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EARLY-EFFECT OF BARIATRIC SURGERY (SCOPINARO METHOD) ON INTESTINAL HORMONES AND ADIPOKINES IN INSULIN RESISTANT WISTAR RAT

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Bariatric surgery consists in duodenal exclusion from the food passage in obese patients with coexistent type 2 diabetes. Nowadays bariatric surgery is considered the most effective method of glycemic index normalization and insulin resistance reduction. Recent results on obese and non-obese rats showed remission of type 2 diabetes symptoms within few days after the surgery. The aim of the present work was to analyze the mechanisms of neuro-hormonal regulation responsible for early normalization of metabolic syndrome after bariatric surgery. In present study the concentration of selected intestinal hormones and adipokines in blood plasma and gastrointestinal tissues were analyzed. Study was conducted on Wistar rats. Animals were divided into three groups (each n=6): control (SH) shame-operated rats; animals in which visceral fat tissue was extracted (LP); and rats in which Scopinaro bariatric surgery was performed (BPD). Immunochemistry analysis of blood plasma showed decrease of insulin concentration in BPD and LP and increase of polypeptide YY (PYY) in BPD group as compared to the control. In duodenal mucosa homogenates the tendency to reduce insulin in LP and BPD group, and increase PYY and visfatin in BPD group was observed. Histometry analysis showed reduction of mucosa thickness in excluded segments of gastrointestinal tract in BPD group as compared to the SH and LP. Concluding, model studies on rats allowed better understanding of mechanisms important for early normalization of glycemic index and insulin resistance reduction in rats.

Key words: *apelin, bariatric surgery, diabetes type 2, glycemic index, insulin, obesity, polypeptide YY, glucagon like peptide-1*

INTRODUCTION

Bariatric surgeries, which were originally intended to treat morbid obese patients with body mass index (BMI) ca. 40, have been proved also as an effective way to treat type 2 diabetes mellitus (T2DM) (1). The effectiveness of bariatric surgery in abolishing T2DM depends on the type of surgical procedure and ranges from 43 to 98% (2). The procedures with malabsorbtive component like, Roux-en-Y gastric bypass (RYGB), biliopancreatic diversion with duodenal switch (BPD-DS) are considered effective. However, the biliopancreatic diversion (BPD) and creation of gastric sleeve bariatric procedure (Scopinaro method) is considered the gold standard procedure with the highest efficacy for the treatment of T2DM patients (3).

Surprisingly, it was found that bariatric procedures diminished insulin resistance and T2DM manifestation just a few days after the surgery, whereas the reduction of body mass proceeded gradually for several months, indicating involvement of distinct mechanisms (4, 5). In addition, in lean patients malabsorbtive bariatric procedures were demonstrated to be effective in abolishing T2DM as well (6). It seems therefore that relief of symptoms of T2DM is weight loss-independent. Long-term post-bariatric observations showed, that remission of T2DM has a permanent character (3). The mechanisms by which bariatric procedures improve glycemia are still unclear. There are two main theories to explain the effect of the bariatric surgery with malabsorbtive component. Both of them assume that the clue is in anatomical modifications of the gastrointestinal tract by surgery, which influence local hormonal and/or neural regulatory mechanisms. The first, foregut hypothesis, assume that preponderant is exclusion of duodenum and proximal part of the jejunum from the contact with digesta. Rubinio *et al*. (7) in lean T2DM Goto-Kakizaki rats demonstrated that exclusion of upper gut led to euglycemic state. In addition, after reestablishing food passage the diabetic phenotype returned in examined rats. The second, hindgut hypothesis suggests that the effect of T2DM remission after malabsorbtive bariatric procedures is a result of increased incretin hormone, glucagon like peptide-1 (GLP-1), release by L-cells from the distal part of the small intestine in response to high digesta influx. Koopmans *et al.* (8) showed that ileal interposition in Goto-Kakizaki rats with the proximal jejunum improved glucose homeostasis after 30 days. Several studies revealed increased plasma ghrelin, polypeptide YY (PYY) and GLP-1 and reduced plasma gastric inhibitory polypeptide (GIP) after malabsorbtive procedures (9).

