

# Low serum PYY is linked to insulin resistance in first-degree relatives of subjects with type 2 diabetes

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## Abstract

Low circulating peptide YY (PYY) levels are reported in obese and type II diabetic subjects and results from PYY knockout animals suggests that PYY deficiency may have a causative role in the etiology of obesity and type 2 diabetes. Here, our aims were to determine whether people with a genetic predisposition to developing type 2 diabetes and obesity differ from otherwise similar subjects without such family history, in fasting or meal-related PYY levels, fasting insulin, insulin secretion (HOMA-B) and insulin sensitivity. We also investigated whether PYY ablation affects the intrinsic ability of islets to secrete insulin, which may be a contributing factor to the hyperinsulinemia observed in PYY knockout mice.

Healthy female first-degree relatives of people with type 2 diabetes were matched for age, gender and BMI to control subjects but had significantly lower insulin sensitivity ( $p < 0.05$ ). Relatives also had significantly lower fasting serum PYY levels than controls ( $p < 0.05$ ), but their PYY response to a high fat meal (4250 kJ, 73% fat) was not significantly different. Fasting PYY level correlated positively with glucose infusion rate ( $r = 0.713$ ,  $p = 0.002$ ) and fasting adiponectin ( $r = 0.5$ ,  $p = 0.02$ ). Islets of Langerhans from PYY knockout mice were found to hypersecrete insulin in response to 25 mM glucose ( $p < 0.05$ ). These data demonstrate that lack of PYY enhances insulin secretion from the Islets of Langerhans and that low fasting PYY levels are associated with insulin resistance in humans. Together, these findings suggest that low circulating levels of PYY could contribute to hyperinsulinemia and insulin resistance, and possibly contribute to subsequent development of obesity and type 2 diabetes.

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**Keywords:** PYY; Hyperinsulinemia; Insulin; Insulin resistance; Insulin sensitivity; Type II diabetes; First-degree relatives

## 1. Introduction

Peptide YY (PYY) is an enteroendocrine hormone implicated in the pathogenesis of human and rodent

obesity (Batterham et al., 2002; Roth et al., 2005; Alvarez Bartolome et al., 2002). It is a member of the neuropeptide Y family, and is secreted from the L cells of the gastrointestinal tract in response to food (Batterham et al., 2002). There are two endogenous forms of PYY: PYY1-36 and PYY3-36. The latter is produced by removal of two N terminal amino acids from the full-length form by an enzyme called dipeptidyl peptidase-IV (DPP-IV) (Renshaw and Batterham, 2005).

In humans, PYY3-36 is the main form produced postprandially, contributing to approximately 63% of circulating PYY in the fed state and only 37% in the fast-

*Abbreviations:* AUC, area under the curve; BMI, body mass index; DPP-IV, dipeptidyl peptidase-IV; DXA, dual-energy X-ray absorptiometry; GIR, glucose infusion rate; HOMA-B, HOMA-Beta; PYY, peptide YY; RIA, radioimmunoassay.

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ing state (Grandt et al., 1994). Circulating PYY levels increase within 15 min of ingesting a meal, peak at approximately 60 min and remain elevated for up to 6 h (Adrian et al., 1985). Dietary fat is the most potent stimulant of PYY release (Adrian et al., 1985).

Recent studies have investigated the effects of PYY3-36 on satiety, food intake and body weight in animals and humans (Batterham et al., 2003; Batterham et al., 2002). It appears that PYY3-36 acts on the arcuate nucleus in the hypothalamus to reduce food intake and body weight, possibly via inhibiting NPY neurons and stimulating POMC expressing neurons via Y2 receptors (Batterham et al., 2002). It has also been demonstrated that PYY3-36 acts via regions of the brainstem, the area postrema and nucleus tractus solitarius, to reduce food intake by inducing an aversive response (Halatchev and Cone, 2005).

Since PYY reduces food intake and body weight, and because some studies report reduced circulating PYY concentrations in obese people, it is hypothesized that low circulating PYY levels are an etiological factor in the development of obesity. Indeed, obese children (Roth et al., 2005) and morbidly obese patients (Alvarez Bartolome et al., 2002) have significantly lower fasting PYY levels than non-obese subjects. However, whether circulating PYY levels contribute to or result from obesity cannot be determined from cross-sectional studies in subjects already widely different in adiposity. One study reported lower postprandial PYY levels in obese compared to lean adolescents, with no difference in fasting PYY levels between groups (Stock et al., 2005), and another found no differences in fasting PYY levels or PYY secretion in response to oral glucose between lean and obese people, also contradicting previous reports that fasting PYY was inversely correlated with BMI (Kim et al., 2005). Interestingly, this study also highlighted that females produce a larger PYY response to a meal than male participants (Kim et al., 2005).

