Established maternal obesity in the rat reprograms hypothalamic appetite regulators and leptin signaling at birth

Running head: maternal obesity and leptin at birth

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Abstract

Objective: Key appetite regulators and their receptors are already present in the fetal hypothalamus, and may respond to hormones such as leptin. Intrauterine food restriction or hyperglycemia, can reprogram these circuits, possibly predisposing individuals to adverse health outcomes in adulthood. Given the global obesity epidemic, maternal overweight and obesity is becoming more prevalent. Previously we observed rapid growth of pups from obese dams during the suckling period. However, it is unclear whether this is due to alterations in leptin and hypothalamic appetite regulators at birth.

Design: Female Sprague Dawley rats were fed palatable high-fat-diet or chow for 5 weeks to induce obesity before mating. The same diet continued during gestation. At day 1 after birth, plasma and hypothalamus were collected from male and female pups.

Measurements: Body weight and organ mass were recorded. Leptin and insulin levels were measured in the plasma by radioimmunoassay. Hypothalamic mRNA expression of neuropeptide Y, pro-opiomelanocortin, leptin receptor and its downstream signal STAT3 were measured using real-time PCR.

Results: Body and organ weights of pups from obese dams were similar to those from lean dams, across both genders. However, plasma leptin levels were significantly lower in offspring from obese dams (male: 0.53 ± 0.13 vs. 1.05 ± 0.21 ng/ml; female: 0.33 ± 0.09 vs. 2.12 ± 0.57 ng/ml, respectively; both $P < 0.05$). Hypothalamic mRNA expression of neuropeptide Y, pro-opiomelanocortin, leptin receptor and STAT3 were also significantly lower in pups from obese dams.

Conclusion: Long-term maternal obesity, together with lower leptin levels in pups from obese dams may contribute to the lower expression of key appetite regulators on day 1 of life, suggesting altered intrauterine neuron development in response to intrauterine overnutrition, which may contribute to eating disorders later in life.

Key words: maternal obesity, leptin, newborn, NPY, programming

The central neural network regulating appetite is present before birth in rodents and higher-order mammals. In the rat, the differentiation of the neuronal systems responsible for appetite and energy expenditure begins during the last week of gestation, with development continuing until weaning (1). The development and maturation of these pathways depend on the "leptin surge", which appears between postnatal days 6 to 14, characterized by a sudden increase in circulating leptin levels (2).

Leptin, also termed the ob protein, is produced predominantly by adipocytes, and early onset obesity is seen in the absence of either leptin protein or its receptor (3). The key signaling form, the long form of the leptin receptor (Ob-Rb), is found in hypothalamic areas involved in appetite and metabolic regulation (4). The intracellular domain of Ob-Rb binds to signal transducer and activator of transcription (STAT)3, to induce expression of a signaling inhibitor, suppressor of cytokine signaling (SOCS)3 (4). In adult rodents, leptin decreases fasting-induced hyperphagia, and reduces normal daily food intake, body weight and fat accumulation (4), by suppressing the hypothalamic orexigenic peptides, neuropeptide Y (NPY) and agouti-related protein (AgRP), and stimulating the anorexigenic peptides, pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (5).

However, in the pre-weaning rodent, leptin plays a different role to that in the adult animal, which appears to be independent of its role in energy homeostasis (2, 6). Exogenous leptin does not reduce body weight, milk intake, or metabolic rate during the first two postnatal weeks (7), rather it can actually promote voluntary eating and hyperphagia during this period (8). This is of importance, as neonatal rodents need to maximize food intake and also maintain a high thermoregulatory metabolic rate to favour survival. The differentiation of the neuronal systems that regulate appetite and energy expenditure is not complete until weaning (1).

Increased circulating leptin during the “leptin surge” supports the development and maturation of the central neurons involved in appetite and energy metabolism regulation (2). Leptin deficiency in *ob/ob* mice results in a lower hypothalamic neuron density and innervation, which is thought to contribute to the obese phenotype (6, 9). The high postnatal “leptin surge” due to restricted intrauterine nutrition has been shown to be linked to an increased expression of hypothalamic NPY peptide, which may be responsible for increased weight gain and adiposity when animals are fed a high-fat diet (HFD) after weaning (10).

