

CLINICAL STUDY

Effect of somatostatin infusion on peptide YY secretion: studies in the acute and recovery phase of anorexia nervosa and in obesity

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Abstract

Objective: Changes in many gastrointestinal peptides, including the anorexigenic peptide YY (PYY), which is produced by L cells, occur in both anorexia nervosa (AN) and obesity (OB). High PYY levels are present in AN, whereas in morbid OB fasting and postprandial PYY secretion is blunted. Somatostatin (somatotropin release-inhibiting factor (SRIF)) reportedly inhibits plasma PYY concentrations in animals and healthy humans, but the effect of a SRIF infusion on spontaneous PYY secretion in AN and OB is unknown.

Methods: A total of 18 young women, seven with acute AN (A-AN), four with AN in the recovery phase (R-AN), and seven with morbid OB, were studied. All subjects underwent an infusion of SRIF (9 µg/kg i.v./h, over 60 min), with blood samples drawn before and at different time intervals after SRIF administration. Plasma PYY levels were measured at each time point.

Results: SRIF significantly inhibited plasma PYY concentrations in R-AN and OB, without affecting PYY titers in A-AN. In OB, the inhibitory effect of SRIF also persisted at 90 min. Withdrawal of SRIF infusion in R-AN resulted in a prompt restoration of basal plasma PYY levels, whereas termination of SRIF infusion in OB was followed by a slower increase of PYY titers toward baseline levels. After infusion, PYY Δ area under the curve (Δ AUC) in R-AN was significantly higher than those in A-AN and OB patients. A significant difference in PYY Δ AUC between A-AN and OB was present.

Conclusions: These results suggest the existence of a hypo- and hyper-sensitivity of L cells to the inhibitory effect of SRIF in A-AN and OB respectively.

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Introduction

Somatostatin (somatotropin release-inhibiting factor (SRIF)) is composed of two principal bioactive molecular forms, SRIF14 and SRIF28, derived through differential processing of a common prosomatostatin precursor (1). The gastrointestinal tract provides the largest source of SRIF and shows relatively selective distribution of the two molecular forms (2, 3).

The biological effects of SRIF are mediated by five distinct receptors (SSTR1–5) that are widely expressed in the CNS and the periphery, including the gastrointestinal tract (4–18).

Peptide YY (PYY) is a 36-amino acid peptide synthesized and released into the circulation from specialized enteroendocrine cells, called L cells, predominantly located in the distal gastrointestinal tract (19).

In this study, two main forms of PYY have been described: PYY_{1–36} and PYY_{3–36} (20). Importantly,

PYY_{3–36} is the main circulating form of the peptide both in the fasted and in the fed states (21–23).

Circulating PYY levels increase promptly in response to nutrient ingestion, caloric load, food consistency, and nutrient composition (22, 24, 25). The initial postprandial rise in circulating PYY occurs within 15 min of food ingestion, before nutrients have reached the L cells of the distal gastrointestinal tract, implying the existence of a neural or hormonal mechanism in this release process. Typically, plasma PYY peak occurs 1–2 h postprandially, followed by a plateau phase of several hours (26).

Peripheral administration of PYY reduces food intake in rodents and normal-weight humans (27). Reportedly, the anorectic effect of PYY is preserved in patients with morbid obesity (OB), who under baseline conditions exhibit reduced fasting and postprandial levels of the peptide (28). Along this line, a negative association between PYY concentrations and markers of adiposity

has been reported in some, but not all, studies in adults, children, and infants (28–34).

In contrast to OB subjects, increased fasting and meal-stimulated PYY levels are present in patients with anorexia nervosa (AN), suggesting that the peptide may play a pathophysiological role in this eating disorder (34–37).

Previous studies in animal models have shown that SRIF is a potent inhibitor of PYY secretion (10). In addition, SRIF has been reported to inhibit PYY postprandial response also in healthy subjects (38), but the effect of SRIF on PYY secretion in AN and OB is presently unknown.

Therefore, based on the foregoing, we studied the effect of a SRIF14 infusion on spontaneous PYY release in patients with AN, in the acute phase (A-AN) or recovery phase (R-AN) of the disease, and in OB subjects.

Materials and methods

Subjects and methods

A total of 18 young women (seven patients with A-AN, aged 19–33 years; four with R-AN, aged 17–32 years; and seven with morbid OB, aged 17–29 years) were studied.

