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ORIGINAL ARTICLE

Abnormal postprandial PYY response in insulin sensitive nondiabetic subjects with a strong family history of type 2 diabetes

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Objective: Gut-derived hormone peptide YY (PYY) is low in subjects with obesity and type 2 diabetes (T2D). However, it is unknown whether this is a primary defect or a consequence of metabolic disturbances. In this study, we aimed to assess whether low fasting and postprandial PYY secretion is an early defect, potentially promoting the development of obesity and T2D, and whether it is modified by macronutrient content.
Design: Prospective cross-sectional cohort study.
Subjects: Nine individuals with a strong family history of T2D (REL) and seven age and adiposity matched individuals with no family history of T2D (CON).
Interventions: Metabolic studies including hyperinsulinemic-euglycemic clamp, dual X-ray absorptiometry and two meal tests containing 1000 kcal with an either high fat (76%) or high carbohydrate (76%) content.
Main outcome measures: Fasting and postprandial PYY levels were measured and analyzed for potential correlations with markers for adiposity and insulin resistance.
Results: Insulin sensitivity was not different between REL and CON. Fasting glucose, insulin, triglycerides and PYY were also not different between groups. However, the postprandial incremental area under curve (AUC) of PYY was significantly lower in REL after the high carbohydrate (HCHO) meal ($+27.3$ vs $+60.6$% increase from baseline, $P=0.038$). The AUC of insulin during HCHO meal correlated negatively with both AUC and fasting level of PYY ($r=-0.58$ and -0.60, respectively, $P<0.05$).
Conclusions: A blunted postprandial PYY secretion is observed in a very early stage in the development of T2D in genetically susceptible individuals. This defect precedes the presence of insulin resistance and adiposity, and could therefore predispose to the development of T2D.
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Keywords: gastrointestinal hormones; type 2 diabetes; prediabetes; metabolism; PYY

Introduction

The underlying mechanisms in the development of insulin resistance and type 2 diabetes (T2D) are still poorly understood. Clinical studies to detect pathophysiological mechanisms are often limited by many confounding factors when metabolic abnormalities like hyperglycemia and dyslipidemia are already present. Therefore, relatives of individuals with T2D present an ideal human model to test the mechanism of early disease development, as about two-thirds of subjects with two first degree relatives with TD2 mellitus will eventually develop glucose intolerance, and can hence be tested at a very early stage of the process.[1] Insulin resistance can usually be detected early in these subjects and is strongly associated with increased visceral fat mass and intramyocellular lipids.[2–4]

However, the molecular basis of this early insulin resistance is still not completely elucidated, and disturbance of fat metabolism and energy homeostasis has been suggested.[4,5]

Peptide YY (PYY) is a gut-derived hormone secreted by the L cells located in the colon in response to food. PYY1-36 binds and activates all known Y receptors (Y1, Y2, Y4, Y5 and

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Y6) with variable affinities. PYY is quickly processed by dipeptidyl peptidase IV to form PYY3-36 which preferentially binds to Y2 and also Y5 receptors, but has very low affinity for all other Y receptors. Both PYY1-36 and PYY3-36 bind to the Y2 receptor with high affinity. Interestingly, PYY levels are low in obesity and T2D, and the postprandial PYY response is blunted. Furthermore, IV administration of PYY3-36 has a strong anorexic effect inhibiting food intake in humans.[6–9] These studies suggest that PYY has important effects as a satiety signal in the hypothalamus, where the active form PYY3-36, which is the most abundant form of circulating PYY, preferentially binds to the Y2 receptor over the Y1 receptor. Although the association of low PYY and obesity has been demonstrated, it is as yet unclear if this is a primary causative mechanism of the disease, or an adaptive change in response to other confounding factors.

In this study, we took advantage of our model of prediabetes, investigating subjects at high risk for T2D at a very early stage, to detect if gut-derived hormones, and more specifically PYY, are abnormal in the early stage before obesity and hyperglycemia have developed, and therefore possibly play a primary role.

Methods

Experimental subjects

By public advertisement, we recruited nine sedentary, nonsmoking and nondiabetic individuals with a strong family history of T2D (at least two first degree relatives with T2D (REL)), and seven individuals without any family history of T2D (CON). Exclusion criteria were a weight change of >2 kg within the last 6 months, any medication which influences insulin sensitivity or blood pressure, and a personal history of T2D or cardiovascular disease. The study protocol was approved by the Human Research Ethics Committee at St Vincent's Hospital, and written informed consent was obtained from all subjects prior to the study.

