

Sex-dependent regulation of hypothalamic neuropeptide Y-Y₁ receptor gene expression in moderate/high fat, high-energy diet-fed mice

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In this study we investigated whether long-term consumption of a moderate/high fat (MHF), high-energy diet can affect the gene expression of the Y₁ receptor (Y₁R) for neuropeptide Y (NPY) in the dorsomedial (DMH), ventromedial (VMH), arcuate (ARC) and paraventricular (PVN) hypothalamic nuclei of male and female *Y₁R/LacZ* transgenic mice, carrying the murine Y₁R promoter linked to the *LacZ* gene. MHF diet-fed male mice showed an increased consumption of metabolizable energy that was associated with a significant increase in body weight as compared with chow-fed controls. In parallel, consumption of a MHF diet for 8 weeks significantly decreased *Y₁R/LacZ* transgene expression in the DMH and VMH of male mice whereas no changes were found in the ARC and PVN. Leptin treatment reduced body weight of both MHF diet- and chow-fed male mice but failed to prevent the decrease in *Y₁R/LacZ* transgene expression apparent in the DMH and VMH of male mice after 8 weeks of MHF diet intake. Conversely, no significant changes of metabolizable energy intake, body weight or hypothalamic β -galactosidase expression were found in MHF diet-fed female *Y₁R/LacZ* transgenic mice. A gender-related difference of *Y₁R/LacZ* transgenic mice was also observed in response to leptin treatment that failed to decrease body weight of both MHF diet- and chow-fed female mice. Results herein demonstrate that *Y₁R/LacZ* FVB mice show a sexual dimorphism both on energy intake and on nucleus-specific regulation of the NPY Y₁R system in the hypothalamus. Overall, these results provide new insights into the mechanism by which diet composition affects the hypothalamic circuit that controls energy homeostasis.

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The hypothalamus plays a critical role in integrating neural and hormonal signals that influence whole body energy balance, which, when altered, could result in obesity (Schwartz *et al.* 2000). Among those factors that play a major role in regulating body weight, adiposity and energy metabolism are leptin and neuropeptide Y (NPY) (Sahu, 2003; Kalra & Kalra, 2004). Leptin, a hormone produced by adipocytes, acts as a feedback signal to the hypothalamus to reduce food intake and stimulate thermogenesis (Campfield *et al.* 1995; Halaas *et al.* 1995). An increase in body fat increases leptin levels that, in turn, reduces food intake, while a decrease in body fat leads to decreased levels of the circulating hormone and to a stimulation of food intake (Elmquist *et al.* 1998). Mutations that result in leptin deficiency, or in leptin resistance, are associated with massive obesity (Elmquist *et al.* 1998).

NPY is found in high concentration in the hypothalamus of both rodents and humans and it exerts

opposite effects of those of leptin (Chronwall *et al.* 1985; Hokfelt *et al.* 1998). NPY has a potent ability to stimulate feeding, reduce energy expenditure and induce obesity and it opposes the anorectic effect of leptin (Stanley *et al.* 1986; Kalra *et al.* 1991). Hypothalamic NPY-containing neurons express leptin receptors and leptin was shown to negatively regulate arcuate (ARC) NPY gene expression (Stephens *et al.* 1995). Increased hypothalamic NPY activity has been suggested in some forms of genetic obesity linked to defective leptin signalling, including the *ob/ob*, *db/db* and *fa/fa* Zucker rats (Wilding *et al.* 1993; Schwartz *et al.* 1996; Kim *et al.* 2000) and genetic depletion of NPY attenuates obesity in *ob/ob* mice (Erickson *et al.* 1996).

NPY Y₁, Y₂ and Y₅ receptors are expressed in the hypothalamic sites involved in the regulation of ingestive behaviour and energy balance (Parker & Herzog, 1999). Studies in genetically modified mice and pharmacological studies revealed that the Y₁ receptor (Y₁R) plays a crucial role in the control of energy homeostasis (Herzog, 2003;

Pedrazzini, 2004; Eva *et al.* 2006). The potent anorectic effect of selective Y_1R antagonists suggests that Y_1R is physiologically involved in appetite regulation (Kanatani *et al.* 1996; Wieland *et al.* 1998). Mutant mice lacking the Y_1R feed and grow normally but exhibit a significantly reduced feeding response to NPY or to the NPY receptor agonist PYY3-36 (Kanatani *et al.* 2000).

During negative energy balance, for example during food restriction, exercise, thyrotoxicosis, lactation or anorexia, there is an increase in the synthesis of NPY in the ARC and of the peptide concentration in NPYergic terminals (Dryden *et al.* 1994; Ishii *et al.* 2003; Bi *et al.* 2005; Chance *et al.* 2007) that are associated with the down-regulation of Y_1R in the PVN (for review see Eva *et al.* 2006), suggesting that the level of NPY signalling is strongly influenced by the nutritional status. A number of investigators have examined the effect of diet-induced obesity on the hypothalamic NPY system, but no single conclusion can be made from these studies. In diet-induced obesity models, when there is positive energy balance, ARC NPY mRNA and protein content increase (Levin & Dunn-Meynell, 1997; Huang *et al.* 2003), decrease (Wilding *et al.* 1992; Bergen *et al.* 1999; Hansen *et al.* 2004) or remain unchanged (Wilding *et al.* 1992), and alteration of hypothalamic NPY gene expression was shown to be dependent on the genetic background of the different strains of rodents and on the type of treatment. Similarly, conflicting results have been reported on the expression levels of the Y_1R subtype which was found to be either decreased (Beck *et al.* 2001), increased (Schaffhauser *et al.* 2002) or unchanged (Widdowson, 1997; Huang *et al.* 2003) in the hypothalamus of genetically obese and obesity prone rodents. Moreover, it is unclear whether diet composition may affect the expression of hypothalamic Y_1R receptors (Schaffhauser *et al.* 2002; Huang *et al.* 2003). These discrepancies possibly reflect the limitation of analyses done on whole hypothalamus and further studies are needed to show region-specific changes of Y_1R expression in different hypothalamic nuclei.