Clinical and hormonal characteristics of the study subjects are shown in Table 1. All AN patients met the diagnostic criteria for AN according to the Diagnostic and Statistical Manual of Mental Disorders IV-TR (39). Patients were defined having R-AN when, after a complex program adopted for AN, consisting of nutritional rehabilitation, individual psychotherapy, family therapy, and behavioral counseling in both inpatient and outpatient setting, they gained a stable low normal weight, and became eumenorrheic for at least 6 months (40); for R-AN patients, the duration of the R-AN was 15.8 ± 4.0 months. All subjects gave informed consent to participate in the study, which had been approved by the local ethics committee. OB subjects (body mass index (BMI) $> 30 \text{ kg/m}^2$) had no history or actual evidence of endocrine or psychiatric disorders and had not been taking medications in the previous 6 months. Being eumenorrheic, all patients with R-AN and OB were studied in the early follicular

phase of the menstrual cycle. Patients with A-AN were amenorrheic.

After an overnight fast, an indwelling (i.v.) cannula was inserted in both forearms at 0800 h for separate blood sampling and drug administration. SRIF (SRIF14; Stilamin; Serono, Rome, Italy) was infused i.v. in 50 ml normal physiological saline at a rate of $9 \mu\text{g/kg per h}$ over 60 min (0–60 min of the study), with blood samples drawn at –30, 0, 30, 45, 60, 90, 105, 120, 135, and 150 min. Plasma PYY levels were measured at each time interval.

The dose of SRIF was chosen based on its effectiveness to inhibit GH secretion in A-AN (41). Anyway, serum GH levels were measured before, during, and after SRIF infusion (i.e. at 0, 30, and 60 min).

Baseline plasma levels of estradiol (E_2), free triiodothyronine (FT_3), free thyroxine (FT_4), TSH, IGF1, and immunoreactive insulin (IRI) were measured once at –30 min before SRIF infusion.

Total plasma PYY, including both PYY_{1-36} and PYY_{3-36} , was measured by a commercially available RIA for PYY (Linco Research, Saint Charles, MO, USA). The sensitivity of the method was 10 pg/ml ; intra- and inter-assay coefficients of variation (CVs) were 2.9 and 7.1% respectively.

Serum GH levels were estimated by chemiluminescence immunoassay (Nichols Advantage, Nichols Institute Diagnostics, San Juan Capistrano, CA, USA); sensitivity of the assay is 0.01 ng/ml , intra- and inter-assay CVs were 4.4 and 8.6% respectively. Serum IGF1 levels were estimated by chemiluminescence immunoassay (Nichols Advantage, Nichols Institute Diagnostics); sensitivity of the assay was $6 \mu\text{g/l}$, intra- and inter-assay CVs were 4.2 and 6.3% respectively. Serum IRI was measured by electrochemiluminescence immunoassay (ECLIA; Roche Diagnostics GmbH); sensitivity of the assay was $0.2 \mu\text{U/ml}$; intra- and inter-assay CVs were 4.2 and 8.2%, respectively; and normal range in our laboratory was $2.6\text{--}24.9 \mu\text{U/ml}$. Serum TSH was measured by ECLIA (Roche Diagnostics); sensitivity of the assay was 0.005 mU/l ; intra- and inter-assay CVs were 5.4 and 8.7%, respectively; and normal range was $0.27\text{--}4.2 \text{ mU/l}$. Serum FT_4 was measured by ECLIA (Roche Diagnostics); sensitivity of the assay was 0.30 pmol/l ; intra- and inter-assay CVs were 4.8 and 6.6%, respectively; and normal range $12\text{--}22 \text{ pmol/l}$. Serum FT_3 was measured by ECLIA (Roche Diagnostics);

Table 1 Demographic and hormonal characteristics of the study subjects. Data are expressed as mean \pm s.e.m.

