Adaptive Changes of Bowel in Rats with Various Length of Thiry-Vella Fistulas

Farklı Uzunluklarda Thiry-Vella Fistül Oluşturulmuş Ratlarda İntestinal Adaptif Değişiklikler

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Abstract
Purpose: The aim of the current experimental study was to determine interaction between functional and dysfunctional bowel segments in terms of structural changes in the gut mucosa.
Materials and Methods: Twenty-two male rats were divided into four groups in which following operations were performed respectively: Group 1; sham operation, Group 2; 5% Thiry-Vella fistula, Group 3; 80% Thiry-Vella fistula, and Group 4; 5% Thiry-Vella fistula plus 75% bowel resection. At the end of the 14 days, ileum in continuity and Thiry-Vella fistula samples were examined histologically.
Results: Mucosal thickness in ileal samples in continuity were 605±24 µ, 615±14 µ, 731±16 µ, 860±44 µ, in Groups 1, 2, 3 and 4, respectively. Group 3 and Group 4 were significantly different from other groups ($p<0.05$). Mucosal thickness in Thiry-Vella fistula samples were 335±5.4 µ, 425±7.7 µ, 528±42 µ in Group 2, 3 and 4, respectively. Each group was significantly different from eachother ($p<0.05$).
Conclusion: While intestinal adaptive changes related to SBS in continuity were not occurred apparently in Group 3, ileal mucosal thickness increased significantly in Group 4. Long segment Thiry-Vella fistula might have decreased adaptation in functional bowel probably by consuming of systemic factors.

Key words: **Biological adaptation; Intestinal bypass;Short bowel syndrome.**

Özet
Amaç: Bu deneysel çalışmanın amacı, fonksiyonel ince barsak ile nonfonksiyonel ince barsak arasındaki karşılıklı etkileşime bağlı yapısal değişiklikleri incelemekti.
Gereç and Yöntem: Otuz iki adet sıçan 4 gruba ayrıldı. Grup 1'e sham operasyonu, Grup 2'ye %5 Thiry-Vella fistül, Grup 3'e %80 Thiry-Vella fistül ve Grup 4'e %5 Thiry-Vella fistül+%75 barsak rezeksiyonu yapıldı. Fonksiyonel ileum ve Thiry-Vella fistül örnekleri 14ncü günün sonunda histolojik olarak incelendi.
Bulgular: Fonksiyonel ileal örnelerin mukozal kalınlıkları sırasıyla Grup 1; 605±24 µ, Grup 2; 615±14 µ, Grup 3; 731±16 µ ve Grup 4; 860±44 µ, idi. Grup 3 ve 4 diğer iki gruptan anlamlı olarak farklıydı ($p<0.05$). Thiry-Vella fistül örneklerindeki mukozal kalınlıklar ise Grup 2; 335±5,4 µ, Grup 3; 425±7,7 µ ve Grup 4; 528±42 µ idi. Her grup istatistiki olarak diğer gruplardan farklıydı ($p<0.05$).
Sonuç: Kısa barsak sendromuyla ilişkili intestinal adaptif değişiklikler, Grup 3 teki fonksiyonel barsakta belirgin olarak görülmezken, ileal mukozal kalınlık Grup 4 deki sıçanların ileumlarında belirgin olarak artmıştı. Sonuç olarak, uzun segment Thiry-Vella fistül, muhtemelen sistemik faktörleri tüketerek, fonksiyonel barsağın adaptasyonunu azaltmış olabilir.

Anahtar kelimeler: **Biyolojik adaptation; İntestinal bypass; Kısa Barsak Sendromu.**

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Introduction

Intestinal adaptation after bowel resection is a multi-factorial process that results in growth and increase functional capacity of the residual bowel. Many factors such as luminal nutrients, hormones, pancreaticobiliary secretions, and neural pathways in the gastrointestinal tract mediate intestinal adaptation after bowel resection (1). It was demonstrated that isolated loops of small bowel distant from the luminal stream results mucosal atrophy (2). The purpose of this study was to determine interaction between functional bowel and Thiry-Vella Fistula (TVF) in terms of structural adaptive changes in the gut mucosa.

