Heightened fasting response of orexigenic but not anorexigenic neuropeptides by maternal obesity

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Running title: maternal obesity promotes orexigenic response

Abstract

Maternal obesity due to long-term high-fat diet (HFD) consumption leads to faster growth in offspring during suckling, and increased adiposity at 20 days of age. Decreased expression of the orexigenic neuropeptide Y (NPY) and increased anorexigenic proopiomelanocortin (POMC) mRNA expression were observed in the fed state. However, hunger is the major drive to eat and hypothalamic appetite regulating neuropeptides change in response to meals. Therefore it is important to compare both satiated and fasting states. Female Sprague Dawley rats (8 weeks old) were fed a cafeteria-style HFD (15.33kJ/g) or chow for 5 weeks before mating, and the same diet continued throughout gestation and lactation. At postnatal day 20, male pups were killed either after overnight fasting or in the fed state. Milk intake was more than doubled in pups from obese dams compared with those from lean dams. Pups from obese dams had higher hypothalamic mRNA expression of POMC and NPY Y1 receptor, but lower hypothalamic leptin receptor, melanocortin-4 receptor and its downstream target single-minded gene 1 (Sim1) in the fed state. Overnight fasting reduced circulating glucose, insulin, and leptin levels and increased hypothalamic NPY Y1 receptor mRNA in pups from both lean and obese dams. Hypothalamic NPY and agouti-related protein were only increased by fasting in pups from obese dams. At weaning, suppressed brain orexigenic signals in offspring from obese dams were significantly upregulated after overnight fasting, while anorexigenic signaling was impaired in these animals. This may contribute to increased milk intake and faster growth during suckling.

Key words: fasting, obesity, NPY, AgRP, hyperphagia

Introduction

Appetite is regulated by a complex homeostatic network comprising central and peripheral components to maintain the balance between energy intake and energy expenditure (1, 2). As a result, in most adults, adiposity and body weight remain remarkably constant over relatively long periods, despite huge variations in daily food intake and energy expended. The hypothalamus is considered the main integrator and processor of peripheral metabolic information. The flow of signals regulating metabolism within the brain relies on neuronal interactions that regulate the production or release of neurotransmitters (3). The arcuate nucleus in the ventral hypothalamus contains neurons of different functional domains; neurons expressing orexigenic peptides neuropeptide Y (NPY) and agouti-related protein (AgRP), and those expressing anorexigenic peptide pro-opiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript, interact with each other (1). Input from hormones reflecting body adiposity and energy balance (eg. the adipose derived hormone leptin and pancreatic hormone insulin) is integrated within the hypothalamus to regulate feeding.

One of the major public health concerns in this century is the medical consequences stemming from the global obesity epidemic attributed to over-consumption of energy dense food and a sedentary lifestyle (4). Studies on genetic mutant mice indicate that overexpression of orexigenic neuropeptides contributes to obesity or hyperphagia phenotype. The expression of the powerful feeding stimulator NPY in the hypothalamus is increased by several fold in leptin deficient *ob/ob* and leptin receptor deficient *db/db* mice, as well as in agouti obese mice, and there is also marked overexpression of AgRP in both *ob/ob* and *db/db mice* (5, 6). However, in more physiologically relevant dietary obese rodent models, particularly those employing palatable

diets, there is an apparent discrepancy between a hyperphagic phenotype and the hypothalamic expression of both orexigenic and anorexigenic appetite regulators. In both rats and mice, long term high-fat-diet (HFD) feeding induced marked increases in plasma leptin levels, with reduced concentrations of hypothalamic NPY and increased peptide cleavage product of POMC, α-melanocyte-stimulating hormone (α-MSH) (7-9). It seems that hyperphagia in these animals was not regulated by these essential central regulators.

Caloric deprivation or hunger invokes a complex hormonal and neural response. Under normal physiological conditions, NPY concentrations are only elevated before the introduction of food and, thereafter, decrease significantly during the course of eating (10). NPY mRNA expression is increased in response to fasting or chronic food restriction, and decreased within 6-24 hours of *ad libitum* refeeding (10-14). AgRP expression is also further increased in the obese *ob/ob* and *db/db* mouse during fasting (5, 6, 13). POMC mRNA levels are reduced markedly in fasted animals and restored by refeeding, or increased by exogenous administration of leptin (13, 15). Hunger is the major drive for feeding behavior. Therefore, it is important to understand how obesity affects the changes of these hypothalamic neuropeptides and circulating hormones in the hungry state.

The obesity epidemic is affecting the next generation, as increasing numbers of women are entering pregnancy with elevated adiposity (16). Intrauterine over-nutrition due to long-term maternal obesity has been shown to predispose individuals to overeating, increased adiposity and glucose intolerance (17, 18). However, the underlying neural mechanisms are still unclear. Previously, we found a reduced hypothalamic NPY and increased POMC mRNA expression,

along with increased plasma leptin and insulin levels in offspring from obese dams at postnatal day 20 (19), which is similar to the changes in adult-onset dietary-obesity (7-9). However, this was in the free-feeding state. It is unknown how these markers will respond to overnight fasting. We hypothesized that in offspring from obese dams, the increase in hypothalamic orexigenic neuropeptides in response to overnight fasting would be greater than that in offspring from lean dams, leading to caloric overconsumption.

