



Universiteit  
Leiden  
The Netherlands

## **Insulin sensitivity : modulation by neuropeptides and hormones**

Hoek, A.M. van den

### **Citation**

Hoek, A. M. van den. (2006, April 26). *Insulin sensitivity : modulation by neuropeptides and hormones*. Haveka B.V., Alblasserdam. Retrieved from <https://hdl.handle.net/1887/4372>

Version: Corrected Publisher's Version

License: [Licence agreement concerning inclusion of doctoral thesis in the Institutional Repository of the University of Leiden](#)

Downloaded from: <https://hdl.handle.net/1887/4372>

**Note:** To cite this publication please use the final published version (if applicable).

# Chapter 4

## **PYY<sub>3-36</sub> reinforces insulin action on glucose disposal in mice fed a high fat diet.**

Anita M. van den Hoek<sup>1,2\*</sup>, Annemieke C. Heijboer<sup>1,3\*</sup>, Eleonora P.M. Corssmit<sup>3</sup>,  
Peter J. Voshol<sup>1,3</sup>, Johannes A. Romijn<sup>3</sup>, Louis M. Havekes<sup>1,2,4</sup> and Hanno Pijl<sup>3</sup>.

\* both authors contributed equally

<sup>1</sup> TNO-Prevention and Health, <sup>2</sup> Department of Internal Medicine, <sup>3</sup> Department of  
Endocrinology and Metabolic Diseases, <sup>4</sup> Department of Cardiology.

Diabetes 53:1949-1952, 2004



## Abstract

PYY<sub>3-36</sub> is released by the gut in response to nutrient ingestion. It modulates the activities of orexigenic neuropeptide Y (NPY) neurons and anorexigenic pro-opiomelanocortin (POMC) neurons in the hypothalamus to inhibit food intake. As both NPY and POMC have been shown to also impact insulin action, we wondered whether PYY<sub>3-36</sub> could improve insulin sensitivity. To address this question, we examined the acute effect of intravenous PYY<sub>3-36</sub> on glucose and free fatty acid (FFA) flux during a hyperinsulinemic euglycemic clamp in mice maintained on a high fat diet for 2 weeks prior to the experiment. We also evaluated the effects of PYY<sub>3-36</sub> infusion on glucose uptake in muscle and adipose tissue in this experimental context. In basal conditions, none of the metabolic parameters was affected by PYY<sub>3-36</sub>. In hyperinsulinemic conditions, glucose disposal was significantly increased in PYY<sub>3-36</sub>-infused- compared with vehicle-infused mice ( $103.8 \pm 10.9$  vs.  $76.1 \pm 11.4$   $\mu\text{mol}/\text{min}/\text{kg}$ , respectively,  $P=0.001$ ). Accordingly, glucose uptake in muscle and adipose tissue was greater in PYY<sub>3-36</sub>-treated animals, although the difference with controls did not reach statistical significance in adipose tissue (muscle:  $2.1 \pm 0.5$  vs.  $1.5 \pm 0.5$   $\mu\text{mol}/\text{g}$  tissue,  $P=0.049$ ; adipose tissue:  $0.8 \pm 0.4$  vs.  $0.4 \pm 0.3$   $\mu\text{mol}/\text{g}$  tissue;  $P=0.08$ ). In contrast, PYY<sub>3-36</sub> did not impact insulin action on endogenous glucose production or FFA metabolism. These data indicate that PYY<sub>3-36</sub> reinforces insulin action on glucose disposal in mice fed a high fat diet, through a mechanism that is independent of food intake and body weight. In contrast, it leaves glucose production and lipid flux largely unaffected in this experimental context.

## Introduction

Peptide YY (PYY) belongs to a family of peptides that is critically involved in the regulation of appetite. It is synthesized in specialized cells (L-cells) that are found primarily in the distal gastro-intestinal tract. Circulating PYY levels rise within 15 minutes after a meal in proportion to the amount of calories ingested and remain elevated for about 6 hours<sup>1</sup>. The two natural forms of this peptide, PYY<sub>1-36</sub> and PYY<sub>3-36</sub>, have opposing effects on food intake<sup>2</sup>: PYY<sub>1-36</sub> stimulates appetite whereas PYY<sub>3-36</sub> (the major circulating form) inhibits feeding<sup>3-5</sup>.

