Prenatal influences on susceptibility to diet-induced obesity are mediated by altered neuroendocrine gene expression

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Abstract

The escalating rates of obesity and type 2 diabetes have reached pandemic proportions. It has been proposed that the risk of developing metabolic disorders in adult life is influenced by environmental factors, which operate during the early periods of development. We have previously shown that an interaction between the prenatal and the postnatal dietary environment amplifies the propensity towards diet-induced obesity, although the mechanisms are unclear. In the present study, we investigated the interaction between prenatal undernutrition and postnatal high-fat nutrition on key genes of the hypothalamic appetite regulatory network. Pregnant Wistar rats were fed a standard chow diet either *ad libitum* (AD) or at 30% of AD intake throughout gestation (UN). From weaning, female AD and UN offspring were fed either a standard chow (ADC *n*=8, UNC *n*=8) or a high-fat diet (45% kcal as fat; ADHF *n*=8, UNHF *n*=8) *ad libitum* for the remainder of the study. At 24 weeks of age, body composition was assessed by dual energy X-ray

absorptiometry analysis and total RNA was extracted from whole rat hypothalami. Real-time PCR was performed to characterise pro-opiomelanocortin (POMC), neuropeptide Y (NPY), agouti-related protein (AgRP) and OBRb gene expression at the mRNA level. Our results demonstrate that the amplification of postnatal obesity develops as a consequence of an interaction between prenatal undernutrition and postnatal high-fat nutrition. This phenotype also shows significant alterations in POMC, NPY, AgRP and OBRb gene expression together with elevations in circulating levels of both plasma leptin and insulin. These findings are consistent with the predictive adaptive response hypothesis that neuroendocrine development during fetal life may be based on predictions about postnatal environmental conditions. Increased susceptibility to diet-induced obesity develops if a mismatch between the anticipated and the actual conditions are encountered.

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Introduction

Obesity, a key risk factor for cardiovascular disease and type 2 diabetes, is a major health concern, particularly in Western societies. An understanding of the biological processes and mechanisms that promote obesity in childhood and adulthood is therefore imperative for the development of strategies for prevention and intervention. Although genetic and adult lifestyle factors are known to contribute to obesity, recent epidemiological, clinical and experimental studies suggest that the nutritional environment during the prenatal period may have important consequences on metabolic energy regulatory systems in later life (Barker & Osmond 1986, Ravelli *et al.* 1999, Vickers *et al.* 2000).

Much of our current knowledge of the importance of prenatal nutrition has been derived from animal studies. In the rodent, the impact of maternal nutrition has been shown to induce long-term effects on behavioural, anthropometric and metabolic functions (Freinkel 1980, Lucas 1991). Since the developing fetus is sensitive to environmental conditions that

modulate ontogenic pathways, it is thought the maternal metabolic consequences of prenatal undernutrition may modify the developing neural systems that control energy homeostasis in the fetus (Levin 2000).

Leptin plays a key role in energy homeostasis and acts on discrete neuroendocrine pathways within the hypothalamus to reduce food intake and body fat content (Mercer *et al.* 1996, Elmquist *et al.* 1998b). Leptin is secreted mainly from adipose tissue and acts both in the brain and in the peripheral tissues. Five alternatively spliced forms of the leptin receptor are produced (OBRa–e), of which only OBRb has a long cytoplasmic region that is required for signal transduction. Moreover, evidence is accumulating to suggest that obesity is associated with central leptin resistance (Flier 2004). For this reason, investigations over the past decade have focused on determining the mechanisms involved in the action of leptin on hypothalamic neural circuitry systems involved in energy homeostasis.

Key targets of leptin action are the orexigenic neurons within the hypothalamic arcuate nucleus that co-express the

peptides neuropeptide Y (NPY) and agouti-related protein (AgRP), an antagonist of the melanocortin (MC) peptides at the MC-3 and -4 receptors (MC3/4R) (Cowley *et al.* 2001). These neurons express OBRb (Mercer *et al.* 1996, Elmquist *et al.* 1998a) and are inhibited by leptin. When leptin or its receptor levels (OBRb) are deficient, AgRP levels are elevated five- to tenfold in obesity (Ollmann *et al.* 1997, Shutter *et al.* 1997). NPY, the most potent orexigenic peptide, is elevated in response to reduced levels of leptin (Kalra *et al.* 1999) and produces hyperphagia, weight gain and the endocrine/metabolic characteristics of obesity (Heinrichs *et al.* 1998).

