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Original article

Metabolic homeostasis is maintained in myocardial hibernation by adaptive changes in the transcriptome and proteome

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ABSTRACT

A transgenic mouse model for conditional induction of long-term hibernation via myocardium-specific expression of a VEGF-sequestering soluble receptor allowed the dissection of the hibernation process into an initiation and a maintenance phase. The hypoxic initiation phase was characterized by peak levels of K(ATP) channel and glucose transporter 1 (GLUT1) expression. Glibenclamide, an inhibitor of K(ATP) channels, blocked GLUT1 induction. In the maintenance phase, tissue hypoxia and GLUT1 expression were reduced. Thus, we employed a combined "-omics" approach to resolve this cardioprotective adaptation process. Unguided bioinformatics analysis on the transcriptomic, proteomic and metabolomic datasets confirmed that anaerobic glycolysis was affected and that the observed enzymatic changes in cardiac metabolism were directly linked to hypoxia-inducible factor (HIF)-1 activation. Although metabolite concentrations were kept relatively constant, the combination of the proteomic and transcriptomic dataset improved the statistical confidence of the pathway analysis by 2 orders of magnitude. Importantly, proteomics revealed a reduced phosphorylation state of myosin light chain 2 and cardiac troponin I within the contractile apparatus of hibernating hearts in the absence of changes in protein abundance. Our study demonstrates how combining different "-omics" datasets aids in the identification of key biological pathways: chronic hypoxia resulted in a pronounced adaptive response at the transcript and the protein level to keep metabolite levels steady. This preservation of metabolic homeostasis is likely to contribute to the long-term survival of the hibernating myocardium.

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1. Introduction

Hibernating myocardium as defined by Rahimtoola et al. [1] refers to resting LV dysfunction due to reduced coronary blood flow that can be partially or completely reversed by myocardial revascularization and/or by reducing myocardial oxygen demand. This endogenous mechanism of cell survival is a potent cardioprotective response that preserves myocardial viability under hypoxia [1,2]. Unlike myocardial infarction, the hibernating myocardium does not undergo cell

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death, but can be salvaged and its function partially or fully restored upon reperfusion [3]. The adaptive mechanisms by which hibernating myocardium survives during chronic ischemia remain to be elucidated [2,4–6].

Recently, May et al. [7] established a bitransgenic system for conditional and reversible loss of vascular endothelial growth factor (VEGF) function in the heart. Inducible cardiac expression of a soluble decoy receptor sequestered VEGF. As a result of the VEGF blockade, the myocardium was subject to a reversible microvascular deficit and experienced chronic hypoxia, which recapitulates all the hallmarks of long-term myocardial hibernation. Cardiomyocytes showed reduced contraction, but remained viable and preserved their potential of full recovery. Importantly, this animal model resembled myocardial hibernation without accompanying cell death and inflammation, two confounding factors in models of ischemia that complicate comparisons with an -omics approach.

Abbreviations: DIGE, difference in-gel electrophoresis; 2-DE, two-dimensional gel electrophoresis; ¹H-NMR, proton nuclear magnetic resonance spectroscopy; LC-MS/ MS, liquid chromatography tandem mass spectrometry.

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In the present study, we performed a proteomic and metabolomic analysis of hearts after 6 weeks of VEGF blockade. We have previously demonstrated the usefulness of this combined approach to assess enzyme and corresponding metabolite changes in preconditioned [8,9] as well as cardioprotected hearts [10]. The combination of proteomics with metabolomics provides a platform for phenotyping transgenic mice at a molecular level [11,12] as protein changes tend to complement alterations in gene expression and metabolite levels provide the integrated "read-out" of the transcriptomic and proteomic variation. The mouse model of conditional VEGF blockade now offers an opportunity to investigate the hibernation program at the mRNA, protein and metabolite level and to demonstrate the feasibility of combining different "-omics" datasets in unsupervised network analyses.

2. Materials and methods

Detailed methodology is provided in the online data supplement. A bi-transgenic system for heart-specific expression of the ligand binding domain of soluble VEGF receptor 1 (sVEGF-R1) was used for the present study [7]. For proteomic and metabolomic analysis, induction of sVEGF-R1 in double-transgenic mice was carried out postnatally by tetracycline withdrawal for 6 weeks as previously described [7]. For *in vivo* inhibition of K(ATP) channels, glibenclamide (0.3 mg/kg bolus i.p.; Sigma Chemical Corporation) was administered as a single dose to 2-week-old mice and RNA was harvested after 24 h.

Key techniques involved adaptations of previously published protocols, including those for difference in-gel electrophoresis (DIGE) [10], liquid chromatography tandem mass spectrometry (LC-MS/MS) [10], proton nuclear magnetic resonance spectroscopy (¹H-NMR) [13], immunoblotting [10], real-time PCR (qPCR) [7] and hypoxyprobe[™] staining [7]. The Affymetrix Genechip mRNA expression analysis data were previously described by May et al. [7]. The network representation with the Cytoscape software and the pathway analysis with the MetaCore[™] systems biology analysis suite (GeneGo Inc., St. Joseph, MI) is explained online. Protocols for proteomics are available on our website at http://www.vascular-proteomics.com.

3. Results

3.1. Initiation and maintenance phase

The conditional system of VEGF blockade allowed a dissection of the hibernation process into two distinct phases: an initiation phase with induction of K(ATP) channels and GLUT1 and a maintenance phase with reduced tissue hypoxia (Fig. 1A). K(ATP) channels represent a union between a member of the inward rectifier Kir family and the ABC superfamily (ATP binding cassette). The latter provides two binding sites, one for SUR (sulfonylurea, epitomized by glibenclamide) and the other for ATP [3]. The subunits SUR2A and Kir6.2 are particularly abundant in cardiomyocytes. After an initial



Fig. 1. Initiation and maintenance phase. (A) Immunohistochemical staining for hypoxia (HypoxyprobeTM) in the hibernating subendocardium after 3 weeks (3W-ON) and 7 weeks (7W-ON) of VEGF blockade. Brown staining indicates areas of hypoxia. Reduced hypoxyprobe staining was observed after 7 weeks (7W-ON) compared to 3 weeks (3W-ON) of VEGF blockade. Images are representative of 3 independent experiments. (B) qPCR analysis of GLUT1 and K(ATP) channels after 2, 3 and 5 weeks of VEGF blockade (2W-ON, 3W-ON, 5W-ON). Note that the maximum in SUR2A and Kir6.2 expression antedates peak levels of CLUT1. SUR2A: ATP-binding cassette; Kir6.2: potassium inwardly rectifying channel; *p-value<0.05, **p-value<0.01, $n \ge 3$ per group. (C) qPCR analysis of FOXO1, a transcription factor regulating K(ATP) channel expression (n = 3). (D) Effect of glibenclamide, an inhibitor of K(ATP) channels, on GLUT1 gene expression in 2-week-old mice ($n \ge 4$ per group).

upregulation within the first 2 weeks of VEGF blockade, SUR2A and Kir6.2 showed lower expression during pro-longed hypoxia (Fig. 1B). This biphasic response was mirrored by the expression pattern of Foxo1, a key transcription factor regulating K(ATP) channel expression (Fig. 1 C). Interestingly, peak values of SUR2A and Kir6.2 (2W-ON) antedated peak levels of glucose transporter 1 expression (GLUT1, 3W-ON) (Fig. 1B). To explore whether this temporal association reflects a causal relationship, glibenclamide, a K(ATP) channel inhibitor was administered to 2-week-old mice (2W-ON). A single injection of glibenclamide attenuated GLUT1 expression within 24 h (Fig. 1D). Survival was not affected at this time point.

3.2. Proteomics and transcriptomics

The observed reduction of tissue hypoxia during the maintenance phase may result from decreased oxygen consumption or increased oxygen supply with the latter being unlikely given the rarefaction of the microvasculature under VEGF blockade. To provide insights into protein changes, control and transgenic hearts (6W-ON) were compared by DIGE. A representative image of the cardiac proteome as separated by two-dimensional gel electrophoresis (2-DE, pH 3-10 nonlinear) is presented in Fig. 2. Principal component analysis (PCA) and hierarchical clustering were applied to the entire proteomic dataset (7 gels per group) to identify the dominant trends and reveal differentially expressed proteins (Supplemental Figure 1). Examples illustrating the quantitative accuracy of the DIGE approach are shown in Supplemental Figure 2. The protein spots were excised, subject to in-gel tryptic digestion, identified by LC-MS/MS analysis (Table 1 and Supplemental Table I) and mapped to our previously published microarray dataset [7] (Fig. 3). Overall, there was a good correlation between mRNA and protein fold induction (Pearson correlation coefficient = 0.6395, $p \le 0.0001$), in particular for genes displaying a



Fig. 2. Protein expression during the maintenance phase. Protein extracts from control and hibernating hearts after 6 weeks of VEGF blockade (6W-ON) were quantified using DIGE. Differentially expressed spots were numbered and identified by LC-MS/MS (Table 1).

significant change in both the transcriptomic and proteomic datasets (Supplemental Table II).

3.3. Changes in net expression

The hibernating myocardium was characterized by increased expression of HIF target genes, reduced levels of mitochondrial enzymes involved in beta-oxidation and adaptive changes in cardiac glucose and energy metabolism (Fig. 3) [7]. As part of the fetal reprogramming [14], gene expression of glucose transporter 1 (Glut1, +3.4-fold, FDR 0.003), natriuretic peptide precursor type A and B (Nppa and Nppb, +3.4and +2.5-fold, FDR 0.009 and 0.057, respectively), myosin heavy polypeptide 7 (Myct1, +8.0-fold, FDR 0.033) and pyruvate dehydrogenase kinase, isoenzyme 1 (Pdk1, +2.3-fold, FDR 0.036) was induced. The proteomic investigation confirmed a concordant upregulation of lactate dehydrogenase (Fig. 4A) and several glycolytic enzymes, among which fructose-biphosphate aldolase, glyceraldehyde-3-phosphate dehydrogenase, and pyruvate kinase are known HIF targets sensitive to hypoxia. Mitochondrial enzymes related to lipid metabolism, i.e., longchain specific acyl-CoA dehydrogenase, short chain specific 3-hydroxyacyl-CoA dehydrogenase, and delta3,5-delta2,4-dienoyl-CoA isomerase, were downregulated in the hibernating myocardium alongside creatine kinase and adenosine kinase, which contribute to energy homeostasis.

3.4. Changes in post-translational modifications

For two myofilament proteins there was discordant regulation at the mRNA and protein level (Fig. 3). Myosin regulatory light chain 2 (MLC2) was resolved as a charge train with different isoelectric points (pI) by 2-DE [15]. The differentially expressed spot (spot 43, Fig. 2) showed a shift towards a more acidic pI, indicative of phosphorylation. This was subsequently confirmed by mobility shift detection of phosphorylated proteins (Phos-tag, Fig. 4B). Similarly, there were no significant differences in protein abundance of cardiac troponin I (TnI) as the observed change was due to decreased phosphorylation in hibernating hearts (Fig. 4C). Other proteins that showed differential expression on 2-DE gels without corresponding alterations in mRNA transcripts are known to be susceptible to oxidative stress, i.e., aldose reductase has a cysteine residue as regulator of its kinetic and inhibition properties; protein disulfide isomerases are redox-sensitive chaperones responsible for the rearrangement of disulfide bonds; and peroxiredoxin 1 and 2 have redox-active cysteins as their main antioxidative component. Oxidation of their cysteine residues makes these proteins more acidic resulting in a charge shift on 2-DE gels without altering the net expression of these cytosolic antioxidants [16,17] (Fig. 4D). In contrast, levels of mitochondrial manganese SOD (SOD2) were reduced although cytosolic copper-zinc SOD (SOD1) was similar in control and hibernating hearts (Fig. 4D). Thus, besides changes in net expression, hibernation was associated with alterations in post-translational modifications of myofilament and redoxsensitive proteins, which can be interrogated by using a proteomics approach.

