

# The Effect of Vagotomy on Small Intestinal Anastomosis Healing In Rats

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## ABSTRACT

Gastrointestinal anastomotic healing depends on collagen dynamics, microcirculation, and immune responses. The vagus nerve modulates these via the cholinergic anti-inflammatory pathway. This study evaluated the effects of truncal vagotomy on jejunal anastomotic healing in rats.

Forty male Wistar Albino rats were divided into five groups: control, day 4 anastomosis, day 4 vagotomy + anastomosis, day 7 anastomosis, and day 7 vagotomy + anastomosis. Standard end-to-end jejunal anastomoses were performed; vagotomy groups underwent transection of anterior and posterior vagal trunks. Healing was assessed by bursting pressure (cm H<sub>2</sub> O) and tissue hydroxyproline (µg/mg).

No mortality or macroscopic leakage occurred. Mean bursting pressures were 87.0 ± 4.75, 95.37 ± 7.72, 98.25 ± 9.37, 109.25 ± 12.66, and 123.37 ± 4.27 cm H<sub>2</sub> O in Groups I–V, with a significant increase in the 7-day vagotomy group ( $p < 0.001$ ). Hydroxyproline levels were 0.512 ± 0.223, 0.539 ± 0.025, 0.549 ± 0.023, 0.539 ± 0.025, and 0.584 ± 0.023 µg/mg; Group V was significantly higher than Groups IV and III ( $p < 0.001$  and  $p = 0.038$ ). At day 4, bursting pressure increased without a parallel rise in hydroxyproline, whereas both parameters increased significantly at day 7.

Truncal vagotomy enhanced jejunal anastomotic healing at day 7 by increasing collagen deposition and mechanical strength. Disruption of vagal tone appears to modulate inflammatory and fibroblastic responses, influencing the temporal pattern of healing. Further mechanistic and clinical studies are warranted.

**Keywords:** Vagotomy, Jejunum, Anastomosis, Bursting pressure, Hydroxyproline, Wound healing

## Introduction

The integrity and long-term function of gastrointestinal anastomoses rely on the coordinated interaction of several biological and technical factors, including collagen synthesis and degradation, local perfusion, immune modulation, and meticulous surgical technique. These determinants act within three overlapping and time-dependent phases of wound repair: inflammation, proliferation, and remodeling (1–4). In the first 3–4 days after anastomosis, neutrophils and macrophages infiltrate the tissue. Oxidative stress and increased MMP-2/9 activity cause significant collagen breakdown, producing the lowest point in the mechanical strength curve. (2–6). In the early proliferative stage, fibroblasts and myofibroblasts become activated, granulation tissue forms, and angiogenesis increases type III collagen synthesis. After approximately one week, the maturation of collagen fibers—particularly the

rise in the type I to type III ratio and increased cross-linking—gradually restores tensile resistance (2–5,7–9).

The bursting pressure test reflects the composite mechanical endurance of the anastomotic line, whereas hydroxyproline concentration indicates the overall collagen content. Nonetheless, since tensile strength is also dependent on collagen quality—such as fiber orientation, cross-linking density, and the relative proportions of collagen subtypes—hydroxyproline levels alone do not always correlate perfectly with mechanical resistance (2–5,7–10).

The autonomic nervous system, through integration with the enteric network, governs gastrointestinal motility, secretion, vascular tone, and mucosal immunity (11,12). Among its components, the vagus nerve exerts major influence over inflammatory regulation via the “cholinergic anti-inflammatory pathway,” a

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mechanism whereby acetylcholine suppresses the release of proinflammatory mediators such as TNF- $\alpha$  (tumor *necrosis* factor) through activation of  $\alpha$ 7-nicotinic acetylcholine receptors on macrophages (13–15). Surgical division of the anterior and posterior vagal trunks—termed truncal vagotomy—abolishes this cholinergic regulation and consequently alters sympathovagal balance, splanchnic microcirculation, motility, and epithelial barrier function (12,16–18). Although the physiological consequences of vagotomy on intestinal perfusion and neural signaling have been well documented, its influence on anastomotic repair remains incompletely defined and appears to depend on the temporal phase of healing (16–20).

Conceptually, attenuation of vagal activity may initially intensify inflammatory reactions and adhesion formation, potentially strengthening the provisional extracellular matrix and facilitating collagen deposition. Conversely, disturbances in motility or blood flow may create hypoxic stress that promotes MMP-mediated collagen degradation and delays structural restoration (2–5,7–10,19–23). Because jejunal anastomoses are commonly performed in upper gastrointestinal surgery, elucidating the relationship between vagal tone and postoperative tissue repair carries substantial translational importance (1,4,8–10,19–23).

