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ALTERATIONS OF THE MUCOSA AND INTERSTITIAL CELLS OF CAJAL IN THE JEJUNUM OF RATS WITH AN EXPERIMENTAL MODEL OF TYPE 2 DIABETES MELLITUS

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Type 2 diabetes mellitus represents a global health problem that leads to progressive disturbances in the structure and function of the gastrointestinal tract, including alterations of the intestinal mucosa and interstitial cells of Cajal (ICC), which play a key role in regulating gastrointestinal neuromuscular activity. The aim of this study was to examine early histological and morphometric changes of the jejunum, as well as changes in the numerical areal density of ICC in an experimental model of type 2 diabetes mellitus induced by streptozotocin and nicotinamide (STZ+NA) in rats. Six weeks after diabetes induction, hematoxylin–eosin (HE) staining revealed preserved basic mucosal architecture in both groups, but with pronounced differences in morphometric parameters. The STZ+NA group demonstrated significant increases in villus height

and crypt depth, which were quantitatively confirmed by statistically significant differences compared with the control group ($p<0.001$). Immunohistochemical staining using the anti–c-Kit antibody (EP10) showed reduced ICC immunoreactivity, decreased network integrity, and a significantly lower numerical areal density of ICC in the diabetic group ($p<0.001$). These findings indicate that early stages of type 2 diabetes lead to parallel yet divergent remodelling of the epithelial and interstitial compartments of the jejunum, which may represent early histological markers of diabetic enteropathy and contribute to a better understanding of gastrointestinal dysfunction in diabetic patients.

Key words: diabetes mellitus, intestinal villi, diabetic gastroenteropathy, interstitial cells of Cajal, c-Kit

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PROMENE MUKOZE I BROJA INTERSTICIJALNIH ĆELIJA KAHALA U JEJUNUMU PACOVA SA EKSPERIMENTALNIM DIJABETESOM TIP 2

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Dijabetes melitus tipa 2 predstavlja globalni zdravstveni problem koji dovodi do progresivnih poremećaja u funkciji gastrointestinalnog trakta, uključujući promene intestinalne sluzokože i intersticijalnih ćelija Kahala (IĆK), koje imaju ključnu ulogu u regulaciji neuromišićne aktivnosti creva. Cilj ovog istraživanja bio je da se ispitaju rane histološke i morfometrijske promene jejunuma, kao i promena numeričke arealne gustine IĆK u eksperimentalnom modelu dijabetesa tipa 2 kod pacova. Nakon šest nedelja od indukcije dijabetesa, histološka analiza bojena HE metodom pokazala je očuvanu osnovnu arhitektoniku sluzokože u obe grupe, ali i izražene razlike u morfometrijskim parametrima. U dijabetesnoj grupi uočeno je varijabilno povećanje visine

intestinalnih resica i dubine Lieberkühnovih kripti, što je kvantitativno potvrđeno statistički značajnim razlikama u odnosu na kontrolnu grupu ($p<0,001$). Imunohistohemijsko bojenje korišćenjem anti-c-Kit antitela (EP10) ukazalo je na smanjenu imunoreaktivnost IĆK, redukovanu umreženost i značajno smanjenu numeričku arealnu gustinu ovih ćelija u dijabetesnoj grupi ($p<0,001$). Dobijeni rezultati ukazuju da rani stadijum dijabetesa tipa 2 dovodi do paralelnog, ali divergentnog remodelovanja epitelnog i intersticijalnog kompartmenta jejunuma, što može predstavljati rane histološke markere dijabetesne enteropatije i doprineti razumevanju poremećaja intestinalne funkcije kod dijabetesnih pacijenata.

Ključne reči: dijabetes melitus, crevne resice, dijabetesna gastroenteropatija, intersticijalne ćelije Kahala, c-Kit

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INTRODUCTION

Diabetes mellitus is no longer regarded solely as an endocrine disorder but is now recognized as a systemic disease that affects nearly all organ systems (1). Its complications involve vascular, neural, and visceral structures, making it one of the most complex challenges in modern medicine (2). While alterations in blood vessels, kidneys, and peripheral nerves have traditionally been the primary focus of research, the digestive tract was long regarded as a passive participant in diabetic disorders (3). However, contemporary studies indicate that the gastrointestinal system plays an active role in the development and progression of diabetic dysfunction (4). Enteropathic manifestations such as delayed gastric emptying, bloating, diarrhoea, and constipation constitute a major group of complications that markedly reduce patients' quality of life (5). However, the microscopic and histological changes that precede these clinical manifestations remain incompletely elucidated.