3.5. Metabolomic analysis

Among the differentially expressed spots were proteins with established links to cardiac K(ATP) channels, including 3 glycolytic enzymes (pyruvate kinase, triose phosphate isomerase and GAPDH) known to be physically associated with cardiac K(ATP) channels [18,19] and contributors to cardiac energy shuttling, including creatine kinase, adenosine kinase and lactate dehydrogenase [20]. We therefore assessed cardiac metabolism after 6 weeks of VEGF blockade by ¹H-NMR spectroscopy. Representative spectra of cardiac metabolite extracts are shown in Fig. 5. Quantitative data are provided in Table 2. Overall, the metabolite changes were not pronounced, but

Table 1

Differentially expressed proteins identifications by tandem mass spectrometry (LC-MS/MS)

Bit Construction Construc	No.	Protein identity	Fold change hibernating vs control	P-value (t-test)	P-value (FDR)	SWISS PROT accession number	Theoretical pI/MW	Observed pI/MW	No of identified peptides	Sequence coverage (%)
B Process-bip/bape base induces A +1.12 4.4-65 0.007 AUDOL,MOUSE 8.4 / 32. 8.7 / 4.6 7.2 5.2 / 3.6 7.3 / 3.6 <th< td=""><td></td><td>Glucose metabolism</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></th<>		Glucose metabolism								
40 Thicoghophate isonerase +1.43 0.0001 0.0038 TPS_MOUSE 7.1 / 266 7.9 / 218 16 S2.00 41 Thicoghophate isonerase +1.31 0.002 0.0035 TPS_MOUSE 7.1 / 266 47 / 1265 41 / 1265 12 5.2.00 16 Private Kinace, isonym M1/M2 +1.24 0.0040 0.24 RYTM_MOUSE 7.4 / 57.7 7.9 / 6.03 12 32.000 16 Private Kinace, isonym M1/M2 +1.24 0.0002 0.0044 0.97A/M0USE 7.4 / 57.7 7.4 / 57.7 8.1 / 60.01 12 30.0007 17 Private Kinace, isonym M1/M2 +1.24 0.0026 0.0014 0.0085 7.4 / 57.7 8.1 / 60.01 30.0107 17 Private Kinace, isonym V1/M2 +1.24 0.0026 0.0014 0.0014 0.0015 6.3 / 56.7 8.7 / 50.7 7.4 / 67.7 8.1 / 60.1 30.001 12 AppLCo Addroponase, long-chan specific -1.22 0.0021 0.021 HOH_MOUSE 8.1 / 7.1 / 80.1 1.1 / 12 30.0101	28	Fructose-bisphosphate aldolase A	+1.52	4.4e-05	0.0027	ALDOA_MOUSE	8.4 / 39.2	8.7 / 41.6	22	52.50%
11 Tricosphosphare isonerase +1.35 0.0002 0.0005 7.1/266 7.9/218 16 5.20% 11 Caperalide/pice/signalize isonerase +1.41 1.1 e 65 0.0010 Cip-MOLES 8.1/215 12 8.6/357 9 2.80% 12 Caperalide/pice/signalize isonerase +1.41 1.1 e 65 0.0010 Cip-MOLES 8.1/215 12 8.6/357 9 2.80% 17 Private isone, isonym M1N2 +1.24 0.002 0.008 0.014 0.008 7/4/377 8.1/60.0 19 2.900% 18 Private isone, isonym M1N2 +1.24 0.002 0.001 0.008 0.014 0.008 0.014 0.008 0.014 0.008 0.014 0.008 6.014 0.008 6.014 0.008 6.014 0.008 6.014 0.008 6.014 0.008 6.014 0.008 6.014 0.008 6.014 0.008 6.014 0.008 6.014 0.008 6.014 0.008 6.014 0.008 6.014 0.008 6.014 0.008 6.014 0.008 6.014	40	Triosephosphate isomerase	+1.43	0.0001	0.0038	TPIS_MOUSE	7.1 / 26.6	7.5 / 22.0	5	20.50%
42 Ticsephosphate isomerase +2.13 1.2e-07 3.1e-5 TPS_MOUSE 7.1/26.6 8.1/21.7 12 5.80% 16 Optivale divergenate +1.11 1.1e-60 0.000 CUPMONE 7.1/26.6 8.1/21.7 12 5.80% 16 Optivale kinase, isonym M1M2 +1.24 0.0002 0.0044 0.004 0.0015 7.4/17.7 7.4/0.03 22 3.90% 18 Pyrivale kinase, isonym M1M2 +1.24 0.0025 0.0014 0.004 0.004 0.004 0.004 0.004 0.004 0.004 0.004 0.004 0.004 0.004 0.004 0.004 0.004 0.004 0.004 0.004 0.005 1.1 0.000 0.001	41	Triosephosphate isomerase	+1.35	0.0002	0.0055	TPIS_MOUSE	7.1 / 26.6	7.9 / 21.8	16	58.20%
11 Citycraldebydes-3-phosphare +1.41 1.1e-49 0.0010 G2P_MOUSE 8.1 / 477 8.6 / 327 9 2.8.802 16 Pryrrate binase, incorum M1M2 +1.23 0.040 0.24 RPTM_MOUSE 7.4 / 577 7.9 / 60.3 1.9 33000 16 Pryrrate binase, incorum M1M2 +1.56 0.0008 0.014 00PA, MOUSE 8.7 / 457 1.5 33000 21 Autom reflectave 1.10 0.0018 0.014 00PA, MOUSE 8.7 / 450 2 3.3 1.1 38.007 22 Autom reflectave 1.12 0.001 0.001 0.001 7.6 / 25.0 7.6 / 45.0 2 5.127 23 Autom reflectave -1.23 0.003 0.021 HOH, MOUSE 8.7 / 45.0 2 5.127 24 Pelta3-settra2.4-decorpt-CoA -1.23 0.002 0.022 DEE3 5.127 5.128 5.1 / 45.0 2 5.137 25 Avainabe deflydregenase, intechondrial -1.23 0.002 0.028<	42	Triosephosphate isomerase	+2.13	1.2e-07	3.1e-5	TPIS_MOUSE	7.1 / 26.6	8.1 / 21.6	12	51.80%
Inc. Inc. <th< td=""><td>31</td><td>Glyceraldehyde-3-phosphate</td><td>+1.41</td><td>1.1e-05</td><td>0.0010</td><td>G3P MOUSE</td><td>8.1 / 47.7</td><td>8.6 / 35.7</td><td>9</td><td>28.80%</td></th<>	31	Glyceraldehyde-3-phosphate	+1.41	1.1e-05	0.0010	G3P MOUSE	8.1 / 47.7	8.6 / 35.7	9	28.80%
16 Private finance, issegree M1APZ +1.23 0.000 0.24 Private, M0KISE 7.4, 17.7 7.9, 16.00 15 230 7.700 17 Private, finance, issegree M1APZ +1.24 0.0000 0.0048 R/Private, M0KISE 7.4, 17.7 82, 16.00 15 0.0008 18 Private, finance, issegree M1APZ +1.24 0.0007 0.0044 0.778, M0KISE 7.4, 17.7 82, 16.00 13 0.0018 19 Addres reflective +1.24 0.0026 0.0011 ADR_MOKISE 7.6, 17.80 2.2 3.5, 12 14 Ligid incretability +1.29 0.002 0.021 D.011 D.014AUDISE 7.6, 14.01 1.0308 Adres 7.6, 14.01 10.3018 Adres 7.6, 14.01 10.3018 Adres 7.6, 14.01 10.3018 Adres 7.6, 14.01 10.3018 Adres 7.6, 14.01 1.0308 Adres 7.6, 14.01 10.3018 Adres 7.6, 14.01 10.3018 Adres 7.6, 14.01 1.0308 Adres 7.6, 14.01 10.3018 Adres 7.6, 14.01 1.3308 1.3018 Adres 7.6, 14.01 1.3308		dehvdrogenase					,	,		
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Image: Component apha suburit Image: Control	24	Pyruvate dehydrogenase E1	+1.66	0.0008	0.014	ODPA_MOUSE	85/432	77/487	15	30.00%
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Jack Structure Regimes Fractions File Structure	33	I-Lactate dehydrogenase A chain	+1.21	7.8e-05	0.0038	IDHA MOUSE	76/295	82/333	11	30.10%
India metadalam India metadalam India sector India s	55	E Lactate denyarogenase i cham	1.25	7.00 05	0.0050	EDIN_MOODE	7.0 / 25.5	0.2 / 55.5		30.10/0
25 Apj-CoA debydrogenase, long-chain specific -1.23 0.013 0.028 26/47.3 67/45.0 2 5.128 14 belad.5 66/12.4/eleonyt-cenzyme A debydrogenase, in-1.23 0.002 0.028 ECIII.MOUSE 8.6/36.1 7.1/29.3 4 10.108 20 finition and matchindmain -1.23 0.002 0.021 IKCHI_MOUSE 8.1/61.3 8.0/55.9 14 25.108 20 Mainto actimational matchindmain -1.23 0.002 0.028 MICL_MOUSE 8.1/61.3 8.0/75.9 14 25.108 20 Maintore mitorbandinal mitorbandinal mitorbandinal -1.23 0.0024 0.008 MICL_MOUSE 8.1/61.3 8.0/75.9 14 25.108 21 Mainto mitorbandinal mitorbandinal -1.20 0.0021 0.078 AICCM_MOUSE 8.1/61.3 8.0/75.9 14 25.108 23 Ontitit bydrase, mitochondrial -1.20 0.021 0.078 AICCM_MOUSE 8.1/81.4 8.4 13 14.802 24 Adensine kinase mitochondrial -1.20 0.021 <		Linid metabolism								
34 Deftx3.5-defx2.4-defx2.4-defx3.ex, micro-fx1 -1.23 0.003 0.028 ECHI_M0USE 7.6 / 36.1 7.1 / 29.3 4 10.105 35 Hydroxysey-compare A dehydrogenase, mitochondrial -1.22 0.002 0.021 HCDH_M0USE 8.8 / 34.5 8.6 / 32.0 12 22.00% 20 Clutamate dehydrogenase 1, mitochondrial -1.23 0.022 0.082 DHE3_MOUSE 8.1 / 61.3 8.0 / 56.9 14 25.10%, 20 Clutamate dehydrogenase 1, mitochondrial -1.23 0.022 0.082 DHE3_MOUSE 8.1 / 61.3 8.0 / 56.9 14 25.10%, 20 Omitime animatransferse, mitochondrial -1.20 0.021 0.078 ACON_MOUSE 8.1 / 85.4 8.0 / 80.0 6 7.95% 36 Areoniate hydratase, nitochondrial -1.20 0.021 0.078 ACON_MOUSE 8.1 / 85.4 8.0 / 80.0 6 7.95% 2 Adenosine kinase -1.25 0.029 0.093 ADK_MOUSE 7.2 / 31.1 6.4 / 40.0 2 3.88% 2 Creatine kinase Mrope -1.22 0.0021 0.076 ROM_M	25	Acyl-CoA dehydrogenase long-chain specific	-1 29	0.013	0.061	ACADI MOUSE	85/479	76/450	2	5 12%
25 Examples Interpretation Interpretation Interpretation Interpretation 25 Hydroxysop-concurves A debydrogenase, - -1.22 0.002 0.021 HCDH_MOUSE 8.8 / 34.5 8.6 / 32.0 1.2 22.00% 26 Marino and metabolism -1.23 0.002 0.082 DHE3_MOUSE 8.1 / 61.3 8.0 / 56.9 14 25.10% 26 Gatamas debydrogenase I, mitochondrial -1.21 0.0004 0.008 MCCA_MOUSE 8.1 / 61.3 8.0 / 56.9 14 25.10% 27 Orbithine aminotradivation -1.23 0.0021 0.078 ACOM_MOUSE 8.1 / 64.4 8.0 / 80.0 6 7.55% 28 Arteristic Marka -1.22 0.021 0.078 ACOM_MOUSE 8.1 / 64.4 8.0 / 80.0 6 7.2 / 9.1 8.1 / 64.4 13 14.80% 29 Adventistic Marka -1.24 0.020 0.020 ACOM_MOUSE 6.1 / 43.0 8.0 / 83.0 6 4.3 / 50.0 6 7.2 / 9.1 / 8.1 8.4 / 54.00	3/	Delta 3 5-delta 2 4-dienovi-CoA	_1.23	0.013	0.001	FCH1 MOUSE	76/361	71/203	2	10.10%
35 hydroxynerwyner Adebydrogenase: -1.22 0.002 0.021 HCDH_MOUSE 8.8 / 34.5 8.6 / 32.0 12 22.005 20 Glutamate delydrogenase: -1.23 0.002 0.082 DHEJ_MOUSE 8.1 / 61.3 8.0 / 56.9 14 25.10% 20 Glutamate delydrogenase: -1.23 0.002 0.082 DHEJ_MOUSE 8.1 / 61.3 8.0 / 56.9 14 25.10% 21 Omthine aminotransferase, mitochondrial -1.20 0.021 0.078 ACON_MOUSE 6.2 / 48.3 6.6 / 48.3 2 4.783 23 Ornthine aminotransferase, mitochondrial -1.29 0.021 0.078 ACON_MOUSE 8.1 / 54.4 8.0 / 80.0 6 7.95% 36 Arrey antase gamma chain, mitochondrial +1.27 0.021 0.078 ACON_MOUSE 7.2 / 98.1 8.4 / 84.3 13 14.80% 27 Addensine hinase M, type -1.24 0.002 0.003 ADK_MOUSE 7.2 / 98.1 8.4 / 84.3 13 14.80% 28 Adressine hinase M, type -1.22 0.002 0.003 ADK_MOUSE 7.2 / 98.1	74	icomerase mitechondrial	-1.25	0.005	0.028	LCITI_IVIOUSL	7.0 / 50.1	7.1 / 25.5	7	10.10%
3-3 multicologistic -1.22 0.02 0.021 PLDM_MOUSE 8// 94.3 8// 94.3 8// 94.3 12 22.00x 20 Anito add metabolism alglab chain, mutochondrial alglab chain, mutochondrial alglab chain, mutochondrial alglab chain, mutochondrial -1.21 0.002 0.082 DHE3.M0USE 8.1 / 61.3 8.0 / 56.9 14 25.108 23 Omithine aminochondrial alglab chain, mutochondrial alglab chain, mutochondrial -1.20 0.021 0.078 ACOL_MOUSE 8.1 / 85.4 8.0 / 80.0 6 7.95% 5 Aconitate hydratase, mitochondrial +1.22 0.021 0.078 ACOL_MOUSE 8.1 / 85.4 8.0 / 80.0 6 7.95% 6 Aconitate hydratase, nitochondrial +1.27 0.021 0.078 ACOL_MOUSE 8.1 / 85.4 8.0 / 80.0 6 7.95% 6 Aconitate hydratase, nitochondrial +1.27 0.021 0.078 ACOL_MOUSE 8.1 / 82.4 8.3 / 82.4 4.3 14.80% 3.1 / 82.0 5 16.4 4.6 2 3.88% 3.00% 5 17	25	Isomerase, initochonunai	1 22	0.002	0.021	UCDU MOUSE	00/245	86/220	10	22.00%