Murri *et al.* (10) demonstrated that pathogenesis of obesity and insulin resistance-related obesity may be associated with chronic mild inflammation present in adipose tissue and changes of adipokin secretion such as adiponectin or leptin and plasma

level of these hormones may be changed few days after bariatric procedure, nevertheless no changes in fat body mass were found. In last decade a number of new adiopokins like resistin, omentin, visfatin and vaspin was discovered. Their role in alleviating insulin resistance was already described (11) but not following bariatric surgeries.

The aim of present study was to study the early effect of bariatric surgery (Scopinaro method) on a selected number of gut and adipose tissue hormones in order to find the initial factors responsible for restoring insulin sensitivity. Previous studies focused mostly on blood plasma concentrations of hormones potentially involved in alleviating insulin resistance in the tissues. Thereby ignoring possible neurohormonal mechanisms working on a tissue level, including brain-gut axis (12). It is known, that a number of gut hormones, involving cholecystokinin (CCK) (13), leptin (14), ghrelin (15), obestatin (16), and apelin (17) work through the vagal nerves. Moreover, their participation in neurohormonal mechanisms may not be reflected by increased concentration in the circulating blood as it was shown for CCK (13). Therefore in the present study we measured changes in gut hormones in the small intestinal mucosa tissue. Our interest was directed toward ghrelin, apelin and PYY (recognized incretin hormone), which plasma levels are markedly affected after bariatric surgery (18). The rationale for studying visfatin was based on insulin-mimetic effects by binding visfatin to the insulin receptor to stimulate glucose uptake and increase insulin sensitivity (19, 20). Garcia-Fuentes *et al*., (21) reported that in humans plasma visfatin levels were increased 7 months after bariatric surgery, and were positively correlated with visceral fat loss.

MATERIALS AND METHODS

Animals

The animal studies were approved by the Local Ethical Committee. A total of 30 Wistar male rats 3 weeks old were purchased from the Medical University (Bialystok). Animals were housed in individual cages in a light- and temperature-controlled room with free access to standard food and water.

Animal preparation for surgery procedures

Starting from 1 week after arrival, rats were feed with high-caloric diet for 3 weeks, time sufficient to obtain central insulin resistance due to reduced insulin signaling (22, 23, 24). The diet consisted of standard chow diet (Labofeed H, Wytwornia Pasz „Morawski", Kcynia, Poland) with the inclusion of butter and lard (fat consisted 20% diet). 7-week-old rats (ca. 350 g of body weight, b. wt.) were weighted, fasted overnight and randomly divided into three groups: sham operated (SH), visceral fat tissue extracted (LP) and rats underwent biliopancreatic diversion (BPD) and gastric sleeve formation according to Scopinaro (25, 26). Surgery was performed under general anaesthesia induced by atropine (Atropinum Sulphuricum, Farmapol, Poland, 0.05 mg/kg b. wt., s.c.) and ketamine/medetomidine (CP-Pharma, Germany, 15–25/0.25–0.5 mg/kg b. wt., i.p, i.m.). Smaller doses of ketamine and medetomidine were repeated during the surgery to maintain steady anesthesia. Body temperature was continuously controlled and maintained by heating pad (Braintree Scientific Inc, USA) during surgery.

Experimental protocol

Sham surgery (SH group) consisted of laparotomy followed by 30 min exposition of internal organs to air. After that the laparotomy was closed with two-layer sutures. Surgery in LP group consisted of laparotomy followed by omentectomy and visceral peritesticular fat extraction (80% fat removed). Scopinaro surgery (BPD group) consisted of excising 2/3 of the stomach and creating horizontal gastric sleeve near lesser curvature, and transecting small intestine 50 cm from the ileo-cecal junction. The proximal end of duodenum was closed and the end of the proximal part of the intestine was anastomosed side-to-side to the distal part of the intestine around 20 cm from the ileo-cecal junction to create a common limb. The distal part of the intestine was anstomosed side-to-side to the stomach to create the alimentary limb (*Fig. 1*).

After surgery animals received analgesic, tolfedine (Vetoquinol Biowet, Poland) 0.1 ml/animal, s.c. and antibiotic Baytril (Bayer, Germany) 0.1 ml/animal treatment for 3 days. Promptly after surgery warm 5% glucose was administrated s.c. First day after surgery rats received no food, and tap water was offered *ad libitum*. On the next days, rats received soaked standard chow diet and tap water *ad libitum*.

