# Insulin sensitivity : modulation by the gut-brain axis

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# Chapter 6

## Chronic $PYY_{3-36}$ treatment ameliorates insulin resistance in C57BL6-mice on a high fat diet

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*Submitted*

## ABSTRACT

***Aims/hypothesis.*** **$PYY_{3-36}$ is a gut-derived hormone, that acts on hypothalamic nuclei to modulate energy metabolism. We recently showed, that $PYY_{3-36}$ acutely reinforces insulin action on glucose disposal in insulin resistant mice. However the long-term effects of $PYY_{3-36}$ on insulin sensitivity are still unknown.**

***Methods.*** **To adress this question, we examined the effects of chronic $PYY_{3-36}$ administration (2.5 µg/day s.c. for 7 days) on glucose turnover during a hyperinsulinemic-euglycemic clamp in C57BL6 mice maintained on a high fat diet for 16 weeks before the experiment. In addition, metabolic efficacy of continuous *vs.* intermittent administration of $PYY_{3-36}$ was evaluated.**

***Results.*** **Under hyperinsulinemic conditions, glucose disposal was significantly increased in $PYY_{3-36}$ treated mice *vs.* vehicle-treated mice (78.8 ± 13.3 *vs.* 63.4 ± 15.5 µmol/min/kg, respectively, *P*=0.012). Tissue specific glucose uptake was significantly increased in adipose tissue (0.5 ± 0.2 *vs.* 0.2 ± 0.1 µmol/ g tissue; *P*=0.006), but not in muscle (2.2 ± 1.4 *vs.* 1.6 ± 0.8 µmol/ g tissue for $PYY_{3-36}$ and vehicle-treated animals, respectively, *P*=0.38) of $PYY_{3-36}$ treated animals. In contrast, insulin action on endogenous glucose production was not significantly affected. Furthermore, none of these metabolic parameters were affected by the mode of $PYY_{3-36}$ administration (continuous or intermittent).**

***Conclusions/interpretation.*** **Chronic $PYY_{3-36}$ administration enhances the ability of insulin to promote glucose disposal, whereas it does not significantly affect endogenous glucose production in C57BL6 mice maintained on a high fat diet for 16 weeks. In addition, this study shows that continuous and intermittent administration are equally effective in this respect.**

## INTRODUCTION

The metabolic syndrome comprises a cluster of anomalies that increase the risk of cardiovascular disease and type 2 diabetes mellitus: hyperglycemia, abdominal obesity, hypertriglyceridemia, hypertension and low levels of high-density lipoprotein (HDL) cholesterol [1-3]. Insulin resistance may underlie the majority of these pathologies [4] and therapies that effectively reinforce insulin action may therefore ameliorate the risk profile of metabolic syndrome patients [5;6].

Diet-induced obese, insulin resistant C57BL6-mice have increased levels of neuropeptide Y (NPY) and decreased levels of pro-opiomelanocortin (POMC) in hypothalamic nuclei [7-9]. These features of hypothalamic neural circuits may be involved in the pathogenesis of the metabolic syndrome, as intracerebroventricular (icv) administration of NPY or antagonists of POMC induce insulin resistance [10-13]. Therefore, antagonists of NPY and/or agonists of POMC signalling may be useful tools in the clinical management of this syndrome. Peptide $YY_{3-36}$ ($PYY_{3-36}$) is released in response to food intake by L-cells in the distal gastrointestinal tract. It acts via Y2 receptors on NPY neurons in the arcuate nucleus to inhibit NPY neuronal activity and thereby activates adjacent POMC neurons [14;15]. We recently found that $PYY_{3-36}$ administration acutely reinforces insulin action on glucose disposal through a mechanism that is independent of food intake and body weight [16]. This finding suggests that $PYY_{3-36}$ may be used as a therapeutic tool in the clinical management of insulin resistance and the metabolic syndrome. However, the metabolic effects of long-term $PYY_{3-36}$ administration are currently unknown, and waning of early impact may occur during chronic treatment through down regulation of receptor expression or function [17;18]. Therefore, the aim of this study was to investigate the long-term effects of $PYY_{3-36}$ on insulin action by administering $PYY_{3-36}$ subcutaneously for 7 days in mice fed a high-fat diet, and quantifying the effects on glucose production and disposal during a hyperinsulinemic euglycemic clamp study. As the physiology of $PYY_{3-36}$ entails intermittent release in response to food intake, we also examined whether continuous and intermittent administration of $PYY_{3-36}$ impact glucose metabolism differentially in this experimental context.