Human and animal studies suggest that low circulating levels of PYY may be involved in the etiology of type 2 diabetes and associated obesity. Intravenous infusion of PYY3-36 for several hours in diet-induced insulin-resistant mice enhanced insulin sensitivity, as assessed by hyperinsulinemic-euglycemic clamp (van den Hoek et al., 2004). Moreover, a 4-week subcutaneous infusion of PYY3-36 by osmotic pump in diabetic fatty Zucker rats also improved glycemic indices (HbA1c and fructosamine) (Pittner et al., 2004). Recently it has been demonstrated that diet-induced obese mice and rats that were administered PYY3-36 subcutaneously for 28 days consecutively displayed increased glucose tolerance compared to vehicle-treated animals, although this was not statistically significant (Vrang et al., 2006). Diet-induced obese rats administered the highest dose of PYY3-36 showed significantly lower fasting serum insulin levels than fasted vehicle-treated mice. Moreover, PYY3-36 dose-dependently

decreased the area under the insulin response curves after glucose injection in diet-induced obese rats (Vrang et al., 2006). Together these animal studies imply an important role of PYY on both insulin sensitivity and glycemic control. Genetic linkage studies suggest a common Ag72Thr variant in the human PYY gene is modestly associated with type 2 diabetes (Torekov et al., 2005). Furthermore, the circulating PYY response to a meal was blunted in people with type 2 diabetes compared to BMI-matched control subjects, although fasting PYY levels were significantly increased in subjects with diabetes (English et al., 2004). Nonetheless, confusion still exists as to whether low fasting and/or meal-induced levels of PYY are a cause or a consequence of type 2 diabetes or associated obesity.

To study the potential etiological role of PYY in the development of obesity or type 2 diabetes before the disease developed, we investigated fasting and meal-induced circulating PYY levels in healthy women who have first degree relatives with type 2 diabetes mellitus. These women have a strong genetic predisposition to type 2 diabetes and obesity. Two out of three such relatives eventually develop diabetes by glucose tolerance testing (Kahn et al., 1969), and the majority of people with type 2 diabetes become overweight or obese (Drivsholm and Olivarius, 2005). The relatives were compared with a matched control group without any family history of diabetes. Any detectable differences between groups (before excess adiposity or type 2 diabetes developed) could imply low PYY plays a part in the development of obesity and/or the pathophysiology of type 2 diabetes.

Following our previous observations that PYY ablation in mice leads to hyperinsulinemia *in vivo*, particularly in response to a bolus injection of glucose (Boey et al., 2006), our aims were to address whether hyperinsulinemia in PYY knockout animals was a result of a change in the intrinsic ability of their islets to secrete insulin. Here, we isolated islets from PYY knockout and wild type animals and evaluated insulin secretion and beta cell function under basal, moderate and high concentrations of glucose in order to determine the overall effect of PYY deficiency on islet function (Boey et al., 2006). Our hypothesis was that there is a predisposing defect in PYY secretion prior to the development of type 2 diabetes, which influences the later increased adiposity. This defect may also contribute to glucose intolerance, possibly through effects on insulin secretion and/or insulin sensitivity.

## 2. Research design and methods

### 2.1. Subjects

A total of 21 healthy, sedentary, non-smoking female volunteers, aged 20–45 years, without cardiovascular

disease, hypertension, endocrine disorders, or dyslipidemia were recruited. The relative group ( $n=12$ ) had two first-degree relatives (at least one parent) with type 2 diabetes, or one first-degree relative and a personal history of gestational diabetes, (as previously described (Kriketos et al., 2004; Kriketos et al., 2005)). The control group ( $n=9$ ) had no family history of diabetes. All were premenopausal and not pregnant at the time of the study. Subjects were studied at the follicular phase stage of the menstrual cycle. All subjects had normal glucose tolerance by oral glucose tolerance testing. The study received approval by the St Vincent's Hospital Human Research Ethics Committee and all subjects provided written informed consent.

