Supplemental Material

**Serum metabolic profiling in rheumatic heart disease and degenerative aortic stenosis**

**Running title:** Metabolomics in RHD and calcific aortic stenosis

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**Table S1**. Gradient conditions for reverse phase metabolites separation

|  |  |  |
| --- | --- | --- |
| Time (Min) | Flow (mL/Min) | % A |
| 2 | 0.4 | 98 |
| 45 | 0.4 | 5 |
| 47 | 0.4 | 5 |
| 47.1 | 0.4 | 98 |
| 60 | 0.4 | 98 |

**Table S2**. Summary of the pathway mapping by the metabolites detected and different in RHD and controls.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | Total | Hits | Pathway coverage (%) | Raw p | FDR adj. p | Impact | Mapped metabolites |
| Glycerophospholipid metabolism | 36 | 2 | 13.89 | 0.00000572 | 0.000481 | 0.26961 | LysoPC(16:1), LysoPE(0:0/22:5) |
| Linoleic acid metabolism | 5 | 1 | 40 | 0.000641 | 0.026903 | 1 | Linoleic acid |
| Valine, leucine, and isoleucine biosynthesis | 8 | 2 | 25 | 0.001768 | 0.049509 | 0 | Isoleucine, Valine |
| Aminoacyl-tRNA biosynthesis | 48 | 3 | 6.25 | 0.006411 | 0.13464 | 0 | Valine, Isoleucine, Tryptophan |
| Arachidonic acid metabolism | 36 | 1 | 5.56 | 0.034843 | 0.58536 | 0 | 15S-HETE |
| Valine, leucine, and isoleucine degradation | 40 | 2 | 5 | 0.042331 | 0.59263 | 0 | Isoleucine, Valine |

**Table S3**. Summary of the pathway mapping by the metabolites detected and different in AS and controls.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | Total | Hits | Pathway coverage (%) | Raw p | FDR adj. p | Impact | Mapped metabolites |
| Glycerophospholipid metabolism | 36 | 2 | 16.67 | 0.000000268 | 0.0000225 | 0.42215 | LysoPC(17:0/0:0), PA(8:0/13:0),  |
| Phosphatidylinositol signalling system | 28 | 1 | 7.14 | 0.02505 | 0.7014 | 0.09899 | PA(8:0/13:0) |
| Arachidonic acid metabolism | 36 | 1 | 5.56 | 0.040064 | 0.71151 | 0 | 15S-HETE |

**Table S4**. Summary of the pathway mapping by the metabolites detected and different in RHD and AS.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | Total | Hits | Pathway coverage (%) | Raw p | FDR adj. p | Impact | Mapped metabolites |
| Glycerophospholipid metabolism | 36 | 2 | 16.67 | 0.0000004.39 | 0.0000369 | 0.28311 | DG(a-15:0/i-12:0/0:0), LysoPE(0:0/22:5) |
| Glycine, serine, and threonine metabolism | 33 | 2 | 6.06 | 0.036404 | 0.92684 | 0.05034 | Betaine, Creatine |
| Valine, leucine, and isoleucine degradation | 40 | 2 | 5 | 0.055169 | 0.92684 | 0 | Acetoacetate, Valine |

**Table S5**. Summary of the metabolites’ Spearman’s or Point-Biserial correlations analyses with LVEF, LVMI, LVEDV, LVESV, LA area, and valves calcification after adjusting for sex, age, race, BMI, hypertension, diabetes, and batch effects in patients with rheumatic heart disease .

