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PYY transgenic mice are protected against diet-induced and genetic obesity

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Abstract

The gut-derived hormone, peptide YY (PYY) reduces food intake and enhances satiety in both humans and animals. Obese individuals also have a deficiency in circulating peptide YY, although whether this is a cause or a consequence of obesity is unclear. Our aims were to determine whether peptide YY (PYY) over-expression may have therapeutic effects for the treatment of obesity by altering energy balance and glucose homeostasis. We generated PYY transgenic mice and measured body weight, food intake, temperature, adiposity, glucose tolerance, circulating hormone and lipid concentrations and hypothalamic neuropeptide levels (neuropeptide Y; proopiomelanocortin, and thyrotropin-releasing hormone) under chow and high-fat feeding and after crossing these mice onto the genetically obese leptin-deficient *ob*/*ob* mouse background. PYY transgenic mice were protected against diet-induced obesity in association with increased body temperature (indicative of increased thermogenesis) and sustained expression of thyrotropin-releasing hormone in the paraventricular nucleus of the hypothalamus. Moreover, PYY transgenic mice crossed onto the genetically obese *ob*/*ob* background had significantly decreased weight gain and adiposity, reduced serum triglyceride levels and improved glucose tolerance compared to *ob*/*ob* controls. There was no effect of PYY transgenic over expression on basal or fasting-induced food intake measured at 11–12 weeks of age. Together, these findings suggest that long-term administration of PYY, PYY-like compounds or agents that stimulate PYY synthesis *in vivo* can reduce excess adiposity and improve glucose tolerance, possibly via effects on the hypothalamo–pituitary–thyroid axis and thermogenesis.

Keywords: Peptide YY; Transgenic mouse; Adiposity; Thermogenesis

1. Introduction

Type 2-diabetes mellitus and its complications are affecting increasing numbers of people at alarmingly younger ages. This is exacerbated by the current epidemic of obesity, a major risk factor for type 2-diabetes. Although weight loss in overweight or obese individuals significantly reduces the risk or severity of type 2 diabetes, currently the most effective treatments for obesity involve surgical interventions that not only pose medical risks and complications, but are also expensive and unfeasible for many, considering these procedures are generally performed on subjects with a body mass index over 35. Investigating the predisposing and protective factors in the etiology of obesity is vital for finding

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long-term treatments for obesity and reducing the incidence of type 2 diabetes.

Gut-derived hormones have been of major interest as possible targets for the treatment of obesity in light of their marked effects on satiety and food intake. One of these hormones is peptide YY (PYY), which belongs to a family of peptides including neuropeptide Y (NPY) and pancreatic polypeptide (PP), all of which are known to have potent effects on feeding and energy balance via their unique interactions with G-protein-coupled Y receptors (Y1, Y2, Y4, Y5, y6) (Blomqvist and Herzog, 1997; Stanley et al., 1986; Ueno et al., 1999).

PYY is predominantly produced by endocrine L cells of the lower gastrointestinal tract and is also expressed in alpha cells of the islets of Langerhans as well as in the stomach and brainstem (Pieribone et al., 1992). Two forms of PYY exist in the circulation: the full length PYY1-36, and the shortened form PYY3-36, cleaved from secreted PYY1-36 by the cell surface enzyme dipeptidyl peptidase IV (Lundberg et al., 1982). PYY1-36 binds to all known Y receptors, albeit to each with differing affinities, whereas PYY3-36 preferentially binds the Y2 receptor and to a lesser extent the Y5 receptor (Blomqvist and Herzog, 1997). Obese subjects have significantly reduced circulating levels of PYY (Batterham et al., 2003). Moreover, it has been suggested that this is due to a deficiency in PYY release from the colon (Le Roux et al., 2005). In light of these observations, and considering that low fasting serum PYY levels are seen in non-obese subjects with a high genetic predisposition towards subsequent development of obesity on account of a family history of type 2 diabetes mellitus (Boey et al., 2006a), it is likely that low levels of circulating PYY may not simply be a consequence of obesity but a predisposing factor to the development of obesity.

Recent studies have demonstrated acute affects of PYY1-36 and PYY3-36 in inhibiting food intake in animals and in man (Adams et al., 2004; Batterham et al., 2002; Challis et al., 2003; Chelikani et al., 2004, 2005; Halatchev et al., 2004; Riediger et al., 2004). It is thought that after a meal, PYY3-36, the main form of PYY circulating postprandially, acts on the arcuate nucleus of the hypothalamus. Specifically, PYY3-36 binds to Y2 receptors on NPY neurones, thereby inhibiting the orexigenic effect of these neurons and indirectly stimulating the action of anorexigenic neurons producing POMC (proopiomelanocortin, the precursor to the anorexigenic alpha melanocyte stimulating hormone) (Batterham et al., 2002). In contrast, other work has suggested that PYY3-36 not only acts via Y2 receptors to inhibit NPY neurones but also directly inhibits POMC neurones via Y2 receptors (Acuna-Goycolea and van den Pol, 2005; Fetissov et al., 2004). In light of these findings, it has been proposed that the PYY3-36-mediated inhibition of food intake occurs primarily through the inhibition of NPY neurones. Other studies also show that intraperitoneally injected PYY3-36 activates neurones in the area postrema and nucleus tractus solitarius in rodents, which may be another mechanism by which PYY3-36 influences food intake (Bonaz et al., 1993; Halatchev and Cone, 2005).

PYY appears to play a long-term role in regulating food intake, body weight and body composition. A four-week continuous infusion of PYY3-36 in diet-induced obese mice reduced cumulative food intake, weight gain and adiposity (Pittner et al., 2004). In *ob/ob* mice and *fa/fa* rats, on the other hand, four-week continuous infusion of PYY3-36 decreased body weight gain in association with a transient reduction in food intake observed during the first 4 days of infusion only (Pittner et al., 2004). These findings suggest that PYY may also influence body weight and body composition via effects independent of changes in food intake. Indeed, seven day subcutaneous administration of PYY3-36 to diet-induced obese mice transiently reduced food intake and also led to maintenance of mass-specific energy expenditure despite concurrent anorexia, reduced respiratory quotient (indicating increased fat oxidation) and led to significantly decreased adiposity (Adams et al., 2006). Moreover, we and others have previously shown that PYY ablation results in obesity in mice (Batterham et al., 2006; Boey et al., 2006b).

Collectively, these studies suggest that PYY plays a major role in influencing long-term energy balance via effects on food intake and possibly also on metabolic processes, and that long-term elevation of PYY levels may attenuate obesity. However, studies investigating the effects of PYY administration have been limited to a maximum of 4-week duration, which is also not representative of PYY secretion *in vivo*. Furthermore, the mechanisms underlying PYY-induced effects on food intake, metabolic rate, body weight and adiposity have not yet been elucidated. Answers to these caveats are of relevance to human health since pharmacological treatments for obesity are administered for months, not weeks, and because new obesity treatments may involve enhancing endogenous PYY release with meals or episodic dosing strategies.

In order to test the hypothesis that elevated PYY might result in long-term reductions in food intake, body weight and adiposity, we generated PYY transgenic mice to over-express murine PYY. These mice were studied under chow fed conditions as well as under diet- and genetically induced obesogenic conditions to determine whether long-term endogenous PYY over-expression may have an anti-obesity effect. Moreover, we aimed to elucidate mechanisms for any possible effects of PYY over-expression on energy homeostasis by investigating thermogenesis, the hypothalamo–pituitary–thyroid axis as well as glucose homeostasis.