Another group of leptin-responsive neurons within the arcuate nucleus are the anorexigenic pro-opiomelanocortin (POMC) neurons, which also express leptin receptors, but in contrast to NPY/AgRP neurons, are stimulated by leptin (Cowley *et al.* 2001). These POMC neurons produce several neuropeptides derived from the POMC-polypeptide precursor, adrenocorticotrophin, β -endorphin, as well as the MCs α -, β - and γ -melanocyte-stimulating hormone (MSH), which are ligands for the MC3/4R (Vergoni *et al.* 1986, Yaswen *et al.* 1999, Kask *et al.* 2000).

In relation to our present investigation, previous studies have shown alterations in NPY neurons in weanling and adult offspring of gestational diabetic rat dams, and in weanling offspring of perinatal low-protein fed rat dams (Plagemann *et al.* 1999, 2000). Recent research in fetal mice of protein-restricted rat dams during pregnancy, has also shown alterations in mRNA levels for the appetite regulatory peptides (POMC, NPY and AgRP) and the leptin receptor isoforms (Terroni *et al.* 2005), lending support to the hypothesis that changes in the nutritional status of the pregnant mother can alter the offspring's neuroendocrine system. However, the long-term consequences of maternal undernutrition during pregnancy on neuroendocrine gene expression in adult offspring are currently unknown, particularly when a nutrient-rich condition of high caloric density is encountered postnatally.

We have previously shown in rats that maternal undernutrition during gestation leads to increased adiposity and high circulating leptin and insulin concentrations in adult offspring (Vickers *et al.* 2000). Furthermore, combining prenatal undernutrition with postnatal high-fat nutrition exacerbated obesity development and further amplified hyperleptinaemia and hyperinsulinaemia. The aim of the present study was, therefore, to assess whether increased susceptibility to diet-induced obesity in offspring of undernourished mothers is related to alterations in neuroendocrine gene expression involved in the regulation of energy homeostasis.

Materials and Methods

The present study utilised a well-characterised model of maternal undernutrition in the rat (Vickers *et al.* 2005).

In brief, virgin Wistar rats (aged 100 ± 5 days) were time mated using a rat oestrous cycle monitor to assess the stage of oestrous of female animals prior to introducing the male. Following confirmation of mating by the presence of spermatozoa in a vaginal lavage, each dam was housed individually in standard cages containing wood shavings as bedding and free access to water. All rats were housed in the same room with a constant temperature maintained at 25°C and a 12 h light:12 h darkness cycle. Dams were randomly assigned to one of two nutritional groups, fed either *ad libitum* (AD) or were undernourished at 30% of AD intake (UN) of a standard rat chow diet, throughout gestation. After birth, pups were weighed and litter size was adjusted to eight pups per litter to assure adequate and standardised nutrition until weaning. Pups from undernourished mothers were cross-fostered onto dams that had received AD feeding throughout pregnancy. From weaning, female offspring were fed either a standard chow (ADC $n=8$, UNC $n=8$) or a high-fat diet (45% kcal as fat; ADHF $n=8$, UNHF $n=8$) *ad libitum* for the remainder of the study. At 24 weeks of age, body composition was assessed using dual energy X-ray absorptiometry (DEXA). Following an overnight food withdrawal, animals were euthanised by decapitation under halothane anaesthesia. Truncal blood was immediately collected into heparinised vacutainers stored on ice (4°C), centrifuged and plasma collected for analysis. Whole rat brains were rapidly removed and placed on dry ice for slow freezing. Using a previously chilled rat brain matrix and consistent landmarks, one sagittal cut was made rostral to the optic chiasma and another sagittal cut was made rostral to the pons to produce three-thirds of whole brain, with the middle-third encasing the entire hypothalamic region. Tissues were then stored at -80°C until analysis. All procedures were approved by the Animal Ethics Committee of the University of Auckland.

Hypothalamic blocks encasing the regions of the arcuate, paraventricular, dorso- and ventromedial nuclei were isolated from the frozen dissected middle-third of whole brain, using appropriate co-ordinates of dissection (Bregma levels -1.3 to -4.3 mm; Paxinos & Watson 1986).

