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BRIEF COMMUNICATION

Bariatric Surgery

The anorexigenic peptide neurotensin relates to insulin sensitivity in obese patients after BPD or RYGB metabolic surgery

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Abstract

Neurotensin is a peptide with effects on appetite and intestinal lipid absorption. Experimental data suggest a role in glucose homeostasis, while human data is missing. Here, 20 morbidly obese subjects either underwent biliopancreatic diversion with duodenal switch (BPD), or Roux-en-Y gastric bypass (RYGB) in a randomized fashion. Before and 1 year after surgery, anthropometric data, body composition, clinical biochemistry, insulin sensitivity by means of euglycemic hyperinsulinemic clamps (HEC) and fasting plasma proneurotensin 1–117 were analyzed. Plasma proneurotensin increased significantly more 1 year after BDP than RYGB ($P = 0.028$), while weight loss was comparable. After metabolic surgery, proneurotensin correlated positively with insulin sensitivity (M-value) ($r = 0.55$, $P < 0.001$), while an inverse relationship with fasting glucose, HOMA-IR and HbA1c was observed ($P < 0.05$ for all components). After adjustment for age and gender, proneurotensin and BMI remained independently related with delta of M-value ($\beta = 0.46$ and $\beta = 0.51$, $P < 0.05$, resp.). From these data we conclude that proneurotensin positively correlates with insulin sensitivity uniquely after weight loss induced by metabolic surgery in humans. BDP leads to a stronger increase in the anorexigenic peptide compared to RYGB.

Introduction

Obesity is a worldwide epidemic and related to type 2 diabetes (T2D) [1]. Metabolic surgery leads to T2D remission [1], but bariatric interventions are indicated only in a minority of obese subjects. Therefore, identifying key mediators, by which metabolic surgery exerts its beneficial effects on glucose metabolism, could represent a promising strategy to develop less invasive therapeutic approaches.

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Insulin resistance is the pathophysiological root cause of T2D and it is well accepted that circulating organokines, such as adiponectin, modulate insulin action [2]. Neurotensin, a peptide predominantly produced in specialized enteroendocrine cells of the small intestine, is associated with insulin resistance in rodents [3, 4]. In humans, fasting concentrations of proneurotensin, a stable stoichiometric precursor fragment of neurotensin in plasma, are related to incident T2D [5]. Proneurotensin also correlated with the risk of developing obesity in later life [4] and with mortality [5]. Metabolic surgery is known to modulate proneurotensin in both, animals and humans [6–8]. However, to the best of our knowledge, proneurotensin has never been investigated in relation to in vivo gold standard measures of human insulin sensitivity. Therefore, in a pilot study in morbidly obese subjects, we aimed at evaluating the effect of metabolic surgery on fasting plasma concentrations of proneurotensin, and on potential associations with insulin sensitivity as evaluated by the hyperinsulinemic euglycemic glucose clamp (HEC) technique.

Methods

We studied a subset from the hypoglycaemia trial (ClinicalTrials.gov Identifier: NCT01581801) undergoing HEC procedure. Informed consent was obtained, the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the Catholic University of the Sacred Heart Ethical Committee (A1534/CE/2012).

The study was performed as a monocenter prospective controlled randomised clinical trial. Inclusion criteria were as follows: Ability to understand the study protocol and to give written informed consent, age of 25–65 years, BMI ≥ 35 kg/m^2 in combination with typical complications (i.e., sleep apnoea, orthopaedic complications, etc.). Exclusion criteria comprised previous bariatric intervention, manifest cancer, diabetes mellitus or major cardiac, respiratory and gastrointestinal disease and significant hormonal disorders. Twenty eligible subjects were included. Included subjects randomly underwent either BPD or RYGB surgery [1, 2]. Baseline anthropometric measures, body composition, energy intake, medical history, physical examination, clinical chemistry and insulin sensitivity were evaluated and fasting blood samples were taken before and 1 year after surgery. Body composition was measured by dual-energy X-ray absorptiometry using a Lunar Prodigy^{TM} whole-body scanner [2]. Energy consumption was estimated by using structured food intake interviews before and 1 year after surgery. Insulin sensitivity was analyzed by HEC [2] and glucose disposal [expressed as insulin-mediated glucose uptake (*M*-value)] was calculated from the glucose infusion rate during the last 40 min. of the steady-state period [2]. Surrogates of insulin resistance, i.e., homeostasis-model assessment of insulin resistance (HOMA-IR), were calculated. Clinical and metabolic biochemistry was analyzed by standard methods in the certified Catholic University of the Sacred Heart Hospital laboratory. Proneurotensin was measured in fasting plasma specimens that were stored frozen at −80 °C using a chemiluminometric sandwich immunoassay to detect a proneurotensin precursor fragment (pro-NT 1–117) as described previously [9]. The limit of detection of this assay is 10 pmol/l with a coefficient of variation < 20% for inter-assay comparisons and <10% for intra-assay [10].

