ONLINE SUPPLEMENT

Effects of Perhexiline-Induced Fuel Switch on the Cardiac Proteome and Metabolome

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Online Materials and Methods

**Mice.** Mice were fed on a normal dry chow diet containing 4.5% fat by weight (0.02% cholesterol), kept on a light/dark (12/12h) cycle at 22°C receiving food and water *ad libitum*. The diets of mice fed on perhexiline were prepared by homogenising their food and adding the crushed drug Pexsig® (Perhexiline Maleate 100mg, Sigma) in a ratio of 1mg to 1g of mouse food with subsequent freeze-drying of the preparation. All procedures were performed according to protocols approved by the Institutional Committee for the Use and Care of Laboratory Animals. Hearts of C57BL/6 mice used for proteomic and metabolic analyses were rinsed thoroughly with cold PBS to remove any blood components and frozen in liquid nitrogen until they were pulverised with mortar and pestle. Plasma concentrations of perhexiline and hydroxy-perhexiline (OH-perhexiline) were measured by high-performance liquid chromatography (Dr. Alan Hutchinson, Toxicology Lab, LLanduch, UK).

**Difference in-gel electrophoresis (DIGE).** Pulverised heart tissue was incubated in 2DE lysis buffer (9.5M urea, 2% w/v CHAPS, 2% v/v Pharmalyte pH 3-10, 1% w/v DTT, protease inhibitors) for 0.5 hr at RT. After centrifugation at 13,000rpm for 15min, the supernatant was collected, proteins were precipitated (2D Clean-up kit, Bio-Rad) and resuspended in DIGE buffer (8M urea, 4% w/v CHAPS, 30mM TrisCl, pH=8.5). Protein concentrations were normalised using the Bradford assay. Samples were labelled with fluorescent dyes Cy3 and Cy5 with Cy2 being reserved for the labelling of the internal standard. Incubation with the dyes was done at a dye/protein ratio of 400pmol/100µg for 30 min on ice with the reaction being quenched with 10mM L-lysine (L8662, Sigma) for 15 min. Samples were mixed in 2x buffer (8M urea, 4% w/v CHAPS, 2% w/v DTT, 2% v/v Pharmalyte pH 3-10) and a volume of sample calculated to have a protein content of 50µg was diluted in
rehydration solution (8M urea, 0.5% w/v CHAPS, 0.2% w/v DTT, and 0.2% v/v Pharmalyte pH 3-10) and loaded onto a IPG strip for isoelectric focusing (18cm, pH 3-10NL, GE healthcare) for overnight rehydration. IPG strips were focused overnight for 64.6 kVhrs using a gradient programme at 20°C. Strips were equilibrated and run on top of a 12% SDS gel without stacking gel until the blue dye front reached the end of the gel. Fluorescent images of gels were obtained by scanning with an Ettan DIGE Imager (GE healthcare). Differentially expressed spots showing statistical significance (p<0.05) were filtered by using the DeCyder® software (Version 6.5, GE healthcare). Gels were then silver stained (Plus one silver staining kit, GE healthcare) and spots were excised for analysis by mass spectrometry [1, 2].

**Tandem mass spectrometry.** Excised gel spots were subjected to in-gel tryptic digestion with an Investigator ProGest (Genomic Solutions) robotic digestion system with subsequent lyophilisation. Freeze-dried samples were resuspended in 20µl of 0.05% trifluoroacetic acid. Samples were then identified via separation by nano-flow liquid chromatography on a reverse-phase column (PepMap100, 25cm, Dionex) interfaced to a high-performance linear ion trap mass spectrometer (LTQ XL, Thermo Fisher). Spectra were collected for analysis and searched through mouse protein databases using SEQUEST (Bioworks Browser version 3.2, Thermo Fisher Scientific) and imported into Scaffold software (Proteome software).

**Phosphate-affinity gel electrophoresis (Phos-tag®)**. For phosphate-affinity gel electrophoresis, 50 µg of protein extracts were separated on 10% polyacrylamide gels containing 50 µM Phos-tag® (Wako Chemicals GmbH) and 50 µM MnCl$_2$ (Sigma) [1]. After electrophoresis, gels were soaked in transfer buffer with 1mM EDTA for 10 min, then in transfer buffer for another 10 min prior to blotting onto a polyvinylidene fluoride (PVDF) membrane. Membranes were probed with anti-PDH
subunit E1 alpha monoclonal antibody (Invitrogen, 459400).