## MATERIAL AND METHODS

*Animals*

Male C57BL6 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 16 weeks to induce insulin resistance. After 15 weeks of high fat diet, osmotic minipumps (Alzet minipump, model 2001, Charles River, Maastricht, The Netherlands) were placed subcutaneously in the back region under light isoflurane anesthesia. All mice received

a saline (n = 15) or $PYY_{3-36}$ (2.5 µg/day, n = 5) infusion via the osmotic minipump at a rate of 0.5 µl/h for 7 days. In addition, daily subcutaneous injections (50 µl at 09.00 am) of saline or $PYY_{3-36}$ (2.5 µg) were given, where mice receiving continuous $PYY_{3-36}$ treatment were additionally injected with saline, and mice receiving saline by minipump were assigned to receive either saline (n = 8) or $PYY_{3-36}$ (n = 7) by injection. Thus, glucose kinetics were determined in 2 experimental groups: 1) receiving saline and 2) receiving $PYY_{3-36}$, where $PYY_{3-36}$ was administered continuously by minipump or intermittently by daily subcutaneous injection. 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*

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 [19]. First, basal rates of glucose turnover were measured by giving a primed (0.7 µCi) continuous (1.2 µCi/h) infusion of $^{14}$C-glucose (Amersham, Little Chalfont, U.K.) 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 ~3.5 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 µl) 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, non-esterified fatty acids (NEFA), insulin and $PYY_{3-36}$ concentrations and $^{14}$C-glucose specific activities.

To assess insulin-mediated glucose uptake in individual tissues, 2-deoxy-D-[$^3$H] glucose (2-[$^3$H]DG; Amersham, Little Chalfont, UK) was administered as a bolus (1µCi), 40 minutes before the end of the clamp experiments. 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 NEFA were determined using commercially available kits (Instruchemie, Delfzijl, The Netherlands and Wako, Neuss, Germany). Plasma insulin and $PYY_{3-36}$ concentrations were measured by a mouse insulin ELISA and $PYY_{3-36}$ RIA (Mercodia, Uppsala, Sweden; Phoenix pharmaceuticals, Belmont, CA, USA; sensitivity of 1 pg/µl for the $PYY_{3-36}$ RIA). Total plasma $^{14}$C-glucose was determined in 7.5 µl plasma and in

supernatants after trichloroacetic acid (20%) precipitation and water evaporation to eliminate tritiated water.

*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 [19-21].

*Calculations*

Turnover rates of glucose (µmol/min/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 $^{14}$C-glucose (dpm/µmol). 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 adiopose tissue was calculated from tissue 2-DG content, corrected for plasma specific activity and expressed as µmol 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 means ± SD.

## RESULTS

*Animals*

Body weight did not differ between $PYY_{3-36}$ and vehicle-infused animals (after 7 days of $PYY_{3-36}$/saline administration: 28.0 ± 3.7 gram in the $PYY_{3-36}$ group and 28.3 ± 1.5 gram in the control group, P=0.68). Overnight food intake was measured at day 2 and day 5 of $PYY_{3-36}$/saline administration and was similar in both groups (day 2: 2.37 ± 0.68 vs. 2.32 ± 0.33 gram, P=0.96; day 5: 2.76 ± 0.54 vs. 2.75 ± 0.43 gram, P=0.97 in $PYY_{3-36}$ and vehicle-treated animals, respectively). Furthermore, body weight and overnight food intake was not different in groups receiving continuous or intermittent $PYY_{3-36}$ treatment (body weight: 29.5 ± 3.9 vs. 26.9 ± 3.4 gram, P=0.20; food intake day 2: 2.14 ± 0.98 vs. 2.48 ± 0.56 gram, P=0.38; day 5: 2.53 ± 0.69 vs. 2.92 ± 0.39 gram, P=0.27 for continuous and intermittent administration, respectively).

