The loss of ERE-dependent ER signaling potentiates the effects of maternal high-fat diet on energy homeostasis in female offspring on a high-fat diet

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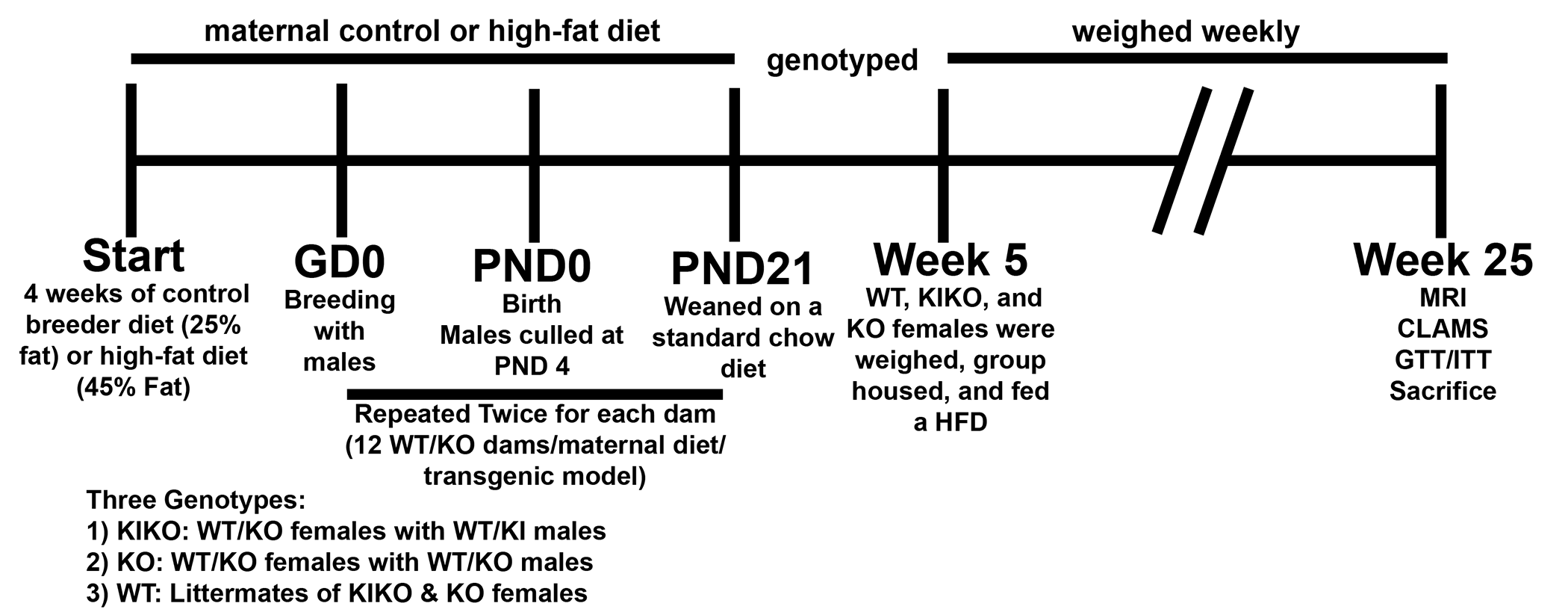
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**Supplemental Data**

**Supplemental Figure S1**

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**Supplemental Figure S1.** Timeline of the experimental design for maternal high-fat diet and assessment of adult female offspring energy and glucose homeostasis.

