In vivo and in vitro degradation of peptide YY3–36 to inactive peptide YY3–34 in humans

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PYY is present in plasma in two major molecular forms, the 36-amino-acid form, PYY1–36, and the 34-amino-acid NH2-terminally truncated form, PYY3–36, which is formed by cleavage of the two NH2-terminal amino acids by the enzyme dipeptidyl peptidase-4 (DPP-4) (28). DPP-4 is present in both a soluble form and as a transmembrane protein in endothelial, epithelial, and lymphoid tissue. DPP-4 cleaves and inactivates, e.g., glucose-dependent insulinotropic polypeptide and GLP-1 with short plasma half-lives of 5 and 1–2 min, respectively (15, 23, 36).

Anorexigenic effects of PYY3–36 have been demonstrated in several studies with intravenous administration of PYY3–36 (6, 16, 32, 34), whereas infusions of PYY1–36 have no effect on food intake (34), suggesting that the cleavage of PYY1–36 to PYY3–36 is not as complete and rapid as for GLP-1 (34). The half-life of PYY1–36 has been determined as 9.1 min (5). Studies of PYY degradation in vitro have identified various enzymes involved in PYY metabolism besides the NH2-termini truncation by DPP-4. PYY is also NH2 terminally degraded by aminopeptidase P, which cleaves off the NH2-terminal tyrosine residue, by the metalloendopeptidase meprin β, and neutral endopeptidase 24-11 (NEP 24.11) with major cleavage sites between residues 10 and 11 and 29–30, respectively (2, 28). PYY and NPY are COOH terminally amidated and therefore considered relatively protected from COOH-terminal degradation. However, COOH-terminal degradation of NPY, rendering the peptide inactive on the Y1- and Y2-receptors, has been demonstrated in vitro (1, 9, 22, 24). As the sequences of NPY and PYY are 70% homologous, we speculated that PYY might also be prone to COOH-terminal degradation. PYY3–36 infusions in pigs yielding near physiological concentrations showed PYY to be degraded COOH terminally to PYY3–34, which was not active on the Y2 receptor, and the peptide was degraded with a half-life ($t_{1/2}$) of 3.6 min (35). Studies of NPY-Y1/Y2 receptor interactions showed that an intact COOH-terminal pentapeptide is crucial for the binding and activation of the receptors (27). The COOH-terminal pentapeptides are identical in PYY and NPY, indicating that COOH-terminal degradation of PYY will attenuate or ameliorate the binding to and activation of the Y2 receptor.

In this study, we investigated whether COOH-terminal degradation of PYY with a formation of PYY3–34 also occurs in humans and determined the elimination rates of PYY1–36 and PYY3–36 following intravenous administration of PYY1–36 and PYY3–36. Sitagliptin, a competitive DPP-4 inhibitor, was administered orally before one of the infusion days to inhibit NH2-terminal degradation of the peptide.

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PEPTIDE YY (PYY) is a gastrointestinal peptide hormone, released into the circulation in response to food intake (3, 4, 8, 18), belonging to the pancreatic polypeptide family together with neuropeptide Y (NPY) and pancreatic polypeptide. These are all 36-amino-acid peptides characterized by a high content of tyrosine, proline, and arginine and a hairpin structure, the so-called “PP-fold” (17, 21).

PYY is synthesized and released from endocrine L-cells in the intestinal mucosa, often together with proglucacon-derived peptides, namely glucagon-like peptide-1 (GLP-1), GLP-2, oxyntomodulin, and glicentin. PYY immunoreactive cells are found primarily in ileum and colon with increasing density distally (4).
METHODS

Participants. Eight normal-weight [BMI: 22.1 ± 0.4 kg/m² (mean ± SE); age: 23.1 ± 1.2 yr (mean ± SE)], healthy, weight-stable, Caucasian men with no evidence of gastrointestinal or renal disease participated in a randomized, single-blinded, cross-over study with intravenous PYY infusions for 2 h on four separate days of either 1) 1.6 pmol·kg⁻¹·min⁻¹ PYY₁₋₃₆, 2) 1.6 pmol·kg⁻¹·min⁻¹ PYY₁₋₃₆ after DPP-4 inhibition, 3) 0.8 pmol·kg⁻¹·min⁻¹ PYY₃₋₃₆, or 4) an equivalent volume of saline using a precision infusion pump (P2000; IVAC Medical Systems, Hampshire, United Kingdom). The doses were chosen to mimic postprandial PYY levels after gastric bypass and were similar to those used in previous human studies with PYY infusions (6, 7, 16, 26a, 32, 34, 37). The minimum washout period between study days was 1 wk. Written and oral informed consent was obtained from all participants, and, following approval by the Committees on Health Research Ethics for the Capital Region of Denmark and by the Danish Data Protection Agency, the study was conducted in accordance with the Declaration of Helsinki II. The trial was registered at clinicaltrials.gov as NCT 02493895.