**Table 1.** Plasma parameters under basal or hyperinsulinemic conditions in overnight fasted mice that received $PYY_{3-36}$ (n=12) or vehicle (n=8) for 7 days.

| | Basal | | Hyperinsulinemic | |
|---|---|---|---|---|
| | Vehicle | $PYY_{3-36}$ | Vehicle | $PYY_{3-36}$ |
| Glucose (mmol/l) | 7.7 ± 1.3 | 8.4 ± 1.5 | 8.4 ± 1.2 | 9.4 ± 0.8 |
| NEFA (mmol/l) | 1.1 ± 0.2 | 0.9 ± 0.2 | 0.6 ± 0.1 | 0.5 ± 0.1 |
| Insulin (ng/ml) | 0.7 ± 0.3 | 0.7 ± 0.4 | 3.2 ± 0.9 | 3.6 ± 0.8 |

Data are the means ± SD. * <0.05 *vs.* vehicle

*Plasma parameters*

Plasma glucose, NEFA, and insulin concentrations in basal and hyperinsulinemic conditions are shown in table 1. Plasma glucose and insulin concentrations did not differ between vehicle and $PYY_{3-36}$ treated animals under basal and steady state clamp conditions. Furthermore, continuous and intermittent $PYY_{3-36}$ administration had similar impact on these parameters, except for the plasma glucose levels under basal conditions, which were slightly but significantly higher in the group that received continuous $PYY_{3-36}$ administration (basal glucose: 9.3 ± 0.9 vs. 7.7 ± 1.5 mmol/l, P=0.048; hyperinsulinemic glucose: 9.9 ± 0.8 vs. 9.1 ± 0.6 mmol/l, P=0.073; basal insulin: 0.9 ± 0.4 vs. 0.5 ± 0.3 ng/ml, P=0.073; hyperinsulinemic insulin: 3.9 ± 1.0 vs. 3.4 ± 0.6 ng/ml, P=0.43). Plasma NEFA concentrations were slightly, but significantly, lower in $PYY_{3-36}$ treated mice in basal (P=0.025) and steady state clamp (P=0.031) conditions, where continuous and intermittent $PYY_{3-36}$ administration did not have differential effects (basal NEFA: 0.9 ± 0.3 vs. 0.9 ± 0.1 mmol/l, respectively, P=0.76; hyperinsulinemic NEFA: 0.5 ± 0.1 vs. 0.4 ± 0.1 mmol/l, respectively, P=0.073). Plasma $PYY_{3-36}$ concentrations in basal and hyperinsulinemic conditions were below the

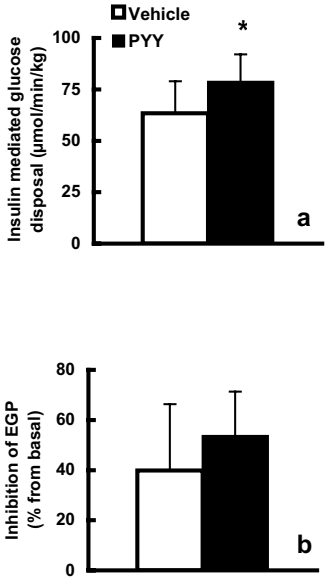


**Fig 1.** Insulin mediated glucose disposal (a) and inhibition of endogenous glucose production (EGP) by insulin (b) in overnight fasted mice before (basal) and during (hyperinsulinemic) a hyperinsulinemic euglycemic clamp study. Prior to the clamp experiment the animals received $PYY_{3-36}$ (n=12) or vehicle (n=8) for 7 days. Values represent the means ± SD. *P<0.05 *vs.* vehicle.

detection level in all groups (<1 pg/µl), except for the basal condition of the mice that received $PYY_{3-36}$ administration (3.7 ± 0.8 pg/µl).

