

Postmortem metabolomics of insulin intoxications and the potential application to find hypoglycemia-related deaths

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Supplement Appendix S1: R code for XCMS pre-processing and CAMERA annotation

```
library("xcms")
library("CAMERA")
xset<-xcmsSet(method="centWave",
ppm=30,peakwidth=c(3,20),snthresh=3, noise=1000)
xset<-group(xset, mzwid=0.05,bw=3)
xset2<-retcor(xset,method="obiwarp",response=10,plottype="d")
xset2<-group(xset2, mzwid=0.05,bw=3,minfrac=0.5)
xset3<-fillPeaks(xset2)
an<-xsAnnotate(xset3)
anF<-groupFWHM(an, perfwhm=0.6)
anC<-groupCorr(anF)
anFI<-findIsotopes(anC)
anFA<-findAdducts(anFI, polarity="positive")
write.csv(getPeaklist(anFA), file = "result_table.csv")
```

Supplement Table S1: XCMS and CAMERA parameters

| Step | Parameter | Value |
|-----------|-----------|-------------|
| xcmsSet | method | centWave |
| | ppm | 30 |
| | peakwidth | 3, 20 |
| | snthresh | 3 |
| | noise | 1000 |
| group - 1 | method | peakDensity |
| | mzwid | 0.05 |
| | bw | 3 |
| retcor | method | obiwarp |
| | response | 10 |
| | plottype | d |
| group - 2 | method | peakDensity |
| | mzwid | 0.05 |
| | bw | 3 |
| | minfrac | 0.5 |
| CAMERA | perfwlm | 0.6 |

Supplement Table S2: Positive drug and alcohol use found in postmortem toxicological screening for cases included in hypo, hyper, and control groups. Hyper and Control group positivity does not reflect any diagnosed contributing factor to cause of death.

| | Hypo (n = 19) | Hyper (n = 19) * | Control (n = 38) * |
|---------------------|---------------|------------------|--------------------|
| Drug positive (any) | 74% | 63% | 66% |
| • Sleep and anxiety | 53% | 32% | 39% |
| • Antidepressants | 26% | 37% | 42% |
| • Opioids | 16% | 11% | 3% |
| • Amphetamine | 11% | 5% | 3% |
| Alcohol positive | 16% | 11% | 32% |

Frequency (%) of positive drug/alcohol findings from toxicological screening performed. Note, the frequencies included poly-drug usage. Drug positive (any) findings are listed, together with frequencies of four common drug classes found positive in samples.

* in Hyper and Control groups the drug/alcohol usage identified was **NOT** diagnosed as a contributing factor to cause of death by forensic pathologists, and may only reflect therapeutic drug use and/or normal consumption of alcohol.

Supplement Table S3: Unidentified discriminating features associated with the *hypo* group.

