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Changes in the mucosa of the Roux-limb after gastric bypass surgery

Running title: Roux-limb mucosa after RYGBP

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Abstract

Aims: Roux-en-Y gastric bypass surgery is the most efficient treatment of morbid obesity but the mechanisms of action are still poorly understood. The aim of this study was to explore the Roux-limb mucosa after gastric bypass surgery, focusing on basic morphology and inflammation.

Methods and Results: Jejunal mucosal samples from the Roux-limb were gathered from eight patients at time of surgery and six to eight months post-surgery. Histological evaluation of inflammation and morphometric investigations were performed, mitotic frequency was assessed using immunohistochemistry and inflammatory markers and Angiotensin (Ang) II receptors were detected using western blot.

Mitotic frequency increased and villous surface area decreased in the Roux-limb mucosa but no signs of active inflammation were observed after surgery. Protein analyses showed increased levels of nicotinicamide adenine dinucleotidephosphate (NADPH)-oxidase, myeloperoxidase (MPO) and the Ang II type 1(AT₁) receptor after surgery, whereas the levels of inducible nitric oxide synthase (iNOS), nitrotyrosine and the Ang II type 2(AT₂) receptor remained constant

Conclusion: These results indicate that the phenotype of the jejunal mucosa changes once exposed to un-digested food and the increased microbial load in the Roux-limb after surgery.

Introduction

Roux-en-Y gastric bypass surgery (RYGBP) is one of the most efficient long term obesity treatments with regard not only to weight-loss but also morbidity and even mortality¹. The reasons for this success are however debated, including a wide range of theoretical explanations^{2,3}. The Roux-en-Y method markedly changes the order of gastrointestinal continuity. The intraluminal milieu of the jejunal segment which is positioned as the proximal end of the Roux-limb, thus becoming the primary recipient of oral intake, is vastly altered with an augmented microbial⁴ and mechanical load as well as the chemical effects of undigested food. Few studies have focused on the juxta-mucosal milieu and the mucosa of the small gastric pouch and Roux-limb after surgery. The questions primarily addressed in the studies performed so far are related to the risk of bacterial over-growth, stomal ulcers and cancer development following surgery⁵⁻⁷. Questions concerning mucosal function and adaptation after RYGBP are rarely addressed in literature. The aim of the present study was therefore to explore changes in the mucosa of the food-receiving Roux-limb. This was done six to eight months postoperatively once the surgical construction was regarded to have reached a steady state. The investigation assessed mucosal morphology including mitotic activity, inflammation as well as presence of radical forming enzymes and pro-inflammatory components of the renin-angiotensin system.

Methods

The study was approved by the Ethics committee of Gothenburg University and by the Regional Ethical Review Board in Gothenburg and was performed in accordance with the Declaration of Helsinki. All patients signed an informed consent form before inclusion into the study.

Surgery and sampling

Patients either went through a first time laparoscopic RYGBP (5patients) or a conversion from vertical banded gastroplasty (VBG) to RYGBP (3patients). The latter group was deemed suitable for inclusion as the VBG does not alter the gastro-intestinal continuity and the area of the jejunum analysed in this study is left intact by the VBG surgical procedure. Patients were re-operated due unsatisfactory weight reduction or complications such as severe dumping symptoms, nausea and frequent vomiting after the first surgical intervention. Patients **that were** re-operated **upon** due to acute surgical complications were not considered for inclusion. The operative technique included an antecolic-antegastric Roux-en-Y construction with a 10 to 20ml gastric pouch. The gastro-entero-anastomosis was constructed using a straight 45mm stapler and complementary hand-suturing thus creating a wide open gastro-entroanastomosis. A full-thickness specimen of the jejunal wall was resected between the gastro-entero and the entero-entero anastomosis as the bowel loop was divided to create the Roux-en-Y construction (Figure 1). The mucosa/submucosa was separated from the muscularis propria by means of sharp dissection. Mucosal tissue specimens were snap-frozen in liquid nitrogen and kept frozen (-70°C) for later analysis of protein expression. Full-thickness specimens were fixed in buffered 4% formaldehyde, dehydrated and embedded in paraffin for histological and immunohistochemical analyses. Between six to eight months after surgery the patients underwent an endoscopic examination of the Roux-limb. Mucosal biopsies were collected approximately 8 cm distal to the stoma (Figure 1) in close proximity to where the pre-operative tissue sample was taken. Three biopsies were taken for Western blot analyses and an additional four biopsies for histology as described above. Information on the patients is found in Table 1.