Procedures

Subjects attended the clinical research facility on three separate visits and each visit was at least 3 days but no more than 14 days apart. On the first visit, subjects fasted for 10-h overnight and body weight was measured in a hospital gown and height measured by a stadiometer. Insulin sensitivity was then measured by 2-h hyperinsulinemic-euglycemic clamp (50 mU m^{-2} min^{-1}), according to previously described methods.[10] Briefly, two IV cannulae were inserted, one for infusion of regular insulin (Novo Nordisk, NSW, Australia) and 25% glucose (Baxter, NSW, Australia) and the other was placed retrograde in the contralateral hand for blood withdrawal with the hand placed in a heating pad. A variable infusion of exogenous glucose was given to maintain glucose concentrations at 5.0 mmol l^{-1}. Following the clamp, body composition was measured by DXA (Lunar DPX-Lunar Radiation, Madison WI, USA). At visits 2 and 3, subjects attended the clinical research facility at 0800 hours to measure various factors in response to a meal following a fasting period of 10 h. These visits were identical except for the type of meal consumed. At each visit, subjects were weighed and a IV cannula was inserted into the antecubital vein. A blood sample was taken (-60 min) and subjects were rested in the supine position for 30 min. Resting metabolic rate and respiratory quotient were determined over a 30 min period (Deltatrac, Datex, Helsinki, Finland).

Another baseline blood sample was taken just before subjects were fed a standard 1000 kcal meal that was high in either fat (HFAT, 76%) or carbohydrate (HCHO, 76%). The meals were held constant for protein (15%) and were randomly assigned to visit 2 or 3. Additional blood samples were taken 30, 60, 120 and 240 min after the meal was completed.

The updated Homeostasis Model Assessment (HOMA) 2 was used to estimate steady-state β-cell function (%B) as percentages of a normal reference population and insulin resistance using fasting glucose and insulin levels.[11]

Biochemical analysis

Serum insulin and plasma PYY were measured by commercial available radioimmunoassays (Linco Research, St Charles, USA). The PYY kit detected both PYY1-36 and PYY3-36, the latter being the major isoform of circulating PYY. In our hands, intra-assay variability of PYY was 6% between 60 and 80 pmol l^{-1}. All samples were measured in a single kit, avoiding between-assay variability. For PYY measurements, 500K IU ml^{-1} aprotinin (Sigma, Sydney, Australia) was added to the EDTA tubes before blood samples were taken. Fasting serum was assayed for total and high-density lipoprotein (HDL) cholesterol, triglycerides (all by enzymatic colorimetry, Roche, Indiana, USA) and LDL were calculated by the Friedewald equation. Free fatty acids (FFA) were measured by enzymatic colorimetry assay (Wako, Osaka, Japan).

Statistical analysis

Data are presented as means ± s.e.m. unless otherwise stated. Statistics were analyzed with Statistica 6.0 (StatSoft Inc., Tulsa, OK, USA). Baseline samples from visits 2 and 3 were averaged to determine differences between groups and were tested by one-way analysis of variance or Mann–Whitney *U*-test as appropriate. Correlations were performed using Pearson's correlation coefficient. Significance was set at $P<0.05$.

All applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research. The study was approved by the Hospital Human Research and Ethics Committee, and informed consent was obtained by all subjects before commencement.

Results

Baseline characteristics

Both groups were closely matched for age and BMI. Baseline characteristics are given in Table 1. Importantly, both groups were similar for % body fat mass, fasting lipids, glucose and insulin, and importantly all parameters were within the normal range.

In contrast to a previous study in relatives,[10] both groups had similar insulin sensitivity, as measured by hyperinsulinemic-euglycemic clamp. This might be explained by the fact that our subjects were studied earlier in the evolution of the life-long process.

Postprandial serum factors

In response to the HCHO and the HFAT meal, serum glucose increased similarly in both groups (Figure 1a). The insulin response, measured as incremental area under the curve (iAUC), was significantly higher in REL following the HCHO ($P=0.02$) and the HFAT meal ($P=0.01$) as compared to controls (Figure 1b). Serum triglycerides increased significantly during the HFAT meal ($P<0.001$) with no differences between groups, and remained unchanged during the HCHO meal in both groups (data not shown).