Materials and Methods

1. Maternal obesity

Outbred female Sprague Dawley rats (8 weeks, Animal Resources Centre, WA, 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 equal average body weight. The control group was exposed to laboratory chow (Gordon's Specialty Stockfeeds, NSW, Australia, 11kJ/g, energy 14% as fat, 21% as protein, 65% as carbohydrate), while the second group was presented with a palatable-HFD (15.33 kJ/g, average energy 34% as fat (saturated fat 17%), 18% as protein, 50% as carbohydrate). Briefly, the HFD consisted of high-fat modified chow (laboratory chow, milk powder, sweetened condensed milk and saturated animal fat) and highly palatable cafeteria-style food such as meat pies, cakes and biscuits (4-5 different types/day) of known caloric content (7, 8, 19). Female rats were fed either chow or HFD for 5 weeks before mating with males (10 weeks) obtained from the same source. Dams continued on the same diet after giving birth and during lactation. The study was approved by the Animal Ethics Committee of the University of New South Wales.

2. Fasting and sample collection

Dams littered normally. At postnatal day 19, half of the male pups from each litter were separated from their mothers at 17:00h and housed with their litter mates. They were allowed free access to water without food. The other rats remained with the dams. The following morning, pups fasted overnight were harvested between 8:00-9:00h, and those who were not fasted between 9:00-11:00h. Rats were deeply anesthetized (ketamine/xylazine 180/32mg/kg, intraperitoneal). After measurement of naso-anal (N-A) length, blood was collected by cardiac puncture and glucose was measured immediately (Accu-Chek® meter; Roche Diagnostics, Nutley, NJ, USA). Plasma was stored at -20°C for hormone measurements. Pups were killed by decapitation and 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. The hypothalamus was removed following a coronal section at the rostral level of the optic chiasm and at the caudal level of the hypothalamic sulcus. The hypothalamic area was dissected by cutting at the level of the hypothalamic sulcus, then cutting just above the top of the third ventricle. Body fat (brown adipose tissue (BAT), epididymal, retroperitoneal, and mesenteric fat) was dissected and weighed, as well as organs (heart, liver, and kidney) and skeletal muscles (extensor digitorum longus (EDL), soleus and tibialis). Tibia length was measured as a marker of growth.

3. Milk intake measurement

Milk intake was measured in a separate cohort of animals, since separation of pups from their mothers causes stress to both dams and pups. At postnatal day 10, body weights of pups were recorded after 2 hours separation from their mothers. Weight was recorded 2 hours after return to the mothers. Weight increases of pups after refeeding served as an indirect measure of milk

ingestion, represented as ml drunk.

4. Plasma leptin and insulin measurements

Plasma leptin and insulin concentrations were measured using commercially available radioimmunoassay kits (Linco, MO, USA). The insulin resistance index of pups was estimated by the homeostasis model assessment (HOMA) parameter: HOMA = fasting plasma insulin (ng/ml) x fasting plasma glucose (mM)/(22.5 x 0.0417); the greater the HOMA value, the greater the level of insulin resistance (20).

5. Quantitative real-time PCR

Total RNA was isolated from the whole hypothalamus of males and females using TriZol reagent (Invitrogen Australia Pty Limited, Melbourne, VIC, 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, Madison, WI, 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, Eppendorf AG, Hamburg, Germany). The target mRNA was 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.

6. Statistical methods

Results are expressed as mean ± SEM. Body weight of dams and HOMA of pups were analyzed by *Student's* unpaired *t* test. Other data were analyzed using two-way ANOVA followed by post hoc Fisher's Least Significance Difference (LSD) tests.

Results

Maternal effect

At day 1 of life, pups born from obese dams were 10% heavier than those from lean dams (Table 1, $p<0.05$). At postnatal day 10, the 2h milk intake was more than doubled in pups from obese dams (1.05±0.10 vs. 0.40±0.08 ml in offspring from obese and lean dams respectively, $p<0.05$ n=22 in each group). In the free feeding state, maternal obesity resulted in nearly doubled body weight in offspring at 20 days compared with those from lean dams (Table 1). Pups from obese dams were significantly larger as shown by 14% increase in body length and 8% increase in tibia length (Table 1). All the major organs (liver, kidney, pancreas and heart) and fat pads (BAT, retroperitoneal, epididymal, and mesenteric fat) sampled were significantly heavier in pups from obese dams, as well as the weights of skeletal muscles. Remarkably, retroperitoneal and epididymal fat masses were more than 9 times and 5 times greater in pups from obese dams. When standardized by body weight, all these differences remained significant. Maternal obesity had little impact on blood glucose and plasma insulin levels in offspring (Fig 1a, 1b), whereas the plasma leptin levels were more than tripled in pups from obese dams (Fig 1c).

When animals were in the fed state, hypothalamic NPY and AgRP mRNA expression in pups from obese dams were slightly lower than that in pups from lean dams, without reaching statistical significance (NPY $p=0.07$, Fig 2a, 2b), while POMC mRNA expression was

significantly increased in pups from obese dams (Fig 2c). NPY Y1 receptor expression (Fig 3a) was increased in pups from obese dams, with no significant changes in mRNA expression of the receptor of α-MSH, melanocortin 4 receptor (MC4R) ($p=0.07$, Fig 3b). However, mRNA expression of the downstream target of MC4R, single-minded gene (Sim1), was significantly reduced by 45% in pups from obese dams ($p<0.05$, Fig 3c). mRNA expression of Ob-Rb and STAT3 was also significantly reduced by 40% in pups from obese dams ($p<0.05$, Fig 4).