Therefore, the primary aim of this study was to examine the effect of long-term exposure to a high-energy, moderate/high fat (MHF) diet on the Y_1R gene expression in four hypothalamic nuclei (ARC, PVN, DMH and VMH) that are involved in the regulation of energy balance (Schwartz *et al.* 2000). To this purpose we used, as a model, transgenic mice ($Y_1R/LacZ$) carrying the murine Y_1R promoter linked to the *LacZ* gene, that were created on a FVB/n background (Oberto *et al.* 1998). We previously demonstrated that changes in energy balance during pregnancy, fasting, leptin treatment or glucose administration can modulate $Y_1R/LacZ$ transgene expression in a tissue-specific manner, suggesting that changes in transgene expression may reflect changes of Y_1R steady state and therefore alteration of NPY Y_1R signal transduction (Zammaretti *et al.* 2001; Oberto *et al.* 2003;

Eva *et al.* 2006). In addition, since gender differences in leptin responsiveness have been reported for FVB mice (Harris *et al.* 2002), both female and male mice were included in the study.

Methods

Experimental animals

$Y_1R/LacZ$ mice from transgenic line 62 of our breeding colony (Oberto *et al.* 1998) were employed in this study. $Y_1R/LacZ$ transgenic mice were created and maintained on a FVB inbred background and were genetically identical. Mice were housed conventionally with a 12 h light–dark cycle and with constant temperature ($21 \pm 2^\circ\text{C}$). Animal care and handling throughout the experimental procedure were in strict accordance with the European Community Council Directive, 24th November 1986 (86/609/EEC) and the protocol was approved by the Animal Investigation Committee of the Ministero dell'Università e della Ricerca Scientifica e Tecnologica.

Treatments

Three-week-old male (initial body weight 11–14 g) or female (initial body weight 7–12 g) $Y_1R/LacZ$ transgenic mice were studied. The animals were randomly assigned to four groups: two groups (of chow-fed males, chow-fed females) had free access to standard mouse chow (Morini, S. Polo D'Enza, Re, Italy), which contains 2.63 kCal g^{-1} with 60% of the metabolizable energy content as carbohydrates, 7% as fibre, 26% as protein and 8% as fat. The remaining two groups of animals (MHF diet-fed males and MHF diet-fed females) were fed with a highly palatable MHF diet (slightly modified diet reported by Lauterio *et al.* 1994; Harlan Italy, S. Pietro al Natisone, Udine, Italy). This diet contains 3.56 kCal g^{-1} with 45% of the metabolizable energy content as carbohydrates, 7% as fibre, 18% as protein and 31% as fat. This dietary model was chosen for two main reasons. First, it is relatively low in fat compared with other models and thus is more in line with the average diet in developed countries consisting of 30–45% of energy from fat. Second, given that the MHF diet enables identification of susceptible and obesity-resistant populations (Lauterio *et al.* 1994; Lauterio *et al.* 1999), this dietary model can be useful to study gender-dependent differences in the hypothalamic circuit that controls feeding behaviour and obesity.

After an 8 week period, each group of mice (chow-fed males, chow-fed females, MHF diet-fed males and MHF diet-fed females) was further divided into two subgroups and treated for 3 days with a single daily intraperitoneal injection of $1 \mu\text{g g}^{-1}$ murine recombinant leptin (LEP; Sigma-Aldrich, Milano, Italy) or saline. All mice were killed by cervical dislocation immediately after the treatment at the end of the day 3. Brains were quickly removed, placed

in 10% embedding medium (Bio-optica, Milano, Italy) in PBS, frozen on crushed dry ice, and stored at -80°C until assayed.

Body weight and food consumption determination

Body weight was measured at 16.00 h on the 7th day of each week throughout experiments. After a 8 week period, the groups of chow-fed males, chow-fed females, MHF diet-fed males and MHF diet-fed females treated with leptin were further weighed daily at 16.00 h immediately after the hormone administration.

Food intake was monitored in three independent experiments. All animals from each group of mice (chow-fed males, chow-fed females, MHF diet-fed males and MHF diet-fed females) were housed in standard cages (each containing 5–6 animals of the same group). Mean food consumption (mean grams per mouse) was determined at 1 week intervals throughout experiments by measuring on the 7th day of each week, at 16.00 h, the amount of food consumed per cage and dividing it by the number of mice housed in the cage. The amount of food consumed per cage during these time intervals was estimated by subtracting the residual food recovered from each cage from the total amount presented. After the measurement the mice were given fresh quantities of their diets. Caloric intake was obtained by multiplying the mean food intake per mouse by diet metabolizable energy and results were expressed as mean kilocalories per mouse.