Histology and morphometry

A skilled **histopathologist** (Vieth, M) evaluated signs of mucosal inflammation in coded four-micron sections stained with eosin-hematoxylin, of the full-thickness specimens from time of surgery and the endoscopically retrieved mucosal post-surgery biopsies, respectively. A cell count of immunologically active cells was also performed: mucosal lymphocytes, eosinophilic granulocytes and plasma cells found in the lamina propria were counted as cells per high power field. Intraepithelial lymphocytes were counted per 100 epithelial cells. The same sections were used for morphometry. The presence of villi in the small intestine results in an amplification of the mucosal surface area. The total area of the mucosal surface in relation to the relatively flat muscularis mucosae was assessed by the grid-line intersection method. Slides stained with PAS according to McManus were used for quantitative analyses of the volume density of the lamina propria in per cent of the total volume of the mucosa, and of the goblet cells as per cent of the total epithelial volume, using the point-counting method. Detailed descriptions of these methods have previously been published by Bancroft⁸.

Immunohistochemical demonstration of mitoses

Four-micron sections of paraffin embedded tissue samples were picked up on glass slides, rehydrated, and boiled for 10 min in 50mM borate buffer pH 8.0. After rinsing in PBS, endogenous peroxidase was blocked by 20min exposure to 1% H₂O₂ in PBS. Renewed rinsing in PBS was followed by treatment with 5% normal goat serum (Dako, Glostrup, Denmark), and exposure for 1 h at room temperature to the primary antibody Rb- α -Ki-67 (Biocare Medical, Concord, CA, USA) diluted 1:100 in PBS containing 0.2% triton. Negative controls were incubated with PBS with 0.2% triton. Following renewed rinsing in PBS, the sections were treated for 30min with a secondary antibody, EnVision Rabbit (Dako), and rinsed in 0.05 M Tris pH 7.6. Immunoreactivity was visualized by the DAB method, and sections were contrasted for 30sec in Mayer's hematoxylin. Human mammary gland tumour

served as a positive control. After dehydration and treatment with xylene, coverslips were mounted. In order to estimate the percentage of labelled epithelial cells, morphometry was carried out using an x100 oil immersion objective lens. Swaths of sections that displayed the entire thickness of the epithelium (from the bottom of the crypts of Lieberkühn to the surface of the mucosa) were inspected; the regions to be studied were selected by systematic sampling with a random start. Approximately 1000 cells were counted per tissue sample and results are given as % stained cells.

Western blot

The specimens were thawed and homogenised on ice (Polytron, Kinematica AG, Switzerland) in buffer A (10% glycerol, 20mmol/L Tris-HCl pH 7.3, 100mmol/L sodium chloride, 2mmol/L phenylmethylsulfonyl fluoride, 2mmol/L EDTA, 2mmol/L EGTA, 10mmol/L sodium orthovanadate, 10mg/mL leupeptin, and 10mg/mL aprotinin). Centrifugation was performed at 30,000 g for 30min at 4°C. The pellet was resuspended in buffer B (1 % NP-40 (Sigma-Aldrich, Stockholm, Sweden) in buffer A) and subsequently stirred at 4°C for one hour before centrifugation at 30,000 g for 30min at 4°C. The supernatant was analysed for protein content using the Bradford method⁹ and stored at -70°C for further analysis. Total protein samples were diluted in SDS buffer and heated at 70°C for 10min. before they were loaded on a NuPage 10% Bis-Tris gel, and electrophoresis run using a MOPS buffer (Invitrogen AB, Lidingo, Sweden). One lane of each gel was loaded with a prestained molecular weight standard (SeeBlue, Invitrogen AB). A positive control was loaded on each gel. After the electrophoresis the proteins were transferred to a polyvinylidene difluoride transfer membrane, Hybond, 0.45µm, RPN303F, (Amersham, Buckinghamshire, UK) using an iBlot (Invitrogen AB). Membranes were then incubated with polyclonal specific antibodies directed at the AT₁-receptor, (AT₂)-receptor, MPO, NADPH-oxidase (p47^{phox}-subunit), nitrotyrosine and iNOS respectively. An alkaline phosphatase conjugated goat anti-rabbit IgG antibody and

CDP-Star (Tropix, Bedford, MA, USA) as a substrate were used to identify immunoreactive proteins by means of chemiluminescence. **Detailed information on positive controls and antibodies can be found as supplementary data.** Images were captured by a Chenidox XRS cooled CCD camera, and semi-quantification was performed using Quantity One software (Bio-Rad Laboratories, Hercules, USA). All samples compared were run on the same gel and all gels were run in duplicates.

Statistics

As all included individuals acted as their own controls, all groups of observations were considered as dependent and statistically significant differences were identified using Wilcoxon signed rank test. A p-value ≤ 0.05 was considered to be of statistical significance.

Results

General remarks

Samples from all eight patients were gathered pre and post surgery and each individual served as its own control. The median weight loss in the eight patients during the postoperative period was 24.8% (range 7.2 to 32.9%) of the pre-operative weight rendering the eight patients a median BMI of 30.1 (range 28.1 to 37.9) as compared to 40.3 (range 32.4 to 44.9) prior to surgery (Table 2). The endoscopic evaluation showed no signs of esophagitis, gastritis or stomal ulcers and the inspection of the Roux-limb showed a normal mucosal appearance in all eight patients.