Fasting effect

Overnight fasting led to 18% and 12% reductions in body weight in pups from lean and obese dams respectively (Table 1). Of the organs sampled, only liver was affected by fasting, with reductions of 35% and 30% in pups from lean and obese dams, respectively ($p< 0.05$ fasting effect, Table 1). Overnight fasting also caused some reduction in fat and muscle mass, however, significance was only observed in mesenteric fat and EDL (Table 1). Blood glucose and insulin concentrations were significantly reduced to similar levels in pups from lean and obese dams after overnight fasting (Fig 1a, 1b). HOMA was not different between pups from lean (1.34±0.26) and obese dams (1.59±0.25). Plasma leptin was reduced by almost 80% in pups from both lean and obese dams, remaining 3 times higher in the latter (Fig 1c).

After overnight fasting, hypothalamic NPY mRNA expression was not significantly altered in pups from lean dams, but was markedly increased in pups from obese dams, to a similar level to that of pups from lean dams (Fig 2a). Although hypothalamic AgRP mRNA expression was increased by 20% in pups from lean dams, it did not reach statistical significance, while the expression was nearly doubled in pups from obese dams ($p<0.05$, fasting effect). POMC mRNA

expression was not altered in either group. Hypothalamic Y1 receptor mRNA was increased in response to a fast by 77% and 65% in pups from lean and obese dams respectively; however, the expression in pups from obese dams was 30% higher than that in pups from lean dams after fasting (Fig 3a). MC4R was significantly reduced in pups from lean dams, which was not observed in pups from obese dams (Fig 3b). Sim1 mRNA expression was also reduced by nearly 40% after overnight fasting in pups from lean dams, with no change in pups from obese dams (Fig 3c). Hypothalamic Ob-Rb mRNA expression tended to be reduced by fasting in pups from lean dams, with no change in pups from obese dams (Fig 4a). STAT3 mRNA expression was only significantly reduced by fasting in pups from lean dams ($p<0.05$).

Discussion

Maternal obesity is a significant risk factor for childhood obesity. Human studies suggest that intrauterine factors can be more important than genetic factors in changing the gene expression in response to HFD, which may have a longstanding influence postnatally. Hunger is the major drive for eating behaviour in both humans and animals. This study investigated the impact of pre-existing maternal obesity on offspring central appetite regulators, and the response to overnight fasting.

Long-term maternal obesity led to greater body weight, larger body size, and more severe fat deposition in offspring at weaning, which is consistent with our earlier observations (19). This could partly due to increased milk intake during the suckling period, since there was no significant difference in birth weight between pups from lean and obese dams. As a result, plasma leptin was significantly increased. As an adaptation to the increased adiposity and plasma

leptin concentration, when animals were in a satiated state, the anorexigen POMC was significantly upregulated in offspring from obese dams, which is similar to the changes observed in adult onset dietary obese animals (21). However, Y1 receptor mRNA was also upregulated in the non-fasted state. The NPY Y1 receptor not only mediates the feeding effects of NPY, but also regulates energy expenditure (22). Increased NPY Y1 receptor expression may lead to reduced energy expenditure in the satiated state.

One of the novel observations in the current study is that hypothalamic Sim1 expression was significantly lower in pups from obese dams, and MC4R showed a similar pattern, in the face of increased POMC expression. MC4R and Sim1 are co-expressed in neurons in the hypothalamic paraventricular nucleus (PVN), a region rich in terminals releasing NPY, AgRP and α-MSH and thus critical for appetite regulation (23, 24). Absence of Sim1 is associated with hyperphagic obesity in both humans and rodents (25) and overexpression of Sim1 can normalize food intake in agouti yellow obese mice and diet-induced obesity (26). Sim1 heterozygous mice have been reported to be resistant to melanocortin signaling (27). These mice displayed normal energy expenditure and elevated hypothalamic POMC expression, yet are hyperphagic. The appetite suppressive effect of melanocortin agonist melanotan-2 was also blunted in these mice (27). Therefore, Sim1 is suggested to be the essential downstream target for MC4R signaling in feeding regulation (26). In this study, the appetite regulation in offspring from obese dams showed similar phenotype as Sim1 heterozygous mice. Thus we suggest that maternal “junk eating” inhibits anorexigenic melanocortin signaling via both MC4R and its downstream target Sim1. Increased NPY Y1 receptor together with low Sim1 in the satiated state may also drive feeding behavior in offspring from obese dams even if they are not hungry.

Changes of appetite regulators in the satiated state were not substantial enough to explain increased milk intake in offspring from obese dams. NPY, AgRP and POMC can change in response to energy deficiency to induce foraging and feeding behaviour, which is necessary for survival. Thus it is of importance to compare the physiological changes in hungry state between pups from lean and obese dams. Rats are nocturnal animals and consume the majority of daily intake during the night. Thus food deprivation overnight should be strong enough to trigger significant changes.

