Supplemental Information

Figure S1: Experimental outline to determine the critical timing of maternal high fat feeding on metabolic programming in the offspring

Female C57Bl/6 virgin mice were fed with either a normal chow diet (NCD) or a high-fat diet (HFD) from the age of 4 to 11 weeks (wks) of age. Breedings with male C57Bl/6 mice were set up during the 11th week of age. During gestation, mice were maintained on the same diet that they had received before. At the day of birth (DOB) of the offspring, litter size was reduced to 6-7 animals per mother and half of the NCD-fed mothers were exposed to a HFD, while half of the HFD-fed mothers were exposed to a NCD during lactation. After weaning, all offspring was exposed to a NCD until 8 weeks of age, after which each group of offspring was divided into groups either receiving a NCD or a HFD until 20 weeks of age. In total, this resulted in 8 groups of offspring differing in the prenatal and postnatal maternal diet and in the diet of the offspring after week 8. Light blue lines indicate NCD-feeding and dark blue lines indicate HFD-feeding.

Figure S2: Maternal HFD-feeding exclusively during lactation predisposes the offspring for metabolic disorders – also in females

The following metabolic parameters were analyzed in all eight groups of female offspring. (A) Body weight (BW) on (i) normal chow diet (NCD) or (ii) high fat diet (HFD) after 8 weeks of age (n_{NCD}=11*vs*9*vs*5*vs*6 and n_{HFD}=13*vs*10*vs*9*vs*6), (B) body fat content (n_{NCD}=11*vs*9*vs*5*vs*5 and n_{HFD}=13*vs*10*vs*9*vs*5) and (C) perigonadal fat pad weight

(n_{NCD}=11*vs*9*vs*5*vs*5 and n_{HFD}=13*vs*10*vs*9*vs*6) at 20 weeks of age, (D) fasted serum leptin levels (n_{NCD}=8*vs*8*vs*5*vs*5 and n_{HFD}=9*vs*8*vs*7*vs*4) and (E) homeostatic model assessment indices of insulin resistance (HOMA-IR) (n_{NCD}=8*vs*8*vs*5*vs*5 and n_{HFD}=9*vs*8*vs*8*vs*5) at the age of 15 weeks and (F) glucose tolerance tests (GTT) at the age of 15 weeks on i) NCD and ii) HFD (n_{NCD}=11*vs*9*vs*4*vs*5 and n_{HFD}=13*vs*10*vs*9*vs*5). Data are presented as mean ± SEM, *p < 0.05. **p < 0.01. ***p < 0.001 versus all other groups within the same diet after the age of 8 weeks, if not indicated otherwise.

Figure S3: Maternal HFD-feeding during lactation does not induce hypothalamic inflammation in the offspring

Quantitative real-time PCR analysis of hypothalamic *tumor necrosis factor* (*Tnf*), *interleukin 6* (*Il6*) and *interleukin 1 beta* (*Il1b*) mRNA expression of NCD/NCD and NCD/HFD offspring at 3 and 20 weeks of age on a NCD. Data are presented as mean ± SEM.

Figure S4: Milk composition is highly enriched in mothers exposed to HFD during lactation

(A) Maternal body weight changes between postnatal day 4 (P4) and P19, (B) glucose, (C) insulin and (D) leptin levels, as well as (E) non-esterified free fatty acids (FFA) content in the milk on P19 in mothers consistently exposed to a NCD during gestation

and lactation (NCD/NCD) and mothers fed a HFD starting during lactation (NCD/HFD). Data are presented as mean ± SEM, *p < 0.05. **p < 0.01.

Figure S5: Maternal HFD-feeding during lactation persistently impairs axonal projections of ARH neurons to the DMH and LH independent of POMC-specific insulin signaling

Images and quantification of α-melanocyte-stimulating hormone (α-MSH) and agouti-related-peptide (AgRP) immunoreactive fibers at 20 weeks of age innervating (A) the dorsomedial nucleus of the hypothalamus (DMH; $n_{\alpha\text{-MSH}}$=5 for all groups and n_{AgRP}=5*vs*4*vs*4*vs*5); and (B) the lateral hypothalamic area (LH; $n_{\alpha\text{-MSH}}$=4*vs*5*vs*5*vs*5 and n_{AgRP}=5*vs4vs*5*vs*5) in NCD/NCD ctrl, NCD/NCD $POMC^{\Delta IR}$, NCD/HFD ctrl and NCD/HFD $POMC^{\Delta IR}$ offspring. White boxes indicate area of quantification. 3V = third ventricle, fx = fornix. Scale bar = 100 µm. Data are presented as mean ± SEM, *p < 0.05, **p < 0.01 versus all other groups of offspring, unless otherwise indicated.

Figure S6: POMC-specific IR-deficiency does not affect GLP-1 or FFA concentration in NCD/HFD offspring

(A) Serum glucagon-like peptide 1 (GLP-1) and (B) non-esterified free fatty acid (FFA) concentration under basal conditions and 5 minutes after intravenous glucose injection in NCD/HFD ctrl and NCD/HFD $POMC^{\Delta IR}$ offspring at 15 weeks of age after a 16 hour fasting period. Data are presented as mean ± SEM.