Supplementary data

for article "Metabotyping Patients' Journeys Reveals Early Predisposition to Lung Injury after Cardiac Surgery"

Raluca Georgiana Maltesen, Bodil Steen Rasmussen, Shona Pedersen, Munsoor Ali Hanifa, Sergey Kucheryavskiy, Søren Risom Kristensen, Reinhard Wimmer

Table S1 A prolonged surgical procedure affects the metabolome. *Association between metabolome and duration of surgical procedure assessed by partial least square (PLS) regression. Both the calibrated (Cal) and cross-validated (CV) results are provided. At 4 hours post-CPB the association was weaker, suggesting that the metabolome had begun normalizing after prolonged surgical stress. No significant CV association was observed at 8 and 20 hourspostoperatively. Models with low predicted association are marked in grey.*

	CABG (196 ± 50 min.)		CPB (64 ± 29 min.)		Ischemia (33±19 min.)	
	R^2 Cal	R^2 CV	R^2 Cal	R^2 CV	R^2 Cal	R^2 CV
0 hour	-	-	0.94	0.76	0.92	0.75
2 hours	0.9	0.75	0.87	0.67	0.72	0.54
4 hours	0.82	0.6	0.7	0.41	0.7	0.44
8 hours	0.68	0.44	0.7	0.36	0.61	0.41
20 hours	0.74	0.39	0.55	0.32	0.63	0.38

Table S2 Metabolite time profiles in both left atrium (LA) and pulmonary artery (PA) samples. *Mean change in percentage was calculated using the formula: (post-CPB ─ pre-CPB)/pre-CPB*100. Significance was assessed by means of factorial ANOVA with Tukey's post hoc test for multiple comparisons. Abbreviations: TMAO, trimethylamine-N-oxide; N-Ac-Gal, N-acetyl-galactosamine; N-Ac-Glc, N-acetyl-glucosamine; FA, fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; DAG, diacylglycerol; PEP, phosphoenolpyruvate; 4.60ppm*, unassigned metabolite; DAGPL, diacylglycerophosphocholine; PC, phosphatidylcholine; GPC, glycerophosphocholine; SM, sphingomyelin.*

Metabolites	0 hour		2 hours		4 hours		8 hours		20 hours		p-value	
	LA	PA	**LA**	PA	**LA**	PA	**LA**	PA	**LA**	PA	**LA**	PA
Trigonelline	**-15**	-17	**-32**	-38	**-45**	-46	**-50**	-53	**-49**	-63	**0.010**	0.001
Formate	**2**	1	**-13**	-15	**-20**	-20	**-19**	-21	**-16**	-14	**<0.0001**	<0.0001
Inosine	**-14**	-18	**-27**	-25	**-28**	-29	**-24**	-25	**-24**	-30	**<0.0001**	<0.0001
Hypoxanthine	**-13**	-12	**-6**	-4	**-9**	-7	**4**	0	**12**	8	**<0.0001**	<0.0001
Adenine	**6**	12	**56**	45	**51**	47	**67**	67	**65**	54	**<0.0001**	<0.0001
Tryptophan	**-25**	-22	**-29**	-28	**-28**	-27	**-19**	-22	**-10**	-10	**<0.0001**	<0.0001
Histidine	**-1**	-1	**-9**	-11	**-14**	-15	**-13**	-14	**-14**	-16	**<0.0001**	<0.0001
3-Metylhistidine	**4**	4	**11**	9	**6**	6	**14**	15	**36**	32	**<0.0001**	<0.0001
1- Metylhistidine	**-7**	-10	**-7**	-11	**-12**	-14	**-5**	-9	**6**	0	**<0.0001**	<0.0001
Phenylalanine	**4**	4	**1**	-1	**1**	-1	**-4**	-6	**8**	6	**0.03**	0.02
Uric acid	**-6**	-8	**-13**	-17	**-13**	-16	**-2**	-6	**24**	17	**<0.0001**	<0.0001
Tyrosine	**4**	3	**-13**	-15	**-15**	-16	**-18**	-19	**-13**	-16	**<0.0001**	<0.0001
Glucuronate	**5**	6	**15**	13	**9**	9	**30**	26	**100**	94	**<0.0001**	<0.0001
Xanthine	**-16**	-16	**-26**	-26	**-24**	-25	**-14**	-16	**5**	3	**<0.0001**	<0.0001
L-Dopa	**0**	-1	**-8**	-10	**-11**	-12	**-11**	-12	**-7**	-9	**<0.0001**	<0.0001
Fumarate	**2**	1	**-11**	-13	**-9**	-9	**7**	-1	**8**	3	**0.04**	0.21
Uridine	**4**	3	**-5**	-7	**-9**	-9	**-14**	-15	**-23**	-28	**<0.0001**	<0.0001
Urea	**3**	1	**4**	1	**5**	4	**-4**	-6	**-24**	-27	**<0.0001**	<0.0001
MUFA	**-25**	-25	**49**	46	**77**	76	**76**	75	**63**	59	**<0.0001**	<0.0001
TAG	**-7**	-9	**123**	123	**159**	162	**146**	147	**136**	134	**<0.0001**	<0.0001