PYY measurements

The PYY responses in the two groups by meal are shown in Figure 2. During the HCHO meal PYY peaked after 30 min, whereas during the HFAT meal the peak was reached only after 120 min.

PYY levels were not different between groups in the fasting state (CON 63.9 ± 2.2 pmol l^{-1}, REL 59.2 ± 1.9 pmol l^{-1}, $P=0.13$; Figure 3a). However, during the HCHO meal there was a significantly lower PYY response in the REL (iAUC: 3880 ± 710 min $\times$ pmol l^{-1}, $+27.3\%$ increase from baseline) as compared to the CON (iAUC: 9287 ± 2570 min $\times$ pmol l^{-1}, $+60.6\%$ increase from baseline, $P=0.038$; Figure 3b). The PYY response by group was not statistically different for the HFAT meal ($P=0.2$).

The AUC of insulin during HCHO meal correlated negatively with both fasting PYY (Figure 4a) and the AUC of PYY (Figure 4b) during HCHO meals ($r=-0.60$ and -0.58, respectively, $P<0.05$). PYY levels at 120 min after a HCHO meal correlated negatively with mean fasting glucose ($r=-0.53$, $P<0.05$). In our cohort, PYY levels were not related to any marker for adiposity or insulin sensitivity.

Discussion

Decreased levels of PYY have been associated with obesity, but its role in the development of T2D is unknown. In this study we aimed to determine whether healthy individuals with a genetically high risk for T2D show abnormalities in fasting and postprandial PYY levels prior to development of hyperinsulinemia and hyperlipidemia.

Table 1 Baseline characteristics of study participants with no family history of T2D (CON) and a strong family history of T2D (REL) following a 10-h overnight fast

	CON	*REL*
Sex (M/F)	2/5	2/7
Age (y)	41 ± 7	46 ± 6
Weight (kg)	75.5 ± 14.6	70.1 ± 9.0
BMI (kg m^{-2})	26.5 ± 5.7	26.6 ± 5.3
Total fat (%)	36 ± 10	38 ± 12
Glucose (mmol l^{-1})	4.38 ± 0.20	4.72 ± 0.38
Insulin (mU ml^{-1})	10.2 ± 3.0	11.3 ± 3.7
Total-C (mmol l^{-1})	4.6 ± 0.8	4.9 ± 0.7
HDL-C (mmol l^{-1})	1.2 ± 0.2	1.3 ± 0.2
LDL-C (mmol l^{-1})	2.9 ± 0.5	3.0 ± 0.6
Triglyceride (mmol l^{-1})	1.3 ± 0.4	1.2 ± 0.6
GIR (μmol min^{-1} per kg FFM)	78.7 ± 22.8	73.2 ± 24.5
HOMA2-IR	1.27 ± 0.37	1.43 ± 0.46
HOMA2-%B	149 ± 27	140 ± 40

Abbreviations: BMI, body mass index; C, cholesterol; CON, participants with no family history of T2D; F, female; FFM, fat-free mass; GIR, glucose infusion rate during hyperinsulinemic-euglycemic clamp; HDL, high-density lipoproteins; HOMA, Homeostasis Model Assessment; LDL, low-density lipoprotein; M, male; REL, strong family history of T2D. Means ± s.d. No statistically significant differences between groups.