The intrauterine environment exerts an important role in fetal development and determination of risk of obesity and metabolic disorders such as glucose intolerance and hypertension in later life (11). Intrauterine undernutrition related catch up growth and increased risk of obesity has been thoroughly studied to date. However, given the global obesity epidemic, there is a need to understand the impact of maternal overnutrition on the fetal hypothalamic circuitry involved in energy homeostasis. There are increasing numbers of women entering pregnancy are overweight or obese (12). Intrauterine overnutrition due to maternal obesity has been linked childhood obesity. Few laboratories have studied the neuroendocine impact of maternal obesity due to long-term high-fat diet (HFD) feeding on offspring (13, 14).

Previously, we observed that offspring from obese dams were born with similar body weights as those from lean dams, whereas their weight gain accelerated soon after birth, becoming significantly heavier than those from lean dams after postnatal day 10 (13). However, little is known regarding how intrauterine overnutrition due to maternal HFD feeding reprograms metabolism, especially hypothalamic appetite regulators in the pups, to promote growth shortly after birth. Thus we hypothesized that at day 1 of life, pups born from obese dams would have increased plasma leptin and expression of hypothalamic NPY, but reduced POMC

mRNA expression, promoting increased milk intake, leading to rapid growth immediately after birth.

Materials and Methods

Maternal obesity

Virgin outbred female Sprague Dawley rats (8 weeks, Animal Resources Centre, Perth, Australia), were housed at 20 ± 2°C, and maintained on a 12:12 h light/dark cycle (lights on at 06:00h). Rats were assigned to two groups of similar average body weight. The control group was exposed to standard laboratory chow (Gordon's Specialty Stockfeeds, NSW, Australia) providing 11 kJ/g (14% energy as fat), while the second group was presented with a palatable cafeteria HFD providing 15.33 kJ/g (34% energy as fat). Briefly, the HFD consisted of high-fat modified chow (standard chow, milk powder, sweetened condensed milk and saturated animal fat) and highly palatable cafeteria style food such as cakes and biscuits (4-5 different food types per day) of known caloric content. Fresh food was provided daily. Female rats were exposed to either chow or HFD for 5 weeks before mating with male rats (aged 9 weeks) obtained from the same source. The current study was approved by the Animal Ethics Committee of the University of New South Wales.

2. Sample collection

On day 1 after birth, pups of both genders were separated from their mothers and killed immediately by decapitation. Trunk blood was collected and blood glucose was measured (Accu-Chek® glucose meter; Roche Diagnostics, USA). Separated plasma was stored at -20°C for hormone measurements. The whole hypothalamus was dissected and snap frozen in liquid nitrogen, and stored at -80°C for determination of mRNA expression of genes of interest. Heart, liver, and stomach were dissected and weighed.

3. Plasma leptin and insulin measurements

Plasma leptin and insulin concentrations were measured using commercially available radioimmunoassay kits (Linco, Missouri, USA), according to the manufacturers' instructions. The detection limits for leptin and insulin were 0.125 and 0.05ng/ml respectively.

4. Quantitative real-time PCR

Total RNA was isolated from individual hypothalamus of males and females using TriZol reagent (Invitrogen Australia Pty Limited, Australia) according to the manufacturer's instructions. The purified total RNA was used as a template to generate first-strand cDNA synthesis using M-MLV Reverse Transcriptase, RNase H Minus, Point Mutant Kit (Promega, USA). Applied Biosystem probe/primers (Foster City, CA, USA) that were pre-optimized and validated were used for quantitative real-time PCR (Eppendorf Realplex 2, Germany). The sequence of probes provided by the manufactor is listed in Table 1. The target gene probes were labeled with FAM and 18s rRNA was labeled with VIC. Thus gene expression was quantified in a single multiplexing reaction, where our gene of interest was standardized to housekeeping gene (18s rRNA). An individual sample from the control group was then arbitrarily assigned as a calibrator against which all other samples are expressed as fold difference.