Materials and Methods

The experimental protocol was approved by the local animal ethics review committee of Celal Bayar University. Thirty two male Sprague-Dawley type rats weighing 260 to 320g were used in this study. The animals were maintained on pellet food and water ad libitum. Rats were divided into 4 groups containing 8 rats in each. The rats fasted overnight with free access to water. All surgical procedures were performed via midline abdominal incision under ketamine (50 mg/kg) and xylazine HCl (8 mg/kg) anesthesia.

The small bowel was measured along the antimesenteric border and transection of ileum 10 cm proximal from cecum and then reanastomosis was done with interrupted 5-0 silk sutures in Group 1 (Sham group). In Group 2 (5%TVF), the small intestine divided 10 to 15 cm proximal to the cecum, leaving the vascular supply intact. The two ends of 5 cm length segment were exteriorized from the left abdominal wall through double muco-cutaneous stomata. Intestinal continuity was re-established with the end-to-end anastomosis between the remaining jejunum and ileum. In Group 3 (80%TVF), the small intestine divided 10 cm distal to the ligament of Treitz and 10 cm proximal to the cecum, leaving the vascular supply intact. The two ends of this segment were exteriorized as a TVF. Each 10 cm length proximal and distal bowel remnants were anastomosed end-to-end. In Group 4 (5%TVF + %75 resection), 5 cm length ileal segment which is 10 to 15 cm proximal to the cecum was exteriorized as a TVF. Each 10 cm length proximal and distal remnants were anastomosed end to end after 75% bowel resection. Intestinal continuity was restored by end-to-end anastomosis. Rats were weighted after surgical procedures (weight I). All surgical procedures and histogical sampling areas were represented in Fig 1.

After the operations, all rats were weighed and received lactated Ringer's solution (40 mL/kg) subcutaneously, and allowed free access to water. Postoperative second day, rats were allowed chow ad libitum. End of the 14 days, rats killed with high dose ketamin HCl after weighed (weight II). Ileal samples were taken 5cm proximal from the cecum. TVF samples were taken 2 cm distant from distal end of the TVF.

Histological Examination. After being washed with saline solution 0.9%, the excised tissues were stored in buffered 10% formalin solution for 3 days. The material was sliced into 5µ sections, which were stained with hematoxylin and eosin (HE). The video camera transferred the image from the microscope to the computer screen. For the morphometric study of intestinal mucosa, "BAB Bs200Pro Image Processing and Analysis System" was used. The height of each villus was manually delimited with a computer mouse from its apex to the transition into the crypt zone. The studied intestinal mucosa area comprised the entire mucosal thickness from the apex of the villus to the muscularis mucosae. The measurements were expressed in microns. The analysis was by under 100x magnification using specimens in which the 10 consecutive villi were perpendicular to the muscularis mucosae. Villus height (from the top of the villus to the villus-crypt junction) and total mucosal thickness (from the top of the villus to the muscularis mucosae) were obtained.

Statistical Analysis. Statistical analyses were accomplished by using SPSS computer program (version 13.0). All results were reported as means ± S.E. The comparison of the results from the various experimental groups and their corresponding controls was carried out using a one-way analysis of variance (ANOVA) followed by pairwise multiple comparison procedures (Tukey test). The differences were considered significant when $P<0.05$.

Results

Table I and Table II show mucosal thickness, villus length and weight values for all groups. Mucosal thicknesses of ileum in continuity were 605±24 µ, 615±14 µ, 731±16 µ, 860±44 µ in the group 1, 2, 3 and 4 respectively. The group 3 and the group 4 were significantly different from other groups ($P<0.05$). Villi lengths of all the groups were in accordance with mucosal thicknesses. Fig 2 shows histological appearance of ileum and TVF samples.