Group	Age (years)	BMI (kg/m^2)	Dis. dur (years)	PYY (pg/ml)	E_2 (pmol/l)	FT_3 (pmol/l)	FT_4 (pmol/l)	TSH (mU/l)	IRI ($\mu\text{U/ml}$)	IGF1 ($\mu\text{g/l}$)
A-AN	22.6 ± 1.7	$13.8 \pm 0.3^*$	4.1 ± 1.6	127.87 ± 6.98	$39.4 \pm 3.4^*$	$2.4 \pm 0.4^*$	12.4 ± 0.4	2.4 ± 0.4	$2.9 \pm 0.7^*$	$57.1 \pm 7.67^*$
R-AN	24.0 ± 2.1	19.0 ± 0.8	1.3 ± 0.3	114.53 ± 10.88	123.7 ± 31.2	4.3 ± 0.5	12.8 ± 1.5	1.3 ± 0.2	8.6 ± 2.9	155.9 ± 12.98
OB	21.5 ± 1.8	$37.4 \pm 1.5^{*\dagger}$	–	$109.71 \pm 7.75^\dagger$	$289.2 \pm 85.2^{*\dagger}$	$4.7 \pm 0.3^\dagger$	14.3 ± 0.6	2.2 ± 0.2	$16.9 \pm 3.3^{*\dagger}$	$122.4 \pm 14.60^\dagger$

* $P < 0.01$ versus R-AN; $^\dagger P < 0.01$ versus A-AN. Dis. dur, disease duration.

sensitivity of the assay was 0.4 pmol/l; intra- and inter-assay CVs were 5.1 and 9.0%, respectively; and normal range was 2.8–7.1 pmol/l. Serum E_2 was measured by ECLIA (Roche Diagnostics); sensitivity of the assay was 18.4 pmol/l; intra- and inter-assay CVs were 4.9 and 6.2%, respectively; and normal range for the follicular phase of the menstrual cycle was 90.1–716 pmol/l.

No adverse effects were recorded during the test.

Statistical analysis

A specific statistical software package (Prism 5, GraphPad Software, San Diego, CA, USA) was used for data analysis and graphing.

Results are expressed as mean \pm s.e.m. To facilitate comparison of the PYY secretory profiles during the test, plasma PYY concentrations were expressed either as absolute values or as PYY Δ area under the curve (Δ AUC; Δ AUC detected after withdrawal of SRIF infusion minus the value of the plasma PYY level at t_{60} multiplied by 90 min in each subject). To demonstrate the effectiveness of the infused dose of SRIF, the SRIF-induced inhibition of serum GH levels was calculated as Δ AUC (i.e. AUC detected during SRIF infusion minus the value of the serum GH level at t_{60} multiplied by 60 min in each subject).

Plasma PYY concentrations were compared within each group and among all groups using a two-way ANOVA with repeated measures followed by the *post hoc* Tukey's test. One-way ANOVA was used to compare PYY Δ AUCs of the three groups as well as the other demographic and hormonal characteristics of the study subjects. Correlations between baseline PYY levels or PYY Δ AUCs and the other parameters were performed by linear regression analysis.

A *P* value of 0.05 was chosen as statistically significant.

Results

Table 1 shows the mean values \pm s.e.m. for age, BMI, PYY, E_2 , FT₃, FT₄, TSH, IGF1, and IRI in A-AN, R-AN, and OB women. For A-AN and R-AN, the duration of the condition is also reported.

Serum GH levels were significantly inhibited by SRIF infusion in A-AN and R-AN (GH at 0 vs 60 min: 4.17 ± 0.85 vs 0.90 ± 0.41 ng/ml in A-AN and 4.92 ± 2.89 vs 0.48 ± 0.18 ng/ml in R-AN, $P < 0.01$; Δ AUC: -122.30 ± 2.88 ng/ml \times min in A-AN and -176.70 ± 50.64 ng/ml \times min in R-AN), whereas no statistically significant difference was found in serum GH levels in OB before and after SRIF infusion (0.07 ± 0.02 vs 0.05 ± 0.01 ng/ml; Δ AUC: -20.00 ± 11.10 ng/ml \times min), indicating the effectiveness of the infused dose of SRIF.

Baseline plasma PYY concentrations were significantly higher in A-AN than in OB (127.9 ± 7.0 vs

109.7 ± 7.8 pg/ml, $P < 0.001$), and the same was true for R-AN patients (114.5 ± 10.9 pg/ml), although the difference did not reach statistical significance. Baseline plasma PYY concentrations were lower in OB than in R-AN patients, but with no statistical significance (Table 1).

Plasma PYY concentrations from 30 up to 150 min were significantly lower in R-AN than in A-AN ($P < 0.01$) and remained persistently higher in A-AN than in OB patients ($P < 0.001$ at all time points). No differences were found in PYY profiles of R-AN and OB patients, except for plasma PYY concentrations at t_{90} ($P < 0.05$; Fig. 1).