5. Statistical methods

Results are expressed as mean ± SEM. Data were analysed by Shafario Wilks test for normality (Graphpad Prism 5). If data were normally distributed, they were analyzed by *Student's* unpaired *t* test. If they were not, they were log transformed to normal distribution and then analyzed by *Student's* unpaired *t* test.

Results

Before the dietary intervention began, body weights between the groups of females were similar (chow-fed 183.0 ± 3.8 g vs. HFD-fed 183.9 ± 4.3 g). Before mating, HFD-fed female rats were 29% heavier than the chow-fed rats (295.2 ± 11.1 g vs. 229.5 ± 7.54 g, respectively; $P < 0.05$). At sacrifice, body weight of obese dams was 30% greater than that of lean dams ($P< 0.05$, Table 2). Although the increase in liver weight was not significant, heart weight was significantly increased by 14% in HFD-fed dams compared with chow-fed dams. BAT, retroperitoneal, mesentery and uterine fat masses were about 3 times greater in the HFD-fed dams (Table 2). Blood glucose and plasma insulin concentrations also appeared higher in the obese dams; however, this did not reach statistical significance. Plasma triglycerides and leptin levels were significantly increased by 3 and 1.7 times respectively in the obese rats ($P< 0.05$, Table 2). The average litter size for chow-fed mothers was 9.9 and 10.0 for HFD-fed dams.

At day 1, the male pups from lean dams were 11% heavier than the female pups (Tables 3, 4). There were no significant differences in body weight or organ weights in response to maternal HFD feeding across both genders (Tables 3, 4). In pups from lean dams, insulin and leptin concentration of females was higher than males. In both genders, blood glucose and plasma insulin of pups from obese dams were slightly higher than those from lean dams without reaching statistical significance. Interestingly, plasma leptin levels were significantly lower in pups from obese dams versus lean dams in both genders, and this was more marked in females ($P < 0.05$, maternal diet effect in both genders, Tables 3, 4). Across both dietary groups, plasma leptin was also positively correlated with day 1 body weight in both genders (male: $r = 0.64$, $n = 16$, $P = 0.008$; female, $r = 0.60$, $n = 16$, $P = 0.02$).

The Ct value of the housekeeping gene 18s was not changed by maternal overnutrition across genders (male pup: lean dam 16.13 ± 0.15 vs. obese dam 16.29 ± 0.40; female pup: lean dam 15.88 ± 0.28 vs. obese dam 15.67 ± 0.18, n = 5-6). Both male and female pups responded to maternal overnutrition in a similar fashion, and changes in hypothalamic mRNA expression were also similar across genders. In male pups, mRNA expression of hypothalamic NPY, POMC, melanocortin 4 receptor (MC4R), leptin receptor (Ob-Rb), STAT3, SOCS3, and the fuel sensor, mammalian target of rapamycin (mTOR), were significantly lower in the pups from obese dams compared to those from lean dams ($P < 0.05$, Figs 1, 2); whereas AgRP mRNA expression was at a similar level between the two groups (Fig 1B). In female pups, mRNA expression of NPY, MC4R, and Ob-Rb were significantly reduced in pups from obese dams ($P < 0.05$, Fig 3A, 3E, 4A). There was some reduction in AgRP, POMC, and SOCS3 expression; however these did not reach statistical significance (Fig 3B, 3D, 4C). mTOR and STAT3 mRNA expression was not altered by maternal obesity in female pups.

Discussion

Here we describe, for the first time, significantly reduced plasma leptin levels, as well as downregulation of its receptor and downstream signal STAT3, in postnatal day 1 offspring from obese dams. Contrary to our hypothesis, reduced expression of the key hypothalamic appetite regulating peptides, NPY and POMC was observed in offspring from obese dams. These data demonstrate a major impact of maternal overnutrition on neuroendocrine appetite regulation in newborn offspring.