Protocol. Participants were instructed to fast from 22:00 the night before each test day but were allowed to drink water. Before one of the two PYY₁₋₃₆ test days, participants were instructed to take 100 mg Januvia (sitagliptin) 10 h and 1 h before start of the infusion, respectively. On each test day, participants were weighed and placed in a reclined position in a hospital bed; two catheters were inserted into antecubital veins, one for infusion and one for blood sampling. After collection of two basal blood samples, the infusion was started and continued for 2 h. Blood was sampled at t = −15, 0, 60, 90, and 120 min relative to start of the infusion as well as frequently after the infusion was discontinued at t = 122, 125, 130, 135, 140, 150, 165, 195, and 240 min. At t = 0, 60, 90, 120, 135, 165, and 240 min 100-mm visual analog scales (VAS) were employed for rating of appetite, nausea, and abdominal pain, and blood pressure and heart rate were measured.

Infusions. PYY peptides (>97% pure, structure and purity verified by mass spectrometry, sequence, and high-performance liquid chromatography analysis) were purchased from Bachem (Bubendorf, Switzerland), dissolved in sterilized water containing 2% human serum albumin (human albumin 20%; CSL Behring, Marburg, Germany), subjected to sterile filtration, and dispensed into vials under aseptic conditions. Vial content was tested for sterility and bacterial endotoxins (Central Pharmacy, Herlev Hospital, Denmark). Vials were kept at −20°C until the experimental days.

Blood samples and analysis. Blood was collected 1) into chilled EDTA tubes containing a DPP-4 inhibitor (0.01 mmol/l valine-pyrrolidone, final concentration) and 500 KIE aprotinin (Trasylol, final concentration); they were immediately centrifuged and plasma stored at −20°C until analysis; 2) into Eppendorf tubes containing EDTA, which were immediately centrifuged and the contents used for analysis of plasma glucose using YSI model 2300 STAT plus (YSI, Yellow Springs, OH).

Degradation of PYY₁₋₃₆ in vitro. Blood was collected from five healthy participants into chilled heparinized tubes. Synthetic human PYY₁₋₃₆ (0.6 pmol/ml, final concentration, Bachem) was incubated in blood and plasma at 37°C. Samples were taken regularly (t = 0, 0.5, 1, 2, 3, 4, 6, 8, 21, and 24 h), mixed with EDTA, aprotinin (500 KIE/ml), and the DPP-4 inhibitor valine-pyrrolidone (0.01 mmol/l, final concentration), and frozen instantly for the blood samples immediately after centrifugation and separation of plasma.

Peptide measurements. Peptide levels in plasma samples from the infusion study were determined using three different in-house PYY radioimmunoassays (RIA). RIA of total PYY in plasma was performed using a monoclonal antibody MAB8850 (clone RPY-B12; Abnova, Taipei, Taiwan), which reacts equally well with PYY₁₋₃₆ and PYY₃₋₃₆. Synthetic human PYY₃₋₃₆ (Bachem) was used as standard and ¹²⁵I-labeled PYY₁₋₃₆ (NEX341; Perkin Elmer, Waltham, MA) as tracer. Assay buffer was 80 mmol/l sodium phosphate buffer, pH 7.5, containing, in addition, 0.1% (wt/vol) human serum albumin (Calbiochem, San Diego, CA), 10 mmol/l EDTA, and 0.6 mmol/l thiomersal (Sigma, St. Louis, MO). PYY₁₋₃₆ was measured with a COOH-terminal-specific antiserum (code no. 2940) raised in white rabbits against residues PYY₂₃₋₃₆ coupled to keyhole limpet hemocyanin. Synthetic porcine PYY₃₋₃₆ (Genscript, Piscataway, NJ) was used as standard, and the tracer was synthetic human PYY₃₋₃₆. ¹²⁵I-labeled using stoichiometric chloramine-T method (20). The assay detects both PYY₁₋₃₄ and PYY₃₋₃₄ and shows negligible cross reaction (<1%) with P-YY₂₃₋₃₆. Assay buffer was 0.1 M Tris, pH 8.5, containing 0.2% (wt/vol) human serum albumin, 20 mmol/l EDTA, and 0.6 mmol/l thiomersal. PYY₃₋₃₆ was measured using an NH₂-terminal specific antibody (1067-HK; Millipore, Billerica, MA). ¹²⁵I-labeled PYY₃₋₃₆ (T-059-02; Phoenix Peptide, Belmont, CA) as tracer, and synthetic PYY₃₋₃₆ (H-8585, Bachem) as standard. The assay buffer was 0.1 M Tris.