1000000000000000000000000000000000000	30	Hydroxyacyi-coenzynne A denydrogenase,	-1.22	0.002	0.021	HCDH_WOUSE	8.8 / 34.3	8.6 / 32.0	12	22.00%
20 Anito acid metabolism Guanance delpdição acibavylase acidamete delpdição acibavylase acidamete delpdição acibavylase acidamete delpdição acibavylase acidamete delpdição acibavylase acidameter delpdição acidavylase acidameter delpdição acidavylase acidameter delpdição acidavylase acidameter delpdição acidavylase acidameter delpdição acidavylase acidameter delpdição acidavylase acidameter delpdição acidameter delpdição acidameter acidameter acidameter delpdição acidameter delpdição acidameter d		mitochondrial								
Antice delay increases I, mitochondrial -1.23 0.022 0.082 DHE3_MOUSE 8.1 / 61.3 8.0 / 56.9 14 25.10% 23 Ornithine aminochondrial -1.21 0.004 0.008 MCCA_MOUSE 7.7 / 7.3 8.0 / 7.4.1 11 13.00% 23 Ornithine aminochondrial +1.42 9.8e-08 3.1e-5 0AT_MOUSE 6.2 / 48.3 6.6 / 48.3 2 4.783 5 Aconate hydratase, mitochondrial -1.20 0.021 0.078 ACOC_MOUSE 2.1 / 3.4 6.0 / 80.0 6 7.95% 6 Aconate hydratase, cytoplasmic -1.22 0.021 0.078 ACCC_MOUSE 2.1 / 3.28 9.1 / 27.1 5 15.108 2 Adtensite hydratase, printecholom -1.22 0.022 0.028 ACCC_MOUSE 7.2 / 1.1 6.4 / 46.0 2 3.88% 27 Creatine kinase 4, mitochondrial +1.24 0.005 0.009 ACM 8.1 / 6.1.3 8.0 / 43.8 24 5.1 0% 4 Portein disalified-somerase +1.		Aming and materialism								
20 Cultarinate deligningenade 1, internominat -1.21 0.0024 0.082 Diff. JAUCKE 8.1 / 5.4 8.0 / 56.9 14 2.5.05 20 Methylcrononyl-Go. Carbonylae -1.21 0.0004 0.008 8.1 / 6.1 8.1 / 5.4 8.0 / 50.9 14 13.05 21 Ornthine animotrandrease, mitchondrial +1.42 9.8e-08 3.1e-5 0.AT_MOUSE 6.1 / 8.3 6.6 / 48.3 2 4.78% 25 Aconitate hydratase, mitchondrial -1.20 0.021 0.078 ACON_MOUSE 8.1 / 8.4 8.0 / 80.0 6 7.95% 36 ATP synthase samma chain, mitchondrial +1.27 0.021 0.078 ACON_MOUSE 8.1 / 8.4 8.0 / 80.0 6 7.95% 22 Adensize kinase 4, mitchondrial +1.27 0.021 0.078 ACON_MOUSE 7.2 / 9.1 8.1 / 8.4 8.3 8.1 14.000 23 Adensize kinase 4, mitchondrial +1.27 0.021 0.078 ACON_MOUSE 7.2 / 7.1 5.1 7.0 / 7.2 5.8 5.0.053 24 Adensize kinase 4, mitchondrial +1.27 0.002 <	20	Amino acid metabolism	4.00	0.000	0.000	DUES MOURE	0.4. (01.0	00/500		25 1 000
A Methylcrotonogi-LoA Carboxylase 1.21 0.0004 0.008 MICLA,MOUSE 1.7 / 19.3 8.0 / 1.4 1 13.802 23 Ornithine animothondrial +1.42 9.88-08 3.1e-5 OAT_MOUSE 6.2 / 48.3 6.6 / 48.3 2 4.78% 5 Aconitate hydratase, mitochondrial -1.20 0.021 0.078 ACON_MOUSE 6.1 / 85.4 8.0 / 80.0 6 7.95% 6 Aconitate hydratase, mitochondrial +1.27 0.021 0.078 ACON_MOUSE 7.1 / 85.4 8.0 / 80.0 6 7.95% 6 Aconitate hydratase, mitochondrial +1.27 0.021 0.078 ACO_MOUSE 7.2 / 31.1 6.4 / 46.0 2 3.88% 6 Adensylate kinase 4, mitochondrial +1.23 0.002 0.0055 ROX1_MOUSE 7.2 / 31.1 6.4 / 46.0 2 3.88% 7 Creatine kinase 4, mitochondrial +1.24 0.005 0.0005 ROX1_MOUSE 5.2 / 21.8 5.7 / 32.5 8 3.50.0% 4 Protein disulfide-isomerase	20	Giutamate denydrogenase 1, mitochondriai	-1.23	0.022	0.082	DHE3_MOUSE	8.1/61.3	8.0 / 56.9	14	25.10%
a) pho chain, mitochoodrial +1.42 9.8e-08 3.1e-5 OVT_MOUSE 6.2 / 48.3 6.6 / 48.3 2 4.785 5 Aconitate hydratase, mitochondrial -1.20 0.021 0.078 ACON_MOUSE 7.2 / 98.1 8.4 / 84.8 13 14.805 5 Aconitate hydratase, mitochondrial +1.27 0.021 0.078 ATPC_MOUSE 9.1 / 92.8 8.1 / 85.4 8.0 / 80.0 6 7.953 5 Adressine hydratase, mitochondrial +1.27 0.021 0.078 ATPC_MOUSE 9.1 / 92.8 9.1 / 92.8 9.1 / 92.8 9.1 / 92.8 9.1 / 92.8 9.1 / 92.8 9.1 / 92.8 9.1 / 92.8 9.1 / 92.8 9.1 / 92.8 9.1 / 92.8 9.1 / 92.8 9.1 / 92.8 9.1 / 92.8 9.1 / 92.8 9.1 / 92.8 9.1 / 92.8 9.1 / 92.8 9.1 / 92.28 8.3 / 92.2 8.3 / 92.2 8.3 / 92.2 8.3 / 92.2 8.3 / 92.2 8.3 / 92.2 8.3 / 92.2 8.3 / 92.2 8.3 / 92.2 8.3 / 92.2 8.3 / 92.2 8.3 / 92.2 8.3 / 92.2 8.3 / 92.2 8.3 / 92.2 8.3 / 92.1 8.3 / 92.2 8.3 / 92.1 8.3 / 92.2 8.3 / 92.1 8.3 / 92.2 8.3 / 92.2	/	Methylcrotonoyl-CoA carboxylase	-1.21	0.0004	0.008	MCCA_MOUSE	7.7 / 79.3	8.0 / 74.1	11	13.80%
23 Ornthine aniochasterase, mitochondrial +1.42 9.84-08 3.1e-5 OAT_MOUSE 6.2 / 48.3 6.6 / 48.3 2 4.78s TCA cycle Aconitate hydratase, mitochondrial -1.20 0.021 0.078 ACON_MOUSE 8.1 / 85.4 8.0 / 80.0 6 7.95% 6 Aconitate hydratase, cytoplasmic -1.29 0.021 0.078 ACOC_MUSE 7.2 / 98.1 8.4 / 48.4 13 14.80% 22 Adenosine kinase -1.25 0.029 0.093 ADK_MOUSE 7.2 / 31.1 6.4 / 46.0 2 3.88% 7 Creatific kinase M-type -1.25 0.002 0.005 KCRM_MOUSE 6.6 / 43.0 8.0 / 43.8 2.4 54.10% 49 Adensylate kinase A, mitochondrial +1.23 0.001 0.005 PROX1_MOUSE 8.3 / 22.2 8.7 / 18.3 11 42.20% 44 Peroxiredoxin 1 +1.33 0.0010 0.005 PROX1_MOUSE 8.3 / 22.2 8.7 / 18.3 11 42.20% 41 Protein disulfide-isomerase A3 +1.43 5.7-06 0.00061 PDU3_MOUSE 6.3 / 6.5 / 60.5		alpha chain, mitochondrial								
TCA cycle Aconitate hydratase, mitochondrial -1.29 0.021 0.078 ACON, MOUSE 8.1 / 854 8.0 / 80.0 6 7.95% 36 Arm Synthase gamma chain, mitochondrial +1.27 0.021 0.078 ACOC, MOUSE 7.2 / 98.1 8.0 / 80.0 6 1 14.80% 36 Ard Synthase gamma chain, mitochondrial +1.27 0.021 0.078 ACOC, MOUSE 7.2 / 98.1 8.1 / 85.4 8.4 / 84.8 13 15.10% 22 Adenosine kinase -1.25 0.029 0.090 ADK,MOUSE 7.2 / 31.1 6.4 / 45.0 2 3.88% 54.10% 44 Protein kinase M-type -1.22 0.002 0.020 KCRM,MOUSE 6.7 / 45.1 8.0 / 48.8 24 54.10% 48 Peroxinedoxin 1 +1.35 0.0002 0.0056 PRDX1,MOUSE 8.3 / 22.2 8.7 / 18.3 11 42.00% 41 Protein disulfide-isomerase +1.23 0.011 0.014 PDIA3,MOUSE 6.5 / 60.3 6 11.90% 42 Protein disulfide-isomerase A3 +1.43 3.8e-10 31e-05 GRP78,MOUS	23	Ornithine aminotransferase, mitochondrial	+1.42	9.8e-08	3.1e-5	OAT_MOUSE	6.2 / 48.3	6.6 / 48.3	2	4.78%
TCA cycle TCA cycle TCA cycle Aconitate hydratse, mitochondrial -1.29 0.021 0.078 ACON, MOUSE 8.1 / 85.4 8.0 / 80.0 6 7.95% 6 Acronitate hydratse, cytoplasmic -1.29 0.034 0.095 ACOC, MOUSE 9.1 / 27.1 5 14.80% 22 Adenosine kinase -1.25 0.029 0.093 ADK, MOUSE 7.2 / 31.1 6.4 / 46.0 2 3.88% 27 Creatine kinase 4, mitochondrial +1.24 0.005 0.090 KCMM, MOUSE 7.0 / 25.1 7.9 / 23.5 8 35.00% 4 Adensylate kinase 4, mitochondrial +1.23 0.0002 0.0055 PRDX1_MOUSE 8.3 / 22.2 8.7 / 18.3 11 42.20% 44 Peroxiredoxin 2 +1.23 0.011 0.056 PRDX1_MOUSE 6.0 / 56.6 6.5 / 60.5 6 11.00% 14 Protein disulfide-isomerase A3 +1.43 5.7e-06 0.0006 PDIA3_MOUSE 6.1 / 22.4 8.0 / 73.6 21 33.70% 74 <										
5 Aconitate hydratsse, rincchondrial -1.20 0.078 ACON,MOUSE 8.1 / 85.4 8.0 80.0 6 7.25% 36 ACTP synthase gamma chain, mitochondrial +1.27 0.021 0.078 ACON,MOUSE 7.2 / 9.8.1 8.4 / 84.8 8.1 11.430% 36 ATP synthase gamma chain, mitochondrial +1.27 0.021 0.078 ACMOUSE 7.2 / 9.8.1 5.4 / 64.0 2 3.88% 37 Creatine kinase -1.22 0.002 0.020 KCRM,MOUSE 7.2 / 9.1.1 6.4 / 46.0 2 3.88% 37 Creatine kinase M-type -1.22 0.002 0.020 KCRM,MOUSE 7.0 / 25.1 7.9 / 25.5 8 35.00% 40 Adenytate kinase 4, mitochondrial +1.23 0.0002 0.0056 PRDX1_MOUSE 8.2 / 22.8 8.7 / 18.3 11 42.20% 44 Proxinedoxin 1 +1.135 0.0002 0.0056 PRDX1_MOUSE 5.2 / 21.8 5.0 / 18.0 11.90% 41 Protein disulfide-isomerase A + 1.43 5.0 - 0100 0.014 PDIA3_MOUSE 6.1 / 22.0 6.1 / 20.0 11.90%		TCA cycle								
6 Aconitate hydratase, cytoplasmic -1.29 0.034 0.095 ACCC_MOUSE 7.2 / 9.1 8.4 / 8.4 13 14.80% 36 ATP synthase gamma chain, mitochondrial +1.27 0.021 0.078 ATPC_MOUSE 7.2 / 9.1 5 15.10% 22 Adenosine kinase -1.25 0.029 0.093 ADK_MOUSE 7.2 / 3.1 6.4 / 46.0 2 3.88% 22 Adenosine kinase -1.25 0.020 0.020 KCRM_MOUSE 7.2 / 3.1 6.4 / 46.0 2 3.88% 49 Adenosine kinase +1.22 0.002 0.020 KCRM_MOUSE 7.0 / 25.1 7.9 / 23.5 8 35.00% Antioxidants - - +1.23 0.011 0.056 PRDX1_MOUSE 5.2 / 7.18 11 42.20% 44 Peroxiredoxin 1 +1.32 0.0011 0.014 PDIA1_MOUSE 5.2 / 7.18 5.0 / 73.6 21 33.70% 74 Atpa crystalin B chain +1.48 3.8e-10 3.1e-05 CRP78_MOUSE 5.1 / 72.4 5.0 / 73.6 21 33.70% 7 <td< td=""><td>5</td><td>Aconitate hydratase, mitochondrial</td><td>-1.20</td><td>0.021</td><td>0.078</td><td>ACON_MOUSE</td><td>8.1 / 85.4</td><td>8.0 / 80.0</td><td>6</td><td>7.95%</td></td<>	5	Aconitate hydratase, mitochondrial	-1.20	0.021	0.078	ACON_MOUSE	8.1 / 85.4	8.0 / 80.0	6	7.95%
36 ATP synthase gamma chain, mitrochondrial + 1.27 0.021 0.078 ATPC_MOUSE 9.1 / 22.8 9.1 / 27.1 5 15.10% Energy metabolism Energy metabolism Creatine kinase - 1.25 0.029 0.093 ADK_MOUSE 7.2 / 31.1 6.4 / 46.0 2 3.88% 27 Creatine kinase M-type - 1.22 0.002 0.020 KCRM_MOUSE 7.0 / 25.1 7.9 / 23.5 8 35.00% 48 Peroxiredoxin 1 + 1.23 0.010 0.095 PRDX1_MOUSE 8.3 / 72.2 8.7 / 18.3 11 42.20% 44 Peroxiredoxin 2 + 1.23 0.011 0.056 PRDX1_MOUSE 5.2 / 21.8 5.0 / 18.4 6 2.4 / 70% 9 Protein disulfide-isomerase A3 + 1.43 5.7 e-06 0.0061 PDI3_MOUSE 6.1 / 72.4 5.0 / 73.6 21 33.70% 74 Alpha crystalin B chain + 1.46 9.6 e-05 0.0020 RCRAB_MOUSE 6.1 / 72.4 5.0 / 73.6 21 33.70% 74 Alpha crystalin B chain + 1.46 9.6 e-05 0.0020 NCRAB_MOUSE 6.1	6	Aconitate hydratase, cytoplasmic	-1.29	0.034	0.095	ACOC_MOUSE	7.2 / 98.1	8.4 / 84.8	13	14.80%