Total RNA was isolated from half of each homogenised rat hypothalami using Trizol/chloroform extraction as directed by the manufacturer's instructions (Invitrogen). RNA was purified using the Qiagen midi column purification kit (Qiagen), quantified by nanodrop meter and assessed for integrity using the RNA 6000 Nano Labchip (Agilent Technologies, Santa Clara, CA, USA). TaqMan real-time PCR (qRT-PCR) was performed for each sample using the ABI PRISM 7900HT Sequence Detection System (Perkin-Elmer Applied Biosystems, Foster City, CA, USA). The PCRs were one cycle at 50°C for 2 min and 95°C for 10 min. The amplification was followed by 40 cycles of denaturation at 95°C for 15 s and annealing at 60°C for 1 min. TaqMan gene expression assays and standard reagents (Applied Biosystems) were used for qRT-PCR of each sample for POMC (Assay ID: Rn00595020_m1), NPY (Assay ID: Rn00561681_m1), OBRb (Assay ID: Rn00561465_m1) and 18S rRNA

(endogenous control). Oligonucleotides and TaqMan probe were designed for AgRP using the ABI Prism Primer Express (v. 2.0) software (Applied Biosystems) based on published sequences obtained from the GenBank database (GenBank accession number AF206017; forward: 5'-TTCCCA-GAGTTCTCAGGTCTAAGTC-3'; reverse: 5'-GGATC-TAGCACCTCTGCCAAA-3' and TaqMan probe: 5'-FAM-AAGAAGACAGCAGCAGAC-3'). Standard curves were prepared for POMC, NPY, AgRP, OBRb and the endogenous control 18S rRNA. For each experimental sample, the relative concentrations of each gene and endogenous control were determined from the appropriate standard curve. The quantification of gene expression for each experimental sample was then obtained by normalising the relative concentration of each gene to the amount of endogenous control.

Statistical analyses

All data are expressed as means $\pm$ S.E.M. ($n=8$ per group). The data were transformed to satisfy the criteria of ANOVA as follows: POMC was square root transformed, whereas OBRb, leptin and insulin data were log transformed. Analyses were conducted using the SigmaStat v. 3.1 statistical package (Jandel Scientific, San Rafael, CA, USA). Differences between groups were determined by two-way factorial ANOVA (prenatal undernutrition and postnatal high-fat diet as factors), followed by Bonferroni *post hoc* analysis. For those differences where the *F*-value was statistically significant, Bonferroni protected *t*-tests were used to search, *post hoc* for the significant differences between means within the specific groups. Statistical significance was accepted at $P<0.05$.

Results

Prenatal undernutrition had no effect on hypothalamic POMC gene expression in chow-fed UN offspring when compared with AD offspring. However, UN offspring fed a high-fat diet postnatally had increased POMC expression when compared with AD high-fat-fed animals ($P<0.05$, $t=3.080$; Fig. 1). High-fat nutrition resulted in a significant increase in POMC expression ($P<0.05$, $t=3.733$) in UN offspring, but postnatal diet had no effect on AD offspring gene expression. There was a significant interaction between prenatal undernutrition and postnatal high-fat nutrition on increased POMC expression in UNHF offspring ($P<0.01$, $F=10.020$).

Similarly, there was no difference in NPY mRNA expression between UN and AD chow-fed offspring with prenatal undernutrition. In offspring exposed to prenatal undernutrition and fed the high-fat diet postnatally, NPY expression was increased when compared with ADHF offspring ($P<0.05$, $t=3.193$; Fig. 2). In UN but not AD

Figure 1 POMC mRNA expression relative to 18S rRNA (arbitrary units) in AD or UN animals fed either a chow or a high-fat (HF) diet postnatally. *Significant versus all other groups. AD/UN $\times$ diet interaction $P<0.01$. Data are means $\pm$ S.E.M., $n=8$ per group.

offspring, NPY expression was significantly increased with high-fat nutrition when compared with UN offspring on chow diet ($P<0.05$, $t=3.184$). As observed with POMC, there was also a significant interaction between prenatal undernutrition and postnatal high-fat diet on increased NPY expression in UNHF offspring ($P<0.05$, $F=5.332$).

AgRP gene expression was significantly reduced by exposure to prenatal undernutrition in chow-fed offspring ($P<0.05$, $t=2.058$; Fig. 3). In offspring exposed to prenatal undernutrition and fed the high-fat diet postnatally, AgRP expression was decreased when compared with ADHF offspring ($P<0.05$, $t=2.058$). High-fat nutrition had no significant effect on AgRP mRNA expression in either UN or AD offspring, although there was a trend toward lower AgRP mRNA levels in both ADHF and UNHF offspring when compared with chow-fed controls.