PYY<sub>3-36</sub> reduces food intake by acting on appetite regulation centers in the hypothalamus<sup>3,6</sup>. Specifically, PYY<sub>3-36</sub> is an agonist of the presynaptic NPY Y2

receptor (Y2R), which inhibits NPY neuronal activity in the arcuate nucleus and thereby activates adjacent pro-opiomelanocortin (POMC) neurons<sup>3</sup>. In addition to their critical role in the control of feeding, both NPY and POMC affect insulin action. Intracerebroventricular infusion of NPY induces hyperinsulinemia and insulin resistance in rats<sup>7,8</sup>. In contrast, central administration of  $\alpha$ -melanocyte stimulating hormone ( $\alpha$ -MSH), a splice-product of the POMC peptide, enhances insulin sensitivity<sup>9</sup>. In view of the fact that PYY<sub>3-36</sub> inhibits NPY- and activates POMC neuronal activity, we wondered whether it could improve insulin sensitivity “directly” (i.e. through a mechanism independent of food intake and body weight). To address this question, we infused PYY<sub>3-36</sub> intravenously and quantified its acute effects on glucose and fatty acid flux during a hyperinsulinemic euglycemic clamp in mice without access to food during the experiment.

### Methods

**Animals.** Male C57BL/6J mice were housed in a temperature-controlled room on a 12-hour light-dark cycle and were fed a high fat diet (43 energy% fat derived from bovine lard) with free access to water for 2 weeks to induce insulin resistance. All animal experiments were performed in accordance with the regulations of Dutch law on animal welfare and the institutional ethics committee for animal procedures approved the protocol.

**Hyperinsulinemic euglycemic clamp.** Thirty-six mice were fasted overnight with food withdrawn at 05.00 pm the day prior to the study. The next day, hyperinsulinemic euglycemic clamps were performed as described earlier<sup>10</sup>. PYY or vehicle was administered as a primed (0.15  $\mu$ g) continuous (0.25  $\mu$ g/h) intravenous (i.v.) infusion during the whole experiment (basal and hyperinsulinemic period). In one series of experiments glucose turnover was quantified and in another series FFA turnover was determined. First, basal rates of glucose or FFA turnover were measured by giving a primed (p) continuous (c) infusion of <sup>3</sup>H-glucose (p: 0.7  $\mu$ Ci, c: 1.2  $\mu$ Ci/h) (Amersham, Little Chalfont, U.K.) or <sup>14</sup>C-palmitate (p: 1.8  $\mu$ Ci, c: 3  $\mu$ Ci/h) (Amersham, Little Chalfont, U.K) respectively for 80 min. Subsequently, insulin was administered in a primed (4.1 mU) continuous (6.8 mU/h) i.v. infusion for 2 to 3 hours to attain steady state circulating insulin levels of ~6 ng/ml. A variable infusion of 12.5% D-glucose was used to maintain euglycemia (measured at 10 min intervals via

tail bleeding, Freestyle, TheraSense, Disetronic Medical Systems BV, Vianen, The Netherlands). Blood samples (75  $\mu$ l) were taken during the basal period (after 60 and 80 minutes) and during the clamp period (when glucose levels were stable and 20 and 40 minutes later) for determination of plasma glucose, FFA and insulin concentrations and  $^3\text{H}$ -glucose and  $^{14}\text{C}$ -palmitate specific activities.

To assess insulin-mediated glucose uptake in individual tissues, 2-deoxy-D- [ $^3\text{H}$ ] glucose (2- [ $^3\text{H}$ ]DG; Amersham, Little Chalfont, UK) was administered as a bolus (1  $\mu\text{Ci}$ ), 40 minutes before the end of the clamp experiments in which FFA turnover was measured. At the end of the clamp, mice were sacrificed and muscle and adipose tissue were isolated and frozen in liquid nitrogen for subsequent analysis.