**High-resolution NMR spectroscopy.** For metabolomic analysis, cardiac metabolites were extracted in 6% perchloric acid [2]. All hearts were from the same litter of mice and harvested and processed at the same time. Storage times of the extracts were identical. Neutralized extracts were freeze-dried and reconstituted in deuterium oxide (D₂O). One-half milliliter of each extract was placed in 5-mm nuclear magnetic resonance tubes. Proton magnetic resonance spectroscopy (¹H-NMR) spectra were obtained using a Bruker 600-MHz spectrometer (Bruker BioSpin GmbH, Rheinstetten, Germany) as previously described [3, 4]. The water resonance was suppressed by using gated irradiation centered on the water frequency. Fifty microliters of 5 mM sodium 3-trimethylsilyl-2,2,3,3-tetradeuteropropionate (TSP) in D₂O was added to the samples for chemical shift calibration and quantification. Immediately before the ¹H-NMR analysis, the pH was readjusted to 7 with perchloric acid or potassium hydroxide.

**Statistical and bioinformatic analysis.** Principle components analysis (PCA) was performed in R, where the principle components were identified by a singular value decomposition of the data [5]. Pearson correlations were calculated between each of the metabolites and the associated False Discovery Rate (FDR) was applied for each using the q-value method [6]. The functional enrichment analysis of Gene Ontology (GO) and KEGG Pathway terms was performed on the selected proteins showing differences after perhexilene treatment using DAVID [7]. The enrichment calculations used a Fishers Exact P-value and multiple testing corrections were applied using the False Discovery Rate (AFDR) [8]. Terms with an FDR <5% were deemed to be significant. The hierarchical clustering of the metabolites was performed in R using the ‘Manhattan’ distance. The ‘Manhattan’ distance metric is
based on the absolute difference between the two vectors of measurements [5]. The approximately unbiased (AU) values were calculated with multiscale bootstrap resampling and the bootstrap probability (BP) values were calculated via standard bootstrap resampling. The corresponding AU and BP values were calculated at each branch of the cluster dendrogram, where a value of 95% in Figure 2C corresponds to a significance level equal to $p<0.05$. Each resampling was run with a bootstrap sample size of 10,000. Metabolite profiles were interrogated using the context likelihood of relatedness (CLR) between all possible metabolite pairs.

Computational modeling. For computational modeling, we used an existing and well-validated proteome-scale model of heart mitochondria metabolism [9, 10], slightly modified to address a small earlier error in the mass-balancing of complex IV of the respiratory chain. The model did not contain any kinetic data, but instead consisted of the stoichiometric coefficients of every reaction in the human heart mitochondrial metabolic network, obtained from the existing literature and curated databases [9, 10]. Simulations assumed that the network was at steady-state. In other words, if $S$ was the matrix of stoichiometric coefficients and $v$ was a column vector of unknown reaction fluxes, we sought to characterize all solutions that satisfied $Sv = 0$. We also applied linear inequalities to many of the elements of $v$ (i.e. upper and lower limits of substrate exchange), based on literature values. Greater detail regarding the model itself can be found in [9, 10]. We chose the mitochondria model for this project because it is small enough to be tractable using random sampling techniques, yet still encompasses the salient features of central carbon metabolism in the human heart.

The final model comprised 195 reactions, 235 metabolites and 25 exchange reactions. Uniform sampling of the solution space was carried out using a random walk algorithm (accelerated-centering hit-and-run [11]) as described in [9] and as
implemented in the COBRA toolbox [12]. For each sampling experiment, 20,000 warm-up points were generated, from which an approximate centre was estimated; thence 50,000 points were computed with a 1,000 point skip between samples. We simulated perhexiline treatment by constraining C16-C20 fatty acid uptake to <25% of the median values obtained by sampling the control model. Simulations were conducted using the COBRA toolbox [12] and Tomlab CPLEX solver (Tomlab Optimization, Miami, FL), both in Matlab R2012a (MathWorks, Natick, MA). All other computation was conducted in either Matlab or Microsoft Excel.
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Online Figures

Supplemental Figure 1. A quantized (5-colour) heatmap showing differences in median flux between control and simulated-perhexiline conditions. All comparisons are perhexiline vs. control (for example, 'down' indicates 'flux down in perhexiline vs control').
GREEN: strongly suppressed
LIGHT GREEN: modestly suppressed
YELLOW: no change
ORANGE: moderately increased
RED: strongly increased

Supplemental Figure 2. Flux probability density functions from computer simulations of mitochondrial metabolism.
Red = perhexiline, blue = control.
(A) EX glc(e) = glucose exchange (negative value indicates uptake)
(B) EX glu-L(e) = L-glutamate exchange
(C) LDH L = L-lactate dehydrogenase (negative value indicates pyruvate production)
(D) PDHm = pyruvate dehydrogenase
(E) CSm = citrate synthase
(F) FUMm = mitochondrial fumarase
Supplemental Table 1: List of differentially expressed proteins in perhexiline-treated hearts as compared to controls.