Energy metabolism -1.25 0.029 0.093 ADK,MOUSE 7.2 / 31.1 6.4 / 46.0 2 3.88% 22 Creatine kinase 4, mitochondrial + 1.24 0.005 0.090 KCRM_MOUSE 66 / 33.0 8.0 / 43.8 24 3.88% 49 Adensylate kinase 4, mitochondrial + 1.24 0.005 0.090 KCRM_MOUSE 63 / 32.0 8.0 / 18.4 64 35.00% 44 Peroxitedoxin 1 + 1.35 0.0002 0.0055 PRDX1_MOUSE 5.2 / 71.8 5.0 / 18.4 6 24.70% 9 Protein disulfide-isomerase A3 + 1.43 5.7e-06 0.0014 PDIA_J.MOUSE 6.0 / 56.6 6.5 / 60.5 6 11.90% 1 78 kDa glucose-regulated protein + 1.48 3.8e-10 3.1e-05 CRP73_MOUSE 6.1 / 72.0 5.0 / 73.6 2.1 33.70% 1 78 kDa glucose-regulated protein + 1.46 9.6e-05 0.003 CRP3_MOUSE 6.1 / 23.0 6.1 / 23.0 6.1 / 23.0 6.1 / 23.0 6.1 / 23.0 6.1 / 23.0 6.1 / 23.0 <td>36</td> <td>ATP synthase gamma chain, mitochondrial</td> <td>+1.27</td> <td>0.021</td> <td>0.078</td> <td>ATPG_MOUSE</td> <td>9.1 / 32.8</td> <td>9.1 /27.1</td> <td>5</td> <td>15.10%</td>	36	ATP synthase gamma chain, mitochondrial	+1.27	0.021	0.078	ATPG_MOUSE	9.1 / 32.8	9.1 /27.1	5	15.10%
Energy metabolism Energy metabolism 22 Adenosine kinase -1.25 0.029 0.093 ADK_MOUSE 7.2/31.1 6.4/45.0 2 3.88% 27 Creatine kinase M-type -1.22 0.002 0.020 KCRM_MOUSE 6.6/43.0 8.0/43.8 24 54.10% 48 Peroxiredoxin 1 +1.35 0.0002 0.0055 PRDX1_MOUSE 5.3/22.2 8.7/18.3 11 42.20% 44 Peroxiredoxin 1 +1.35 0.010 0.056 PRDX1_MOUSE 5.3/22.2 8.7/18.3 11 42.20% 44 Peroxiredoxin 2 +1.32 0.011 0.056 PRDX1_MOUSE 5.3/22.1 8.0/18.4 6 4.70% 9 Protein disulfide-isomerase +1.43 5.7e-06 0.00061 PDIA1_MOUSE 6.3/26.6 6.5 / 60.5 6 11.90% 7.4 Dab glucose-regulated protein +2.18 3.8e-10 3.1e-05 CRY8_MOUSE 6.3/20.1 8.0/17.6 10 45.10% 9 Hoat-shock protein boad plucose-r										
22 Adenosine kinase -125 0.029 0.093 ADK_MOUSE 7.2/31.1 6.4/450 2 3.88% 27 Creatine kinase M-type -1.22 0.002 0.020 KCRM_MOUSE 6.6/430 8.0/438 24 54.10% 49 Adenylate kinase 4, mitochondrial +1.24 0.005 0.090 KAD4_MOUSE 7.0/25.1 7.9/23.5 8 35.00% 48 Peroxiredoxin 1 +1.35 0.001 0.056 PRDX1_MOUSE 8.3/22.2 8.7/18.3 11 42.20% 44 Peroxiredoxin 1 +1.32 0.011 0.056 PRDX1_MOUSE 8.3/22.2 8.5/14.6 24.70% 9 Protein disulfide-isomerase A3 +1.43 5.7e-06 0.00061 PDA3_MOUSE 6.0/56.6 6.5/60.5 6 11.90% Chaperones - - 7.8 kDa glucose-regulated protein +2.18 3.8e-10 3.1e-05 GRY3_MOUSE 6.1/23.0 6.1/23.0 6.1/23.0 6.1/23.0 6.1/23.0 6.1/23.0 6.1/23.0 6.1/23.0 6.1/23.0 6.1/23.0 6.1/23.0 6.1/23.0 6.1/23.0 6.1/23.0 <td></td> <td>Energy metabolism</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>		Energy metabolism								
27 Creatine kinase M-type -1.22 0.002 KCRM_MOUSE 6.6 / 43.0 8.0 / 43.8 24 54.10% 49 Adenylate kinase M-type +1.24 0.005 0.090 KAD4_MOUSE 7.0 / 25.1 7.9 / 23.5 8 35.00% 41 Peroxiredoxin 1 +1.35 0.0002 0.0055 PRDX1_MOUSE 8.3 / 22.2 8.7 / 18.3 11 42.20% 42 Peroxiredoxin 2 +1.23 0.011 0.056 PRDX1_MOUSE 5.2 / 21.8 5.0 / 18.4 6 2.470% 9 Protein disulfide-isomerase A3 +1.43 5.70-66 0.00061 PDIA3_MOUSE 5.1 / 72.4 5.0 / 73.6 21 33.70% 14 Protein disulfide-isomerase A3 +1.43 5.70-66 0.00061 PDIA3_MOUSE 6.1 / 23.0 6.1 / 22.2 2 9.09% 17 Rkop glucose-regulated protein +2.18 3.8e-10 3.1e-05 CRP78_MOUSE 6.1 / 23.0 6.1 / 22.0 6.1 / 23.0 6.1 / 22.0 2 9.09% 38 Heat-shock protein beta-1 (27 / Ka) +1.26 0.0003 0.0071 TNNI3_MOUSE 6.1 / 23	22	Adenosine kinase	-1.25	0.029	0.093	ADK_MOUSE	7.2 / 31.1	6.4 / 46.0	2	3.88%
49 Adenylate kinase 4, mitochondrial +1.24 0.005 0.090 KAD4_MOUSE 7.0 / 25.1 7.9 / 23.5 8 35.00% Antioxidants - - Antioxidants -	27	Creatine kinase M-type	-1.22	0.002	0.020	KCRM_MOUSE	6.6 / 43.0	8.0 / 43.8	24	54.10%
Antioxidants 48 Peroxiredoxin 1 +1.35 0.0002 0.0055 PRDX1_MOUSE 8.3 / 22.2 8.7 / 18.3 1.1 42.20% 44 Peroxiredoxin 2 +1.32 0.011 0.056 PRDX2_MOUSE 5.2 / 21.8 5.0 / 18.4 6 24.70% 59 Protein disulfide-isomerase +1.32 0.011 0.016 PDIA1_MOUSE 6.0 / 56.6 6.5 / 60.5 6 11.90% Chaperones Chaperones 17 78.Kbg alguose-regulated protein +2.18 3.8e-10 3.1e-05 CRY78_MOUSE 5.1 / 72.4 5.0 / 73.6 21 33.70% 47 Alpha crystallin B chain +1.46 9.6e-05 0.0038 CRYAB_MOUSE 6.1 / 22.2 2 9.09% 8 T-complex protein bt.at-1 (27 kDa) +1.36 0.002 10.20 HSPBI_MOUSE 6.1 / 23.0 6.1 / 22.2 2 9.09% 43 Myosin regulatory light chain 2, -1.34 0.018 0.073 MLRV_MOUSE 4.7 / 18.8 4.6 / 16.5 11 46.40% 44 Protein in cardiac muscle	49	Adenylate kinase 4, mitochondrial	+1.24	0.005	0.090	KAD4_MOUSE	7.0 / 25.1	7.9 / 23.5	8	35.00%
Antioxidans 48 Peroxiredoxin 1 +1.35 0.0002 0.0055 PRDX1_MOUSE 8.3 / 222 8.7 / 18.3 11 42.20% 44 Peroxiredoxin 2 +1.23 0.011 0.056 PRDX2_MOUSE 5.2 / 21.8 5.0 / 18.4 6 24.70% 9 Protein disulfide-isomerase +1.32 0.001 0.014 PPIA1_MOUSE 6.8 / 57.1 4.6 / 59.9 5 8.64% 1 Protein disulfide-isomerase A3 +1.43 5.7e-06 0.00061 PDIA3_MOUSE 5.1 / 72.4 5.0 / 73.6 21 33.70% 47 Alpha crystallin B chain +1.46 9.6e-05 0.0038 CRYAB_MOUSE 6.1 / 23.0 6.1 / 23.0 6.1 / 23.0 6.1 / 23.0 6.1 / 23.0 6.1 / 23.0 6.1 / 23.0 6.1 / 23.0 10 19.20% 39 Heat-shock protein beta-1 (27 kDa) +1.25 0.006 0.040 TCP2_MOUSE 6.7 / 57.8 7.9 / 64.3 10 19.20% 43 Myosin regulatory light chain 2, ventricular/cardiac muscle isoform -1.34 0.018 0.073 MLRV_MOUSE 4.7 / 18.8 4.6 / 16.5 11										
44 Peroxiredoxin 1 +1.35 0.0002 0.0055 PRDXL,MOUSE 8.3 / 22.2 8.7 / 18.3 11 42.20% 44 Peroxiredoxin 1 +1.23 0.011 0.056 PRDXL,MOUSE 5.2 / 21.8 5.0 / 18.4 6 24.70% 9 Protein disulfide-isomerase +1.32 0.001 0.014 PDIA1_MOUSE 5.2 / 21.8 5.0 / 18.4 6 24.70% 14 Protein disulfide-isomerase A3 +1.43 5.7e-06 0.00061 PDIA3_MOUSE 6.0 / 56.6 6.5 / 60.5 6 11.90% Chaperones 1 78 kDa glucose-regulated protein +2.18 3.8e-05 0.002 GRYAB_MOUSE 6.1 / 72.4 5.0 / 73.6 21 33.70% 39 Heat-shock protein beta-1 (27 kDa) +1.36 0.002 0.020 HSPB1_MOUSE 6.1 / 23.0 6.1 / 23.2 2 9.09% 8 T-complex protein beta-1 (37 kDa) +1.25 0.006 0.040 TCPZ_MOUSE 6.1 / 23.0 6.1 / 23.0 6.1 / 23.0 6.1 / 23.0 6.1 / 23.0 6.1 / 23.0 6.1 / 23.0 6.1 / 23.0 6.1 / 23.0 6.1 /		Antioxidants								
44 Peroxiredoxin 2 +1.23 0.011 0.056 PRDx2_MOUSE 5.2 / 21.8 5.0 / 18.4 6 24.70% 9 Protein disulifici-isomerase A3 +1.32 0.001 0.014 PDIA1_MOUSE 4.8 / 57.1 4.6 / 59.9 5 8.644% 1 Protein disulifici-isomerase A3 +1.43 5.7e-66 0.00061 PDIA3_MOUSE 6.0 / 56.6 6.5 / 60.5 6 1.1 / 05.0 7 Apha crystalin B chain +1.46 9.6e-05 0.0038 CRYA8_MOUSE 6.8 / 75.8 7.0 / 75.6 21 33.70% 7 Apha crystalin B chain +1.46 9.6e-05 0.0038 CRYA8_MOUSE 6.1 / 23.0 6.1 / 22.2 2 9.09% 8 T-complex protein 1, zeta subunit +1.25 0.006 0.040 TCP2_MOUSE 4.7 / 18.8 4.6 / 16.5 11 46.40% 43 Myofilaments	48	Peroxiredoxin 1	+1.35	0.0002	0.0055	PRDX1_MOUSE	8.3 / 22.2	8.7 / 18.3	11	42.20%
9Protein disulfide-isomerase $+1.32$ 0.0010.014PDIAL_MOUSE 4.8 / 57.1 4.6 / 59.9 5 $8.64x$ 14Protein disulfide-isomerase A3 $+1.43$ $5.7e-06$ 0.00061PDIA_MOUSE 6.0 / 56.6 6.5 / 60.5 6 $11.90x$ 17Rk Da glucose-regulated protein $+2.18$ $3.8e-10$ $3.1e-05$ GRP78_MOUSE 5.1 / 72.4 5.0 / 73.6 21 $33.70x$ 47Alpha crystalin B chain $+1.46$ $9.6e-05$ 0.0038 GRYAB_MOUSE 6.1 / 23.0 6.1 / 22.2 2 $9.09x$ 39Heat-shock protein beta-1 (27 kDa) $+1.36$ 0.002 0.020 HSPB1_MOUSE 6.7 / 57.8 7.9 / 64.3 10 $19.20x$ 43Myofilaments Myosin regulatory light chain 2, ventriculat/cardiac muscle -1.67 0.003 0.071 TNNI3_MOUSE 9.6 / 21.2 9.4 / 21.0 2 $9.48x$ 11Desmin $+1.38$ $3.5e-05$ 0.0024 DESM_MOUSE 5.2 / 53.4 5.2 / 56.4 26 $50.70x$ 12Desmin $+1.50$ $3.3e-07$ $5.8e-05$ DESM_MOUSE 5.2 / 53.4 5.2 / 56.4 26 $50.70x$ 12Desmin $+1.76$ 0.001 0.016 APA1_MOUSE 5.2 / 53.4 5.2 / 56.4 26 $50.70x$ 12Desmin $+1.20$ 0.001 0.056 TBB2MOUSE 5.2 / 53.4 5.2 / 56.4 26 $50.70x$ 13Fibrinogen parma chain $+1.20$	44	Peroxiredoxin 2	+1.23	0.011	0.056	PRDX2_MOUSE	5.2 / 21.8	5.0 / 18.4	6	24.70%
14 Protein disulfide-isomerase A3 +1.43 5.7e-06 0.00061 PDIA3_MOUSE 6.0 / 56.6 6.5 / 60.5 6 11.90% 1 78 kDa glucose-regulated protein +2.18 3.8e-10 3.1e-05 GRP78_MOUSE 5.1 / 72.4 5.0 / 73.6 21 33.70% 47 Alpha crystalin B chain +1.46 9.6e-05 0.0038 CRYA8_MOUSE 6.1 / 23.0 6.1 / 22.2 2 9.09% 8 T-complex protein heta-1 (27 kDa) +1.25 0.006 0.040 TCPZ_MOUSE 6.7 / 57.8 7.9 / 64.3 10 19.20% 43 Myofilaments Myosin regulatory light chain 2, ventricular/cardiac muscle isoform -1.34 0.018 0.073 MLRV_MOUSE 4.7 / 18.8 4.6 / 16.5 11 46.40% 43 Myosin regulatory light chain 2, ventricular/cardiac muscle isoform -1.67 0.0003 0.0071 TNN13_MOUSE 5.2 / 51.4 5.2 / 56.4 26 50.70% 11 Desmin +1.38 3.5e-05 0.0024 DESM_MOUSE 5.2 / 51.4 5.2 / 56.4 26 50.70% 12 Desmin +1.45 0.011	9	Protein disulfide-isomerase	+1.32	0.001	0.014	PDIA1_MOUSE	4.8 / 57.1	4.6 / 59.9	5	8.64%
Chaperones 1 78 kDa glucose-regulated protein + 2.18 3.8e-10 3.1e-05 GRP78_MOUSE 5.1 / 72.4 5.0 / 73.6 21 33.70% 47 Alpha crystallin B chain + 1.46 9.6e-05 0.0038 HSPB_MOUSE 6.8 / 20.1 8.0 / 17.6 10 45.10% 39 Heat-shock protein beta-1 (27 kDa) + 1.25 0.000 0.020 HSPB_LMOUSE 6.7 / 57.8 7.9 / 643 10 19.20% 43 Myosin regulatory light chain 2, ventricular/cardiac muscle isoform -1.34 0.018 0.073 MLRV_MOUSE 4.7 / 18.8 4.6 / 16.5 11 46.40% 7 Troponin I, cardiac muscle -1.67 0.0003 0.0071 TNNI3_MOUSE 5.2 / 53.4 5.2 / 56.4 2.6 50.70% 11 Desmin + 1.38 3.5e-05 0.0024 DESM_MOUSE 5.2 / 53.4 5.2 / 56.4 2.6 50.70% 12 Desmin + 1.50 3.3e-07 5.8e-05 DESM_MOUSE 5.2 / 53.4 5.3 / 56.4 27 51.60% 14 Tobulin beta-2 C chain + 1.70 0.001 0.016	14	Protein disulfide-isomerase A3	+1.43	5.7e-06	0.00061	PDIA3_MOUSE	6.0 / 56.6	6.5 / 60.5	6	11.90%
$ \begin{array}{c} Chaperones \\ \hline Chaperones \\ \hline Chaperones \\ \hline Chaperones \\ \hline Chapterones \\ \hline Chapterone \\$										
