Supporting Information

for

Effects of Workers Exposure to Nanoparticles Studied by NMR Metabolomics

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Content

Table S1. Basic characteristics of the samples studied. Data are reported as mean ± standard deviation, or count.

		pre-shift (n=20)	post-shift (n=20)	control (n=20)
age	years	41.8 ± 11.3		43.3 ± 11.5
female		5		7
male		15		13
working with NPs	years	6.5 ± 3.9		-
daily exposure	h/day	1.7 ± 1.0		-
exposure on the sampling day	min	156.3 ± 61.9		-
weight	kg	87.5 ± 23.0		77.7 ± 16.7
height	cm	176.1 ± 9.9		175.7 ± 8.8
BMI	kg/m2	28.0 ± 6.2		25.1 ± 4.8
alcohol comsumption	Yes : No	18 : 2		17 : 3
smoking	Yes : No	1 : 19		2 : 18
physical activity	Yes : No	15 : 5		13 : 7
medication	Yes : No	9 : 11		8 : 12
Letrox (thyroid hormone)	Yes : No	2 : 18		1 : 19
Tulip (statin)	Yes : No	1 : 19		1 : 19
Vit E	Yes : No	0 : 20		2 : 18
Warfarin (anticoagulant)	Yes : No	0 : 20		1 : 19
Antihypertensives	Yes : No	5 : 15		3 : 17
Vit B	Yes : No	2 : 18		2 : 18
Vit C	Yes : No	4 : 16		1 : 19
bronchial pneumonia	Yes : No	15 : 5		10 : 10
hypothyroidism	Yes : No	3 : 17		2 : 18
cardiac arrhythmia	Yes : No	2 : 18		1 : 19
hypertension	Yes : No	10 : 10		6 : 14
hypercholesterolemia	Yes : No	5 : 15		3 : 17
gout	Yes : No	2 : 18		1 : 19
urinary tract infection	Yes : No	4 : 16		4 : 16
factor V Leiden	Yes : No	1 : 19		0 : 20
hyperbilirubinemia	Yes : No	3 : 17		0 : 20
asthma	Yes : No	1 : 19		1 : 19
allergic rhinitis	Yes : No	9 : 11		9 : 11
cough	Yes : No	5 : 15		1 : 19
shortness of breath	Yes : No	2 : 18		0 : 20
cold	Yes : No	1 : 19		1 : 19
FVC	L	4.33 ± 1.02	4.33 ± 0.91	4.38 ± 1.05
FEV	L	3.19 ± 0.95	3.73 ± 0.81	3.85 ± 0.98
LTB4	pg/mL	36.2 ± 11.5	36.0 ± 11.0	26.2 ± 6.8
LTD4	pg/mL	30.0 ± 5.1	27.2 ± 4.0	26.3 ± 3.4
LTE4	pg/mL	39.0 ± 7.3	39.9 ± 7.7	34.5 ± 5.7
LXB4	pg/mL	59.2 ± 4.1	60.3 ± 6.8	64.5 ± 4.3
IL 9	pg/mL	14.7 ± 1.0	15.9 ± 1.4	16.3 ± 1.4
IL 10	pg/mL	33.9 ± 2.2	36.2 ± 2.8	35.0 ± 2.8
MDA	ng/mL	23.3 ± 6.1	28.0 ± 9.4	18.5 ± 4.6

		pre-shift (n=20)	post-shift (n=20)	control (n=20)
HNE	ng/mL	15.3 ± 5.2	27.7 ± 11.7	19.7 ± 5.2
HHE	ng/mL	18.9 ± 6.0	26.8 ± 12.8	20.8 ± 4.4
C6-C13 aldehydes (even no. of C)	ng/mL	4.4 ± 3.4	5.0 ± 3.6	2.2 ± 1.0
C6-C13 aldehydes (odd no. of C)	ng/mL	6.2 ± 3.7	6.6 ± 4.3	3.1 ± 1.3
8-isoprostane	pg/mL	32.2 ± 11.2	33.4 ± 11.8	25.2 ± 5.8
8-OHdG	pg/mL	28.8 ± 7.1	29.0 ± 6.4	21.5 ± 7.5
8-OHG	pg/mL	37.1 ± 11.6	37.2 ± 11.5	21.2 ± 9.1
5-OHMeU	pg/mL	28.0 ± 6.5	26.9 ± 5.9	20.7 ± 6.2
o-Tyr	pg/mL	32.1 ± 9.6	32.2 ± 9.3	23.3 ± 8.4
3-ClTyr	pg/mL	33.8 ± 10.6	33.6 ± 10.3	24.0 ± 7.2
NOTyr	pg/mL	58.6 ± 10.3	58.0 ± 9.7	52.8 ± 7.6

