SUPPLEMENTARY MATERIAL

Impact of metabolic stress induced by diets, aging and fasting on tissue oxygen consumption

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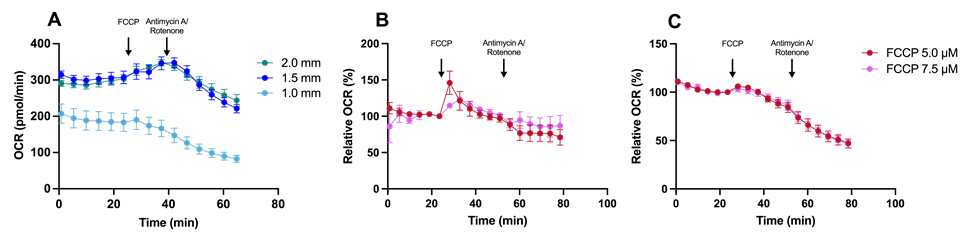
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**Supplementary Figures**

**Supplementary Figure S1: Testing of experimental conditions for tissue measurements. (A)** Evaluation of different punch sizes (1.0 mm, 1.5 mm, 2.0 mm) for measurement of oxygen consumption rate (OCR) in liver. **(B-C)** FCCP titration experiments in epididymal white adipose tissue (eWAT, B) and liver (C).

**Supplementary Figure S2: 3D printing of micro-restrainer.** **(A)** Micro-restrainer were printed with green UV-sensitive resin (Type basic, Anycubic) with a layer height of 25 µm utilizing an Anycubic Photon 3D printing device. **(B)** A simple CAD model was developed to allow rapid printing of multiple restrainer per run.

**Supplementary Figure S3: Reproducibility.** Comparison of two independent groups (1st and 2nd group) of naïve, young adult male BL/6J mice. **(A)** Body weight. **(B)** Fat mass and lean mass. Data represent mean ± SEM. n = 7 1st, n = 5 2nd group.

**Supplementary Figure S4: OCR measurements in mice with different genetic strain backgrounds (BL/6J vs BL/6N)**. **(A)** Basal OCR in liver. **(B)** Maximal OCR in liver. **(C)** Spare capacity in liver. **(D)** Basal OCR in epididymal white adipose tissue (eWAT). **(E)** Maximal OCR in eWAT. **(F)** Spare capacity in eWAT. Statistical significance was calculated using a two-tailed unpaired Student *t* test (A-E) or Mann-Whitney test (F). Data represent the mean ± SEM. n = 12 BL/6J, n = 5 BL/6N.

**Supplementary Figure S5: Lean mass, organ weights, protein analysis and gene expression after 12 weeks of diet. (A)** Lean mass during 12 weeks of standard-control diet (STD), high-sucrose diet (HSD), high-fat diet (HFD) or western diet (WD). **(B)** Organ weights after 12 weeks of STD, HSD, HFD or WD. **(C)** Representative blot of OXPHOS analysis complex I – V, TFAM and GAPDH in liver. **(D)** Quantification of western blot analysis of the respiratory chain complexes and TFAM in liver. **(E-J)** qPCR analysis of *Tfam* and *Opa1* in eWAT (E) and hepatic *Acaca* (F), *FASN* (G), *Ppara* (H), *Cd36* (I) and *G6pc* (J) expression after 12 weeks of STD, HSD, HFD or WD normalized to 18s. (D) Relative protein abundance of complex I (CI: NDUFB8), complex II (CII: SDHB), complex III (CIII: MTCO1), complex IV (CIV: UQCRC2), complex V (CV: ATP5A) and TFAM normalized to GAPDH (37 kDa) as loading control. *\*p* ≤ 0.05, *\*\*p* ≤ 0.01. Statistical significance was calculated using one-way ANOVA with Tukey´s multiple comparisons test (B (tested per tissue), F-J), or two-way ANOVA with Bonferroni multiple comparisons test (A, D, E). Data represent the mean ± SEM. n = 5 STD, n = 6 HSD/HFD/WD (A-R). AU, arbitrary units; iWAT, inguinal white adipose tissue; pWAT, perirenal white adipose tissue; OPA1, mitochondrial dynamin like GTPase; TFAM, Transcription Factor A, Mitochondrial.