Chronic hyperglycemia initiates a cascade of pathophysiological processes that alter the microstructure of the intestinal wall (6). Disturbed microcirculation, oxidative stress, and dysregulated growth factors affect epithelial proliferation, differentiation, and regeneration, leading to progressive alterations in mucosal architecture (7). Changes in villus height and the depth of Lieberkühn crypts reflect an imbalance between the absorptive and proliferative compartments of the mucosa (8,9). Such morphometric deviations occur throughout the small intestine, but tend to be most pronounced in specific segments (10). Among these, the jejunum stands out due to its extensive absorptive surface area, rich microvasculature, and high epithelial turnover rate (11,12). Despite this, most studies have focused on the duodenum and ileum, whereas morphological and functional changes in the jejunum remain considerably less documented (12,13). This segment, however, is particularly sensitive to early microvascular and oxidative disturbances associated with diabetes, making it an excellent region for detecting initial, potentially reversible stages of mucosal remodelling (10). Morphometric evaluation of villus height and crypt depth, therefore, enables more accurate identification of early structural changes that precede permanent damage to the intestinal wall (8,9).

Although epithelial alterations are important indicators of early injury, a comprehensive assessment of diabetic enteropathy requires examination of the deeper layers of the intestinal wall as well. Beyond mucosal changes, diabetes also induces interstitial alterations within these layers, primarily affecting interstitial cells of Cajal (ICC)—a heterogeneous population of c-Kit–positive cells that plays a key role in the gastrointestinal neuromuscular network (14,15). Early in the course of diabetic gastroenteropathy, ICC undergo reductions in number and disruptions in organization, preceding complications such as enteric neuropathy and smooth muscle myopathy (16,17). These alterations impair smooth muscle contractility and contribute to gastrointestinal dysmotility, which represents a central pathophysiological mechanism of diabetic enteropathy (3,18,19). Evaluating mucosal and interstitial changes together therefore provides a more complete understanding of early structural alterations of the intestinal wall and the potential relationship between epithelial and neuromuscular disturbances in diabetes (20).

The aim of this study was to examine early mucosal changes—villus height and crypt depth—in rats with type 2 diabetes mellitus, as well as the density of interstitial cells of Cajal in the muscular layer of the jejunum, and to determine whether these changes reflect a shared early pattern of intestinal wall remodelling in type 2 diabetes.

MATERIAL AND METHODS

Experimental design

The study was conducted at the Biomedical Research Centre and the Department of Histology and Embryology of the Faculty of Medicine at the University of Niš. The experimental protocol was approved by the Faculty's Ethics Committee (No. 12-519/7), and all procedures were performed in accordance with the National Guidelines for the Welfare of Experimental Animals and the Faculty's internal regulations on laboratory animal care.

The study included 24 adult male Wistar rats, ten weeks old and weighing 230–250 g, which were randomly assigned to two groups (n = 12). The animals were housed under standard laboratory conditions (22 ± 2 °C, 12/12-h light–dark cycle) with *ad libitum* access to food and

water. In the diabetic group (STZ+NA), type 2 diabetes mellitus was induced using a combination of nicotinamide (110 mg/kg; Sigma-Aldrich, Germany), administered 15 minutes before streptozotocin (45 mg/kg; Sigma-Aldrich, Germany) dissolved in 0.1 M citrate buffer (pH 4.5). Streptozotocin was administered in the morning hours without prior fasting, according to validated STZ+NA protocols for inducing type 2 diabetes. The control group received an equivalent intraperitoneal injection of physiological saline.

