**Supplemental Data**

**Muscle mitochondrial stress adaptation operates independently of endogenous FGF21 action**

Mario Ost**1,6,\***, Verena Coleman**1,6**, Anja Voigt**1**, Evert M. van Schothorst**2**, Susanne Keipert**3**, Inge van der Stelt**2**, Sebastian Ringel**1**, Antonia Graja**4**, Thomas Ambrosi**4**, Anna P. Kipp**5**, Martin Jastroch**3**, Tim J. Schulz**4**, Jaap Keijer**2**, and Susanne Klaus**1**

1Research Group Physiology of Energy Metabolism, German Institute of Human Nutrition, Nuthetal, 14558

2Human and Animal Physiology, Wageningen University, Wageningen, 6708

3Helmholtz Diabetes Center, Helmholtz Zentrum München, Neuherberg, 85764

4Research Group Adipocyte Development, German Institute of Human Nutrition, Nuthetal, 14558

5Department of Molecular Toxicology, German Institute of Human Nutrition, Nuthetal, 14558

6M.O. and V.C. contributed equally to this work

**Figure S1**

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**Figure S1: Negligible role of muscle mitochondrial stress-induced FGF21 on bone mass, body weight gain and whole body energy expenditure****.**

Phenotype data from male WT, FGF21-/-, TG, and TG/FGF21-/- mice fed low-fat (LFD) or high-fat diet (HFD) for 24 weeks (wks) from 16 wks of age. (A) Body length of 40 weeks old mice; (n= 9-11 per group). (B) Bone mineral density (BMD) (n=10-11 per group) and representative H&E histology of bone (femur, bars represent 250µm) of 40 wks old mice. (C) Lean body mass at 40wks of age (left panel) and body weight gain (right panel) from week 16 to week 40 of age of male mice from (A) (n=10-11 per group). (D) Fat depot weights of epididymal white adipose tissue (eWAT), subcutaneous WAT (sWAT) and interscapular brown adipose tissue (iBAT) of 40 wks old mice (n=9-11 per group). (E) Body temperature of 40 weeks old mice; (n= 5-11 per group). (F-H) Absolute energy expenditure (kJ) per day (d), 24h profile and total energy expenditure normalized to body mass at 36 weeks of age (n=9-11 per group). All data are mean + SEM, means with different letters are significantly different.

**Figure S2**

**C:\Users\donner\Desktop\Figure S_TV3 wk22 phenotype.tifFigure S2: FGF21-independent whole body metabolic adaptations suring HFD intervention from 8 up to 24 wks of age.**

Phenotype data of male WT, FGF21-/-, TG, and TG/FGF21-/- mice fed low-fat (LFD) or high-fat diet (HFD) for 16 weeks (wks) from 8 wks of age. (A-C) Body mass, lean body mass and fat mass from week 8 to wk 24 of age of male mice (n=9-10 per group). (D) Plasma FGF21 concentration at wk 24 of age (n= 5-10 per group). (E) Body length of 24 weeks old mice; (n= 9-10 per group). (F+G) Organ (Quad, quadriceps muscle) and fat depot weights of epididymal white adipose tissue (eWAT), subcutaneous WAT (sWAT) and interscapular brown adipose tissue (iBAT) of 24 wks old mice (n=9-10 per group). (H-K) Blood glucose and plasma insulin levels (basal, postabsorptive state), blood glucose and plasma insulin levels during an oral glucose tolerance test (oGTT) at wk 22 of age (n=9-10 per group). All data represent mean + SEM; statistical significance is denoted by \*p<0.05, \*\*p<0.01, \*\*\*p<0.001 (color-coding of asterisks represents statistical differences between the genotypes: light-grey, vs. WT; black, vs. TG, orange, vs. TG/FGF21-/-); means with different letters are significantly different; #, not detectable.

**Figure S3**

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**Figure S3: Adipose tissue characterization of male mice.**

Data of male WT, FGF21-/-, TG, and TG/FGF21-/- mice fed low-fat (LFD) or high-fat diet (HFD) for 24 weeks (wks) from 16 wks of age and young male littermates fed LFD at 8 wks of age. (A) Representative H&E histology of epididymal WAT (eWAT) of 40 wks old mice from Figure 1A. (B+C) Cytochrome c oxidase (COX) and citrate synthase (CS) activity of interscapular brown adipose tissue (iBAT) in 40 wks old mice (n=4-5 per group). (D+E) SCA1+/Lin-(CD45/CD31) progenitor cell analysis of iBAT stromal vascular fraction (SVF) from male littermates at 8 wks of age (n= 3-4 per group). All data are mean + SEM, means with different letters are significantly different.