The present study was designed to examine the time-dependent effects of truncal vagotomy on jejunal anastomotic healing in rats. By measuring bursting pressure and tissue hydroxyproline, we aimed to test the hypothesis that withdrawal of vagal cholinergic control enhances collagen accumulation and mechanical strength during the proliferative and early remodeling stages, with limited benefit during the initial inflammatory phase (2–5,7–10,13–15,19–23).

## Materials and Methods

This experimental study was conducted at the Zonguldak Karaelmas University Faculty of Medicine Experimental Animal Laboratory with ethical approval (No. 2010-01-028/01, dated January 28, 2011). Forty male Wistar Albino rats (20 weeks, 270–370 g; mean  $320 \pm 27.7$  g) were housed under controlled environmental conditions with ad libitum access to standard chow and water. The rats were randomly divided into five groups:

Group I (control, day 0), Group II (day 4 anastomosis), Group III (day 4 vagotomy +

anastomosis), Group IV (day 7 anastomosis), and Group V (day 7 vagotomy + anastomosis).

All procedures were performed between 09:00 and 12:00 under clean, non-sterile conditions by a single surgeon to minimize technical variability. After shaving and preparing the abdomen with 10% povidone-iodine, a midline laparotomy was performed (Figure 1). The esophagus was identified (figure 2), and the jejunum was localized (figure 3). In vagotomized groups, anterior and posterior vagal trunks were dissected and transected (figure 4–5).

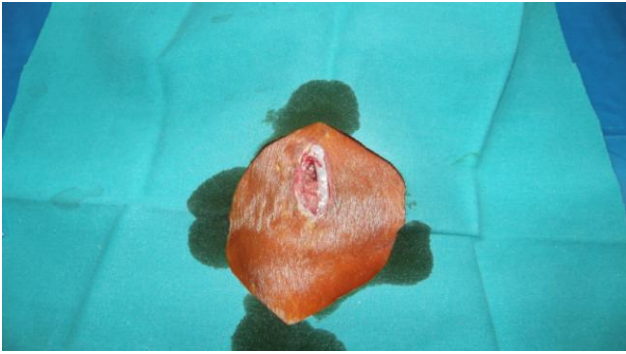
Standardized end-to-end jejunal anastomoses were created using eight single inverting 6/0 polypropylene sutures. To prevent dehydration, 3 mL of normal saline was instilled intraperitoneally, and the abdominal wall was closed with a single-layer running 3/0 silk suture. Standard diet and water were resumed 6 hours postoperatively. Anesthesia was induced with intramuscular ketamine hydrochloride (50 mg/kg).

**Measurement of Bursting Pressure:** During reoperation, the anastomotic segment was carefully exposed, preserving adhesions. After clamping the distal lumen, a 6F catheter was inserted proximally and secured with 2/0 silk. The lumen was perfused with saline at 50 mL/h via an Abbott perfusion pump, with pressure monitored using a Transpac IV transducer and Petas KMA 460R monitor. The pressure at the sudden drop in the curve or appearance of leakage was recorded as the bursting pressure (cm H<sub>2</sub>O).

**Biochemical Analysis:** Frozen tissue samples (–80°C) were lyophilized, homogenized, and processed for hydroxyproline assay. After hydrolysis, aliquots were reacted with chloramine-T and Ehrlich's reagent, incubated at 50°C for 90 minutes, and absorbance was measured at 560 nm. Hydroxyproline concentrations were calculated using a standard curve (0.2–1.6  $\mu$ g range) and expressed as  $\mu$ g/mg tissue (Graph 1-2).

All rats were sacrificed on postoperative days 4 and 7 under the same anesthesia via intracardiac exsanguination. Segments containing 2 cm proximal and distal to the anastomosis were resected for biochemical analysis.

**Statistical Analysis:** Normality was assessed using the Shapiro–Wilk test and Q–Q plots. Parametric and nonparametric data were expressed as mean  $\pm$  SD or median (IQR), respectively. Group comparisons were performed using Student's t-test or Mann–Whitney U test, and one-way ANOVA or Kruskal–Wallis test as appropriate. Variance homogeneity was tested by