| No. | Protein Name                                      | UniProt ID          | Calculated pl/Mw (kD) | Average Ratio* | T-test (p-Value) | Number of unique peptides | Number of unique spectra | Number of total spectra | Sequence Coverage |
|-----|--------------------------------------------------|---------------------|-----------------------|-----------------|-----------------|--------------------------|-------------------------|------------------------|-------------------|
|     | **Glycolysis**                                   |                     |                       |                 |                 |                          |                         |                         |                   |
| 25  | Alpha-enolase                                    | ENOA_MOUSE          | 6.37/47.14            | -1.25           | 0.020           | 5                       | 6                       | 7                      | 20.0%             |
| 35  | L-lactate dehydrogenase B chain                  | LDHB_MOUSE          | 7.96/36.57            | 1.26            | 4.9E-4          | 4                       | 4                       | 4                      | 10.5%             |
| 36  | L-lactate dehydrogenase B chain                  | LDHB_MOUSE          | 7.96/36.57            | 1.76            | 7.6E-6          | 4                       | 4                       | 5                      | 12.0%             |
| 22  | Pyruvate dehydrogenase E1 component subunit alpha, mitochondrial | ODPA_MOUSE          | 8.49/43.23           | -1.42           | 0.0039          | 5                       | 5                       | 6                      | 13.6%             |
| 24  | Pyruvate dehydrogenase E1 component subunit alpha, mitochondrial | ODPA_MOUSE          | 8.49/43.23           | -1.43           | 7.8E-4          | 4                       | 5                       | 7                      | 11.0%             |
| 26  | Pyruvate dehydrogenase E1 component subunit alpha, mitochondrial | ODPA_MOUSE          | 8.49/43.23           | -3.23           | 3.2E-4          | 14                      | 15                      | 18                     | 25.6%             |
| 28  | Pyruvate dehydrogenase E1 component subunit alpha, mitochondrial | ODPA_MOUSE          | 8.49/43.23           | -1.34           | 6.0E-4          | 4                       | 4                       | 4                      | 10.5%             |
| 29a | Pyruvate dehydrogenase E1 component subunit alpha, mitochondrial | ODPA_MOUSE          | 8.49/43.23           | -2.19           | 2.5E-8          | 11                      | 12                      | 16                     | 27.7%             |
| 30  | Pyruvate dehydrogenase E1 component subunit alpha, mitochondrial | ODPA_MOUSE          | 8.49/43.23           | -1.36           | 5.3E-4          | 7                       | 7                       | 10                     | 18.5%             |
| 32  | Pyruvate dehydrogenase E1 component subunit alpha, mitochondrial | ODPA_MOUSE          | 8.49/43.23           | -1.75           | 5.3E-4          | 17                      | 21                      | 35                     | 39.7%             |
| 33  | Pyruvate dehydrogenase E1 component subunit alpha, mitochondrial | ODPA_MOUSE          | 8.49/43.23           | 2.02            | 6.9E-8          | 15                      | 19                      | 31                     | 35.9%             |
| 34  | Pyruvate dehydrogenase E1 component subunit alpha, mitochondrial | ODPA_MOUSE          | 8.49/43.23           | 1.51            | 9.3E-7          | 6                       | 6                       | 7                      | 15.4%             |
| 41  | Triosephosphate isomerase                        | TPIS_MOUSE          | 5.56/26.71           | -1.33           | 0.0090          | 3                       | 3                       | 3                      | 14.9%             |
|     | **Lipid Metabolism**                             |                     |                       |                 |                 |                          |                         |                         |                   |
| 29b | Acyl-coenzyme A thioesterase 2,                  | ACOT2_MOUSE         | 5.88/49.66           | -2.19           | 2.5E-8          | 5                       | 7                       | 46                     | 35.0%             |
| Number | Protein Name                                      | Gene Symbol | Value   | p Value | FDR  | Z-score | Fold Change | Protein Function                                |
|--------|--------------------------------------------------|-------------|---------|---------|------|----------|-------------|-----------------------------------------------|