After overnight fasting, body weight, liver and muscle masses, together with blood glucose and plasma leptin and insulin levels were significantly reduced in pups from both lean and obese dams. The effect of fasting on body weight is related to the growth rate at weaning age, and interruption of nocturnal feeding. Since excess glucose is stored as glycogen in the liver and muscle and used as fuel during times of energy deficiency, the reduced liver and muscle mass could reflect mobilization of stored glycogen to maintain minimum glucose levels.

The major finding of the current study is the differential changes in appetite regulatory neurotransmitters induced by overnight fasting in pups from lean and obese dams. An 80% reduction in plasma leptin levels, and its downstream signal, STAT3, after fasting in pups from both lean and obese dams suggests reduced actions of leptin in this situation. One direct outcome is to reduce the expression of anorexigenic and increase orexigenic neurotransmitters, to promote feeding when food becomes available. In pups from lean dams, although POMC was not altered by fasting, both MC4R and Sim1 were downregulated, suggesting the anorexigenic pathway was

switched off. While mRNA expression of NPY and AgRP was not significantly increased by overnight fasting in pups from lean dams, they were doubled in pups from obese dams, suggesting they are hyperresponsive to food withdrawal and may have shorter meal intervals than those from lean dams; further work would be required to confirm this. In adult rats, NPY concentrations are elevated before a meal and decreased significantly during the course of eating, while α-MSH counteracts NPY to inhibit feeding (28). It is also of interest to observe an upregulation of hypothalamic Y1 receptor mRNA after overnight fasting in both diet groups. Few studies have measured Y1 receptor expression, with one report of reduced Y1 receptor number, distribution and mRNA expression in the arcuate nucleus during fasting (29). This may reflect regional differences. In the current study, we measured total hypothalamic Y1 receptor mRNA expression, which includes arcuate nucleus and paraventricular nucleus. Increased Y1 receptor can promote the orexigenic effects of NPY.

In pups from obese dams after overnight fasting, NPY and AgRP mRNA expression were increased to similar levels as those in pups from lean dams, while the upregulation of Y1 receptor mRNA expression was greater than that in pups from lean dams. This increased response of orexigenic peptides and receptor could be due to more pronounced reduction in leptin levels on fasting. Higher Y1 receptor mRNA can facilitate NPY to exert stronger orexigenic effects even when fasting NPY mRNA levels were similar between groups. Although the adaptive response of MC4R and Sim1 observed in pups from lean dams was absent in those from obese dams, their basal expression levels were already low in the latter. This suggests that the anorexigenic signalling is less responsive to change in feeding states in these offspring. In other words, after refeeding, POMC and its downstream signalling cascade would not increase to

terminate eating. Moreover, AgRP is a potent selective antagonist of MC4R, inhibiting binding of α-MSH (6, 30), while NPY can inhibit the melanocortin system via Arc Y1 receptors (21, 31, 32). Normalized hypothalamic NPY and AgRP mRNA expression and upregulated Y1 receptor mRNA expression after overnight fasting can further weaken the already reduced anorexigenic effects of the melanocortin pathway in offspring from the obese dams. This may help to explain the greater milk intake of pups from obese dams.

Reduced baseline Ob-Rb and its downstream STAT3 may be an initial step towards leptin resistance commonly observed in dietary obese animals and humans. Indeed it has been shown previously that maternal obesity led to leptin resistance in offspring even before they were exposed to HFD (33). This explains why in the satiated state, the high circulating leptin levels in offspring from obese dams did not significantly inhibit both NPY and AgRP. Although Ob-Rb and STAT3 were not significantly altered after fasting, there was even more marked reduction in plasma leptin levels during fasting. As stated above, this may contribute to the increased NPY and AgRP mRNA expression.

Conclusion

In day 20 offspring, pre-existing maternal obesity increased hypothalamic mRNA expression of the orexigenic regulators, NPY and AgRP to levels similar to offspring of lean dams during fasting, and led to a heightened response of Y1 receptor to fasting,. It also inhibited the downstream signals of the melanocortin system, comprising MC4R and Sim1, in the satiated state and made MC4R and Sim1 less responsive to changes in feeding state in these offspring.

These changes could promote hyperphagia and reduced meal interval in offspring from obese dams, contributing to their obesity.

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Reference:

1 Wynne K, Stanley S, McGowan B, Bloom S. Appetite control. *J Endocrinol* 2005;184:291-318.

2 Schwartz MW, Woods SC, Porte DJ, Seeley RJ, Baskin DG. Central nervous system control of food intake. *Nature* 2000;404:661-71.

3 Horvath TL, Diano S, Tschop M. Brain circuits regulating energy homeostasis. *Neuroscientist* 2004;10:235-46.

4 West DB, York B. Dietary fat, genetic predisposition, and obesity: lessons from animal models. *Am J Clin Nutr* 1998;67:505-12S.

5 Shutter JR, Graham M, Kinsey AC, Scully S, Luthy R, Stark KL. Hypothalamic expression of ART, a novel gene related to agouti, is up-regulated in obese and diabetic mutant mice. *Genes Dev* 1997;11:593-602.

6 Ollmann MM, Wilson BD, Yang YK, et al. Antagonism of central melanocortin receptors in vitro and in vivo by agouti-related protein. *Science* 1997;278:135-8.