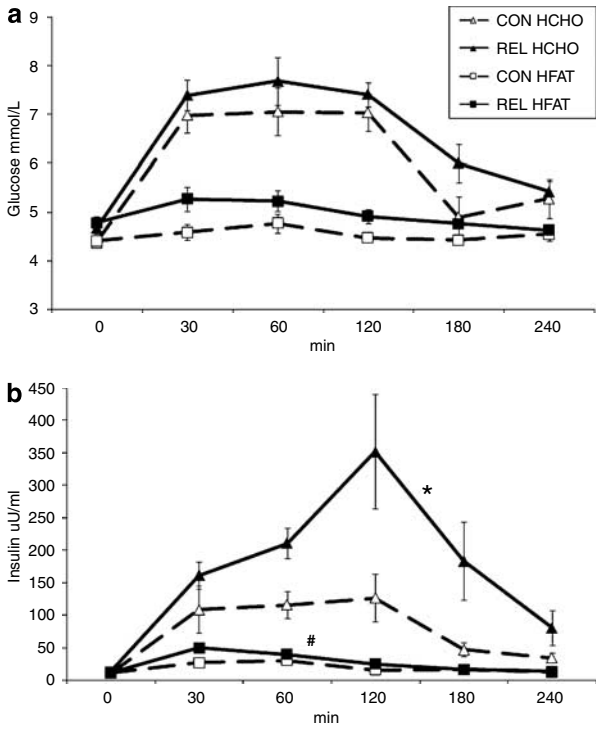

Figure 1 Serum levels of (**a**) glucose and (**b**) insulin in response to the HCHO and HFAT meal in individuals with a strong family history of diabetes (REL) vs matched control subjects (CON). Error bars = s.e.m. Significant differences in incremental area under the curve between groups: $^{*}P=0.02$, $^{\#}P=0.01$. HCHO, high carbohydrate meal; HFAT, high-fat meal.

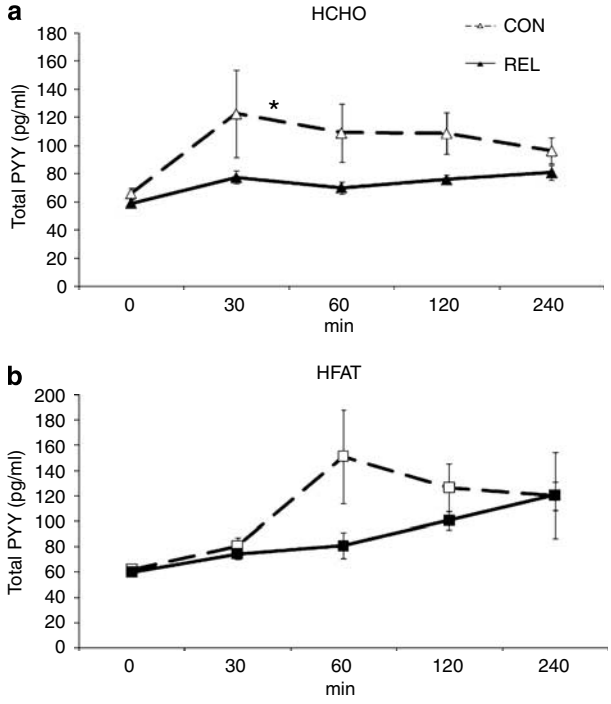

Figure 2 Peptide YY (PYY) secretion in response to HCHO (**a**) and HFAT meals (**b**) in individuals with a strong family history of diabetes (REL) vs matched control subjects (CON). Incremental area under the curve between groups. *$P<0.038$. HCHO, high carbohydrate meal; HFAT, high-fat meal.