| 47     | Apolipoprotein A-I                               | APOA1 MOUSE | 5.64/30.59 | 1.86    | 0.0016 | 20       | 24          | 40                                           | 52.7% |
| 52     | Fatty acid-binding protein                       | FABP4 MOUSE | 8.53/14.65 | 1.80    | 1.2E-4 | 9        | 15          | 23                                           | 47.0% |
| 53     | Fatty acid-binding protein                       | FABP4 MOUSE | 8.53/14.65 | 2.21    | 6.5E-5 | 7        | 8           | 8                                            | 52.3% |
|        | **Energy Metabolism**                             |             |          |         |      |          |             |                                               |       |
| 44     | Adenylate kinase isoenzyme 4, mitochondrial      | KAD4 MOUSE  | 7.02/25.06 | 1.28    | 0.0079 | 4        | 5           | 5                                            | 21.1% |
| 21     | Creatine kinase B-type                           | KCRB MOUSE  | 5.40/42.71 | -1.27   | 9.6E-4 | 12       | 13          | 18                                           | 41.7% |
| 38     | Isocitrate dehydrogenase [NAD] subunit alpha, mitochondrial | IDH3A MOUSE | 6.27/39.64 | -1.25   | 5.4E-3 | 11       | 14          | 16                                           | 27.6% |
|        | **Protein Metabolism**                           |             |          |         |      |          |             |                                               |       |
| 23     | 2-oxoisovalerate dehydrogenase subunit alpha, mitochondrial | ODBA_MOUSE | 8.14/50.37 | -1.27   | 0.025  | 3        | 3           | 3                                            | 10.2% |
| 27     | 2-oxoisovalerate dehydrogenase subunit alpha, mitochondrial | ODBA_MOUSE | 8.14/50.37 | -2.31   | 3.2E-7 | 3        | 3           | 4                                            | 8.4%  |
| 39     | Elongation factor Ts, mitochondrial              | EFTS_MOUSE  | 6.61/35.33 | -1.30   | 4.0E-6 | 3        | 3           | 3                                            | 10.5% |
| 31     | Elongation factor Tu, mitochondrial              | EFTU_MOUSE  | 7.23/49.51 | -1.22   | 9.0E-6 | 19       | 28          | 53                                           | 40.5% |
|        | **Antioxidants**                                 |             |          |         |      |          |             |                                               |       |
| 45     | Glutathione S-transferase P 1                    | GSTP1 MOUSE | 7.69/23.61 | -1.25   | 3.7E-4 | 4        | 4           | 6                                            | 24.8% |
| 48     | Peroxiredoxin-2                                  | PRDX2 MOUSE | 5.20/21.78 | 1.24    | 0.0017 | 2        | 2           | 2                                            | 13.6% |
|        | **Cytoskeletal/Structural**                      |             |          |         |      |          |             |                                               |       |
| 46     | Cysteine and glycine-rich protein 3              | CSRP3 MOUSE | 8.90/20.89 | -1.32   | 0.0027 | 5        | 6           | 7                                            | 28.9% |
| 40     | F-actin-capping protein subunit beta             | CAPZB MOUSE | 5.47/31.35 | -1.24   | 0.0034 | 2        | 2           | 2                                            | 9.0%  |
| 1      | LIM domain-binding protein 3                     | LDB3 MOUSE  | 7.96/76.43 | -1.23   | 8.1E-4 | 2        | 3           | 4                                            | 3.5%  |
| 2      | LIM domain-binding protein 3                     | LDB3 MOUSE  | 7.96/76.43 | -1.30   | 0.0014 | 5        | 6           | 6                                            | 10.0% |
| 3      | LIM domain-binding protein 3                     | LDB3 MOUSE  | 7.96/76.43 | -1.22   | 3.5E-4 | 11       | 12          | 16                                           | 21.6% |
|   | Protein Name | Accession | U00788 | U00787 | U00786 | U00785 | U00784 | U00783 | U00782 | U00781 | U00780 | U00779 | U00778 |
|---|-------------|-----------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 4 | LIM domain-binding protein 3 | LDB3_MOUSE | 7.96/76.43 | -1.20 | 0.0025 | 13 | 16 | 19 | 25.4% |
| 37 | PDZ and LIM domain protein 1 | PDLI1_MOUSE | 6.38/35.77 | -1.22 | 1.5E-4 | 7 | 7 | 8 | 21.1% |
| 13 | Tubulin beta-2A chain | TBB2A_MOUSE | 4.78/49.91 | 1.56 | 7.0E-4 | 11 | 11 | 16 | 23.4% |
| 14 | Tubulin beta-2A chain | TBB2A_MOUSE | 4.78/49.91 | 1.41 | 2.2E-4 | 18 | 19 | 35 | 38.2% |
| 15 | Tubulin beta-2A chain | TBB2A_MOUSE | 4.78/49.91 | 1.20 | 0.0044 | 15 | 17 | 37 | 32.4% |
| 5 | Vimentin | VIME_MOUSE | 5.05/53.69 | 1.44 | 5.0E-5 | 3 | 3 | 4 | 7.9% |