### 3. Protocol

Subject characteristics (weight, BMI, percent body fat, abdominal fat, and fasting serum levels of insulin, glucose, and adiponectin) were determined as described previously (Kriketos et al., 2004; Kriketos et al., 2005). Percent body fat was determined by whole body DXA (Lunar DPX-Lunar Radiation Corporation, Madison, Wisconsin, USA, software version 1.35). Serum insulin and adiponectin were measured by radioimmunoassays (Linco, St Charles, MO). Adiponectin levels were measured in light of previous findings that plasma adiponectin levels strongly correlate with whole body insulin sensitivity (Weyer et al., 2001). Visceral and subcutaneous abdominal fat was determined by four T1 weighted axial abdominal MRI scans (5 mm thickness) between L1/2 and L4/5 intervertebral discs using imaging software NIH Image 1.62 (National Institute of Health, Bethesda, MD). Glucose tolerance was measured by a 75-g oral glucose tolerance test, and insulin sensitivity was assessed by euglycaemic-hyperinsulinemic clamp (Kriketos et al., 2004). Clamps were performed 1-week before the meal test. Briefly, one cannula was placed in an antecubital vein for infusion of insulin (Novo Nordisk, NSW, Australia) and glucose (Baxter, NSW, Australia). A second cannula was placed retrograde in a dorsal vein of the contralateral hand for blood withdrawal, and the hand was placed in a heating pad. Insulin was infused at 50 mU/m<sup>2</sup>/min and arterialized glucose was measured at 10-min intervals (YSI 2300, Yellow Springs, OH), and a variable infusion of exogenous glucose was given to maintain glucose concentrations at 5 mmol/L.

As high fat gives greater PYY stimulus and in order to evaluate the postprandial PYY response to a meal, subjects were fasted for a minimum of 10 h before consuming a meal containing ~80 g of dietary fat with an energy content of 4250 kJ. The meal was composed of 19 g carbohydrate, 40 g saturated fat and 47 g protein consisting of cheese (44 g), beef (100 g), fried egg (118 g), copha (coconut oil; 8 g), cracker biscuit (12.5 g) cream

(fat > 48%) (25 g), strawberries (130 g) and macadamia nuts (20 g); total 458 g (Peake et al., 2003). PYY is secreted in proportion to calories ingested, particularly calories from fat, so we therefore used a high calorie meal that was high in fat to stimulate PYY output, in order to magnify differences between groups. The meal was prepared on the same morning and consumed within 20 min, accompanied by 600 mL of water. Blood was collected via an antecubital vein 10 min and 0 min prior to the meal and at 30, 60, 120 and 360 min after completion of meal. Complete blood samples in response to the meal were available on a subset of subjects (8 controls, 9 relatives).

### 4. PYY radioimmunoassay

PYY was measured as follows: serum samples were frozen immediately at  $-80^{\circ}\text{C}$ . Aprotinin was added immediately on thawing (0.3 TIU aprotinin/mL serum). Serum aliquots (500  $\mu\text{L}$ ) were acidified with 1% TFA and then loaded onto Sep-pak C18 columns (Phenomenex Torrance CA USA) that had been equilibrated with 60% acetonitrile in 1% TFA followed by 1% TFA in water. Columns were washed twice with 1% TFA in water and the peptide was eluted with 60% acetonitrile in 1% TFA. Samples were air-dried overnight, frozen in liquid nitrogen and freeze-dried. The lyophilised powder was resuspended in the buffer provided in the Human PYY radioimmunoassay kit (Phoenix Pharmaceuticals, Belmont, California, USA).

Total human PYY including both PYY1-36 and PYY3-36 was measured by a commercially available radioimmunoassay kit containing 125I-labelled human PYY and antibody against human PYY that exhibits 100% cross-reactivity for both forms of PYY.