**Supplemental Table S1. Primer sequences**

|  |  |  |  |
| --- | --- | --- | --- |
| Gene Name | Forward Primer | Reverse Primer | Accession # |
| *Actb* | GCCCTGAGGCTCTTTTCCA | TAGTTTCATGGATGCCACAGGA | NM\_007393.3 |
| *Agrp* | CTCCACTGAAGGGCATCAGAA | ATCTAGCACCTCCGCCAAA | NM\_007427.2 |
| *Cart* | GCTCAAGAGTAAACGCATTCC | GTCCCTTCACAAGCACTTCAA | NM\_013732 |
| *Dgat2* | ACTCTGGAGGTTGGCACCAT | GGGTGTGGCTCAGGAGGAT | NM\_026384.3 |
| *Esr1* | GCGCAAGTGTTACGAAGTG | TTCGGCCTTCCAAGTCATC | NM\_007956 |
| *Fas* | GGGTTCTAGCCAGCAGAGTC | TCAGCCACTTGAGTGTCCTC | NM\_007988.3 |
| *G6pase* | GCCTCCTGTCGGATACAGAA | TGCACCGCAAGAGCATT | [NM\_008061.4](http://www.ncbi.nlm.nih.gov/nuccore/NM_008061.4) |
| *Gapdh* | TGACGTGCCGCCTGGAGAAA | AGTGTAGCCCAAGATGCCCTTCAG | NM\_008084.2 |
| *Hprt* | GCTTGCTGGTGAAAAGGACCTCTCGAAG | CCCTGAAGTACTCATTATAGTCAAGGGCAT | NM\_013556 |
| *Insr* | GTGTTCGGAACCTGATGAC | GTGATACCAGAGCATAGGAG | NM\_010568 |
| *Kiss1* | TGATCTCAATGGCTTCTTGGCAGC | CTCTCTGCATACCGCGATTCCTTT | NM\_178260 |
| *Lepr* | AGAATGACGCAGGGCTGTAT | TCCTTGTGCCCAGGAACAAT | NM\_146146.2 |
| *Npy* | ACTGACCCTCGCTCTATCTC | TCTCAGGGCTGGATCTCTTG | NM\_023456 |
| *Pepck* | AGCGGATATGGTGGGAAC | GGTCTCCACTCCTTGTTC | NM\_011044.2 |
| *Pomc* | GGAAGATGCCGAGATTCTGC | TCCGTTGCCAGGAAACAC | NM\_008895 |
| *Srepb1* | TTGATAGAAGACCGGTAGCGC | CAGCTCAGAGCCGTGGTGA | NM\_0114803 |

**Supplemental Figure S2.**

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**Supplemental Figure S2. A:** Average dailyRER as a function of body weight. ANCOVA: no significance. **B:** Daytime energy expenditure (kCal/hr)as a function of body weight. ANCOVA: maternal diet: F (1, 53) = 9.42, P < .01. **C:** Nighttime energy expenditure (kCal/hr)as a function of body weight. ANCOVA: maternal diet: F (1, 53) = 13.48, P < .001.

**Supplemental Table S2. Arcuate and liver gene expression**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Gene Name | Treatment | WT | KIKO | KO |
| ARC *Insr* | Control | 0.97 ± 0.29 | 1.39 ± 0.51 | 1.69 ± 0.51 |
|  | HFD | 2.23 ± 0.70**a** | 0.52 ± 0.12**\*\*** | 0.24 ± 0.08**a,\*\*** |
| ARC *Lepr* | Control | 0.91 ± 0.14 | 1.54 ± 0.42 | 0.45 ± 0.06**#** |
|  | HFD | 0.96 ± 0.15 | 2.42 ± 0.57**\*\*\*** | 0.40 ± 0.07**####** |
| Liver *Insr* | Control | 1.03 ± 0.14 | 1.10 ± 0.45 | 0.99 ± 0.11 |
|  | HFD | 1.45 ± 0.23 | 0.65 ± 0.20 | 1.22 ± 0.17 |
| Liver *Lepr* | Control | 0.96 ± 0.09 | 0.86 ± 0.08 | 0.75 ± 0.05 |
|  | HFD | 1.08 ± 0.13 | 0.75 ± 0.11\* | 067 ± 0.03**\*** |