Leptin is produced in multiple sites apart from white adipose tissue, including the stomach, placenta, and certain fetal organs such as the heart, bone and cartilage (15-20). Breast milk

can also provide the newborn with an important source of leptin (21, 22). However, in this study, 1-day old pups were only fed for 24 hours. Thus leptin from milk is unlikely to markedly affect their circulating leptin levels at this time point. It is well accepted that in humans gestational diabetes causes high leptin levels in offspring, while the opposite is observed in small for gestational age babies (23, 24). This may be due to the difference in adiposity, because in the human embryo, leptin is mainly sourced from developing fat cells (25). However, adipose tissue was hardly detectable in newborn pups from either obese or lean dams in our study. The placenta can produce leptin, with a small amount (1.6%) released into the fetal circulation (25, 26). It has been documented that maternal circulating leptin crosses the blood-placental barrier into the fetal circulation and plays an important role in fetal development (27). While elucidating the mechanism underlying the reduced circulating leptin in offspring of obese dams requires further work, it is likely that a high level of maternal leptin reduced its production from fetal organs, leading to lower plasma leptin shortly after birth. Another explanation is that high maternal circulating triglyceride levels may reduce leptin transport at the blood-placenta barrier to cause fetal deficiency, since triglycerides decrease leptin transport at the blood-brain barrier (28). Further studies are needed to confirm this. The low leptin at postnatal day 1 seems at odds with the similar body weight and organ mass between pups from obese and lean dams since leptin is important for fetal growth (25). Pups from obese dams would receive higher levels of nutrients, such as triglycerides, from the maternal circulation to support their growth. Interestingly, unchanged or lower birth weight has been observed in offspring from obese mothers in genetically obese-prone and palatable cafeteria HFD induced obese rats (29-31), which may be due to the relatively immature developmental state of rodents at birth.

Newborn humans with either high or low leptin levels at birth have a higher risk of developing obesity and type 2 diabetes compared with those who have normal leptin levels (23, 32). We did find that pups from obese dams had greater weight gain during the suckling period and lay down fat tissue at a faster rate than those from lean dams after birth, resulting in 10 times greater retroperitoneal fat mass at day 7 after birth. At this time, plasma leptin levels were increased compared pups from lean dams (unpublished data), which may be due to the richer maternal milk or increased fat mass (30). The increase in both adiposity and circulating leptin levels in offspring from obese dams persisted until postnatal day 20 (13).

Newborn leptin levels are suggested to be correlated with levels of insulin and insulin-like growth factor-1 (33). In the current study, insulin levels were higher in the female pups than male pups, as recorded previously in the literature (34). However it was not significantly changed by maternal obesity. It has been suggested that leptin signaling pathways in the neonatal hypothalamus do not develop until after the 6^{th} postnatal day (7, 35), and the expression of Ob-Rb does not affect leptin penetrating the blood brain barrier (36); whereas a population of arcuate Ob-Rb expressing neurons can be directly accessed by circulating leptin (37). Downregulated STAT3 mRNA expression in male pups at day 1 may lead to a lower responsiveness to leptin in the offspring of obese dams even if they obtain leptin from maternal milk or adipocytes and other organs later on. On the other hand, the reduction in SOCS3 expression observed may compensate for the reduced Ob-Rb and STAT3. It is interesting to see the gender difference on hypothalamic STAT3 expression, which was not reduced in the female pups as in the males. Cross talk between leptin and insulin pathways has been well documented, as insulin can increase the phosphorylation of STAT3 either directly or via leptin (38, 39). Plasma insulin was increased by 61% in female offspring from obese dams compared with those from lean dams. Although this did not reach statistical

significance, the increased insulin level may contribute to the hypothalamic STAT3 mRNA expression in female pups from obese dams.