### 5. Islet studies

All research and animal care procedures were approved by the Garvan Institute/St Vincent's Hospital Animal Experimentation Ethics Committee. Wild type and *PYY*<sup>-/-</sup> animals generated as described previously (Boey et al., 2006) were group-housed and fed *ad libitum* under conditions of controlled temperature (22  $^{\circ}\text{C}$ ) and illumination (12 h light cycle, light onset at 7.00 am). Four 13-week old wild type and 4 age-matched PYY knockout mice were anaesthetized and their islets of Langerhans isolated with liberase (Roche Diagnostics, Indianapolis, IN, USA) digestion of the pancreas followed by purification with a Ficoll–Paque density gradient (Amersham Biosciences, Uppsala, Sweden), as described elsewhere (Kjorholt et al., 2005). Two to three batches of 5 islets were incubated in Krebs Ringer buffer supplemented with 2.8, 16.7 or 25 mM glucose for 1 h at 37  $^{\circ}\text{C}$ . Insulin was measured in an aliquot of the buffer

by radioimmunoassay (Linco Research, St Charles, Missouri, USA) with rat insulin standard.

## 6. Statistical analysis

Data are expressed as means  $\pm$  SEM unless otherwise stated. Statistical analyses were performed using STATVIEW 5 (SAS Institute Inc., Cary NC). Subjects' physical characteristics were compared between groups using one-way ANOVA. Postprandial responses to the meal were analysed by repeated measures ANOVA, and as area under the curve (AUC) by the trapezoidal method. Insulin secretion was estimated using the homeostasis model assessment, HOMA-Beta (HOMA-B), which approximates the steady state beta cell function for the duration of the euglycaemic-hyperinsulinemic clamp, using the fasting serum glucose and insulin levels (Matthews et al., 1985). Correlations between continuous variables were examined by simple linear regression analyses. A  $p$ -value of 0.05 or less was considered significant.

## 7. Results

### 7.1. Comparison of adiposity, metabolic parameters, serum adiponectin and PYY levels in controls and diabetic relatives

There were no significant differences between controls and relatives with respect to age or BMI (Table 1). Visceral fat was similar between groups, although relatives had non-significantly higher subcutaneous body fat (Table 1). Fasting insulin, glucose and adiponectin levels

were also comparable between controls and relatives (Table 1). As we have shown previously, relatives were significantly less insulin sensitive than the controls, indicated by the significantly lower clamp glucose infusion rate (GIR) (Table 1) (Kriketos et al., 2004). Fasting PYY was significantly lower in the relative group compared to controls (Table 1).

### 7.2. Serum PYY response after a high fat meal in controls and diabetic relatives

While the PYY response to a meal appeared flatter in the relatives versus controls (Fig. 1), the difference was not statistically significant when assessed with repeated measures ANOVA ( $p=0.13$ ). PYY area under the curve was not different between the groups ( $p=0.34$ ).

#### 7.2.1. Serum PYY levels in relation to GIR, fasting insulin, HOMA-B and serum adiponectin levels

There were no significant relationships between fasting serum PYY and BMI or other anthropometric indices in the overall group (% fat and BMI are shown in Fig. 2). Fasting serum PYY was significantly related to insulin sensitivity (GIR) (Fig. 2a). Moreover, there was an inverse relationship between fasting serum PYY and fasting serum insulin (Fig. 2b). There was also an inverse relationship between fasting serum PYY and HOMA-B (Fig. 2c) and a positive relationship with serum adiponectin (Fig. 2d). No correlations were observed between the area under the curve PYY and the area under the curve insulin response to a meal.

There was one relative subject with a BMI of 32 and low PYY levels in comparison to the rest of the relatives group (Fig. 2f). Correlation analyses were repeated with

Table 1  
Physical characteristics of controls and relatives of people with type 2 diabetes

	Controls	Relatives	$p$ -value
$N$	9	12	
Age (y)	28.9 $\pm$ 2.5	29.4 $\pm$ 2.2	0.88
Weight (kg)	60.2 $\pm$ 2.3	69.6 $\pm$ 5.0	0.14
BMI (kg/m <sup>2</sup> )	22.0 $\pm$ 1.0	25.5 $\pm$ 1.6	0.09
Body fat (%)	31 $\pm$ 3	37 $\pm$ 3	0.11
Total abdominal fat (L)	2.0 $\pm$ 0.5	2.8 $\pm$ 0.4	0.24
Visceral fat (L)	0.4 $\pm$ 0.1	0.5 $\pm$ 0.1	0.37
Subcutaneous fat (L)	1.6 $\pm$ 0.5	2.3 $\pm$ 0.4	0.23
GIR ( $\mu$ mol/kgFFM/min)	61.0 $\pm$ 5.6	45.0 $\pm$ 4.6	0.04
HOMA-beta	173 $\pm$ 24	192 $\pm$ 16	0.52
Fasting PYY (pmol/L)	30.1 $\pm$ 5.7	17.5 $\pm$ 5.7	0.05 <sup>a</sup>
Fasting insulin (mU/ $\mu$ l)	8.6 $\pm$ 1.2	12.4 $\pm$ 2.0	0.15
Fasting glucose (mM)	4.6 $\pm$ 0.1	4.8 $\pm$ 0.2	0.35
Fasting adiponectin ( $\mu$ g/ml)	21.9 $\pm$ 1.9	18.4 $\pm$ 2.2	0.26