On the day 4 after surgery the rats were killed by barbiturate (Thiopental, Polfa S.A, Polska) overdose, blood was withdrawn from the heart, and duodenum, proximal and distal part of jejunum and ileum were collected for further histology and hormone RIA analyses.

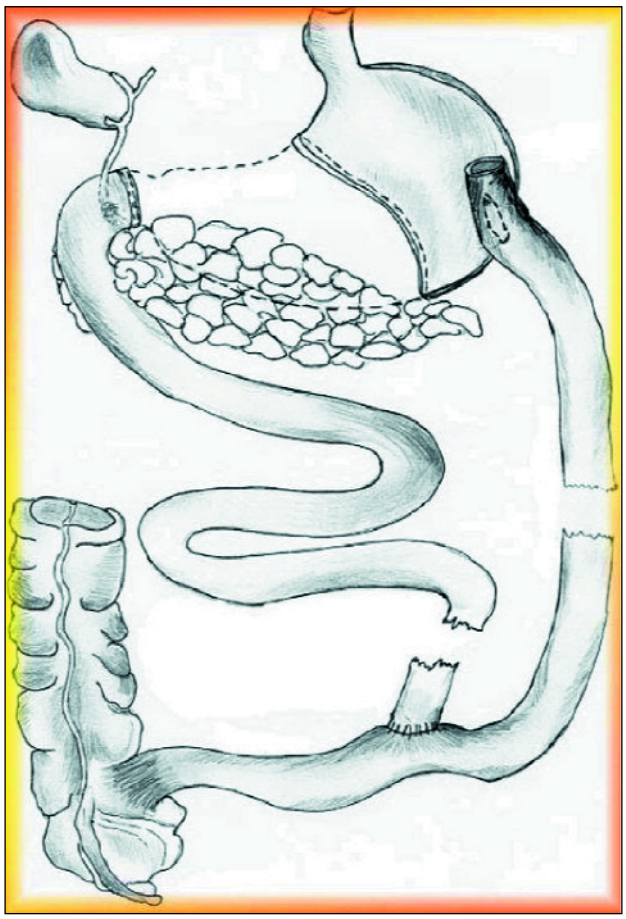

Fig. 1. Billiaro-pancreatic diversion according to Scopinaro consisted of creating gastric sleeve, and transecting small intestine 50 cm from the ileo-cecal junction. The proximal end of duodenum was closed, and the end of the proximal part of the intestine was anastomosed side-to-side to the distal part of the intestine to create a common limb. The distal part of the intestine was anastomosed side-to-side to the stomach to create the alimentary limb.

Histometry analysis

Whole thickness 1.5 cm segments of duodenum, proximal (25%), mid (50%) and distal (75%) jejunum and ileum were rinsed with cold PBS and fixed in 4% buffered formaldehyde and then stored in ethanol. Subsequently, the samples were embedded in paraffin, and serial histological 5 µm sections were stained with hematoxylin and eosin for morphometric analysis under the optical microscope BX61 (OLYMPUS Sp z o. o., Poland) coupled *via* a camera to PC computer. Morphometric analysis included measurements of the length of villi, crypts, mucosal thickness, and muscle layer thickness. For each intestine segment, 4–5 slides were analyzed, and minimum 10 well-oriented villi and crypts were measured using Cell^P software (OLYMPUS). Subsequently, the thickness of mucosa and muscle layer was measured using identical procedure.

