

Physiology

Does gut hormone PYY₃₋₃₆ decrease food intake in rodents?

Arising from: R. L. Batterham *et al.* *Nature* 418, 650–654 (2002)

Batterham *et al.* report that the gut peptide hormone PYY₃₋₃₆ decreases food intake and body-weight gain in rodents¹, a discovery that has been heralded as potentially offering a new therapy for obesity. However, we have been unable to replicate their results. Although the reasons for this discrepancy remain undetermined, an effective anti-obesity drug ultimately must produce its effects across a range of situations. The fact that the findings of Batterham *et al.*¹ cannot easily be replicated calls into question the potential value of an anti-obesity approach that is based on administration of PYY₃₋₃₆.

We have been unable to observe an acute or chronic decrease in food intake or in body weight following peripheral administration of PYY₃₋₃₆ to rodents (for full details, see the authors' website at www.pyyobesity.com). We repeated and extended the original studies¹, peripherally and/or centrally administering PYY₃₋₃₆ in equivalent concentrations to rodents from eight different strains in twelve independent laboratories (our methods and results are summarized in Table 1).

Food intake was measured continuously or hourly for up to 24 hours and daily for up to 10 days in 'acute' and 'chronic' experiments. In 37 of 39 experiments, PYY₃₋₃₆ either did not change or increased food intake compared with control-treated rodents (Table 1; see also Fig. 1a). In two continuous-infusion experiments, food intake did decrease, but only transiently; no effects were found on meal size or number, although we observed a transient increase in feeding behaviour that lasted for 10 min.

We found that the anorexigenic agents MT-II (a melanocortin-receptor agonist) and naloxone (an opioid-receptor antagonist) decreased food intake ($P=0.001$) in rats that had not responded to PYY₃₋₃₆, as did the gut-satiety hormone cholecystokinin ($P=0.05$) (see Fig. 1b, c and the authors' website). This indicated that the animals and conditions that we used were capable of revealing known anorectic effects.

Body weight was measured for up to 10 days following peripheral treatment with PYY₃₋₃₆ (Table 1; see also Fig. 1d). In 13 of 14 experiments, body weight either did not change or increased compared with that of control-treated rodents; only in one case (*Ob/Ob*^{-/-} mouse) was there a transient decrease in body weight (Table 1). Lean-muscle mass did not change, and adipose-tissue mass either increased or did not change.

We found that there was a significant reduction in the animals' locomotor activity and rearing and sniffing behaviour following PYY₃₋₃₆ administration; overall energy