In this pilot study, data were analyzed with IBM SPSS 15.0 (Chicago, Ill, USA). Data are given as mean ± SEM if not otherwise stated. Spearman's rank correlation coefficient was used for correlation analyzis, Wilcoxon rank and Mann Whitney U test for evaluating group differences. Linear associations were examined using a backward least-square regression analysis. Significance level was defined as two-sided $P < 0.05$.

Results

Baseline characteristics and effects of surgery after 1 year including anthropometry, energy consumption, and measures of glucose metabolism are given in Table 1. As expected, surgical intervention had favourable effects not only on caloric intake and on body weight, but also on metabolic profile, particularly on insulin sensitivity and fasted glucose levels ($P < 0.05$, respectively; Table 1).

Plasma proneurotensin markedly increased 1 year after metabolic surgery ($P < 0.001$; Suppl.Fig.1). By stratifying by RYGB vs. BPD we observed similar reductions in caloric intake (−2945 ± 173 kcal/d for RYGB vs. −2983 ± 300 kcal/d in BPD; $P = 0.97$) and no significant differences in body weight loss (−34.1 ± 5.3 kg for RYGB vs. −39.9 ± 6.3 kg in BPD; $P = 0.20$) and BMI (−11.4 ± 2.0 kg/m^2 for RYGB vs. −14.1 ± 2.2 kg/m^2 in BPD; $P = 0.11$), while the increase in fasting plasma proneurotensin was more pronounced after BPD ($P = 0.028$;Suppl.Fig.2), as was the increase in *M*-value (1.4 ± 0.2 mg/kg/min. for RYGB vs. 2.8 ± 0.3 mg/kg/min. in BPD; $P < 0.001$).

No association of baseline fasting plasma proneurotensin with energy intake, BMI, body weight, percent BF, fasting glucose, glycated haemoglobin (HbA1c), insulin resistance, insulin sensitivity (*M*-value in HEC) and blood lipids was observed ($P > 0.05$,respectively, data not shown). However, 1 year after surgery analysis of pre- and post surgery data showed an inverse correlation of energy consumption, body weight and BMI with proneurotensin concentrations in plasma ($r = -0.57$, $P < 0.001$, $r = -0.40$, $P = 0.011$ and $r = -0.44$, $P = 0.004$, respectively). Moreover, a significant

Table 1 Anthropometric and clinical chemistry data before and after bariatric surgery induced weight loss