178 kDa glucose-regulated protein $+2.18$ 3.8e-103.1e-05GRP78_MOUSE5.1 / 72.45.0 / 73.62133.70%47Alpha crystallin B chain $+1.46$ 9.6e-050.003CRYAB_MOUSE6.8 / 20.18.0 / 17.61045.10%9Heat-shock protein beta-1 (27 kDa) $+1.36$ 0.0020.020HSPB1_MOUSE6.7 / 57.87.9 / 64.31019.20%8T-complex protein 1, zeta subunit $+1.25$ 0.0060.040TCPZ_MOUSE6.7 / 57.87.9 / 64.31019.20%43Myosin regulatory light chain 2, ventricular/cardiac muscle isoform -1.67 0.0030.0071TNNI3_MOUSE9.6 / 21.29.4 / 21.029.48%11Desmin $+1.38$ 3.5e-050.0024DESM_MOUSE5.2 / 53.45.2 / 56.42650.70%12Desmin $+1.45$ 0.0110.056TBB2C_MOUSE5.2 / 53.45.3 / 56.42751.60%10Tubulin beta-2C chain $+1.75$ 0.0010.016APA1_MOUSE5.6 / 30.65.4 / 20.4619.70%13Fibrinogen gamma chain $+1.22$ 0.020.23FIBs_MOUSE5.6 / 49.45.7 / 53.836.65%2Serotransferrin $+1.34$ 0.0020.023RFE_MOUSE5.6 / 76.777.2 / 73.836.65%3Serotransferrin $+1.45$ 0.0110.016APA1_MOUSE5.6 / 90.65.4 / 20.4619.70%14Serotransferrin <td< td=""><td></td><td>Chaperones</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></td<>		Chaperones								
47 Alpha crystallin B chain + 1.46 9.6e-05 0.0038 CRYAB_MOUSE 6.8 / 20.1 8.0 / 17.6 10 45.10% 39 Heat-shock protein beta-1 (27 kDa) + 1.25 0.006 0.000 TCPZ_MOUSE 6.1 / 23.0 6.1 / 22.2 2 9.99% 8 T-complex protein 1, zeta subunit + 1.25 0.006 0.000 TCPZ_MOUSE 6.7 / 57.8 7.9 / 64.3 10 19.20% 43 Myosin regulatory light chain 2, ventricular/cardiac muscle -1.34 0.018 0.071 TNNI3_MOUSE 4.7 / 18.8 4.6 / 16.5 11 46.40% 7 Troponin 1, cardiac muscle -1.67 0.0003 0.0071 TNNI3_MOUSE 5.2 / 53.4 5.2 / 56.4 26 50.70% 12 Desmin + 1.50 3.2e-07 5.8e-05 DESM_MOUSE 5.2 / 53.4 5.3 / 56.4 27 51.60% 10 Tubulin beta-2C chain + 1.45 0.011 0.056 TBB2C_MOUSE 5.4 / 20.4 6 19.70% 13 Fibrinogen beta chain + 1.75 0.001 0.012 FIB8_MOUSE 6.7 / 54.7 6.8 / 58.9	1	78 kDa glucose-regulated protein	+2.18	3.8e-10	3.1e-05	GRP78_MOUSE	5.1 / 72.4	5.0 / 73.6	21	33.70%
39 Heat-shock protein beta-1 (27 kDa) + 1.36 0.002 0.020 HSPB1_MOUSE 6.1 / 23.0 6.1 / 22.2 2 9.09% 43 Myofilaments Myosin regulatory light chain 2, ventricular/cardiac muscle isoform -1.34 0.018 0.073 MLRV_MOUSE 4.7 / 18.8 4.6 / 16.5 11 46.40% 37 Troponin I, cardiac muscle isoform -1.67 0.003 0.0071 TNNI3_MOUSE 9.6 / 21.2 9.4 / 21.0 2 9.48% 11 Desmin + 1.38 3.5e-05 0.0024 DESM_MOUSE 5.2 / 53.4 5.2 / 56.4 26 50.70% 12 Desmin + 1.50 3.3e-07 5.8e-05 DESM_MOUSE 5.2 / 53.4 5.2 / 56.4 26 50.70% 10 Tubulin beta-2C chain + 1.70 0.001 0.016 APA1_MOUSE 5.6 / 30.6 5.4 / 20.4 6 19.70% 13 Fibrinogen patterins + 1.72 0.001 0.016 APA1_MOUSE 5.6 / 30.6 5.4 / 20.4 6 19.70% 14 14.45 0.011 0.016 APA1_MOUSE 5.6 / 30.6 5.4 / 20.4	47	Alpha crystallin B chain	+1.46	9.6e-05	0.0038	CRYAB_MOUSE	6.8 / 20.1	8.0 / 17.6	10	45.10%
8 T-complex protein 1, zeta subunit + 1.25 0.006 0.040 TCPZ_MOUSE 6.7 / 57.8 7.9 / 64.3 10 19.20% 43 Myofilaments Myosin regulatory light chain 2, ventricular/cardiac muscle isoform - 1.34 0.018 0.073 MLRV_MOUSE 4.7 / 18.8 4.6 / 16.5 11 46.40% 37 Troponin I, cardiac muscle -1.67 0.0003 0.0071 TNNI3_MOUSE 9.6 / 21.2 9.4 / 21.0 2 9.48% 11 Desmin + 1.38 3.5e-05 0.0024 DESM_MOUSE 5.2 / 53.4 5.2 / 56.4 26 50.70% 12 Desmin + 1.50 3.3e-07 5.8e-05 DESM_MOUSE 5.2 / 53.4 5.3 / 56.4 27 51.60% 10 Tubulin beta-2C chain + 1.45 0.011 0.056 TBB2C_MOUSE 5.6 / 30.6 5.4 / 20.4 6 19.70% 13 Fibrinogen gamma chain + 1.22 0.020 OL12 FIBB_MOUSE 6.7 / 54.7 6.8 / 58.9 10 21.00% 13 Fibrinogen gamma chain + 1.22 0.020 OL23 FIBG_MOUSE 6.9 / 76.7	39	Heat-shock protein beta-1 (27 kDa)	+1.36	0.002	0.020	HSPB1_MOUSE	6.1 / 23.0	6.1 / 22.2	2	9.09%
Myofilaments Myosin regulatory light chain 2, wentricular/cardiac muscle isoform -1.34 0.018 0.073 MLRV_MOUSE 4.7 / 18.8 4.6 / 16.5 11 46.40% 37 Troponin I, cardiac muscle isoform -1.67 0.0003 0.0071 TNNI3_MOUSE 9.6 / 21.2 9.4 / 21.0 2 9.48% Intermediate filaments, Microtubules 11 Desmin +1.38 3.5e-05 0.0024 DESM_MOUSE 5.2 / 53.4 5.2 / 56.4 26 50.70% 12 Desmin +1.45 0.011 0.056 TBB2C_MOUSE 5.2 / 53.4 5.3 / 56.4 27 51.60% 10 Tubulin beta-2C chain +1.45 0.011 0.056 TBB2C_MOUSE 4.8 / 50.4 4.9 / 74 8 1840% Plasma proteins Apolipoprotein A-I +1.70 0.001 0.016 APA1_MOUSE 5.6 / 30.6 5.4 / 20.4 6 19.70% 13 Fibrinogen gamma chain +1.22 0.02 0.23 FIBE_MOUSE 6.9 / 76.7 77.2 / 7.7 15 24.70% Mixtures -1.32 <t< td=""><td>8</td><td>T-complex protein 1, zeta subunit</td><td>+1.25</td><td>0.006</td><td>0.040</td><td>TCPZ_MOUSE</td><td>6.7 / 57.8</td><td>7.9 / 64.3</td><td>10</td><td>19.20%</td></t<>	8	T-complex protein 1, zeta subunit	+1.25	0.006	0.040	TCPZ_MOUSE	6.7 / 57.8	7.9 / 64.3	10	19.20%
Myofilaments ventricular/cardiac muscle isoform -1.34 0.018 0.073 MLRV_MOUSE 4.7 /18.8 4.6 / 16.5 11 46.40% 37 Troponin I, cardiac muscle isoform -1.67 0.003 0.0071 TNN13_MOUSE 9.6 / 21.2 9.4 / 21.0 2 9.48% 10 Intermediate filaments, Microtubules -1.83 3.5e-05 0.0024 DESM_MOUSE 5.2 / 53.4 5.2 / 56.4 26 50.70% 12 Desmin +1.38 3.5e-05 0.011 0.056 DESM_MOUSE 5.2 / 53.4 5.3 / 56.4 27 51.60% 12 Desmin +1.45 0.011 0.056 DESM_MOUSE 5.2 / 53.4 5.3 / 56.4 27 51.60% 14 Desmin +1.75 0.011 0.056 TBB2C_MOUSE 5.6 / 30.6 5.4 / 20.4 6 19.70% 15 Fibrinogen parta chain +1.75 0.001 0.012 FIBB_MOUSE 5.6 / 30.6 5.4 / 20.4 6 19.70% 15 Fibrinogen gamma chain +1.32 0.02 0.23 FIBC_MOUSE 5.6 / 30.6 5.4 / 20.4 6 19		r r					,	,		
43 Myosin regulatory light chain 2, ventricular/cardiac muscle isoform -1.34 0.018 0.073 MLRV_MOUSE 4.7 /18.8 4.6 / 16.5 11 46.40% 37 Troponin I, cardiac muscle isoform -1.67 0.0003 0.0071 TNNI3_MOUSE 9.6 / 21.2 9.4 / 21.0 2 9.48% Intermediate filaments, Microtubules 11 Desmin +1.38 3.5e-05 0.0024 DESM_MOUSE 5.2 / 53.4 5.2 / 56.4 26 50.70% 12 Desmin +1.45 0.011 0.056 TBB2C_MOUSE 5.2 / 53.4 5.3 / 56.4 27 51.60% 10 Tubulin beta-2C chain +1.45 0.011 0.056 TBB2C_MOUSE 5.6 / 30.6 5.4 / 20.4 6 19.70% 15 Fibrinogen beta chain +1.75 0.001 0.012 FIBB_MOUSE 6.7 / 54.7 6.8 / 58.9 10 21.00% 13 Fibrinogen gamma chain +1.22 0.02 0.23 FIBC_MOUSE 6.9 / 76.7 77.2 / 7.7 15 24.70% 2 Serotransferrin +1.34 0.002 0.019 TRFE		Myofilaments								
11 body function	43	Myosin regulatory light chain 2.	-1.34	0.018	0.073	MLRV MOUSE	4.7 /18.8	4.6 / 16.5	11	46.40%
37 Troponin I, cardiac muscle -1.67 0.0003 0.0071 TNNI3_MOUSE 9.6 / 21.2 9.4 / 21.0 2 9.48% Intermediate filaments, Microtubules 11 Desmin +1.38 3.5e-05 0.0024 DESM_MOUSE 5.2 / 53.4 5.2 / 56.4 26 50.70% 12 Desmin +1.50 3.3e-07 5.8e-05 DESM_MOUSE 5.2 / 53.4 5.3 / 56.4 27 51.60% 10 Tubulin beta-2C chain +1.45 0.011 0.056 TBB2C_MOUSE 5.6 / 30.6 5.4 / 20.4 6 19.70% 15 Fibrinogen beta chain +1.75 0.001 0.012 FIBB_MOUSE 6.7 / 54.7 6.8 / 58.9 10 21.00% 13 Fibrinogen gamma chain +1.22 0.02 0.23 FIBG_MOUSE 6.9 / 76.7 77.2 / 7.7 15 24.70% 2 Serotransferrin +1.34 0.001 0.018 TRFE_MOUSE 6.9 / 76.7 77.2 / 7.8 40 57.00% 4 Serotransferrin +1.34 0.001 0.018 TRFE_MOUSE 6.9 / 76.7 77.2 / 7.		ventricular/cardiac muscle isoform					,			
11 Desmin +1.38 3.5e-05 0.0024 DESM_MOUSE 5.2 / 53.4 5.2 / 56.4 26 50.70% 12 Desmin +1.50 3.3e-07 5.8e-05 DESM_MOUSE 5.2 / 53.4 5.3 / 56.4 27 51.60% 10 Tubulin beta-2C chain +1.45 0.011 0.056 TBB2C_MOUSE 5.6 / 30.6 5.4 / 20.4 6 19.70% 15 Fibrinogen beta chain +1.75 0.001 0.012 FIBB_MOUSE 6.7 / 54.7 6.8 / 58.9 10 21.00% 13 Fibrinogen gamma chain +1.22 0.02 0.23 FIBE_MOUSE 6.9 / 76.7 77.2 / 7.3 3 6.65% 2 Serotransferrin +1.34 0.001 0.018 TRFE_MOUSE 6.9 / 76.7 77.2 / 7.7 15 24.70% 3 Serotransferrin +1.34 0.001 0.018 TRFE_MOUSE 6.9 / 76.7 77.2 / 7.9 34 45.90% 4 Serotransferrin +1.34 0.002 0.019 TRFE_MOUSE 6.9 / 76.7 77.2 / 7.9 34 45.90% 19 Mixt	37	Troponin L cardiac muscle	-167	0.0003	0.0071	TNNI3 MOUSE	96/212	94/210	2	9.48%
Intermediate filaments, Microtubules + 1.38 3.5e-05 0.0024 DESM_MOUSE 5.2 / 53.4 5.2 / 56.4 26 50.70% 12 Desmin + 1.50 3.3e-07 5.8e-05 DESM_MOUSE 5.2 / 53.4 5.3 / 56.4 27 51.60% 10 Tubulin beta-2C chain + 1.45 0.011 0.056 TBB2C_MOUSE 5.4 / 20.4 6 19.70% 38 Apolipoprotein A-I + 1.70 0.001 0.016 APA1_MOUSE 5.6 / 30.6 5.4 / 20.4 6 19.70% 15 Fibrinogen beta chain + 1.75 0.001 0.012 FIBB_MOUSE 6.7 / 54.7 6.8 / 58.9 10 21.00% 13 Fibrinogen gamma chain + 1.22 0.02 0.23 FIBG_MOUSE 6.9 / 76.7 77.2 / 7.8 3 6.65% 2 Serotransferrin + 1.34 0.001 0.018 TRFE_MOUSE 6.9 / 76.7 77.2 / 7.8 40 57.00% 3 Serotransferrin + 1.34 0.002 0.019 TRFE_MOUSE 6.9 / 76.7 77.2 / 7.8 40 57.00% 4 Serotran	57	hoponini i, curulae musele	1.07	0.0005	0.0071	IIIIII5_IIIOOSE	5.6 / 21.2	5.1/21.0	2	5.10/0
11 Desmin +1.38 3.5e-05 0.0024 DESM_MOUSE 5.2 / 53.4 5.2 / 56.4 26 50.70% 12 Desmin +1.50 3.3e-07 5.8e-05 DESM_MOUSE 5.2 / 53.4 5.3 / 56.4 27 51.60% 10 Tubulin beta-2C chain +1.45 0.011 0.056 TBB2C_MOUSE 4.8 / 50.4 4.9 / 57.4 8 18.40% Plasma proteins 38 Apolipoprotein A-I +1.70 0.001 0.016 APA1_MOUSE 5.6 / 30.6 5.4 / 20.4 6 19.70% 15 Fibrinogen beta chain +1.75 0.001 0.012 FIBB_MOUSE 6.7 / 54.7 6.8 / 58.9 10 21.00% 13 Fibrinogen gamma chain +1.22 0.02 0.23 FIBG_MOUSE 5.5 / 49.4 5.7 / 53.8 3 6.65% 2 Serotransferrin +1.34 0.001 0.018 TRFE_MOUSE 6.9 / 76.7 77.2 / 7.7 15 24.70% 3 Serotransferrin +1.34 0.002 0.019 TRFE_MOUSE 6.9 / 76.7 77.2 / 7.9 34 </td <td></td> <td>Intermediate filaments Microtubules</td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td> <td></td>		Intermediate filaments Microtubules								
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A transferase 1, mitochondrial		Succinyl-CoA:3-ketoacid-coenzyme				SCOT1_MOUSE	8.7 / 60.0	8.0 / 58.5	8	18.70%
		A transferase 1, mitochondrial								