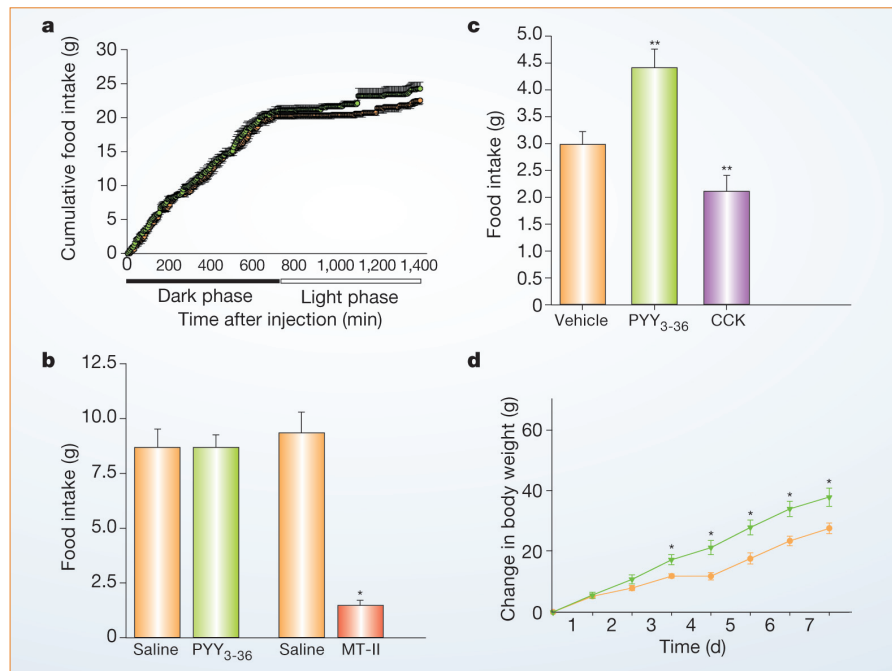


Figure 1 Lack of inhibitory effect of PYY₃₋₃₆ on food intake in rodents. Animals were acclimated to test conditions for 5–28 days (Table 1), fed normally and showed no signs of stress. **a–d**, Testing of human PYY₃₋₃₆ (**a**, **b**, **d**) and rat PYY₃₋₃₆ (**c**). **a**, PYY₃₋₃₆ (intraperitoneal (i.p.) dose of 3 $\mu\text{g kg}^{-1}$, $n=10$; green circles) did not decrease food intake over 24 hours in individually housed Wistar rats compared with vehicle-injected controls (orange circles). **b**, The melanocortin-receptor agonist MT-II (3 mg kg^{-1}), but not PYY₃₋₃₆ (i.p., injected just before lights turned off; 100 $\mu\text{g kg}^{-1}$), decreased 4-hour food intake in Wistar rats ($n=18$). **c**, PYY₃₋₃₆ (i.p., 5 $\mu\text{g kg}^{-1}$) increased food intake after overnight fasting (30 min, $P<0.05$, $n=10$) in Sprague–Dawley rats; cholecystokinin (CCK; i.p., 6 $\mu\text{g kg}^{-1}$) decreased food intake ($n=14$). **d**, PYY₃₋₃₆ (i.p., twice a day at 50 $\mu\text{g kg}^{-1}$; green line) increased body weight compared with saline control (orange line) over a 7-day treatment period in Sprague–Dawley rats ($n=8$). Error bars show standard error of the mean; * $P<0.001$; ** $P<0.05$.

expenditure tended to decrease (not significant; 6 mice received PYY₃₋₃₆), as did faecal caloric content (not significant; 8 mice received PYY₃₋₃₆). These changes favour a positive energy balance and ultimately promote body-fat gain. Experiments usually had equal or greater statistical power (number of animals in a group) compared with those of Batterham *et al.*¹.

Batterham *et al.* do not describe in detail the adaptation or habituation of their animals¹, so we used our own established and more extensive adaptation protocols. We carefully adapted our animals to experimental conditions for at least five days and excluded stress-related bias in these animals by standard assay of plasma corticosteroids and hypothalamic *c-fos* expression in the paraventricular nucleus area, or by monitoring feeding² and other stress-sensitive behaviour (results not shown).

Batterham *et al.* used human PYY₃₋₃₆ (supplied by Bachem) for their rodent studies (personal communications); we ran our tests with human or rodent PYY₃₋₃₆ from several suppliers (Bachem, P&E GmbH, Polypeptide Laboratories, Neosystems, Eli Lilly). The relative molecular mass of the peptide was confirmed upon delivery and in the injection

solution either before or after experiments (by high-performance liquid chromatography and/or mass spectrometry).

The biological activity of PYY₃₋₃₆ was confirmed *in vivo* by determining the increase in food intake following intracerebroventricular administration (through Y1/Y5 receptors³) and the delay in gastric emptying following intraperitoneal administration (through Y2 receptors⁴). These assays test the functional integrity of the peptide and show that its activity is not species specific. Y2-receptor binding was assayed⁵ in addition to confirm the activity and integrity of the fresh and left-over PYY₃₋₃₆.

To our knowledge, only one group not associated with the authors of refs 1 and 6 has so far replicated parts of the original study. However, this group^{7,8} found no effect of PYY₃₋₃₆ on body weight or on chronic food intake, and no effect on acute food intake without specific pre-fasting. Others have studied the longer peptide PYY₁₋₃₆ (ref. 9) or focused on the cardiovascular effects of PYY₃₋₃₆ (ref. 10). On the basis of the results available so far in rodents, we believe that there is no conclusive evidence that PYY₃₋₃₆ is a physiological satiety factor with the potential to treat obesity.