β -Galactosidase staining

$Y_1R/LacZ$ expression was determined by β -galactosidase staining of mice brain coronal sections, as previously described (Zammaretti *et al.* 2001; Oberto *et al.* 2003). Briefly, frozen brains were cut on a cryostat at -20°C and $25\ \mu\text{m}$ sections were collected on clean slides starting from a level corresponding to the end of the anterior commissure. Sections were dehydrated with acetone–chloroform (1:1), air dried and shortly fixed in 2.5% glutaraldehyde in PBS (each step for 5 min on ice), and incubated overnight at 37°C in a solution containing $1\ \text{mg ml}^{-1}$ of 5-bromo-4-chloro-3-indolyl-beta-D-galactopyranoside (X-gal; Inalco, Milano, Italy), 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide, 2 mM MgCl_2 and 0.01% Triton X-100 in PBS. After washing in water, sections were counterstained with nuclear fast red, dried and coverslipped with DPX mounting medium (Fluka, Buchs, Switzerland).

Quantification of transgene expression as determined by β -galactosidase histochemistry

The expression of the transgene appears as blue dots. Quantification of the $Y_1R/LacZ$ transgene expression was

made by computer-assisted morphometrical analysis as previously described (Zammaretti *et al.* 2001; Oberto *et al.* 2003). Sections were counterstained with neutral fast red and hypothalamic nuclei were identified on the basis of the mouse brain atlas of Franklin and Paxinos (Franklin & Paxinos, 1997). For each mouse, two standardized sections of comparable levels of the PVN (around bregma -0.70 to $-0.82\ \text{mm}$), rostral ARC (around bregma -1.34), rostral DMH (around bregma -1.46), rostral VMH (around bregma -1.22) were chosen. Selected sections were placed on a Leica Diaplan microscope, observed by means of a $\times 10$ objective, and the corresponding image was transferred, via a black and white CCD camera (PCO, VC44, Keilheim, Germany) to a digitizing board (Scion LG-3, Scion Co., Frederick, MD, USA) placed in a PowerPC 8200 Macintosh computer. Acquisition and analysis of the images were performed using the software NIH-Image (version 1.62, a freeware by W. Rasband, NIH, Bethesda, MA, USA). Sections were observed and digitized first by using a built-in green filter to better identify the nuclei extension. A line, drawn following the boundaries of the selected nuclei, defined the area of interest (AOI, Figs 3 and 4). The same section was then digitized using a built-in red filter obtaining a strong enhancement of the histochemical signal, but losing the definition of the nuclear boundaries. The AOI selected on the first image was finally superimposed on the second image to delimit the region in which dots should be counted. Using a manual thresholding method, dots were selected and the corresponding image was binarized. For each animal and nucleus the cumulative number of dots and the cumulative areas of the analysed sections were considered to obtain the density expression of the transgene expressed as dots per μm^2 .

Data analysis

A repeated measures analysis of variance (ANOVA) was used to compare mean body weight over time and the appropriate contrasts were analysed by unpaired *t* test. Food consumption, energy intake and transgene expression were examined using one-way ANOVA and Newman–Keuls test for multiple comparisons. All data are expressed as means \pm s.e.m. and the level of statistical significance was set at $P < 0.05$ for all comparisons.

Results

Effects of the MHF diet on $Y_1R/LacZ$ mice body weight

A different response of male and female mice to ingestion of MHF diet for 8 weeks was observed. In males, MHF diet-fed mice consumed the same amount of food, as compared with chow-fed mice, during the 8 weeks of treatment (Fig. 1A), resulting in a significant increase

in their cumulative energy intake (Fig. 1B). Analysis of variance for repeated measurements detected a significant effect of the diet on body weight of MHF diet-fed mice as compared with chow-fed ones (Fig. 2A). However, despite their tendency to gain weight more rapidly than controls, *Y₁R/LacZ* mice only poorly responded to the MHF diet, being 6–8% heavier of chow-fed controls at the end of the experiment.

Conversely, in females, MHF diet-fed mice had a significantly lower food intake than chow-fed mice (Fig. 1A), and no significant effects of diet were evident when the cumulative intake of metabolizable energy was calculated (Fig. 1B). Moreover the ingestion of the MHF diet did not significantly increase body weight of females during the 8 weeks of treatment (Fig. 2B).

The subcutaneous injection of $1 \mu\text{g g}^{-1}$ of leptin for 3 days decreased the body weight of both chow-fed ($P = 0.009$) and MHF diet-fed ($P = 0.053$) male mice. Conversely, the same treatment failed to affect the body weight of chow- or MHF diet-fed female mice (Table 1). The treatment with saline for 3 or 7 days failed to affect body weight of chow-fed male and female mice (data not shown; Zammaretti *et al.* 2001).

