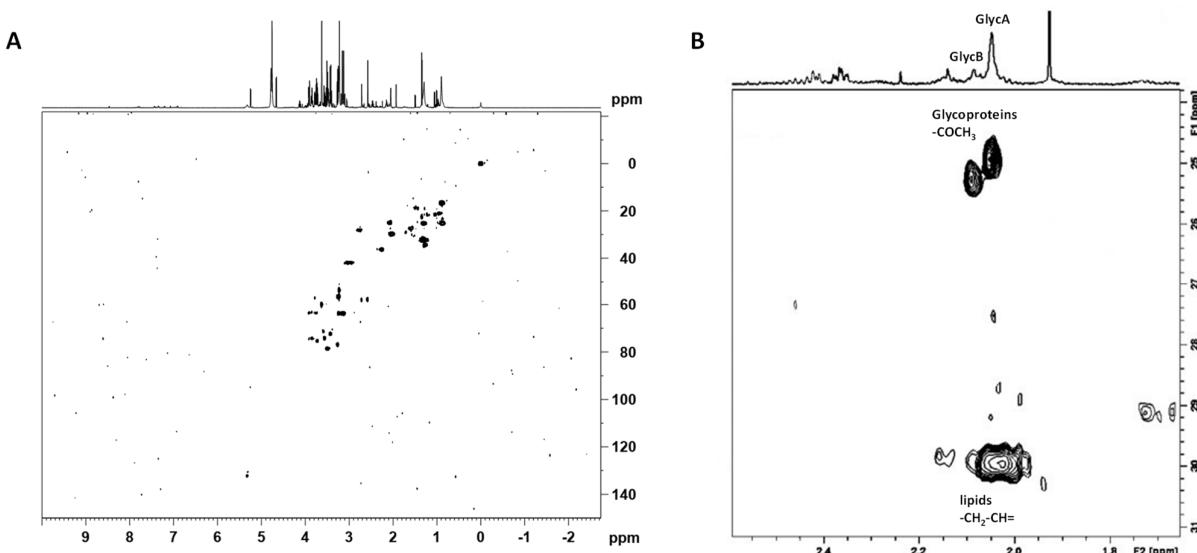


Figure S1. **A)** 600MHz ^1H - ^{13}C HSQC spectrum of human serum and **B)** relative expansion in the region 2.5-1.5 ppm (for ^1H) and 31-20 ppm (for ^{13}C); -COCH₃ acetyl groups of glycoproteins, GlycA and GlycB [19].

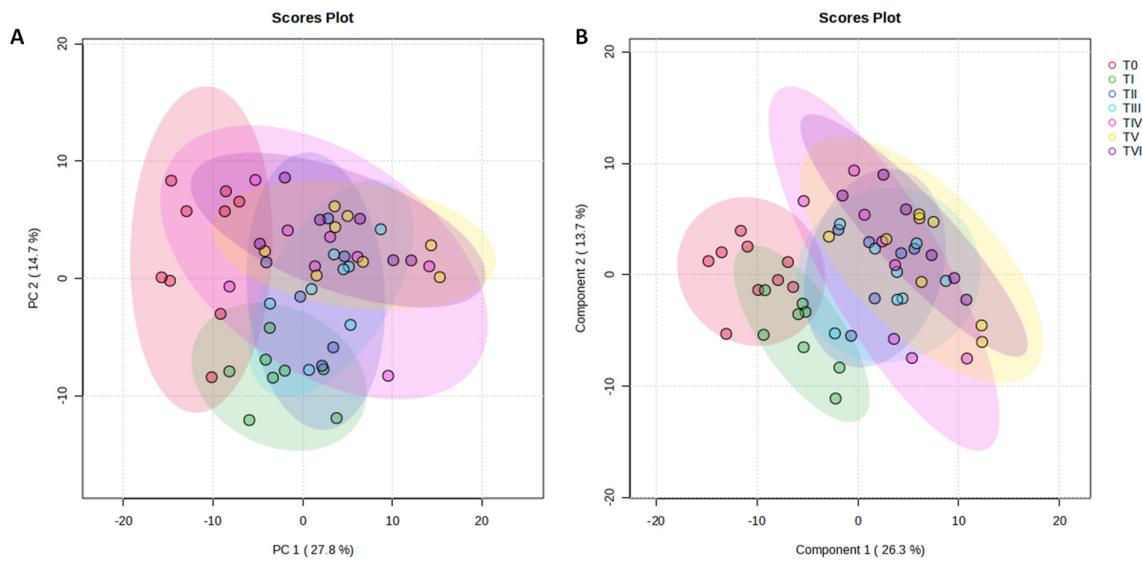


Figure S1. **A)** PCA and **(B)** PLS-DA score plots obtained from the explorative (PCA model) and supervised (PLS-DA model) analyses performed on the whole spectral data. From statistical analyses, relatively higher levels of glucose, glutamate and N-acetyl glycoproteins (GlycA) and relative low content 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.