Radioimmunoassays and ELISA

Twenty cm segments of duodenum, proximal and distal part of jejunum and ileum were cut along, and exposed mucosa was rinsed with cold PBS. Mucosa was gently scraped with glass slide, frozen in liquid nitrogen and stored at –80°C until analyses. Mucosa samples were thawed, homogenized in cold distilled water (1 g mucosa : 5 ml cold distilled water), centrifuged for 5 min at 4°C 1000 g, and supernatant aliquots were taken for further analysis. Tissue and serum samples were analyzed by commercial radioimmunoassay (RIA) or ELISA assays. Insulin was analyzed by rat insulin RIA kits (Millipore, Billerica, MA, USA) using purified rat insulin as standard. The detection limit was 0.081 ng/mL, and inter/intraassay variation 10.1 and 9.5%, respectively. Apelin-36 was measured by RIA kit (Phoenix Pharmaceuticals, CA, USA) using rat apelin as standard. The detection limit was 20 pg/ml and inter/intraassay variation 3.8–10.8/2.7–5.8%, respectively. PYY was measured by RIA kit (Millipore, Billerica, MA, USA) using rat PYY as standard. The detection limit was 15.6 pg/ml, and inter/intraassay variation 3.9/7.6% respectively. Active ghrelin was analyzed by ELISA kit (Millipore, MA, USA) using rat/mouse ghrelin as standard. The detection limit was 8 pg/ml, and inter/intraassay variation 9.81/4.90%, respectively. Visfatin was analyzed by ELISA kit (USCN Life Science Inc., USA) using rat visfatin as standard. The detection limit was 2.5 ng/ml, and inter/intraassay variation 5.5/11%, respectively.

Statistical analysis

The data are expressed as mean and standard errors of mean (S.E.M.). One-way parametric ANOVA followed by Tukey-Kramer post-test were used to test statistical differences between the animal groups (InStat® v.3.06, GraphPad Software Inc., USA). In all statistical analyses $p<0.05$ was taken as the level of significance.

RESULTS

Body mass and biochemistry parameters in plasma

There were no differences in body mass 4 days after BPD and LP as compared to SH group (*Table 1*). The daily food intake was not different between the groups and increased from 0 to 2% of the b. wt. from the first to fourth day after surgery, respectively on day 1 it was 0%, day 2–1%, and day 3–2%. Fasting plasma glucose level represented a significant decrease in LP and BPD group as compared to SH group. No statistically significant differences were found in triglycerides and cholesterol between the groups (*Table 1*).

Plasma hormones concentrations

Plasma concentrations of hormones are summarized in *Table 2*. Insulin concentration in BPD group was significantly lower as compared to sham operated (SH) controls. No significant differences were found in ghrelin concentration. Plasma apelin was significantly reduced in BPD as compared to SH, however, plasma PYY showed over 10-fold increase as compared co SH controls. The concentration of visfatin was significantly lower after BPD as compared to SC and LP groups. In rats with visceral fat extraction, plasma insulin was significantly reduced as compared to sham operated control (*Table 2*).

Tissue hormone concentration

Concentration of hormones in small intestine mucosa homogenates is summarized in *Table 3*. No significant differences were found in insulin and active ghrelin. Apelin concentration in duodenal tissue was significantly higher in BPD as compared to SH, whilst in the jejunum it showed a tendency toward increase. Tissue PYY concentration showed gradient along the gut (low in duodenum and, respectively, high and very high in proximal and distal jejunum) both in SH and BPD groups. However, in BPD rats PYY concentration was significantly higher as compared to SH, in particular in the jejunum. Interestingly, in visceral fat extracted group there was no PYY gradient in mucosa tissue, since tissue PYY was high in the duodenum and proximal intestine as compared to SH controls. Visfatin concentration was significantly increased in proximal and distal jejunum in BPD group as compared to SH. No differences were observed in the duodenum tissues. Visceral fat extraction had no effect on visfatin concentration in small intestinal mucosa (*Table 3*).

Table 1. Body mass (g) and biochemical characteristic of shame operated (SH), visceral fat extracted (LP) and Scopinaro-treated (BPD) rats on day 4 after the surgery (mean ±S.E.M.). Rats were fed with high caloric diet for 3 weeks prior to surgery.

	SH	LP	BPD[1]	P
Body mass (g)	331±15	310±23	304±19	0.58
Glucose (mmol/ml)	12.75±1.62[a]	9.79±1.65[b]	6.71±1.52[c]	<0.0001
Trigliceride (mmol/ml)	1.06±0.11	0.93±0.25	0.91±0.25	0.59
Cholesterol (mmol/ml)	1.74±0.32	1.74±0.4	1.44±0.22	0.47

[abc] different letters in superscript indicate statistical significance. Parametric one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test ($P<0.05$). [1]In BPD group "proximal and distal jejunum" correspond to original anatomical position of segments before the surgery - proximal segments corresponds therefore to "enzymatic limb" and the distal segment to the "alimentary limb" see: description of Scopinaro surgery in *Experimental protocol*.