Table 1 **Effects of PYY₃₋₃₆ on food intake and body weight in rodents**

Species (male)	Administration route	Study	Dose	Total number of animals (controls)*	Food intake	Body weight
Wistar rat	i.c.v.	Acute	10 µg 5 µl ⁻¹	14 (5)	Increase (~200%)	ND
			2 µg 3 µl ⁻¹ , 4 µg 3 µl ⁻¹ , 8 µg 3 µl ⁻¹	32 (8)	Increase (~300%)	ND
	i.p.	Acute	0.3 µg 100 g ⁻¹	10 (5)	No effect	ND
			100 µg kg ⁻¹	16 (8)	No effect	ND
			100 µg kg ⁻¹	24 (12)	No effect	ND
			100 µg kg ⁻¹	24 (8)	No effect	ND
			3 µg, 30 µg, 100 µg (twice a day)	32 (8)	No effect	ND
			100 µg kg ⁻¹	24 (6)	No effect	ND
Chronic (2 d)	Chronic (2 d)	5 µg kg ⁻¹	102 (32)	Increase	ND	
		30 µg kg ⁻¹ , 100 µg kg ⁻¹ , 300 µg kg ⁻¹	32 (8)	No effect	ND	
Sprague-Dawley rat	i.v.	Chronic (2 d)	5 µg 100 g ⁻¹ (twice a day)	16 (8)	No effect	No effect
	i.p.	Acute	3 µg 100 g ⁻¹ , 10 µg 100 g ⁻¹	72 (24)	No effect	No effect
			3 µg 100 g ⁻¹	96 (24)	No effect	ND
			0.3 nmol kg ⁻¹ , 1 nmol kg ⁻¹ , 3 nmol kg ⁻¹ , 10 nmol kg ⁻¹	5 × 8 (8)	Transient decrease†	ND
	Chronic (7 d)	Chronic (7 d)	5 µg 100 g ⁻¹ (twice a day)	16 (8)	Increase (~7%)	Increase (~35%)
Long Evans rat	i.c.v.	Acute	0.2 nmol, 1 nmol	24 (8)	No effect‡, increase§	No effect
			30 µg kg ⁻¹ , 100 µg kg ⁻¹ , 300 µg kg ⁻¹	32 (8)	No effect	No effect
	i.p.	Acute	30 µg kg ⁻¹	10 (5)	No effect	No effect
			5 µg 100 g ⁻¹	16 (8)	ND	No effect
Chronic (5 d)	Chronic (5 d)	5 µg 100 g ⁻¹	16 (8)	ND	No effect	
		5 µg 100 g ⁻¹	16 (8)	ND	No effect	
Lister hooded rat	i.p.	Acute	3 µg kg ⁻¹ , 30 µg kg ⁻¹ , 100 µg kg ⁻¹	4 × 10 (10)	No effect	No effect
NMRI mouse	i.p.	Acute	50 µg kg ⁻¹ , 100 µg kg ⁻¹ (twice a day)	18 (6)	No effect	No effect
		Chronic (10 d)	100 µg kg ⁻¹ (twice a day)	14 (7)	Increase (~20%)	Increase (~5%)
C57BL/6 mouse	s.c.	Chronic (7 d)	1 mg kg ⁻¹	17 (9)	Transient decrease¶	No effect
			1 nmol kg ⁻¹ , 3 nmol kg ⁻¹	3 × 6 (6)	No effect	ND
	i.p.	Acute	1 nmol kg ⁻¹ , 3 nmol kg ⁻¹	4 × 6 (12)	No effect	ND
Ob/Ob ^{-/-} mouse	s.c.	Chronic (7 d)	1 mg kg ⁻¹	17 (9)	Transient decrease¶	Transient decrease#
NZO mouse	i.p.	Chronic (8 d)	100 µg kg ⁻¹	10 (4)	No effect	No effect