**Table S1**

**Microarray TOP 30 most significant upregulated genes**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Gene**  **Symbol** | **Probe**  **Name** | **Gene**  **Name** | **Genbank**  **Accession** | **UniGene**  **ID** | **EntrezGene**  **ID** |
| **Hpdl** | A\_51\_P317214 | **4-hydroxyphenylpyruvate dioxygenase-like** | NM\_146256 | Mm.279897 | 242642 |
| **Kng1** | A\_55\_P2003414 | **Kininogen 1** | NM\_023125 | Mm.2160 | 16644 |
| **Ppargc1b** | A\_55\_P1993168 | **Peroxisome proliferative activated receptor, gamma, coactivator 1 beta** | NM\_133249 | Mm.415302 | 170826 |
| **Adck3** | A\_51\_P221651 | **AarF domain containing kinase 3** | NM\_023341 | Mm.38330 | 67426 |
| **Cpt1b** | A\_55\_P1956882 | **Carnitine palmitoyltransferase 1b, muscle** | NM\_009948 | Mm.227738 | 12895 |
| **Fabp3** | A\_55\_P1974367 | **Fatty acid binding protein 3, muscle and heart** | NM\_010174 | Mm.388886 | 14077 |
| **Cox7a1** | A\_51\_P148612 | **Cytochrome c oxidase subunit VIIa 1** | NM\_009944 | Mm.423030 | 12865 |
| **Cox8b** | A\_52\_P423814 | **Cytochrome c oxidase subunit VIIIb** | NM\_007751 | Mm.3841 | 12869 |
| **Pdk4** | A\_51\_P350453 | **Pyruvate dehydrogenase kinase, isoenzyme 4** | NM\_013743 | Mm.235547 | 27273 |
| **Plin5** | A\_55\_P2104259 | **Perilipin 5** | NM\_001077348 | Mm.254985 | 66968 |
| **Ppara** | A\_55\_P2071596 | **Peroxisome proliferator activated receptor alpha** | NM\_011144 | Mm.212789 | 19013 |
| **Letmd1** | A\_51\_P486512 | **LETM1 domain containing 1** | NM\_134093 | Mm.275272 | 68614 |
| **Acaa2** | A\_51\_P125260 | **Acetyl-Coenzyme A acyltransferase 2 (mitochondrial)** | NM\_177470 | Mm.245724 | 52538 |
| **Acadvl** | A\_55\_P1991906 | **Acyl-Coenzyme A dehydrogenase, very long chain** | NM\_017366 | Mm.18630 | 11370 |
| **Hadhb** | A\_55\_P2073294 | **Hydroxyacyl-Coenzyme A dehydrogenase** | NM\_001289798 | Mm.291463 | 231086 |
| **Otop1** | A\_55\_P2078143 | **Otopetrin 1** | NM\_172709 | Mm.204765 | 21906 |
| **Cidea** | A\_51\_P199168 | **Cell death-inducing DNA fragmentation factor, alpha subunit-like effector A** | NM\_007702 | Mm.449 | 12683 |
| **Slc27a2** | A\_55\_P2077153 | **Solute carrier family 27 (fatty acid transporter), member 2** | NM\_011978 | Mm.290044 | 26458 |
| **Pank1** | A\_51\_P263591 | **Pantothenate kinase 1** | NM\_023792 | Mm.24742 | 75735 |
| **Ucp1** | A\_51\_P426353 | **Uncoupling protein 1 (mitochondrial, proton carrier)** | NM\_009463 | Mm.4177 | 22227 |
| **Elovl3** | A\_51\_P324633 | **Elongation of very long chain fatty acids-like 3** | NM\_007703 | Mm.21806 | 12686 |
| **Acot11** | A\_55\_P2096043 | **Acyl-CoA thioesterase 11** | NM\_025590 | Mm.222956 | 329910 |
| **Atp4b** | A\_55\_P1959909 | **ATPase, H+/K+ exchanging, beta polypeptide** | NM\_009724 | Mm.154039 | 11945 |
| **Elovl6** | A\_51\_P463440 | **ELOVL family member 6, elongation of long chain fatty acids (yeast)** | NM\_130450 | Mm.314113 | 170439 |
| **Rap1gap** | A\_55\_P2039225 | **Rap1 GTPase-activating protein** | NM\_001081155 | Mm.180763 | 110351 |
| **Aspg** | A\_52\_P338956 | **Asparaginase homolog (S. cerevisiae)** | NM\_001081169 | Mm.77133 | 104816 |
| **Acsm3** | A\_51\_P487175 | **Acyl-CoA synthetase medium-chain family member 3** | NM\_212441 | Mm.334199 | 20216 |
| **Chchd10** | A\_51\_P269084 | **Coiled-coil-helix-coiled-coil-helix domain containing 10** | NM\_175329 | Mm.333849 | 103172 |
| **Dusp4** | A\_51\_P518051 | **Dual specificity phosphatase 4** | NM\_176933 | Mm.170276 | 319520 |
| **Alb** | A\_51\_P160713 | **Albumin** | NM\_009654 | Mm.16773 | 11657 |