Blood glucose levels were assessed 3–7 days after induction using a handheld glucometer (Accu-Chek Performa, Roche Diagnostics, USA). Diabetes was confirmed in animals with glucose levels above 8.3 mmol/L. Throughout the six-week experimental period, all rats were monitored for body weight, food and water intake, and overall condition. At the end of the study, the animals were deeply anaesthetized with ketamine (100 mg/kg) and euthanized by exsanguination followed by bilateral thoracotomy, in accordance with institutional guidelines for humane euthanasia.

Histological and morphometric analysis

Jejunal samples were rinsed with physiological saline immediately after euthanasia and fixed in 10% neutral-buffered formalin for 24 hours. After routine histological processing, the tissues were sectioned at 5 µm using a microtome (Leica RM2235, Germany). The sections were mounted onto poly-L-lysine–coated slides and incubated in a thermostat at 64 °C. Hematoxylin and eosin (H&E) staining was used for general histological evaluation. Microscopic assessment was performed using an Olympus BX50 microscope equipped with a Leica DFC295 camera (Leica Microsystems, France). Various magnifications were used to evaluate the overall architecture of the intestinal wall, the morphology of the mucosal and submucosal layers, and the integrity of the Lieberkühn crypts and intestinal villi. Morphometric analysis focused on villus height (VH) and crypt depth (CD). Measurements were performed on digital microphotographs captured at ×100 magnification, using ImageJ v1.53k (NIH, USA) after calibration with a micrometric scale. For each histological section, 10–15 representative villi and their corresponding Lieberkühn crypts were analyzed across five different microscopic fields per animal. Only centrally oriented, longitudinally sectioned, intact villi—with clearly defined contours, appropriate alignment along the longitudinal

axis, and a visible luminal tip were included, together with their structurally preserved corresponding crypts. Villus height was measured from the villus–crypt junction, where the villous epithelium transitions into the crypt epithelium, to the villus tip. Crypt depth was measured from the crypt opening to its base. All analyses were performed according to recommended guidelines for standardized histomorphological evaluation of the intestinal mucosa, ensuring reproducibility and comparability with previous experimental studies in rodents and humans (21).

Immunohistochemical analyses

Immunohistochemical detection of interstitial cells of Cajal (ICC) was performed using a primary rabbit monoclonal c-Kit antibody (clone EP10; Abcam, Cambridge, UK; Ab32363) at a 1:100 dilution, prepared with the EnVision™ FLEX Antibody Diluent DM830 (catalogue no. K8006; Dako, Denmark). After incubation with the secondary antibody EnVision™ FLEX SM802 (catalogue no. K8000; Dako, Denmark) for 45 minutes at room temperature, the immune complexes were visualized using the Dako REAL EnVision™ Detection System with DAB chromogen (catalogue no. K5007; Dako, Denmark). The sections were then counterstained with Mayer's hematoxylin, dehydrated through a graded ethanol series, cleared in xylene, and mounted in Canada balsam. Mast cells in the jejunum served as an internal positive control for c-Kit immunoreactivity and consistently demonstrated strong expression across all staining series. Negative controls were processed in parallel, omitting the primary antibody.

Quantification of interstitial cells of Cajal (ICC)

The total number of c-Kit–immunopositive ICC, without differentiation of individual subtypes, was quantified across the entire muscular layer of the jejunal wall, and the results were expressed as numerical areal density (NA; number of cells/mm^2). ICC were counted manually to avoid misidentification of mast cells, which also express c-Kit. Identification of ICC was based on their characteristic morphological features, such as elongated or spindle-shaped cell bodies, the presence of dendritic processes, or a network-like arrangement, depending on the subtype—and on their typical localization within the muscular layer. Particular attention was given to

distinguishing ICC from mast cells, which are smaller, round, and exhibit uniformly stained basophilic cytoplasm.

Statistical analyses

Data were presented as mean ± standard deviation (SD). Normality was assessed using skewness and kurtosis coefficients, whose values fell within the reference range (–2 to +2), indicating an approximately normal distribution. The Student's t-test was used for comparisons between groups, while the Mann–Whitney U test was used when normality was not met. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using SPSS Statistics v20 (IBM, Chicago, USA) and the Real Statistics Add-in for Microsoft Excel 2013.