	Pre bariatric surgery	Post bariatric surgery	Mean Δ	*P*-value
RYGB/BPD (%)	–	50/50	–	–
n (% male)	20 (70)	–	–	–
IFG/T2D (%)	10/0	0/0	–	–
Age (years)	43 ± 2	–	–	–
Body weight (kg)	153.2 ± 6.1	116.1 ± 5.7	−37.0 ± 4.0	<0.001
BMI (kg/m²)	52.3 ± 1.9	39.5 ± 1.7	−12.8 ± 1.5	<0.001
Body fat (%)	50.0 ± 2.1	42.7 ± 2.6	−7.5 ± 1.9	0.016
Lean body mass (kg)	64.2 ± 3.6	62.1 ± 3.4	−2.1 ± 0.2	0.032
Energy intake (kcal/d)	5033 ± 238	2069 ± 160	−2964 ± 168	<0.001
Fasting glucose (mg/dl)	88.5 ± 2.0	72.9 ± 2.6	−15.6 ± 2.1	<0.001
Fasting plasma insulin (μU/ml)	13.7 ± 2.0	8.2 ± 1.2	−5.5 ± 2.0	n.s.
HbA1c (mmol/mol)	40.2 ± 0.7	37.1 ± 0.6	−2.9 ± 0.8	n.s.
HbA1c (%)	5.8 ± 0.1	5.5 ± 0.1	−0.3 ± 0.1	n.s.
HOMA-IR	3.3 ± 0.5	1.6 ± 0.2	−1.7 ± 0.5	0.032
Hepatic-IR index	2.4 ± 0.03	2.3 ± 0.03	−0.1 ± 0.03	n.s.
M-value (mg/kg/min.)	3.4 ± 0.2	5.4 ± 0.3	+ 2.1 ± 0.2	<0.001
SS plasma insulin (μU/ml)	45.3 ± 11.2	15.9 ± 4.6	−29.4 ± 6.6	0.13
γGT (U/l)	40.7 ± 5.1	16.3 ± 1.7	−24.3 ± 4.4	<0.001
Triacylglycerides (mg/dl)	124.5 ± 14.1	112.1 ± 14.2	−12.4 ± 7.3	n.s.
LDL cholesterol (mg/dl)	113.2 ± 6.7	78.2 ± 6.9	−37.9 ± 9.4	0.048

Data are given as means ± SE or absolute values

Group comparisons were performed by using Wilcoxon rank test with post hoc Bonferroni adjustment. Before Bonferroni correction significant differences were observed concerning HbA1c ($P = 0.004$), Hepatic-IR index ($P = 0.005$), fasting insulin levels ($P = 0.005$) and steady state plasma insulin concentrations ($P = 0.008$)

BMI body mass index, *BPD* biliopankreatic diversion, *γGT* γ-glutamyl transaminase, *HbA1c* glycated hemoglobin 1c, *Hep-IR* hepatic insulin resistance, *HOMA-IR* homeostasis model assessment of insulin resistance, *IFG* impaired fasting glucose, *kcal* kilocalories, *LDL* low density lipoprotein, *n.s.* not significant, *RYGB* Roux-en-Y gastric bypass, *SS* steady state, *T2D* type 2 diabetes mellitus

positive relationship of combined pre and post surgery fasting proneurotensin with M-value, the gold standard read out of insulin sensitivity, became highly significantly apparent ($r = 0.55$, $P < 0.001$; Fig.1). When adjusting for main confounders (i.e., BMI, age gender) by means of backward linear regression analysis with delta of M-value as dependent variable, we observed an expected correlation with combined pre- and post surgery BMI ($\beta = 0.51$, $P = 0.010$), but there was also an independent association with combined plasma proneurotensin data in the last model ($\beta = 0.46$, $P = 0.018$; adjusted $R^2 = 0.41$, $P = 0.005$ for the model). Furthermore, combined pre and post surgery plasma proneurotensin was inversely related to fasting glucose ($r = -0.54$, $P < 0.001$; Fig. 1b), HOMA-IR ($r = -0.37$, $P = 0.020$; Fig. 1c) and HbA1c ($r = -0.44$, $P = 0.005$; Fig. 1d).

Discussion

The major finding of our study is that proneurotensin is positively associated with in insulin sensitivity as assessed by the hyperinsulinemic euglycemic clamp after bariatric surgery. Interestingly, the association became only apparent after weight loss due to metabolic surgery. Our findings further provide evidence for varying effects of diverse metabolic surgery techniques on fasting plasma proneurotensin.

A recent human study linked fasting proneurotensin to T2D and cardiovascular mortality [5]. This relationship to strong clinical endpoints could indicate a prominent role of the proneurotensin-neurotensin system in energy homeostasis. Neurotensin is expressed in brain and gastrointestinal tract, with an established effect on food intake [6]. It is therefore tempting to speculate that the observed increase in proneurotensin after metabolic surgery contributes to reduced food intake after the surgical intervention, and by this mechanism, improves insulin sensitivity indirectly. In contrast, in a previous report of Li et al., the absence of neurotensin was protective against insulin resistance, a condition that was reversible by pharmacological substitution of the peptide [4]. In line with the latter finding, fasting proneurotensin was reported to be positively associated with incident T2D in a human