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Calcification rs | p-value | Calcification raw rs | p-value | LVMI (g/m2) rs | p-value | LVMI (g/m2) raw rs | p-value | LVEDV (ml) rs | p-value | LVEDV (ml) raw rs | p-value | LVESV (ml) rs | p-value | LVESV raw (ml) rs | p-value | LVEF (%) rs | p-value | LVEF (%) raw rs | p-value | LA Area (cm2) rs | p-value | LA area raw (cm2) rs | p-value |
| Cholestoric acid-Deoxycholate | -0.03 | 0.903 | 0.15 | 0.419 | -0.12 | 0.556 | -0.13 | 0.523 | -0.17 | 0.410 | -0.17 | 0.339 | 0.20 | 0.326 | 0.19 | 0.283 | -0.64 | 0.000 | -0.55 | 0.001 | 0.28 | 0.151 | 0.18 | 0.319 |
| Cholestoric acid/Deoxycholate | 0.01 | 0.964 | -0.21 | 0.230 | 0.07 | 0.724 | 0.08 | 0.707 | 0.13 | 0.522 | 0.12 | 0.499 | -0.35 | 0.077 | -0.26 | 0.150 | 0.57 | 0.002 | 0.53 | 0.001 | -0.34 | 0.084 | -0.24 | 0.186 |
| Propionylcarnitine/Acetoacetate | -0.19 | 0.360 | -0.10 | 0.565 | -0.57 | 0.003 | -0.57 | 0.003 | -0.47 | 0.014 | -0.18 | 0.317 | -0.13 | 0.534 | -0.18 | 0.324 | -0.26 | 0.193 | -0.12 | 0.500 | -0.02 | 0.933 | -0.09 | 0.609 |
| Butyrylcarnitine | -0.09 | 0.662 | 0.12 | 0.489 | 0.05 | 0.820 | 0.04 | 0.846 | -0.04 | 0.842 | 0.10 | 0.572 | 0.11 | 0.598 | 0.24 | 0.183 | -0.54 | 0.004 | -0.38 | 0.028 | 0.32 | 0.101 | 0.15 | 0.418 |
| 13-Docosenamide | 0.14 | 0.505 | 0.15 | 0.419 | -0.12 | 0.571 | -0.12 | 0.567 | -0.35 | 0.071 | -0.09 | 0.612 | 0.13 | 0.529 | 0.16 | 0.375 | -0.50 | 0.008 | -0.41 | 0.018 | 0.36 | 0.063 | 0.31 | 0.084 |
| (acetoacetate+deoxycholate+cholestonic)/(Carnitines) | -0.14 | 0.488 | -0.19 | 0.297 | 0.08 | 0.709 | 0.08 | 0.687 | 0.28 | 0.165 | 0.16 | 0.373 | -0.13 | 0.511 | -0.15 | 0.420 | 0.46 | 0.016 | 0.43 | 0.012 | -0.35 | 0.078 | -0.30 | 0.094 |