OBRb mRNA expression was not significantly different between AD and UN offspring fed either the standard chow or the high-fat diet. However, high-fat nutrition in UN offspring resulted in a significant increase in OBRb expression ($P<0.05$, $t=2.704$; Fig. 4), but was without effect on AD offspring expression. There was a significant

Figure 2 NPY mRNA expression relative to 18S rRNA (arbitrary units) in AD or UN animals fed either a chow or a high-fat (HF) diet postnatally. *Significant versus all other groups. AD/UN $\times$ postnatal diet interaction $P<0.05$. Data are means $\pm$ S.E.M., $n=8$ per group.

Figure 3 AgRP mRNA expression relative to 18S rRNA (arbitrary units) in AD or UN animals fed either a chow or a high-fat (HF) diet postnatally. * $P < 0.05$. Data are means $\pm$ S.E.M., $n = 8$ per group.

interaction between prenatal undernutrition and postnatal high-fat diet on increased OBRb expression in UNHF offspring ($P < 0.05$, $F = 5.419$).

Plasma leptin ($P < 0.01$, $t = 4.386$) and insulin concentrations ($P < 0.01$, $t = 4.894$) were significantly different by prenatal undernutrition in UN when compared with AD offspring fed postnatal high-fat nutrition, but not fed chow nutrition (Table 1). There was a significant interaction between prenatal undernutrition and high-fat diet on increased plasma leptin ($P < 0.01$, $F = 8.585$) and increased plasma insulin concentrations ($P < 0.01$, $F = 11.949$) in UNHF offspring. With high-fat nutrition, there was a significant increase in plasma leptin concentrations in both AD ($P < 0.05$, $t = 4.220$) and UN ($P < 0.05$, $t = 8.364$) groups. Furthermore, within UN ($P < 0.05$, $t = 5.904$) groups only, was there a significant increase in plasma insulin concentrations with high-fat nutrition.

Total fat mass (Table 1) was not significantly different between UNC and ADC offspring, but was significantly increased in UNHF when compared with ADHF offspring ($P < 0.05$, $t = 4.791$). In both AD ($P < 0.05$, $t = 3.347$) and

Figure 4 OBRb mRNA expression relative to 18S rRNA (arbitrary units) in AD or UN animals fed either a chow or a high-fat (HF) diet postnatally. * $P < 0.05$. AD/UN $\times$ postnatal diet interaction $P < 0.05$. Data are means $\pm$ S.E.M., $n = 8$ per group. NS, not significant.

UN ($P < 0.05$, $t = 8.510$) groups, high-fat nutrition resulted in a significant increase in fat mass. There was a significant interaction between prenatal undernutrition and high-fat diet on increased fat mass in UNHF offspring ($P < 0.01$, $F = 13.327$).

Caloric intake (Table 1) was increased by prenatal undernutrition in UN when compared with AD offspring fed either a standard chow diet ($P < 0.05$, $t = 2.944$) or a high-fat diet ($P < 0.05$, $t = 2.944$). High-fat nutrition had no effect on caloric intake in both AD and UN animals.

Discussion

The hypothalamic neuropeptide network plays a key role in the regulation of energy homeostasis. Furthermore, it has been suggested that early nutritional influences may impact on the normal development and maturation of this neuroendocrine network for the organism's immediate survival (Levin 2000). Yet, these early alterations may have no future adaptive value if a different postnatal environment is encountered (Gluckman & Hanson 2004), resulting in an increased susceptibility to developing obesity. It is known from previous rodent studies using models of gestational diabetic dams, gestational protein-deprived and perinatal protein-deprived dams that changes in the early (prenatal and neonatal) nutritional status of the pregnant mother can alter the offspring's neural system at fetal, weanling and adult age (Plagemann *et al.* 1999, 2000, Terroni *et al.* 2005). However, to date, no studies have been undertaken to investigate the consequences of gestational maternal undernutrition on the offspring's neural system.

Using a well-established rodent model of maternal undernutrition (Vickers *et al.* 2005), the present study investigated in adult female offspring the effects of and interactions between prenatal maternal undernutrition and postnatal high-fat diet on hypothalamic POMC, NPY, AgRP and OBRb gene expression and susceptibility to obesity development. We were unable to quantitate POMC, NPY, AgRP and OBRb protein expression by western blot analysis, since the immunoreactive signals were very weak and below the level of detection.