Top 30 most significant regulated genes using whole genome microarray analysis (ANOVA FDR p<0.0005 followed by FC TG over TG/FGF21-/-) from Figure 2F.**Table S2**

**Microarray TOP 30 pathway enrichment analysis**

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Enrichment analysis report** | |  |  | |  | |  | |  |
| **Enrichment by Pathway Maps** | |  | **FCranked\_TG vs. TG/FGF-/- mice from Figure 2F-H** | | | | | | |
| **#** | **Maps** | **Total** | **p-value** | **FDR** | | **In Data** | | **Network Objects from Active Data** | |
| 1 | [**Oxidative phosphorylation**](http://portal.genego.com/cgi/imagemap.cgi?id=920) | 105 | 3.787E-38 | 2.416E-35 | | 45 | | NDUFS2, ATP5F1, Cytochrome c, COX VIc, CYC1, NDUFS7, UQCRQPC, UQCRH, NDUFAB1, NDUFA1, ATPK, NDUFB9, NDUFA8, MTND4L, NDUFA10, NDUFA9, NDUFB6, UQCRB, NDUFB8, NDUFS8, MTND6, UQCRC2, COX III, UQCR10, NDUFS1, NDUFA3, UQCRFS1, NDUFA6, NDUFS6, NDUFV1, NDUFB5, COX Va, ATP5A, NDUFV2, NDUFA5, UQCRC1, COX Vb, MTND3, COX I, ATP5I, NDUFV3, COX II, COX VIIb-1, ATP5D, COX VIIa-2 | |
| 2 | [**Ubiquinone metabolism**](http://portal.genego.com/cgi/imagemap.cgi?id=884) | 74 | 2.202E-17 | 7.026E-15 | | 24 | | NDUFS2, NDUFS7, NDUFAB1, NDUFA1, NDUFB9, NDUFA8, MTND4L, NDUFA10, NDUFA9, NDUFB6, NDUFB8, COQ6, NDUFS8, MTND6, NDUFS1, NDUFA3, NDUFA6, NDUFS6, NDUFV1, NDUFB5, NDUFV2, NDUFA5, MTND3, NDUFV3 | |
| 3 | [**Brown adipocyte differentiation**](http://portal.genego.com/cgi/imagemap.cgi?id=3287) | 39 | 3.752E-10 | 7.979E-08 | | 13 | | Cytochrome c, DIO2, UCP1, PERC, COX IV-1, PPARGC1 (PGC1-alpha), ELOVL3, CIDE-A, TrkC, EVA1, COX Vb, PPAR-alpha, OTOP1 | |
| 4 | [**Tricarbonic acid cycle**](http://portal.genego.com/cgi/imagemap.cgi?id=814) | 51 | 1.426E-07 | 2.274E-05 | | 12 | | SUCLG1, FUMH, IDH3, ODO2, CISY, SUCB1, IDH3A, IDH3G, IDH3B, SCS-A, ODO1, ACON | |
| 5 | [**Adiponectin in pathogenesis of T2D (type 2 diabetes**](http://portal.genego.com/cgi/imagemap.cgi?id=5951)**)** | 29 | 4.131E-07 | 5.272E-05 | | 9 | | FASN, ACACB, PPARGC1 (PGC1-alpha), ACACA, TORC2, H-FABP, ACADM, PPAR-alpha, CPT-1B | |
| 6 | [**Adiponectin in regulation of metabolism**](http://portal.genego.com/cgi/imagemap.cgi?id=4733) | 43 | 1.797E-06 | 1.911E-04 | | 10 | | Cytochrome c, SOD2, PPARGC1 (PGC1-alpha), ACACA, TORC2, H-FABP, ACADM, PPAR-alpha, CPT-1B, mTOR | |
| 7 | [**PPAR regulation of lipid metabolism**](http://portal.genego.com/cgi/imagemap.cgi?id=632) | 42 | 1.225E-05 | 1.117E-03 | | 9 | | UCP1, CPT II, HADHA, Fatty acid-binding protein, CAC, ACADM, PPAR-alpha, Acetyl-CoA acyltransferase, CPT-1B | |