Although birth weight and organ masses were not affected by maternal obesity, our data suggest that the low circulating leptin in pups from obese dams regulates the production of hypothalamic neurotransmitters involved in energy homeostasis. Unlike adult animals where low plasma leptin is associated with increased NPY, in newborn rats, leptin may stimulate the production of hypothalamic neuropeptides or neurite growth. Key hypothalamic neurotransmitters were significantly altered at birth by intrauterine overnutrition. In pups from obese dams, production of hypothalamic appetite regulatory peptides (NPY, POMC), the fuel sensor (mTOR), and MC4R were reduced, together with lower expression of leptin receptors and its downstream signaling system. It is interesting to observe no alteration in AgRP mRNA expression between groups. Brain AgRP mRNA expression appeared at gestation day 14 in rats, however the level was dramatically decreased to undetectable level at gestation day 18 (40). Thus, it is possible that in newborns, AgRP reappears and thus was less affected by the degree of maternal nutrition. The relative importance of NPY and AgRP in voluntary suckling needs further investigation. In adult animals, hypothalamic NPY and POMC are downregulated in the face of HFD feeding, whereas MC4R mRNA expression is upregulated (41-43). In the current study, mRNA expression of MC4R was reduced by maternal obesity. MC4R is critical for normal control of food intake (44), as MC4R-selective agonists cause dose dependent hypophagia and weight loss, whereas MC4R-selective antagonists or MC4-R knockout cause markedly increased food intake obesity (45, 46). Thus the reduction in MC4R mRNA and unchanged AgRP expression, alongside reduced POMC mRNA, in offspring of obese dams may promote hyperphagia. mTOR is a newly identified hypothalamic sensor of fuels such as amino acids and glucose, whose activation leads to reduced food

intake and body weight gain; inhibition of mTOR can also diminish the effects of leptin (47). Reduced hypothalamic mTOR mRNA expression in male newborns from obese dams may affect nutrient sensing and thus increase milk intake as we observed in postnatal day 10 pups (Chen and Morris, unpublished data). However, here mTOR was differently programmed in the female pups. We suggest that it could be consistent with unchanged STAT3, since leptin can directly activate mTOR (47).

After birth, maternal milk will start to supply leptin to the pup, and with the differentiation of adipocytes, endogenous production of leptin will also catch up in pups from obese dams. Whether this will over compensate and cause a higher "leptin surge" leading to faster growth during the suckling period is under further investigation. Another important question is whether these hypothalamic adaptations to maternal obesity are long-lasting. However, our results demonstrate that marked changes in key hypothalamic appetite regulators at postnatal day 1 are induced by maternal obesity, which may promote overeating in offspring. Low neonatal plasma leptin in leptin deficient *ob/ob* mice and following intrauterine nutrition restriction in rats resulted in disturbed neural development during suckling period and obesity at adulthood (48, 49), while leptin injection during the early postnatal period ameliorated the obese phenotype (48, 50), suggesting early postnatal leptin supplementation may reverse the detrimental impact of maternal obesity in offspring.

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Table 1: TaqMan probe sequence (Applied Biosystem, Foster City, USA) used for real time-PCR.

Gene	NCBI gene references	FAM-labeled Probes (5'→ 3')	Applied Biosystem Assay
Ob-Rb	NM_012596.1	TTAATTTCCAAAAGCCTGAAACATT	Rn01433205_m1
mTOR	XM_213539.4	AACCTGATCGCCTGTGGCTCCATGA	Rn01464435_g1
MC4R	NM_013099.2	AGCAGAAGCCTGATTCCACTGTTTA	Rn01491866_s1
NPY	NM_012614.1	GCCCGCCCGCCATGATGCTAGGTAA	Rn00561681_m1
POMC	NM_139326.2	AAGCAACCTGCTGGCTTGCATCCGG	Rn00595020_m1
SOCS3	NM_053565.1	ACCCCCGGAGCACGCAGCCAGTGCC	Rn00585674_s1
STAT3	NM_012747.2	ACCCAGGTAGTGCTGCCCCTTACCT	Rn00562562_m1