The results were obtained after habituation of all animals, which were individually housed. Briefly, animals were adapted to single-housed test conditions and diet, non-interrupted 12-hour light cycles and handling, including body-weight measurements and saline injections for 5–28 days before study onset. Food intake in the pre-study habituation phase was monitored to verify that it had stabilized and did not differ from the food intake of the control group during testing. Bedding was screened for spilt food and noise was kept to a minimum. Animals were monitored for evidence of stress (for example, poor grooming, defensive postures, discoloration around eyes and nose). Although the results of a few experiments were in agreement with those of Batterham *et al.*, that number (assuming all tests performed at a 2-tailed 0.05 significance level) was not significantly greater than expected by chance ($P=0.255$ for food intake), whereas the number in the other direction was significantly greater than would be expected by chance ($P=0.016$). For further details, see the authors' website at www.pyobesity.com.

ND, not determined; i.c.v., intracerebroventricular administration; i.p., intraperitoneal administration; s.c., continuous administration by subcutaneously implanted osmotic minipump.

* The total number of animals comprises treated animals and controls.

† Transient decrease (about 70%) at 30 min only at the dose 1 nmol kg⁻¹ only. No effect with any other dose or at any other time point.

‡ No effect at 0.2 nmol dose.

§ Increase (about 60%) at 1 nmol dose.

|| No effect on total 1-h intake, but significant increase (about 23–50%) in duration of feeding behaviour in the first 10 min of test.

¶ Transient decrease (about 30%) in first 3 d, then no effect.

Decrease (about 2%) on first day, then no effect.

M. Tschöp^{1,2}, T. R. Castañeda^{1,2}, H. G. Joost²,
C. Thöne-Reineke³, S. Ortmann^{2,4}, S. Klaus²,
M. M. Hagan⁵, P. C. Chandler⁵,
K. D. Oswald⁵, S. C. Benoit¹, R. J. Seeley¹,
K. P. Kinzig⁶, T. H. Moran⁶, A. G. Beck-
Sickinger⁷, N. Koglin⁷, R. J. Rodgers⁸,
J. E. Blundell⁸, Y. Ishii⁸, A. H. Beattie⁸,
P. Holch⁸, D. B. Allison⁹, K. Raun¹⁰,
K. Madsen¹⁰, B. S. Wulff¹⁰, C. E. Stidsen¹⁰,
M. Birringer¹¹, O. J. Kreuzer¹¹,
M. Schindler¹², K. Arndt¹², K. Rudolf¹²,
M. Mark¹², X. Y. Deng¹³, D. C. Withcomb¹³,
H. Halem¹⁴, J. Taylor¹⁴, J. Dong¹⁴, R. Datta¹⁴,
M. Culler¹⁴, S. Craney¹⁵, D. Flora¹⁵,
D. Smiley¹⁵, M. L. Heiman¹⁵

¹Department of Psychiatry, University of Cincinnati Genome Research Institute, Cincinnati, Ohio 45237, USA

Ohio 45237, USA

e-mail: tschoemh@ucmail.uc.edu

²German Institute of Human Nutrition, Potsdam-Rehbrücke 14558, Germany; ³Center for Cardiovascular Research, Institute for Pharmacology and Toxicology, Charité University Hospital, Berlin 10115, Germany; ⁴Institute for Zoo and Wildlife Research, Berlin 10315, Germany; ⁵Department of Psychology, University of Alabama at Birmingham, Birmingham, Alabama 35294, USA; ⁶Department of Psychiatry and Behavioral Science, Johns Hopkins University School of Medicine, Baltimore, Maryland 21205, USA; ⁷Institute of Biochemistry, University of Leipzig, Leipzig 04103, Germany; ⁸Behavioural Pharmacology Laboratory, School of Psychology, University of Leeds, Leeds LS2 9JT, UK; ⁹Section on