**a** = compared to Control within genotype, **\*** compared to WT, **#** = compared to KIKO. (**a/\*/#** = P < .05;

**b/\*\*/##** = P < .01; **c/\*\*\*/###** = P < .001; **d/\*\*\*\*/####** = P < .0001

**Supplemental Table S3. ANOVA statistics for each physiological endpoint**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Endpoint** | **Genotype** | **Maternal Diet** | **Time** | **Interactions** |
| weight 5 wks | F(2,58)=7.42, P<.01 | F(1,58)=38.58, P<.0001 |  |  |
| weight 25 wks | F(2,54)=11.87, P<.0001 | F(1,54)=17.09, P<.001 |  | F(2,54)=5.06, P<.01 |
| fat mass | F(2,54)=8.45, P<.001 | F(2,54)=21.87, P<.0001 |  | F(2,54)=7.75, P<.01 |
| lean mass |  |  |  |  |
| V.O2 | F(2,108)=15.17, P<.0001 | F(1,108)=4.02, P<.05 | F(1,108)=27.01, P<.0001 | g\*m: F(2,108)=3.56, P<.05 |
| V.CO2 | F(2,108)=9.70, P<.001 | F(1,108)=5.06, P<.05 | F(1,108)=12.88, P<.001 | g\*m: F(2,108)=3.08, P<.05 |
| RER |  |  |  |  |
| Heat (energy expenditure) | F(2, 108)=9.60, P<.001 | F(1,108)=4.72, P<.05 | F(1,108)=8.25, P<.01 |  |
| X | F(2,108)=38.55, P<.0001 |  | F(1,108)=127.4, P<.0001 | g\*m: F(2,108)=3.36, P<.05  g\*t: F(2,108)=26.57, P<.0001  m\*t: F(1,108)=4.69, P<.05 |
| Z | F(2,108)=12.56, P<.0001 |  | F(1,108)=69.56, P<.0001 | g\*t: F(2,108)=10.32, P<.0001 |
| Triglycerides |  |  |  |  |
| Fasting Glucose |  |  |  | F(2,53)=3.51, P<.05 |
| GTT | F(2,52)=9.83, *P*<.001 | F(1,52)=5.25, *P*<.05 | F(6,312)=105.1, *P*<.0001 | g\*t: F(12,312)=4.31, *P*<.0001 |
| GTT AUC | F(2,52)=10.24, P<.001 | F(1,52)=4.42, P<.05 |  |  |
| ITT | F(2,53)=7.68, *P*<.01 | F(1,53)=4.87, *P*<.05 | F(5,265)=122.1, *P*<.0001 | g\*t: F(10,265)=3.71, *P*<.001 |
| ITT AUC | F(2,53)=7.54, P<.01 |  |  |  |
| E2 | F(2,36)=16.28, P<.0001 |  |  | F(2,36)=4.60, P<.05 |
| insulin | F(2,52)=22.70, P<.0001 | F(1,52)=20.87, P<.0001 |  | F(2,52)=16.71, P<.0001 |
| leptin |  |  |  |  |
| IL-6 |  | F(1,52)=8.77, P<.01 |  | F(2,52)=4.35, P<.05 |
| MCP-1 |  | F(1,52)=9.24, P<.01 |  |  |
| TNF |  |  |  |  |

Interaction terms are defined as follows: g\*m = genotype\*maternal diet; m\*t = time\*maternal diet; g\*t = genotype\*time.

**Supplemental Table S4. ANOVA statistics for arcuate nucleus gene expression**

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| **Arcuate** | **Genotype** | **Maternal Diet** | **Interactions** |
| *Agrp* | F(2,54)=3.32, P<.05 |  | F(2,53)=10.86, P<.001 |
| *Cart* | F(2,54)=7.70, P<.01 |  |  |
| *Esr1* | F(2,54)=13.92, P<.0001 |  |  |
| *Insr* |  |  | F(2,54)=6.37, P<.01 |
| *Kiss1* |  |  | F(2,54)=9.823, P<.001 |
| *Lepr* | F(2,54)=16.15, P<.0001 |  |  |
| *Npy* | F(2,54)=11.54, P<.0001 |  |  |
| *Pomc* | F(2,54)=12.95, P<.0001 |  | F(2,54)=4.11, P<.05 |

**Supplemental Table S5. ANOVA statistics for liver gene expression**

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| --- | --- | --- | --- |
| **Liver** | **Genotype** | **Maternal Diet** | **Interactions** |
| *Dgat2* |  |  |  |
| *Esr1* | F(2,54)=53.06, P<.0001 | F(1,54)=11.59, P<.01 | F(2,54)=13.16, P<.0001 |
| *Fas* |  | F(1,55)=7.21, P<.01 |  |
| *G6pc* | F(2,55)=17.51, P<.0001 |  |  |
| *Insr* |  |  |  |
| *Lepr* | F(2,54)=6.70, P<.01 |  |  |
| *Pepck* | F(2,54)=3.47, P<.05 |  | F(2,54)=5.55, P<.01 |
| *Srebp1* | F(2,54)=8.93, P<.001 |  |  |