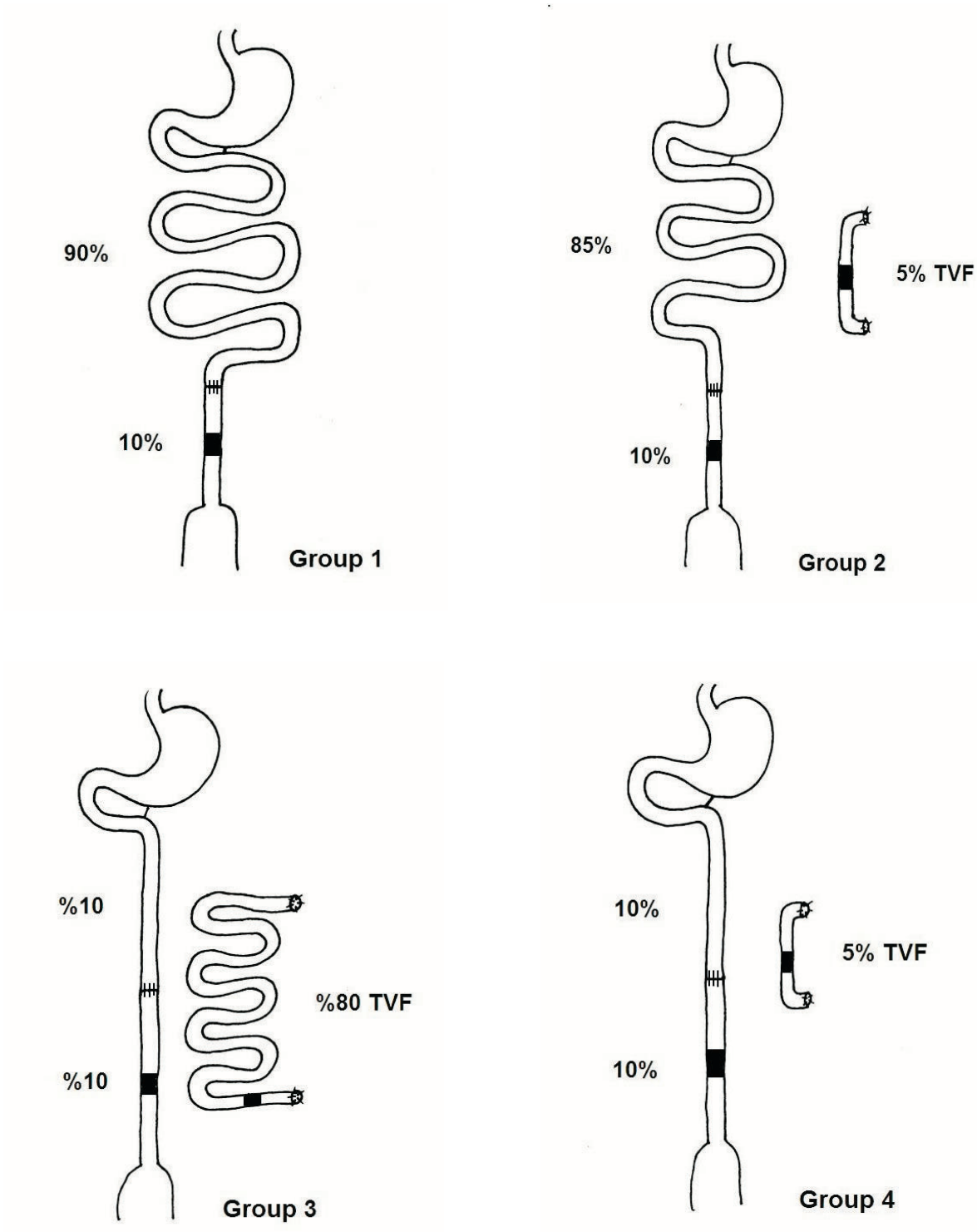

Figure 1. Diagrammatic representations of all surgical procedures and sites of histological sampling (black).