(continued on next page)

Table 1 (continued)

No.	Protein identity	Fold change hibernating vs control	P-value (t-test)	P-value (FDR)	SWISS PROT accession number	Theoretical pI/MW	Observed pI/MW	No of identified peptides	Sequence coverage (%)
	Cytosol aminopeptidase				AMPL_MOUSE	6.7 / 56.0	8.0 / 58.5	5	13.30%
	Mixtures								
21	Mixture:	-1.24	0.008	0.046					
	Beta-enolase				ENOB_MOUSE	6.7 / 47.0	8.1 / 50.3	7	14.50%
	Fumarate hydratase, mitochondrial				FUMH_MOUSE	9.1 / 54.2	8.1 / 50.3	3	5.72%
26	Mixture	-1.23	0.009	0.049					
	Creatine kinase M-type				KCRM_MOUSE	6.6 / 43.0	7.8 / 44.2	22	50.90%
	Acyl-CoA dehydrogenase,				ACADL_MOUSE	8.5 / 47.9	7.8 / 44.2	18	40.90%
	long-chain specific								
30	Mixture:	+1.36	0.002	0.0055					
	Glyoxylate reductase/				GRHPR_MOUSE	7.6 / 35.3	8.2 / 36.5	10	23.50%
	hydroxypyruvate reductase								
	Glyceraldehyde-3-phosphate				G3P_MOUSE	8.5 / 35.7	8.2 / 36.5	8	29.10%
	dehydrogenase								
32	Mixture:	-1.32	0.0008	0.014					
	Four and a half LIM domains protein 2				FHL2_MOUSE	7.3 / 32.1	8.0 / 33.5		
	L-Lactate dehydrogenase A chain				LDHA_MOUSE	7.6 / 29.5	8.0 / 33.5	6	15.10%
45	Mixture:	+1.33	0.008	0.046					
	NADH dehydrogenase [ubiquinone]				NDUBA_MOUSE	8.4 / 20.9	8.8 / 19.6	8	40.30%
	1 beta subcomplex subunit 10								
	Cysteine and glycine-rich protein 3				CSRP3_MOUSE	8.9 / 20.8	8.8 / 19.6	3	24.70%
46	Mixture:	+1.30	0.007	0.043					
	Cysteine and glycine-rich protein 3				CSRP3_MOUSE	8.9 / 20.8	8.6 / 19.3	6	39.20%
	Glutathione S-transferase P1				GSTP1_MOUSE	8.1 / 23.5	8.6 / 19.3	5	37.60%