*Glucose turnover*

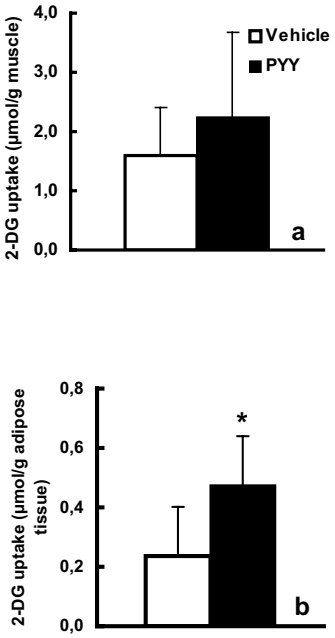


**Fig 2.** Muscle-specific (a) and adipose tissue-specific (b) glucose uptake under hyperinsulinemic conditions in overnight fasted mice that received $PYY_{3-36}$ (n=11) or vehicle (n=7) for 7 days. Values represent the means ± SD. *P<0.05 vs. vehicle.

In basal conditions, glucose disposal was similar in $PYY_{3-36}$ and vehicle-treated mice (52.0 ± 10.5 *vs.* 50.4 ± 10.4 µmol/min/kg, respectively, P=0.68). The rate of glucose infusion necessary to maintain euglycemia during insulin infusion was significantly higher in $PYY_{3-36}$ treated mice than in vehicle-treated animals (54.0 ± 11.4 *vs.* 33.4 ± 11.6 µmol/min/kg, *P*=0.000), indicating that chronic $PYY_{3-36}$ administration enhances whole body insulin sensitivity. Continuous and intermittent administration of $PYY_{3-36}$ had similar effects on the glucose infusion rate (54.7 ± 9.2 *vs.* 53.6 ± 10.2 µmol/min/kg, respectively, P=0.27). Hyperinsulinemia increased glucose disposal in both groups. However, the disposal rate was significantly higher in $PYY_{3-36}$ treated animals compared with vehicle-treated controls (78.8 ± 13.3 *vs.* 63.4 ± 15.5 µmol/min/kg, respectively, *P*=0.012, Figure 1a) and was similar in animals treated by continuous and intermittent administration (81.2 ± 13.8 *vs.* 77.1 ± 13.7 µmol/min/kg, respectively, P=0.64). Endogenous glucose production was similar in $PYY_{3-36}$ and vehicle-treated mice in basal conditions and was suppressed by insulin to the same extent in both groups (by 54 ± 18 *vs.* 40 ± 26% from basal in $PYY_{3-36}$ *vs.* vehicle treated groups, respectively; *P*=0.27, Figure 1b), where percent inhibition did not differ between animals receiving continuous or intermittent $PYY_{3-36}$ treatment. (52 ± 25 *vs.* 55 ± 12% from basal, respectively, P=0.53).

*Tissue-specific glucose uptake*

Insulin-mediated 2-deoxy-glucose uptake was measured in muscle and adipose tissue. In muscle, 2-deoxy-glucose was similar in both groups (2.2 ± 1.4 *vs.* 1.6 ± 0.8 µmol/ g tissue for $PYY_{3-36}$ and vehicle-treated animals, respectively, *P*=0.38). In adipose tissue 2-deoxy-glucose uptake was significantly increased in $PYY_{3-36}$ treated animals compared with vehicle treated mice (0.5 ± 0.2 *vs.* 0.2 ± 0.1 µmol/ g tissue; *P*=0.006, Figure 2).

## DISCUSSION

Here we show that chronic $PYY_{3-36}$ administration improves whole body insulin sensitivity of glucose metabolism in C57BL6 mice maintained on a high fat diet for 16 weeks. In particular, $PYY_{3-36}$ treatment enhances the ability of insulin to promote glucose disposal via mechanistic routes that are independent of food intake or body weight. In addition, this study documents that continuous and intermittent administration of $PYY_{3-36}$ reinforce insulin action to a similar extent.