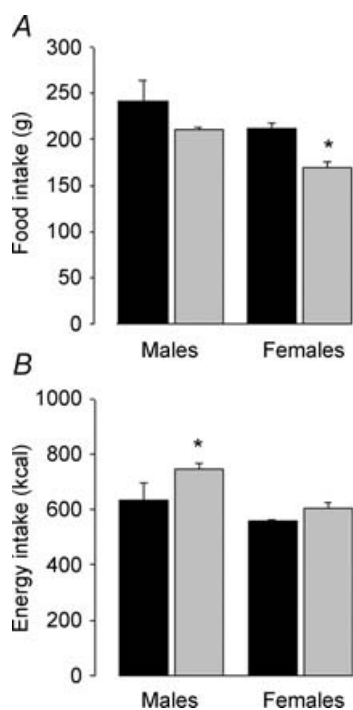


Figure 1. Cumulative food intake (A) and caloric intake (B) of *Y₁R/LacZ* transgenic male or female mice fed either standard chow (black bars) or MHF diet (grey bars) for 8 weeks

Data are the sum of mean grams per mouse (A) and mean kilocalories per mouse (B) that were determined on the 7th day of each week and are means \pm s.e.m. from three experiments ($N = 5-6$). One-way ANOVA; A, $F_{3,8} = 6.062$, $P = 0.019$. * $P < 0.05$ versus chow-fed females and MHF diet-fed males, by Newman-Keuls test; B, $F_{3,8} = 6.043$ $P = 0.019$. * $P < 0.05$ versus chow-fed males and MHF diet-fed females, by Newman-Keuls test.

Effects of the MHF diet on *Y₁R/LacZ* transgene expression in the VMH and DMH

Y₁R/LacZ transgene expression was determined by histochemical β -galactosidase staining of brain coronal sections using the chromogenic substrate X-gal. Ingestion of MHF diet for 8 weeks decreased β -galactosidase staining in VMH (Fig. 3A and C) and DMH (Fig. 4A and C) of male mice as compared with chow-fed mice. Leptin treatment ($1 \mu\text{g g}^{-1}$ for 3 days) failed to abrogate this effect (Figs 3D and 4D). Quantitative analysis (summarized in Fig. 5) demonstrated that 8 week ingestion of the MHF diet decreased β -galactosidase expression in VMH and DMH of male mice by 25% and 22%, respectively, as compared with chow-fed mice. Conversely, no significant changes of β -galactosidase staining were observed in VMH and DMH of MHF diet-fed female mice (Fig. 5B). Leptin treatment failed to modify *Y₁R/LacZ* expression in the VMH and DMH of chow-fed or MHF diet-fed male and female mice (Fig. 5).

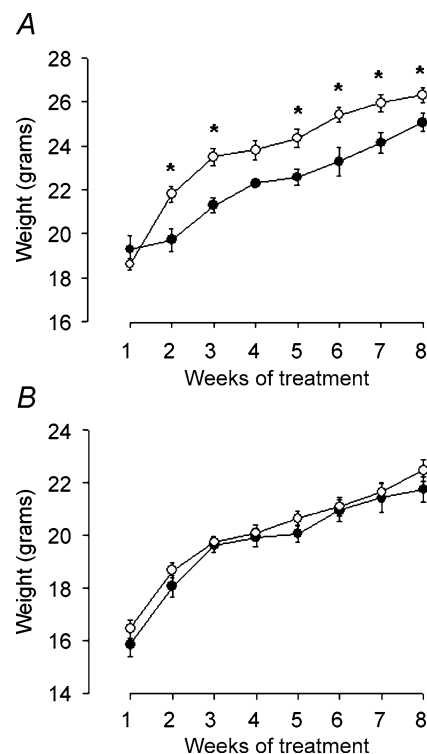


Figure 2. Weight changes of *Y₁R/LacZ* transgenic male (A) or female (B) mice fed a MHF diet (○) compared with controls fed standard chow (●)

Body weight was determined on the 7th day of each week and data are means \pm s.e.m. of values from 7 (chow-fed males), 20 (MHF diet-fed males), 14 (chow-fed females) or 28 (MHF diet-fed females) mice. One-way ANOVA for repeated measurements: A, $F_{1,25} = 6.725$ $P = 0.0157$. * $P < 0.05$ versus chow-fed mice, by unpaired *t* test; B, $F_{1,40} = 0.854$, $P = 0.361$.

Table 1. Effect of the treatment with $1 \mu\text{g g}^{-1}$ of murine recombinant leptin for 3 days on the body weight of $Y_1R/LacZ$ transgenic male or female mice fed with standard chow or with a MHF diet for 8 weeks

Days of treatment	Males weight (g)		Females weight (g)	
	Chow-fed mice	MHF-fed mice	Chow-fed mice	MHF-fed mice
d0	22.69 ± 0.35	26.77 ± 0.44	21.74 ± 0.62	23.18 ± 0.91
d3	$21.82 \pm 0.45^*$	25.09 ± 0.74^a	21.22 ± 0.56	22.68 ± 0.96

Data are the mean \pm s.e.m. from 6 (chow-fed males), 8 (chow-fed females), 9 (MHF fed males) and 12 (MHF fed females) determinations. One-way ANOVA for repeated measurements: males, overall effect: $F_{1,13} = 30.06$, $P = 0.001$; effect of leptin treatment, $F_{1,1} = 7.42$, $P = 0.017$. $^*P < 0.01$ versus chow-fed mice at d0; $^aP = 0.053$ versus MHF-fed mice at d0; females, overall effect: $F_{1,18} = 1.384$, $P = 0.25$.