Free and bound moieties were separated with plasma-coated charcoal (E. Merck, Darmstadt, Germany). All plasma samples were extracted with 70% ethanol (final concentration) to remove unspecific, cross-reacting substances. The recovery of synthetic PYY₃₋₃₆, and PYY₃₋₃₄ added to plasma before extraction and assay was 71.5 ± 2.6% (mean ± SE) in the total PYY assay, 61.5 ± 5.5% in the NH₂-terminal specific assay, and 68.0 ± 3.1% in the COOH-terminal specific assay. For all assays, the experimental detection limits were <5 pmol/l, and the intra-assay coefficient of variation was below 6% when evaluated at a concentration of 40 pmol/l.

Total PYY and PYY₁₋₃₆ concentrations in samples from in vitro incubations were measured using two commercially available RIA kits (cat no. PYY-67HK and PYY-66HK, Millipore).

Transfections and phosphatidylinositol assay. COS-7 cells were grown at 10% CO₂ and 37°C in Dulbecco’s modified Eagle’s medium with Glutamax (Invitrogen, Taastrup, Denmark) to which was added 10% fetal bovine serum, 180 U/ml penicillin, and 45 μg/ml streptomycin (PenStrep). Cotransfection of the hNPY receptor and promiscuous chimeric G protein Gqi4myr (26) was performed using the calcium phosphate precipitation method, as previously described (25). The use of cotransfection with the Y2 receptor and the chimeric G protein Gqi4myr shuffles the Ga₅ signal to a Gα₅ signal and thereby enables measurement of activation (formation of IP3) rather than inhibition of cAMP. The cotransfected COS-7 cells were incubated for 24 h with 5 μCi/ml [myo-³H]-inositol in growth medium, and the assay was carried out as described previously (29). Three independent experiments with duplicate measurements were performed. PYY₁₋₃₆ was used as a positive control, and both agonistic as well as antagonistic effects of PYY₁₋₃₄ and PYY₃₋₃₄ were tested.

Calculations and statistics. Descriptive data are stated as means ± SE, and P values of <0.05 are considered significant. All data analysis was done with Prism for Windows (version 6.05; GraphPad Software, San Diego, CA).

During PYY infusions, basal concentration was calculated as the mean of PYY determinations at t = −15 and 0 min, steady-state concentration as mean of concentrations at t = 60, 90, and 120 min. PYY₁₋₃₆ concentrations corrected for NH₂-terminal degradation were estimated as total PYY immunoreactivity (IR) = PYY₁₋₃₆ IR. On the PYY₃₋₃₆ infusion day, intact PYY₃₋₃₆ levels were estimated as total PYY IR = PYY₃₋₃₆.

The T₀ of PYY was calculated using the One-Phase Decay model of GraphPad Prism 6. Memory clearance rate (MCR) was calculated as the infused rate of PYY divided by incremental steady-state concentrations. The apparent distribution volume was calculated as the infused rate of PYY divided by incremental steady-state concentrations. The apparent distribution volume was calculated as the infused rate of PYY divided by incremental steady-state concentrations. The apparent distribution volume was calculated as the infused rate of PYY divided by incremental steady-state concentrations.
The total area under the curve (AUC) during infusion for VAS ratings was calculated using the trapezoidal rule and analyzed using one-way ANOVA. AUCs for PYY infusions were compared with AUC for saline infusion using one-way ANOVA and Bonferroni correction for multiple comparisons.