**Serum proteins**

|   | Protein Name | Accession | U00788 | U00787 | U00786 | U00785 | U00784 | U00783 | U00782 | U00781 | U00780 | U00779 | U00778 |
|---|-------------|-----------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 11 | Alpha-1-antitrypsin 1-1 | A1AT1_MOUSE | 5.44/46.00 | 1.28 | 0.036 | 5 | 7 | 9 | 13.3% |
| 12 | Alpha-1-antitrypsin 1-1 | A1AT1_MOUSE | 5.44/46.00 | 1.48 | 0.0014 | 9 | 11 | 11 | 24.2% |
| 17 | Fibrinogen beta chain | FIBB_MOUSE | 6.68/54.75 | 1.45 | 8.5E-5 | 4 | 4 | 4 | 10.6% |
| 18 | Fibrinogen beta chain | FIBB_MOUSE | 6.68/54.75 | 1.81 | 2.1E-4 | 16 | 19 | 25 | 38.3% |
| 19 | Fibrinogen beta chain | FIBB_MOUSE | 6.68/54.75 | 1.26 | 0.0077 | 6 | 6 | 8 | 14.1% |
| 16 | Fibrinogen gamma chain | FIBG_MOUSE | 5.54/49.39 | 1.45 | 4.8E-5 | 9 | 11 | 16 | 21.1% |
| 59 | Hemoglobin subunit alpha | HBA_MOUSE | 7.97/15.09 | 2.31 | 7.8E-4 | 10 | 18 | 82 | 76.1% |
| 60 | Hemoglobin subunit alpha | HBA_MOUSE | 7.97/15.09 | 2.49 | 4.9E-4 | 7 | 12 | 37 | 62.7% |
| 61 | Hemoglobin subunit alpha | HBA_MOUSE | 7.97/15.09 | 2.38 | 9.4E-4 | 6 | 11 | 33 | 62.7% |
| 62 | Hemoglobin subunit alpha | HBA_MOUSE | 7.97/15.09 | 2.06 | 2.4E-5 | 3 | 5 | 15 | 25.4% |
| 63 | Hemoglobin subunit alpha | HBA_MOUSE | 7.97/15.09 | 2.22 | 1.7E-4 | 5 | 6 | 15 | 54.2% |
| 51 | Hemoglobin subunit beta-1 | HBB1_MOUSE | 7.13/15.84 | 2.92 | 1.8E-4 | 5 | 6 | 11 | 42.9% |
| 55 | Hemoglobin subunit beta-1 | HBB1_MOUSE | 7.13/15.84 | 2.60 | 3.8E-4 | 5 | 7 | 12 | 44.9% |
| 56 | Hemoglobin subunit beta-1 | HBB1_MOUSE | 7.13/15.84 | 2.46 | 0.0013 | 7 | 14 | 33 | 54.4% |
| 57 | Hemoglobin subunit beta-1 | HBB1_MOUSE | 7.13/15.84 | 2.34 | 5.6E-4 | 8 | 18 | 88 | 54.4% |
| 58 | Hemoglobin subunit beta-1 | HBB1_MOUSE | 7.13/15.84 | 2.50 | 7.6E-4 | 9 | 16 | 52 | 54.4% |
| 50 | Myoglobin | MYG_MOUSE | 7.06/17.07 | 1.20 | 8.8E-4 | 2 | 3 | 3 | 14.9% |
| 6 | Serum albumin | ALBU_MOUSE | 5.75/68.69 | 1.26 | 0.022 | 27 | 34 | 50 | 46.9% |
| 7 | Serum albumin | ALBU_MOUSE | 5.75/68.69 | 1.41 | 0.0014 | 44 | 70 | 127 | 61.5% |
| 8 | Serum albumin | ALBU_MOUSE | 5.75/68.69 | 1.36 | 0.0040 | 54 | 83 | 161 | 70.7% |
| 9 | Serum albumin | ALBU_MOUSE | 5.75/68.69 | 1.40 | 0.0013 | 38 | 58 | 107 | 62.5% |
| 10 | Serum albumin | ALBU_MOUSE | 5.75/68.69 | 1.37 | 0.0017 | 29 | 40 | 63 | 50.7% |
| 49 | Transthyretin | TTHY_MOUSE | 5.77/15.78 | 1.24 | 0.012 | 4 | 4 | 5 | 25.2% |

**Others**

|   | Protein Name | Accession | U00788 | U00787 | U00786 | U00785 | U00784 | U00783 | U00782 | U00781 | U00780 | U00779 | U00778 |
|---|-------------|-----------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 20 | cAMP-dependent protein kinase type I- | KAP0_MOUSE | 5.27/43.19 | -1.24 | 8.0E-4 | 6 | 6 | 7 | 19.4% |
| No. | Protein                              | Accession  | Ratio   | p-value | fold change | PERHEXILINE |
|-----|-------------------------------------|------------|---------|---------|-------------|--------------|
| 43  | Carbonic anhydrase 1                | CAH1_MOUSE| 6.44/28.32 | 1.50    | 5.3E-4      | 5            | 7            | 22.6%        |
| 42  | Carbonic anhydrase 2                | CAH2_MOUSE| 6.49/29.03 | 1.91    | 0.0014      | 4            | 6            | 7            | 14.6%        |
| 54  | D-dopachrome decarboxylase          | DOPD_MOUSE| 6.09/13.08 | 1.51    | 2.9E-4      | 4            | 4            | 4            | 44.9%        |