**Fig. 1.** Preoperative skin preparation and surgical field setup



**Fig. 2.** Laparotomy and identification of the esophagus

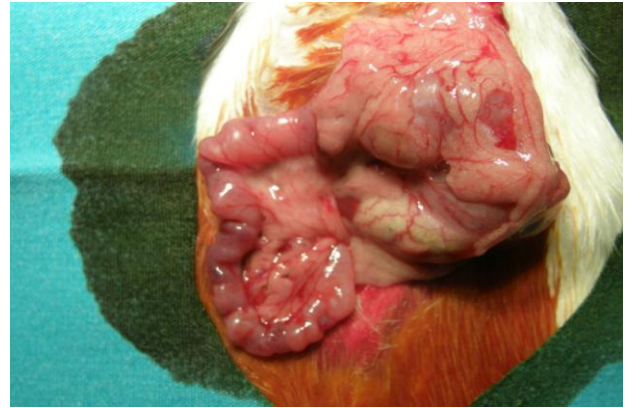
Levene's test; Welch correction was applied when needed. Post-hoc analyses used Tukey HSD or Dunn tests (Holm–Bonferroni correction). Repeated measures were analyzed using two-way ANOVA or mixed-effects models; Greenhouse–Geisser correction was applied for sphericity violations. Significance was set at  $\alpha = 0.05$  (two-tailed). Analyses were performed by a blinded researcher using standard statistical software.

## Results

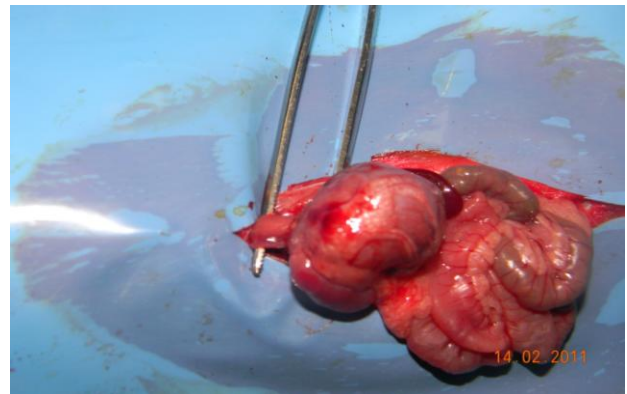
No mortality or gross leakage occurred. All bursts occurred at the anastomotic line.

Mean bursting pressures (cm H<sub>2</sub> O) were: Group I,  $87.00 \pm 4.75$ ; Group II,  $95.37 \pm 7.72$ ; Group III,  $98.25 \pm 9.37$ ; Group IV,  $109.25 \pm 12.66$ ; Group V,  $123.37 \pm 4.27$ . The 7-day vagotomy group (V) had significantly higher pressures than Groups IV ( $p = 0.003$ ) and III ( $p < 0.001$ ).

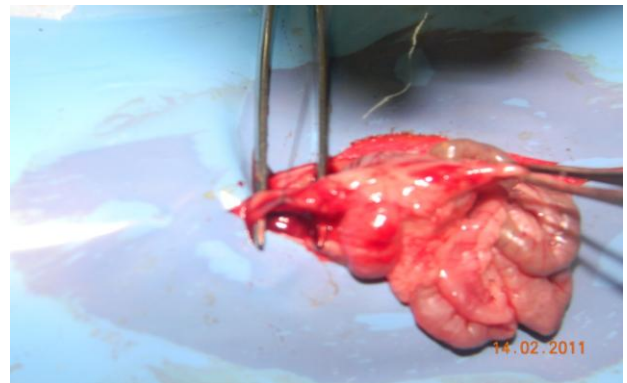
Hydroxyproline levels ( $\mu\text{g}/\text{mg}$ ) were: Group I,  $0.512 \pm 0.223$ ; Group II,  $0.539 \pm 0.025$ ; Group III,  $0.549 \pm 0.023$ ; Group IV,  $0.539 \pm 0.025$ ; Group V,  $0.584 \pm 0.023$ . Group V showed significantly higher levels than Groups IV ( $p < 0.001$ ) and III ( $p = 0.038$ ). At day 4, increases in bursting pressure did not parallel hydroxyproline, but both parameters rose markedly at day 7,