* *Placement of table 1*

Morphometry

The enlargement of the luminal surface area due to the villi structure (described as the total area of the mucosal surface in relation to the relatively flat area of the muscularis mucosae) was markedly lower in all individuals after surgery ($p=0.012$) (Figure 2a). The result of the morphometrical analysis was supported by the histological evaluation, which described a mild

to moderate villous hypotrophy. No differences were detected regarding volume density of the lamina propria (Figure 2b) or of the numerical density of the goblet cells (Figure 2c).

Mitoses

In per-operative mucosal specimens very few epithelial cells exhibited immunoreactivity to Ki67, which was used as a marker of mitoses. There was a significant increase in the proportion of labelled cells after surgery as compared to the per-operative situation ($p=0.012$) (Figure 2d). Most of the labelled cells were found close to the bottom of the crypts of Lieberkühn. In the lamina propria only a small proportion of cells displayed immunoreactivity to Ki67 with no obvious difference between specimens taken per-and post-operatively. No cells were stained in the negative controls whereas the positive controls had a high proportion of labelled cells. (Figure 3)

Histological signs of inflammation

No signs of active inflammation, defined as presence of neutrophils, macrophages and intramucosal oedema, were detected in the jejunal mucosa at time of surgery, or in the Roux-limb six to eight months after surgery. The number of mucosal lymphocytes was significantly ($p=0.036$) (Figure 4a) lower after surgery as was the number of intraepithelial lymphocytes ($p=0.012$) (Figure 4b). No significant differences were detected in the amount of plasma cells (Figure 4c) or eosinophiles (Figure 4d) in the mucosa.

Biochemical signs of inflammation

A significant increase of NADPH oxidase protein expression ($p=0.012$) (Figure 5a) was detected as well as a significant increase of MPO protein expression ($p=0.025$) (Figure 5b). No significant differences in per to post-surgical expression of iNOS (Figure 5c) or nitrotyrosine (Figure 5d) were detected. In all eight individuals the expression of AT₁-receptor protein was higher after surgery than at time of surgery ($p=0.012$) (Figure 6a)

whereas no significant difference in AT₂-receptor protein expression could be detected (Figure 6b).

Discussion

When investigated six to eight months after RYGBP surgery the intestinal mucosa of the Roux-limb exhibited a vastly altered appearance as compared to the per-operative state. Firstly, the epithelial architecture was changed towards shorter villi, resulting in a reduced surface area. Secondly the mitotic activity in the epithelium was up-regulated. Thirdly, despite the absence of histological inflammation, there were clear signs of an increased oxy-radical forming capacity as indicated by the amplified levels of MPO and NADPH-oxidase and fourthly the expression of the pro-inflammatory active AT₁-receptor was elevated.

The most intuitive reflection is that the changed juxta-mucosal milieu achieved by the RYGBP initiates adaptive changes of the intestinal mucosal phenotype in the proximal Roux-limb. The morphometric findings in the present study displayed decreased height of villi and increased mitotic frequency suggesting an increased epithelial cell loss at the tips of the villi with compensatory cell replication in the crypts of Lieberkühn and supporting the theory of a adaptive change of the phenotype. A decrease in villous surface area of the jejunum has previously been demonstrated after experimental gastro-jejunostomy in rats¹⁰. Similarly, vascularised ileal segments of cat intestine displayed alterations of villous surface area and mitotic frequency when positioned as part of the colon, urinary bladder or jejunum¹¹. The changed character of the luminal contents apparently influences the intestinal mucosal morphology although the mechanism behind this adaptation remains to be elucidated. The Roux-limb is, in addition to undigested nutrients, subjected to an increased microbial load as the protection normally offered by the gastric acid is abolished by the RYGBP surgical construction. Sundbom et al has shown that the pharyngeal bacterial flora can be cultured

from Roux-limb samples subsequent to RYGBP surgery⁴. Despite the increased bacterial load no histological signs of active inflammation were detected in the early postoperative period, indicating that the Roux-limb was not infected but colonised by the naso-pharyngeal bacterial flora⁴. The histological assessment in the present study performed six to eight months after surgery was also deficient of signs of inflammation in the Roux-limb mucosa as indicated by the lack of local oedema and infiltrating neutrophilic granulocytes. Csendes et al showed similar results in a study of the Roux-limb two years after gastric by-pass surgery⁵. Despite the absence of endoscopic and histological signs inflammation in the Roux-limb, Sundbom et al⁴ detected increased mucosal levels of defensin-5, suggesting an antimicrobial protective response to the bacterial presence. In the present study the oxy-radical-promoting enzymes MPO and NADPH-oxidase were significantly up-regulated in the Roux-limb mucosa after surgery, whereas levels of nitrotyrosine and iNOS, as markers of the peroxynitrite formation, were unaltered. The formation of the reactive cytotoxic radical peroxynitrite constitutes an important part of the host defence system and is mainly the result of a reaction between superoxide and nitric oxide, two radicals with extremely short biological half-time which are difficult to assess. Increased expression of oxy- and nitroradical forming enzymes are commonly used as indicators of tissue reactions of infection or mechanical trauma¹². In the present study only a selective up-regulation of the oxy-radical forming system was displayed. For example, the semi-quantitative immunoblotting of NADPH-oxidase (NOX¹³), represented by the organizer subunit p47phox of the phagocytic NADPH-oxidase NOX2¹³ showed a significant postoperative increase. As the phagocytic cells believed to harbour NOX2 did not increase in the Roux-limb after surgery the increased expression of p47phox either occurred in resident phagocytes in other cell types¹⁴⁻¹⁷. NOX derived reactive oxygen species like superoxide have, in addition to being part of the innate defences¹⁸, also been shown to influence mucosal cell proliferation and apoptosis in *e.g.* the colon¹⁹. The altered architecture