RESULTS

The mean blood glucose level in the control group was 6.42 ± 0.58 mmol/L, whereas in the STZ+NA group it was significantly higher, reaching 12.09 ± 1.21 mmol/L. The difference between the groups was highly statistically significant ($p < 0.001$), confirming successful induction of diabetes in the STZ+NA model.

Morphological characteristics of the jejunum

Histological evaluation of H&E-stained jejunal sections showed that the overall architecture of the intestinal wall was preserved in both experimental groups, with clearly defined mucosal, submucosal, muscular, and serosal layers. In the control group, intestinal villi were well-oriented, slender, elongated, and uniform in height, with a clearly visible central lacteal and a well-organized epithelial lining composed of absorptive and goblet cells. Lieberkühn crypts were narrow, regularly arranged, and distinctly separated from the muscularis mucosae. In the diabetic group, more pronounced alterations of the mucosal layer were observed. Villi appeared irregularly arranged, variable in height, with partial epithelial shortening and expansion of the interstitium. A slight

increase in the number of goblet cells was noted, and Lieberkühn crypts were wider, deeper, and more densely distributed. No differences were observed in the overall thickness of the intestinal wall or the muscular layer between the two groups, and the structure of the submucosa and serosa remained preserved (Figure 1).

Morphometric analyses

Morphometric analysis showed that diabetic rats exhibited marked changes in both evaluated parameters. The mean villus height (VH) was 442.26 ± 53.81 µm (n = 12) in the diabetic group and 382.33 ± 51.81 µm (n = 12) in the control group (Figure 2). Student's t-test confirmed a highly significant difference between the groups ($p < 0.001$). The mean depth of the Lieberkühn crypts (CD) was 132.74 ± 21.51 µm in the diabetic group and 109.32 ± 12.27 µm in the control group. These results indicate that the crypt zone, which represents the proliferative portion of the mucosa, was significantly deeper in diabetic rats ($p < 0.001$) (Figures 1 and 2).

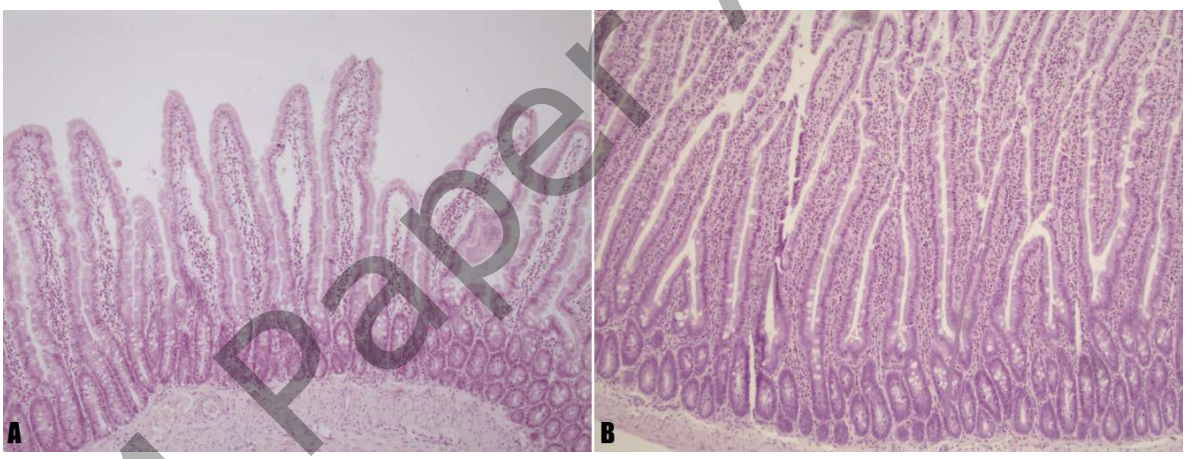

Figure 1. Hematoxylin–eosin (H&E) staining of rat jejunum in the control (A) and STZ+NA (B) groups (×100).

A) Control group with preserved mucosal architecture, tall and slender intestinal villi, and well-formed Lieberkühn crypts. B) STZ+NA group showing irregularly shaped villi with variability in height and enlarged Lieberkühn crypts, accompanied by a slight increase in goblet cell number compared to controls.