Statistical Genetics, Department of Biostatistics and Clinical Nutrition Research Center, University of Alabama at Birmingham, Birmingham, Alabama 35294, USA; ¹⁰Novo Nordisk A/S, Discovery, Maalov 2760, Denmark; ¹¹Peptides and Elephants GmbH, Potsdam-Rehbrücke 14558, Germany; ¹²Boehringer-Ingelheim Pharma GmbH and Co. KG, Biberach 88397, Germany; ¹³Division of Gastroenterology, Hepatology and Nutrition, Department of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania 15213, USA; ¹⁴Biomeasure Incorporated/Ipsen Group, Milford, Massachusetts 01757, US; ¹⁵Eli Lilly & Co. Research Laboratories, Indianapolis, Indiana 46285, USA
doi:10.1038/nature02665

1. Batterham, R. L. *et al.* *Nature* **418**, 650–654 (2002).

2. Halford J. C. *et al.* *Pharmacol. Biochem. Behav.* **61**,

- 159–168 (1998).
- Kanatani, A. *et al. Endocrinology* **141**, 1011–1016 (2000).
 - Allen, J. M. *et al. Digestion* **30**, 255–262 (1984).
 - Amersham Biosciences, RPNQ0085, Neuropeptide Y SPA binding assay (Y2).
 - Halatchev, I. G. *et al. Endocrinology* **145**, 2585–2590 (2004).
 - Challis, B. G. *et al. Biochem. Biophys. Res. Commun.* **311**, 915–919 (2003).
 - Challis, B. G. *et al. Proc. Natl Acad. Sci. USA* **101**, 4695–4700 (2004).
 - Asakawa, A. *et al. Gastroenterology* **124**, 1325–1336 (2003).
 - Nordheim, U. & Hofbauer, K. G. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **286**, R138–R142 (2004).

Competing financial interests: declared. (Several of the authors consult with, and have received financial support from, companies and institutions, including pharmaceutical firms, that may have active programmes relating to PYY_{3–36}, of which we are not aware. Several of these companies have obesity-treatment development programmes and may therefore, from some perspectives, be considered competitors to companies pursuing PYY_{3–36}.)

Batterham et al. reply — The results of Tschöp *et al.*¹ on the lack of effect of peripheral administration of PYY_{3–36} on food intake in rodents are at odds with both the published literature^{2–8} (our Table 1) and with earlier data generated by the Tschöp laboratory.

It has been shown that PYY_{3–36} does not inhibit food intake in animals whose appetite is reduced by stress⁵ and this seems the likeliest explanation for the different findings now reported by Tschöp *et al.* The results of Tschöp *et al.* may also be undermined by their failure to measure the blood concentrations of PYY_{3–36} that were achieved following peripheral administration. Were they sufficient to result in inhibition of food

intake? In addition, their meta-analysis does not include earlier data generated in Tschöp's laboratory that confirm our findings⁹ (M.T., personal communication; data available from S.R.B. on request).

Tschöp *et al.* do not challenge the large body of appropriate mechanistic data that includes the inhibitory effect on food intake of intra-arcuate Y2-receptor (Y2R) agonists or PYY_{3–36}, changes in hypothalamic neuropeptide messenger RNA, the analysis of rodent hypothalamic explants and studies in Y2R-knockout mice^{4,9}. The human studies showing that PYY_{3–36} inhibits food intake^{9,10} are also uncontested.

Peripherally administered PYY_{3–36} inhibits food intake in procedure-acclimatized