and increased mitotic activity noted in the Roux-limb might be partly mediated by an increased superoxide production.

The present investigation also demonstrated an increase of AT₁-receptor protein in the Roux-limb mucosa after surgery, whereas the level of AT₂-receptor protein expression remained constant. The renin-angiotensin system (RAS) is best known for its effects on hemodynamic regulation and body fluid homeostasis but is also implicated in inflammation, wound healing and tissue growth and differentiation²⁰⁻²². Especially activation the AT₁-receptor has been shown to promote inflammation^{23, 24} and experimental data link AT₁-receptor signalling to NADPH-oxidase- as well as MPO-activity^{25 26}. Furthermore it has been demonstrated that AT₁-receptor activity promotes apoptosis in intestinal epithelial cells after small bowel resection in mice²⁷. It can thus be speculated that the observed up-regulation of the AT₁-receptor directly influences the oxy-radical forming enzymes in the Roux-limb mucosa and maybe even the histologically noted adaptive changes. AT₁-receptor antagonists, which are well established as pharmacological antihypertensive treatment, could be used in future studies to elucidate the role of RAS in the altered mucosal constitution induced by RYGBP.

In summary: The present investigation demonstrates that the appearance of the Roux-limb mucosa has changed six to eight months after RYGBP surgery as depicted by a flattened villous surface area and an increased mitotic frequency. No histological signs of inflammation were present but increased expression of proinflammatory proteins, NADPH-oxidase, MPO and AT₁-receptor was found. These results indicate that the phenotype of the jejunal mucosa changes from a digestive-absorptive character into an appearance more suited for food reception/transportation and tissue-defence when it becomes positioned as the Roux-limb.

1. Sjostrom L, Narbro K, Sjostrom CD, *et al.* Effects of bariatric surgery on mortality in Swedish obese subjects. *N Engl J Med.* 2007;**357**;741-52.
2. Rubino F, Gagner M, Gentileschi P, *et al.* The early effect of the Roux-en-Y gastric bypass on hormones involved in body weight regulation and glucose metabolism. *Ann Surg.* 2004;**240**;236-42.
3. le Roux CW, Welbourn R, Werling M, *et al.* Gut hormones as mediators of appetite and weight loss after Roux-en-Y gastric bypass. *Ann Surg.* 2007;**246**;780-5.
4. Sundbom M, Elphick DA, Mahida YR, *et al.* Alteration in human defensin-5 expression following gastric bypass surgery. *J Clin Pathol.* 2007;**60**;1029-34.
5. Csendes A, Smok G, Burgos AM. Endoscopic and histologic findings in the gastric pouch and the Roux limb after gastric bypass. *Obes Surg.* 2006;**16**;279-83.
6. Melstrom LG, Bentrem DJ, Salvino MJ, *et al.* Adenocarcinoma of the gastroesophageal junction after bariatric surgery. *Am J Surg.* 2008;**196**;135-8.
7. Machado JD, Campos CS, Lopes Dah Silva C, *et al.* Intestinal bacterial overgrowth after Roux-en-Y gastric bypass. *Obes Surg.* 2008;**18**;139-43.
8. Bancroft JD, Stevens A, Turner DR. Theory and Practice of Histological techniques. 4th ed; 1996.
9. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem.* 1976;**72**;248-54.
10. Stenling R, Helander HF. Stereological studies on the rat small intestinal epithelium. II. Effects of antrectomy and antral exclusion. *Cell Tissue Res.* 1983;**228**;563-71.
11. Philipson B. Morphology in the cat ileal mucosa following construction of an ileal reservoir or transposition of patches to different locations. *Scand J Gastroenterol.* 1975;**10**;369-77.
12. Bentz BG, Simmons RL, Haines GK, 3rd, Radosevich JA. The yin and yang of nitric oxide: reflections on the physiology and pathophysiology of NO. *Head Neck.* 2000;**22**;71-83.
13. Bedard K, Krause K-H. The NOX family of ROS-generating NADPH oxidases: Physiology and Pathophysiology. *Physiol Rev.* 2007;**87**;245-313.
14. Serrano F, Kolluri NS, Wientjes FB, Card JP, Klann E. NADPH oxidase immunoreactivity in the mouse brain. *Brain Res.* 2003;**988**;193-8.
15. Gorlach A, Brandes RP, Nguyen K, *et al.* A gp91phox containing NADPH oxidase selectively expressed in endothelial cells is a major source of oxygen radical generation in the arterial wall. *Circ Res.* 2000;**87**;26-32.