Intestinal histometry analysis

A significant reduction of villi length and tunica mucosa and tunica muscularis thickness was found in the duodenum of BPD and LP rats as compared to SH (*Table 4*). The crypt depth was not different between 3 experimental groups. Similar results were found in the proximal jejunum. In the distal jejunum and ileum, however, the results were opposite, namely in BPD group the villi were longer and mucosa and muscularis were thicker as compared to SH group (*Table 4*).

DISCUSSION

Previous studies shown long-term effects of bariatric procedures with malabsorbtive component on intestinal hormones and adipokines (27) secretion. However, only few studies analyzed short-term changes in plasma hormones, whereas these changes might play the key role in understanding the mechanisms of diabetes type 2 remission after bariatric surgery independent of weight loss. Short-term changes in plasma levels of intestinal GIP, GLP-1 and leptin were observed (5, 28). In contrast to conclusions by Yip *et al*. (29) our data suggest that there are other mechanisms involved rather than caloric restrictions. Our data demonstrate significant changes in plasma concentrations of insulin, apelin, PYY and visfatin, and for the first time reveals major changes in intestinal mucosa apelin, PYY, and visfatin concentrations just 4 days after BPD. Interestingly, during 4 postoperative days changes were evident in intestinal histometry parameters, meaning that food entering directly to alimentary loop and bypassing the enzymatic loop led to switch in villi length and gut thickness in BPD as compared to

Table 2. Concentration of hormones in blood plasma (pg/ml) in shame operated (SH), visceral fat extracted (LP) and Scopinaro-treated (BPD) rats fed with high caloric diet for 3 weeks prior to surgery (mean ± S.E.M.).

Concentration	SH	LP	BPD[1]	P
Insulin	14.94±6.47[a]	5.30±2.96[b]	2.30±1.60[b]	0.008
Ghrelin	549±117	–	439±165	>0.05
Apelin	1120±250[a]	2470±1680[a]	990±380[b]	0.006
PYY	8.33±8.05[a]	21.67±11.00[a]	108.4±40.1[b]	0.0008
Visfatin	98.4±13.9[a]	103.2±19.7[a]	55.9±15.3[b]	0.0001

Statistical evaluation was done using parametric one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test. Different letters (a, b) in superscript depict statistical difference ($p<0.05$). [1]In BPD group "proximal and distal jejunum" correspond to original anatomical position of segments before the surgery - proximal segments corresponds therefore to "enzymatic limb" and the distal segment to the "alimentary limb" see: description of Scopinaro surgery in *Experimental protocol*.

Table 3. Selected hormones concentration in intestinal mucosa homogenates (pg/g tissue) in shame operated (SH), visceral fat extracted (LP) and Scopinaro-treated (BPD) rats fed with high caloric diet for 3 weeks prior to surgery (mean ± S.E.M.).

	SH	LP	BPD[1]	P
Insulin (pg/g tissue)				
Duodenum	3.16±1.62	1.51±0.58	1.80±1.69	0.319
Proximal jejunum	3.30±1.04	1.59±1.33	4.22±3.89	0.104
Distal jejunum	2.28±0.87	1.74±1.67	1.82±0.41	0.924
Ghrelin (pg/g tissue)				
Duodenum	1120±80	1470±420	2350±2480	0.727
Proximal jejunum	910±530	1360±630	1590±340	0.335
Distal jejunum	600±210	440±200	690±120	0.106
Apelin (pg/g tissue)				
Duodenum	5860±250[a]	9080±4390[a]	16420±920[b]	0.003
Proximal jejunum	5680±200	10980±5330	11540±4820	0.057
Distal jejunum	5750±310	10480±5340	12230±4870	0.09
PYY (pg/g tissue)				
Duodenum	89±5[a]	403±86[b]	165±41[ab]	0.003
Proximal jejunum	123±72[a]	880±355[ab]	2570±792[b]	0.018
Distal jejunum	187±59[a]	295±127[a]	3340±320[b]	0.0001
Visfatin (pg/g tissue)				
Duodenum	1.84±1.29	3.65±2.22	3.59±1.76	0.417
Proximal jejunum	3.12±0.50[a]	4.50±2.33[a]	22.10±3.86[b]	0.0005
Distal jejunum	6.60±3.97[a]	7.07±2.27[a]	25.84±11.71[b]	0.0001