Data is expressed as mean  $\pm$  SD.

<sup>a</sup> Log transformed.

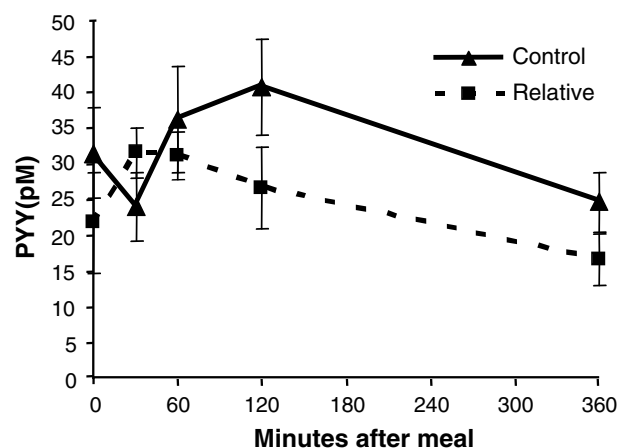


Fig. 1. PYY levels in response to a high fat meal in 8 control subjects (control) and 9 relatives of people with type 2 diabetes (relative) for up to 6 h.



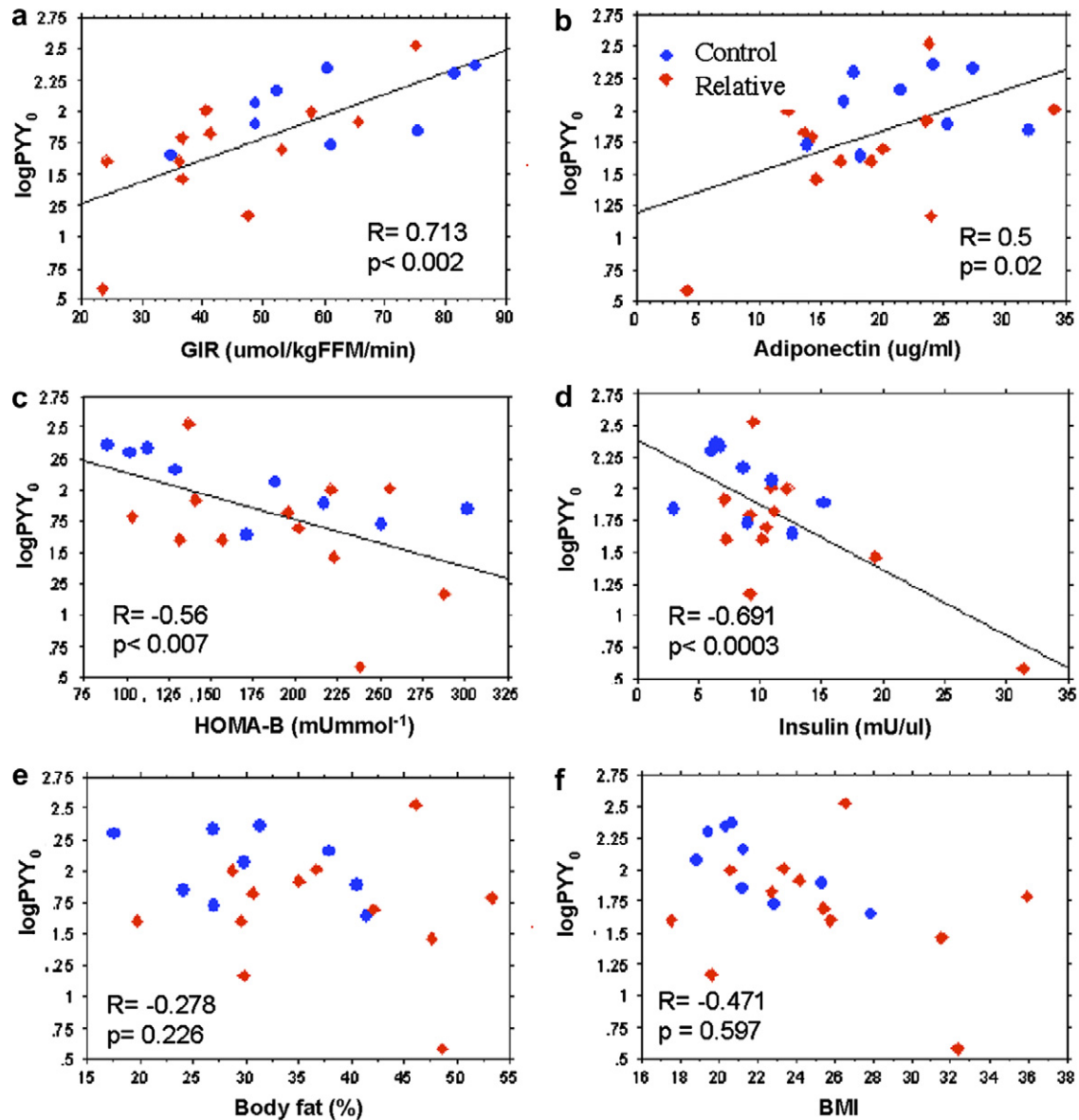


Fig. 2. Correlations between fasting PYY levels ( $PYY_0$ ) and measures of insulin sensitivity and B cell function.

this subject removed and the relationship between fasting PYY and GIR ( $r = 0.659$ ,  $p = 0.001$ ), HOMA-B ( $r = -0.549$ ,  $p = 0.01$ ) and adiponectin ( $r = 0.527$ ,  $p = 0.02$ ) remained, although the correlation between serum PYY levels and fasting serum insulin levels observed with analysis of the whole group was no longer significant ( $r = -0.361$ ,  $p = 0.12$ ).

### 7.3. Glucose-stimulated insulin secretion in islets of Langerhans from PYY knockout mice

The above data show that low fasting serum PYY levels inversely correlate with insulin sensitivity and increased insulin secretion. Moreover, PYY knockout