Mucosal thickness in TVF samples were 335±5.4 μ, 425±7.7 μ, 528±42 μ in Group 2 (Fig 2b), 3 and 4 respectively. Each group was significantly different from one another and sham (P<0.05). Villi lengths of all the groups were in accordance with its mucosal thicknesses.

Table I. Mucosal Thickness and Villus Length of ileal segments.

	Group 1	Group 2	Group 3	Group 4	F (Anova)	P (Anova)
Mucosal Thickness (μ)	605±24	615±14	731±16 [a]	860±44 [b]	24,65	0,0001
Villus Length (μ)	422±19	416±25	582±19 [c]	651±22 [d]	31,05	0,0001

Valuea are mean±S.E. Post Hoc Tukey results: Group 3 (p=0.016 [a]) and Group 4 (p=0.0001[b]) different from Group 1 for mucosal thickness. Group 3 (p=0.001 [c]) and Group 4 (p=0.001 [d]) different from Group 1 for villus length.

Table II. Mucosal Thickness and Villus Length of TVF segments.

	Group 2	Group 3	Group 4	F (Anova)	P (Anova)
Mucosal Thickness (μ)	335±5,4	425±7,7	528±42 [a,b]	15,06	0,0001
Villus Length (μ)	262±5,5	311±4,2	390±30 [c,d]	13,00	0,0001

Valuea are mean±S.E. Post Hoc Tukey results: Group 4 different from Group 2 (p=0.0001 [a]) and Group 3(p=0.021 [b]) for mucosal thickness. Group 4 different from Group 2 (p=0.0001 [c]) and Group 3 (p=0.015 [d]) for villus length. TVF: Thiry Vella Fistula

While the rats in Group 1 and 2 gained weight, the rats Group 3 and 4 lost weight (Table II). The weight loss was more prominent in the group 3 then the group 4 (P<0.05).

Table III. Weight changes of all groups

	Group 1	Group 2	Group 3	Group 4	F (Anova)	P (Anova)
Weight I (gr)	289±7,3	281±8,1	267±8,2	287±10,1	1,19	0,261
Weight II (gr)	303±8,1	292±9,9	234±6,4[a,b,c]	270±10,9	11,5	0,0001
Weight chances (%)	4,5±2,3	4,5±2,7	-12,3±2,4[d,e,f]	-5.9 ± 2.9	34,6	0,0001

Valuea are mean±S.E. Post Hoc Tukey results: Group 3 different from Group 1 (p=0,0001 [a]) , Group 2 (p=0,01 [b]) and Group 4 (p=0,048 [c]) for weight II. Group 3 different from Group 1 (p=0,0001[d]), Group 2 (p=0,001 [e]) and Group 4 (p=0,019 [f]) for weight changes.