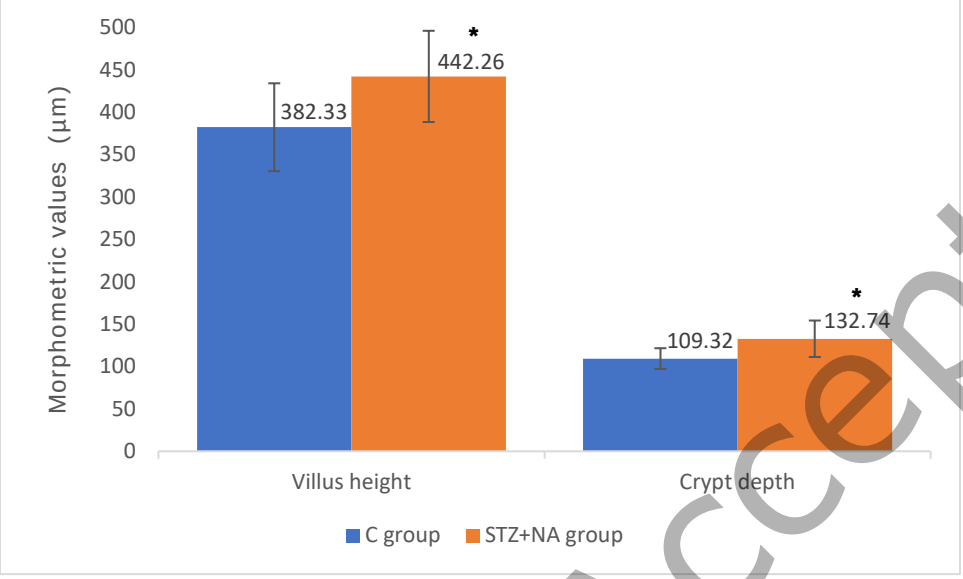

Figure 2. Mean villus height and crypt depth ($\bar{x}$ ± SD) in the control (C) and STZ+NA groups. The asterisk (*) indicates a statistically significant difference compared with the control group ($p < 0.001$).

Immunohistochemical analyses of interstitial cells of Cajal (ICC)

Immunohistochemical staining for c-Kit (CD117) demonstrated the presence of immunopositive ICC within the muscular layer of the jejunal wall in both groups, distributed between smooth muscle cells and around the submucosal and myenteric plexuses. In the control group, ICC were clearly identifiable, spindle-shaped, and characterized by long cytoplasmic processes forming a dense network along the muscle fibers (Figure 3A, B). In the diabetic group, ICC were fewer in number, more sparsely distributed, showed reduced immunoreactivity, and exhibited partially shortened processes, indicating decreased functional activity (Figure 3C, D). The numerical areal density of ICC was 87.80 ± 36.13/mm² (n = 12) in the diabetic group and 119.47 ± 37.44/mm² (n = 12) in the control group. Due to non-normal data distribution, intergroup differences were assessed using the Mann–Whitney U test, which confirmed high

statistical significance (U = 3825.5; z = 6.13; p < 0.001). The median ICC count was 91.38 in the diabetic group and 113.7 in the control group, demonstrating a significant reduction in ICC number in diabetes (p < 0.001) (Table 1). This represents an approximate 27% decrease in numerical areal density.

Table 1. Numerical areal density (N_A) of interstitial cells of Cajal (ICC; cells/mm^2) in the jejunal muscular layer of control (C) and STZ+NA groups

Group	**N**	**N_A ($\bar{x}$ ± SD), cells/mm^2**
Control	12	119.47+37.44
STZ+NA	12	87.80+36.13
Statistic	_	U = 3825.5; z = 6.13; p < 0.001

N_A – numerical areal density, N – number of animals, $\bar{x}$ – mean, SD – standard deviation, U –Mann–Whitney test, z – standardized test statistic, p – significance level