Lack of effects of ingestion of the MHF diet on $Y_1R/LacZ$ transgene expression in PVN and ARC

Quantitative analysis of $Y_1R/LacZ$ transgene expression in PVN and ARC demonstrated that exposure of

$Y_1R/LacZ$ male mice to the MHF diet for 8 weeks did not induce significant changes on β -galactosidase expression (Fig. 6A). Leptin treatment also failed to affect β -galactosidase expression in the PVN and ARC of chow-fed and MHF diet-fed male mice (Fig. 6A). Similarly,

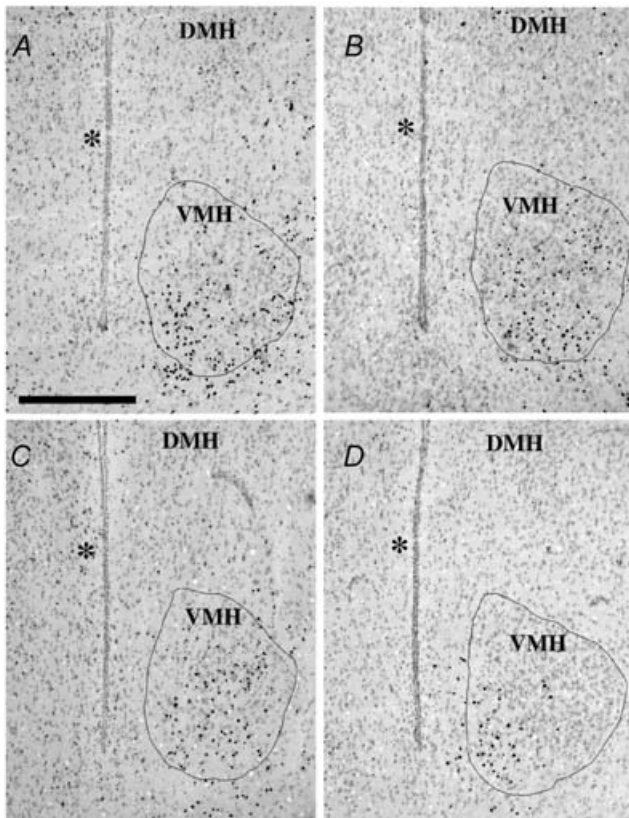


Figure 3. Coronal sections illustrating different expression of the $Y_1R/LacZ$ transgene in the rostral level of the ventromedial (VMH) nucleus of male mice from different experimental groups A, standard chow-fed male mouse; B, standard chow-fed male mouse treated for 3 days with $1 \mu\text{g g}^{-1}$ of murine recombinant leptin; C, MHF diet-fed male mouse; D, MHF diet-fed male mouse treated for 3 days with $1 \mu\text{g g}^{-1}$ of murine recombinant leptin. Pictures were digitized using a red filter to enhance the histochemical staining. The closed lines in each picture represent the area of interest (AOI) for counting the number of positive dots. The AOI was drawn following the boundaries of the ventromedial nucleus (VMH) on the green filter-digitized image. DMH, dorsomedial nucleus. * indicates third ventricle; scale bar, $400 \mu\text{m}$.

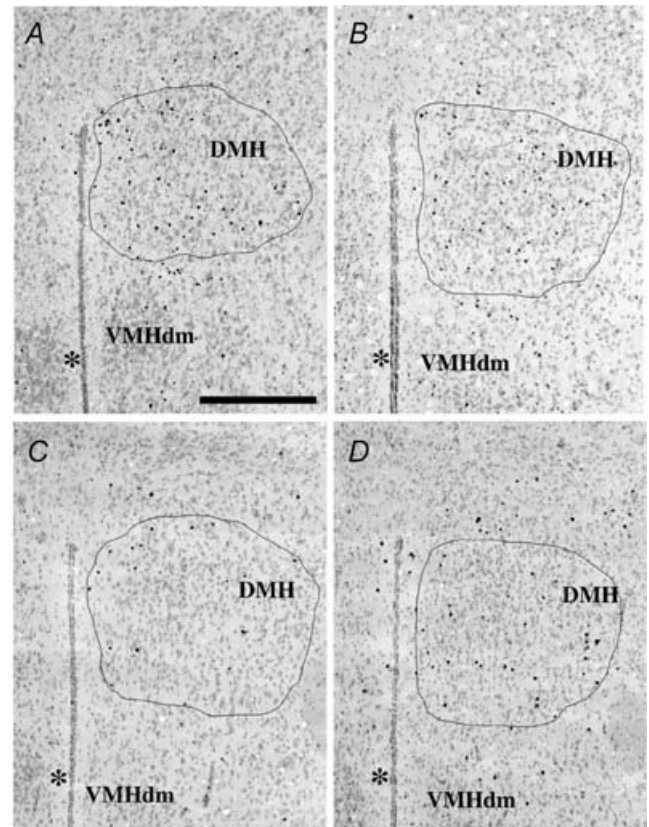


Figure 4. Coronal sections illustrating different expression of the $Y_1R/LacZ$ transgene in the rostral level of the dorsomedial (DMH) nucleus of male mice from different experimental groups A, standard chow-fed male mouse; B, standard chow-fed male mouse treated for 3 days with $1 \mu\text{g g}^{-1}$ of murine recombinant leptin; C, MHF diet-fed male mouse; D, MHF diet-fed male mouse treated for 3 days with $1 \mu\text{g g}^{-1}$ of murine recombinant leptin. Pictures were digitized as described in Fig. 3. The AOI was drawn following the boundaries of the dorsomedial nucleus (DMH) on the green filter-digitized image. VMHdm, ventromedial hypothalamic nucleus, pars dorsomedialis. * indicates third ventricle; scale bar, $400 \mu\text{m}$.

neither the exposure to MHF diet nor the leptin treatment affected transgene expression in the PVN and ARC of female $Y_1R/LacZ$ mice (Fig. 6B).