| Inosine-Valine | 0.12 | 0.547 | 0.15 | 0.397 | -0.08 | 0.692 | -0.11 | 0.595 | -0.13 | 0.510 | 0.07 | 0.688 | 0.00 | 0.989 | 0.02 | 0.899 | -0.05 | 0.786 | 0.03 | 0.852 | 0.46 | 0.017 | 0.37 | 0.032 |
| 7-Hoca | -0.09 | 0.670 | 0.10 | 0.591 | -0.26 | 0.206 | -0.27 | 0.190 | -0.28 | 0.152 | -0.19 | 0.293 | 0.13 | 0.505 | 0.11 | 0.550 | -0.45 | 0.017 | -0.35 | 0.043 | 0.19 | 0.352 | 0.09 | 0.638 |
| LysoPC(22:6/0:0) | -0.25 | 0.216 | 0.01 | 0.939 | 0.02 | 0.935 | 0.01 | 0.960 | -0.09 | 0.648 | 0.07 | 0.692 | 0.40 | 0.040 | 0.34 | 0.053 | -0.43 | 0.024 | -0.37 | 0.036 | 0.26 | 0.196 | 0.17 | 0.337 |
| Keto-hydroxypimelic acid | -0.05 | 0.827 | 0.06 | 0.730 | 0.42 | 0.032 | 0.42 | 0.036 | 0.40 | 0.037 | 0.26 | 0.150 | 0.41 | 0.031 | 0.37 | 0.034 | -0.03 | 0.880 | -0.11 | 0.532 | 0.07 | 0.725 | 0.01 | 0.948 |
| LA/3-Formylindole | 0.17 | 0.402 | 0.03 | 0.878 | 0.10 | 0.621 | 0.10 | 0.642 | -0.07 | 0.734 | -0.13 | 0.477 | -0.16 | 0.425 | -0.17 | 0.340 | 0.20 | 0.317 | 0.23 | 0.196 | 0.41 | 0.035 | 0.38 | 0.028 |
| Propionylcarnitine\*Acetoacetate | -0.07 | 0.725 | 0.09 | 0.618 | -0.41 | 0.036 | -0.41 | 0.040 | -0.39 | 0.042 | -0.18 | 0.329 | -0.14 | 0.501 | -0.18 | 0.326 | -0.13 | 0.507 | -0.07 | 0.685 | -0.11 | 0.588 | -0.17 | 0.356 |
| Deoxycholate\*Cholestoric acid | 0.09 | 0.667 | -0.19 | 0.297 | 0.20 | 0.334 | 0.20 | 0.331 | 0.25 | 0.202 | 0.16 | 0.384 | -0.12 | 0.536 | -0.06 | 0.749 | 0.40 | 0.040 | 0.36 | 0.041 | -0.19 | 0.333 | -0.09 | 0.636 |
| LysoPE(0:0/22:5) | -0.01 | 0.949 | 0.01 | 0.939 | 0.18 | 0.392 | 0.17 | 0.404 | 0.14 | 0.490 | 0.04 | 0.844 | 0.40 | 0.036 | 0.39 | 0.025 | -0.38 | 0.049 | -0.33 | 0.065 | 0.29 | 0.149 | 0.13 | 0.486 |