**Supplementary Figure S6:** **Organ weights of different age and diet groups.** Organ weights of **(A)** young (18 weeks) vs. matured (30 weeks) male mice after 12 weeks of high-fat diet (HFD). **(B)** young (18 weeks) vs. age-old (2 years old) male mice. **(C)** Overnight fasted vs. fed male mice. *\*\*p* ≤ 0.01, *\*\*\*p* ≤ 0.001. Statistical significance was calculated using a two-tailed unpaired Student *t* test (tested per tissue) (A, B (iWAT, kidney, spleen)) or Mann-Whitney test (B (pWAT), C). Data represent the mean ± SEM. n = 6 young HFD, n = 5 matured HFD (A); n = 12 young, n = 6 age-old (B); n = 12 (C). iWAT, inguinal white adipose tissue; pWAT, perirenal white adipose tissue.

**Supplementary Figure S7:** Uncoupling protein 1 (*Ucp1*) expression in brown adipose tissue (BAT) of young (18 weeks) vs. age old (2 years old) mice normalized to 18s. *\*\*p* ≤ 0.01. Statistical significance was calculated using Mann-Whitney test. Data represent the mean ± SEM. n = 5 young, n = 6 age-old. AU, arbitrary units.

******Supplementary Figure S8: Comparison of fed and fasted male mice. (A)** Spare capacity in epididymal white adipose tissue. *\*p n* ≤ 0.05. Statistical significance was calculated using a two-tailed unpaired Student *t* test. Data represent the mean ± SEM. n = 6. (**B**)Representative electron microscopy images of hepatocytes for mitochondria analysis. Magnification, 5000x. 11.2 x 7.4 µm, red scale represents 1 µm. White M, mitochondria.

**A**

**B**

**Supplementary Tables**

**Supplementary Table S1. Regular maintenance chow (RD):** **Rat/Mouse-Maintenance, Ssniff**.

|  |  |  |  |
| --- | --- | --- | --- |
| **Composition** |  | **Composition** | **(%)** |
| Fat | 9 kJ% | **Amino acids** | **(%)** |
| Protein | 24 kJ% | Lysine | 1.10 |
| Carbohydrates | 67 kJ% | Methionine | 0.38 |
|  |  | Cystine | 0.35 |
| **Crude Nutrients** | **(%)** | Met+Cys | 0.73 |
| Crude protein (N x 6.25) | 19.0 | Threonine | 0.72 |
| Crude fat | 3.3 | Tryptophan | 0.25 |
| Crude fibre | 4.9 | Arginine | 1.19 |
| Crude ash | 6.4 | Histidine | 0.49 |
| Starch | 35.2 | Valine | 0.92 |
| Sugar | 5.3 | Isoleucine | 0.79 |
| N free extracts | 54.2 | Leucine | 1.39 |
|  |  | Phenylalanine | 0.89 |
| **Minerals** | **(%)** | Phe+Tyr | 1.50 |
| Calcium | 1.00 | Glycine | 0.89 |
| Phosphorus | 0.70 | Glutamic acid | 4.22 |
| Ca / P | 1.43 : 1 | Aspartic acid | 1.84 |
| Sodium | 0.24 | Proline | 1.31 |
| Magnesium | 0.22 | Serine | 1.01 |
| Potassium | 0.92 | Alanine | 0.87 |
|  |  |  |  |
| **Fatty acids** | **(%)** | **Vitamins** | **per kg** |
| C 12:0 | ---- | Vitamin A | 25,000 IU |
| C 14:0 | 0.01 | Vitamin D3 | 1,500 IU |
| C 16:0 | 0.45 | Vitamin E | 135 mg |
| C 18:0 | 0.09 | Vitamin K (as MNB) | 20 mg |
| C 20:0 | 0.01 | Thiamine (B1) | 86 mg |
| C 16:1 | 0.01 | Riboflavin (B2) | 32 mg |
| C 18:1 | 0.62 | Pyridoxine (B6) | 31 mg |
| C 18:2 | 1.76 | Cobalamin (B12) | 150 µg |
| C 18:3 | 0.23 | Nicotinic acid | 153 mg |
|  |  | Pantothenic acid | 59 mg |
| **Trace elements** | **per kg** | Folic acid | 10 mg |
| Iron | 186 mg | Biotin | 710 µg |
| Manganese | 68 mg | Choline | 1,370 mg |
| Zinc | 91 mg |  |  |
| Copper | 15 mg |  |  |
| Iodine | 2.1 mg |  |  |
| Selenium | 0.3 mg |  |  |