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2. Research design and methods

2.1. PYY transgenic mouse generation

All research and animal care procedures were approved by the Garvan Institute/St. Vincent's Hospital Animal Experimentation Ethics Committee. A 129/SvJ mouse genomic BAC clone containing the PYY gene and flanking sequences was obtained from Prof. Dominic Withers (UCL, UK). The 130 kb clone was mapped and various fragments subcloned. A 10.5 kb *Spe*I fragment containing 6 kb 5′-flanking sequence, the entire PYY gene and 3 kb 3′-flanking sequence was chosen as the transgene construct (Fig. 1A). In order to identify the transgene the *Nsi*I restrictions site in the position 9832 of the *Spe*I fragment was mutated to a *Bgl*II restriction site. A 300 bp probe for screening located between nucleotides 7470 and 7770 of the *Spe*I fragment was generated by PCR. Purified DNA of the modified *Spe*I fragment was injected into oozytes from C57BL/6 mice and reintroduced into pseudo-pregnant mice. Southern analysis of DNA obtained from tail tips of offspring from these mice revealed two positive founders carrying the PYY transgene. Wild type C57/BL6 mice were used as controls (Animal Resource Centre, Perth, Australia).

Mice genotype was confirmed on DNA obtained from tail tips with PCR using the combination of oligonucleotides *mPYY-R* (5′-GGTACAAGTATGTG-TAGGTGC-3′) and *mPYY-S* (5′-GAACTCACTCTG-TAGACCAG-3′). The conditions for all PCR analyses were 35 cycles of 94 °C for 45 s, 58 °C for 1 min and 63 °C for 1 min. Insertion of the *Bgl*2 site was confirmed by digesting the PCR product overnight with restriction enzyme *Bgl*2. Mice genotype was also confirmed by Southern blot analysis of *Bg*l2 digested DNA employing a probe generated by PCR using oligonucleotidess *mPYY-G* (5′-GAATTCCCTGTAATGCTG-3′) and *mPYY-H* (5′- CCCTCCAAGACCTAGGAAA-3′). All of the mice used in experiments were shown by both PCR and Southern blot analysis to carry the PYY transgene.

PYY transgenic mice were also crossed onto mice of mixed C57BL/6/129/SvJ background, heterozygous for the *ob*/*ob* gene (Jackson Laboratories, ME, USA). Homozygosity of the *ob* locus in *ob*/*ob* mice was confirmed using PCR with oligonucleotides mOb-2 (5′-GAG-TCAAGCATTGTGGAGT-3′) and mOb-3 (5′-CAG-TCGGTATCCGCCAAG-3′) using 35 cycles of 94 °C for 45 s, 61 °C for 1 min and 72 °C for 25 s followed by digestion with the restriction enzyme *Ddel* (Roche Diagnostics, Mannheim, Germany) at 37 °C overnight.

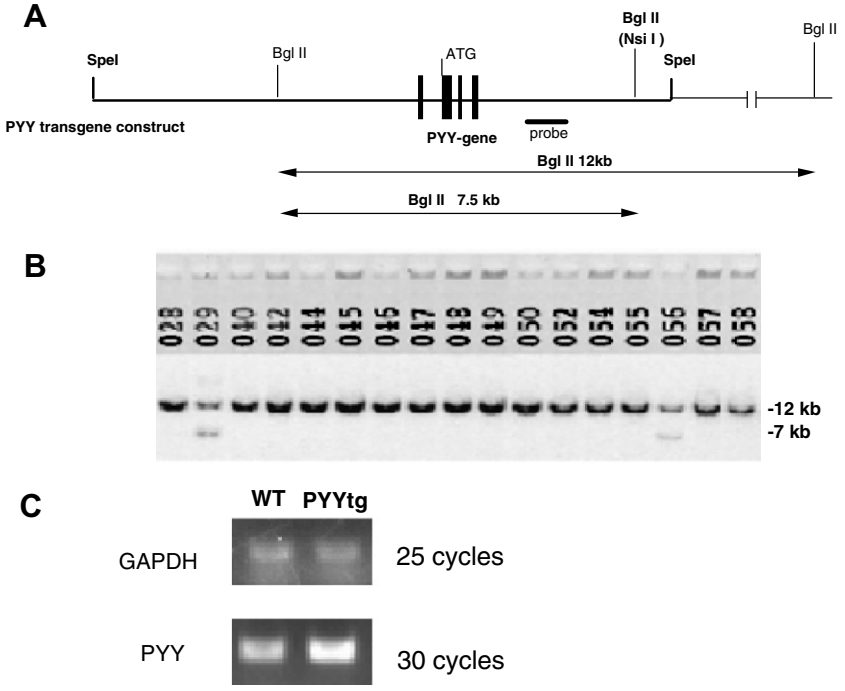

Fig. 1. Generation of *PYY* transgenic mice. PYY transgene construct and screening strategy. (A) A 10 kb SacI fragment (bold line) containing the entire mouse PYY gene is used as a transgene. For screening purposes, an Nsi I site has been mutated to a Bgl II site distinguishing between the 14 kb WT band and the 7.2 kb transgene band when Probe A is used. (B) Southern analysis of DNA from *PYYtg* mice. Positive transgenic mice exhibit the 14 and 7 kb fragment. (C) Representative pictures of GAPDH and PYY expression in wild type and *PYYtg* mice using RT-PCR.

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2.2. Staining and measurement of PYY positive cells

Whole pancreas and segments of the colon were fixed and stained for PYY as described previously (Boey et al., 2006b). Six to eight random pancreatic sections obtained from whole pancreas from four male wild type and 4 PYY transgenic mice were stained for PYY. Briefly, tissues fixed in 4% paraformaldehyde (PFA) in PBS overnight at 4 °C were transferred to 70% ethanol before being processed and embedded in paraffin. Sections (7 μm) were cut and mounted onto charged slides. Sections were deparaffinised in xylene and rehydrated from 100% ethanol to water. Slides were incubated in 1% H_2O_2 in methanol for 20 min, rinsed in PBS and blocked with 20% normal goat serum in PBS for 20 min. Excess blocking solution was removed before applying the rabbit anti-human PYY antiserum (Peninsula Laboratories, CA, USA) (1:1000) for 1 h at room temperature. Slides were rinsed in PBS before incubation with goat anti-rabbit IgG-horse radish peroxidase conjugated antibody (Zymed Laboratories, San Francisco, CA, USA) (1:1000) for 30 min at room temperature. Slides were washed in PBS and treated with the diaminobenzidine DAKO (Carpinteria, CA, USA) for 5 min. Slides were rinsed in water, counterstained in haematoxylin (30 s), rinsed in water, dipped in lithium carbonate and rinsed in water again. Sections were dehydrated through graded ethanols and xylene before coverslipping. Using a ProgRes 3008 camera (Zeiss, Jena, Germany) mounted on a Zeiss Axiophot microscope, the number of PYY positive cells per islet was counted using a 20× objective lens. Islet size was determined using Leica IM 1000 version 1.20 software (Leica Microsystems, Heerbrig, Switzerland). The average number of PYY positive cells per mm^2 islet was calculated.

2.3. Reverse transcriptase PCR

Whole mouse colon rapidly excised from animals killed by cervical dislocation at 10:00–12:00 h was cleaned and immediately frozen in liquid nitrogen. Total RNA was extracted using Trizol (Invitrogen, CA, USA) according to the manufacturer's instructions. RNA was treated with DNase (Promega, Madison, USA) prior to RNeasy Mini clean-up (Qiagen, Victoria, Australia) following the manufacturers protocol. RNA quality and concentration was determined using the Agilent 2100 bioanalyser (Agilent Technologies, Palo Alto, CA, USA).

Total RNA (1 μg) was denatured at 65 °C for 5 min in the presence of 1 μl oligo$(dT)_{20}$ and 1 μl dNTP, and cDNA was synthesized according to the manufacturers instructions (Invitrogen, CA, USA).

GAPDH and PYY expression were determined using RT-PCR. In brief, a 25 μl PCR reaction volume containing cDNA (1 μl), 0.25 μl primers for GAPDH (Forward 5′ ACTTTGTCAAGCTCATTTCC 3′; Reverse 5′ TGCAGCGAACTTTATTGATG 3′) or PYY (Forward 5′ ACGGTCGCAATGCTGCTAAT 3′; Reverse 5′ AAGGGGAGGTTCTCGCTGTC) (100 pmol/μl), 2.5 μl 10xPCR Buffer, 3 μl $MgCl_2$, 0.25 μl dNTPs (25 mM), 0.5 μl Taq polymerase (Roche Diagnostics, Castle Hill, NSW) made up to 25 μl with water was reacted using conditions starting with 94 °C for 5 min followed by the optimized conditions (GAPDH: 94 °C for 40 s, 57 °C 40 s, 72 °C 45 s, 25 cycles; PYY: 94 °C for 40 s, 65 °C 40 s, 72 °C 45 s, 30 cycles).