We have shown that based on prenatal influences alone, UNC offspring did not develop an obese phenotype, even though they demonstrate a small, but significant increase in food intake. Circulating leptin and insulin levels remained unchanged, and there were no significant differences in POMC, NPY and OBRb mRNA expression, but there was a significant downregulation in AgRP mRNA expression.

Furthermore, we have been able to demonstrate that based on prenatal influences within offspring fed postnatal high-fat nutrition (UNHF versus ADHF), UNHF offspring develop significant increases in both fat mass and food intake, as well as an increase in circulating plasma levels of both leptin and insulin. With respect to mRNA expression, this is accompanied by increases in both POMC and NPY, a decrease in

Table 1 Comparison of fasting plasma leptin and insulin levels, per cent total body fat dual energy X-ray absorptiometry (DEXA) and caloric intake (calories consumed per day per gram body weight). Data are means $\pm$ S.E.M., $n=8$ per group

	Leptin (ng/ml)	Insulin (ng/ml)	Total fat mass (%)	Caloric intake (kcal/g body weight)
AD chow	11.08 $\pm$ 1.46	0.28 $\pm$ 0.04	31.59 $\pm$ 2.53	0.180 $\pm$ 0.01
AD high fat	30.00 $\pm$ 4.93	0.37 $\pm$ 0.06	41.73 $\pm$ 1.94	0.192 $\pm$ 0.02
UN chow	11.38 $\pm$ 1.05	0.26 $\pm$ 0.02	30.46 $\pm$ 1.18	0.203 $\pm$ 0.01
UN high fat	83.50 $\pm$ 18.90	2.25 $\pm$ 0.92	56.24 $\pm$ 2.61	0.215 $\pm$ 0.03
Prenatal effect	NS	NS	NS	$P<0.05$
Postnatal diet effect	$P<0.05$	$P<0.05$	$P<0.05$	NS
Interaction	$P<0.01$	$P<0.01$	$P<0.05$	NS

NS, not significant.

AgRP, but no significant change in OBRb. Interestingly, with postnatal high-fat feeding ADHF offspring did not show any significant differences in POMC, NPY, AgRP and OBRb expression or caloric intake. However, they showed a significant increase in total fat mass and circulating plasma levels of both leptin and insulin. Postnatal high-fat nutrition induced a significant increase in POMC, NPY and OBRb expression, but no change in AgRP expression in UNHF offspring. Furthermore, these offspring developed a significant increase in circulating plasma levels of both leptin and insulin and a significant increase in total fat mass without any change in caloric intake.

According to the predictive adaptive response hypothesis, a developing organism adjusts its physiology to be appropriate for its predicted mature environmental range, such that a mismatch between the developmental and the adult environment may lead to an increased risk of disease development (Gluckman & Hanson 2004). Our data support this theory and suggest that changes in energy regulatory systems of the hypothalamus may be key pathways that modify susceptibility to obesity development.

Our present findings also suggest that exposure to prenatal undernutrition results in adult dysregulation of appetite homeostasis and reduced AgRP mRNA expression, possibly via central leptin resistance, which we have previously shown can occur in the absence of obesity and hyperleptinaemia and is associated with increased plasma C-peptide and triglyceride levels (Krechowiec *et al.* 2006). We have also demonstrated that, while exposure to prenatal undernutrition can increase food intake, the development of amplified obesity characterised by excessive fat mass accrual requires a critical interaction with postnatal high-fat nutrition.

In diet-induced obesity, AD and UN offspring fed postnatal high-fat nutrition have increased fat mass in association with hyperleptinaemia and hyperinsulinaemia, with no change in food intake. This suggests that exposure to high-fat nutrition determines susceptibility to obesity development, via mechanisms independent of increased food intake. Decreased transport of leptin into the brain has been suggested as one mechanism causing central leptin resistance in diet-induced obesity (Van Heek *et al.* 1997, Banks *et al.* 1999). However,

central leptin gene therapy fails to overcome leptin resistance in diet-induced obesity (Wilsey *et al.* 2003), suggesting downstream signalling defects as possible primary causes of leptin resistance. In the present study, we were unable to detect changes in POMC, NPY, AgRP and OBRb mRNA expression in ADHF offspring, as reported previously (Harrold *et al.* 1999, Wang *et al.* 2002). The mechanisms contributing to diet-induced obesity in this study may therefore be primarily metabolic in nature, involving stimulation of fat synthesis, a reduction in activity level (Vickers *et al.* 2003), fat oxidation, energy expenditure and thermogenesis. Moreover, the possible interplay of other hypothalamic neuropeptides, which function independently of leptin, such as melanin-concentrating hormone, and also galanin and the orexins, which are highly responsive to dietary fat (Leibowitz & Wortley 2004) may have contributed a role in mediating the metabolic mechanisms of diet-induced obesity in AD and UN offspring.