| 8 | [**Propionate metabolism**](http://portal.genego.com/cgi/imagemap.cgi?id=824) | 66 | 1.711E-05 | 1.365E-03 | | 11 | | SUCLG1, DCMC, ACACB, ACACA, ACAA2, HADHA, ACADM, SCS-A, Acetyl-CoA acyltransferase, HADHB, HCDH | |
| 9 | [**Mitochondrial unsaturated FAO (fatty acid beta-oxidation**](http://portal.genego.com/cgi/imagemap.cgi?id=833)**)** | 45 | 2.218E-05 | 1.572E-03 | | 9 | | ACSL6, DCI, ACAA2, HADHA, ACSL5, ACADM, Acetyl-CoA acyltransferase, HADHB, HCDH | |
| 10 | [**Mitochondrial long chain FAO (fatty acid beta-oxidation**](http://portal.genego.com/cgi/imagemap.cgi?id=832)**)** | 83 | 3.148E-05 | 2.008E-03 | | 12 | | CPT II, ACSL6, ACADL, ACAA2, HADHA, ACSL5, ACADM, ACADVL, Acetyl-CoA acyltransferase, CPT-1B, HADHB, HCDH | |
| 11 | [**Bile acids regulation of glucose and lipid metabolism via FXR**](http://portal.genego.com/cgi/imagemap.cgi?id=3089) | 37 | 3.517E-05 | 2.040E-03 | | 8 | | FASN, ACACB, ACACA, ME1, NIPK, APOC2, PPAR-alpha, F16P | |
| 12 | [**Mitochondrial dysfunction in neurodegenerative diseases**](http://portal.genego.com/cgi/imagemap.cgi?id=3232) | 59 | 2.035E-04 | 9.987E-03 | | 9 | | SDHA, Cytochrome c, SOD2, SDHB, ANT, ODP2, IP3 receptor, ACON, PPIF | |
| 13 | [**Triacylglycerol metabolism**](http://portal.genego.com/cgi/imagemap.cgi?id=838) | 59 | 2.035E-04 | 9.987E-03 | | 9 | | AK1BA, ACSL6, PLCC, PLCA, GPD2, ACSL5, GLPK, PLCB, GPD1 | |
| 14 | [**Beta adrenergic receptors in brown adipocyte differentiation**](http://portal.genego.com/cgi/imagemap.cgi?id=3289) | 37 | 2.663E-04 | 1.214E-02 | | 7 | | DIO2, UCP1, FASN, PPARGC1 (PGC1-alpha), C/EBPbeta, Adenylate cyclase, PPAR-alpha | |
| 15 | [**Butanoate metabolism**](http://portal.genego.com/cgi/imagemap.cgi?id=2397) | 63 | 3.386E-04 | 1.440E-02 | | 9 | | DCMC, ACACB, ACACA, ACAA2, ACADM, SCS-A, Acetyl-CoA acyltransferase, ACSS1, HCDH | |
| 16 | [**Sirtuin6 regulation and functions**](http://portal.genego.com/cgi/imagemap.cgi?id=6935) | 64 | 3.820E-04 | 1.523E-02 | | 9 | | SOD2, ELOVL6, FASN, PPARGC1 (PGC1-alpha), PDK4, ACACA, AMPK beta subunit, USP10, LDHB | |
| 17 | [**Glycolysis and gluconeogenesis**](http://portal.genego.com/cgi/imagemap.cgi?id=930) | 66 | 4.825E-04 | 1.811E-02 | | 9 | | HXK2, PFKP, ENO1, PFKL, PGAM1, ENO, PKM2, F16P, GPI | |
| 18 | [**Peroxisomal branched chain fatty acid oxidation**](http://portal.genego.com/cgi/imagemap.cgi?id=834) | 83 | 6.515E-04 | 2.309E-02 | | 10 | | FACVL1, CPT II, ACADL, PCCB, ACAA2, HADHA, Acetyl-CoA acyltransferase, CPT-1B, ACOX2, HADHB | |