Discussion

In the present study we examined the effect of a moderate to high fat, high-energy diet on the Y_1R gene expression in the hypothalamus of $Y_1R/LacZ$ transgenic mice, which were created and maintained on a FVB/n inbred mouse strain. Although the susceptibility of the FVB mouse strain to obesity is not well studied, it was previously reported that FVB mice fed with a high fat diet become obese and show an increase in circulating leptin that is correlated with body weight and adiposity, indicative of leptin resistance (Frederich *et al.* 1995; Martin *et al.* 2006).

Data presented here show that, in $Y_1R/LacZ$ transgenic male mice, consumption of a MHF diet for 8 weeks promotes a significant increase of body weight that is associated with a decrease of Y_1R gene expression in the DMH and VMH. Conversely, Y_1R gene expression in the ARC and PVN was not altered in this obese model, despite the well documented role of ARC NPY neurons in

feeding regulation. In addition, the decreased expression of $Y_1R/LacZ$ transgene was only observed in MHF diet-fed male mice but not in MHF diet-fed females, which failed to gain weight over the course of the experiment, suggesting that the susceptibility to develop obesity of male mice correlates with changes in the Y_1R gene expression in the VMH and DMH.

NPY neurons of ARC, DMH and VMH belong to complex pathways involved in the control of feeding behaviour and they can be differentially regulated in response to hyperphagia and excessive body weight gain (Beck, 2006). In animal models of obesity with a deficiency in leptin signalling, ARC NPY system is up-regulated (for review see Beck, 2006; Eva *et al.* 2006). Conversely, in other rodent models of genetic obesity, for instance those where the hypothalamic melanocortin signalling is reduced, NPY expression was found to be decreased in ARC and increased in DMH and VMH (Kesterson *et al.* 1997; Guan & Van der Ploeg, 1998; Bi *et al.* 2001). Additionally, diet-induced obese mice showed a profound increase in the NPY mRNA expression both in the DMH and the VMH, and a decrease in the ARC (Guan *et al.* 1998a). It has been postulated that the activation of NPY signalling in the DMH and VMH

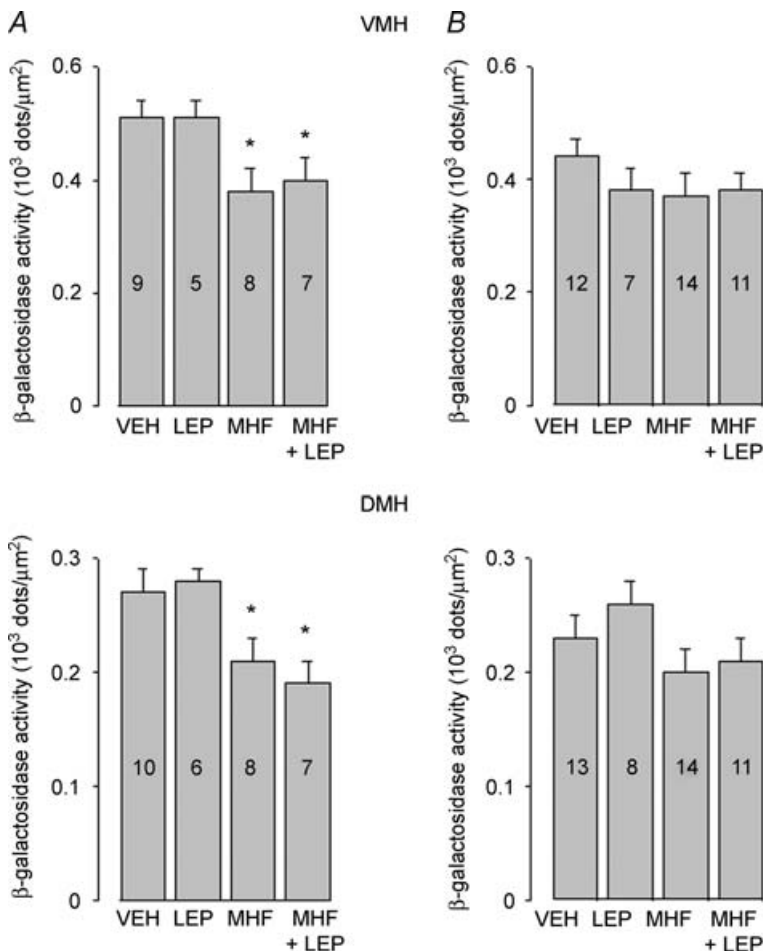


Figure 5. Effect of exposure to a MHF diet for 8 weeks on β -galactosidase in the ventromedial (VMH) (upper panel) and dorsomedial (DMH) (lower panel) of 11-week-old male (A) and female (B) $Y_1R/LacZ$ transgenic mice