7 Hansen MJ, Jovanovska V, Morris MJ. Adaptive responses in hypothalamic neuropeptide Y in the face of prolonged high-fat feeding in the rat. *J Neurochem* 2004;88:909-16.

8 Morris MJ, Chen H, Watts R, Shulkes A, Cameron-Smith D. Brain neuropeptide Y and CCK and peripheral adipokine receptors: temporal response in obesity induced by palatable diet. *Int J Obes* 2008;32:249-58.

9 Fam BC, Morris MJ, Hansen MJ, et al. Modulation of central leptin sensitivity and energy balance in a rat model of diet-induced obesity. *Diabetes, obesity & metabolism* 2007;9:840-52.

10 Kalra SP, Dube MG, Sahu A, Phelps CP, Kalra PS. Neuropeptide Y secretion increases in the paraventricular nucleus in association with increased appetite for food. *Proc Natl Acad Sci USA* 1991;88:10931-5.

11 Beck B, Jhanwar-Uniyal M, Burlet A, Chapleur-Chateau M, Leibowitz SF, Burlet C. Rapid and localized alterations of neuropeptide Y in discrete hypothalamic nuclei with feeding status. *Brain Res* 1990;528:245-9.

12 Brady LS, Smith MA, Gold PW, Herkenham M. Altered expression of hypothalamic neuropeptide mRNAs in food-restricted and food-deprived rats. *Neuroendocrinology* 1990;52:441-7.

13 Swart I, Jahng JW, Overton JM, Houpt TA. Hypothalamic NPY, AGRP, and POMC mRNA responses to leptin and refeeding in mice. *Am J Physiol Regul Integr Comp Physiol* 2002;283:R1020-6.

14 Bi S, Robinson BM, Moran TH. Acute food deprivation and chronic food restriction differentially affect hypothalamic NPY mRNA expression. *Am J Physiol Regul Integr Comp Physiol* 2003;285:R1030-6.

15 Schwartz MW, Seeley RJ, Woods SC, et al. Leptin increases hypothalamic pro-opiomelanocortin mRNA expression in the rostral arcuate nucleus. *Diabetes* 1997;46:2119-23.

16 Mokdad AH, Serdula MK, Dietz WH, Bowman BA, Marks JS, Koplan JP. The spread of the obesity epidemic in the United States, 1991-1998. *JAMA* 1999;282:1519-22.

17 Caluwaerts S, Lambin S, van Bree R, Peeters H, Vergote I, Verhaeghe J. Diet-induced obesity in gravid rats engenders early hyperadiposity in the offspring. *Metabolism* 2007;56:1431-8.

18 Bayol SA, Farrington SJ, Stickland NC. A maternal 'junk food' diet in pregnancy and lactation promotes an exacerbated taste for 'junk food' and a greater propensity for obesity in rat offspring. *Br J Nutr* 2007;98:843-51.

19 Chen H, Simar D, Lambert K, Mercier J, Morris MJ. Intrauterine and postnatal overnutrition differentially impact appetite regulators and fuel metabolism. *Endocrinology* 2008;149:5348-56.

20 Schaalan M, El-Abhar HS, Barakat M, El-Denshary ES. Westernized-like-diet-fed rats: effect on glucose homeostasis, lipid profile, and adipocyte hormones and their modulation by rosiglitazone and glimepiride. *J Diabetes Complications* 2008: doi:10.1016/j.jdiacomp.2008.02.003.

21 Hansen MJ, Schioth HB, Morris MJ. Feeding responses to a melanocortin agonist and antagonist in obesity induced by a palatable high-fat diet. *Brain Res* 2005;1039:137-45.

22 Kushi A, Sasai H, Koizumi H, Takeda N, Yokoyama M, Nakamura M. Obesity and mild hyperinsulinemia found in neuropeptide Y-Y1 receptor-deficient mice. *Proc Natl Acad Sci USA* 1998;95:15659-64.

23 Balthasar N, Dalgaard LT, Lee CE, et al. Divergence of melanocortin pathways in the control of food intake and energy expenditure. *Cell* 2005;123:493-505.

24 Holder JL, Jr., Zhang L, Kublaoui BM, et al. Sim1 gene dosage modulates the homeostatic feeding response to increased dietary fat in mice. *Am J Physiol Endocrinol Metab* 2004;287:E105-13.

25 Baskin DG. Single-minded view of melanocortin signaling in energy homeostasis. *Endocrinology* 2006;147:4539-41.

26 Kublaoui BM, Holder JL, Jr., Tolson KP, Gemelli T, Zinn AR. SIM1 overexpression partially rescues agouti yellow and diet-induced obesity by normalizing food intake. *Endocrinology* 2006;147:4542-9.

27 Kublaoui BM, Holder JL, Jr., Gemelli T, Zinn AR. Sim1 haploinsufficiency Impairs melanocortin-mediated anorexia and activation of paraventricular nucleus neurons. *Mol Endocrinol* 2006;20:2483-92.

28 Ahima RS, Saper CB, Flier JS, Elmquist JK. Leptin regulation of neuroendocrine systems. *Front Neuroendocrinol* 2000;21:263-307.

29 Cheng X, Broberger C, Tong Y, et al. Regulation of expression of neuropeptide Y Y1 and Y2 receptors in the arcuate nucleus of fasted rats. *Brain Res* 1998;792:89-96.