2.4. Measurement of food intake and bodyweight

Male C57/BL6 wild type, PYYtg, ob/ob (a gift from M. Cleasby, Garvan Institute, Sydney Australia) and PYYtg/ob animals originating from three to four different breeding pairs were housed under conditions of controlled temperature (22 °C) and illumination (12 h light cycle, lights on at 07:00 h) ($n = 6$–12). Unless otherwise stated, all mice were fed a normal chow diet ad libitum (6% calories from fat, 21% calories from protein, 71% calories from carbohydrate, 2.6 kcal/g, Gordon's Speciality Stock Feeds, Yanderra, NSW, Australia). One group of wild type and PYYtg mice were fed a high fat diet (46% calories from fat, 21% calories from protein, 33% calories from carbohydrate, 4.72 kcal/g) from 4 to 5 weeks of age onwards. The high fat diet, made in-house, was based on the composition of Rodent Diet Catalogue Number D12451 (Research Diets, New Brunswick, NJ), with the exception that safflower oil and copha were used in place of soybean oil and lard, respectively. Body weight was monitored weekly. At 11 weeks of age, non-fasted food and water intake were measured over four consecutive days in individually housed mice. Actual food intake was calculated as the weight of pellets taken from the food hopper minus the weight of food spilled in the cage. The weight of spilled food was determined as the 24-h increase in weight of the cage bedding, after removing all feces and air-drying to eliminate weight changes due to urine and water bottle drips. At 12 weeks of age, mice were fasted for 24 h and the amount of food removed from the hopper was measured at 1, 2, 8, 24, 48 and 72 h after re-introduction of food. Actual food intake was determined as described above after 24, 48 and 72 h of refeeding, and body weight was tracked at the same time each day before and after the 24-h fast.

2.5. Temperature measurements

Rectal temperature was measured at 16:00 h for 3 days consecutively in 10-week-old mice. *PYYtg* and wild type mice were placed in a hinged restraining cylinder and a rectal temperature probe (Physitemp Instruments,

NJ, USA) attached to the BAT-10 Multipurpose Thermometer (Physitemp Instruments, NJ, USA) was inserted and left in place until the temperature reading stabilized. Temperatures were measured in *PYYtg*/*ob* and *ob*/*ob* mice without a hinged restraining cylinder due to size.

2.6. Glucose tolerance tests

At 13 weeks of age, mice were fasted for 24 h prior to intraperitoneal glucose tolerance tests (1 g/kg). Serial blood samples were collected from the tail for determination of serum glucose and insulin levels. Area under the curve (AUC) was calculated between 0 and 120 min after glucose injection and expressed as mM glucose per minute.

2.7. Tissue collection and analysis

Wild type, *PYYtg*, *ob*/*ob* and *PYYtg*/*ob* mice (16 weeks of age) were killed by cervical dislocation and decapitation between 12:00 and 14:00 h for collection of trunk blood. Brains were immediately removed and frozen on dry ice. White adipose tissue depots (right inguinal, right epididymal or periovarian (gonadal), right retroperitoneal and mesenteric) were removed and weighed.

2.8. Serum analyses

Total serum PYY concentrations were determined using a radioimmunoassay kit from Phoenix Pharmaceuticals, Belmont, CA, USA as previously described (Boey et al., 2006a). Serum glucagon levels were measured by radioimmunoassay kits from Linco Research (St. Louis, MO, USA), insulin levels were measured using an ELISA Kit from Mercodia (Uppsala, Sweden) or Linco Research, serum free T4 and testosterone concentrations were measured with kits from ICN Biomedicals (Costa Mesa, CA, USA), serum IGF-1 was determined using an RIA Kit from Bioclone (Marrickville, NSW, Australia), serum glucose was determined with a glucose oxidase assay kit (Trace Scientific, Melbourne, Australia), and serum triglyceride and free fatty acid levels were determined with kits from Roche Diagnostics, Mannheim, Germany and Wako, Osaka, Japan, respectively.

2.9. In situ hybridization

Coronal brain sections (20 μm) were cut on a cryostat (Leica CM3050S, Leica, Wetzlar, Germany) and thaw-mounted on Superfrost® slides (Menzel-Glaser, Braunschweig, Germany). Matching sections from the same coronal brain area of PYY transgenic and wild type mice (five mice per group) were assayed together using DNA oligonucleotides complementary to mouse NPY (5′-GAGGGTCAGTCCACACAGCCCCATTCGCTT-GTTACCTAGCAT-3′); mouse POMC (5′-TGGCT-GCTCTCCAGGCACCAGCTCCACACATCTATGG AGG-3′); and mouse thyrotropin-releasing hormone (TRH; 5′-AACCTTACTCCTCCAGAGGTT CCCT-GACCCAGGCTTCCAGTTGTG-3′) as described previously (Sainsbury et al., 2002). NPY and POMC expression were determined in the arcuate nucleus and TRH was determined in the paraventricular nucleus of the hypothalamus.

For evaluation of mRNA levels in scattered neurons, images from dipped sections were digitized using a ProgRes 3008 camera (Zeiss, Jena, Germany) mounted on a Zeiss Axiophot microscope. Silver grain density over single neurons was evaluated using NIH-Image 1.61 software (written by Wayne Rasband and available at zippy.nimh.nih.gov). Background labeling was uniform and never exceeded 5% of specific signal.

2.10. Statistical analyses

Analyses were done using two-factor ANOVA to assess differences due to genotype and diet. When the effect of genotype was significant, or when there was a significant genotype-diet interaction effect, Fisher's post hoc tests were used to determine differences amongst groups. Results for body weight, lean and fat mass, food intake and differences in serum glucose and insulin levels during glucose tolerance tests were compared among groups using two-factor repeated measures ANOVA followed by Fisher's post hoc tests. StatView version 4.5 (Abacus Concepts Inc., CA, USA) was used for all statistical analyses and $p < 0.05$ was accepted as being statistically significant.

3. Results

3.1. Increased embryonic lethality in PYY transgenic mice

Of the 150 offspring derived from oozytes injected with a construct containing the murine PYY gene under its endogenous mouse promoter, only two mice carrying the *PYY* transgene were identified by southern blot analyses (Fig. 1B). The low success rate in obtaining PYY over-expressing mice suggests that strong expression of PYY may be embryonic lethal, which has been confirmed by a recent report that CMV driven PYY overexpression in embryonic life leads to neural tube defects (Yuzuriha et al., 2007). RT-PCR confirmed the over-expression of PYY mRNA in our PYY transgenic mice (Fig. 1C). Serum concentrations of PYY were elevated in fat-fed transgenic mice (40.6 ± 5.9 pM versus 24.4 ± 2.8 pM in fat-fed wild types, means ± SEM of 3–5 mice per group), albeit this difference did not reach

statistical significance. Nevertheless, quantification of alpha cells in the islets of Langerhan, known to produce PYY (Lundberg et al., 1982), clearly demonstrated that under conditions of high-fat feeding, the number of PYY-positive alpha cells in the islets of Langerhans was significantly higher in PYY transgenic mice compared to wild type mice (Table 1). In this regard it is noteworthy that high-fat feeding increases alpha cell size, volume and number (Cerf et al., 2005).