mice exhibit high glucose-induced serum insulin levels (Boey et al., 2006). We therefore investigated whether the negative correlation between PYY levels and HOMA-B in our human study, as well as the high circulating insulin levels observed in PYY knockout mice, could be explained by effects of PYY deficiency on islet function. At a non-insulin-stimulating dose of glucose (2.8 mM), insulin secretion is comparable between islets taken from wild type and PYY KO mice (Fig. 3). In contrast, at the insulin-stimulating doses of 16.7 mM and 25 mM glucose, islets taken from PYY KO mice secrete 3- to 4-fold more insulin than islets taken from wild types, significantly so at the higher glucose dose (Fig. 3).

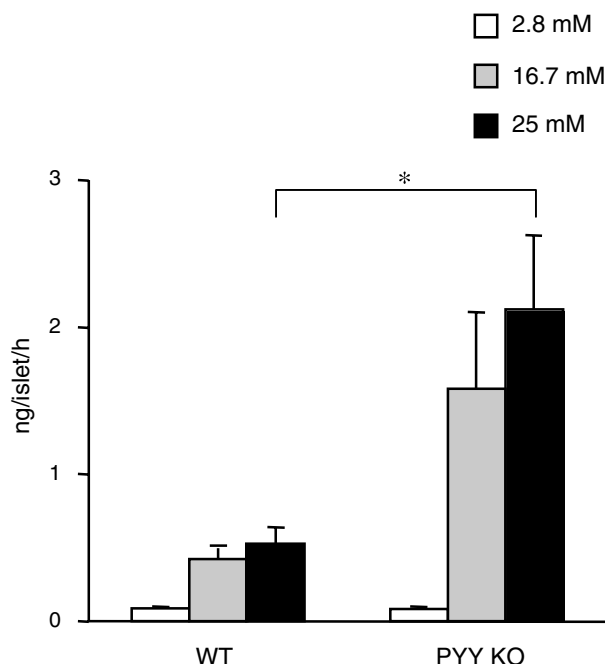


Fig. 3. Insulin secretion from isolated mouse pancreatic islets from wild type and PYY knockout mice in the presence of 2.8, 16.7 and 25 mM glucose.