These data corroborate our previous findings, which unveil similar acute effects of $PYY_{3-36}$ administration on insulin action [16], and support the emerging concept of neural circuits controlling fuel flux, independent of their impact on food intake and body weight. In addition, our data indicate that the effects of $PYY_{3-36}$ on glucose metabolism do not wane during chronic treatment, which suggests that this peptide may be a novel asset in the battle against insulin resistance and the metabolic syndrome.

Although $PYY_{3-36}$ enhanced insulin-induced glucose disposal, it did not significantly affect the ability of insulin to inhibit endogenous glucose production. Nonetheless, we can not exclude the possibility that the experimental group size may have limited the statistical power necessary to detect a subtle influence of $PYY_{3-36}$ on hepatic glucose metabolism. Alternatively, $PYY_{3-36}$ exerts differential, tissue specific, effects on insulin action.

The mechanism by which $PYY_{3-36}$ affects insulin-mediated glucose metabolism remains to be elucidated. Perhaps, $PYY_{3-36}$ modulates insulin action via the hypothalamic Y2 receptor, in analogy with the mechanism guiding its effects on appetite. Y2-receptor mediated inhibition of NPY and stimulation of POMC neuronal activity by $PYY_{3-36}$ potentially reinforces insulin action on glucose metabolism indeed [10;11;13].

Circulating $PYY_{3-36}$ levels in fasting conditions remained below the level of detection (< 1 pg/μl) during continuous treatment, and rose to 3.7 ± 0.8 pg/μl approximately one hour after i.p injection. During hyperinsulinemia (3-3.5 hours after injection), $PYY_{3-36}$ levels were undetectable by our assay in all animals. Thus, in spite of the fact that continuous $PYY_{3-36}$ treatment did not produce measurable plasma concentrations and intermittent administration induced a merely transitory increase of circulating $PYY_{3-36}$, both treatments significantly facilitated insulin mediated glucose disposal in high fat fed animals. Relatively few papers report plasma $PYY_{3-36}$ concentrations in rodents. Postprandial levels may be in the range of 112 pmol/L (~ 0.4 pg/μl) and 0.18 pg/μl in freely feeding normal weight rats and mice respectively [14;22], whereas fasting levels are considerably lower, as $PYY_{3-36}$ is primarily released in response to food intake [14;23]. Plasma $PYY_{3-36}$ concentrations in high fat fed mice are unknown, but may be significantly reduced, as obese humans have clearly diminished circulating $PYY_{3-36}$ levels [24]. Taken together, our data suggest, that even a relatively low dose of $PYY_{3-36}$ (in view of the low circulating $PYY_{3-36}$ levels during treatment)

can reinforce insulin action. Further dose-response experiments are warranted to evaluate the potential efficacy of $PYY_{3-36}$ in the treatment of the metabolic syndrome.

Food intake and body weight were not affected by $PYY_{3-36}$ administration in our study. These findings agree with data from Challis *et al.*, indicating that 7 days of $PYY_{3-36}$ administration did not affect food intake and body weight in $POMC^{-/-}$ and wild type mice [25]. In contrast, Batterham *et al.* reported that $PYY_{3-36}$ acutely inhibits food intake [14], an observation that could not be reproduced by Tschöp and coworkers [26;27]. To take this issue further, we compared the acute effects of a single intraperitoneal (2.5 µg) injection of $PYY_{3-36}$ (n = 8) or vehicle (n = 8) at 09.00 am on food intake in our animals, and found that cumulative food intake in 4 hours after injection was significantly inhibited by 21% in overnight fasted mice (P=0.028), whereas subsequent feeding over 24 hours was not affected by $PYY_{3-36}$. These data suggest that this dose of $PYY_{3-36}$ has a short-term inhibitory impact on food intake in overnight fasted C57BL6 mice, whereas consumption over 24 hours is not affected, probably as a result of a rebound compensatory increase of appetite [14;15].

In conclusion, the present study shows that chronic $PYY_{3-36}$ administration reinforces insulin action on glucose disposal in mice maintained on a high fat diet, whereas it also tends to enhance the ability of insulin to suppress endogenous glucose production. These observations suggest that $PYY_{3-36}$ or potential analogues may be a useful treatment for insulin resistance and the metabolic syndrome.

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