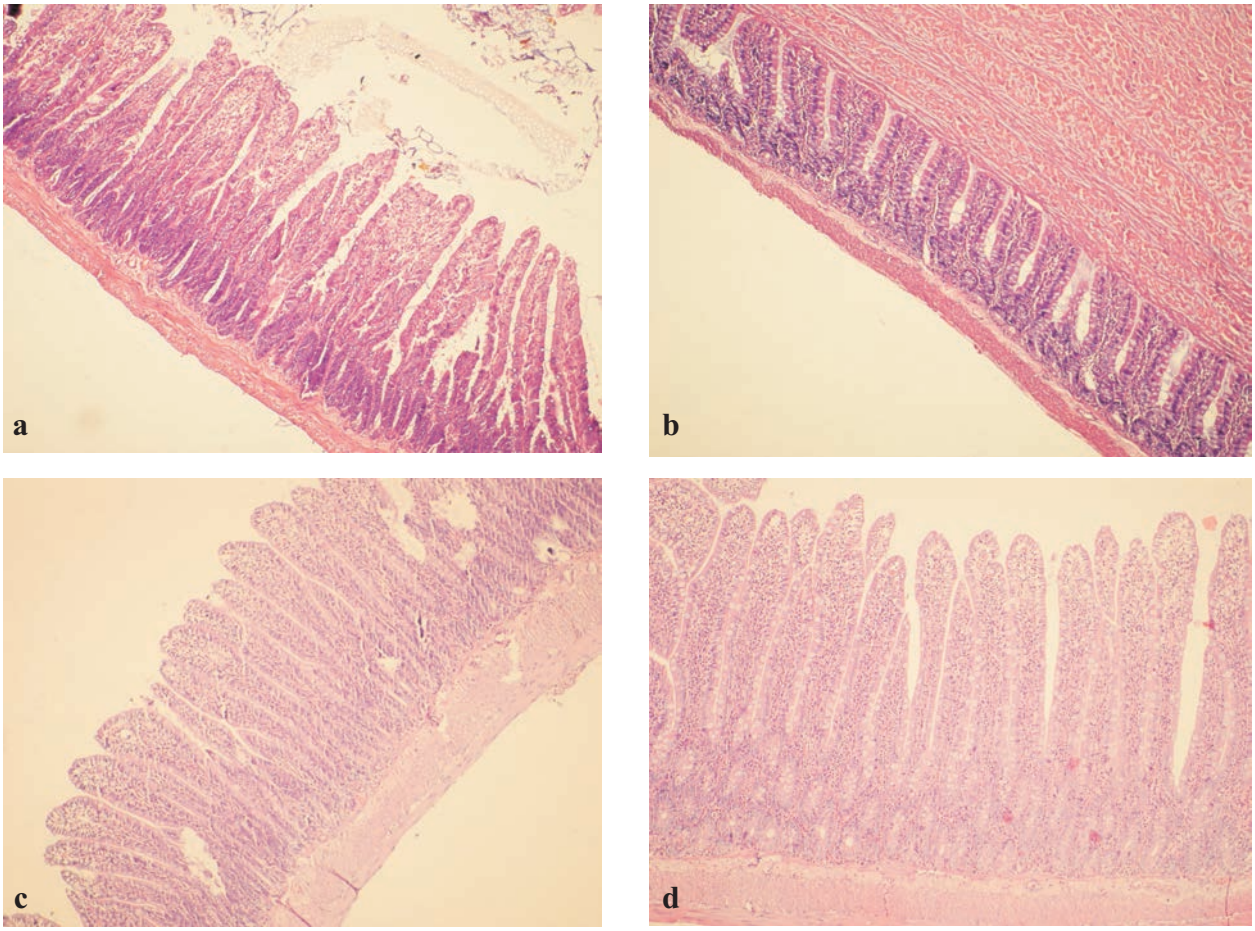

Figure 2. (a) Sham group, (b) 5% TVF in group 2, (c) distal ileum in continuity of group 3 and, (d) distal ileum in continuity of group 4. (HE, x100).

Discussion

Resection of half or more of the small bowel is termed short bowel syndrome (SBS) that associated with diarrhea, steatorrhea, dehydration, electrolyte imbalances, malabsorption and malnutrition (3, 4, 5). Survival of patients with short bowel syndrome associated with the adaptation capacity of the residual bowel (4). Post-resectional adaptation is characterized by mucosal hypertrophy and hyperplasia, dilatation and elongation of the remnant bowel, and increase digestive and absorptive capacity of per unit length (6, 7, 8, 9). Adaptation of residual bowel is influenced by many factors, including luminal nutrients, pancreaticobiliary secretions and hormones such as gastrin, enteroglucagon (10, 11, 12) insulin-like growth factor and epidermal growth factor (13). Luminal factors including nutrients and pancreaticobiliary secretions play very important role for stimulating mucosal growth and absorptive function after bowel resection (14, 15). Truly, it was demonstrated that short segment isolated loops of small bowel distant from the luminal stream results a significant mucosal atrophy (2). Optimum adaptation requires both intra-luminal factors and enterotrophic hormones (16). The mechanism of increased enterocyte proliferation in short gut syndrome is unclear. Suggested mechanism is that malabsorbed nutrients and pancreaticobiliary secretions release growth factors from distal bowel; these enter the systemic circulation and so are delivered to the whole gut (17, 18). The purpose of this study was to determine interaction between functional bowel and various length of defunctionalized intestinal loop in terms of mucosal structural changes.