| Identifier | m/z | rt | Identifier | m/z | rt |
|------------|---------|---------|------------|---------|---------|
| M310T112 | 310.057 | 112.91 | M163T485 | 163.075 | 485.58 |
| M194T124 | 194.118 | 124.96 | M366T546 | 366.271 | 546.42 |
| M195T125 | 195.121 | 125.67 | M343T546 | 343.293 | 546.55 |
| M270T127 | 270.144 | 127.065 | M406T555 | 406.203 | 555.255 |
| M334T149 | 334.185 | 149.21 | M407T555 | 407.206 | 555.67 |
| M247T152 | 247.128 | 152.76 | M59T555 | 59.0494 | 555.695 |
| M194T160 | 194.117 | 160.155 | M457T560 | 457.335 | 560.12 |
| M178T164 | 178.121 | 164.1 | M456T560 | 456.332 | 560.2 |
| M208T166 | 208.133 | 166.8 | M302T563 | 302.216 | 563.27 |
| M243T170 | 243.17 | 170.29 | M464T577 | 464.337 | 577.43 |
| M194T172 | 194.117 | 172.9 | M372T589 | 372.302 | 589.88 |
| M337T191 | 337.036 | 191.04 | M701T591 | 701.559 | 591 |
| M130T239 | 130.208 | 239.56 | M312T602 | 312.16 | 602.72 |
| M575T246 | 575.34 | 246.45 | M747T606 | 747.593 | 606.79 |
| M210T251 | 210.17 | 251.32 | M701T612 | 701.559 | 612.06 |
| M302T313 | 302.196 | 313.275 | M446T613 | 446.326 | 613.35 |
| M206T369 | 206.139 | 369.59 | M720T622 | 720.567 | 622.02 |
| M250T371 | 250.165 | 371.45 | M301T622 | 301.216 | 622.24 |
| M489T386 | 489.315 | 386.3 | M787T629 | 787.605 | 629.38 |
| M268T391 | 268.211 | 391.87 | M788T629 | 788.615 | 629.505 |
| M533T399 | 533.341 | 399.73 | M450T630 | 450.349 | 630.39 |
| M296T409 | 296.206 | 409.99 | M497T633 | 497.506 | 633.02 |
| M385T414 | 385.267 | 414 | M280T642 | 280.163 | 642.275 |
| M293T436 | 293.195 | 436.4 | M734T643 | 734.569 | 643.9 |
| M294T436 | 294.199 | 436.97 | M813T647 | 813.619 | 647.71 |
| M195T448 | 195.122 | 448.5 | M797T655 | 797.619 | 655.16 |
| M402T462 | 402.284 | 462.56 | | | |

Feature unidentified due to lack of matching m/z values to HMDB database able to provide putative identification in accordance to MSI levels 2/3.

m/z – mass-to-charge ratio; rt – retention time (secs).

Supplement Table S4: Reported causes of death for the test group cases that were screened as the control group, of presumed normoglycemic cases, using a class prediction model based on the insulin intoxication metabolic fingerprint.

| Control prediction, n = 322 (42.8%) | | | |
|--|------------|--|-----------|
| Cause of death | Number | Cause of death | Number |
| Cardiovascular-related | 92 (11.9%) | Asphyxia | 57 (7.3%) |
| <ul style="list-style-type: none"> • Myocardial infarction • Coronary atherosclerosis • Enlarged heart • Cerebral hemorrhage • <i>Other</i> | | <ul style="list-style-type: none"> • Hanging • Drowning • Suffocation | |
| Traumatic injury | 54 (7.0%) | Substance overdose/poisoning | 48 (6.2%) |
| <ul style="list-style-type: none"> • Gunshot • Skull fracture • Brain stem/spinal cord injury • Internal/external injury • <i>Other</i> | | <ul style="list-style-type: none"> • Drug overdose/poisoning • Alcohol overdose/poisoning • Carbon monoxide poisoning | |
| Pulmonary-related | 14 (1.8%) | Digestive system-related | 11 (1.4%) |
| <ul style="list-style-type: none"> • Bronchopneumonia • Lobar pneumonia • Aspiration pneumonia • <i>Other</i> | | <ul style="list-style-type: none"> • Gastrointestinal hemorrhage • Gastrointestinal ulcer • Liver cirrhosis • <i>Other</i> | |
| Central nervous system-related | 6 (0.8%) | Hypothermia | 3 (0.4%) |
| Sepsis | 3 (0.4%) | Anaphylactic shock | 2 (0.3%) |
| Neoplasms | 2 (0.3%) | Ketoacidosis | 1 (0.1%) |
| Kidney inflammation | 1 (0.1%) | Electric shock | 1 (0.1%) |
| Heat exhaustion | 1 (0.1%) | Starvation | 1 (0.1%) |
| Diabetes mellitus with coma | 1 (0.1%) | <i>Undetectable cause of death</i> | 22 (2.8%) |

(%) are total percent of test group (n = 776) used for screening using a class prediction model

Supplement Table S5: Reported causes of death for the test group cases that failed to reach the probability threshold for group screening, using a class prediction model based on the insulin intoxication metabolic fingerprint.