Plasma glucose, heart rate, and blood pressure were analyzed using a repeated-measures two-way ANOVA. When treatment or treatment × time was found to be significant, PYY infusions were compared with placebo at each time point using post hoc testing with Bonferroni correction.

Half-lives of PYY in blood and plasma during in vitro incubation were determined using the One-Phase Decay model of GraphPad Prism 6. The rate of formation of PYY3–34 in plasma was fitted with linear regression and the slope tested for significance vs. zero. PYY3–36 formation was fitted with the One-Phase Association model using Prism 6.

Receptor assay. Sigmoidal dose-response curves were fitted using GraphPad Prism 6.

RESULTS

Degradation of PYY in vivo. Infusion of PYY1–36 increased total PYY IR in plasma from 11.3 ± 1.2 to 130.6 ± 10.1 pmol/l, infusion of PYY1–36 + 2 × 100 mg sitagliptin from 9.8 ± 1.1 pmol/l to 116.0 ± 8.5 pmol/l, and PYY1–36 infusion from 10.9 ± 2.9 to 98.6 ± 8.3 pmol/l (steady-state concentrations, Fig. 1A). Measured with the NH2-terminal-specific PYY3–36 assay, the steady-state plasma concentration during PYY3–36 infusion was 57.7 ± 3.0 pmol/l. Infusion of PYY1–36 increased the COOH-terminal PYY3–34 concentrations from 5.3 ± 0.4 pmol/l to 14.8 ± 1.4 pmol/l with sitagliptin and from 4.9 ± 0.4 pmol/l to 15.3 ± 1.5 pmol/l without. At the PYY3–36 infusion day, COOH-terminal-specific PYY3–34 levels increased from 4.7 ± 0.5 pmol/l to 16.4 ± 1.6 pmol/l. AUCs for PYY3–34 were significantly different from placebo on all PYY infusion days (Fig. 1D).

The elimination half-lives were similar after infusion of PYY1–36 with and without administration of 2 × 100 mg sitagliptin (10.1 ± 0.5 min and 9.4 ± 0.8 min, respectively). The half-life of PYY3–36 was significantly longer, 14.9 ± 1.3 min. The half-life of PYY3–36 determined with the NH2-terminal-specific PYY3–36 assay was 15.5 ± 0.8 min and not different from that determined with the total PYY assay.

When correcting for COOH-terminal degradation (by subtracting PYY3–34 levels from total PYY levels), the elimination half-lives of PYY1–36 were significantly shorter both with and without sitagliptin (8.8 ± 0.4 min and 7.7 ± 0.6 min, respectively). The half-life of PYY3–36 corrected for COOH-terminal degradation was 12.2 ± 1.2 min (Fig. 2A). The half-lives of PYY1–36 corrected for NH2-terminal degradation (calculated as PYY3–36 determination subtracted from total PYY) were 8.2 ± 0.5 when sitagliptin was coadministered and significantly lower (5.0 ± 0.3 min) without sitagliptin (P < 0.0001).

Fig. 1. Total peptide YY (PYY) (A), NH2-terminal-specific PYY3–36 (B), and COOH-terminal-specific PYY3–34 (C) are shown. Placebo (○), PYY1–36 + sitagliptin (○), PYY1–36 (○), and PYY3–36 (○) are shown. Total area under the curves (AUCs) of PYY3–34 concentrations during PYY1–36, PYY1–36, or saline infusions from t = 0 to t = 120 min are shown (D). Differences to placebo were evaluated using repeated-measures 1-way ANOVA followed by Bonferroni correction for multiple comparisons. Data are means ± SE.
MCRs (using total PYY estimates of PYY concentration) were 16.1 ± 1.1 ml·kg⁻¹·min⁻¹ and 14.2 ± 1.0 ml·kg⁻¹·min⁻¹, respectively, when infusing PYY1–36 with and without administration of sitagliptin and significantly lower during PYY3–36 infusion (9.8 ± 0.7 ml·kg⁻¹·min⁻¹) (Fig. 2B). Vdₐₚp was similar for the three different infusions (PYY1–36: 206.0 ± 18.6 ml/kg; PYY1–36 + sitagliptin: 160.9 ± 24.4 ml/kg; PYY3–36: 168.0 ± 11.5 ml/kg) (Fig. 2C).