pI denotes isoelectric point; MW, molecular weight.

Difference in-gel electrophoresis results were reproduced with different biological replicates using reverse-labeling (biological replicates n = 4 for control and hibernating hearts, with technical replicates n = 7). *P*-values for differences between the two groups were derived from unpaired *t*-tests using Decyder software (v6.5, GE healthcare). Corrections for multiple testing were performed by the Benjamini–Hochberg equation, yielding False Discovery rates (FDR). The differentially expressed proteins are numbered in Fig. 2.

the observed reduction of glutamate, glutamine and total creatine in hibernating hearts by ¹H-NMR spectroscopy corresponded to a decrease of mitochondrial glutamate dehydrogenase and creatine kinase in the proteomic dataset (Table 1). Consistent with previous reports in hypoxic rats [21], the observed metabolic differences (lower glutamine levels, but higher aspartate to glutamate concentration ratios) are indicative of a decreased flux through the malate-aspartate shuttle under conditions of oxygen limitation. In addition, choline, taurine, and leucine concentrations were lower in hibernating hearts. Perturbations of glucose concentrations and the adenosine pool (ADP + ATP) failed to reach statistical significance.

3.6. Network analysis

To enable an unbiased analysis at the network level, interactions within the transcriptomics data [7] were first visualized using Cytoscape (Fig. 6A). This analysis revealed 2 major clusters linked by transcription factor 4 and synphilin-1, a protein that is encoded by the SNCAIP gene and contains several protein–protein interaction domains, including an ATP/GTP-binding motif. The role of these genes in cardiac hibernation is currently unknown. The proteomic and metabolomic data were then combined with the transcriptomic data and analyzed at the pathway level either independently, or in combination using the



Fig. 3. Combined proteomic and transcriptomic investigation. Comparison between fold induction of mRNA expression (orange bars, log scaled) and protein changes (blue bars) in hibernating versus control hearts for proteins detected by DIGE (see Table 1). The proteins are grouped according to the GO annotations.



Fig. 4. Protein expression and post-translational modifications. (A) Western blot analysis of GLUT1, LDH and IGFBP2 in hibernating and control hearts (6W-ON). (B, C) Phosphate-affinity gel electrophoresis for mobility shift detection of phosphorylated proteins. Significant changes were detected in the phosphorylation (upper panel, bands marked with an arrow) of myosin regulatory light chain 2 (MLC2, B) and cardiac troponin I (TnI, C) without differences in protein abundance (lower panels). Quantitative data are shown in Supplemental Figure 3. (D) Western blot analysis of anti-oxidant proteins in hibernating and control hearts. Densitometry data for SOD2 are provided in Supplemental Figure 3.

systems biology analysis suite MetaCore™. Results are presented in Supplemental Table III. Although the protein changes were not always consistent with the mRNA changes, protein changes were in the same pathway as transcriptomic changes and both datasets contributed different focus molecules to the pathway analysis (Supplemental Figure 4). Consequently, the combination of the proteomic and transcriptomic dataset significantly improved the statistical confidence of the pathway analysis, with HIF-hypoxia-Akt signaling and glycolysis being the most significant (Fig. 6B). In addition, the combined transcriptomic and proteomic data pointed towards the activation of hydroxyproline production, a pivotal component of collagen synthesis, providing a direct link to cardiac remodeling. Adding the metabolite data did not increase the power of the analysis, as the corresponding metabolite concentrations, i.e., lactate, were kept relatively constant in the hibernating myocardium. "Cardiomyopathies" and "heart failure" were returned as the most prominent linked diseases based on the combined analysis using Metacore[™].

4. Discussion

In the present study, a combined transcriptomic, proteomic and metabolomic approach has been conducted to provide a comprehensive analysis of molecular changes in a mouse model of chronic myocardial hibernation. The different analyses explored different aspects of cellular processes, i.e., microarrays interrogated the transcriptional signal whereas proteomics related to translational and post-translational mechanisms. Therefore, setting analytical considerations aside, differences between the datasets were not only inevitable but also critical in understanding the various aspects of cellular process mechanism and regulation. Interestingly, chronic hypoxia resulted in a pronounced myocardial response at the transcript and the protein level but relatively minor changes in the metabolome indicating that metabolic homeostasis is maintained by adaptive changes in the proteome and the transcriptome.