**Analytical procedures.** Plasma levels of glucose and FFA were determined using commercially available kits (Instruchemie, Delfzijl, The Netherlands and Wako, Neuss, Germany). Plasma insulin and PYY<sub>3-36</sub> concentrations were measured by a mouse insulin ELISA and PYY<sub>3-36</sub> RIA (Alpco, Windham, NH, USA; Phoenix pharmaceuticals, Belmont, CA, USA). Total plasma  $^3\text{H}$ -glucose was determined in 7.5  $\mu$ l plasma and in supernatants after trichloroacetic acid (20%) precipitation and water evaporation to eliminate tritiated water. Total plasma  $^{14}\text{C}$ -palmitate was determined in 7.5  $\mu$ l plasma after extraction of lipids by a modification of Bligh and Dyer's method <sup>11</sup>. Briefly, 7.5  $\mu$ l plasma was dried and resolved in 100  $\mu$ l water. Then 1.1 ml demi-water and 4.5 ml methanol:chloroform (2:1) was added and mixed thoroughly, after which 1.5 ml chloroform was added and mixed and finally, 1.5 ml demi-water was added and mixed. After centrifugation, the chloroform layer was collected and the FFA fraction was separated from other lipid components by thin-layer chromatography (TLC) on silica gel plates.

**Tissue analysis.** For determination of tissue 2-DG uptake, the homogenate of muscle and adipose tissue was boiled and the supernatant was subjected to an ion-exchange column to separate 2-DG-6-P from 2-DG as described previously <sup>10;12;13</sup>.

**Calculations.** Turnover rates of glucose and FFA ( $\mu\text{mol}/\text{min}/\text{kg}$ ) were calculated during the basal period and in steady-state clamp conditions as the rate of tracer infusion (dpm/min) divided by the plasma specific activity of  $^3\text{H}$ -glucose or  $^{14}\text{C}$ -palmitate (dpm/ $\mu\text{mol}$ ). The ratio was corrected for body weight. EGP was calculated as the difference between the tracer-derived rate of glucose appearance and the glucose infusion rate.

Tissue-specific glucose uptake in muscle and adipose tissue was calculated from tissue 2-DG content, corrected for plasma specific activity and expressed as  $\mu\text{mol}$  per gram of tissue.

**Statistical analysis.** Differences between groups were determined by Mann-Whitney non-parametric test for 2 independent samples. A P-value  $< 0.05$  was considered statistically significant. All values shown represent mean  $\pm$  SD.

## Results

**Animals.** Mice were 16-18 weeks old during the experiments. Body weight was  $23.3 \pm 1.2$  gram in the control group and  $22.8 \pm 1.4$  gram in the PYY group.

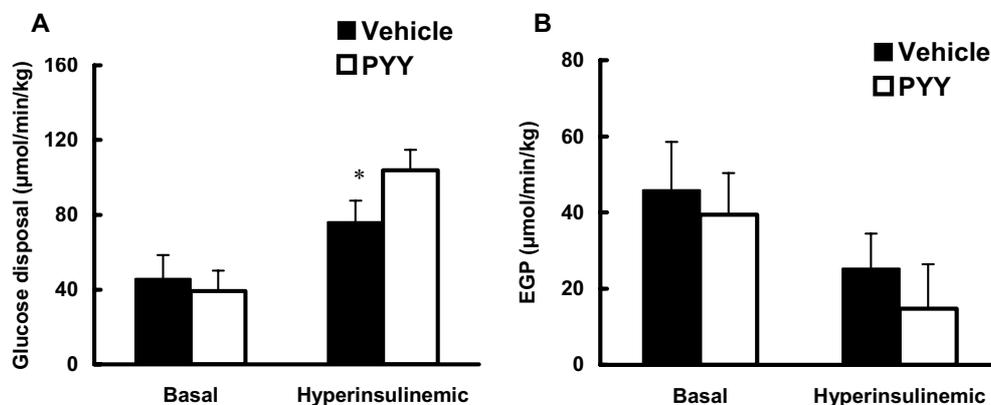
**Plasma parameters.** Plasma glucose, FFA, insulin and PYY<sub>3-36</sub> concentrations in basal and hyperinsulinemic conditions are shown in table 1. In the basal state, plasma parameters did not differ between PYY- and vehicle-infused animals, except for the plasma PYY<sub>3-36</sub> concentration. In steady state clamp conditions, plasma insulin and glucose concentrations were similar in both groups. Hyperinsulinemia suppressed FFA levels to a similar extent in PYY- and vehicle-infused mice.