VAS. Infusions of PYY1–36 and PYY3–36 did not significantly influence VAS rating AUCs for hunger, satiety, or abdominal pain. On the PYY3–36 infusion day, participants felt nauseous, and 50% vomited. Consistent with this observation, the AUC of nausea VAS ratings for the PYY3–36 infusion was significantly greater than the corresponding AUC on the placebo day (P = 0.0049) (Figs. 3D and 4).

Glucose/pulse/blood pressure. Plasma glucose concentrations were significantly higher during the infusion of PYY3–36 compared with placebo and also toward the end of the PYY1–36 infusion (Fig. 5A). Heart rate was not significantly different during any of the infusions (Fig. 5B), but there was a tendency for an increased heart rate during PYY3–36 infusion and toward the end of the PYY1–36 infusion (P = 0.091). Infusion of PYY did not alter systolic and diastolic blood pressure (Fig. 5, C and D).

Degradation of PYY in vitro. In blood in vitro at 37°C, PYY, measured as total PYY, was degraded with t½ of 22.8 ± 8.0 h ± 3.6 ± 0.6 h when correcting for NH₂-terminal degradation. The concentrations of PYY3–36 and PYY3–34 peaked after 4 and 6 h, respectively, and hereafter the levels of the two PYY moieties declined.

In plasma, no degradation of total PYY could be detected, but, when we corrected for NH₂-terminal degradation, intact PYY1–36 had a t½ of 8.6 ± 3.6 h (Fig. 6). PYY3–34 formation followed a linear model with a slope of 4.08 ± 0.81 pmol·l⁻¹·h⁻¹ (significantly different from zero, P < 0.0001). PYY3–36 followed pseudo-first-order association kinetics with a rate constant of 0.22 ± 0.04 per h and a plateau of 268 ± 23.4 pmol/l.

PYY signaling in COS-7 cells. The biological activities of hPYY3–36, hPYY1–34, and hPYY3–34 were tested in COS-7
cells transiently transfected with the human Y2 receptor and the chimeric G protein Gqi4myr, using IP turnover measurements. The human Y2 receptor was activated by hPYY 3–36 with an EC50 value of 3.5 nmol/l, but neither PYY 1–34 nor PYY3–34 had agonistic or antagonistic activity on the receptor (Fig. 7).

**DISCUSSION**

This study examined the pharmacokinetics and degradation of intravenously administered PYY1–36 and PYY3–36 in healthy normal-weight men. The results reveal that 1) both PYY moieties are degraded from the COOH terminus to form PYY1/3–34; 2) human PYY3–34 has no agonistic or antagonistic effects on the human Y2 receptor; and 3) PYY3–36 has a longer half-life than PYY 1–36.

Previous studies have shown that in vitro incubations of NPY in human plasma resulted in cleavage of the COOH-terminal amino acid and dipeptide (1, 22). We have demonstrated that PYY3–36 is significantly degraded to PYY3–34 in the pig and also shown that the liver is involved in the COOH-terminal truncation (35). Here we extend these findings to humans and demonstrate formation of PYY1/3–34 after infusion of both PYY 1–36 and PYY3–36, although to a somewhat lesser extent than in anesthetized pigs. At the PYY 3–36 infusion day, COOH-terminal-specific PYY3–34 levels amounted to about 28% of NH2-terminal specific PYY3–36 levels during steady state. As we have not measured the intact COOH terminus of 1/3–36, we cannot exclude that the COOH terminus is degraded by other enzymes yielding different PYY moieties. Furthermore, as we cannot inhibit the COOH-terminal degradation, we cannot determine the role that COOH-terminal degradation plays in the overall inactivation of PYY. A method for exact measurements of active PYY 3–36 levels (e.g., sandwich ELISA or LC-MS) would therefore be very valuable in the interpretation of the physiological effects of PYY. In vitro degradation of NPY in human plasma has indicated that NPY is COOH terminally cleaved to primarily NPY 3–35, and only trace amounts of NPY3–34 have been identified (1). As in pig blood and plasma, PYY was COOH terminally degraded in human blood and plasma in vitro. As currently used assays are unlikely to discriminate between COOH-terminally degraded and intact PYY, PYY levels are probably generally overestimated. The only way to overcome this problem is to use a sandwich ELISA with COOH-terminal and NH2-terminal spe-

![Fig. 3. Visual analogue scale (VAS) ratings: AUC during infusion. Hunger (A), satiety (B), abdominal pain (C), and nausea (D) are shown. Data are means ± SE. AUC for PYY infusions were compared with AUC for saline infusion using 1-way ANOVA followed by post hoc tests with Bonferroni correction for multiple comparisons. *Different from placebo.](image)

![Fig. 4. Nausea VAS ratings. Placebo (○), PYY1–36 + sitagliptin (■), PYY1–36 (□), and PYY3–36 (○) are shown. Data are means ± SE.](image)
specific antibodies, but, to our best knowledge, such assays have not been developed so far.