Table 1
The number of PYY-positive cells in the pancreatic islets of Langerhan, food and water intake and serum parameters of chow and high fat-fed wild type and PYY transgenic mice on a lean or obese (*ob/ob*) background. Data represents means ± SEM of 8–12 mice per group

	Wild type	PYYtg
PYY positive cells/size of islet (mm^2)		
Chow	39.4 ± 2.3	39.8 ± 3.8
High-fat	48.9 ± 2.6	58.1 ± 5.8†
Food Intake/24 h (g)		
Chow	4.5 ± 0.3	4.7 ± 0.1
High-fat	3.4 ± 0.5	2.9 ± 0.1
ob/ob	7.3 ± 0.5	7.2 ± 0.5
Water Intake/ 24 h (g)		
Chow	6.3 ± 0.4	5.7 ± 0.3
High-fat	3.1 ± 0.2	3.5 ± 0.2
ob/ob	7.7 ± 0.9	6.2 ± 0.6
Free fatty acids (mM)		
Chow	1.6 ± 0.1	1.9 ± 0.1**
High-fat	1.9 ± 0.1*	1.6 ± 0.1†
ob/ob	1.6 ± 0.1	1.6 ± 0.1
Triglycerides (mM)		
Chow	0.91 ± 0.15	1.83 ± 0.15***
High-fat	1.38 ± 0.22	1.22 ± 0.18†
ob/ob	23.6 ± 1.9	13.8 ± 0.7**
Free T4 (pmol/l)		
Chow	20.9 ± 1.58	20.0 ± 1.8
High-fat	31.23 ± 8.69*	22.55 ± 2.43
Glucagon (pM)		
Chow	25.9 ± 4.6	31.4 ± 6.7
High-fat	22.2 ± 5.1	37.0 ± 6.2
Testosterone (nM)		
Chow	10.8 ± 3.3	14.7 ± 10.0
High-fat	21.5 ± 7.2	15.7 ± 8.0
Glucose (mM)		
Chow	10.4 ± 0.4	9.1 ± 0.4
High-fat	9.7 ± 0.7	9.0 ± 0.4
Insulin (pM)		
Chow	230.0 ± 19.3	324.9 ± 56.5
High-fat	311.1 ± 63.5	311.8 ± 36.1
IGF-1 (ng/ml)		
Chow	35.34 ± 2.62	26.75 ± 4.32*
High-fat	33.51 ± 2.53	27.53 ± 1.00

* $p < 0.05$.
** $p < 0.01$.
*** $p < 0.001$ versus chow-fed wild type mice.
† $p < 0.05$ versus chow-fed *PYYtg* mice.

3.2. Attenuated diet-induced obesity in PYYtg mice with no effects on food intake

There has been controversy as to whether exogenously administered PYY reduces food intake in mice (Tschop et al., 2004). Moreover, there have been limitations in elevating PYY levels using osmotic mini-pumps because this mode of administration does not mimic the secretion of PYY *in vivo* (Pittner et al., 2004). We therefore investigated food intake as well as body weight and adiposity in *PYYtg* mice. Under chow feeding, body weight (Fig. 2A), basal and fasting-induced food intake measured at 11–12 weeks of age (Table 1, Fig. 3A) and adiposity (Fig. 2D and F) were comparable between wild type and PYY transgenic animals. Interestingly, under high-fat feeding PYY transgenic mice showed no significant difference in body weight (Fig. 2B) but were significantly less fat (Fig. 2D and F) and had a significantly higher body temperature (Fig. 2H) than fat-fed wild types in the absence of changes in basal or fasting-induced food intake measured at 11–12 weeks of age (Table 1, Fig. 3B). This increase in temperature suggests that the reduced adiposity observed in high-fat fed PYY transgenic compared to fat-fed wild type mice was attributed by an increase in thermogenesis. In keeping with their lack of change in food intake compared to wild type values, *PYYtg* mice were no different from wild types with respect to water intake (Table 1). High fat-fed *PYYtg* mice lost significantly less body weight than controls after a 24-h fast compared to wild type (Fig. 3D).

3.3. Increased activity of the hypothalamo–pituitary–thyroid axis in PYY transgenic mice

In order to determine whether the decreased fat mass in *PYYtg* mice was accompanied by changes in circulating lipids, free fatty acid and triglyceride levels in PYY transgenic animals on a high-fat diet were analysed. PYYtg mice exhibited significantly lower serum free fatty acid and triglyceride levels in comparison with transgenic animals on a chow diet (Table 1), although chow-fed PYY transgenics on a lean background had higher serum free fatty acid and triglyceride levels than chow-fed wild type controls (Table 1). Circulating thyroid hormone levels (free T4) were also measured as an indication of activity of the hypothalamo–pituitary–thyroid axis, an important regulator of thermogenesis and metabolic rate (Ribeiro et al., 2001). Unlike wild type animals, which showed significantly higher serum free T4 levels on a high-fat diet compared to a chow diet, no such increase in circulating free T4 levels was seen in transgenic mice with high fat feeding (Table 1). There were no differences in serum concentrations of glucagon, testosterone, glucose or insulin between wild type and *PYYtg* animals under conditions of chow- or

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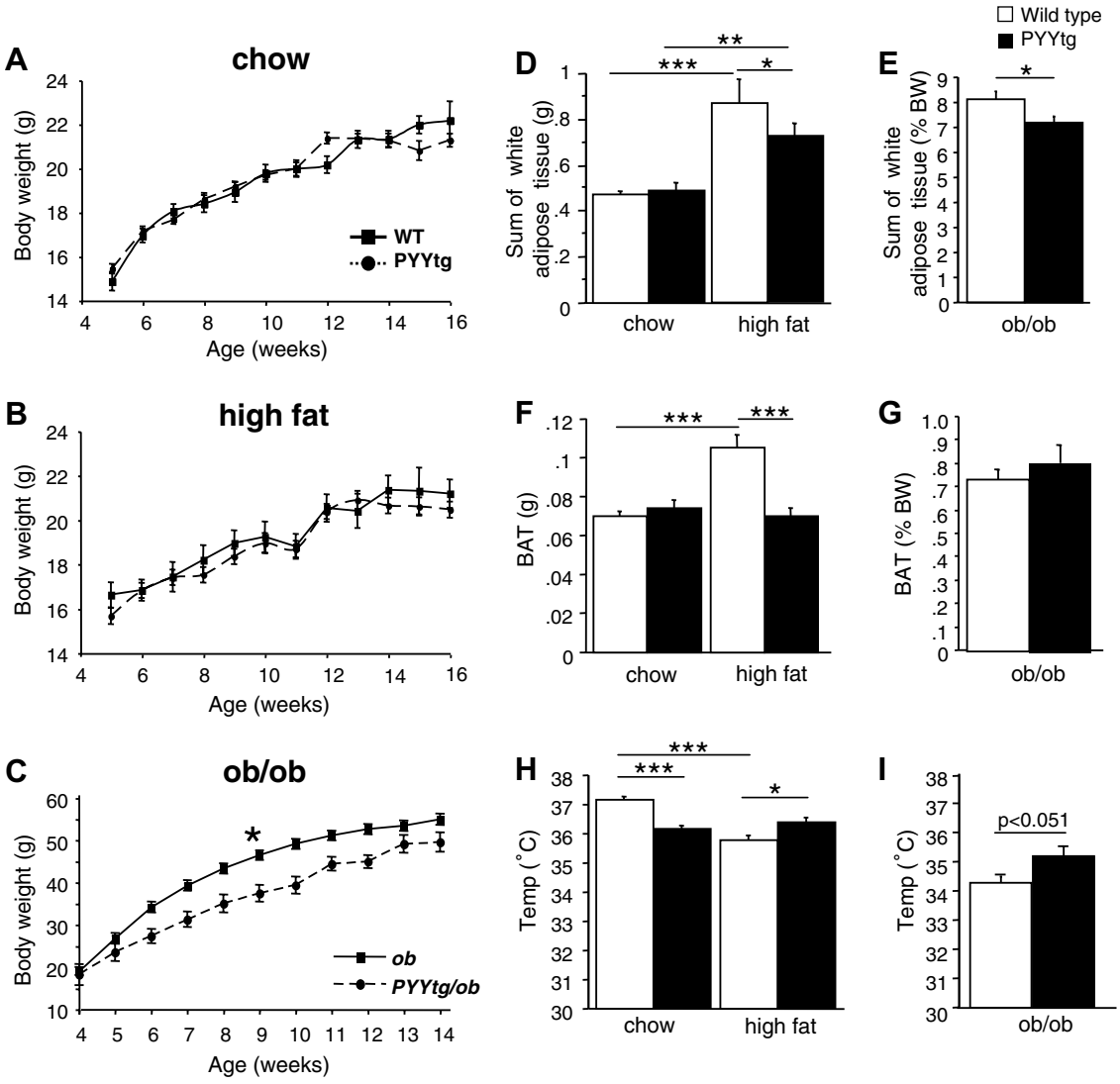