**Table 1.** Plasma parameters in overnight fasted mice that received an i.v.-infusion of PYY or vehicle under basal or hyperinsulinemic conditions. Values represent mean  $\pm$  SD for at least 7 mice per group.

	Basal		Hyperinsulinemic	
	Vehicle	PYY	Vehicle	PYY
Glucose (mmol/l)	$4.9 \pm 0.6$	$5.0 \pm 0.6$	$9.0 \pm 0.9$	$9.2 \pm 2.0$
FFA (mmol/l)	$0.9 \pm 0.2$	$1.0 \pm 0.2$	$0.4 \pm 0.1$	$0.5 \pm 0.1$
Insulin (ng/ml)	$0.3 \pm 0.1$	$0.3 \pm 0.1$	$5.2 \pm 2.9$	$6.7 \pm 2.7$
PYY <sub>3-36</sub> (pg/ $\mu$ l)	$< 1$	$3.8 \pm 0.7$	$< 1$	$3.2 \pm 0.7$

**Glucose turnover.** In the basal condition, glucose disposal was similar in PYY- and vehicle-infused mice ( $39.5 \pm 10.9$  vs.  $45.9 \pm 12.6$   $\mu\text{mol}/\text{min}/\text{kg}$ , respectively;  $P=0.19$ , Figure 1a). The rate of glucose infusion necessary to maintain euglycemia during insulin infusion was significantly higher in PYY-infused mice than in vehicle-infused animals ( $89.1 \pm 7.1$  vs.  $50.9 \pm 13.6$   $\mu\text{mol}/\text{min}/\text{kg}$ ,  $P=0.001$ ), indicating that i.v. PYY<sub>3-36</sub>

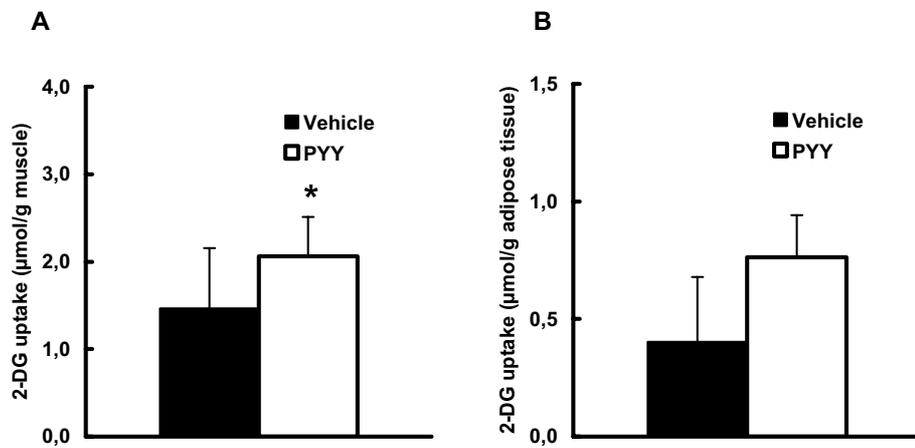
administration acutely enhances insulin sensitivity. Hyperinsulinemia increased glucose disposal in both groups. However, the disposal rate was significantly higher in PYY-infused animals compared with vehicle-infused controls ( $103.8 \pm 10.9$  vs.  $76.1 \pm 11.4 \mu\text{mol}/\text{min}/\text{kg}$ , respectively,  $P=0.001$ , Figure 1a). In contrast, endogenous glucose production (EGP), which was similar in PYY and vehicle treated mice in basal conditions, was suppressed by insulin to the same extent in both groups. (by  $62 \pm 29$  vs.  $42 \pm 18\%$  from basal respectively;  $P=0.30$ , Figure 1b).