**Table S6**. Summary of the metabolites’ Spearman’s or Point-Biserial correlations analyses with LVEF, LVMI, LVEDV, LVESV, LA area, and valves calcification after adjusting for sex, age, race, BMI, hypertension, diabetes, and batch effects in patients with degenerative aortic stenosis.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Calcification rs | p-value | Calcification raw rs | p-value | LVMI (g/m2) rs | p-value | LVMI raw (g/m2) rs | p-value | LVEDV (ml) rs | p-value | LVEDV raw (ml) rs | p-value | LVESV (ml) rs | p-value | LVESV raw (ml) rs | p-value | LVEF (%) rs | p-value | LVEF raw (ml) rs | p-value | LA area (cm2) rs | p-value | LA area raw rs | p-value |
| 10-Nitroelaidic acid | 0.84 | 0.371 | 0.55 | 0.127 | 0.93 | 0.236 | -0.03 | 0.947 | 0.99 | 0.075 | -0.36 | 0.346 | 1.00 | 0.011 | -0.20 | 0.606 | 0.83 | 0.374 | 0.25 | 0.521 | 0.92 | 0.259 | 0.27 | 0.483 |
| 3-Formylindole | 1.00 | 0.049 | 0.55 | 0.127 | 0.96 | 0.184 | 0.35 | 0.359 | 0.86 | 0.345 | -0.02 | 0.965 | 0.78 | 0.431 | 0.09 | 0.824 | 0.32 | 0.793 | -0.33 | 0.385 | 0.48 | 0.678 | 0.64 | 0.061 |
| 3-Formylindole\*LA | -0.44 | 0.707 | -0.41 | 0.272 | -0.62 | 0.572 | -0.60 | 0.087 | -0.80 | 0.411 | -0.34 | 0.371 | -0.87 | 0.325 | -0.08 | 0.841 | -1.00 | 0.037 | -0.30 | 0.437 | -0.99 | 0.077 | -0.30 | 0.425 |
| 3-Formylindole-LA | 0.39 | 0.743 | 0.55 | 0.127 | 0.58 | 0.607 | 0.15 | 0.704 | 0.76 | 0.447 | -0.18 | 0.638 | 0.84 | 0.361 | -0.03 | 0.947 | 1.00 | 0.002 | 0.43 | 0.250 | 0.98 | 0.113 | 0.60 | 0.087 |
| Acetoacetate/Propionylcarnitine | -0.59 | 0.601 | -0.41 | 0.272 | -0.74 | 0.466 | -0.08 | 0.841 | -0.89 | 0.305 | 0.13 | 0.738 | -0.94 | 0.219 | -0.17 | 0.671 | -0.97 | 0.143 | 0.03 | 0.948 | -1.00 | 0.028 | -0.77 | 0.014 |
| acetylcarnitine/Acetoacetate | 0.88 | 0.309 | 0.55 | 0.127 | 0.96 | 0.173 | 0.26 | 0.497 | 1.00 | 0.013 | -0.19 | 0.622 | 0.99 | 0.074 | 0.09 | 0.824 | 0.77 | 0.436 | -0.07 | 0.880 | 0.88 | 0.321 | 0.56 | 0.119 |
| Butyrylcarnitine | 0.98 | 0.141 | 0.55 | 0.127 | 1.00 | 0.005 | 0.29 | 0.454 | 0.97 | 0.155 | -0.10 | 0.806 | 0.93 | 0.242 | 0.20 | 0.606 | 0.58 | 0.604 | 0.37 | 0.336 | 0.72 | 0.489 | 0.55 | 0.126 |
| Butyrylcarnitine\*Acetoacetate | 0.53 | 0.646 | 0.55 | 0.127 | 0.69 | 0.511 | 0.28 | 0.468 | 0.85 | 0.350 | -0.23 | 0.558 | 0.92 | 0.264 | -0.33 | 0.385 | 0.99 | 0.098 | 0.53 | 0.148 | 1.00 | 0.016 | -0.12 | 0.755 |
| Butyrylcarnitine\*Propionylcarnitine | -0.97 | 0.151 | -0.55 | 0.127 | -1.00 | 0.015 | -0.50 | 0.174 | -0.97 | 0.145 | -0.08 | 0.841 | -0.93 | 0.231 | -0.24 | 0.543 | -0.60 | 0.594 | -0.27 | 0.493 | -0.73 | 0.479 | -0.30 | 0.425 |
| Butyrylcarnitine/Acetoacetate | -0.36 | 0.767 | 0.41 | 0.272 | -0.55 | 0.632 | -0.26 | 0.497 | -0.74 | 0.471 | -0.59 | 0.093 | -0.82 | 0.385 | -0.70 | 0.037 | -1.00 | 0.023 | 0.08 | 0.843 | -0.98 | 0.138 | -0.45 | 0.221 |