Table 1 Summary of literature data on PYY_{3–36} and food intake in rodents

Species	Route	Study	Dose	Total number of animals (controls)	Effect
Wistar rats (male)	i.p.	Acute (freely feeding) ⁶	300 µg kg ⁻¹	28 (14)	Reduction in 24-h food intake*
	i.p.	Acute (dark phase) ⁹	3, 10, 30 µg kg ⁻¹	32 (8)	Reduction in 4-h food intake*
	i.p.	Acute (fasted) ⁹	3, 10, 30 µg kg ⁻¹	32 (8)	Reduction in 4-h food intake*
	i.p.	Acute (fasted) ⁹	50 µg kg ⁻¹	24 (12)	Reduction in 24-h food intake**
	i.p.	Chronic (7 d) ⁹	50 µg kg ⁻¹ d ⁻¹ (twice daily)	24 (12)	Reduction in food intake and body-weight gain**
	Intra-arcuate	Acute (fasted) ⁹	0.001, 0.01, 0.1 nmol	32 (8)	Reduction in 2-h food intake*
Diabetic Fatty Zucker rats (male)	Alzet pump	Chronic (28 d) ⁷	30, 100, 300 µg d ⁻¹	44 (14)	Reduction in cumulative food intake at highest two doses** Reduction in HbA1c and fructosamine*
Fatty Zucker obese non-diabetic rats (male)	Alzet pump	Chronic (56 d) ⁷	100 µg kg ⁻¹ d ⁻¹	20 (13)	Reduction in cumulative food intake and body weight*
129/J mice (male)	i.p.	Acute (fasted) ⁴	100 µg kg ⁻¹	16 (8)	Food intake reduction at 6 h*** and 24 h**
	i.p.	Acute (dark phase) ⁴	100 µg kg ⁻¹	16 (8)	Tendency to reduce 24-h food intake (NS)
	i.p.	Acute (freely feeding) ⁴	100 µg kg ⁻¹	20 (10)	Increased POMC mRNA at 6 h** and 24 h** Decreased NPY mRNA at 6 h**
POMC KO mice (male) and WT controls (male)	i.p.	Acute (fasted) ³	100 µg kg ⁻¹	64 (32)	Reduction in 4-h food intake**
	i.p.	Chronic (7 d) ³	100 µg kg ⁻¹ (twice daily)	32 (16)	No effect on food intake or body weight
NIH/Swiss non-obese mice (female)	i.p.	Acute (fasted) ⁷	0.1, 1, 3, 10, 30, 100, 300, 1,000 µg kg ⁻¹	192 (40)	Reduction in 1-h food intake with all doses greater than 3 µg kg ⁻¹ *
Ob/Ob ^{-/-} mice (female)	Alzet pump	Chronic (28 d) ⁷	100, 300, 1,000 µg kg ⁻¹ d ⁻¹	32 (8)	300 and 1,000 µg kg ⁻¹ d ⁻¹ doses led to reduction in cumulative food intake** and reduction in body-weight gain***
C57BL6/J DIO mice (male)	Alzet pump	Chronic (28 d) ⁷	30, 100, 300, 1,000 µg kg ⁻¹	76 (20)	Dose-dependent reduction in cumulative food intake*, body weight*** and reduced adiposity*
C57BL6/J mice (male) ⁵	i.p.	Acute (fasted) unacclimatized ⁵	3, 30, 100 µg kg ⁻¹	20 (5)	No effect on food intake in unacclimatized mice
	i.p.	Acute (fasted) ⁵	3, 30, 100 µg kg ⁻¹	20 (5)	Reduction in 4-h food intake*
	i.p.	Acute (fasted) ⁵	3, 100 µg kg ⁻¹	19 (10)	Reduction in 4-h food intake*
	i.p.	Acute (fasted) ⁵	30, 100 µg kg ⁻¹	19 (10)	Reduction in 4-h food intake*
Y2 receptor KO mice (males) and WT controls (males)	i.p.	Acute (fasted) ⁹	50 µg kg ⁻¹	40 (10)	Reduction of food intake in WT mice * No effect in Y2R KO mice
Ddy mice (male)	i.p.	Acute (fasted) ²	3 nmol	18 (9)	Reduction in 24-h food intake*
	i.p.	Acute (dark phase) ²	3 nmol	16 (8)	Reduction in 24-h food intake*
MC4R KO mice (male) and WT controls (male)	i.p.	Acute (dark phase) ⁵	3, 30, 100 µg kg ⁻¹	70 (36)	Reduction in 4-h food intake
Agouti mice (male) and WT controls (male)	i.p.	Acute (fasted) ⁸	0.02 µmol kg ⁻¹	44 (21)	Reduction in 2-h re-feeding***

Abbreviations: KO, knockout; NS, non-significant; i.p., intraperitoneal; WT, wild type; POMC, pro-opiomelanocortin; MC4R, melanocortin-4-receptor. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.