**Fig. 3.** Localization of the jejunum



**Fig. 4.** Transection of the anterior vagal trunk during truncal vagotomy

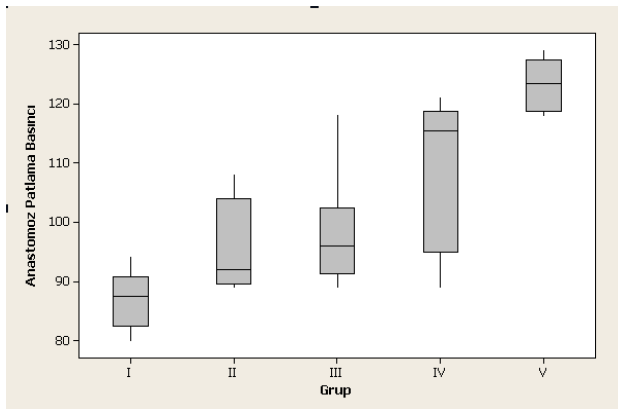
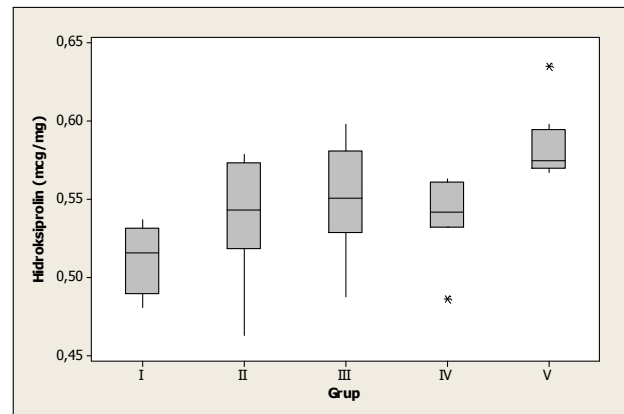


**Fig. 5.** Truncal vagotomy – transection of the posterior vagal trunk

indicating synchronized biochemical and mechanical improvement (Table 1-4).

## Discussion

This study demonstrates that truncal vagotomy enhances jejunal anastomotic healing in a time-dependent manner. While the day-4 increase in bursting pressure was not accompanied by higher hydroxyproline levels, both indices significantly

Graph 1: Bursting pressure values (cm H<sub>2</sub> O)

Graph 2: Hydroxyproline values (µg/mg)

increased at day 7. The early discrepancy likely reflects immature collagen architecture and delayed hydroxyproline accumulation, consistent with the known transition from type III to type I collagen and enhanced cross-linking after the first week (2–5,7–10).

The early mechanical improvement without biochemical correspondence may also result from temporary factors such as collagen fiber orientation or adhesion-related mechanical support. In contrast, the synchronized late-phase increase in both parameters indicates true improvement in collagen quantity and quality (2–5,7–10,21–23).

Disruption of vagal tone may influence inflammatory responses and fibroblast activity, potentially affecting collagen deposition, though the exact molecular mechanisms require further investigation, leading to increased ECM deposition and anastomotic stability (13–15,20–23). Conversely, sympathetic predominance may induce splanchnic vasoconstriction, compromising microcirculation. Hypoxic conditions may stimulate angiogenesis and matrix deposition via HIF-1 $\alpha$  but also promote matrix degradation via excessive MMP-2/9 activity (2–5,7–10,19–23). The current data suggest that, under the controlled surgical and perioperative setting of this study, these effects were collectively directed toward improved healing.

Previous studies show that vagus nerve stimulation (VNS) attenuates systemic inflammation and regulates macrophage activity through  $\alpha 7$ -nAChR (13–15,24–26). However, the effects of vagal enhancement (VNS/cholinergic agonists) versus suppression (vagotomy) on wound healing vary depending on phase and tissue context. Controlled early inflammation may transiently strengthen the anastomosis, whereas long-term collagen maturation requires adequate

perfusion and balanced MMP/TIMP regulation (2–5,7–10,19–23,27–29).

Clinically, integrating neuroimmune modulation with perfusion monitoring (e.g., indocyanine green fluorescence angiography), tissue oxygenation assessment, mechanical stress management, and microbiota/nutritional optimization may improve surgical outcomes (19,21–23,27–29). The role of vagal signaling in glial-immune interactions and epithelial barrier integrity also warrants investigation (11–15,24–26,30).

Limitations include use of a single species and sex, limited sample size and time points (days 0, 4, 7), absence of histopathologic scoring (Masson trichrome, Picrosirius red), immunophenotyping (TNF- $\alpha$ , IL-6/IL-10, HMGB1), macrophage polarization markers (CD68, CD206),  $\alpha 7$ -nAChR expression, and perfusion analysis (laser speckle, Doppler, photoacoustic oximetry). Alternative suture materials, stapled anastomoses, and tension testing were not evaluated (1–5,19,21–23,27). Future studies should incorporate VNS/cholinergic pharmacologic arms, MMP/TIMP balance, collagen type I/III ratio, and structural mapping by second harmonic generation (SHG) microscopy or atomic force microscopy, alongside tensile strength testing for mechanical correlation (2–5,7–10,21–23,27–30).