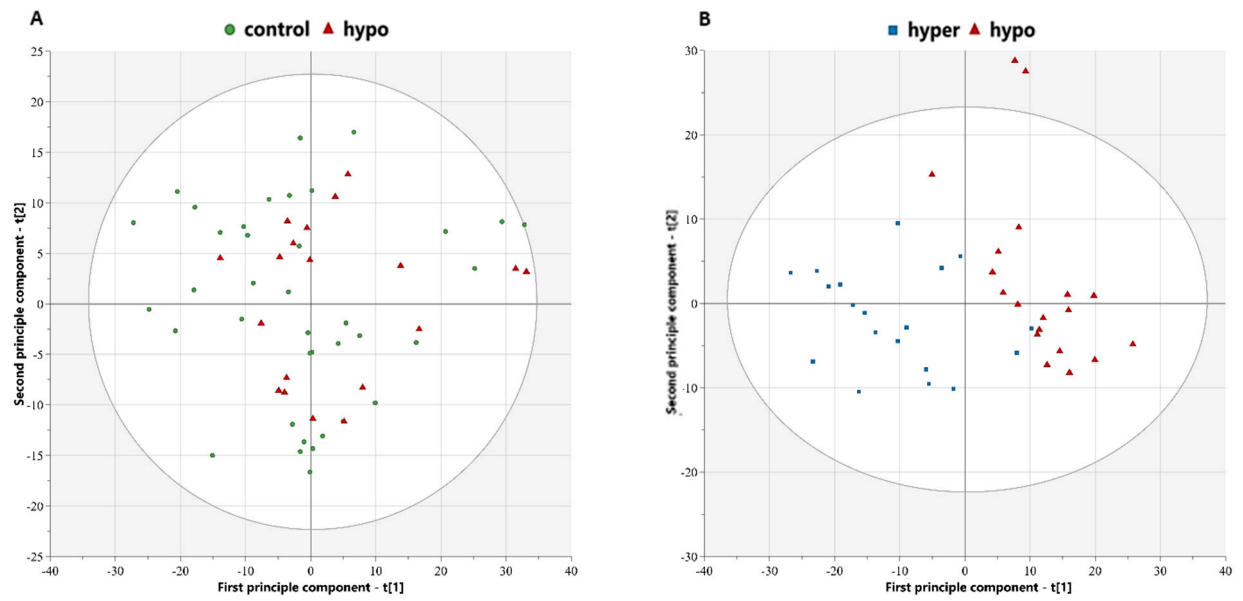
| No class, n = 378 (48.7%) | | | |
|--|-------------|--|------------|
| Cause of death | Number | Cause of death | Number |
| Cardiovascular-related: | 108 (13.9%) | Substance overdose/poisoning | 78 (10.0%) |
| <ul style="list-style-type: none"> • Myocardial infarction • Coronary atherosclerosis • Enlarged heart • Cerebral hemorrhage • <i>Other</i> | | <ul style="list-style-type: none"> • Drug overdose/poisoning • Alcohol overdose/poisoning • Carbon monoxide poisoning | |
| Traumatic injury: | 59 (7.6%) | Asphyxia | 41 (5.3%) |
| <ul style="list-style-type: none"> • Gunshot • Skull fracture • Brain stem/spinal cord injury • Internal/external injury • <i>Other</i> | | <ul style="list-style-type: none"> • Hanging • Drowning • Suffocation | |
| Pulmonary-related: | 22 (2.8%) | Digestive system-related | 13 (1.7%) |
| <ul style="list-style-type: none"> • Bronchopneumonia • Lobar pneumonia • Aspiration pneumonia • <i>Other</i> | | <ul style="list-style-type: none"> • Gastrointestinal hemorrhage • Gastrointestinal ulcer • Liver cirrhosis • <i>Other</i> | |
| Central nervous system-related | 9 (1.2%) | Keto-/acidosis | 8 (1.0%) |
| Hypothermia | 5 (0.6%) | Diabetes mellitus-related | 5 (0.6%) |
| Sepsis/infection | 4 (0.5%) | Neoplasms | 2 (0.3%) |
| Acute renal failure | 1 (0.1%) | Anaphylactic shock | 1 (0.1%) |
| Pregnancy-related | 1 (0.1%) | <i>Undetectable cause of death</i> | 21 (2.7%) |