30 Fong TM, Mao C, MacNeil T, et al. ART (protein product of agouti-related transcript) as an antagonist of MC-3 and MC-4 receptors. *Biochem Biophys Res Commun* 1997;237:629-31.

31 Fuxe K, Tinner B, Caberlotto L, Bunnemann B, Agnati LF. NPY Y1 receptor like immunoreactivity exists in a subpopulation of [beta]-endorphin immunoreactive nerve cells in the arcuate nucleus: a double immunolabelling analysis in the rat. *Neurosci Lett* 1997;225:49-52.

32 Roseberry AG, Liu H, Jackson AC, Cai X, Friedman JM. Neuropeptide Y-mediated inhibition of proopiomelanocortin neurons in the arcuate nucleus shows enhanced desensitization in *ob/ob* mice. *Neuron* 2004;41:711-22.

33 Ferezou-Viala J, Roy A-f, Serougne C, et al. Long-term consequences of maternal high-fat feeding on hypothalamic leptin sensitivity and diet-induced obesity in the offspring. *Am J Physiol Regul Integr Comp Physiol* 2007;293:R1056-62.

Table 1 Body weight and parameters in pups from lean and obese dams at 20 days

	Lean dam		**Obese dam**	
	Baseline	**Fasting**	**Baseline**	**Fasting**
	(n=8)	**(n=9)**	**(n=9)**	**(n=9)**
BW 1d (g) ‡	6.56 ± 0.13		7.21 ± 0.14*	
BW 20d (g)	35.0 ± 1.1	28.7± 0.6	60.6 ±4.4*	53.2 ± 3.2*
N-A (cm)	10.95 ± 0.19	10.43 ± 0.10	12.52 ± 0.24*	12.34 ± 0.17*
Tibia (cm)	2.20 ± 0.04	2.12 ± 0.03	2.38 ± 0.03*	2.26± 0.05*
Liver (mg)	1365.4 ± 70.5	880.3 ± 18. 6#	2439.1 ± 146.5*	1725.8 ± 109.1*#
Kidney (mg)	206.2 ± 5.8	178.2 ± 7.6	326.7 ± 20.2*	295.9 ± 5.8*
Heart (mg)	177.7 ± 6.5	155.1 ± 3.2	333.9 ± 28.2*	313.1 ± 19.1*
BAT (mg)	135.2 ± 5.0	114.3 ± 5.5	287.3 ± 24.3*	218.9 ± 16.2*#
Retroperitoneal fat (mg)	40.9 ± 3.8	33.0 ± 2.5	382.4 ± 67.0*	324.4 ± 50.7*
Epididymal fat (mg)	56.3 ± 5.3	46.7 ± 3.1	320.0 ± 47.4*	273.2 ± 35.9*
Mesenteric fat (mg)	295.4 ± 10.5	245.3 ± 10.5	636.3 ± 40. 4*	552.0 ± 34.4*#
EDL (mg)	13.23 ± 0.79	11.02 ± 0.48#	17.78 ± 0.64*	16.74 ± 0.70*
Soleus (mg)	11.41± 0.70	9.62 ± 0.28	16.62 ± 0.90*	15.63 ± 1.10*
Tibialis (mg)	44.2 ± 2.6	34.9 ± 1.5	69. 6 ± 5.9*	66.5 ± 4.7*

Results are expressed as mean ± S.E.M. Data were analysed by two-way ANOVA, followed by a post hoc LSD test. * $p < 0.05$, maternal effect; # $p < 0.05$, fasting effect. ‡ n = 17, 18 for pups from lean and obese dam respectively

BAT: brown adipose tissue; BW: body weight; EDL: extensor digitorum longus; N-A: naso-anal.

Figure legends:

Figure 1: Blood glucose and plasma insulin and leptin in pups from lean and obese dams at baseline (open bars, n=8) and after overnight fasting (closed bars, n=8). Results are expressed as mean±S.E.M. Data were analysed by two-way ANOVA, followed by a post hoc LSD test. * $p<0.05$, maternal effect; # $p<0.05$, fasting effect.

Figure 2: mRNA expression of hypothalamic NPY (a), AgRP (b) and POMC (c) in pups from lean and obese dams at baseline (open bars, n = 8) and after overnight fasting (closed bars, n=8). Results are expressed as mean±S.E.M. Data were analysed by two-way ANOVA, followed by a post hoc LSD test. * $p<0.05$, maternal effect; # $p<0.05$, fasting effect.

Figure 3: mRNA expression of hypothalamic NPY Y1 receptor, MC4R and Sim1 in pups from lean and obese dams at baseline (open bars, n=8) and after overnight fasting (closed bars, n=8). Results are expressed as mean±S.E.M. Data were analysed by two-way ANOVA, followed by a post hoc LSD test. * $p<0.05$, maternal effect, # $p<0.05$, fasting effect.

Figure 4: mRNA expression of hypothalamic Ob-Rb and STAT3 in pups from lean and obese dams at baseline (open bars, n=8) and after overnight fasting (closed bars, n=8). Results are expressed as mean±S.E.M. Data were analysed by two-way ANOVA, followed by a post hoc LSD test. * $p<0.05$, maternal effect; # $p<0.05$, fasting effect.