High intestinal diversion is sometimes required for neonatal intestinal problems such as multiple intestinal atresia, necrotising enterocolitis and extensive Hirschsprung's disease. We aimed simulate these clinical situations by using a Thiry-Vella Fistulas (TVF) in this study. Isolated and exteriorized loop of small bowel, with its vascular and nervous connections were preserved, facilitates examination of bowel mucosa that is not contact to intra-luminal contents (18, 19). Another similar method to separate luminal stream from gut mucosa is jejunoileal bypass which was used to treatment of morbid obesity in the past (20). There are consensus in published reports that short segment defunctionalized loops go to atrophy because they don't contact to luminal contents which are essential for normal mucosal growth (17, 18). Similarly, in our study the total mucosal thickness and villus height of %5 TVF (Fig 2b) in group 2 decreased significantly ($P<0.05$) when compared with sham group (Fig 2a). In contrast, the 80% TVF of Group 3 rats shown mild atrophy despite the distance from luminal contents; the total mucosal thickness and villus height were higher ($P<0.05$) than the 5% TVF in Group 2 rats. These results were accordance with similar studies (17, 18). Suggested mechanism is that the long segment TVF causes functional short bowel syndrome. Thus the atrophic effect of absence of luminal contents can be attenuated moderately by systemic growth factors released as part of the adaptive response (11, 14). In group 4 rats, the atrophic changes were completely counteracted in 5% TVF. It suggest that systemic trophic factors are more effective on the short segment TVF than on the long segment TVF to prevent mucosal atrophy in existence of short bowel syndrome.

Functional adaptation of the remaining small bowel has been demonstrated in man after intestinal resection. This compensatory adaptation also occurs following jejunoileal bypass for morbid obesity, as indicated by the decrease in the rate of weight loss with time (21, 22). However, effect of this isolated loop to adaptation rate in functional bowel is not known clearly. Our main aim in this study was to determine whether length of defunctionalized intestinal loop effects the adaptation of bowel segment remaining in continuity. We determined that villus height and total mucosal thickness of bowel remaining in continuity of the group 4 were significantly higher than the group 3. The weight changes of rats were also in accordance with histological changes of gut in circuit. Weight loss of the group 3 rats ($12.5 \pm 1,7$ %) more than the group 4 rats ($5.9 \pm 0,7$ %) suggests that the long segment TVF decrease functional adaptation of short bowel in circuit (TableIII). The long segment TVF might have prevented bowel adaptation in continuity probably by consuming of systemic trophic factors.

In the light of histopathological findings, we concluded that atrophic chances of the long segment TVF are more moderate than the short segment TVF. However long segment TVF causes decrease of adaptation in functional bowel and, in existence of SBS, atrophic chances of the long segment TVF are more prominent than the short segment TVF.

References

1. Williamson RC. Intestinal adaptation (second of two parts). Mechanisms of control. N Engl J Med 1978; 298(26): 1444 –1450.

2. Hanson WR, Rijke RPC, Plasier HM, Van Ewijk W, Osborne JW.. The effect of intestinal resection on Thiry-Vella fistulae of jejunal and ileal origin in the rat: evidence for a systemic control mechanism of cell renewal. Cell Tissue Kinet 1977;10(6): 543–555.