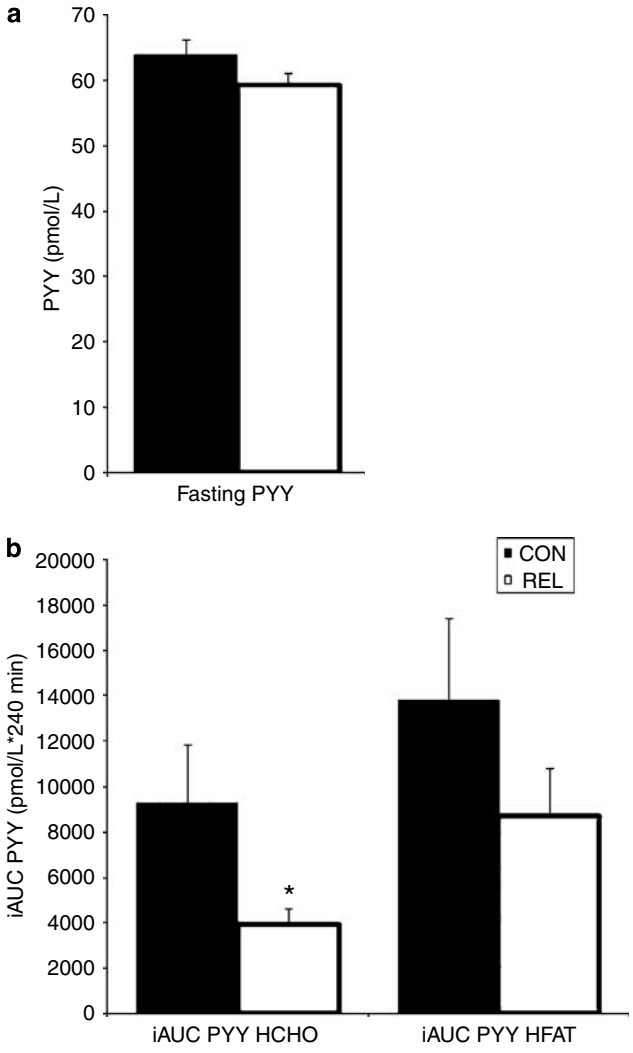

Figure 3 (**a**) Fasting peptide YY (PYY) levels in individuals with a strong family history of diabetes (REL) and matched control subjects (CON). No statistically significant difference between groups ($P=0.13$). (**b**) Incremental area under the curve (iAUC) of PYY response during high carbohydrate (HCHO) and high-fat meals (HFAT). *$P=0.038$.

In this study, subjects having relatives with T2D were well matched for adiposity, but in contrast to earlier studies[10] showed similar levels of insulin sensitivity as compared to control subjects.

This is probably due to the very early stage in disease progression, which allowed us to detect metabolic changes present before insulin resistance is established.

We found that fasting PYY levels were not different between REL and CON (Figure 3a). However, PYY levels were significantly reduced in the REL following an HCHO meal (Figure 3b), whereas there was no difference between groups after a HFAT meal. Even though our study sample size was small, it is even more striking that we still found a clearly blunted PYY response after a HCHO meal in genetically predisposed individuals. It is also important to acknowledge that this change is observed at a stage when the subjects are normal weight and insulin sensitive by clamp and HOMA2-IR (a measure of fasting insulin sensitivity), with no difference between groups, and the group showing the PYY differences is the one where two-thirds on average will later in life have glucose intolerance, 90% being overweight or obese.

PYY3-36 is generated by cleavage of PYY1-36 by dipeptidyl peptidase IV, and has a very short half-life. Some studies have shown that it represents a major part of the total circulating PYY, often measured by commercial radioimmunoassay.[9] Previous studies have demonstrated that total PYY and PYY3-36 responses during the meals are identical. In our study, we also measured the active form PYY3-36, and also found that postprandial responses of total PYY were significantly correlated with PYY3-36 responses in both meals (data not shown). However, the radioimmunoassay specific for PYY3-36 showed a wide intra-assay variability of 20% in our hands, and therefore we report here only total PYY levels as measured by an established and widely used commercial assay.

Low fasting and postprandial PYY levels have been reported in obese individuals,[9,12,13] but there is only one study reporting low PYY levels in prediabetes. We have reported earlier that total PYY was reduced in a similar group