Mice were fed with standard chow and treated for 3 days with saline (VEH); fed with standard chow and treated for 3 days with $1 \mu\text{g g}^{-1}$ of murine recombinant leptin (LEP); fed with a MHF diet for 8 weeks, then treated for 3 days with saline (MHF); fed with a MHF diet for 8 weeks, then treated for 3 days with $1 \mu\text{g g}^{-1}$ of murine recombinant leptin (MHF + LEP). Data are expressed as density of blue dots and are means \pm S.E.M.; numbers within each column indicate the number of animals used for the determination. VMH, one-way ANOVA: A, $F_{1,3} = 3.6764$, $P = 0.0254$. * $P < 0.05$ versus VEH by Newman-Keuls test; B, $F_{1,3} = 1.1757$, $P = 0.331$. DMH, one-way ANOVA: A, $F_{1,3} = 6.1503$, $P = 0.025$. * $P < 0.05$ versus VEH and LEP by Newman-Keuls test; B, $F_{1,3} = 1.5731$, $P = 0.21$.

can be a compensatory mechanism to counteract ARC NPY neuron deficiency, in order to maintain adequate food intake (Beck, 2006).

Indeed, increased neuronal activity in DMH was found to contribute to the development of obesity caused by ingestion of a high fat diet (Bellinger & Bernardis, 2002). Mice susceptible to diet-induced obesity have increased *c-fos* expression in DMH when fed a high fat diet (Xin *et al.* 2000), whereas lesions of rat DMH attenuate high fat diet-induced weight gain (Bellinger & Bernardis, 2002).

In our dietary obesity model, MHF-fed $Y_1R/LacZ$ transgenic male mice consume the same amount of food as compared with chow-fed mice in spite of the higher energy content of MHF diet. Thus, we speculate that MHF diet consumption may activate the anabolic NPYergic neurons both in the VMH and DMH, leading to the down-regulation of the Y_1R .

On the other hand, the fact that we did not observe significant changes of the $Y_1R/LacZ$ transgene expression in the PVN is not surprising, given that in several rodent models of genetic and dietary obesity the expression of the NPY Y_5R subtype was consistently shown to be altered in

the PVN (Widdowson, 1997; Xin & Huang, 1998; Beck *et al.* 2001; Schaffhauser *et al.* 2002). The decrease of $Y_1R/LacZ$ expression in DMH and VMH may therefore only partially reflect alterations of NPY receptors in the hypothalamus of MHF-fed male mice. Measurement of NPY release in VMH, DMH and PVN could provide information on the causal relationship between changes in NPY Y_1R transmission and weight gain in MHF-fed male mice.

Furthermore, since the decrease of Y_1R gene expression was only observed in MHF diet-fed $Y_1R/LacZ$ transgenic mice, that develop obesity over the course of the treatment, the possibility that the down-regulation of Y_1R is secondary to the weight gain cannot be excluded. Recent studies have shown that the expression of hypothalamic Y_1R can be regulated in a nucleus-specific manner, independently of the activity of the NPY system (Chance *et al.* 2007). Thus, the changes in DMH and VMH Y_1R gene transcriptional activity could be an integral part of NPY dysfunction in our dietary obesity model.

Further studies will be needed to identify the role of leptin in the susceptibility of male mice when eating a MHF diet. Previous studies have shown that