| Butyrylcarnitine+Propionylcarnitine | 0.51 | 0.659 | 0.55 | 0.127 | 0.68 | 0.524 | 0.18 | 0.638 | 0.84 | 0.363 | -0.24 | 0.543 | 0.91 | 0.277 | 0.10 | 0.806 | 0.99 | 0.086 | 0.57 | 0.121 | 1.00 | 0.029 | 0.53 | 0.141 |
| Cholestoric acid/Deoxycholate | -0.93 | 0.241 | -0.27 | 0.476 | -0.99 | 0.106 | -0.24 | 0.543 | -1.00 | 0.055 | -0.08 | 0.841 | -0.98 | 0.141 | -0.41 | 0.274 | -0.70 | 0.504 | -0.03 | 0.948 | -0.82 | 0.389 | -0.71 | 0.034 |
| Cholestoric acid-Deoxycholate | -0.97 | 0.150 | 0.41 | 0.272 | -1.00 | 0.015 | 0.01 | 0.982 | -0.97 | 0.146 | -0.24 | 0.543 | -0.93 | 0.232 | -0.60 | 0.087 | -0.59 | 0.595 | 0.42 | 0.270 | -0.73 | 0.480 | -0.32 | 0.398 |
| Deoxycholate\*Cholestoric acid | -0.98 | 0.118 | -0.41 | 0.272 | -1.00 | 0.017 | -0.30 | 0.439 | -0.96 | 0.178 | 0.03 | 0.929 | -0.92 | 0.264 | -0.38 | 0.309 | -0.55 | 0.626 | -0.03 | 0.948 | -0.69 | 0.512 | -0.64 | 0.061 |
| Glucose+AA | -0.34 | 0.782 | 0.00 | 1.000 | -0.53 | 0.647 | -0.39 | 0.297 | -0.72 | 0.486 | -0.44 | 0.231 | -0.81 | 0.400 | -0.04 | 0.911 | -1.00 | 0.038 | 0.33 | 0.385 | -0.97 | 0.152 | -0.08 | 0.841 |
| Indolepropionic acid | -0.38 | 0.749 | -0.14 | 0.725 | -0.57 | 0.614 | -0.50 | 0.174 | -0.76 | 0.453 | -0.39 | 0.297 | -0.84 | 0.367 | -0.06 | 0.876 | -1.00 | 0.005 | -0.28 | 0.463 | -0.98 | 0.119 | 0.03 | 0.947 |
| Inosine\*Valine | 0.42 | 0.727 | -0.14 | 0.725 | 0.60 | 0.592 | 0.39 | 0.297 | 0.78 | 0.431 | 0.53 | 0.141 | 0.86 | 0.345 | 0.13 | 0.738 | 1.00 | 0.017 | -0.10 | 0.810 | 0.99 | 0.097 | -0.11 | 0.772 |
| Inosine/Valine | 0.81 | 0.394 | 0.41 | 0.272 | 0.92 | 0.259 | 0.40 | 0.286 | 0.99 | 0.098 | 0.12 | 0.755 | 1.00 | 0.012 | -0.12 | 0.755 | 0.85 | 0.351 | 0.02 | 0.982 | 0.93 | 0.236 | 0.12 | 0.755 |
| Inosine+Valine | -0.34 | 0.782 | 0.00 | 1.000 | -0.53 | 0.647 | -0.39 | 0.297 | -0.72 | 0.486 | -0.44 | 0.231 | -0.81 | 0.400 | -0.04 | 0.911 | -1.00 | 0.038 | 0.33 | 0.385 | -0.97 | 0.152 | -0.08 | 0.841 |
| Valine+Inosine+Creatine+Betaine | -0.34 | 0.782 | 0.00 | 1.000 | -0.53 | 0.647 | -0.39 | 0.297 | -0.72 | 0.486 | -0.44 | 0.231 | -0.81 | 0.400 | -0.04 | 0.911 | -1.00 | 0.038 | 0.33 | 0.385 | -0.97 | 0.152 | -0.08 | 0.841 |
| lysoPE(17:0) | 0.58 | 0.606 | 0.41 | 0.272 | 0.74 | 0.471 | 0.02 | 0.965 | 0.88 | 0.310 | -0.24 | 0.527 | 0.94 | 0.224 | 0.10 | 0.789 | 0.98 | 0.138 | 0.20 | 0.613 | 1.00 | 0.024 | 0.61 | 0.082 |
| LysoPE(0:0/22:5) | 0.81 | 0.404 | 0.55 | 0.127 | 0.91 | 0.269 | -0.07 | 0.859 | 0.99 | 0.108 | -0.40 | 0.286 | 1.00 | 0.022 | -0.16 | 0.687 | 0.86 | 0.340 | 0.30 | 0.437 | 0.94 | 0.226 | 0.35 | 0.359 |
| Propionylcarnitine/Butyrylcarnitine | -0.36 | 0.764 | 0.27 | 0.476 | -0.55 | 0.629 | -0.02 | 0.965 | -0.74 | 0.468 | -0.26 | 0.497 | -0.83 | 0.382 | -0.54 | 0.134 | -1.00 | 0.020 | 0.07 | 0.880 | -0.98 | 0.134 | -0.57 | 0.106 |