SRIF significantly inhibited plasma PYY concentrations (at 30, 45, and 60 min) in R-AN and OB, without affecting those of A-AN ($P < 0.001$) patients. In OB, the inhibitory effect also persisted at 90 min ($P < 0.001$; Fig. 1).

Percent inhibition of plasma PYY concentrations from t_0 to t_{60} during SRIF infusion was significantly higher in R-AN and OB than in A-AN (29.2 ± 2.3 and $31.5 \pm 3.1\%$ vs $14.5 \pm 4.2\%$, respectively, $P < 0.001$), although no difference was present between R-AN and OB patients (data not shown).

SRIF infusion withdrawal in R-AN resulted in a prompt rise in plasma PYY levels from 81.1 ± 7.9 pg/ml at t_{60} to 119.0 ± 13.5 pg/ml at t_{90} ($P < 0.001$), whereas termination of SRIF infusion in OB was followed by a sluggish rise in plasma PYY levels from 75.2 ± 2.4 pg/ml at 60 min to a maximum of 99.1 ± 12.6 pg/ml at 135 min ($P < 0.001$; Fig. 1).

PYY Δ AUC in R-AN (1674.9 ± 110.0 pg/ml \times min) was significantly higher than that in A-AN (782.3 ± 122.0 pg/ml \times min, $P < 0.001$) and OB (1072.7 ± 222.0 pg/ml \times min, $P < 0.001$) patients. There was

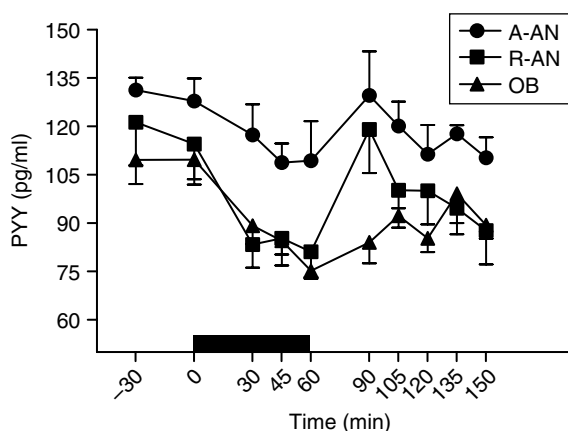


Figure 1 Plasma PYY concentration profiles (mean \pm s.e.m.) of patients with anorexia nervosa in the acute (A-AN) and recovery (R-AN) phases and subjects with obesity (OB) administered a 1 h infusion of SRIF (9 μ g/kg per h i.v.). Bar indicates timing and duration of infusion. See text for intra- and inter-group statistically significant differences.

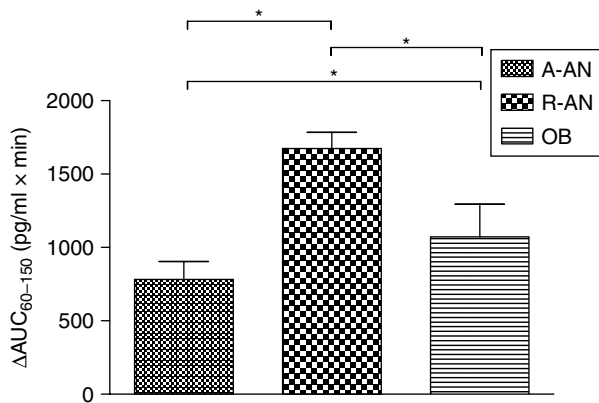


Figure 2 Plasma PYY Δ AUC (mean \pm S.E.M.) of A-AN, R-AN, and OB patients administered a 1 h infusion of SRIF (9 μ g/kg per h i.v.). Δ AUCs were calculated as area under the curve after withdrawal of SRIF infusion minus the value of the plasma PYY level at t_{60} multiplied by 90 min in each subject. Brackets and asterisks indicate groups that are statistically different. * $P < 0.001$.

a significant difference in PYY Δ AUC between A-AN and OB ($P < 0.001$; Fig. 2).

Among the possible correlations between basal PYY levels and PYY Δ AUCs after SRIF infusion withdrawal and clinical or hormone parameters, of interest were the negative correlations between baseline PYY levels and BMI ($r = -0.52$; $P < 0.01$), IRI ($r = -0.61$; $P < 0.05$), or IGF1 ($r = -0.49$; $P < 0.05$; Fig. 3).