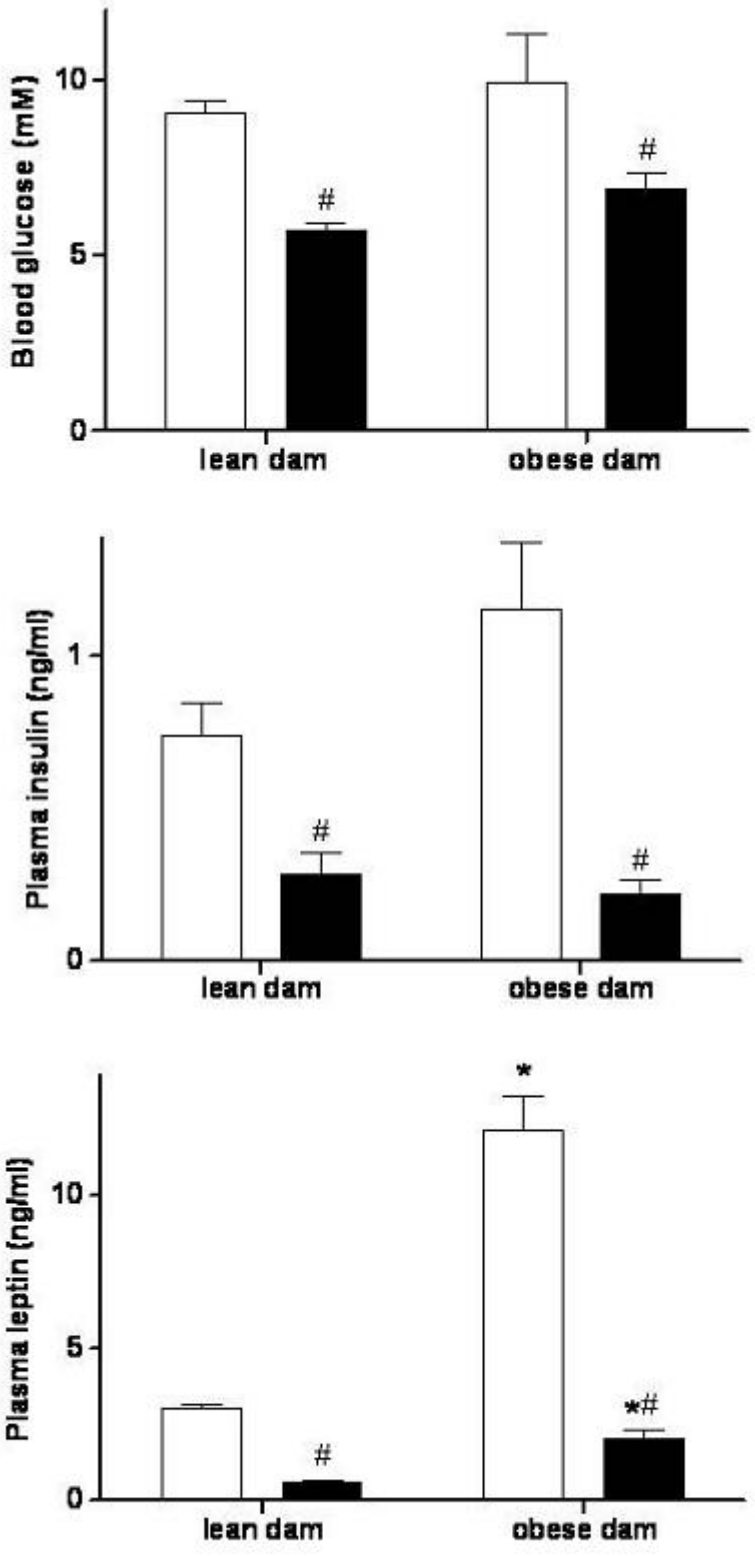
Blood glucose (mM)
10
5
0
#
#
lean dam
obese dam
Plasma insulin (ng/ml)
1
0
#
#
lean dam
obese dam
Plasma leptin (ng/ml)
10
5
0
*
#
*#
lean dam
obese dam

Figure 1

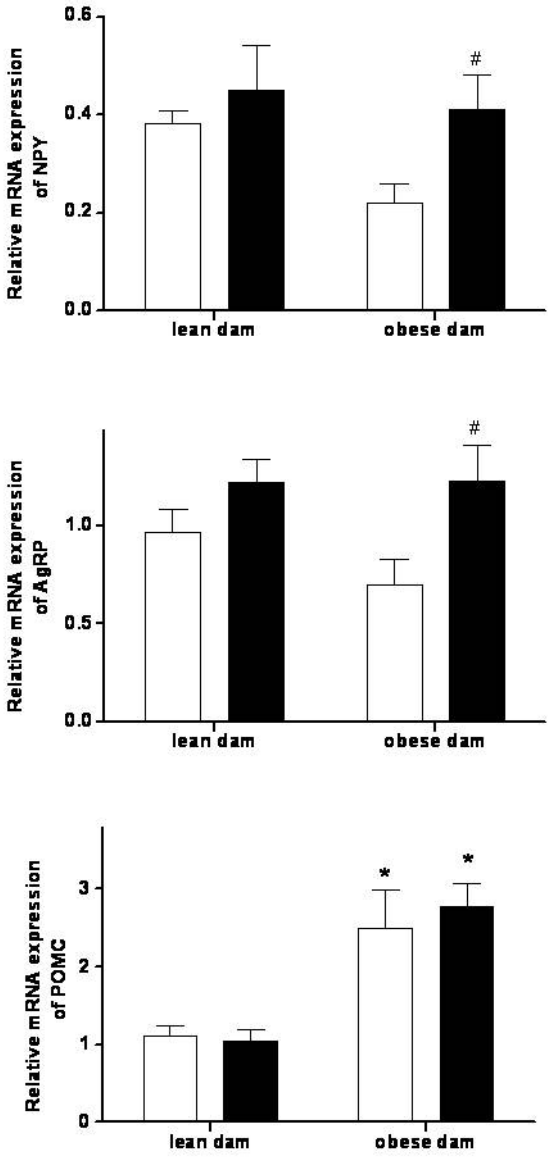
Relative mRNA expression of NPY
0.6
0.4
0.2
0.0
#
lean dam
obese dam
Relative mRNA expression of AgRP
1.0
0.5
0.0
#
lean dam
obese dam
Relative mRNA expression of POMC
3
2
1
0
*
*
lean dam
obese dam