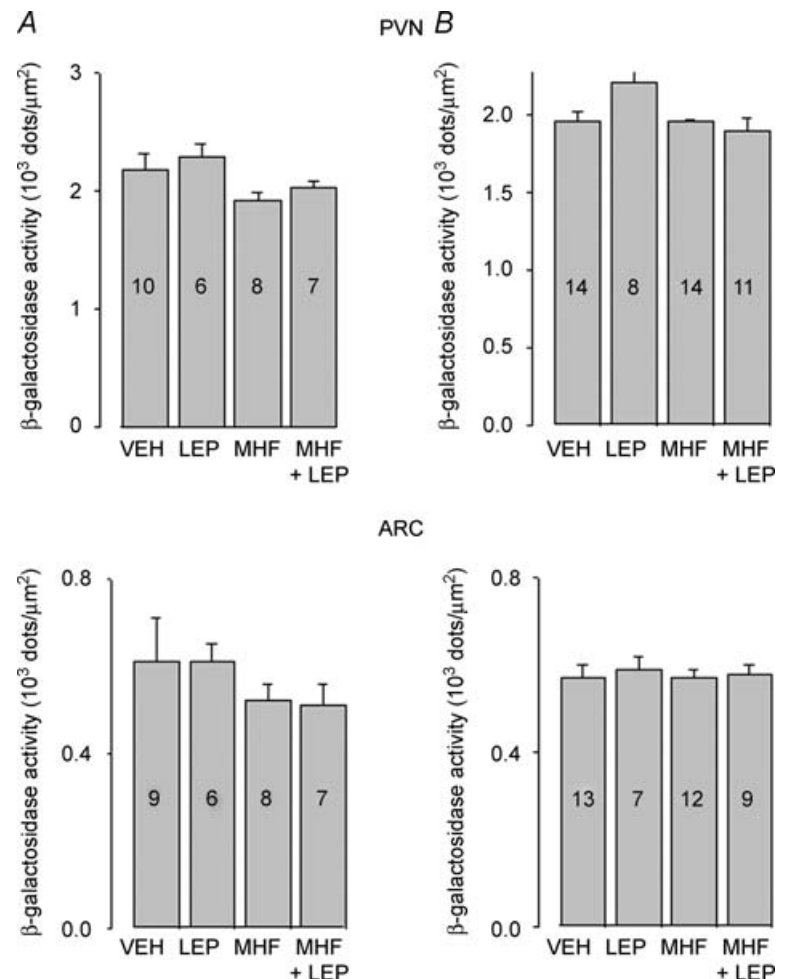


Figure 6. Effect of exposure to a MHF diet for 8 weeks on β -galactosidase in the paraventricular (PVN) (upper panel) and arcuate (ARC) (lower panel) of male (A) or female (B) $Y_1R/LacZ$ transgenic mice

Mice were treated as in Fig. 5. Data are expressed as density of blue dots and are means \pm S.E.M.; numbers within each column indicate the number of animals used for the determination. PVN, one-way ANOVA: A, $F_{1,3} = 2.37$, $P = 0.0927$; B, $F_{1,3} = 2.207$, $P = 0.101$. ARC, one-way ANOVA: A, $F_{1,3} = 0.675$, $P = 0.575$; B, $F_{1,3} = 0.9114$, $P = 0.1768$.

obesity-prone mice exposed to a high fat diet develop leptin resistance progressively, and that leptin insensitivity becomes apparent after 8 weeks of treatment (Lin *et al.* 2000; Martin *et al.* 2006). Accordingly, we showed that leptin treatment decreases body weight of both chow-fed mice and mice fed with a MHF diet for 8 weeks, although, in the latter case, it failed to reach a fully significant effect. Conversely, we were unable to relate the leptin-induced decrease in body weight with Y_1R gene expression since leptin treatment failed to prevent the decrease in $Y_1R/LacZ$ transgene expression in VMH and DMH of MHF diet-fed male mice. This finding could reflect a level of leptin resistance in MHF diet-fed male mice that, in turn, could compromise leptin-dependent regulation of VMH and DMH NPY Y_1R system.

However, given that in MHF-fed mice, leptin levels presumably increase to reflect the energy status of the animals, we cannot exclude the possibility that higher doses of leptin could counteract the reduction of $Y_1R/LacZ$ transgene expression observed in DMH and VMH of male mice.

The second major finding of this study is that, by using a dietary model that enables identification of susceptible and obesity-resistant populations (Lauterio *et al.* 1994; Lauterio *et al.* 1999), we could demonstrate that the FVB mouse strain shows a sexual dimorphism in the response to high fat diet and leptin sensitivity. $Y_1R/LacZ$ transgenic female mice consumed a significantly lower amount of food than chow-fed female mice and MHF diet-fed male mice, and did not show body weight gain or changes of $Y_1R/LacZ$ transgene expression in the hypothalamus. We also demonstrated a gender-related difference in the FVB mouse strain in response to leptin treatment that failed to decrease body weight of both MHF diet- and chow-fed female mice. This observation is in line with previous studies showing that peripheral infusion of leptin produces a greater weight loss in male FVB mice than in females, and it suggests that leptin responsiveness of FVB mice is sex related (Harris *et al.* 2002).

The molecular mechanisms responsible for the sex difference in the effect of both the diet manipulation and leptin administration remain to be clarified. However, we could speculate that this sexual dimorphism may depend on differences in sex hormones and/or in oestrogen receptor isoform distribution. Indeed, steroid environment may affect Y_1R -mediated signalling (Musso *et al.* 2000; Xu *et al.* 2000; Hill *et al.* 2004) as well as the expression of leptin receptors (Bennett *et al.* 1998) in the hypothalamus. Moreover, the expression of NPY and Y_1R can be differentially regulated depending on the region-specific ratio between oestrogen receptor α and oestrogen receptor β (Musso *et al.* 2000; Titolo *et al.* 2006) and this mechanism could mediate, at least in part, the effects of oestrogen on energy balance (Acosta-Martinez *et al.* 2007).

On the other hand, given that gender differences in responsiveness to leptin treatment were reversed for FVB mice compared with those observed in C57BL/6J mice (Sarmiento *et al.* 1997; Harris *et al.* 2003), the smaller response to high energy diet and leptin treatment in female FVB mice might represent a peculiarity specific to the FVB/N mouse strain.

In conclusion, the novel finding of this study is that $Y_1R/LacZ$ FVB mice show a sexual dimorphism in response to a MHE, high-energy diet that affects feeding behaviour and energy intake as well as the expression of hypothalamic Y_1R receptor gene in a nucleus-specific manner.

Although the physiological basis of this regulatory mechanism remains to be determined, our dietary obesity model appears to be useful to better understand the complex mechanisms involved in the gender-dependent regulation of specific gene expression patterns of distinct nuclei in the hypothalamus. The findings presented herein may therefore be of significance for understanding the overall effects of NPY Y_1R transmission in the hypothalamic nuclei involved in feeding behaviour and energy homeostasis.

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