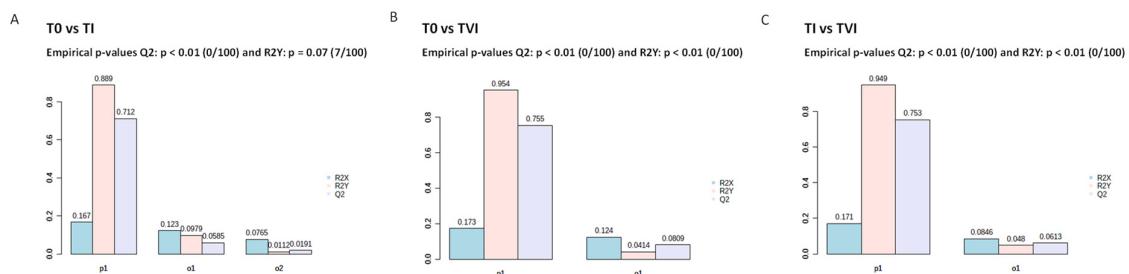


Figure S2. The overview of the OPLS-DA model performance for the selected groups and the permutation test. R^2X and R^2Y refer to the goodness and represent the model interpretation rate. Q^2 refers to the predicted variation (quality of prediction). R^2Y and Q^2 closer to 1 indicate the more stable and reliable model.

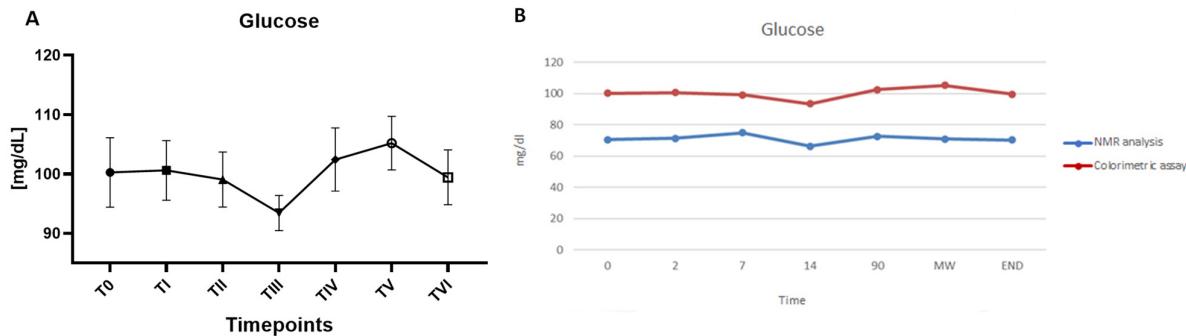


Figure S3. **A)** Colorimetric assay for glucose circulating levels of subjects in correspondence of the different timepoints. Values are represented as mean \pm SEM. Error bar indicates the interindividual fluctuation in the measured concentrations; **B)** Bruker ERETIC measurements used to directly 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, N(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, CH ₂ CH ₂ COOC)/27.53; 2.01(m, CH ₂ 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, CH ₂ CH ₂ COOC)/27.53; 2.01(m, CH ₂ 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 S2. The significant differences of the mean values, for all the time-points, from T0 to subsequent samplings after 7 (TII), 14 (TIII), 90 (TIV), 180 (TV), and 270 (TVI) days after arrival were obtained by one-way ANOVA followed by Tukey's honestly significant differences (HSD) post hoc test and repeated one-way ANOVA (Core Team (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>).

Metabolite assignment	Binned NMR peak (ppm)	p-value	Post hoc tests
Glutamate	2.37	1.61×10^{-10}	t1-t0; t2-t0; t3-t0; t4-t0; t5-t0; t6-t0
Glutamine	2.47	1.36×10^{-9}	t1-t0; t2-t0; t3-t0; t4-t0; t5-t0; t6-t0; t4-t1; t5-t1; t6-t1; t4-t2; t6-t2
Lipids	0.89	7.28×10^{-4}	t5-t0; t6-t0; t5-t1
N-acetyl glycoproteins	2.05	1.09×10^{-8}	t2-t0; t3-t0; t4-t0; t5-t0; t6-t0; t3-t1; t4-t1; t5-t1; t6-t1

Lipids (bin: 0.89)						
Univariate Type II Repeated-Measures ANOVA						
	Sum Sq	num Df	Error SS	den Df	F value	Pr(>F)
(Intercept)	0.072297	1	0.0050777	44	626.4811 < 2.2e-16 ***	
subject	0.002688	7	0.0050777	44	3.3275 0.006252 **	
TIME	0.000000	6	0.0000000	264	NaN	NaN
subject:TIME	0.000000	42	0.0000000	264	NaN	NaN

Glutamate (bin: 2.37)						
Univariate Type II Repeated-Measures ANOVA						
	Sum Sq	num Df	Error SS	den Df	F value	Pr(>F)
(Intercept)	0.011432	1	0.00070641	44	712.0919 <2e-16 ***	
subject	0.000252	7	0.00070641	44	2.2423 0.0485 *	
TIME	0.000000	6	0.0000000	264	NaN	NaN
subject:TIME	0.000000	42	0.0000000	264	NaN	NaN

Glutamine (bin: 2.47)						
Univariate Type II Repeated-Measures ANOVA						
	Sum Sq	num Df	Error SS	den Df	F value	Pr(>F)
(Intercept)	0.0081472	1	0.0005836	44	614.2580 < 2e-16 ***	
subject	0.0002288	7	0.0005836	44	2.4641 0.03184 *	
TIME	0.0000000	6	0.0000000	264	NaN	NaN
subject:TIME	0.0000000	42	0.0000000	264	NaN	NaN

<i>N</i> -acetyl-glycoproteins (bin: 2.05)						
Univariate Type II Repeated-Measures ANOVA						
	Sum Sq	num Df	Error SS	den Df	F value	Pr(>F)
(Intercept)	0.182367	1	0.0030815	44	2603.9466 < 2e-16 ***	
subject	0.000644	7	0.0030815	44	1.3131 0.2668	
TIME	0.000000	6	0.0000000	264	NaN	NaN
subject:TIME	0.000000	42	0.0000000	264	NaN	NaN

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