*A negative or positive ratio indicates a decrease or increase in perhexiline-treated murine hearts compared to controls.*
| GO and KEGG Categories | GO and KEGG Terms | Percentage of proteins | P-Value | FDR | Proteins |
|-----------------------|------------------|------------------------|--------|-----|----------|
| GO Biological Process | GO:0015669~gas transport | 12.50 | 2.21E-06 | 3.09E-03 | CAH2_MOUSE, HBA_MOUSE, HBB1_MOUSE, MYG_MOUSE |
| GO Biological Process | GO:0006906~glycolysis | 12.50 | 9.81E-05 | 1.37E-01 | CAH2_MOUSE, HBA_MOUSE, HBB1_MOUSE |
| GO Biological Process | GO:0006007~glucose catalytic process | 12.50 | 2.12E-07 | 3.03E-04 | CAH2_MOUSE, HBA_MOUSE, HBB1_MOUSE, MYG_MOUSE |
| GO Biological Process | GO:0006096~glycolysis | 12.50 | 1.62E-04 | 2.26E-01 | LDHB_MOUSE, TPIS_MOUSE, ENOA_MOUSE, ODPA_MOUSE |
| GO Biological Process | GO:0006091~generation of precursor metabolites and energy | 15.63 | 1.89E-03 | 2.62E+00 | LDHB_MOUSE, IDH3A_MOUSE, TPIS_MOUSE, ENOA_MOUSE, ODPA_MOUSE |
| GO Biological Process | GO:0006006~glucose metabolic process | 12.50 | 2.90E-03 | 3.99E+00 | LDHB_MOUSE, TPIS_MOUSE, ENOA_MOUSE, ODPA_MOUSE |
| GO Molecular Function | GO:0016836~hydro-lyase activity | 9.38 | 4.28E-03 | 4.71E+00 | CAH2_MOUSE, ENOA_MOUSE, CAH1_MOUSE |
| GO Cellular Component | GO:0005739~mitochondrion | 28.13 | 4.18E-03 | 4.42E+00 | EFTU_MOUSE, LDHB_MOUSE, IDH3A_MOUSE, EFTS_MOUSE, PRDX2_MOUSE, KCRB_MOUSE, ODPA_MOUSE, KAD4_MOUSE, ODBA_MOUSE |
| GO Biological Process | GO:0019220~regulation of phosphate metabolic process | 15.63 | 3.18E-03 | 4.35E+00 | HBB1_MOUSE, FABP4_MOUSE, PRDX2_MOUSE, KAP1_MOUSE, APOA1_MOUSE |
| GO Biological Process | GO:00015671~oxygen transport | 9.38 | 2.33E-04 | 3.11E-01 | HBA_MOUSE, HBB1_MOUSE, MYG_MOUSE |
| GO Molecular Function | GO:0005344~oxygen transporter activity | 9.38 | 3.76E-04 | 4.63E+00 | HBA_MOUSE, HBB1_MOUSE, MYG_MOUSE |
| GO Biological Process | GO:00044275~cellular carbohydrate catalytic process | 12.50 | 2.48E-04 | 3.46E+00 | LDHB_MOUSE, TPIS_MOUSE, ENOA_MOUSE, ODPA_MOUSE |
| GO Biological Process | GO:0006091~generation of precursor metabolites and energy | 15.63 | 3.16E-03 | 4.35E+00 | LDHB_MOUSE, IDH3A_MOUSE, TPIS_MOUSE, ENOA_MOUSE, ODPA_MOUSE |
| GO Cellular Component | GO:0005739~mitochondrion | 28.13 | 4.18E-03 | 4.42E+00 | EFTU_MOUSE, LDHB_MOUSE, IDH3A_MOUSE, EFTS_MOUSE, PRDX2_MOUSE, KCRB_MOUSE, ODPA_MOUSE, KAD4_MOUSE, ODBA_MOUSE |
| GO Biological Process | GO:0019220~regulation of phosphate metabolic process | 15.63 | 3.18E-03 | 4.35E+00 | HBB1_MOUSE, FABP4_MOUSE, PRDX2_MOUSE, KAP1_MOUSE, APOA1_MOUSE |
| GO Biological Process | GO:00015671~oxygen transport | 9.38 | 2.33E-04 | 3.11E-01 | HBA_MOUSE, HBB1_MOUSE, MYG_MOUSE |
| GO Molecular Function | GO:0005344~oxygen transporter activity | 9.38 | 3.76E-04 | 4.63E+00 | HBA_MOUSE, HBB1_MOUSE, MYG_MOUSE |
Supplemental Table 3:
Metabolic profile of perhexiline-treated hearts as compared to controls.