Statistical evaluation was done using parametric one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test. Different letters (a, b) in superscript depict statistical difference ($p<0.05$). [1]In BPD group "proximal and distal jejunum" correspond to original anatomical position of segments before the surgery - proximal segments corresponds therefore to "enzymatic limb" and the distal segment to the "alimentary limb" see: description of Scopinaro surgery in *Experimental protocol*.

Table 4. Histometry analysis (μm) in sham operated (SH), visceral fat extracted (LP) and Scopinaro-treated (BPD) rats fed with high caloric diet for 3 weeks prior to surgery (mean ±S.E.M.).

	SH	LP	BPD[1]	P
Duodenum				
Villi lenght	764±70[a]	542±63[b]	364±6[c]	0.0001
Crypts depth	130±13[a]	103±21[a]	94±21[ab]	0.035
Mucosa thickness	887±85[a]	658±82[b]	459±73[c]	0.0001
Muscle thickness	159±17[a]	105±22[b]	98±14[b]	0.0002
Proximal jejunum				
Villi lenght	641±81[a]	437±62[b]	442±78[ab]	0.005
Crypts depth	114±7[ab]	90±18[a]	98±21[ab]	0.009
Mucosa thickness	759±77[a]	530±72[b]	544±100[a]	0.001
Muscle thickness	120±9	76±9	85±12	0.142
Distal jejunum				
Villi lenght	471±37[a]	317±34[b]	560±77[a]	0.004
Crypts depth	109±7	92±17	124±17	0.459
Mucosa thickness	581±37[a]	410±48[b]	686±77[a]	0.011
Muscle thickness	141±9[a]	88±11[b]	108±18[b]	0.0001
Ileum				
Villi lenght	316±45[a]	279±22[a]	415±61[b]	0.0005
Crypts depth	75±6[a]	83±17[ab]	108±20[b]	0.019
Mucosa thickness	398±55[a]	361±36[a]	523±63[b]	0.0003
Muscle thickness	75±8[a]	81±6[a]	100±14[b]	0.005

Statistical evaluation was done using parametric one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparisons test. Different letters (a, b, c) in superscript depict statistical difference ($p<0.05$). [1]In BPD group "proximal and distal jejunum" correspond to original anatomical position of segments before the surgery - proximal segments corresponds therefore to "enzymatic limb" and the distal segment to the "alimentary limb" see: description of Scopinaro surgery in *Experimental protocol*.

sham operated controls. Importantly, daily food intake and body weight loss was similar in all examined groups.

Recent human studies showed BPD to be the most effective method in normalizing glycaemia that accompanies morbid obesity (BMI>35) (3). In our study, concomitant decreases in plasma insulin and glucose concentrations suggest normalization of glycaemia within 4 postoperative days in BPD rats (*Table 1*) due to improved tissue sensitivity to insulin. It was demonstrated that plasma concentration of apelin correlated with BMI and was twice as high in obese patients compared to lean patients (30). In our study, however, we observed significant decrease in concentration of apelin in plasma 4 days after BPD regardless of weight loss. Soriguer *et al.* (31) demonstrated that obesity is not a determining factor in plasma apelin concentration. Seven months after BPD and RYGB apelin level correlated with the changes in plasma glucose ($r=0.338$, $p=0.038$) and insulin sensitivity ($r= –0.417$, $p=0.043$) while no correlation with body weight was observed.

Increase in PYY concentration after BPD in rats and human was described by several authors to appear approximately 1 month after the procedure (4, 5, 32, 33). Stratis *et al.* (33) measured PYY concentration 1, 3 and 7 days and 1 and 3 months after BPD. They did not observe changes in plasma PYY during the first week after surgery probably due to fasting patients for 5 days after BPD, since PYY secretion increases in response to the nutrients entering distal jejunum and ileum. In our animal model, restricted feeding produced marked elevation of plasma PYY on the 4th postsurgical day. Our results corroborate with studies of le Roux *et al.* (34), who demonstrated increase of plasma PYY in obese patients 2, 4 and 42 days after RYGB in response to restricted feeding (400 kcal). According to the hindgut hypothesis and more recent studies by Boey *et al.* (35), low levels of circulating PYY may contribute to hyperinsulinemia and insulin resistance, therefore it is possible that PYY plays a role in reducing insulin resistance following bariatric surgery.