| 19 | [**Putative pathways for stimulation of fat cell differentiation by BPA**](http://portal.genego.com/cgi/imagemap.cgi?id=5030) | 32 | 7.745E-04 | 2.601E-02 | | 6 | | ERR3, PERC, FASN, PPARGC1 (PGC1-alpha), C/EBPbeta, GPD1 | |
| 20 | [**Glycolysis and gluconeogenesis**](http://portal.genego.com/cgi/imagemap.cgi?id=816) | 46 | 1.054E-03 | 3.364E-02 | | 7 | | AK1BA, HXK2, PFKP, PFKL, KHK, F16P, GPI | |
| 21 | [**Glycolysis and gluconeogenesis**](http://portal.genego.com/cgi/imagemap.cgi?id=818) | 24 | 1.306E-03 | 3.786E-02 | | 5 | | ENO1, PGAM1, ENO, LDHB, PKM2 | |
| 22 | [**Glycolysis and gluconeogenesis / Human version**](http://portal.genego.com/cgi/imagemap.cgi?id=2335) | 24 | 1.306E-03 | 3.786E-02 | | 5 | | ENO1, PGAM1, ENO, LDHB, PKM2 | |
| 23 | [**Signal transduction\_PKA signaling**](http://portal.genego.com/cgi/imagemap.cgi?id=675) | 51 | 1.961E-03 | 5.441E-02 | | 7 | | G-protein alpha-12 family, G-protein alpha-i family, Adenylate cyclase, p90RSK1, PDE4A, G-protein alpha-13, PDE4D | |
| 24 | [**Insulin, IGF-1 and TNF-alpha in brown adipocyte differentiation**](http://portal.genego.com/cgi/imagemap.cgi?id=3292) | 53 | 2.459E-03 | 6.536E-02 | | 7 | | UCP1, FASN, PPARGC1 (PGC1-alpha), C/EBPbeta, PPAR-alpha, mTOR, COX II | |
| 25 | [**Pentose phosphate pathway/ Rodent version**](http://portal.genego.com/cgi/imagemap.cgi?id=2324) | 42 | 3.333E-03 | 8.507E-02 | | 6 | | 6PGL, TKT, TALDO, 6PGD, GPI, RBSK | |
| 26 | [**Development\_ACM2 and ACM4 activation of ERK**](http://portal.genego.com/cgi/imagemap.cgi?id=2516) | 43 | 3.761E-03 | 8.880E-02 | | 6 | | PKC-epsilon, G-protein alpha-i family, Rap1GAP1, p90RSK1, G-protein alpha-o, IP3 receptor | |
| 27 | [**Pentose phosphate pathway**](http://portal.genego.com/cgi/imagemap.cgi?id=822) | 43 | 3.761E-03 | 8.880E-02 | | 6 | | 6PGL, TKT, TALDO, 6PGD, GPI, RBSK | |
| 28 | [**Regulation of acetyl-CoA carboxylase 2 activity in muscle**](http://portal.genego.com/cgi/imagemap.cgi?id=140) | 19 | 3.897E-03 | 8.880E-02 | | 4 | | ACACB, AMPK beta subunit, ACLY, CPT-1B | |
| 29 | [**Fructose metabolism**](http://portal.genego.com/cgi/imagemap.cgi?id=820) | 73 | 4.066E-03 | 8.945E-02 | | 8 | | AK1BA, HXK2, PMM1, PFKP, PFKL, KHK, F16P, GPI | |
| 30 | [**Role of Sirtuin1 and PGC1-alpha in activation of antioxidant defense**](http://portal.genego.com/cgi/imagemap.cgi?id=6600) | 60 | 4.993E-03 | 1.062E-01 | | 7 | | GCL cat, TXNRD2, ERR1, SOD2, PRDX3, PPARGC1 (PGC1-alpha), AMPK beta subunit | |

Pathway enrichment analysis using MetaCore (FC > 1.15, FDR-adjusted p<0.05).