16. Javesghani D, Magder SA, Barreiro E, Quinn MT, Hussain SN. Molecular characterization of a superoxide-generating NAD(P)H oxidase in the ventilatory muscles. *Am J Respir Crit Care Med.* 2002;**165**;412-8.
17. Cheng G, Ritsick D, Lambeth JD. Nox3 regulation by NOXO1, p47phox, and p67phox. *J Biol Chem.* 2004;**279**;34250-5.
18. Rada B, Leto TL. Oxidative innate immune defenses by Nox/Duox family NADPH oxidases. *Contrib Microbiol.* 2008;**15**;164-87.
19. Geiszt M, Lekstrom K, Brenner S, *et al.* NAD(P)H oxidase 1, a product of differentiated colon epithelial cells, can partially replace glycoprotein 91phox in the regulated production of superoxide by phagocytes. *J Immunol.* 2003;**171**;299-306.
20. Okada H, Watanabe Y, Kobayashi T, *et al.* Angiotensin II type 1 and type 2 receptors reciprocally modulate pro-inflammatory/ pro-fibrotic reactions in activated splenic lymphocytes. *Am J Nephrol.* 2004;**24**;322-9.
21. Lucius R, Gallinat S, Rosenstiel P, *et al.* The angiotensin II type 2 (AT2) receptor promotes axonal regeneration in the optic nerve of adult rats. *J Exp Med.* 1998;**188**;661-70.
22. Viswanathan M, Saavedra JM. Expression of angiotensin II AT2 receptors in the rat skin during experimental wound healing. *Peptides.* 1992;**13**;783-6.
23. Ruiz-Ortega M, Ruperez M, Esteban V, *et al.* Angiotensin II: a key factor in the inflammatory and fibrotic response in kidney diseases. *Nephrol Dial Transplant.* 2006;**21**;16-20.
24. Manabe S, Okura T, Watanabe S, Fukuoka T, Higaki J. Effects of angiotensin II receptor blockade with valsartan on pro-inflammatory cytokines in patients with essential hypertension. *J Cardiovasc Pharmacol.* 2005;**46**;735-9.
25. Chan YC, Leung PS. AT1 receptor antagonism ameliorates acute pancreatitis-associated pulmonary injury. *Regul Pept.* 2006;**134**;46-53.
26. Yamamoto E, Tamamaki N, Nakamura T, *et al.* Excess Salt Causes Cerebral Neuronal Apoptosis and Inflammation in Stroke-Prone Hypertensive Rats Through Angiotensin II-Induced NADPH Oxidase Activation. *Stroke.* 2008.
27. Koga H, Yang H, Haxhija EQ, Teitelbaum DH. The role of angiotensin II type 1a receptor on intestinal epithelial cells following small bowel resection in a mouse model. *Pediatr Surg Int.* 2008;**24**;1279-86.

Table 1: Clinical information

Gender	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Total
	♀	♂	♂	♀	♀	♀	♂	♂	♂4♀4
Surgical procedure									
1 st time RYGB / Conversion to RYGB	1 st time	1 st time	1 st time	Conversion	1 st time	Conversion	1 st time	Conversion	n=5 n=3
Age (years)	41	48	39	66	34	54	37	49	Median:44.5
BMI (kg/m²)									
per-op	42.1	40.2	44.9	40.5	39.1	44.3	36.6	32.4	Median:40.3
post-op	32.6	29.3	30.1	29.5	28.1	30.1	30.5	30.1	Median:30.1
Preoperative co-morbidity									
Hypertension	No	Yes	Yes	No	No	No	No	No	n=2
Diabetes	Yes	No	No	No	No	Yes	Yes	No	n=3
Musculo-skeletal problems	No	No	Yes	Yes	No	No	Yes	No	n=3
Respiratory diagnosis	No	Yes	Yes	No	Yes	No	No	No	n=3
No co-morbidity	No	No	No	No	No	No	No	Yes	n=1