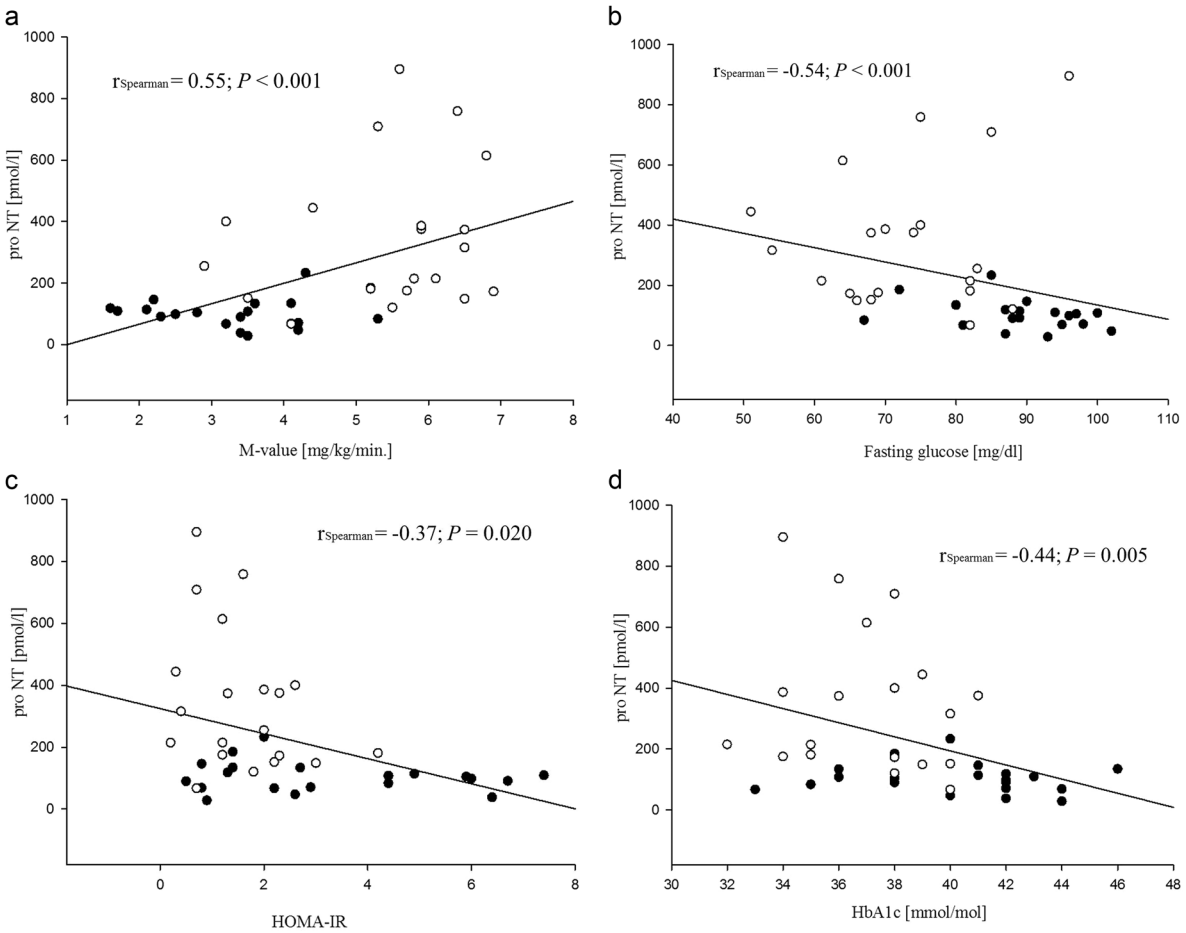

Fig. 1 Correlation of proneurotensin with measures of glucose metabolism. **a** The scatter plot shows the relationship of fasting proneurotensin levels with *M*-value ($r = 0.55$, $P < 0.001$). **b** Correlation of plasma proneurotensin and fasting glucose ($r = -0.54$, $P < 0.001$); **c** Association of plasma proneurotensin with HOMA-IR ($r = -0.37$, $P = 0.020$); **d** Scatter plot showing plasma proneurotensin and HbA1c ($r = -0.44$, $P = 0.005$). Black circles: Pre-surgery data, white circles: Post-surgery data; *HbA1c* glycated hemoglobin 1c, *HOMA-IR* homeostasis model assessment of insulin resistance