PYY1–36 was infused at a double rate compared with PYY3–36, and we therefore expected higher plasma levels of PYY1/3–34 after PYY1–36 infusion. When measuring PYY3–34, it turned out that the plasma levels were comparable after PYY1–36 and PYY3–36 infusion. This discrepancy might be explained by the longer half-life of PYY3–36, or it could be that the NH2-terminal structure influences COOH-terminal degradation, as has been suggested to be the case for in vitro degradation of NPY (22). PYY3–34 was not active on the Y2 receptor, and the COOH-terminal degradation is therefore likely to represent an inactivation and elimination step; however, it cannot at the current stage be excluded that PYY3–34 exerts a physiological effect via a different receptor.

In vitro incubations in plasma and blood showed both COOH-terminal and NH2-terminal degradation of the peptide. The rates of the NH2-terminal dipeptide cleavage with t½ values of 8–9 h in plasma and 3–4 h in blood are similar to the half-lives of GLP-2 (which is also cleaved by DPP-4) in plasma and blood (19) but longer than those reported for GLP-1 (14).

The in vivo half-life of PYY (total PYY IR) was 10.1 ± 0.5 and 9.4 ± 0.8 min with and without sitagliptin, respectively,

Fig. 5. A: plasma glucose time course (treatment × time effect, *P < 0.0001). B: heart rate (no treatment × time effect, P = 0.091). C and D: systolic and diastolic blood pressure (no treatment × time effect, P = 0.57 and P = 0.47, respectively). Placebo (♀), PYY1-36 + sitagliptin (♂), PYY1-36 (○), and PYY3-36 (▲) are shown. Data are means ± SE. Comparisons were made using a 2-way repeated-measures ANOVA. If significant, PYY infusions were compared with placebo by post hoc testing with Bonferroni corrections for multiple comparisons.

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which is in accordance with an earlier determination of PYY half-lives of 9.1 ± 0.3 min (5). The half-life of PYY1–36 appeared to be significantly longer, 14.9 ± 1.3 min, suggesting that the change in tertiary conformation when the NH2-terminal dipeptide is cleaved off alters the affinity of the peptidases involved in PYY metabolism. In accordance with this finding, it was shown by in vitro incubations that PYY3–36 was not prone to degradation by NEP 24.11, whereas PYY1–36 is cleaved by NEP 24.11 with a major hydrolysis site between residues 29–30 (28). 

Vd was determined as ~180 ml/kg, a size equal to the extracellular volume. Adrian et al. (3) determined Vd to be considerably smaller (92 ml/kg), and also MCR (7.2 ml·kg⁻¹·min⁻¹) was lower than MCR found in this study. This difference is obviously due to differences in plasma peak levels in relation to the infused amount. Reported plasma peak concentrations after 0.8 pmol·kg⁻¹·min⁻¹ infusions of PYY vary between ~35 pmol/l and 270 pmol/l (7, 16, 26a, 32, 34, 37), and this discrepancy probably reflects differences in the PYY assay performances and/or preparation of the infusion solutions.

The discrepancy between the PYY levels at the PYY3–36 infusion day determined with the total PYY assay and the PYY3–36 assay reveals an inconsistency. In accordance with this, recovery studies with the two assays showed a higher recovery when analyzing spiked plasma samples with the total PYY assay (71.5 ± 2.6%) compared with the PYY3–36 assay (61.5 ± 5.5%). Differences in antibody affinities or interassay variation may explain this discrepancy.