**Supplementary Table S2. Standard-control diet (STD): S8090-E050, Ssniff.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Composition** |  | **Ingredients** | **g** |
| Fat | 15 kJ% | Casein | 21.000 |
| Protein | 20 kJ% | L-Cystine | 0.250 |
| Carbohydrates | 65 kJ% | Corn Starch | 45.300 |
|  |  | Maltodextrin | 8.720 |
| **Crude Nutrients** | **(%)** | Sucrose | 5.500 |
| Crude protein | 18.5 | Cellulose | 6.000 |
| Crude fat | 6.1 | Vitamin premixture | 1.000 |
| Crude fibre | 6.0 | Mineral premix | 6.000 |
| Crude ash | 4.4 | Soybean Oil | 5.000 |
| Starch | 43.5 | Pork Lard | 1.000 |
| Dextrin | 8.6 | Food dye, Y/G/R/B | 0.030 |
| **Sugar** | 6.5 | Total | 100.000 |

**Supplementary Table S3. High-sucrose diet (HSD): S8090-E052, Ssniff.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Composition** |  | **Ingredients** | **g** |
| Fat | 15 kJ% | Casein | 21.200 |
| Protein | 20 kJ% | L-Cystine | 0.250 |
| Carbohydrates | 65 kJ% | Corn Starch | 3.000 |
|  |  | Maltodextrin | 2.000 |
| **Crude Nutrients** | **(%)** | Sucrose | 54.220 |
| Crude protein | 18.7 | Cellulose | 6.000 |
| Crude fat | 6.2 | Vitamin premixture | 1.000 |
| Crude fibre | 6.0 | Mineral premix | 6.000 |
| Crude ash | 5.4 | Soybean Oil | 5.100 |
| Starch | 2.9 | Pork Lard | 1.000 |
| Dextrin | 2.0 | Food dye, Y/G/R/B | 0.030 |
| **Sugar** | 54.9 | Total | 100.000 |

**Supplementary Table S4. High-fat diet (HFD):** **D12492, Ssniff**.

|  |  |  |  |
| --- | --- | --- | --- |
| **Composition** |  | **Composition** | **(%)** |
| Fat | 60 kJ% | **Amino acids** | **(%)** |
| Protein | 20 kJ% | Lysine | 2.02 |
| Carbohydrates | 20 kJ% | Methionine | 0.86 |
|  |  | Cystine | 0.45 |
| **Crude Nutrients** | **(%)** | Met+Cys | 1.31 |
| Crude protein (N x 6.25) | 24.4 | Threonine | 1.07 |
| Crude fat | 34.6 | Tryptophan | 0.33 |
| Crude fibre | 6.0 | Arginine | 0.95 |
| Crude ash | 5.3 | Histidine | 0.74 |
| Starch | 0.1 | Valine | 1.70 |
| Sugar | 9.4 | Isoleucine | 1.38 |
| N free extracts | 26.3 | Leucine | 2.42 |
|  |  | Phenylalanine | 1.27 |
| **Minerals** | **(%)** | Phe+Tyr | 2.56 |
| Calcium | 0.92 | Glycine | 0.52 |
| Phosphorus | 0.64 | Glutamic acid | 5.50 |
| Ca / P | 1.44 | Aspartic acid | 1.82 |
| Sodium | 0.20 | Proline | 2.80 |
| Magnesium | 0.23 | Serine | 1.46 |
| Potassium | 0.97 | Alanine | 0.81 |
|  |  |  |  |
| **Fatty acids** | **(%)** | **Vitamins** | **per kg** |
| C 12:0 | 0.07 | Vitamin A | 15,000 IU |
| C 14:0 | 0.44 | Vitamin D3 | 1,500 IU |
| C 16:0 | 7.93 | Vitamin E | 150 mg |
| C 18:0 | 4.37 | Vitamin K (as MNB) | 20 mg |
| C 20:0 | 0.11 | Thiamine (B1) | 25 mg |
| C 16:1 | 0.94 | Riboflavin (B2) | 16 mg |
| C 18:1 | 13.97 | Pyridoxine (B6) | 16 mg |
| C 18:2 | 4.64 | Cobalamin (B12) | 30 µg |
| C 18:3 | 0.49 | Nicotinic acid | 47 mg |
|  |  | Pantothenic acid | 55 mg |
| **Trace elements** | **per kg** | Folic acid | 16 mg |
| Iron | 168 mg | Biotin | 300 µg |
| Manganese | 95 mg | Choline | 1,140 mg |
| Zinc | 65 mg |  |  |
| Copper | 13 mg |  |  |
| Iodine | 1.2 mg |  |  |
| Selenium | 0.2 mg |  |  |