Table 2 Parameters of breeders at kill

	Chow-fed dam (n = 10)	**HFD-fed dam (n = 9)**
BW (g)	353.9 ± 10.7	462.4 ± 32.3 *
Liver (g)	12.17 ± 0.44	13.09 ± 0.95
Heart (g)	0.97 ± 0.03	1.11 ± 0.04 *
BAT (g)	0.25 ± 0.05	0.75 ± 0.15 *
Retroperitoneal fat (g)	3.95 ± 1.07	13.39 ± 1.43 *
Mesenteric fat (g)	5.79 ± 1.10	16.58 ± 3.97 *
Uterine fat (g)	7.69 ± 1.40	19.88 ± 2.12 *
Blood glucose (mM)	10.68 ± 0.82	12.35 ± 1.21
Plasma triglycerides (mM)	0.39 ± 0.12	1.71 ± 0.48 *
Plasma insulin (ng/ml)	0.15 ± 0.06	0.37 ± 0.11
Plasma leptin (ng/ml)	8.22 ± 1.55	16.25 ± 1.41 *

Results are expressed as mean ± S.E.M. Data were analysed by unpaired student's *t*-test.

* $P < 0.05$, significantly different from pups of chow-fed dams.

Table 3 Parameters of 1-day-old male pups

	Chow-fed dam (n = 7)	**HFD-fed dam (n = 9)**
BW (g)	6.68 ± 0.57	6.65 ± 0.42
Liver (mg)	286.89 ± 22.68	271.69 ± 16.36
Liver (%BW)	4.57 ± 0.33	4.11 ± 0.14
Heart (mg)	39.64 ± 3.07	43.04 ± 2.77
Heart (%BW)	0.63 ± 0.04	0.65 ± 0.02
Stomach (mg)	314.81 ± 65.85	357.96 ± 53.17
Stomach (%BW)	4.79 ± 0.70	5.15 ± 0.58
Blood glucose	4.47 ± 0.49	4.77 ± 0.36
Plasma insulin (ng/ml)	0.18 ± 0.04	0.21 ± 0.05
Plasma leptin (ng/ml)	1.05 ± 0.21	0.53 ± 0.13*

Results are expressed as mean ± S.E.M. Data were analysed by unpaired student's *t*-test.

* $P < 0.05$, significantly different from pups of chow-fed dam.

Table 4 Parameters of 1-day-old female pups

	Chow-fed dam (n = 9)	**HFD-fed dam (n = 7)**
BW (g)	5.99 ± 0.17	5.94 ± 0.36
Liver (mg)	259.51 ± 10.59	250.15 ± 20.01
Liver (%BW)	4.36 ± 0.21	4.21 ± 0.23
Heart (mg)	37.86 ± 2.25	37.33 ± 3.57
Heart (%BW)	0.63 ± 0.02	0.63 ± 0.03
Stomach (mg)	248.90 ± 31.86	248.15 ± 51.98
Stomach (%BW)	4.12 ± 0.50	4.02 ± 0.61
Blood glucose	4.49 ± 0.41	4.93 ± 0.46
Plasma insulin (ng/ml)	0.36 ± 0.08	0.58 ± 0.17
Plasma leptin (ng/ml)	2.12 ± 0.57	0.33 ± 0.09*

Results are expressed as mean ± S.E.M. Data were analysed by unpaired student's *t*-test.

* $P < 0.05$, significantly different from pups of chow-fed dam.

Figure legends

Fig1: Hypothalamic NPY (A), AgRP (B), mTOR (C), POMC (D) and MC4R (E) mRNA expression in male pups of chow (open bars n = 6) and HFD-fed (closed bars n = 5) dams. Results are expressed as mean ± S.E.M. Data were analysed by unpaired student's *t*-test.

* $P < 0.05$ significantly different from pups of chow-fed dams.

Fig 2: Hypothalamic Ob-Rb (A), STAT3 (B) and SOCS3 (C) mRNA expression in male pups of chow (open bars n = 6) and HFD-fed (closed bars n = 5) dams. Results are expressed as mean ± S.E.M. Data were analysed by unpaired student's *t*-test.

* $P < 0.05$ significantly different from pups of chow-fed dams.