Fig. 2. Body weight, adiposity and thermogenesis in *PYYtg* mice. Bodyweight curves for male wild type and PYY transgenic mice fed a chow diet (A) or high fat diet (B) or crossed on the *ob*/*ob* background (C). Data represents means ± SEM of 6–12 mice per group. $^{*}p < 0.0001$ versus the growth curve for *ob*/*ob* mice. Weight of the summation of (D + E) white adipose tissue depots, (F + G) brown adipose tissue depots represented as absolute values or as percentage of body weight (%BW), and (H + I) temperatures of chow- and high fat-fed wild type and PYY transgenic mice and *PYYtg*/*ob* and *ob*/*ob* mice. Data represents means ± SEM of 8–12 mice per group. $^{*}p < 0.05$, $^{**}p < 0.01$ $^{***}p < 0.001$ versus the comparison shown by horizontal bars.

high fat feeding (Table 1). Interestingly, under chow-fed conditions, serum IGF-1 levels were significantly lower in *PYYtg* animals than in wild types (Table 1).

3.4. PYY transgenic mice have increased thyrotropin-releasing hormone (TRH) expression in the paraventricular nucleus of the hypothalamus

To assess possible central mechanisms for the decreased adiposity and increased thermogenesis observed in *PYYtg* mice, we measured central expression of peptides known to regulate energy balance (TRH, NPY and POMC). Under chow-fed conditions, TRH levels in the paraventricular nucleus were similar between *PYYtg* and wild type animals (Table 2). However, although TRH levels were significantly reduced by high-fat feeding in wild type animals, TRH levels in PYY transgenic animals were unaffected by diet and were significantly higher in high-fat fed *PYYtg* mice compared with high-fat fed wild types (Table 2).

Under chow-fed conditions, expression of NPY in the arcuate nucleus of the hypothalamus was 16% higher in PYY transgenic mice compared to wild types, albeit the difference was not significant, and there was no change in POMC expression observed between the chow-fed groups (Table 2). NPY and POMC expression were not different between high-fat fed groups (Table 2).

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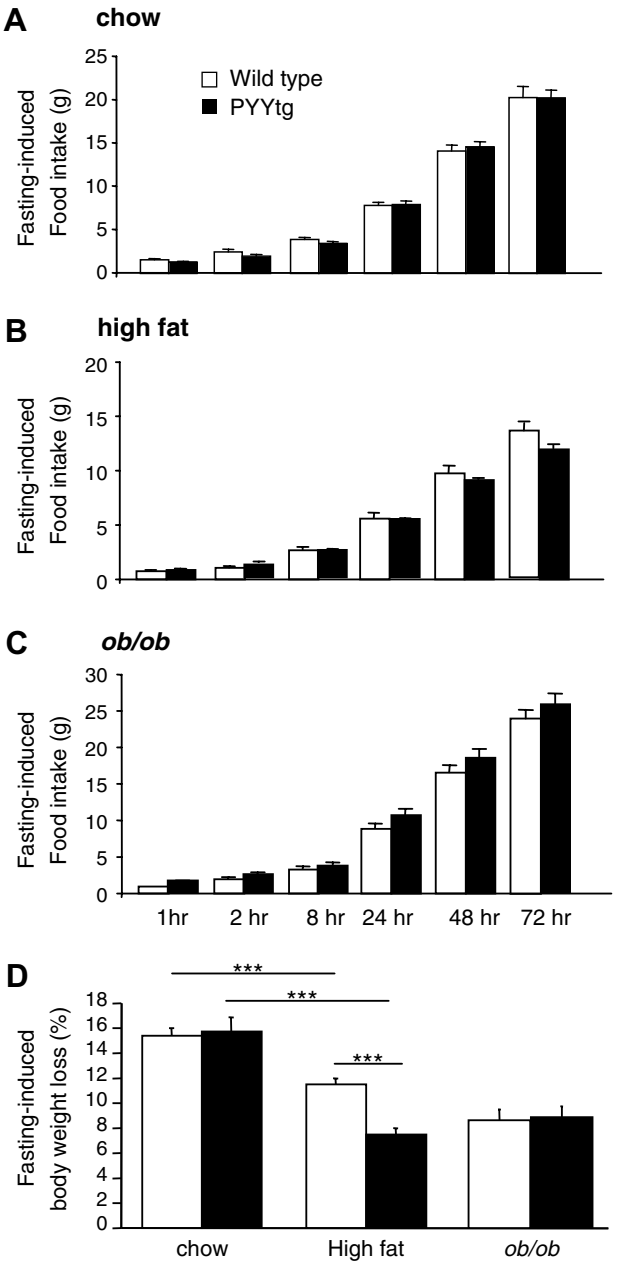

Fig. 3. Fasting-induced food intake and body weight in *PYYtg* mice. The fasting-induced food intake of chow-fed wild type and *PYYtg* mice (A), high fat-fed wild type and *PYYtg* mice (B) and chow-fed *ob/ob* and *PYYtg/ob* mice (C). (D) Percentage body weight loss after a 24 hour fast in chow-fed and high fat-fed lean and obese wild type and *PYYtg* animals. Data represents means ± SEM of 7–12 mice per group. $^{***}p < 0.001$ versus the comparison shown by horizontal bars.

3.5. PYY transgenic expression results in decreased body weight and adiposity in leptin deficient mice

As PYY transgenic mice were protected against the development of diet-induced obesity, we were interested in determining the effect of PYY over-expression on a genetic model of obesity, using *ob/ob* mice. *PYYtg/ob* mice had a significantly lower body weight than *ob/ob* controls (Fig. 2C). Similar to high-fat fed *PYYtg mice*, *PYYtg/ob* mice had significantly less white adipose tissue both in absolute terms (data not shown) and as a percent of body weight (Fig. 2E) and an increased body temperature (Fig. 2I) than *ob/ob* controls, in the absence of changes in basal and fasting-induced food intake (Table 1, Fig. 3C). Consistent with the lack of effect of the PYY transgene on food intake, water intake of *PYYtg/ob* mice was no different from that of *ob/ob* mice (Table 1). However, *PYYtg/ob* mice showed significantly lower serum triglyceride levels than *ob/ob* controls (Table 1), in keeping with the associated decrease in fat mass (Fig. 2E). This demonstrates that *PYYtg* mice have reduced weight and body fat via effects independent of food intake.

3.6. PYYtg/ob mice exhibit improved glucose tolerance

In light of our previous findings that PYY ablation results in basal and glucose-induced hyperinsulinemia and glucose intolerance (Boey et al., 2006b), we hypothesized that *PYYtg* mice would have improved glucose tolerance. Surprisingly, under conditions of chow- and high-fat feeding, fasting serum glucose levels were significantly higher in *PYYtg* compared to wild type mice (Fig. 4A and B). However, serum glucose levels, glucose area under the curve and serum insulin levels during a glucose tolerance test were not significantly different between chow- or fat-fed transgenic and wild type animals on a lean background (Fig. 4A + B, D + E, and G + H).

As *PYYtg/ob* mice showed decreased body weight and fat mass, we hypothesized that PYY over-expression may have additional beneficial effects on the hypeinsulinemia, hyperglycemia and insulin resistance exhibited by *ob/ob* mice. In keeping with this, *PYYtg/ob* mice had significantly lower fasting serum glucose levels and were markedly more glucose tolerant than *ob/ob* animals during the glucose tolerance test (Fig. 4C + F). *PYYtg/ob* mice also exhibited lower serum insulin levels for the duration of the glucose tolerance test (Fig. 4I).