3. Booth IW, Lander AD. Short bowel syndrome. Baillieres Clin Gastroenterol 1998; 12(4):739–773.

4. Sukhotnik I, Siplovich L, Shiloni E, Mor-Vaknin N, Harmon CM, Coran AG. Intestinal adaptation in short-bowel syndrome in infants and children: a collective review. Pediatr Surg Int 2002; 18(4): 258–263.

5. Vanderhoof JA. Short bowel syndrome. Neonat Gastroenterol 1996; 23(2): 377–386.

6. Chaet MS, Arya G, Ziegler MM, Warner BW. Epidermal growth factor enhances intestinal adaptation after massive small bowel resection. J Pediatr Surg 1994; 29(8):1035–1038.

7. Hanson WR, Osborne JW, Sharp JG. Compensation by the residual intestine after intestinal resection in the rat. I. Influence of amount of tissue removed. Gastroenterology 1977; 72(4 Pt 1):692–700.

8. Hanson WR, Osborne JW, Sharp JG. Compensation by the residual intestine after intestinal resection in the rat. II. Influence of postoperative time interval. Gastroenterology 1977; 72(4 Pt 1):701–705.

9. O'Loughlin E, Winter M, Shun A, Hardin JA, Gall DG.. Structural and functional adaptation following jejunal resection in rabbits: Effect of epidermal growth factor. Gastroenterology 1994; 107(1):87–93.

10. Al-Mukhtar MYT, Sagor GR, Ghatei MA, Bloom SR, Wright NA. The role of pancreatico-biliary secretions in intestinal adaptation after resection and its relationship to plasma enteroglucagon. Br J Surg 1983; 70(7):398-400.

11. Johnson LR, Lichtenberger LM, Copeland EM, Dudrick SJ, Castro GA. Action of gastrointestinal structure and function. Gastroenterology 1975; 68(5 Pt 1):1184–1192.

12. Tappenden KA. Mechanisms of enteral nutrient-enhanced intestinal adaptation. Gastroenterology 2006; 130(2 Suppl 1):93–99.

13. Juno RJ, Knott AW, Erwin R, Warner BW. A Serum factor(s) after small bowel resection induces intestinal epithelial cell proliferation: effects of timing, site, and extent of resection. J Pediatr Surg 2000; 38(6): 868–874.

14. Dowling RH. Small bowel adaptation and its regulation. Scand J Gastroenterol Suppl 1982; 74:53–74.

15. Gleeson MH, Cullen J, Dowling RH. Intestinal structure and function after small bowel by-pass in the rat. Clin Sci 1972; 43(6):731–742.

16. Williamson RC, Bauer FL. Evidence for an enterotropic hormone: compensatory hyperplasia in defunctioned bowel. Br J Surg 1978; 65(10):736–739.

17. Albert V, Young GP, Morton CL, Robinson P, Bhathal PS. Systemic factors are trophic in bypassed rat small intestine in absence of luminal contents. Gut 1990; 31(3): 311–316.

18. Hashimoto N, Ohyanagi H. Sytemic factors are trophic in Thiry-Vella loop in absence of luminal contents. Hepatogastroenterology 2002; 49(44):428–431.

19. Kyo U, Higashide S, Evers BM, Ishizuka J, Townsend CM Jr, Thompson JC. Bombesin stimulates mucosal growth in jejunal and ileal Thiry-Vella Fistulas. Ann Surg 1995; 221(5): 602–611.

20. Stein TA, Wise MS, Wise L. Functional adaptation of the intestinal mucosal enzymes after jejunoileal bypass for morbid obesity. Am J Clin Nutr 1978; 31(7): 1143–1148.

21. Fenyo G, Backman L, Hallberg D. Morphological changes of the small intestine following jejuno-ileal shunt in obese subjects. Acta Chir Scand 1976; 142(2):154–159.

22. Solhaug JH. Morphometric studies of the small intestine following jejuno-ileal shunt operation. Scand J Gastroenterol 1976; 1l(2):155–160.