|                  | Control (n=5) | Perhexiline (n=5) | Ratio* | P (t-test) |
|------------------|---------------|-------------------|--------|------------|
| Acetate          | 0.134 (± 0.019) | 0.087 (± 0.009)   | -1.54  | 0.086      |
| Alanine          | 2.122 (± 0.153) | 2.111 (± 0.481)   | -1.01  | 0.984      |
| AMP+ADP+ATP      | 4.454 (± 0.186) | 2.966 (± 0.703)   | -1.50  | 0.102      |
| Aspartate        | 0.367 (± 0.069) | 0.390 (± 0.028)   | 1.06   | 0.787      |
| B-Hydroxybutyrate| 0.106 (± 0.017) | 0.133 (± 0.039)   | 1.25   | 0.557      |
| Carnitine        | 0.581 (± 0.063) | 0.454 (± 0.013)   | -1.28  | 0.137      |
| Choline          | 0.179 (± 0.008) | 0.176 (± 0.021)   | -1.02  | 0.901      |
| Creatine         | 8.979 (± 0.039) | 7.945 (± 0.170)   | -1.13  | 0.003**    |
| Formate          | 4.863 (± 1.313) | 3.092 (± 1.062)   | -1.57  | 0.353      |
| Fumarate         | 0.048 (± 0.014) | 0.048 (± 0.020)   | 1.00   | 0.988      |
| Glucose          | 0.116 (± 0.034) | 0.236 (± 0.085)   | 2.03   | 0.248      |
| Glutamate        | 3.299 (± 0.368) | 2.759 (± 0.302)   | -1.20  | 0.371      |
| Glutamine        | 5.352 (± 0.572) | 3.827 (± 0.436)   | -1.40  | 0.071      |
| Glycine          | 0.548 (± 0.017) | 0.487 (± 0.024)   | -1.13  | 0.081      |
| Glycolic acid    | 0.515 (± 0.147) | 0.303 (± 0.033)   | -1.70  | 0.260      |
| Iso-Leucine      | 0.228 (± 0.035) | 0.190 (± 0.029)   | -1.20  | 0.455      |
| Lactate          | 10.154 (± 0.961) | 10.265 (± 1.842) | 1.01   | 0.960      |
| Leucine          | 0.178 (± 0.016) | 0.174 (± 0.033)   | -1.02  | 0.908      |
| NAD+NADH         | 0.562 (± 0.035) | 0.515 (± 0.016)   | -1.09  | 0.300      |
| Phenylalanine    | 0.071 (± 0.018) | 0.054 (± 0.006)   | -1.31  | 0.447      |
| Phosphocholine   | 0.162 (± 0.019) | 0.127 (± 0.023)   | -1.28  | 0.292      |
| Succinate        | 1.641 (± 0.369) | 1.476 (± 0.300)   | -1.11  | 0.752      |
| Syllo-inositol   | 0.371 (± 0.030) | 0.343 (± 0.029)   | -1.08  | 0.543      |
| Taurine          | 25.680 (± 0.132) | 22.020 (± 0.609) | -1.17  | 0.003**    |
| Tyrosine         | 0.032 (± 0.006) | 0.017 (± 0.005)   | -1.88  | 0.106      |
| Valine           | 0.128 (± 0.013) | 0.108 (± 0.016)   | -1.19  | 0.373      |

*A negative or positive ratio indicates a decrease or increase in perhexiline-treated murine hearts compared to controls. ** indicates significant changes using an unpaired Student’s t-test.
### Supplemental Table 4: Cross Correlation of Cardiac Metabolites