4. Discussion

This study demonstrates the important role PYY has in the regulation of energy homeostasis, since even only modestly increased expression of PYY can significantly reduce body weight and/or adiposity under diet- and genetically-induced obesogenic conditions. This is associated with significantly improved glucose tolerance in genetically obese *ob/ob* mice, changes in the hypothalamo–pituitary–thyroid axis and increased thermogenic activity. Resistance to diet- or genetically-induced obesity in *PYYtg* mice occurred in the absence of significant

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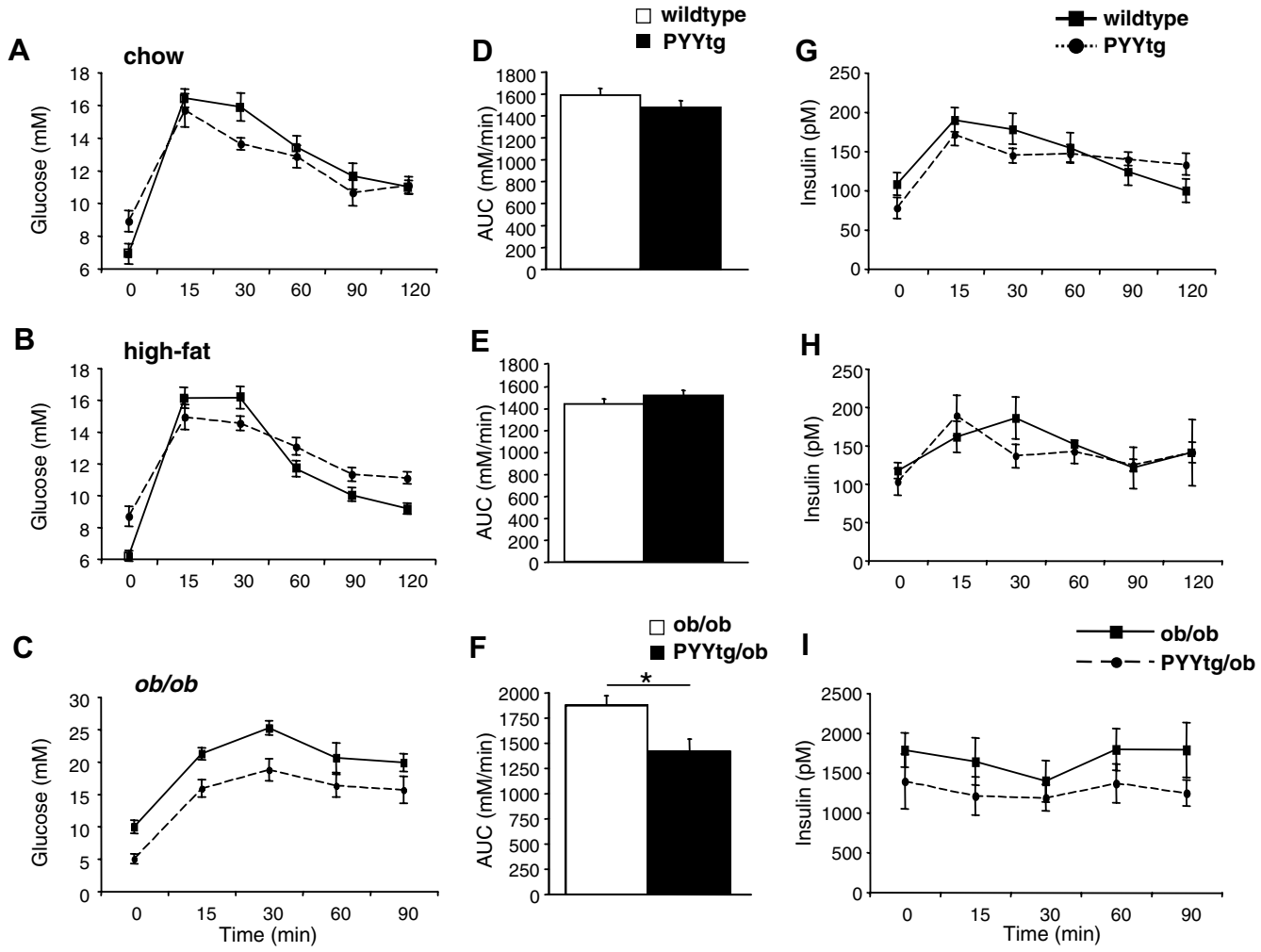

Fig. 4. Glucose homeostasis in *PYYtg* mice. (A–C) Changes in serum glucose levels after ip glucose injection (1.0 mg/kg) in 24 h fasted chow- or high fat-fed male wild type and *PYYtg* mice and in *PYYtg*/*ob* and *ob*/*ob* mice. (D–F) Areas under the glucose response curves (AUC). (G–I) Changes in serum insulin levels after ip glucose injection (1.0 mg/kg) in 24 h fasted chow- or high fat-fed male wild type and PYY transgenic mice and *PYYtg*/*ob* and *ob*/*ob* mice. Data represent means ± SEM of 8–12 mice per group. $^{*}p < 0.05$ versus the comparison shown by horizontal bar.

Table 2
Thyrotropin releasing hormone (TRH) mRNA expression in the paraventricular nucleus of the hypothalamus and neuropeptide Y (NPY) and proopiomelanocortin (POMC) mRNA expression in the arcuate nucleus of the hypothalamus in wild type and *PYYtg* mice

	Wild type	PYYtg
TRH		
Chow	100% ± 8.2	107.4% ± 8.9
High fat	73.6% ± 8.4*	103.6% ± 12.4†
NPY		
Chow	100% ± 8.0	116% ± 6.5
High fat	87.6% ± 9.6	93.4% ± 9.3
POMC		
Chow	100% ± 9.2	91.2% ± 12.1
High fat	90.6% ± 13.1	83.7% ± 3.2

Data represents means ± SEM of 4–5 mice per group.
* $p < 0.05$ versus chow-fed wild type mice.
† $p < 0.05$ versus high-fat fed wild type mice.

effects on basal and fasting-induced food intake measured at 11–12 weeks of age, nor significant changes in NPY and POMC expression in the hypothalamic arcuate nucleus, illustrating the importance of factors other than energy intake in mediating and/or maintaining anti-obesity effects of PYY.

The reduced fat mass of PYY transgenic mice may be explained by changes in the hypothalamo–pituitary–thyroid axis. Indeed, unlike wild type mice, which showed a decrease in TRH expression in the arcuate nucleus in response to high fat diet, PYY transgenic mice on a high fat diet showed no such inhibition of hypothalamic TRH levels. This suggests that *PYYtg* animals retain normal activity of the hypothalamo–pituitary–thyroid axis during high fat feeding, which may be causally associated with the significantly increased temperature and reduced brown adipose tissue mass seen in these

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animals. This finding is in keeping with a recent report that PYY3-36 stimulates thyrotropin (thyroid stimulating hormone) secretion by direct action on the pituitary gland *in vitro*, and significantly increases circulating thyroptropin levels after ip injection in fasted rats (Oliveira et al., 2006). Alternatively, it is possible that the increased TRH expression observed in *PYYtg* mice was a consequence rather than a cause of their resistance to diet-induced obesity: because these mice were resistant to increased adiposity with high-fat feeding, there was no compensatory increase in activity of the thyrotropic axis and circulating free T4 levels, which therefore did not down-regulate TRH levels in the hypothalamus as part of the negative feedback loop. Both potential pathways are consistent with previous studies showing that thyroid hormones increase thermogenesis and decrease brown and white adipose tissue mass (Ribeiro et al., 2001; Silva and Larsen, 1983). It is also possible that the apparent increase in energy expenditure in fat-fed *PYYtg* mice compared to wild type mice (indicated by reduced adiposity in the presence of no significant change in food intake) may be due to increases in free T3 concentrations within peripheral tissues rather than changes in circulating free T4 levels as were measured in this study (Silva and Larsen, 1983).