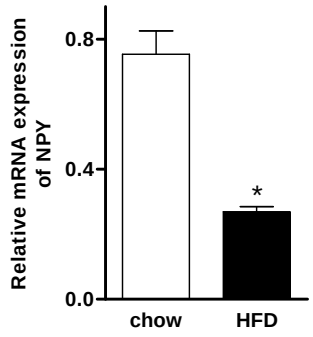
Fig3: Hypothalamic NPY (A), AgRP (B), mTOR (C), POMC (D) and MC4R (E) expression in female pups of chow (open bars n = 6) and HFD-fed (closed bars n = 6) dams. Results are expressed as mean ± S.E.M. Data were analysed by unpaired student's *t*-test.

* $P < 0.05$ significantly different from pups of chow-fed dams.

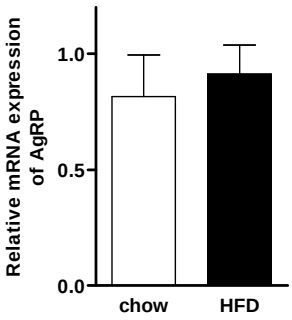
Fig 4: Hypothalamic Ob-Rb (A), STAT3 (B) and SOCS3 (C) mRNA expression in female pups of chow (open bars n = 6) and HFD-fed (closed bars n = 6) dams. Results are expressed as mean ± S.E.M. Data were analysed by unpaired student's *t*-test.

* $P < 0.05$ significantly different from pups of chow-fed dams.

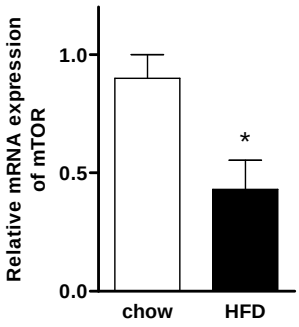
A.


Relative mRNA expression of NPY
0.8
0.4
0.0
*
chow
HFD

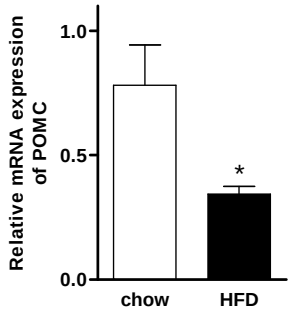
B.


Relative mRNA expression of AgRP
1.0
0.5
0.0
chow
HFD

C.


Relative mRNA expression of mTOR
1.0
0.5
0.0
*
chow
HFD

D.


Relative mRNA expression of POMC
1.0
0.5
0.0
*
chow
HFD

E.

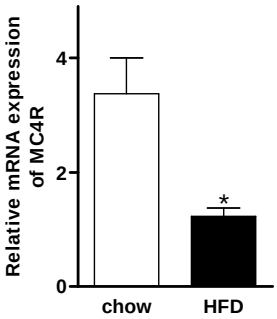

Relative mRNA expression of MC4R
4
2
0
*
chow
HFD

Fig1

A. B. C.

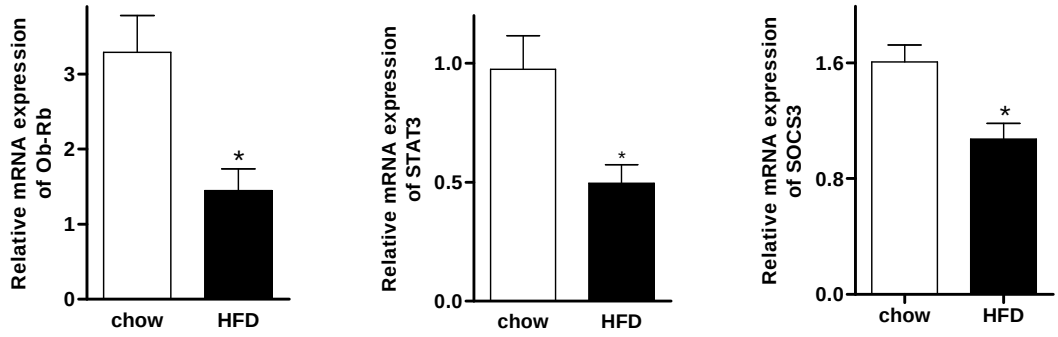
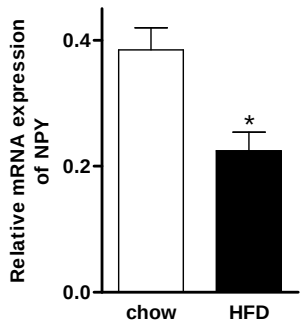
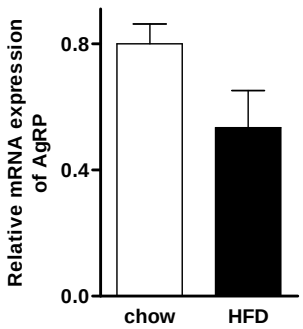