- Figure 1** Roux-en Y gastric by-pass construction. Arrows indicating sites of per-operative sampling and post-operative biopsies respectively.
- Figure 2** Morphometric evaluation of per and postoperative samples, all patients (numbered 1 to 8) act as their own controls and are displayed as individual observations (* denotes a p-value of ≤ 0.05).
- Panel A: Villi derived area amplification, given as hits over the mucosal surface/hits over the muscularis mucosae.
- Panel B: Lamina propria volume density given as % of the mucosa
- Panel C: Goblet cell volume density given as % of the epithelium
- Panel D: Ki67 staining signifying mitotic index given as % of counted epithelial cells
- Figure 3** Immunohistochemistry images depicting Ki67 staining as a marker of mitotic index, background staining with Mayer's hematoxylin.
- Panel A: Ki67 staining in a peroperative sample from patient number 4, arrow indicating stained cells (x20), bar indicating 100 μ m
- Panel B: Ki67 staining in a postoperative biopsy from patient number 4 (x20), bar indicating 100 μ m
- Panel C: Negative control. Peroperative sample from patient number 4 (x20), bar indicating 100 μ m
- Panel D: Negative control. Postoperative biopsy from patient number 4 (x20), bar indicating 100 μ m
- Panel E: Ki67 staining in a peroperative sample from patient number 4 (x40) arrow indicating stained cells, bar indicating 50 μ m
- Panel F: Ki67 staining in a postoperative biopsy from patient number 4 (x40), bar indicating 50 μ m
- Figure 4** Immunocell-count of per and postoperative samples, all patients (numbered 1 to 8) act as their own controls and are displayed as individual observations (* denotes a p-value of ≤ 0.05).
- Panel A: Number of mucosal lymphocytes given as cells per high power field
- Panel B: Number of intraepithelial lymphocytes per 100 epithelial cells
- Panel C: Number of plasma cells given as cells per high power field
- Panel D: Number of eosinophilic granulocytes given as cells per high power field
- Figure 5** Presence of radical forming enzymes analyzed by western blot in per and postoperative samples, all patients (numbered 1 to 8) act as their own controls and are displayed as individual observations (* denotes a p-value of ≤ 0.05).
- Panel A: NADPH-oxidase protein
- Panel B: MPO protein

Panel C: iNOS protein

Panel D: Nitrotyrosine

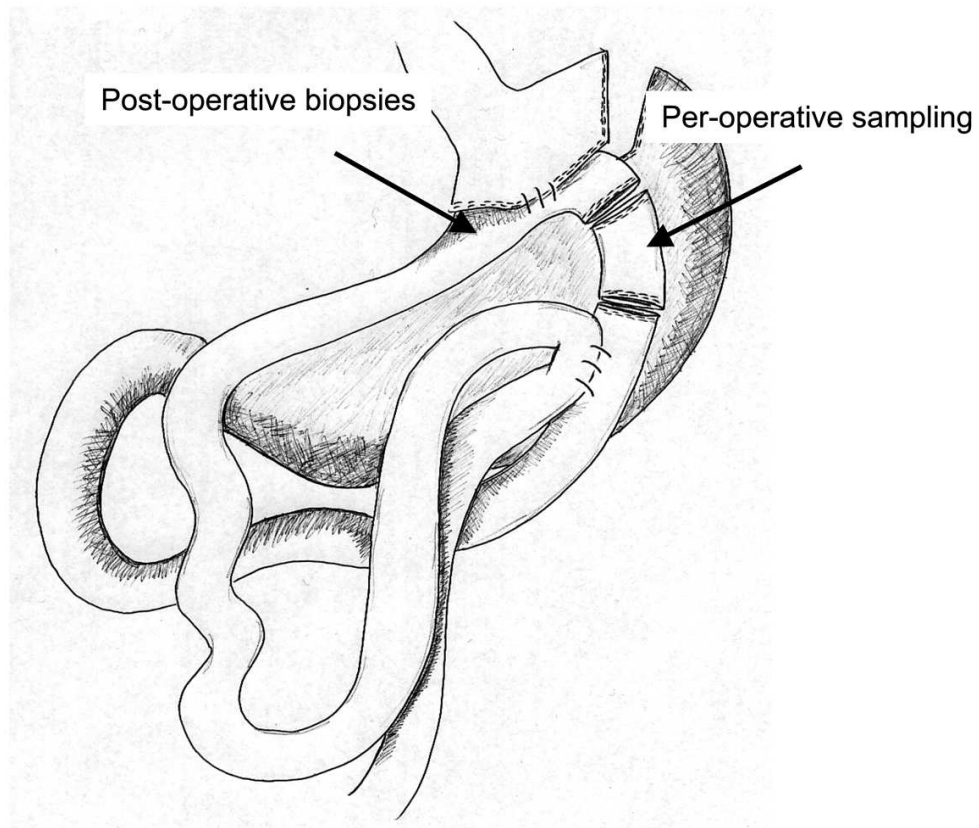
Figure 6 Ang II receptor protein analyzed by western blot in per and postoperative samples, all patients (numbered 1 to 8) act as their own controls and are displayed as individual observations (* denotes a p-value of ≤ 0.05).