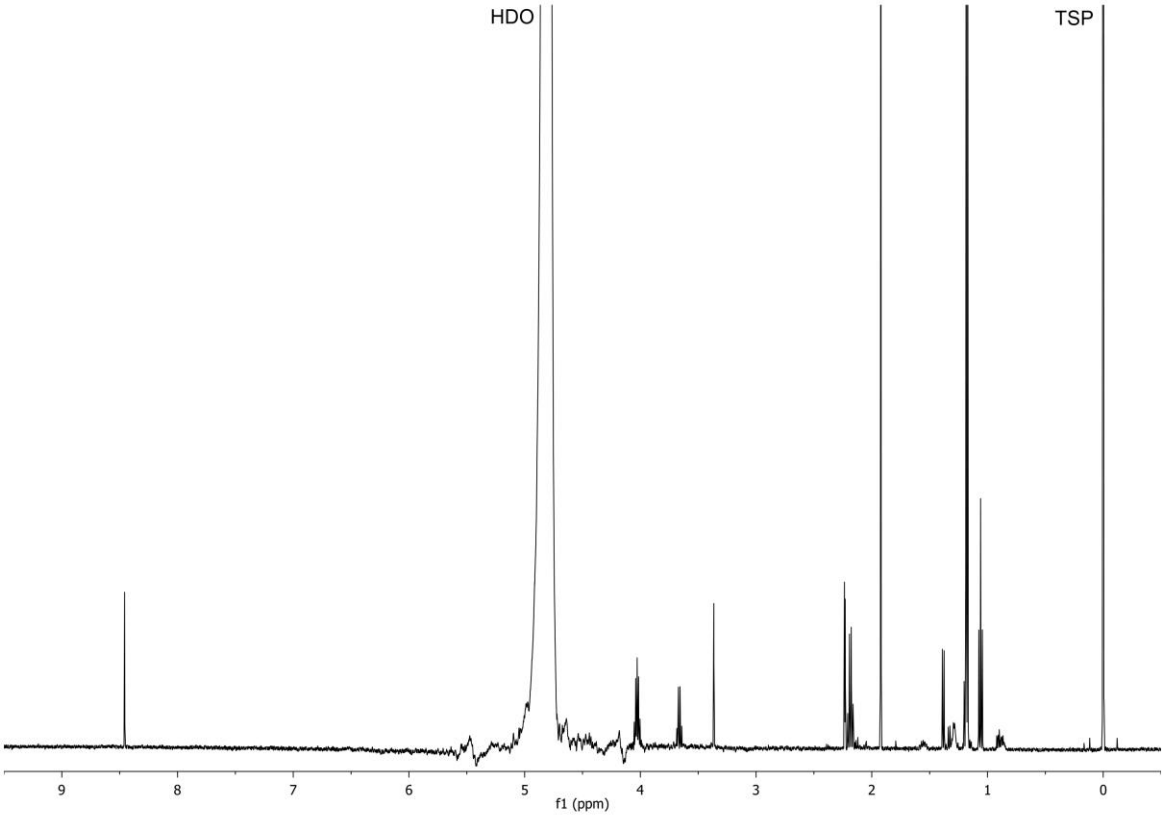


Figure S1. ^{1}H NMR spectrum of a representative EBC sample.

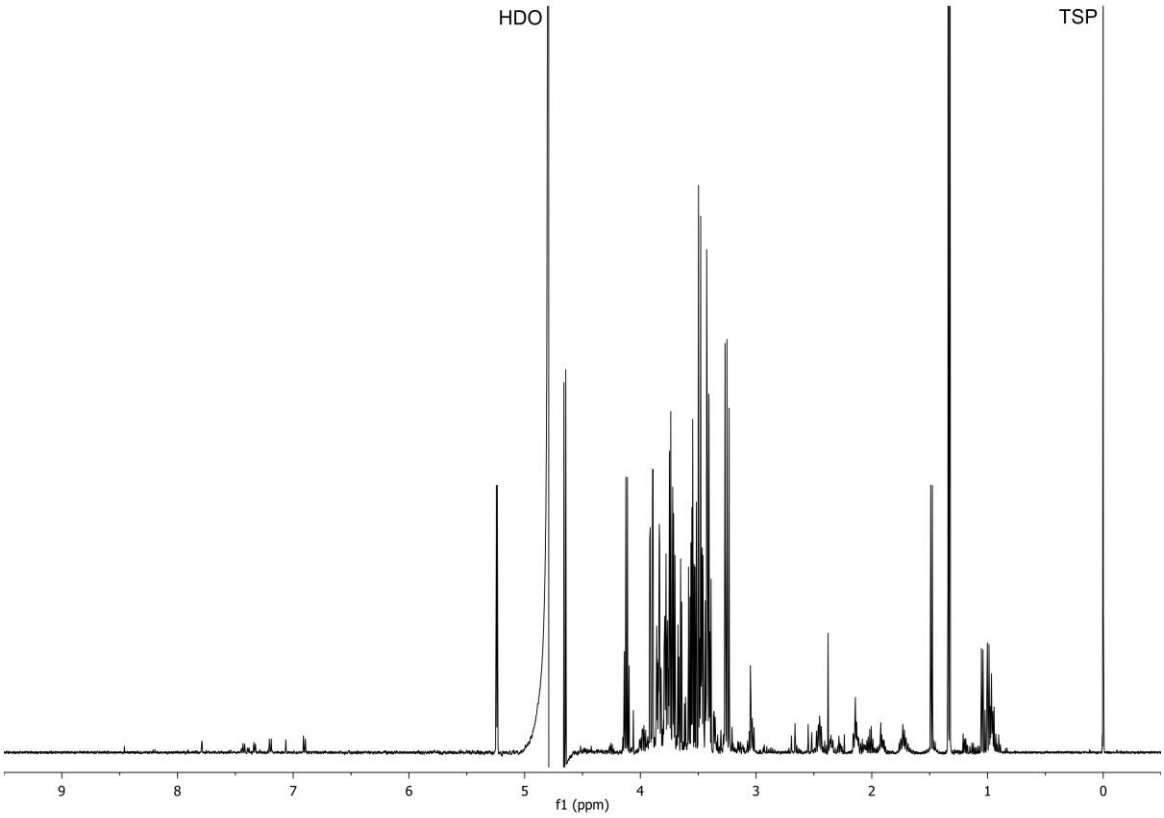


Figure S2. ^{1}H NMR spectrum of a representative blood plasma sample.

Table S2. Adjusted *P*-values after Benjamini-Hochberg correction obtained from Wilcoxon test for EBC samples.

	pre-shift vs HC*	post-shift vs HC*	pre-shift vs post-shift**
Acetate	0.0880	0.1735	1.0000
Acetoin	**0.0010**	**0.0162**	**0.0203**
Acetone	0.0880	**0.0162**	0.5404
Butyrate	0.2036	0.1735	0.6791
Dimethylamine	0.7331	**0.0162**	**0.0025**
Ethanol	0.5021	0.4641	0.6138
Formate	0.1608	0.4232	0.6138
Glycerol	0.0586	0.2842	0.8725
Isobutyrate	0.8287	1.0000	0.6791
Isopropanol	0.1514	**0.0162**	0.1074
Isovalerate	0.1998	0.2842	1.0000
Lactate	**0.0418**	0.2842	0.6138
Methanol	0.0586	0.5463	0.5404
Propionate	**0.0031**	**0.0309**	0.3649
Propylene glycol	0.7649	0.9560	0.8725