(%) are total percent of test group (n = 776) used for screening using a class prediction model

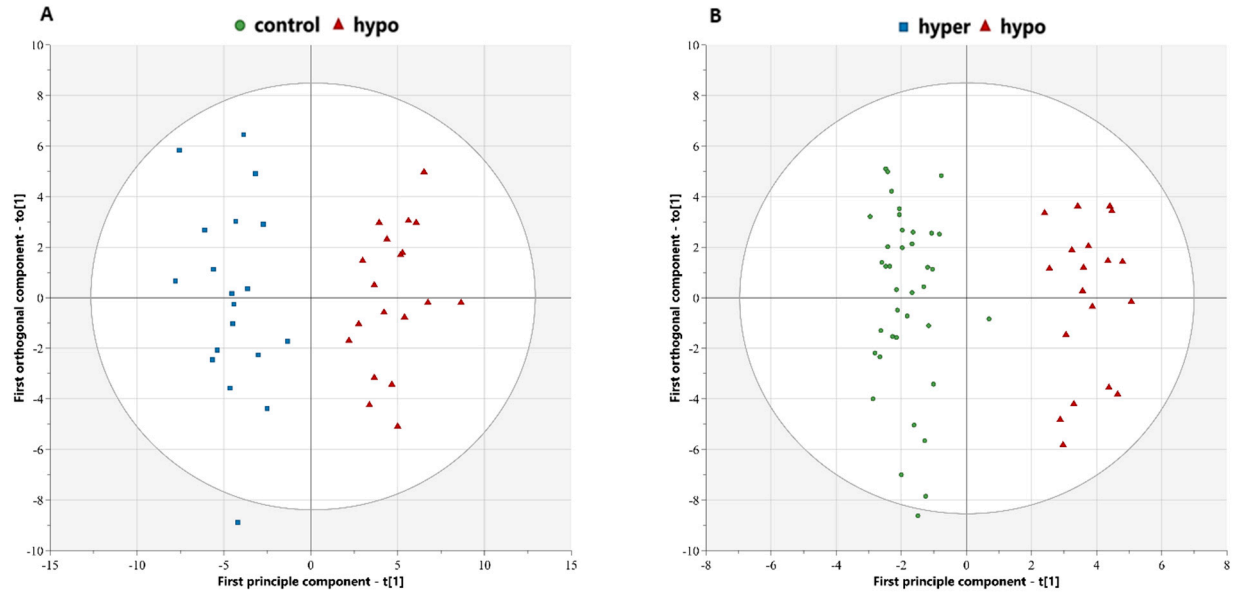
Supplement Table S6: Reported causes of death for the test group cases that were screened into multiple groups, using a class prediction model based on the insulin intoxication metabolic fingerprint.

| Hypo & Control, n = 3 (0.4%) | | Hyper & Control, n = 1 (0.1%) | |
|------------------------------|----------|-------------------------------|----------|
| Cause of death | Number | Cause of death | Number |
| Cerebral hemorrhage | 1 (0.1%) | Cardiac arrest | 1 (0.1%) |
| Drug overdose/poisoning | 1 (0.1%) | | |
| Gastrointestinal hemorrhage | 1 (0.1%) | | |

(%) are total percent of test group (n = 776) used for screening using a class prediction model



Supplement Figure S1: Multivariate modeling using unsupervised principle component analysis (PCA). (A) PCA score plot plotting the first two principle components, of seven, comparing control (green circles) and hypo (red triangles) group. (B) PCA score plot plotting the first two principle components, of five, comparing hyper (blue squares) and hypo (red triangles) groups.



Supplement Figure S2: Multivariate modeling using supervised orthogonal partial least squares-discriminant analysis (OPLS-DA) comparing group separation using only the 85 features found to discriminant the hypo group from control and hyper groups. (A) OPLS-DA score plot comparing control (green circles) and hypo (red triangles) group. (B) OPLS-DA score plot comparing hyper (blue squares) and hypo (red triangles) groups.