## 8. Discussion

This study shows that, prior to the development of type 2 diabetes, women with a strong genetic propensity to type 2 diabetes have significantly lower fasting serum PYY levels than controls without any family history of diabetes. Moreover, fasting serum PYY levels, while not significantly correlated with any measures of adiposity, were positively correlated with insulin sensitivity and adiponectin, and negatively correlated with fasting insulin secretion as assessed by HOMA-B. Consistent with this, islets of Langerhans from PYY knockout mice were found to hyper-secrete insulin in response to high glucose concentrations. High fasting insulin is a better predictor of eventual development of type 2 diabetes than insulin resistance (Weyer et al., 2000). If PYY deficiency or low circulating levels of PYY contributes to the development of insulin hypersecretion and insulin resistance, this could be a putative cause of increased adiposity and insulin resistance with subsequent beta cell failure, as has been suggested in Pima Indians (Weyer et al., 2000).

The observation of a reduction in circulating PYY levels in relatives of people with type 2 diabetes compared to controls is important as these people are healthy and not yet significantly different to controls with respect to BMI, glycemia and hormonal or metabolic parameters. Thus, even in this normal but genetically “enriched” group, differences in circulating PYY levels could still be discerned, although, as stated, not all

subjects are expected to have the genetic abnormalities. Since greater differences in circulating PYY levels have been detected between frankly obese and lean subjects in previous studies (Alvarez Bartolome et al., 2002; Batterham et al., 2003; Roth et al., 2005), it is conceivable that small, very long term deficiencies in PYY may contribute to the development of excess adiposity, but that subsequent differences in food intake and adiposity between lean and already obese subjects may exacerbate such differences. This study highlights an important association between PYY and insulin secretion in both humans and animals, with low circulating levels of PYY predisposing to high insulin secretion.

Our studies also show a relationship between PYY and insulin sensitivity in man (measured by the gold standard, the hyperinsulinemic clamp) that has previously been shown in rodents (van den Hoek et al., 2004). This is consistent with the direct relationship of PYY with adiponectin levels, which predicts both adiposity and insulin sensitivity (Hotta et al., 2000; Weyer et al., 2001).

In addition to our previous findings that PYY knock-out animals exhibit elevated fasting or glucose-induced serum insulin levels in vivo (Boey et al., 2006), this study further demonstrates that lack of PYY alters the intrinsic properties of the Islets of Langerhans such that they hyper-secrete insulin in response to maximal stimulatory glucose concentrations. These islet isolation studies were performed in 13 week old chow-fed PYY knockout animals that were lean, showing that changes in islet function does not arise as a secondary effect of obesity. Indeed, a role for PYY in inhibiting glucose-stimulated insulin secretion has been demonstrated in vivo and in vitro in animals. Intravenously administered PYY has no effect on basal plasma insulin levels, but inhibits glucose-stimulated insulin secretion in mice (Bottcher et al., 1989; Szecowka et al., 1983). In vitro studies also show that PYY dose-dependently inhibits glucose-stimulated but not basal insulin secretion (Karlsson and Ahren, 1996; Nieuwenhuizen et al., 1994). Studies using perfused islets isolated from rats suggest that this is a direct action of PYY on islets (Bertrand et al., 1992). This is supported by work on isolated mouse islets where PYY antiserum potentiates glucose-induced insulin secretion in culture (Karlsson and Ahren, 1996). However, the mechanisms by which PYY regulates insulin secretion still remain unclear.

Together, these data suggest that low circulating levels of PYY are linked to both insulin resistance and insulin hypersecretion. Although this pilot study was performed solely in female subjects to eliminate known variability between genders, further studies with a larger sample size including males would clearly be informative. Nonetheless, PYY may have an important regulatory role in the development of the metabolic abnormalities of type 2 diabetes. We have previously

described, in a similar group of diabetic relatives, a progressive decline in insulin secretion (by HOMA-B) over 6 years, which was predicted only by the gain in central fat, not by any increase in insulin resistance (Kriketos et al., 2003). Further study is indicated to determine whether there is a correlation between low fasting serum PYY levels and the subsequent fat gain, as well as the progressive decline in pancreatic function in these people at high risk for development of obesity and type 2 diabetes.

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