N-Ac-Glc	**5**	4	**17**	16	**26**	27	**41**	41	**60**	59	**<0.0001**	<0.0001
DAG	**15**	13	**6**	8	**4**	6	**12**	12	**19**	14	**0.001**	0.02
PEP	**10**	7	**22**	20	**30**	30	**16**	15	**-21**	-21	**<0.0001**	<0.0001
N-Ac-Gal	**16**	21	**15**	13	**13**	18	**-22**	-23	**-61**	-56	**<0.0001**	<0.0001
β-Glucose	**38**	39	**15**	14	**11**	12	**17**	17	**13**	13	**<0.0001**	<0.0001
4.60 ppm*	**-27**	-25	**-24**	-27	**-18**	-20	**-8**	-12	**27**	31	**<0.0001**	<0.0001
Malic acid	**-7**	-3	**-31**	-33	**-39**	-39	**-39**	-39	**-41**	-37	**<0.0001**	<0.0001
Ascorbate	**2**	10	**-27**	-28	**-27**	-28	**-21**	-22	**-19**	-9	**<0.0001**	<0.0001
DAGPL	**-25**	-19	**-46**	-48	**-47**	-48	**-41**	-43	**-22**	-16	**<0.0001**	<0.0001
PC	**-18**	-17	**-3**	-6	**2**	1	**-1**	-3	**1**	1	**<0.0001**	<0.0001
GPC	**-7**	-7	**-14**	-16	**-14**	-17	**-14**	-16	**-10**	-11	**<0.0001**	<0.0001
Lactate	**36**	33	**5**	0	**6**	2	**-1**	-4	**-7**	-11	**<0.0001**	<0.0001
Glycerol	**11**	10	**26**	20	**34**	32	**1**	-1	**-45**	-46	**<0.0001**	<0.0001
Glycine	**-3**	-4	**-7**	-11	**-12**	-13	**-20**	-21	**-30**	-33	**<0.0001**	<0.0001
α-Glucose	**36**	36	**16**	14	**12**	13	**18**	18	**12**	11	**<0.0001**	<0.0001
Taurine	**-1**	-2	**-10**	-11	**-14**	-13	**-17**	-17	**-25**	-26	**<0.0001**	<0.0001
Choline, SM	**-17**	-18	**-9**	-10	**-3**	-4	**3**	2	**1**	1	**<0.0001**	<0.0001
TMAO	**-4**	-5	**-10**	-12	**-14**	-14	**-15**	-16	**-18**	-21	**0.03**	0.007
Malonate	**-1**	-2	**-4**	-6	**-7**	-6	**-8**	-7	**-7**	-8	**0.80**	0.81
Creatinine	**-2**	-2	**-3**	-3	**-4**	-4	**-4**	-4	**-5**	-6	**0.09**	0.04
Creatine	**31**	33	**29**	26	**19**	23	**15**	12	**8**	9	**<0.0001**	<0.0001
Lipoic acid	**-5**	-5	**-15**	-17	**-39**	-38	**-58**	-59	**-72**	-72	**<0.0001**	<0.0001
PUFA	**-27**	-27	**38**	35	**60**	59	**62**	61	**59**	58	**<0.0001**	<0.0001
Citrate	**28**	30	**27**	25	**41**	41	**22**	22	**2**	2	**0.01**	0.01
Glutamine	**-1**	-1	**-17**	-18	**-24**	-24	**-24**	-24	**-26**	-28	**<0.0001**	<0.0001
Pyruvate	**67**	61	**36**	29	**38**	35	**32**	28	**41**	33	**<0.0001**	<0.0001