Truncal vagotomy significantly enhanced jejunal anastomotic healing in rats, particularly on postoperative day 7, as evidenced by higher bursting pressure and hydroxyproline levels. These results align with potential neuroimmune modulation and alterations in extracellular matrix dynamics. Further mechanistic and clinically oriented investigations incorporating histomolecular and biomechanical endpoints are warranted.

**Conflict of Interest:** No conflict of interest was declared by the authors.

**Table 1:** Bursting Pressure Values (cm H<sub>2</sub> O)

Subject No	Group I	Group II	Group III	GroupIV	GroupV
1	82,000	108,000	118,000	92,000	122,000
2	80,000	92,000	92,000	118,000	118,000
3	87,000	95,000	94,000	116,000	118,000
4	88,000	89,000	101,000	89,000	128,000
5	90,000	107,000	98,000	104,000	129,000
6	91,000	92,000	103,000	119,000	118,000
7	84,000	91,000	91,000	121,000	125,000
8	94,000	89,000	89,000	115,000	121,000
Mean±SD (cm H <sub>2</sub> O)	87,00 ± 4,75	95,37 ± 7,72	98,25 ± 9,37	109,25 ± 12,66	123,37 ± 4,27

**Table 2:** Pairwise Comparisons of Bursting Pressure

Bursting Pressure	N	Mean ± SD	Median	Min – Max	p
Group I-II	8	87,00 ± 4,75	87,50	80– 94	0,015
		95,37 ± 7,72	92	89 – 108	
Group I-III	8	87,00 ± 4,75	87,50	80 – 94	0,005
		98,25 ± 9,37	96	89 – 118	
Group I-IV	8	87,00 ± 4,75	87,50	80 – 94	0,002
		109,25 ± 12,66	115,50	89– 121	
Group I-V	8	87,00 ± 4,75	87,50	80 – 94	<0,001
		123,37 ± 4,27	123,50	118 – 129	
Group IV-V	8	109,25 ± 12,66	115,50	89– 121	0,003
		123,37 ± 4,27	123,50	118 – 129	
Group III-V	8	98,25 ± 9,37	96	89 – 118	<0,001
		123,37 ± 4,27	123,50	118 – 129	
Grup II-III	8	95,37 ± 7,72	92	89 – 108	0,505
		98,25 ± 9,37	96	89 – 118	

**Table 3:** Hydroxyproline Values (µg/mg)

Subject No	Group I	Group II	Group III	GroupIV	GroupV
1	0,537	0,570	0,488	0,486	0,570
2	0,488	0,551	0,528	0,532	0,567
3	0,530	0,574	0,553	0,537	0,570
4	0,495	0,463	0,586	0,563	0,584
5	0,528	0,514	0,532	0,532	0,572
6	0,504	0,579	0,598	0,563	0,635
7	0,481	0,535	0,565	0,546	0,577
8	0,532	0,532	0,549	0,556	0,598
Mean ± SD (µg/mg)	0,512 ±0,223	0,539 ± 0,025	0,549 ± 0,023	0,539 ± 0,025	0,584 ± 0,023

**Table 4:** Pairwise Comparisons of Hydroxyproline

Hydroxyproline	N	Mean ± SD	Median	Min - Max	p
Group I-II	8	0,512 ± 0,223	0,516	0,48 – 0,54	0,065
		0,539 ± 0,025	0,543	0,46 – 0,58	
Group I-III	8	0,512 ± 0,223	0,516	0,48 – 0,54	0,028
		0,549 ± 0,023	0,551	0,49 – 0,60	
Group I-IV	8	0,512 ± 0,223	0,516	0,48 – 0,54	0,021
		0,539 ± 0,025	0,542	0,49 – 0,56	
Group I-V	8	0,512 ± 0,223	0,516	0,48 – 0,54	<0,001
		0,539 ± 0,025	0,543	0,46 – 0,58	
Group II-III	8	0,549 ± 0,023	0,551	0,49 – 0,60	0,721
		0,539 ± 0,025	0,542	0,49 – 0,56	
Group IV-V	8	0,584 ± 0,023	0,575	0,57 – 0,64	<0,001
		0,549 ± 0,023	0,551	0,49 – 0,60	
Group III-V	8	0,584 ± 0,023	0,575	0,57 – 0,64	0,038
		0,549 ± 0,023	0,551	0,49 – 0,60	

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**Ethics Ethics Committee Approval:** The study was approved by the Experimental Medicine and Animal Laboratory of Zonguldak Karaelmas University Faculty of Medicine (Approval Date: January 28, 2011; Approval No: 2010-01-028/01).

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