**Supplementary Table S5.** Detailed overview of age, cohort size and allocation to experiments. ND, normal diet; HSD, high-sucrose diet; WD, western diet; HFD, high-fat diet.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Diet** | **Age** | **Feeding state** | **n** | **Aim** |
| **ND** | 18 weeks | fed | 7 | Baseline measurements |
| 18 weeks | fed | 5 | Reproducibility measurements |
| 23 weeks | fed | 5 | Strain difference (here BL/6N) |
| 96 weeks | fed | 6 | Aging |
| 96 weeks | fasted | 6 | Fasting |
| **HSD** | 18 weeks | fed | 6 | Dietary effects |
| **WD** | 18 weeks | fed | 6 | Dietary effects |
| **HFD** | 18 weeks | fed | 6 | Dietary effects |
| 30 weeks | fed | 5 | Dietary effects |

**Supplementary Table S6.** Standardized lab protocol for tissue OCR/ECAR measurements. AA, antimycin A; mito, mitochondrial; R, Rotenone.

|  |  |  |  |
| --- | --- | --- | --- |
| **Procedure/Step** | **Time (min)** | **Temp. (°C)** | **Specifications** |
| Organ harvest | 5 - 10 | RT | all organs for OCR analysis |
| Biopsy sampling | 3 - 7 | RT | punch size depending on organ |
| Transfer | 5 | 37 | in 96-well in assay medium |
| Micro-restrainment | 10 - 15 | 37 | pre-equilibrated, over every biopsy |
| Protocol initialization | 12 | 37 | equilibration |
| Basal OCR | 4/cycle | 37 | 6 cycles |
| 🡪 FCCP injection | | 37 | 5 µM |
| Maximal OCR | 4/cycle | 37 | 3 cycles |
| 🡪 R/AA injection | | 37 | each 2.5 µM |
| Non-mito OCR | 4/cycle | 37 | 6 cycles |
| Storage | ∞ | -20 | for micro-BCA for normalization |

**Supplementary Table S7.** Primer sequences for real-time qPCR detection of gene expression.

|  |  |  |  |
| --- | --- | --- | --- |
| **Gene** | **PrimerBank ID** | **Orientation** | **Sequence** |
| *18S* |  | Forward | 5’-TTGACGGAAGGGCACCACCAG-3’ |
|  | Reverse | 5’-GCACCACCACCCACGGAATCG-3’ |
| *Acaca* |  | Forward | 5’-CCGATTCATAATTGGGTCTGTGT-3’ |
|  | Reverse | 5’-CCATCCTGTAAGCCAGAGATCC-3’ |
| *Cd36* |  | Forward | 5’-TCGCTTCCACATTTCCTACA-3’ |
|  | Reverse | 5’-GTTGACCTGCAGTCGTTTTG-3’ |
| *Fasn* | 30911099a1 | Forward | 5’- GGAGGTGGTGATAGCCGGTAT -3’ |
| Reverse | 5’- TGGGTAATCCATAGAGCCCAG -3’ |
| *G6Pc* |  | Forward | 5’-CGAGGAAAGAAAAAGCCAAC-3’ |
|  | Reverse | 5’-GGGACAGACAGACGTTCAGC-3’ |
| *Ppara* |  | Forward | 5’-AGACCCTCGGGGAACTTAGA-3’ |
|  | Reverse | 5’-GTGGGGAGAGAGGACAGATG-3’ |
| *Opa1* | 19526960a1 | Forward | 5’-TGGAAAATGGTTCGAGAGTCAG-3’ |
| Reverse | 5’-CATTCCGTCTCTAGGTTAAAGCG-3’ |
| *Tfam* | 6678303a1 | Forward | 5’-GGAATGTGGAGCGTGCTAAAA-3’ |
| Reverse | 5’-ACAAGACTGATAGACGAGGGG-3’ |
| *Ucp1* |  | Forward | 5’-TCTCTGCCAGGACAGTACCC-3’ |
|  | Reverse | 5’-GCTGTTCAAAGCACACAAACA-3’ |