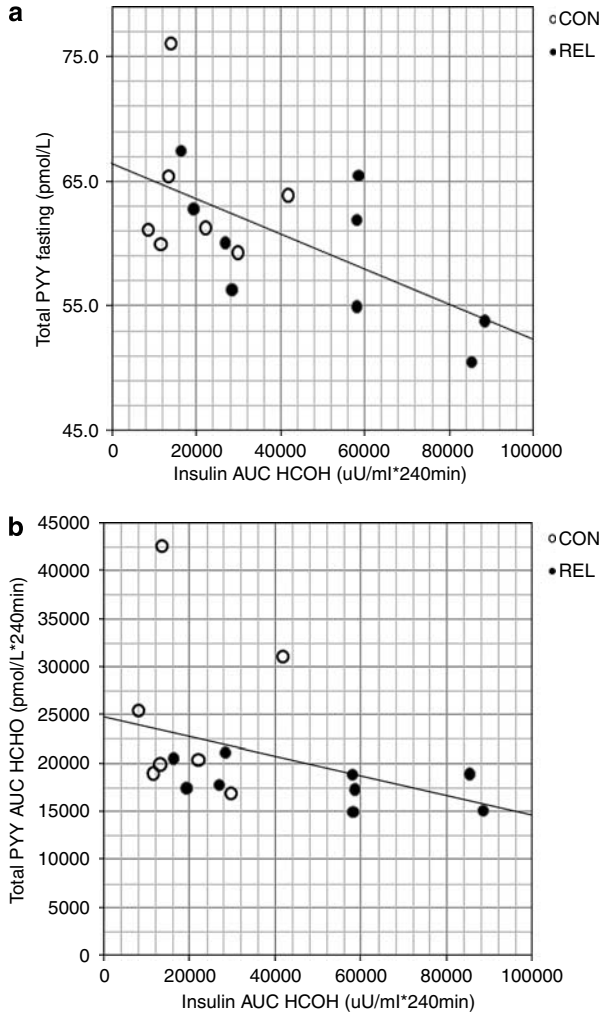

Figure 4 Negative correlation between area under curve (AUC) insulin response during HCHO meals and (**a**) fasting peptide YY (PYY) ($r=-0.60$, $P<0.05$) and (**b**) AUC PYY during HCHO meal ($r=-0.58$, $P<0.05$). ○ = CON, ● = REL. HCHO, high carbohydrate meal; AUC, area under the curve.

of relatives of individuals with T2D,[14] but these studies were only done in females already showing insulin resistance. It was therefore not completely clear whether the low PYY levels were primary or a consequence of insulin resistance. Following the results of the current study, we suggest that reduced postprandial PYY secretion is a primary defect, preceding most other metabolic abnormalities, including insulin resistance.

In our cohort, we found subtle metabolic differences between groups, with a small but significantly higher glucose response during the HFAT meal and higher insulin responses in REL during both meals. This may indicate a higher 'gluconeogenesis setting' to fat input in this population. While a higher insulin response during HCHO meals, as observed in the REL group, occurs in insulin resistance, the glucose infusion rate (GIR) during the hyperinsulinemic-euglycemic clamp (the gold standard measure of insulin sensitivity) was not different between groups, nor was HOMA2-IR which reflects fasting insulin resistance. However, we observed a significant negative correlation between the AUC of insulin response during HCHO meals and fasting PYY (Figure 4a), as well as between AUC insulin and AUC PYY during HCHO meals (Figure 4b). This raises the possibility that low postprandial PYY levels are causing postprandial hyperinsulinemia, which itself could promote obesity. This parallels the observation by Boey *et al.*,[13] where PYY knockout mice showed high insulin levels and hence increased obesity. Transgenic mice that overproduce PYY show the opposite effect and are resistant to high-fat diet or genetically induced obesity.[15] Low levels of PYY could therefore promote obesity not only via the lack of central inhibition of appetite, but also by removing inhibition of insulin secretion at the level of the pancreas. However, whether hyperinsulinemia is involved in the development of obesity is still controversial, despite strong evidence from animal models. Another interesting observation was the negative correlation of PYY levels 120 min after HCHO meals with the mean glucose level. This suggests that despite the absence of any statistical difference in mean glucose, insulin resistance and β-cell function between groups, a very early disturbance in glucose metabolism might be linked to the decline in PYY secretion.

The reason for decreased PYY secretion by the L cells is largely unknown, but a common cause for the secretory defect impacting on PYY and the co-secreted glucagon-like peptide-1 is likely.

Interestingly, there are recent reports that variations in PYY and Y2 receptor genes in humans are highly associated with severe obesity.[16–19] Unfortunately, PYY secretion was not assessed in these studies, but it is possible that these polymorphisms might lower PYY secretion. These findings support our results and hypothesis that a disturbed PYY release could be part of a primary hereditary defect, promoting the further development of obesity and T2D.

This study suggests that reduced PYY precedes development of obesity and further suggests that PYY may serve as an early predictor of T2D. PYY administration efficiently decreases hunger and food intake,[7] and replacement of PYY has been discussed for treatment of obesity. We speculate that agents with a PYY segretagogue effect on L cells, such as glutamine[20] may prevent further metabolic deterioration in individuals susceptible for T2D.

In conclusion, individuals with genetic susceptibility for T2D, which can be considered to be in its earliest stage of disease progression, have a blunted PYY response during high carbohydrate meals. This change occurs before obesity and metabolic abnormalities, such as insulin resistance. Therefore, a defective L-cell response to food intake may be a primary defect, promoting further weight gain and contributing to progression towards overt TD2.

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