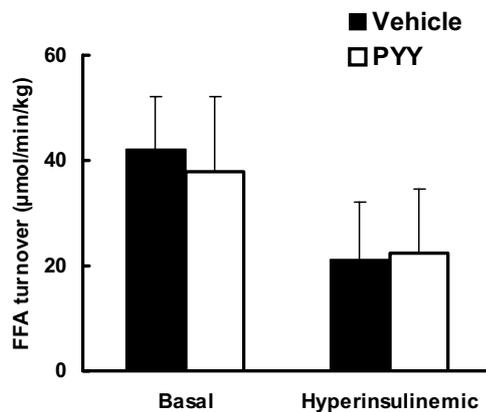
**Figure 1.** Glucose disposal (a) and endogenous glucose production (b) in overnight fasted mice before (basal) and during (hyperinsulinemic) a hyperinsulinemic euglycemic clamp. Prior to and during insulin infusion the animals received an i.v.-infusion of PYY or vehicle. Values represent mean  $\pm$  SD for at least 7 mice per group. \* $P<0.01$  vs. vehicle.

**Tissue-specific glucose uptake.** Insulin-mediated 2-deoxy-glucose uptake in muscle and adipose tissue was greater in PYY-treated animals, but the difference with controls did not reach statistical significance in adipose tissue (muscle:  $2.1 \pm 0.5$  vs.  $1.5 \pm 0.5 \mu\text{mol}/\text{g}$  tissue,  $P=0.049$ ; adipose tissue:  $0.8 \pm 0.4$  vs.  $0.4 \pm 0.3 \mu\text{mol}/\text{g}$  tissue;  $P=0.08$ , Figure 2).

**FFA turnover.** Basal free fatty acid turnover ( $38.0 \pm 14.2$  vs.  $42.3 \pm 9.9 \mu\text{mol}/\text{min}/\text{kg}$ ) was not different between PYY- and vehicle-infused animals and was suppressed to a similar extent by insulin in both groups ( $22.4 \pm 12.3$  vs.  $21.3 \pm 10.9 \mu\text{mol}/\text{min}/\text{kg}$  in PYY- and vehicle-infused animals, respectively; Figure 3).



**Figure 2.** Muscle-specific (a) and adipose tissue-specific (b) glucose uptake under hyperinsulinemic conditions in overnight fasted mice that received an i.v.-infusion of PYY or vehicle. Values represent mean  $\pm$  SD for at least 7 mice per group. \* $P < 0.05$  vs. vehicle.



**Figure 3.** Free fatty acids (FFA) turnover in overnight fasted mice before (basal) and during (hyperinsulinemic) a hyperinsulinemic euglycemic clamp. Prior to and during insulin infusion the animals received an i.v.-infusion of PYY or vehicle. Values represent mean  $\pm$  SD for at least 9 mice per group.

## Discussion

Here we show that intravenous PYY<sub>3-36</sub> administration acutely reinforces insulin action on glucose disposal in overnight fasted mice maintained on a high fat diet. In contrast, PYY<sub>3-36</sub> does not appear to impact the effect of insulin on endogenous glucose production or FFA metabolism in this experimental context.

PYY<sub>3-36</sub> clearly enhanced insulin-induced glucose disposal as determined by tracer dilution methodology. Accordingly, 2-DG uptake in muscle and adipose tissue

in hyperinsulinemic conditions was higher during PYY<sub>3-36</sub> infusion, albeit the difference with control attained statistical significance only for muscle. In contrast, PYY<sub>3-36</sub> did not significantly impact the capacity of insulin to inhibit endogenous glucose production. Insulin action on FFA metabolism was not affected by PYY<sub>3-36</sub> either, as indicated by similar fatty acid turnover rates during hyperinsulinemia in PYY<sub>3-36</sub> and saline infused animals. In light of the current experimental context, these data suggest that PYY<sub>3-36</sub> reinforces the impact of insulin on glucose disposal through a mechanism that is independent of food intake and body weight. In contrast, it appears to leave insulin action on glucose production and FFA turnover largely unaffected.

The plasma PYY<sub>3-36</sub> concentration rose to  $3.2 \pm 0.7$  pg/ $\mu$ l in response to PYY infusion. Relatively few studies have looked at the physiology of circulating PYY<sub>3-36</sub> in rodents. According to a recent paper by Batterham et al.<sup>3</sup>, postprandial PYY<sub>3-36</sub> concentrations amount to 112 pmol/l ( $\sim 0.4$  pg/ $\mu$ l) in freely feeding rats. In a study by Lee et al.<sup>14</sup> plasma PYY levels in fasting mice were 0.18 pg/ $\mu$ l, which accords with our data (table 1). We are not aware of any study measuring postprandial PYY<sub>3-36</sub> concentrations in mice. Thus, the dose of PYY<sub>3-36</sub> we administered may have induced supraphysiological PYY<sub>3-36</sub> levels. Therefore, our data do not allow a definitive inference as to whether the postprandial rise of circulating PYY<sub>3-36</sub> can reinforce insulin action.