In addition to reduced body weight, reduced white adipose tissue mass, reduced circulating triglyceride levels and a tendency to higher body temperatures in the absence of effects on basal- or fasting-induced food intake, *PYYtg*/*ob* mice were significantly more glucose tolerant than *ob*/*ob* controls, with lower serum insulin levels observed for the duration of a glucose tolerance test. This is consistent with our previous findings that PYY knockout animals are equally glucose tolerant but hyperinsulinemic after a glucose challenge (Boey et al., 2006b) and further supports an important long-term role for PYY in regulating glucose homeostasis. Moreover, as low PYY levels are associated with increased insulin secretion and insulin resistance in humans (Boey et al., 2006a), enhanced expression of PYY may improve glucose homeostasis and insulin sensitivity, in keeping with findings from others (van den Hoek et al., 2004; Vrang et al., 2006).

Notably, chow-fed PYY transgenic animals displayed significantly lower serum IGF-1 levels than wild types, consistent with data from PYY knockout mice which show significantly increased serum IGF-1 levels and increased *GHRH* mRNA expression in the ventromedial hypothalamus (VMH) (Boey et al., 2006b). These data – together with findings that growth hormone and IGF-1 enhance PYY expression (Gomez et al., 1996; Wang et al., 2004) – support the notion that PYY is involved in a feedback loop to inhibit the somatotropic axis.

PYY transgenic mice showed no significant decrease in basal or fasting-induced food intake when investigated at 11–12 weeks of age. It was recently shown that whereas continuous infusion of PYY3-36 to obese or diabetic rodent models via osmotic mini pumps results in transient reductions in food intake that persist for only 4–6 days (Adams et al., 2006; Pittner et al., 2004), intermittent administration of PYY3-36 to lean or diet-induced obese rats results in reductions in food intake that are sustained for the entire 10–21-day study period (Chelikani et al., 2006, 2007). It is possible that early PYY-induced reductions in food intake and energy balance may induce compensatory responses to help restore energy homeostasis, or that long-term administration of PYY leads to desensitization and downregulation of Y receptors. Therefore it cannot be excluded that our PYY transgenic animals may have had transient reductions in food intake in early life (e.g. before weaning), and that such anorexia may have led to reductions in body weight and/or adiposity that were maintained throughout the study.

The amount of PYY over-expression in our PYY transgenic animals was not as high as anticipated. However, our findings that only two founder animals were produced, one of which died, and the fact that we found a high frequency of fatalities in either gender of our *PYYtg* breeding pairs indicates that high endogenous PYY expression are disadvantageous during development. Hence, it is likely that only PYY transgenic mice that had modest over-expression of PYY survived. This is supported by a recent study showing that CMV driven PYY over-expression resulted in neural tube defects in mice, a common cause of congenital malformation that leads to infant mortality (Yuzuriha et al., 2007). Nonetheless, our studies demonstrate that even the mild increases in PYY expression observed in our PYY transgenic mice were sufficient to induce significant effects on body weight, adiposity, thermogenesis, activity of the hypothalamo–pituitary–thyroid axis, glucose tolerance and IGF-1 levels. Moreover, the fact that many of the phenotypic characteristics observed in our *PYYtg* mice were *opposite* to effects seen in $PYY^{-/-}$ mice (e.g. fat mass, serum insulin levels in response to glucose as well as serum IGF-1 levels) provides further support that the phenotype observed in our *PYYtg* mice is due to PYY over-expression. Increased PYY expression is evident in the pancreatic islets of high-fat fed PYY transgenic animals but not in chow-fed PYY transgenics suggesting that high fat feeding evokes an increase in endogenous PYY production, which may help to explain why the effects of our PYY transgene were more apparent under conditions of high fat feeding. Alternatively, PYY produced in these mice may be rapidly degraded *in vivo* in compensation for the increased rates of production (Shechter et al., 2005).

In summary, we show that *PYYtg* mice are protected against the development of obesity in high-fat fed and *ob*/*ob* mice in the absence of effects on basal- or fasting-induced food intake when measured at 11–12 weeks

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of age. Similarly, *PYYtg/ob* mice exhibited decreased bodyweight, fat mass and improved glucose tolerance associated with decreased insulin levels. This is consistent with data from PYY knockout mice generated in our laboratory, which were hyperinsulinemic and became fatter than controls with aging or high fat feeding, in the absence of changes in food intake measured at 11–12 weeks of age (Boey et al., 2006b). Additionally, a recent PYY knockout mouse model was also shown to exhibit an obese phenotype, albeit that model also showed significant basal and fasting-induced hyperphagia when measured at 8 weeks of age (Batterham et al., 2006). Together, our findings support the possibility that measures to increase PYY production and secretion may be therapeutic for the treatment of obesity, not only via effects on appetite regulation but possibly also on energy efficiency.

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References

Acuna-Goycolea, C., van den Pol, A.N., 2005. Peptide YY3-36 inhibits both anorexigenic proopiomelanocortin and orexigenic neuropeptide Y neurons: implications for hypothalamic regulation of energy homeostasis. Journal of Neuroscience 25, 10510–10519.

Adams, S.H., Lei, C., Jodka, C., Nikoulina, S.E., Hoyt, J., Gedulin, B., Mack, C.M., Kendall, E.S., 2006. PYY[3-36] administration decreases the respiratory quotient and reduces adiposity in diet-induced obese mice. Journal of Nutrition 136, 195–201.

Adams, S.H., Won, W.B., Schonhoff, S.E., Leiter, A.B., Paterniti Jr., J.R., 2004. Effects of peptide YY[3-36] on short-term food intake in mice are not affected by prevailing plasma ghrelin levels. Endocrinology 145, 4967–4975.

Batterham, R.L., Cohen, M.A., Ellis, S.M., Le Roux, C.W., Withers, D.J., Frost, G.S., Ghatei, M.A., Bloom, S.R., 2003. Inhibition of food intake in obese subjects by peptide YY_{3-36}. The New England Journal of Medicine 349, 941–948.

Batterham, R.L., Cowley, M.A., Small, C.J., Herzog, H., Cohen, M.A., Dakin, C.L., Wren, A.M., Brynes, A.E., Low, M.J., Ghatzel, M.A., Cone, R.D., Bloom, S.R., 2002. Gut hormone PYY_{3-36} physiologically inhibits food intake. Nature 418, 650–654.

Batterham, R.L.H.H., Kapoor, S., Chivers, J.E., Chandarana, K., Herzog, H., Le Roux, C.W., Louise Thomas, E., Bell, J.D., Withers, D.J., 2006. Critical role for peptide YY in protein-mediated satiation and body-weight regulation. Cell Metabolism 4, 223–233.

Blomqvist, A.G., Herzog, H., 1997. Y-receptor subtypes – how many more? Trends in Neurosciences 20, 294–298.

Boey, D., Heilbronn, L., Sainsbury, A., Laybutt, D.R., Kriketos, A.D., Herzog, H., Campbell, L.V., 2006a. Low serum PYY is linked to insulin resistance in first-degree relatives of subjects with type 2 diabetes. Neuropeptides 40, 317–324.

Boey, D., Lin, S., Karl, T., Baldock, P., Lee, N., Enriquez, R., Couzens, M., Slack, K., Dallmann, R., Sainsbury, A., Herzog, H., 2006b. Peptide YY ablation in mice leads to the development of hyperinsulinemia and obesity. Diabetologia 49, 1360–1370.

Bonaz, B., Taylor, I.L., Tache, Y., 1993. Peripheral peptide YY induces c-fos-like immunoreactivity in the rat brain. Neuroscience Letters 163, 77–80.

Cerf, M.E., Williams, K., Nkomo, X.I., Muller, C.J., Du Toit, D.F., Louw, J., Wolfe-Coote, S.A., 2005. Islet cell response in the neonatal rat after exposure to a high-fat diet during pregnancy. American Journal of Physiology 288, 1122–1128.