Discussion

In this study, baseline plasma PYY concentrations were significantly higher in A-AN than in OB; conversely, there were no statistically significant differences in baseline PYY levels between A-AN and R-AN or between OB and R-AN.

Our data confirm those reported in patients with AN (42) and bulimia nervosa (43), in whom PYY concentrations were not significantly different from those present in controls. Either of these studies, like ours, examined a small number of subjects; therefore, lack of power might account for the absence of a statistically significant difference between the groups (A-AN versus R-AN and R-AN versus OB). In this context, in another study, conducted in a larger number of subjects, higher PYY levels were found in AN patients than in healthy girls (36).

R-AN subjects might represent an 'inappropriate' control group and be actually a limit of our study. Weight recovery in AN is reportedly associated per se toward a decrease in PYY levels (36); hence, in our study, the change in PYY levels with weight recovery would probably have been more profound if more weight-recovered patients had been evaluated. Further

studies in a larger population of R-AN patients are mandatory to determine whether a change in BMI predicts an inverse change in PYY levels.

Our data are consistent with previous studies in OB, which have disclosed lower (fasting and postprandial) PYY levels than in controls (23, 28, 29).

Along with the previous considerations, in a simplified way of thinking, this suggests that in OB, low PYY levels may lead to increased food intake, whereas, conversely, in AN high PYY levels may contribute to decreasing food intake (19, 28, 44, 45).

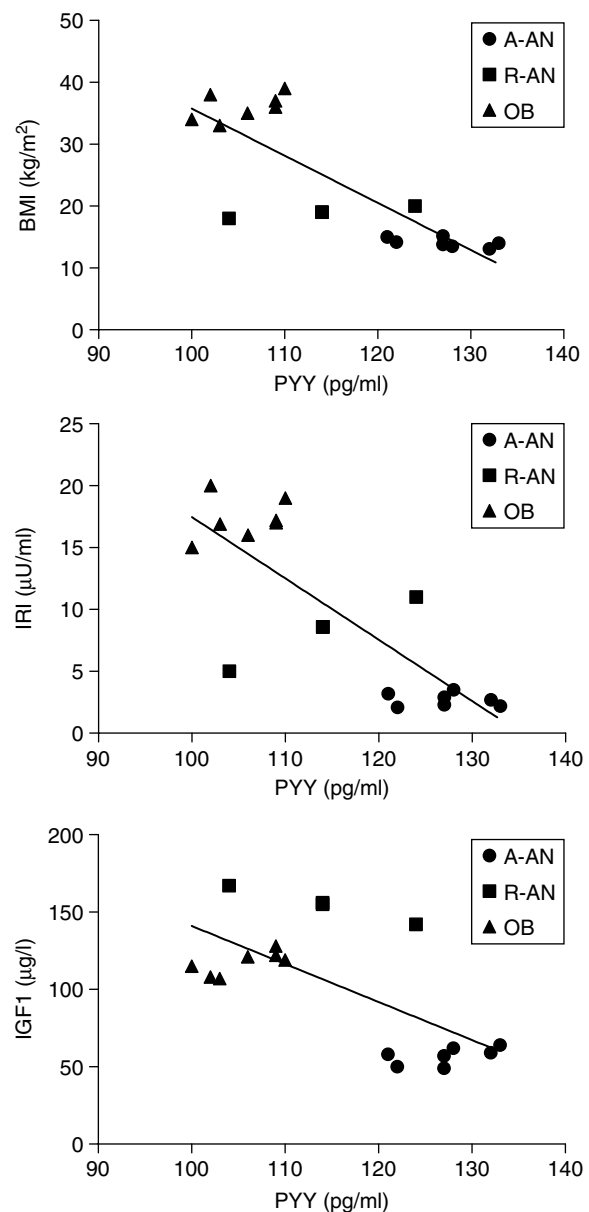


Figure 3 Correlations (for all data) between baseline PYY levels and BMI ($r = -0.52$; $P < 0.01$; top panel), IRI ($r = -0.61$; $P < 0.05$; middle panel), or IGF1 ($r = -0.49$; $P < 0.05$; bottom panel).

SRIF is known to possess a remarkable inhibitory effect on many gastroenteropancreatic hormonal secretions and functions (46).