Postprandial PYY<sub>3-36</sub> release is decreased in obese compared with lean subjects and circulating levels correlate negatively with body mass index. Conversely, intravenous PYY<sub>3-36</sub> infusion significantly reduces food intake in humans<sup>15</sup> and repeated administration of PYY<sub>3-36</sub> attenuates weight gain in rodents<sup>3</sup>. These findings suggest that diminished PYY<sub>3-36</sub> release may contribute to the pathogenesis of obesity in animals and man. The observations presented here allow us to hypothesize that low circulating PYY<sub>3-36</sub> levels may also compromise insulin action in obese subjects. Moreover, perhaps even more important, the data suggest that PYY<sub>3-36</sub> or synthetic analogues of this peptide may be useful tools in the clinical management of obesity and insulin resistance.

It remains to be established whether PYY<sub>3-36</sub> acts through hypothalamic neural circuits (in analogy of the mechanism guiding its effects on appetite) to enhance insulin-mediated glucose disposal. As PYY<sub>3-36</sub> is a high affinity agonist to the Y2R<sup>2</sup> and the distribution of this receptor subtype is largely confined to the central nervous

system (particularly the arcuate nucleus of the hypothalamus)<sup>16</sup>, it is most likely that PYY<sub>3-36</sub> modulates insulin action by activation of Y2R in the brain. As alluded to earlier, Y2R signaling inhibits NPY neuronal activity in the arcuate nucleus of the hypothalamus and thereby activates adjacent POMC neurons<sup>3</sup>. Hypothalamic overexpression of NPY induces obesity and insulin resistance in mice<sup>7;8</sup>, whereas activation of melanocortin receptor subtypes 3 and 4 in the brain enhances insulin sensitivity<sup>9</sup>. Thus, the present data are in keeping with the contention that PYY<sub>3-36</sub> modulates insulin action via hypothalamic Y2R. The downstream mechanisms that actuate the effects of hypothalamic neuronal circuits on muscle and adipose tissue remain to be fully elucidated. At this point, vagotomy was shown to prevent the hyperinsulinemic effects of NPY, which suggests that the autonomic nervous system may be involved<sup>17</sup>. Also, adrenalectomy prevents the obesity syndrome produced by chronic central NPY infusion and reverses the obese phenotype in leptin-deficient ob/ob mice<sup>18;19</sup>, indicating that the pituitary-adrenal ensemble may also serve as a second messenger. Thus, neural and/or endocrine mechanistic routes may convey signals from hypothalamic nuclei to peripheral tissues to orchestrate energy balance and fuel flux. It is conceivable that similar routes partake in the control of insulin action by PYY<sub>3-36</sub>.

In conclusion, PYY<sub>3-36</sub> reinforces insulin action in mice maintained on a high fat diet through a mechanism that is independent of food intake and body weight. In this context, PYY<sub>3-36</sub> appears to predominantly impact insulin-mediated glucose disposal, whereas it leaves insulin action on glucose production largely unaffected. These novel data suggest that PYY<sub>3-36</sub> or synthetic analogues of this peptide may be valuable assets to our armamentarium of drugs designed to battle insulin resistance and type 2 diabetes mellitus.

### **Acknowledgements**

The research described in this paper is supported by the Dutch Scientific Research Council / Netherlands Heart foundation (project 980-10-017 and 907-00-002). This study is conducted in the framework of the "Leiden Center for Cardiovascular Research LUMC-TNO".