*Wilcoxon rank-sum test; **Wilcoxon signed-rank test

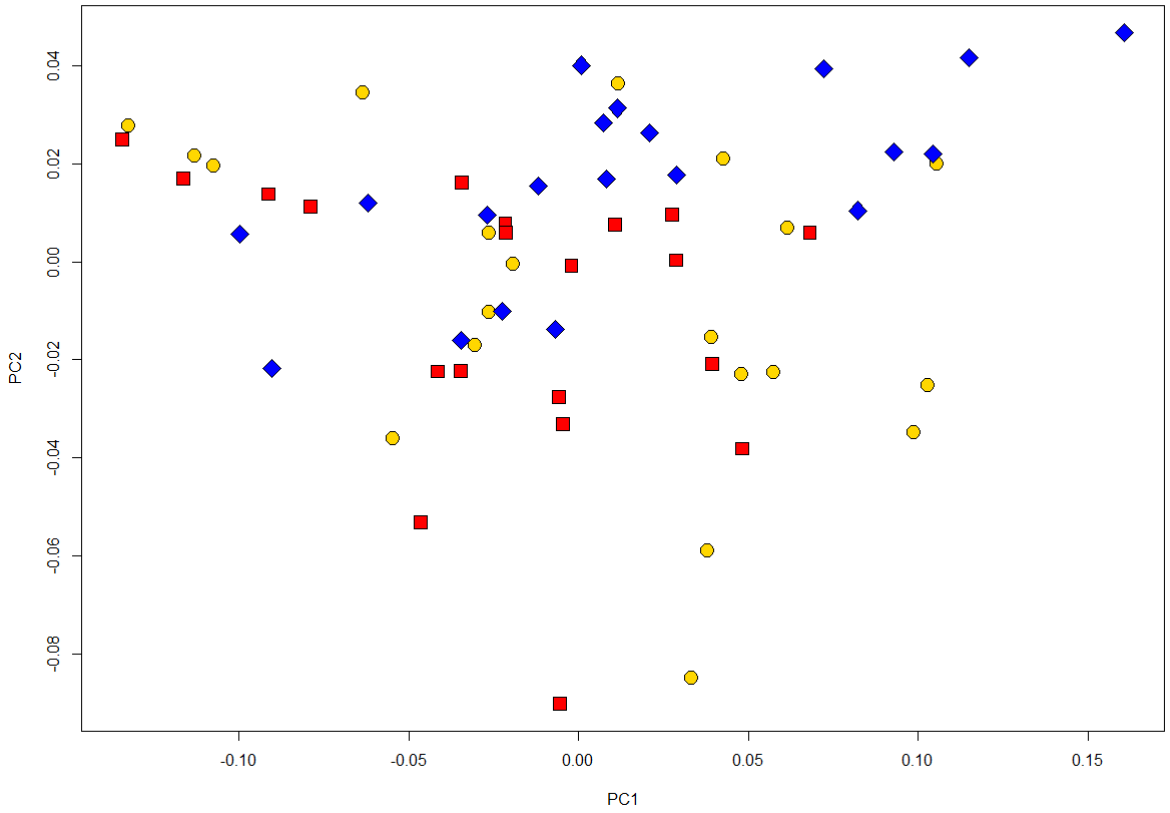


Figure S3. Principal component analysis for EBC samples; pre-shift subjects (yellow circles), post-shift subjects (red squares), and healthy controls (blue diamonds).

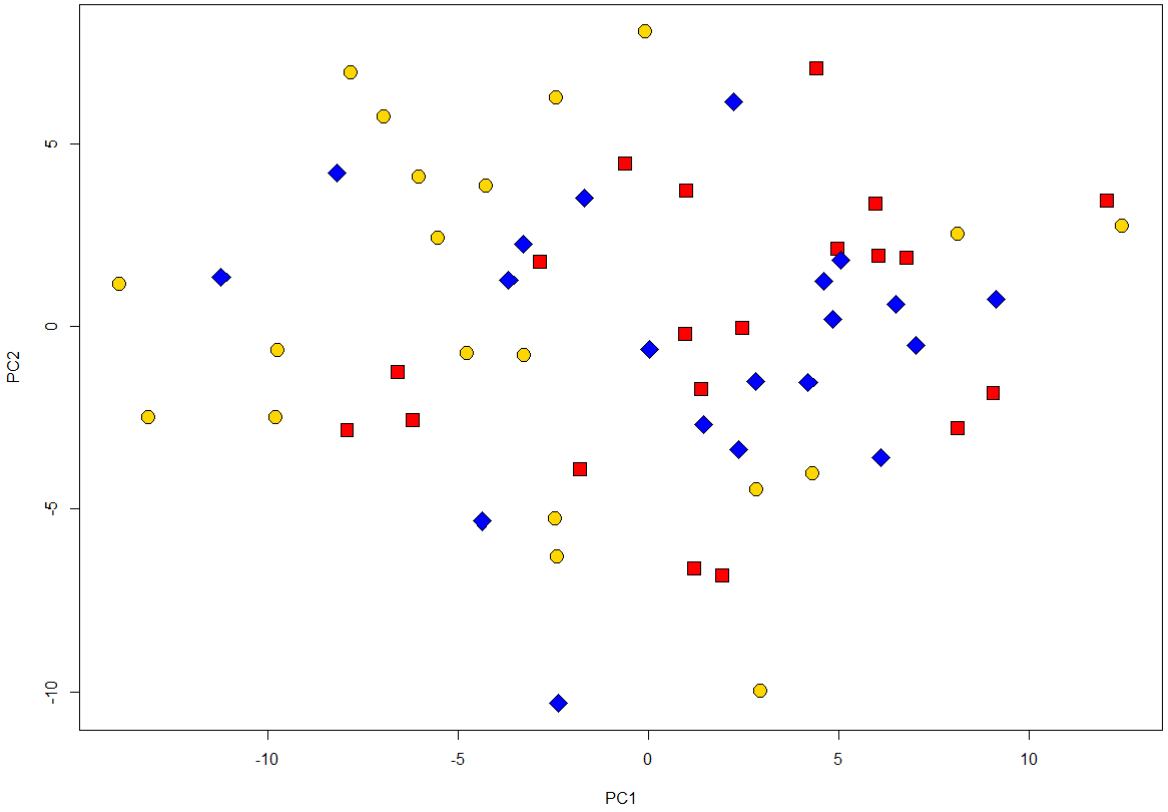


Figure S4. Principal component analysis for blood plasma samples; pre-shift subjects (yellow circles), post-shift subjects (red squares), and healthy controls (blue diamonds).