In our study plasma visfatin was significantly reduced in BPD rats as compared to sham and LP rats. In contrast, Botella-Carretero *et al.* (28) observed elevated plasma visfatin 5–40 months after BPD and RYGB in humans. The increase in plasma visfatin was correlated to the weight loss and reduction in waistline measurements, and the increase appeared to be more significant in diabetic patients. Similar findings were reported by Garcia-Fuentes *et al*. (24) and more recently by Friebe *et al*. (37). We speculate that soon after surgical operation, a yet unidentified mechanism (but rather not originating from visceral fat) associated with remodeling of the gastrointestinal tract by BPD is turned on to inhibit plasma visfatin. It needs further studies to clarify how long plasma visfatin is reduced after surgery, and whether this reduction contributes to conclusion of insulin resistance. In patients with newly diagnosed diabetes Dogru *et al.* (38) showed that plasma visfatin was higher in diabetic patients as compared to non-diabetic controls. Furthermore they found a negative correlation of plasma visfatin with beta-cell function. This strengthen our hypothesis that decrease in plasma visfatin after BPD found in our study can be involved in remission of diabetes type 2 independent of weight loss. Moreover, visfatin concentrations in small intestinal mucosa of BPD rats already started to differentiate from that in controls (*Table 3*).

BPD led to reduction of plasma apelin in examined rats. However, local apelin synthesis in the duodenal mucosa was increased what in turn, might activate some local neurohormonal mechanisms in the duodenal mucosa which involve duodenal cholecystokinin and other gut regulatory peptides (12, 17, 39). On the other hand, major brain-gut axis regulation of the

duodenum and pancreas as well as a sensory input from the upper gut to the brain was disrupted by BPD procedure.

We also observed marked increase in plasma PYY originating from the both proximal and distal small intestine. Increase in PYY concentration in the proximal small intestine being excluded from alimentary passage (enzymatic loop) suggests that the food content is not the only factor that stimulates local secretion of PYY. Possibly local apelin could directly (or indirectly *via* cholecystokinin) stimulate PYY release. Roberts *et al.* (40) showed positive correlation between PYY secretion and secretion of bile salts, glycochenodeoxycholic acid (GCDCA) and taurochenodeoxycholic acid (TCDCA). In the distal part of small intestine, forming "alimentary loop" sharp increase of PYY was observed in response to large amounts of undigested nutrients (fats, proteins, sugars) that appeared after surgical modification of small intestine (41, 42, 43).

Surprisingly, PYY in duodenal mucosa increased also after removing visceral fat in LP group. This could suggest that local secretion of PYY in this part of intestine is regulated by adipokines or other factors from visceral adipose tissue. This type of regulation could explain the reduced levels of PYY in obesity and correlation of PYY concentrations with waist width and BMI (44).

Relatively little is known about visfatin role in the intestinal function. One study shows increase in visfatin mRNA expression in colon tissue in Crohn's disease or ulcerative inflammation of the colon in comparison to healthy patients. Visfatin was also shown to activate human leucocytes, and induce cytokine production. Therefore it was recognized as a new postinflamatory adipokine (45). Recent *in vitro* study in human malignant melanoma ME45 cells demonstrated that visfatin may stimulate inflammatory response through PI3k and nuclear factor-κB pathways (46). Local increase in visfatin concentration 4 days after operation concomitant with reduction of plasma visfatin might therefore reflect inflammatory response to surgical intervention.

Quite surprisingly we did not observe any significant changes in plasma and tissue ghrelin in PPD rats, although our results corroborate with these obtained in rats following gastric sleeve surgery (47).

Concluding, the early effect of biliopacreatic diversion on resuming insulin resistance in rats may be associated with rapid modification of neurohormonal mechanisms involving PYY, apelin and visfatin, rather than with caloric restriction as proposed recently (29).

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