Glutamate	**1**	0	**-2**	-3	**-4**	-5	**-7**	-7	**-5**	-6	**0.21**	0.15
3-HBA	**-17**	-17	**-19**	-20	**-14**	-15	**-5**	-6	**-13**	-14	**0.007**	0.003
Acetoacetic acid	**-23**	-23	**-8**	-8	**0**	1	**9**	8	**-25**	-26	**0.02**	0.02
Acetone	**5**	-1	**108**	96	**139**	132	**146**	134	**97**	87	**<0.0001**	<0.0001
Acetate	**-18**	-20	**-37**	-39	**-44**	-44	**-48**	-48	**-74**	-74	**<0.0001**	<0.0001
Lysine	**-3**	-3	**-9**	-11	**-18**	-19	**-23**	-24	**-30**	-32	**<0.0001**	<0.0001
Proline	**-3**	-3	**-10**	-11	**-18**	-18	**-22**	-23	**-24**	-26	**<0.0001**	<0.0001
Arginine	**-6**	-6	**-9**	-10	**-20**	-20	**-30**	-31	**-39**	-39	**<0.0001**	<0.0001
Free FA	**-29**	-30	**221**	206	**266**	260	**226**	219	**222**	208	**<0.0001**	<0.0001
Alanine	**16**	15	**-3**	-6	**-11**	-11	**-10**	-10	**-10**	-13	**<0.0001**	<0.0001
Ethanol	**-45**	-46	**-31**	-35	**-34**	-32	**-35**	-34	**-36**	-35	**<0.0001**	<0.0001
Isobutyrylglycine	**-12**	-13	**-29**	-30	**-42**	-42	**-55**	-55	**-70**	-71	**<0.0001**	<0.0001
Isobutyrate & Carnitine	**-7**	-7	**-9**	-11	**-16**	-18	**-31**	-32	**-41**	-42	**<0.0001**	<0.0001
Valine	**-3**	-3	**-9**	-11	**-18**	-17	**-25**	-26	**-33**	-35	**<0.0001**	<0.0001
Isoleucine	**-14**	-14	**-28**	-30	**-39**	-39	**-44**	-44	**-33**	-35	**<0.0001**	<0.0001
Leucine	**-7**	-8	**-5**	-7	**-8**	-9	**-11**	-13	**-11**	-14	**0.004**	0.000
Lipoprotein	**-30**	-30	**30**	28	**52**	52	**60**	58	**64**	60	**<0.0001**	<0.0001
Total Cholesterol	**-32**	-35	**1**	0	**20**	18	**33**	30	**38**	28	**<0.0001**	<0.0001

Table S3 Early prediction of hypoxaemia. *Partial least squares (PLS) regression and partial least squares –discriminant analysis (PLS-DA) results. The results show both the calibrated (Cal) and Venetian-Blinds cross-validated (CV) coefficient of association (R^2), specificity, sensitivity, and class error. Each model was built on 100 samples collected from 50 patients from the left atrium and pulmonary artery at one of the mentioned time points. For validation, the Venetian-Blinds cross-validation with 10 segments was applied. 10 consecutive samples were removed and a model was created. Subsequently, the 10 samples were predicted. This procedure was repeated until all samples were removed once. Permutation testing was applied for each model. A p-value ≤ 0.004 was considered significant.*

	PLS regression (Metabolome vs. PaO_2)					**PLS-DA (Metabolome vs. Outcome (hypoxaemia: yes/no))**						
	R^2		**RMSE**		**Permutation**	**Sensitivity**		**Specificity**		**Class error**		**Permutation**
	Cal	**CV**	**Cal**	**CV**	**p-value**	**Cal**	**CV**	**Cal**	**CV**	**Cal**	**CV**	**p-value**
Pre-CPB	0.94	0.707	0.33	0.75	0.004	0.83	0.78	0.95	0.84	0.11	0.19	0.004
0 hour	0.98	0.918	0.15	0.4	<0.001	1	0.88	0.98	0.92	0.01	0.1	<0.001
2 hours	0.98	0.934	0.13	0.36	<0.001	1	0.91	0.98	0.94	0.01	0.1	<0.001
4 hours	0.98	0.954	0.12	0.3	<0.001	0.97	0.88	0.92	0.88	0.06	0.12	<0.001
8 hours	0.98	0.941	0.13	0.34	<0.001	0.97	0.94	0.97	0.92	0.03	0.07	<0.001
20 hours	0.98	0.903	0.19	0.44	<0.001	0.96	0.93	0.97	0.91	0.04	0.08	<0.001