Table S3. Adjusted *P*-values after Benjamini-Hochberg correction obtained from Wilcoxon test for blood plasma samples.

	pre-shift vs HC*	post-shift vs HC*	pre-shift vs post-shift**
2-Hydroxybutyrate	0.5304	0.2657	**0.0024**
2-Hydroxyisobutyrate	0.5304	0.4103	0.8581
2-Hydroxyisovalerate	0.9148	0.8232	0.2688
2-Oxoisocaproate	0.9712	**0.0314**	**0.0024**
3-Hydroxybutyrate	0.5775	0.1846	**0.0024**
3-Hydroxyisovalerate	0.5304	0.2533	0.6905
3-Methyl-2-oxovalerate	1.0000	0.1764	**0.0024**
Acetate	0.5304	0.6726	0.7045
Acetoacetate	0.9850	0.8165	0.6747
Acetone	**0.0060**	**0.0314**	0.2112
Alanine	0.6113	0.4329	0.6747
Arginine	0.5304	0.7425	0.6744
Ascorbate	0.5621	0.5098	1.0000
Asparagine	0.8262	0.9893	0.7196
Betaine	0.4423	0.3703	0.9141

	pre-shift vs HC*	post-shift vs HC*	pre-shift vs post-shift**
Carnitine	0.5304	0.5652	0.7836
Citrate	0.6113	0.8097	0.3499
Creatine	0.8262	0.5652	0.1784
Creatinine	0.9850	0.6858	0.6747
Cystine	0.0652	0.0812	0.9141
Dimethyl sulfone	0.8262	0.7425	0.2688
Dimethylamine	0.9148	0.6858	0.6747
Formate	0.6113	0.6726	0.5609
Glucose	0.5304	0.6072	0.1825
Glutamate	0.0512	**0.0372**	0.5015
Glutamine	0.0652	0.1697	0.7196
Glycine	0.4803	0.4835	0.9141
Glycolate	0.8262	0.4103	**0.0498**
Histidine	0.5775	0.6072	0.7354
Hypoxanthine	0.0563	0.6376	0.6747
Choline	0.8262	0.6726	0.8581
Isobutyrate	0.2182	0.6901	**0.0119**
Isoleucine	0.8262	0.8097	0.6600
Isopropanol	0.2256	0.6428	**0.0123**
Lactate	0.1496	0.6726	**0.0128**
Leucine	0.5621	0.6901	0.8581
Lysine	0.5621	0.4835	0.8581
Mannose	0.5652	**0.0388**	0.1038
Methanol	0.8262	0.9417	0.7045
Methionine	0.9712	0.6483	0.4893
myo-Inositol	0.5304	0.6901	**0.0324**
N.N-Dimethylglycine	0.6113	0.7425	0.6747
O-Acetylcarnitine	0.5304	**0.0388**	0.2444
Ornithine	0.6244	0.8809	0.3604
Phenylalanine	0.6244	0.6072	0.8581
Proline	0.8262	0.8869	0.7045
Propylene glycol	0.8262	**0.0314**	**0.0098**
Pyruvate	0.2256	**0.0314**	0.8581
Sarcosine	0.4938	0.3898	0.9141
Serine	0.6113	0.8097	0.6747
Succinate	0.9148	0.3559	0.2112
Threonine	0.9850	0.3703	0.2444
Trimethylamine	0.8262	0.8748	0.7196
Trimethylamine oxide	0.6113	0.6901	0.6095
Tryptophan	0.5304	0.4801	**0.0024**
Tyrosine	0.5775	0.9146	0.2688
Urea	0.8076	0.7425	0.8581
Valine	0.8262	0.6072	0.5476

*Wilcoxon rank-sum test; **Wilcoxon signed-rank test

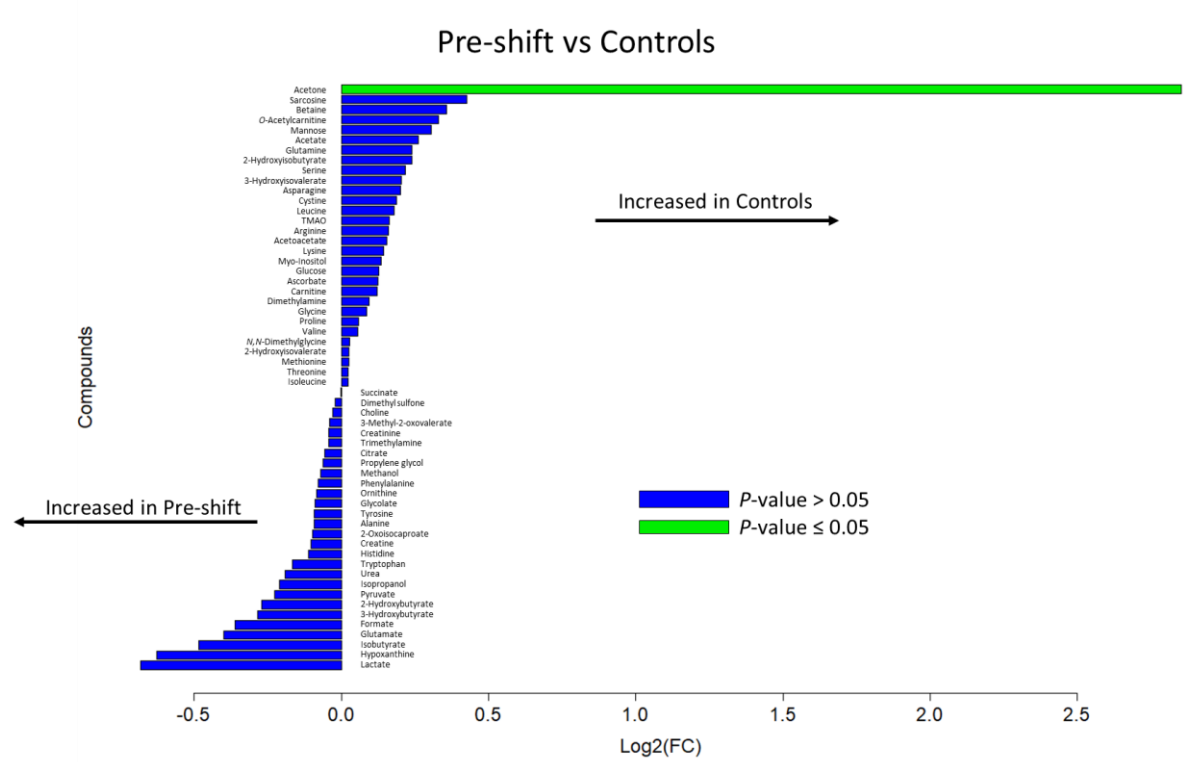


Figure S5. Fold change projection depicting differences in levels of individual metabolites observed in blood plasma samples between pre-shift subjects and healthy controls.