4.1. Integrated pathway analysis to combine "-omics" data

Biological systems are organized in scale-free networks [22]. The promise of systems biology is to characterize these networks and to finally predict their behavior. Despite the comprehensive coverage obtained by whole-genome microarray analysis, additional information can be gained by combining transcriptomic with proteomic data. As demonstrated in this study, proteomics contributed different focus molecules to the protein association networks and the p-value of the top-ranking HIF signaling pathway improved by 2 orders of magnitude in the combined analysis, even though HIF was not the top-ranking pathway in analysis based on the transcriptomic or the proteomic dataset separately. Moreover, proline metabolism and collagen metabolism, a key determinant for cardiac remodeling and cardiomyopathies, had the highest score in the proteomic dataset, but only ranked 22 based on the transcriptomic data. Thus, the bias of proteomics towards high abundant components resulted in a rearrangement of the top scoring pathways with the final top 3 (HIF signaling, glycolysis/glycogenesis, proline and collagen metabolism) being in agreement with the observed reversible fibrosis in this mouse model of hibernation. On the other hand, conventional inference statistics attaches utmost importance to the biggest changes and the absence of a significant change, i.e., for glucose metabolites despite the induction of glycolytic enzymes, does not add to the pathway analysis whereas all that has been shown are differences in net concentrations at the time of measurement. An integrated assessment of enzymes and metabolites helps to highlight potential dynamic adaptations in flux or turnover, but falls short of a metabolic control and flux analysis, which requires a more detailed treatment with respect to definition of control and regulation of metabolism [22-24]. Potential pitfalls include stability and turnover of mRNA, rates of protein synthesis and degradation (peptide chain initiation and elongation as well as activities of the ubiquitin proteasome system and autophagy [7]), and rates of metabolite turnover (e.g., ATP turnover rates). Ultimately, a network of enzyme-catalyzed reactions and ion transport processes is the platform for the interplay of energetic, electrical, Ca²⁺ handling and contractile processes in the heart [25,26]. Without a systems-wide perspective, network behavior can be misinterpreted by relying on transcriptomic, proteomic or metabolomic readouts only.

4.2. Adaptive changes in glycolysis and myofilament phosphorylation

The hallmark of myocardial hibernation is the maintained viability of the dysfunctional hypoxic myocardium. Metabolic activity is sustained by a shift from fatty acid metabolism to glycolysis resulting in an increased glucose uptake with a corresponding accumulation of glycogen, a critical substrate for the ischemic heart [2]. HIF-1 represents a master switch in the metabolic and functional adaptation



Fig. 5. High-resolution ¹H-NMR spectroscopy of cardiac tissue extracts. Representative spectra of the aliphatic region (-0.05 to 4.2 ppm) from control (bottom) and hibernating hearts (top). Quantitative metabolite data are presented in Table 2.

to chronic anaerobic conditions by stimulating glucose metabolism (through GLUT1) and angiogenesis (through VEGF). Upregulation of both HIF-target genes has previously been shown in the human hibernating myocardium [27]. While the recapitulation of known associations validates our approach, the transgenic mice allow us to study HIF-mediated metabolic adaptation without concomitant angiogenic effects. In chronically hibernating myocardium of pigs, persistent regional downregulation of mitochondrial enzymes and upregulation of stress proteins was reported, but no induction of glycolytic enzymes was observed after a 3- to 5-month period [6]. Our study confirms the repression of mitochondrial enzymes and upregulation of anti-oxidant and stress proteins. Compared to the former study in pigs [6], our transgenic mice were subject to shorter periods of ischemia and we

Table 2

Metabolite changes by ¹H-NMR in cardiac tissue extracts.

	Control $(n=3)$	Hibernating $(n=5)$	Fold change	P (t-test)
Leucine	$0.101(\pm 0.005)$	0.075 (±0.005)	0.74	0.016
Isoleucine	0.414 (±0.138)	$0.374(\pm 0.107)$	0.90	0.828
Valine	0.105 (±0.011)	$0.086(\pm 0.008)$	0.82	0.214
Isovalerate	0.123 (±0.034)	0.143 (±0.048)	1.16	0.774
Beta-OH butyrate	0.145 (±0.030)	0.126 (±0.026)	0.87	0.654
Lactate	10.383 (±0.784)	11.689 (±0.648)	1.12	0.255
Alanine	1.680 (±0.273)	1.719 (±0.106)	1.02	0.878
Acetate	0.337 (±0.053)	0.310 (±0.090)	0.92	0.835
Glutamate	3.752 (±0.258)	2.563 (±0.126)	0.68	0.003
Succinate	1.234 (±0.343)	1.087 (±0.119)	0.88	0.638
Glutamine	2.873 (±0.315)	2.000 (±0.186)	0.69	0.042
Aspartate	$0.266(\pm 0.097)$	0.346 (±0.073)	1.30	0.534
Choline	$0.077(\pm 0.005)$	0.051 (±0.004)	0.66	0.006
Phosphocholine	0.173 (±0.027)	0.129 (±0.013)	0.75	0.145
Carnitine	0.546 (±0.091)	0.562 (±0.033)	1.03	0.845
Taurine	22.11 (±1.937)	16.01 (±0.936)	0.72	0.018
Glycine	0.572 (±0.033)	0.704 (±0.082)	1.23	0.282
Creatine	8.349 (±0.937)	6.051 (±0.461)	0.72	0.047
Glycolic acid	0.583 (±0.026)	0.572 (±0.055)	0.98	0.882
Glucose	0.218 (±0.100)	0.309 (±0.061)	1.42	0.438
Fumerate	0.085 (±0.023)	0.073 (±0.012)	0.86	0.622
Tyrosine	0.134 (±0.068)	0.036 (±0.004)	0.27	0.098
Phenylalanine	$0.051(\pm 0.005)$	$0.043 (\pm 0.003)$	0.84	0.217
Adenosine pool	3.419 (±0.357)	2.808 (±0.244)	0.82	0.193
NAD + NADH	$0.344(\pm 0.093)$	$0.360(\pm 0.047)$	1.05	0.875
Formate	0.306 (±0.015)	0.300 (±0.039)	0.98	0.912

Data presented are given in μ mol/g wet weight (mean \pm SE), n = 3 for control and n = 5 for hibernating hearts. *P*-values for differences between the two groups were derived from unpaired *t*-tests (bold numbers highlight significant differences *P*<0.05).

report the induction of an early cardioprotective program characterized by an upregulation of glycolytic enzymes and transient induction of K (ATP) channels. We also measured cardiac metabolites rather than enzymatic activity [6] and demonstrated alterations in the phosphorylation state of myofilmant proteins. Importantly, basal phosphorylation of MLC2 plays a pivotal role in cardiac muscle contraction, and reduced phosphorylation may contribute to the self-protecting cessation of myocardial contraction during hibernation [28]. A decrease in both TnI and MLC2 phosphorylation correlated with enhanced Ca²⁺responsiveness in human failing hearts, while phosphorylation of MLC1 and troponin T isoform expression was unaltered [29,30]. Thus, the cessation of contraction in hibernation is accompanied by a complex interplay between enzymatic changes and alterations in myofilament phosphorylation.

4.3. Adaptive changes in K(ATP) channels and energy metabolism

K(ATP) channels are unique nucleotide sensors that adjust membrane potential in response to intracellular metabolic oscillations. Kir6.2 and SUR2A are the pore-forming and regulatory subunits of the K(ATP) channel complex, respectively. Transition of the SUR subunit from the ATP to the ADP-liganded state promotes K⁺ permeation through Kir6.2 and defines K(ATP) channel activity, which serves a cardioprotective role under ischemic insult [31]. Our data extend these findings by implicating a temporary induction of these metabolic sensors in the cardiac adaptation to chronic hypoxia. Their subsequent suppression may be required for the transition into the maintenance phase of hibernation with reduced metabolic demand, which is supported by the reduction in GLUT1, a hypoxia marker, after administration of glibenclamide. It has previously been proposed that the response of hypoxia tolerant systems to oxygen lack occurs in two phases. The first lines of defense against hypoxia include a balanced suppression of ATP-demand and ATP-supply pathways; this regulation stabilizes (adenylates) at new steady-state levels even while ATP turnover rates greatly decline [32]. Adenosine kinase contributes to energy homeostasis by recovering AMP from adenosine and allows AMP to increase when ATP becomes depleted [33]. The downregulation of this salvage enzyme in hibernation may indicate loss of purines possibly with increased extracellular concentration of adenosine. The hydrolysis of AMP to adenosine has been shown to benefit tissue survival during ischemia by improving the free energy of ATP hydrolysis [34]. Moreover, inhibition of adenosine kinase was protective in a rat model of myocardial infarction [35].



Fig. 6. Bioinformatic analysis. (A) An interaction matrix was constructed using Cytoscape software. The nodes of the differentially expressed transcripts fall into 2 major clusters linked by transcription factor 4 and SNCAIP. (B) For pathway analysis, the transcriptomic, proteomic and metabolomic datasets were combined using the MetaCore™ systems biology analysis suite. Collective bioinformatic interrogation of the 3 different -omic datasets improved the statistical significance (visualized as an increase in the -log (*p*-value)) and resulted in a rearrangement in the ranking of the top scoring pathways (Supplemental Table III).

Interestingly, a chain of adenvlate kinase-catalyzed phosphotranfer reactions has been implicated in the communication of mitochondriagenerated signals to K(ATP) channels [20]. Mitochondrial adenylate kinase 4, identified in this study, contributes to the phosphorylation of AMP, but can only use GTP or ITP as a substrate [36]. It has recently been demonstrated to interact with the mitochondrial inner membrane protein adenine nucleotide translocase, which might be important for its protective benefits under stress conditions [37]. On the other hand, creatine kinase is known to act as a spatial and temporal energy buffer and regulator of pH. Over-expression of the cardiac creatine transporter, however, failed to protect transgenic mice from heart failure despite achieving supraphysiological creatine levels [38]. In fact, the increase in the cardiac creatine content was associated with decreased glycolytic activity [39]. Thus, a reduced creatine pool might be an adaptive mechanism in response to chronic hypoxia [12]. Although the concept of homeostasis reaches far back into the history of experimental physiology [40,41], this is, to our knowledge, the first time that the net effect of hibernation on oxygen balance has been experimentally shown.

4.4. Limitations of the study

No technology can currently resolve the entire complexity of the mammalian proteome and metabolome. While shot-gun proteomic analyses can mine deeper into the proteome, DIGE allows the reliable quantification of differences as low as 10% in protein expression and visualizes the post-translational modifications of intact proteins as shift in isoelectric point or molecular weight. Membrane proteins, however, are not well represented by this technique. Despite a pronounced change in the transcription of the K(ATP) channel components, we were unable to detect K(ATP) channels on 2-DE gels. Furthermore, ¹H-NMR, as employed in the present study, allows the quantification of the major stable metabolites in cardiac tissue extracts. ³¹P-NMR would allow the detection of labile cardiac energetic metabolites, such as phosphocreatine, ATP, inorganic phosphate and intracellular pH, to better clarify the degree of hypoxia.

4.5. Conclusions

In this study, we comprehensively analyze a conditional mouse model of myocardial hibernation by 3 independent "-omics" methodologies. We demonstrate how the integration of corresponding mRNA, protein and metabolite changes by network analysis aids in the identification of key biological pathways that underlie this important cardioprotective phenomenon. The combination of different "-omics" approaches will be indispensable for an integrated phenotyping of transgenic animals [12] and addressing the multiple facets of cardiovascular diseases in a systems biology approach.

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6. Disclosures

There are no conflicts of interests to disclose.

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.yjmcc.2011.02.010.

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