## Reference List

- 1 Adrian TE, Ferri GL, Bacarese-Hamilton AJ, Fuesl HS, Polak JM, Bloom SR. Human distribution and release of a putative new gut hormone, peptide YY. *Gastroenterology* 1985; 89: 1070-1077.
- 2 Grandt D, Schimiczek M, Beglinger C *et al.* Two molecular forms of peptide YY (PYY) are abundant in human blood: characterization of a radioimmunoassay recognizing PYY 1-36 and PYY 3-36. *Regul.Pept.* 1994; 51: 151-159.
- 3 Batterham RL, Cowley MA, Small CJ *et al.* Gut hormone PYY(3-36) physiologically inhibits food intake. *Nature* 2002; 418: 650-654.
- 4 Morley JE, Levine AS, Grace M, Kneip J. Peptide YY (PYY), a potent orexigenic agent. *Brain Res.* 1985; 341: 200-203.
- 5 Hagan MM, Moss DE. Effect of peptide YY (PYY) on food-associated conflict. *Physiol Behav.* 1995; 58: 731-735.
- 6 Challis BG, Pinnock SB, Coll AP, Carter RN, Dickson SL, O'Rahilly S. Acute effects of PYY3-36 on food intake and hypothalamic neuropeptide expression in the mouse. *Biochem.Biophys.Res.Commun.* 2003; 311: 915-919.
- 7 Marks JL, Waite K. Intracerebroventricular neuropeptide Y acutely influences glucose metabolism and insulin sensitivity in the rat. *J Neuroendocrinol* 1997; 9: 99-103.
- 8 Zarjevski N, Cusin I, Vettor R, Rohner-Jeanrenaud F, Jeanrenaud B. Chronic intracerebroventricular neuropeptide-Y administration to normal rats mimics hormonal and metabolic changes of obesity. *Endocrinology* 1993; 133: 1753-1758.
- 9 Obici S, Feng Z, Tan J, Liu L, Karkanas G, Rossetti L. Central melanocortin receptors regulate insulin action. *J.Clin.Invest* 2001; 108: 1079-1085.
- 10 Voshol PJ, Jong MC, Dahlmans VE *et al.* In muscle-specific lipoprotein lipase-overexpressing mice, muscle triglyceride content is increased without inhibition of insulin-stimulated whole-body and muscle-specific glucose uptake. *Diabetes* 2001; 50: 2585-2590.
- 11 Bligh EG, Dyer WJ. A rapid method of total lipid extraction and purification. *Can J Biochem Biophys* 1959; 37: 911-917.
- 12 Rossetti L, Giaccari A. Relative contribution of glycogen synthesis and glycolysis to insulin-mediated glucose uptake. A dose-response euglycemic clamp study in normal and diabetic rats. *J.Clin.Invest* 1990; 85: 1785-1792.
- 13 Goudriaan JR, Dahlmans VE, Teusink B *et al.* CD36 deficiency increases insulin sensitivity in muscle, but induces insulin resistance in the liver in mice. *J.Lipid Res.* 2003; 44: 2270-2277.
- 14 Lee HM, Udipi V, Englander EW, Rajaraman S, Coffey RJ, Jr., Greeley GH, Jr. Stimulatory actions of insulin-like growth factor-I and transforming growth factor-alpha on intestinal neurotensin and peptide YY. *Endocrinology* 1999; 140: 4065-4069.
- 15 Batterham RL, Cohen MA, Ellis SM *et al.* Inhibition of food intake in obese subjects by peptide YY3-36. *N.Engl.J.Med* 2003; 349: 941-948.
- 16 Parker E, Van Heek M, Stamford A. Neuropeptide Y receptors as targets for anti-obesity drug development: perspective and current status. *Eur.J.Pharmacol.* 2002; 440: 173-187.

## Chapter 4

---

- 17 Sainsbury A, Rohner-Jeanrenaud F, Cusin I *et al.* Chronic central neuropeptide Y infusion in normal rats: status of the hypothalamo-pituitary-adrenal axis, and vagal mediation of hyperinsulinaemia. *Diabetologia* 1997; 40: 1269-1277.
- 18 Sainsbury A, Cusin I, Rohner-Jeanrenaud F, Jeanrenaud B. Adrenalectomy prevents the obesity syndrome produced by chronic central neuropeptide Y infusion in normal rats. *Diabetes* 1997; 46: 209-214.
- 19 Makimura H, Mizuno TM, Roberts J, Silverstein J, Beasley J, Mobbs CV. Adrenalectomy reverses obese phenotype and restores hypothalamic melanocortin tone in leptin-deficient ob/ob mice. *Diabetes* 2000; 49: 1917-1923.