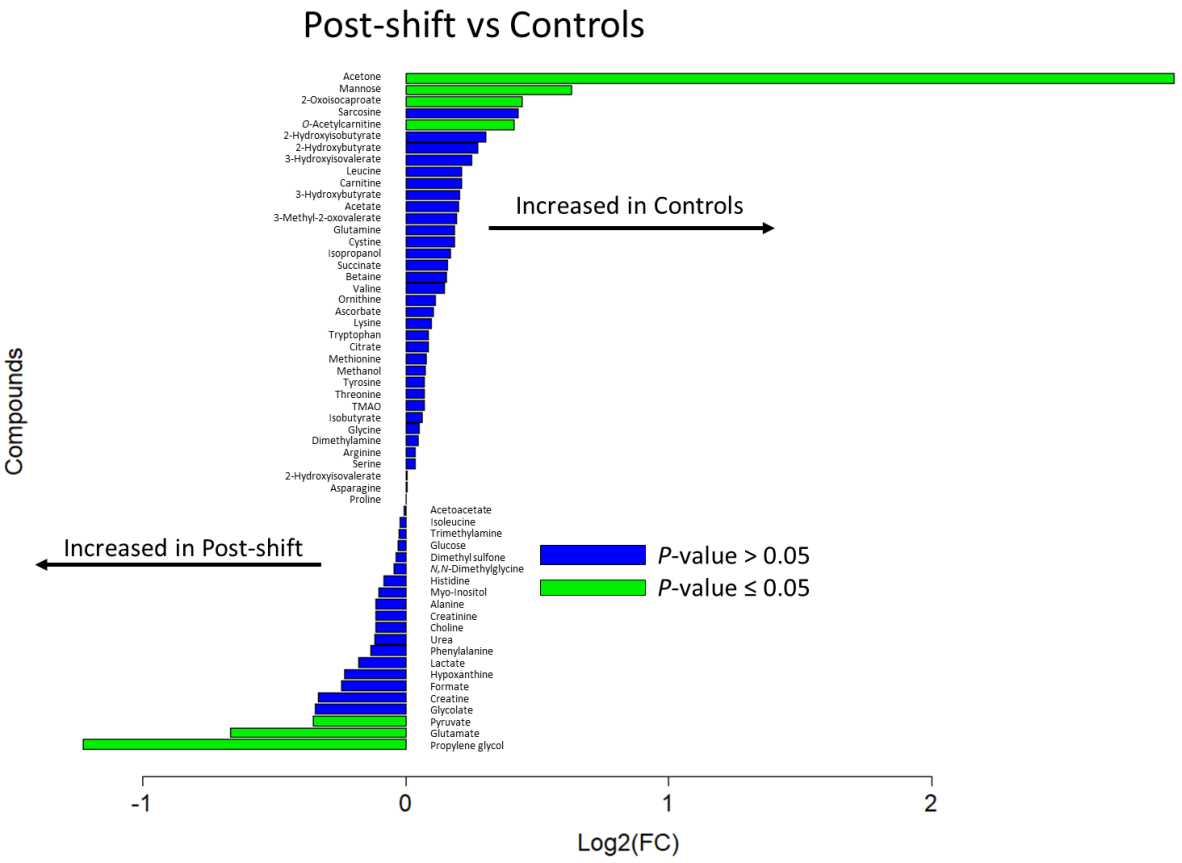


Figure S6. Fold change projection depicting differences in levels of individual metabolites observed in blood plasma samples between post-shift subjects and healthy controls.