This study shows (for the first time) that SRIF inhibits spontaneous secretion of PYY not only in R-AN but also in OB. This finding is consistent with previous studies in animal models and humans, in which SRIF was shown to be a potent inhibitor of fasting and postprandial PYY secretion (10, 38).

At present, the mechanisms underlying the inhibitory effect of SRIF on PYY secretion are unknown, but likely to reflect direct activation of SRIF receptors, which are widely expressed in the gastrointestinal mucosa (4, 5).

SRIF14 has generally been regarded as the only bioactive molecular form of SRIF regulating inhibition of gut endocrine secretion, whereas the inhibitory role of SRIF28 would have been limited to modulating pituitary hormones (15) and insulin (47). In contrast to this view, however, Chisholm & Greenberg (48) have shown that SRIF28 is the principal bioactive molecular form of SRIF mediating inhibition of PYY secretion from rat intestinal cell cultures: in this context, either SRIF molecular form caused dose-dependent inhibition of PYY secretion when stimulated by gastrin-releasing peptide (GRP), the principal hormone regulating PYY release (49). However, SRIF28 was 300-fold more potent than SRIF14 and, interestingly, it would mediate inhibition of GRP-stimulated PYY secretion through activation of SSTR5 (48), the isoform endowed with a higher affinity for SRIF28 than for SRIF14 (6).

In this study, only SRIF14 was administered and an inhibition of ~30% was found on PYY secretion in R-AN and OB, suggesting the involvement of other SSTR isoforms (e.g. SSTR1–4) in these effects. Finally, a direct interaction between SRIF14 and SSTR5 may not be ruled out in our study, as the pharmacological dose of SRIF used in this study was capable of strikingly inhibiting GH secretion in A-AN and R-AN (41).

The main finding of our study was the inability of SRIF to inhibit PYY secretion in A-AN patients and the lack of any rebound rise at the withdrawal of SRIF infusion (i.e. reduced PYY Δ AUC). This pattern contrasts the SRIF-induced inhibition of fasting and postprandial PYY levels of patients with Prader–Willi syndrome, who share with A-AN the hyperghrelinemia (45, 50).

One might hypothesize the existence of an altered sensitivity (hyposensitivity) of L cells to SRIF in A-AN. Alternatively, the unchanged PYY secretion of A-AN patients may be related to a malnourishment-dependent reduction or elevation of an unknown PYY inhibitory or stimulatory factor respectively, resulting in a persistent activation of L cells. A putative candidate could be one of the gastrointestinal hormones that are altered in A-AN (51), ruling out ghrelin, which has an inhibitory effect on PYY secretion (28).

In OB subjects, PYY secretion was promptly inhibited by SRIF, but only slowly returned to baseline levels at the termination of SRIF infusion. In contrast with A-AN, this would imply the existence of a state of hypersensitivity of L cells to SRIF in OB.

The similar percent inhibition of plasma PYY levels in OB and R-AN is not in contrast with this view, because PYY secretion is reduced with fasting and, taking into account the limit of detection of the analytical method we used, it is not further susceptible to inhibition by SRIF. This hypothesis should be verified, in further studies, by evaluating the inhibitory effect of SRIF on the robust PYY release elicited by a high-fat meal.

The altered sensitivity of L cells in OB might be the consequence of an adiposity-related factor, which results in a blunted synthesis and/or release of PYY. This might be the same previously alluded for explaining the findings in A-AN (see above).

Some limitations of this study have to be mentioned. First, only plasma (total) PYY levels were evaluated, disregarding other gastrointestinal peptides that influence PYY secretion (19). Second, no real control group (i.e. healthy, normal-weight subjects) could be recruited; hence, these findings should be considered cautiously. Third, the results were obtained in a reduced number of patients, though the limited spread of data should be positively considered. Finally, measurement of SRIF levels during and after infusion was not performed; therefore, differences in metabolic half-life due to disease-induced changes of plasma peptidase activity may not be ruled out.

In conclusion, elevated PYY secretion in A-AN is unaffected by an SRIF infusion, which, in contrast, inhibits plasma PYY levels in R-AN and OB, with a delayed return to baseline PYY levels in OB. Overall, the existence of a state of hypo- and hypersensitivity of L cells to the inhibitory effect of SRIF in A-AN and OB, respectively, may be envisaged. Though coherent with the results obtained, this interpretation might be too speculative; further studies (also in animals) are, therefore, warranted.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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