| Absolute Value | Leucine | Iso-Leucine | Valine | Oil-Butyrate | Lactate | Alanine | Acetate | Glutamate | Succinate | Glutamine | Aspartate | Choline | Phosphocholine | Taurine | Glycine | Glyceric acid | Glucose | Fumarate | Tyrosine | Phenylalanine | Adenosine nucleotides | NAD+ | NADH | Formate | Sylo-Inositol | Carnitine |
|----------------|---------|-------------|-------|--------------|---------|---------|---------|-----------|-----------|-----------|-----------|---------|---------|---------------|---------|---------|-------------|--------|----------|---------|--------------|-------------------------|------|------|--------|-----------|----------|
| 0.50           | 0.50    | 0.50       | 0.34  | 0.38         | 0.31    | 0.28    | 0.46    | 0.40      | 0.07      | 0.16      | 0.26      | 0.25    | 0.47    | 0.47          | 0.84    | 0.75    | 0.64        | 0.68    | 0.01     | 0.16    | 0.38        | 0.02      | 0.16   | 0.08   |
| 0.02*          | 0.50    | 0.50       | 0.34  | 0.38         | 0.31    | 0.28    | 0.46    | 0.40      | 0.07      | 0.16      | 0.26      | 0.25    | 0.47    | 0.47          | 0.84    | 0.75    | 0.64        | 0.68    | 0.01     | 0.16    | 0.38        | 0.02      | 0.16   | 0.08   |
| 0.02*          | 0.50    | 0.50       | 0.34  | 0.38         | 0.31    | 0.28    | 0.46    | 0.40      | 0.07      | 0.16      | 0.26      | 0.25    | 0.47    | 0.47          | 0.84    | 0.75    | 0.64        | 0.68    | 0.01     | 0.16    | 0.38        | 0.02      | 0.16   | 0.08   |
| 0.02*          | 0.50    | 0.50       | 0.34  | 0.38         | 0.31    | 0.28    | 0.46    | 0.40      | 0.07      | 0.16      | 0.26      | 0.25    | 0.47    | 0.47          | 0.84    | 0.75    | 0.64        | 0.68    | 0.01     | 0.16    | 0.38        | 0.02      | 0.16   | 0.08   |
| 0.02*          | 0.50    | 0.50       | 0.34  | 0.38         | 0.31    | 0.28    | 0.46    | 0.40      | 0.07      | 0.16      | 0.26      | 0.25    | 0.47    | 0.47          | 0.84    | 0.75    | 0.64        | 0.68    | 0.01     | 0.16    | 0.38        | 0.02      | 0.16   | 0.08   |
| 0.02*          | 0.50    | 0.50       | 0.34  | 0.38         | 0.31    | 0.28    | 0.46    | 0.40      | 0.07      | 0.16      | 0.26      | 0.25    | 0.47    | 0.47          | 0.84    | 0.75    | 0.64        | 0.68    | 0.01     | 0.16    | 0.38        | 0.02      | 0.16   | 0.08   |
| 0.02*          | 0.50    | 0.50       | 0.34  | 0.38         | 0.31    | 0.28    | 0.46    | 0.40      | 0.07      | 0.16      | 0.26      | 0.25    | 0.47    | 0.47          | 0.84    | 0.75    | 0.64        | 0.68    | 0.01     | 0.16    | 0.38        | 0.02      | 0.16   | 0.08   |
| 0.02*          | 0.50    | 0.50       | 0.34  | 0.38         | 0.31    | 0.28    | 0.46    | 0.40      | 0.07      | 0.16      | 0.26      | 0.25    | 0.47    | 0.47          | 0.84    | 0.75    | 0.64        | 0.68    | 0.01     | 0.16    | 0.38        | 0.02      | 0.16   | 0.08   |
| 0.02*          | 0.50    | 0.50       | 0.34  | 0.38         | 0.31    | 0.28    | 0.46    | 0.40      | 0.07      | 0.16      | 0.26      | 0.25    | 0.47    | 0.47          | 0.84    | 0.75    | 0.64        | 0.68    | 0.01     | 0.16    | 0.38        | 0.02      | 0.16   | 0.08   |
| 0.02*          | 0.50    | 0.50       | 0.34  | 0.38         | 0.31    | 0.28    | 0.46    | 0.40      | 0.07      | 0.16      | 0.26      | 0.25    | 0.47    | 0.47          | 0.84    | 0.75    | 0.64        | 0.68    | 0.01     | 0.16    | 0.38        | 0.02      | 0.16   | 0.08   |

*The correlation values in bold and denoted with an * are those which were significant at a 10% False Discovery Rate (FDR)
| row | name | role/number | role/number | role/number | role/number |
|-----|------|-------------|-------------|-------------|-------------|
| 50  | *glyceraldehyde-3-phosphate dehydrogenase* | *h<sub>m</sub>* | 2.402 | 5.007 | 5.007 |
| 49  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>m</sub>* | 2.402 | 5.007 | 5.007 |
| 48  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 47  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 46  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 45  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 44  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 43  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 42  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 41  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 40  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 39  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 38  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 37  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 36  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 35  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 34  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 33  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 32  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 31  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 30  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 29  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 28  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 27  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 26  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 25  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 24  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 23  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 22  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 21  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 20  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 19  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 18  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 17  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 16  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 15  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 14  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 13  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 12  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 11  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 10  | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 9   | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 8   | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 7   | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 6   | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 5   | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 4   | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 3   | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 2   | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
| 1   | *glyceraldehyde-3-phosphate dehydrogenase* | *c<sub>c</sub>* | 2.402 | 5.007 | 5.007 |
Supplemental Figure 2