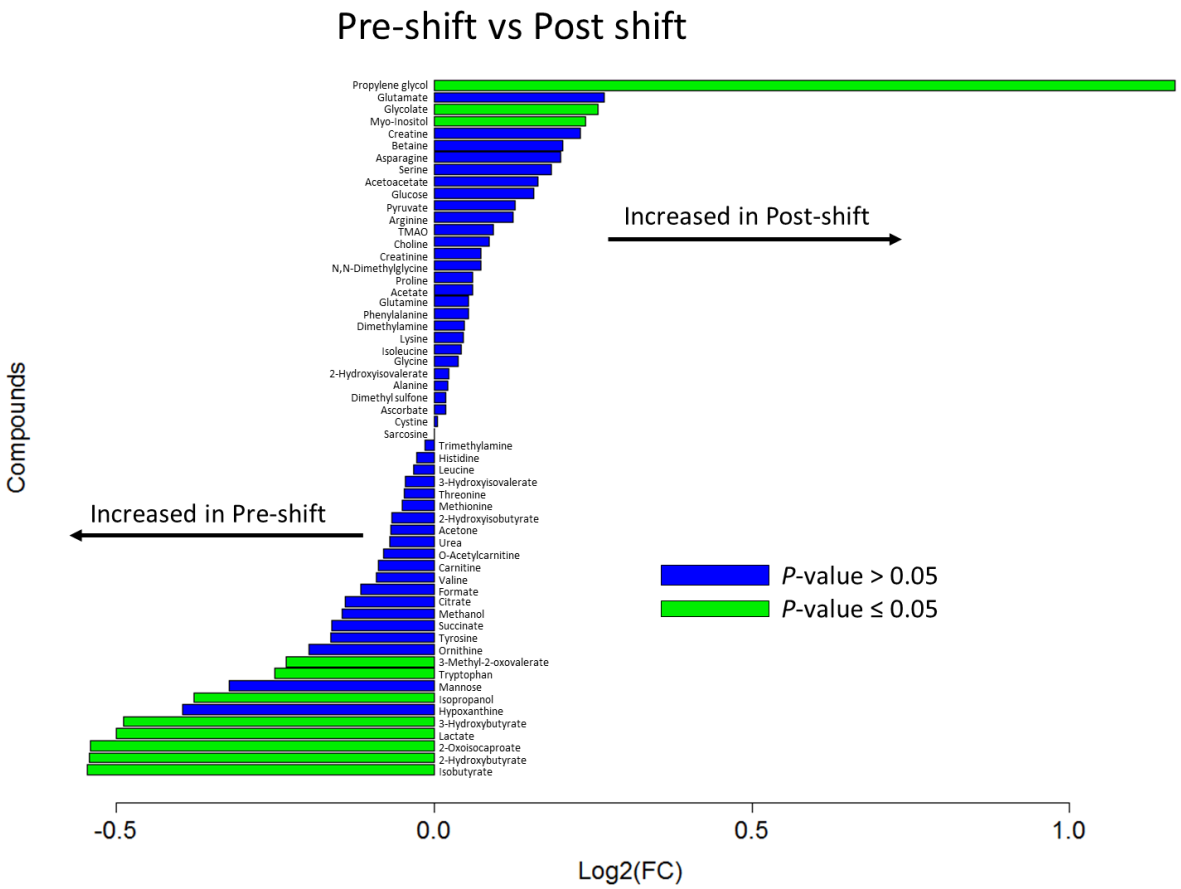
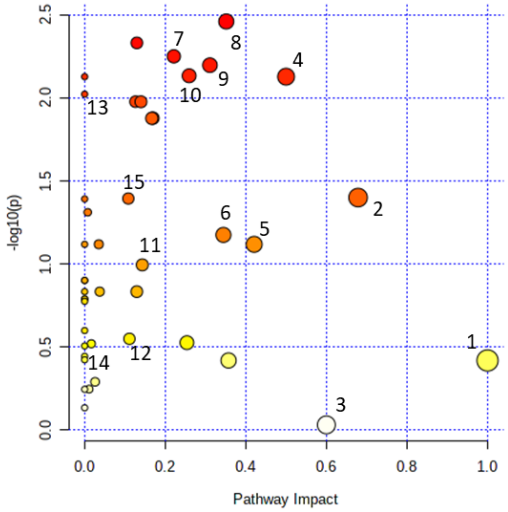
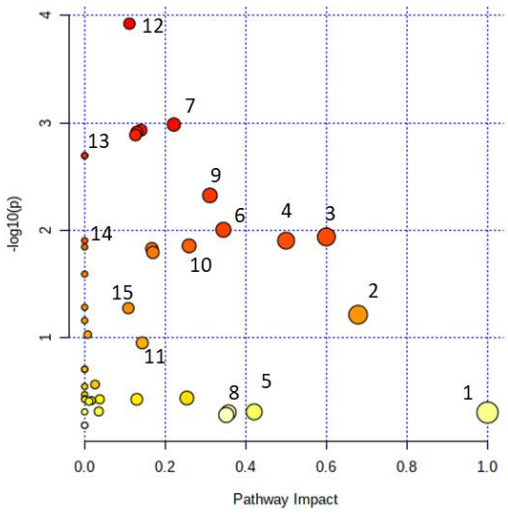


Figure S7. Fold change projection depicting differences in levels of individual metabolites observed in blood plasma samples between pre-shift subjects and post-shift subjects.

a) Pre-shift vs Control



b) Post-shift vs Control



c) Pre-shift vs Post-shift

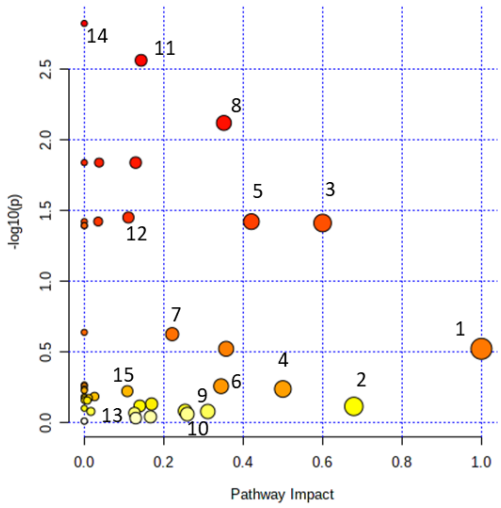


Figure S8. Metabolic pathway analysis of the group discrimination performed on plasma metabolic profiles. The legend to the pathway labelling is given in **Table S3**.

Table S4. Analysis of alteration of the most influenced metabolic pathways performed on plasma metabolic profiles; a) pre-shift vs control, b) post-shift vs control, c) pre-shift vs post-shift

No.	Metabolic pathway	-log10(p)		
		a	b	c
1	Phenylalanine, tyrosine and tryptophane biosynthesis	0.42	0.30	0.52
2	Glycine, serine and threonine metabolism	1.40	1.2	0.12
3	Synthesis and dergradation of ketone bodies	0.03	1.94	1.41
4	D-glutamine and D-glutamate metabolism	2.13	1.90	0.24
5	Starch and sucrose metabolism	1.12	0.42	1.42
6	Arginine and proline metabolism	1.18	2.00	0.26
7	Histidine metabolism	2.25	2.98	0.63
8	Pyruvate metabolism	2.46	0.27	2.12
9	Alanine, aspartate and glutamate metabolism	2.20	2.30	0.08
10	Glyoxylate and dicarboxylate metabolism	2.13	1.85	0.06
11	Tryptophan metabolism	0.99	0.95	2.56
12	Butanoate metabolism	0.55	3.92	1.45
13	Cysteine and methionine metabolism	1.98	2.89	0.07
14	Propanoate metabolism	0.42	1.84	2.82
15	Glutathione metabolism	1.39	1.27	0.23

Table S5. Adjusted *P*-values after Benjamini-Hochberg correction obtained from Wilcoxon test for EBC samples after exclusion of smoking subjects.

	pre-shift vs HC*	post-shift vs HC*	pre-shift vs post-shift**
Acetate	0.0511	0.1368	0.9563
Acetoin	**0.0010**	**0.0183**	**0.0400**
Acetone	0.1254	**0.0183**	0.5165
Butyrate	0.2864	0.1368	0.9563
Dimethylamine	0.6485	**0.0183**	**0.0063**
Ethanol	0.3729	0.4753	0.9563
Formate	0.1254	0.3108	0.8275
Glycerol	0.0557	0.1368	0.9653
Isobutyrate	0.6485	0.9420	0.9563
Isopropanol	0.1254	**0.0183**	0.0618
Isovalerate	0.1872	0.3825	0.9653
Lactate	0.0511	0.3108	0.8006
Methanol	0.0511	0.4753	0.5165
Propionate	**0.0062**	**0.0183**	0.5165
Propylene glycol	0.5446	0.9878	0.9563