Figure 2

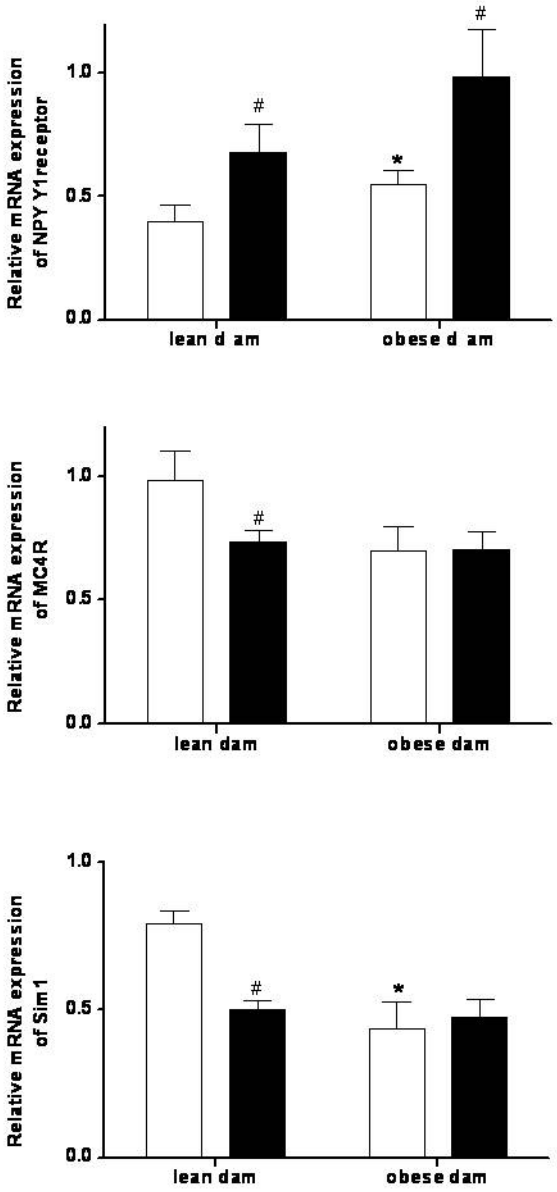
Relative mRNA expression of NPY Y1 receptor
1.0
0.5
0.0
#
#
*
lean d am
obese d am
Relative mRNA expression of MC4R
1.0
0.5
0.0
#
lean dam
obese dam
Relative mRNA expression of Sim 1
1.0
0.5
0.0
#
*
lean dam
obese dam

Figure 3

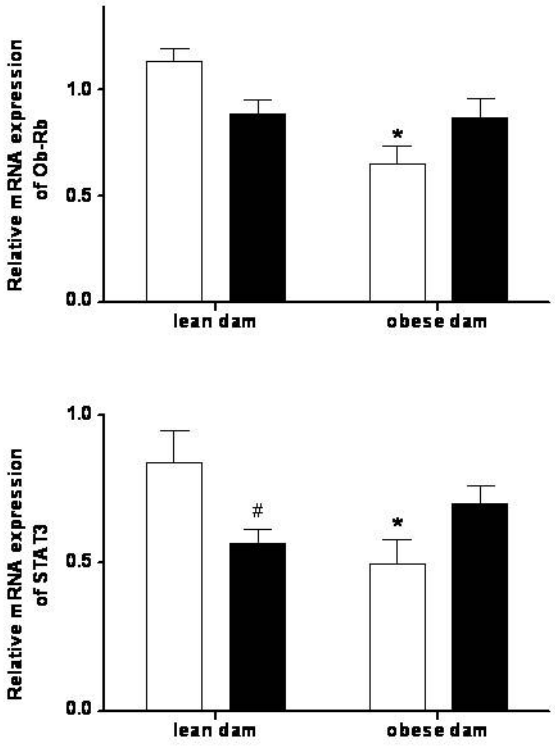
Relative mRNA expression of Ob-Rb
1.0
0.5
0.0
*
lean dam
obese dam
Relative mRNA expression of STAT3
1.0
0.5
0.0
#
*
lean dam
obese dam

Figure 4