Challis, B.G., Pinnock, S.B., Coll, A.P., Carter, R.N., Dickson, S.L., O'Rahilly, S., 2003. Acute effects of PYY_{3-36} on food intake and hypothalamic neuropeptide expression in the mouse. Biochemical and Biophysical Research Communications 311, 915–919.

Chelikani, P.K., Haver, A.C., Reeve, J.R., Kiere, D.A., Reidelberger, R.D., 2006. Daily, intermittent intravenous infusion of peptide YY(3-36) reduces daily food intake and adiposity in rats. American Journal of Physiology 290, R298–R305.

Chelikani, P.K., Haver, A.C., Reidelberger, R.D., 2004. Comparison of the inhibitory effects of PYY(3-36) amd PYY(1-36) on gastric emptying in rats. American Journal of Physiology – Regulatory Integrative and Comparative Physiology 287, R1064–R1070.

Chelikani, P.K., Haver, A.C., Reidelberger, R.D., 2005. Intravenous infusion of peptide YY(3-36) potently inhibits food intake in rats. Endocrinology 146, 879–888.

Chelikani, P.K., Haver, A.C., Reidelberger, R.D., 2007. Intermittent intraperitoneal infusion of peptide YY(3-36) reduces daily food intake and adiposity in obese rats. American Journal of Physiology – Regulatory Integrative and Comparative Physiology 293, R39–R46.

Fetissov, S.O., Byrne, L.C., Hassani, H., Ernfors, P., Hokfelt, T., 2004. Characterization of neuropeptide Y Y2 and Y5 receptor expression in the mouse hypothalamus. Journal of Comparative Neurology 470, 256–265.

Gomez, G., Udupi, V., Qi, X., Lluis, F., Rajaraman, S., Thompson, J.C., Greeley, G.H., 1996. Growth hormone upregulates gastrin and peptide YY gene expression. American Journal of Physiology – Endocrinology and Metabolism 271, E582–E586.

Halatchev, I.G., Cone, R.D., 2005. Peripheral administration of PYY3-36 produces conditioned taste aversion in mice. Cell Metabolism 1, 159–168.

Halatchev, I.G., Ellacott, K.L., Fan, W., Cone, R.D., 2004. Peptide YY3-36 inhibits food intake in mice through a melanocortin-4-receptor-independent mechanism. Endocrinology 145, 2585–2590.

Le Roux, C.W., Batterham, R.L., Aylwin, S.J.B., Patterson, M., Borg, C.M., Wynne, K., Kent, A., Vincent, R.P., Gardiner, J., Ghatei, M.A., Bloom, S.R., 2005. Attenuated peptide YY release in obese subjects is associated with reduced satiety. Endocrinology 147, 3–8.

Lundberg, J.M., Tatemoto, K., Terenius, L., Hellstrom, P.M., Mutt, V., Hokfelt, T., Hamberger, B., 1982. Localization of peptide YY (PYY) in gastrointestinal endocrine cells and effects on intestinal blood flow and motility. Proceedings of the National Academy of Sciences of the United States of America 79, 4471–4475.

Oliveira, K.L., Paula, G.S.M., Costa-e-Sousa, R.H., Souza, L.L., Moraes, D.C., Curty, F.H., Pazos-Moura, C.C., 2006. Peptide YY

(PYY)3-36 modulates thyrotropin secretion in rats. Endocrinology 191, 459–463.

Pieribone, V.A., Brodin, L., Friberg, K., Dahlstrand, J., Soderberg, C., Lahammar, D., Hokfelt, T., 1992. Differential expression of mRNAs for neuropeptide Y-related peptides in rat nervous tissues: possible evolutionary conservation. The Journal of Neuroscience 12, 3361–3371.

Pittner, R., Moore, C., Bhavsar, S., Gedulin, B., Smith, P., Jodka, C., Parkes, D., Peterniti, J., Srivastava, V., Young, A., 2004. Effects of PYY[3-36] in rodent models of diabetes and obesity. International Journal of Obesity 28, 963–971.

Ribeiro, M.O., Carvalho, S.D., Schultz, J.J., Chiellini, G., Scanlan, T.S., Bianco, A.C., Brent, G.A., 2001. Thyroid hormone-sympathetic interaction and adaptive thermogenesis are thyroid hormone receptor isoform-specific. Journal of Clinical Investigations 108, 97–105.

Riediger, T., Bothe, C., Becskei, C., Lutz, T.A., 2004. Peptide YY directly inhibits ghrelin-activated neurons of the arcuate nucleus and reverses fasting-induced c-Fos expression. Neuroendocrinology 79, 317–326.

Sainsbury, A., Schwarzer, C., Couzens, M., Herzog, H., 2002. Y2 receptor deletion attenuates the type 2 diabetic syndrome of ob/ob mice. Diabetes 51, 3420–3427.

Shechter, Y., Tsubery, H., Mironchik, M., Rubinstein, M., Fridkin, M., 2005. Reversible PEGylation of peptide YY3-36 prolongs its inhibition of food intake in mice. FEBS Letters 579, 2439–2444.

Silva, J.E., Larsen, P.R., 1983. Adrenergic activation of triiodothyronine production in brown adipose tissue. Nature 305, 712–713.

Stanley, B.G., Kyrkouli, S.E., Lampert, S., Leibowitz, S.F., 1986. Neuropeptide Y chronically injected into the hypothalamus: a powerful neurochemical inducer of hyperphagia and obesity. Peptides 7, 1189–1192.

Tschop, M., Castaneda, T.R., Joost, H.G., Thone-Reineke, C., Ortmann, S., Klaus, S., Hagan, M.M., Chandler, P.C., Oswald, K.D., Benoit, S.C., Seeley, R.J., Kinzig, K.P., Moran, T.H., Beck-Sickinger, A.G., Koglin, N., Rodgers, R.J., Blundell, J.E., Ishii, Y., Beattie, A.H., Holch, P., Allison, D.B., Raun, K., Madsen, K., Wulff, B.S., Stidsen, C.E., Birringer, M., Kreuzer, O.J., Schindler, M., Arnd, K., Rudolf, K., Mark, M., Deng, X.Y., Withcomb, D.C., Halem, H., Taylor, J., Dong, J., Datta, R., Culler, M., Craney, S., Flora, D., Smiley, D., Heiman, M.L., 2004. Physiology: does gut hormone PYY3-36 decrease food intake in rodents? Nature 430, 1 p following 165; discussion 162 p following 165.

Ueno, H., Inui, A., Iwamoto, M., Kaga, T., Asakawa, A., Okita, M., Fujimiya, M., Nakajima, Y., Ohmoto, Y., Ohnaka, M., Nakaya, Y., Miyazaki, J.I., Kasuga, M., 1999. Decreased food intake and body weight in pancreatic polypeptide-overexpressing mice. Gastroenterology 117, 1427–1432.

van den Hoek, A.M., Heijboer, A.C., Corssmit, E.P.M., Voshol, P.J., Romijn, J.A., Havekes, L.M., Pijl, H., 2004. PYY3-36 reinforces insulin action on glucose disposal in mice fed a high-fat diet. Diabetes 53, 1949–1952.

Vrang, N., Madsen, A.N., Tang-Christensen, M., Hansen, G., Larsen, P.J., 2006. PYY3-36 reduces food intake and body-weight and improves insulin sensitivity in rodent models of diet-induced obesity. American Journal of Physiology 291, R367–R375.

Wang, G., Leiter, A.B., Englander, E.W., Greeley, G.H., 2004. Insulin-like growth factor 1 increases rat peptide YY promotor activity through Sp1 binding sites. Endocrinology 145, 659–666.

Yuzuriha, H., Inui, A., Asakawa, A., Ueno, N., Kasuga, M., Meguid, M.M., Miyazaki, J., Ninomiya, M., Herzog, H., Fujimiya, M., 2007. Gastrointestinal hormones (anorexigenic peptide YY and orexigenic ghrelin) influence neural tube development. Faseb Journal 21, 2108–2112.

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