Blood glucose was significantly higher during the PYY3–36 infusion. Heart rate also tended to be elevated (P = 0.091). In vivo studies in mice (10, 12) and investigations in isolated perfused rat pancreas showed that PYY inhibits insulin secretion, and PYY knockout mice (11) and mice with a deletion of the Y1 receptor (13) were hyperinsulinemic. This points to the role of PYY in glucose homeostasis, but in our study we found no difference between plasma glucose on the saline vs. the PYY1–36 days. Other studies with PYY3–36 infusions in humans reported no effect on glucose levels (7, 27, 28), but Sloth et al. (34) found elevated heart rate during infusion. In our study, nausea VAS ratings were also significantly elevated during PYY3–36 infusion, and we speculate that the elevated blood glucose and heart rate reflect increased sympathoadrenal activity attributable to nausea. On the PYY3–36 infusion day, nausea ratings peaked at t = 60 min with a PYY3–36 concentration of ~55 pM. Although the PYY3–36 concentration remained at about 55–60 pM during the last hour of infusion, the nausea VAS rating decreased, maybe attributable to tachyphylaxis. As the four subjects that vomited all had their first vomiting episode around t = 60 min, it could also be that emptying of the stomach (although they were fasted) relieved the symptoms.

During PYY1–36 infusion, some subjects experienced mild nausea. The nausea VAS rating peaked at t = 90 min with a PYY3–36 concentration of 40 pM. This could indicate that the threshold concentration for nausea is somewhere between 40 and 55 pM but could also reflect a decreased nausea response attributable to tachyphylaxis, as the PYY3–36 level increased at a slower rate during PYY1–36 infusion.

Nausea upon PYY3–36 infusion has been reported by others (16, 26b, 32, 34), and this is likely a confounder with regards to appetite sensations and previously reported reductions in food intake (26b, 34). However, studies of hypthalamic activity upon PYY3–36 administration in both humans and rodents have shown similar patterns as after food intake, indicating that PYY3–36 exerts anorexic effects by modulation of hypothalamic areas (17). Furthermore, these effects are absent in the Y2 receptor knockout mouse, pointing to PYY3–36 acting via the Y2 receptor (33). Degen et al. (16) demonstrated a dose relationship between PYY3–36 administration and food intake, and, during the lowest dose (0.2 pmol·kg⁻¹·min⁻¹), the subjects reduced their food intake and were free from adverse events, indicating that the therapeutic window for PYY3–36 as an obesity drug might be quite narrow.

In agreement with earlier findings, hunger and satiety ratings did not differ significantly at the PYY3–36 infusion days (34).

As a control, we performed PYY determinations on the infusate solutions (collected from the end of the infusion catheter). Unexpectedly, a degradation of PYY1–36 to PYY3–36 in the infusate was discovered. PYY3–36 amounted to ~20% of the total PYY content. This finding could explain the rather high levels of PYY3–36 during the PYY1–36 infusion plus sitagliptin study day and impedes the calculation of a PYY1–36 to PYY3–36 conversion rate but does not influence determination of half-lives of total PYY, PYY3–36, and PYY1–36 (intact NH2-terminus). To investigate this unforeseen degradation, we incubated PYY1–36 with the infusate vehicle at room temperature and tested the albumin used for drug formulation for DPP-4 activity. This experiment revealed that PYY1–36 was degraded to PYY3–36 (~20% of total PYY IR after 4 h at room temperature), and, surprisingly, DPP-4 activity was also detected in the albumin. When one formulates PYY and other DPP-4 substrates for infusion experiments, it is therefore essential to assess the stability of the peptide in the vehicle.

**Conclusion.** A significant formation of the COOH-terminally truncated PYY metabolite, PYY3–34, has been described in pigs, and we here report that this also applies to humans. Human PYY3–34 is not active on the human Y2 receptor, but whether it has other effects remains to be investigated. The formation of this COOH-terminally truncated metabolite of...
PYY significantly shortens the survival time of PYY in the circulation. Therefore PYY levels determined by existing assays do not reflect concentrations of biologically active peptides, and assumptions regarding the physiological functions of PYY at these levels are also inaccurate. Furthermore, in healthy men, PYY\textsubscript{3–36} has a longer half-life than PYY\textsubscript{1–36}.

**Perspectives and Significance**

The study presented here shows that PYY\textsubscript{3–36} has a half-life of \(~15\) min and is subjected to COOH-terminal degradation that eliminates activation of the Y2 receptor. The half-life of 15 min precludes a once-daily dosing regime, and modifications to prolong half-life are probably necessary to ensure compliance and effectiveness. Such modification may include protection (without losing the effect) of the COOH terminus because PYY, as shown here, is subjected to significant COOH-terminal degradation despite the COOH-terminal amidation.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**


**REFERENCES**