Panel A: AT₁-receptor protein

Panel B: AT₂-receptor protein

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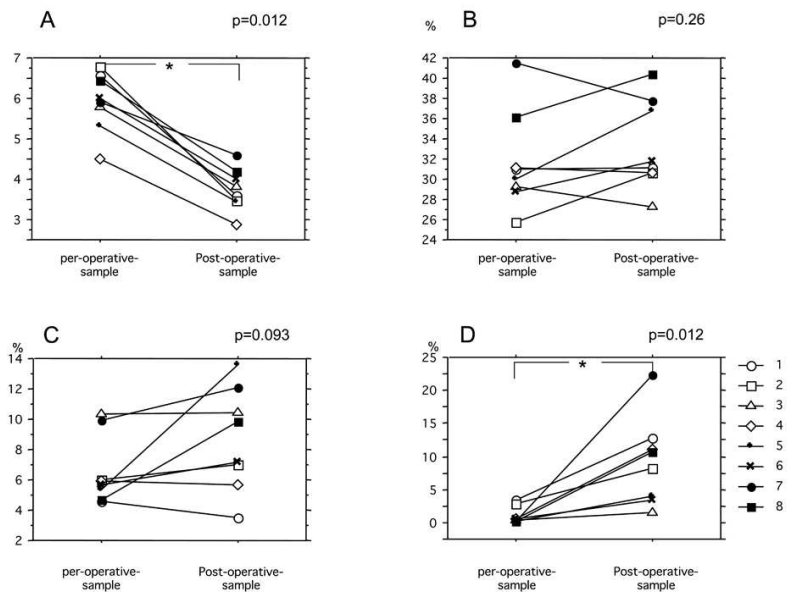
Figure 1