epidemiological study [5]. Yet, we report on a positive relationship with insulin sensitivity and other measures of glucose metabolism, which became exclusively apparent after metabolic surgery, without any significant association with baseline insulin sensitivity. However, in our patients, the observed association of proneurotensin with insulin sensitivity persisted after adjustment for major confounders, such as BMI, and could therefore be indicative of an additional food intake-independent effect of proneurotensin on glucose metabolism. The latter is supported by the observation that although we postoperatively detected a comparable reduction in caloric intake and body weight in our BPD vs. RYGB subjects, the increase in M-value was significantly more pronounced after BPD, in concert with the even more prominent rise in proneurotensin. Therefore, effects on food intake may represent the main, indirect, operating mechanism how proneurotensin effects glucose metabolism in humans, and there may be other complementary effects.

Previous data indicated that neurotensin mediated intestinal lipid absorption [3] and that neurotensin knockout mice had reduced hepatic steatosis together with improved insulin resistance [4, 11]. Therefore, after bariatric surgery, the increase in proneurotensin may reflect an adaptive response to compensate for the malabsorption of lipids [12]. The fact that the increase in proneurotensin was more pronounced after malabsorptive BDP procedure speaks is in favour of this notion. Accordingly, higher proneurotensin levels after metabolic surgery may indicate a higher degree of lipid malabsorption [12]. Reducing dietary lipids improves insulin sensitivity [13, 14]. Other effects of proneurotensin acting on insulin sensitivity in morbidly obese patients undergoing bariatric surgery are unknown so far. The exact mechanisms were beyond the scope of this study and will have to be elucidated by future work.

We observed differential effects of RYGP and BPD on fasting proneurotensin, although subjects were randomly assigned to either technique, matched for age, comparable

in terms of gender distribution and lost similar amounts of body mass. Our data correspond to earlier findings suggesting significantly increased proneurotensin levels at 6, 12 and 24 months after gastric bypass compared to gastric banding or controls [8]. This effect was independent from changes in body weight [8]. Moreover, also Holdstock et al. observed the highest proneurotensin concentrations in RYGB subjects compared to morbid obese and lean subjects [7]. Proneurotensin is mainly produced in specialized cells of the small intestine and it is therefore plausible that diverse bariatric approaches could variably modulate circulating proneurotensin. Indeed, corresponding to available human data, studies in a rat model of RYGB found increased proneurotensin in plasma and the gastrointestinal tract with significant effects on food intake [6]. Whether markedly increased proneurotensin levels after BPD compared to RYGB play a role regarding the more exaggerated effect of BPD vs. RYGP on T2D remains to be investigated. We hypothesize, that in BPD being a significant malabsorptive bariatric operation, which leads to massive lipid malabsorption [12], the neurotensin-mediated intestinal transportation of fatty acids might be enhanced in order to overcome a deficiency of essential fatty acids.

Our study is limited by lack of mechanistic evidence and can therefore not prove causality. Moreover we did not apply stable isotope approaches, which could have helped to detect tissue specific effects on insulin sensitivity [15]. Nevertheless, our data show a consistent relationship of fasting proneurotensin with markers of whole body insulin sensitivity and insulin resistance, and correspond to existing animal data, suggesting a robust association. Furthermore, we studied a limited number of subjects. Otherwise, we report significant and consistent effects, suggesting sufficient power, as in previous studies.

In conclusion, our results support a significant relationship of circulating proneurotensin with insulin sensitivity/insulin resistance in humans after weight loss due to metabolic surgery. Moreover, our data indicate that the malabsorptive BDP procedure increases proneurotensin to a higher degree than RYGB.

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Compliance with ethical standards

Conflict of interest JS is employed by sphingotec GmbH, a company having patent rights in the proneurotensin assay and commercializing it. AB is CEO of sphingotec GmbH and holds shares in this company. The remaining authors declare that they have no conflict of interest.

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