*Wilcoxon rank-sum test; **Wilcoxon signed-rank test

Table S6. Adjusted *P*-values after Benjamini-Hochberg correction obtained from Wilcoxon test for blood plasma samples after exclusion of smoking subjects.

	pre-shift vs HC*	post-shift vs HC*	pre-shift vs post-shift**
2-Hydroxybutyrate	0.5690	0.3136	**0.0049**
2-Hydroxyisobutyrate	0.6298	0.6044	0.9217
2-Hydroxyisovalerate	0.9570	0.9738	0.3572
2-Oxoisocaproate	0.9570	0.0618	**0.0039**
3-Hydroxybutyrate	0.6110	0.2206	**0.0039**
3-Hydroxyisovalerate	0.3328	0.2206	0.8757
3-Methyl-2-oxovalerate	0.9570	0.2717	**0.0039**
Acetate	0.3555	0.5075	0.8032
Acetoacetate	0.9880	0.8908	0.7663
Acetone	**0.0041**	**0.0319**	0.2403
Alanine	0.5690	0.2538	0.6373
Arginine	0.5690	0.8180	0.7478
Ascorbate	0.5690	0.4623	0.9217
Asparagine	0.9570	0.9332	0.7294
Betaine	0.2851	0.2206	0.9217
Carnitine	0.3328	0.3160	0.8919
Citrate	0.6298	0.9332	0.4586
Creatine	0.6110	0.7704	0.2110
Creatinine	0.9880	0.7704	0.6373
Cystine	0.1306	0.1996	0.9217
Dimethyl sulfone	0.9880	0.7314	0.2983

	pre-shift vs HC*	post-shift vs HC*	pre-shift vs post-shift**
Dimethylamine	0.9880	0.8908	0.7110
Formate	0.7997	0.8908	0.4728
Glucose	0.4042	0.6044	0.1846
Glutamate	0.0563	0.0618	0.6305
Glutamine	0.0585	0.1143	0.8215
Glycine	0.5690	0.6254	0.9217
Glycolate	0.7039	0.2717	0.0874
Histidine	0.6298	0.7704	0.7110
Hypoxanthine	**0.0333**	0.6110	0.6373
Choline	0.9570	0.7704	0.8919
Isobutyrate	0.2162	0.8908	**0.0206**
Isoleucine	0.9880	0.7704	0.6373
Isopropanol	0.2072	0.8180	**0.0215**
Lactate	0.0954	0.6928	**0.0168**
Leucine	0.6571	0.8908	0.9217
Lysine	0.5690	0.5075	0.9217
Mannose	0.5690	0.0585	0.1026
Methanol	0.9570	0.9810	0.7848
Methionine	0.9880	0.7704	0.6305
myo-Inositol	0.3328	0.8908	**0.0266**
N.N-Dimethylglycine	0.6387	0.8859	0.6373
O-Acetylcarnitine	0.2999	**0.0319**	0.3461
Ornithine	0.6298	0.9663	0.4320
Phenylalanine	0.6571	0.6180	0.8919
Proline	0.6666	0.9332	0.6373
Propylene glycol	0.9728	0.0618	**0.0168**
Pyruvate	0.1273	**0.0157**	0.9217
Sarcosine	0.3328	0.5075	0.9217
Serine	0.8992	0.9332	0.8397
Succinate	0.9728	0.3136	0.0742
Threonine	0.9880	0.5075	0.3461
Trimethylamine	0.6298	0.7704	0.8578
Trimethylamine oxide	0.9880	1.0000	0.6305
Tryptophan	0.5690	0.4623	**0.0039**
Tyrosine	0.6298	0.9332	0.4450
Urea	0.9570	0.8908	0.9217
Valine	0.9880	0.7704	0.6373

*Wilcoxon rank-sum test; **Wilcoxon signed-rank test

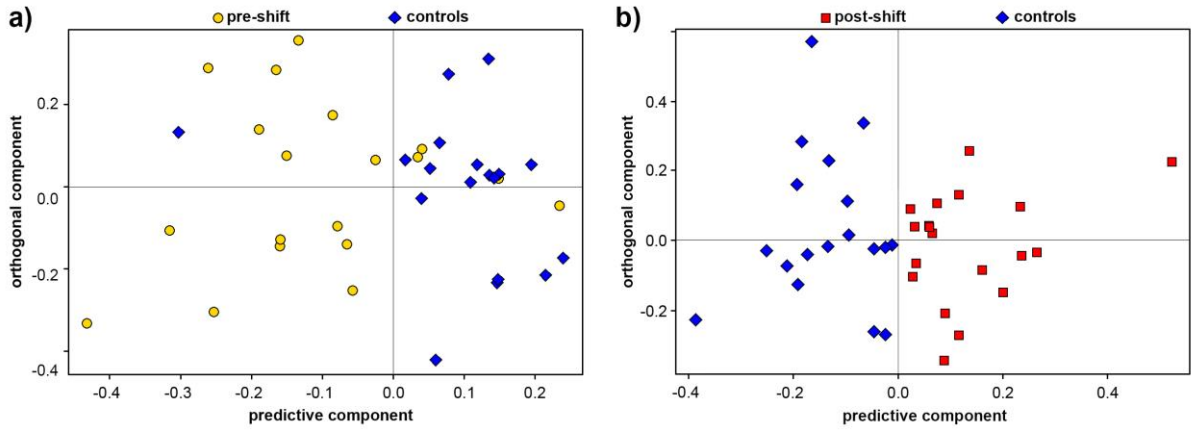


Figure S9. OPLS-DA of pre-shift subjects (yellow circles, 19) and healthy controls (blue diamonds, 18) using 320 bins from EBC samples after exclusion of smoking subjects; Acc. 89.7%, Sen. 82.8%, Spe. 97.0% (a). OPLS-DA of post-shift subjects (red squares, 19) and healthy controls (blue diamonds, 18) using 320 bins from EBC samples after exclusion of smoking subjects; Acc. 85.1%, Sen. 88.7%, Spe. 81.3% (b).