The concurrent hyperleptinaemic and hyperinsulinaemic levels with increased fat accrual in diet-induced obese AD and UN offspring may represent the failure of leptin's metabolic action in normally inhibiting insulin secretion in pancreatic β -cells (Kieffer *et al.* 1997, Seufert *et al.* 1999). This may have subsequently produced the anabolic effect of *de novo* lipogenesis (Kieffer & Habener 2000) and further promotion of adipogenesis in diet-induced obese AD and UN offspring. This positive feedback loop of leptin resistance and compensatory hyperinsulinism results in dysregulation of the adipoinular axis (Vickers *et al.* 2001) and suggests leptin resistance at the level of the pancreas (Breier *et al.* 2001) in diet-induced obese AD and UN offspring.

The failure of hyperleptinaemia to regulate NPY mRNA levels in UNHF offspring in the present study suggests that NPY neurons are less sensitive to leptin's suppressive effects (NPY neuronal resistance). Alternatively, the increase in NPY expression may be the result of inhibited leptin signalling, at the level of reduced signal transducer and activator of transcription-3 (STAT-3) activation, and involving increased expression of suppressor of cytokine signalling-3 (SOCS-3), an inhibitor of leptin signalling (Munzberg *et al.* 2004). Our observation of increased NPY expression, as previously

reported (Gao *et al.* 2002, Huang *et al.* 2004) may function to attenuate catabolic signalling.

Despite increased hyperleptinaemic levels enhancing POMC expression in UNHF offspring, excessive fat mass accrual was not restricted in UNHF offspring resulting in amplification of obesity. Increased POMC mRNA levels have been reported in other diet-induced obesity studies (Zioto-poulou *et al.* 2000, Torri *et al.* 2002). However, it is difficult to interpret changes in POMC mRNA, since α -MSH and β -endorphin have opposing catabolic and anabolic effects on energy homeostasis respectively (Kalra *et al.* 1999). However, it is possible that increased β -endorphin production either as a result of increased POMC expression or by the stimulatory effect of increased NPY expression in UNHF offspring (Horvath *et al.* 1992, Kalra *et al.* 1995) led to a potentiation in its hypothalamic transmission. The resulting outcome is attenuation in catabolic signalling by decreasing sympathetic activity and produced the anabolic effect of increased fat deposition (Leibowitz & Wortley 2004).

The increased expression of OBRb in obese UNHF offspring is not surprising, since POMC and NPY expression are increased and OBRb is co-localised in both these neuronal populations (Elias *et al.* 1999, Lin *et al.* 2000). However, in spite of increased OBRb expression in transducing the satiety effects of hyperleptinaemic signalling by normally decreasing orexigenic peptides, NPY expression was increased in UNHF offspring. As mentioned previously, this could possibly be mediated at the level of reduced STAT-3 activation, and involve increased expression of SOCS-3, an inhibitor of leptin signalling (Munzberg *et al.* 2004), resulting in an attenuation of catabolic signalling. UNHF offspring also showed increased POMC expression suggesting the possibility of an increase in responsiveness of POMC neurons to hyperleptinaemic signalling mediated by increased OBRb. However, the differential effects of increased production of α -MSH and β -endorphin by increased POMC may also contribute to the attenuation of catabolic signalling.

In conclusion, our findings suggest that mismatched early developmental and mature environments determine a higher susceptibility to obesity development through alterations in POMC, NPY, AgRP and OBRb gene expression with hyperleptinaemia and hyperinsulinaemia, which may be associated with both central and peripheral leptin resistance. In addition, our study provides genetic evidence in keeping with the predictive adaptive response theory. Further studies are required to delineate the underlying cellular mechanisms of the prenatal influences on susceptibility to diet-induced obesity within specific regions of the brain.

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