Relative mRNA expression of Ob-Rb
3
2
1
0
*
chow
HFD
Relative mRNA expression of STAT3
1.0
0.5
0.0
*
chow
HFD
Relative mRNA expression of SOCS3
1.6
0.8
0.0
*
chow
HFD

Fig 2

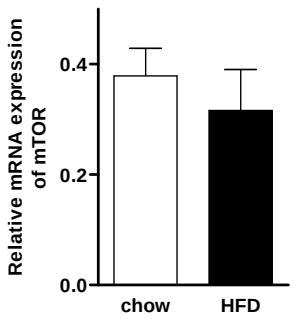
A.


Relative mRNA expression of NPY
0.4
0.2
0.0
*
chow
HFD

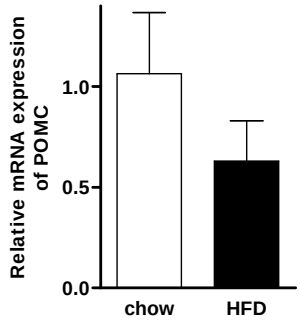
B.


Relative mRNA expression of AgRP
0.8
0.4
0.0
chow
HFD

C.


Relative mRNA expression of mTOR
0.4
0.2
0.0
chow
HFD

D.


Relative mRNA expression of POMC
1.0
0.5
0.0
chow
HFD

E.

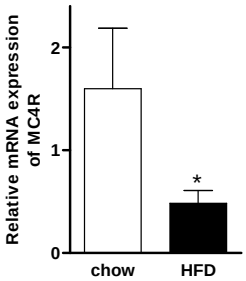

Relative mRNA expression of MC4R
2
1
0
*
chow
HFD

Fig 3

A. B. C.

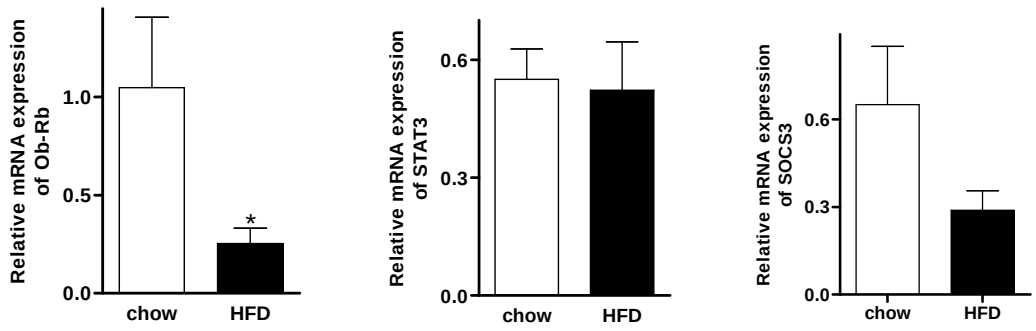

Relative mRNA expression of Ob-Rb
1.0
0.5
0.0
*
chow
HFD
Relative mRNA expression of STAT3
0.6
0.3
0.0
chow
HFD
Relative mRNA expression of SOCS3
0.6
0.3
0.0
chow
HFD

Fig 4