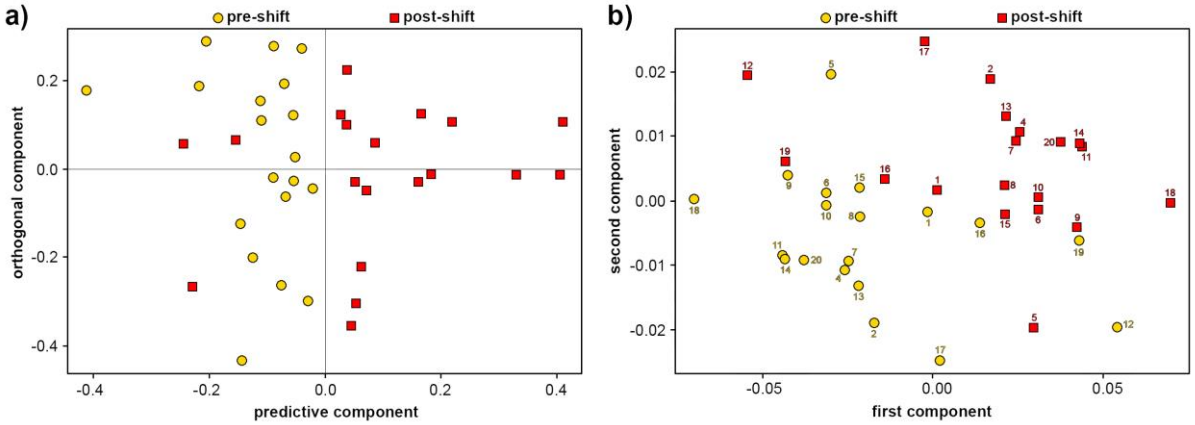


Figure S10. OPLS-DA of pre-shift (yellow circles, 19) and post-shift subjects (red squares, 19) after exclusion of smoking subjects; Acc. 79.6%, Sen. 81.2%, Spe. 78.0% (a). Multilevel partial least squares (mPLS) analysis of pre-shift (yellow circles, 19) and post-shift subjects (red squares, 19) after exclusion of smoking subjects; Acc. 80% (b). Both using 320 bins in each EBC sample.

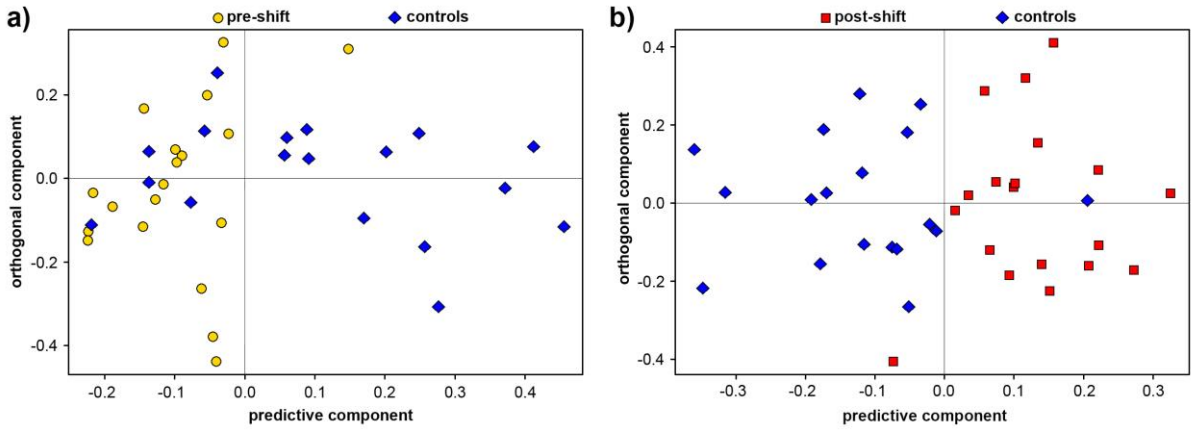


Figure S11. OPLS-DA of healthy controls (blue diamonds, 18) and pre-shift subjects (yellow circles, 19) after exclusion of smoking subjects; Acc. 80.6%, Sen. 90.4%, Spe. 70.2% (a). OPLS-DA of post-shift subjects (red squares, 19) and healthy controls (blue diamonds, 18) after exclusion of smoking subjects; Acc. 87.1%, Sen. 86.6%, Spe. 87.6% (b). Both using 58 normalized metabolites from blood plasma samples.

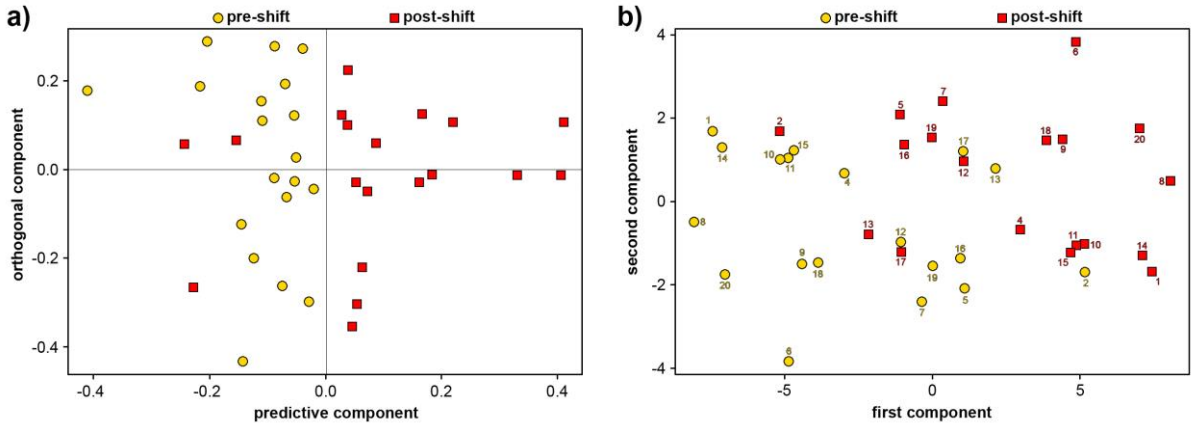


Figure S12. OPLS-DA of pre-shift (yellow circles, 19) and post-shift subjects (red squares, 19) after exclusion of smoking subjects; Acc. 76.3%, Sen. 81.5%, Spe. 71.2% (a). mPLS analysis of pre-shift (yellow circles, 19) and post-shift subjects (red squares, 19) after exclusion of smoking subjects; Acc. 88.5% (b). Both using 58 normalized metabolites from blood plasma samples.