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EW

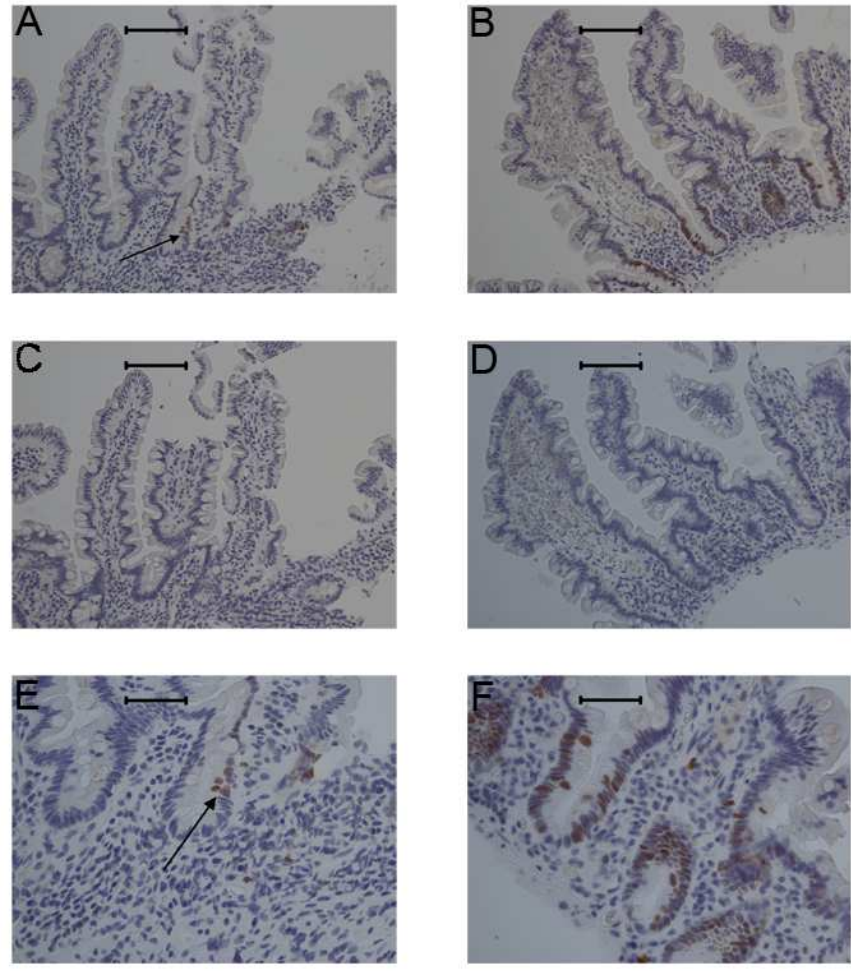
Figure 2



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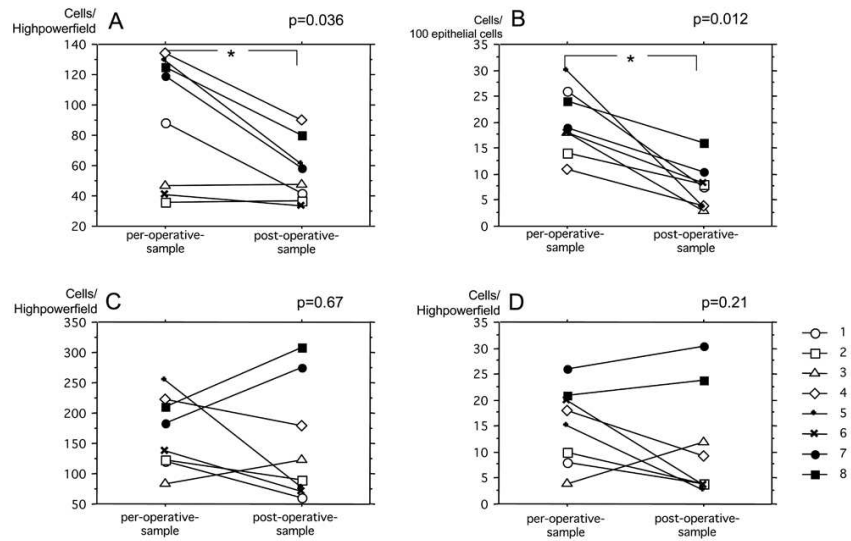
Review

Figure 3



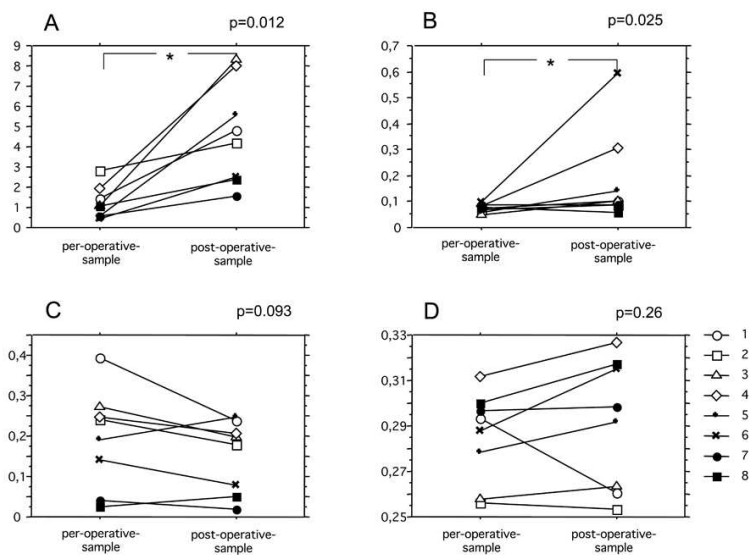
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Figure 4



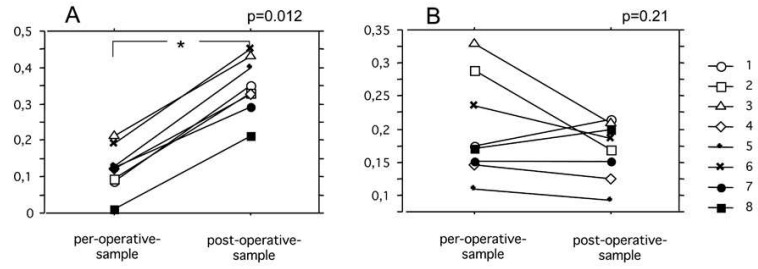
99x77mm (300 x 300 DPI)

Figure 5



99x77mm (300 x 300 DPI)

Figure 6



99x77mm (300 x 300 DPI)

Review

Antibodies and controls used in Western Blot analyses

Target protein	Primary antibody	Secondary antibody	Positive control
MPO	Antimyeloperoxidase 07-496 Lot 24587 Upstate/Millipore	Goat anti rabbit 12-448 Lot 26669 Upstate	HI-60 sc-2209 Lot C 2904 Santa Cruz
Nitrotyrosine	Antinitrotyrosine 06-284 Lot 32601 Upstate/Millipore	Goat anti rabbit sc-2007 Lot L 1505 Santa Cruz	Nitrotyrosine 12-354 Lot 23709 Upstate
iNOS	Transinos N 32030 Lot 16, L 18 TransductionLab/BioSite	Goat anti rabbit sc-2007 Lot L 1505 Santa Cruz	RAW 264.7 sc-2212 Lot D 2007 Santa Cruz
NADPH-oxidase (p47^{phox})	H-195 sc-14015 Lot J 0405 Santa Cruz	Goat anti rabbit sc-2007 Lot L 1505 Santa Cruz	HI-60 sc-2209 Lot 23709 Santa Cruz
AT₁-receptor	N-10 sc-1173 Lot D 2406 Santa Cruz	Goat anti rabbit sc-2007 Lot L 1505 Santa Cruz	Pc-12 sc-2250 Lot D 1805 Santa Cruz
AT₂-receptor	H-143 sc-9040 Lot C 2706 Santa Cruz	Goat anti rabbit sc-2007 Lot L 1505 Santa Cruz	Hep G2 sc-2227 Lot J 1007 Santa Cruz