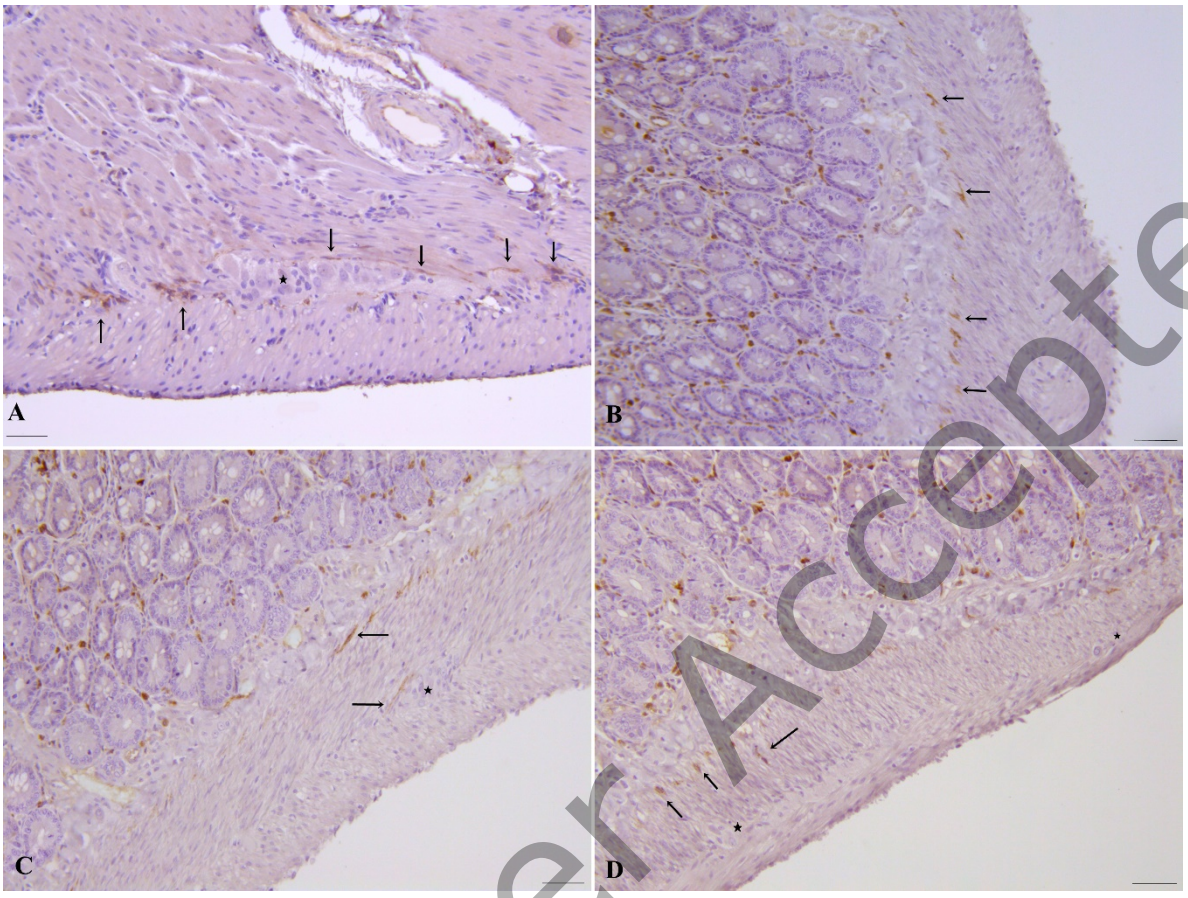

Figure 3. Immunohistochemical staining of c-Kit–positive interstitial cells of Cajal (ICC) in the muscular layer of the rat jejunal wall in the control (A,B) and STZ+NA (C,D) groups (×200). ICC (arrow) are most prominent along the submucosal and myenteric plexus (asterix). In the control group, a continuous, dense ICC network is observed, whereas the diabetic group shows decreased density and discontinuity in ICC distribution, with areas of marked cellular depletion. Scale bar = 50 μm.

DISCUSSION

The results of this study show that, in the early phase of experimentally induced type 2 diabetes mellitus, the jejunum undergoes characteristic remodeling involving both the mucosal and interstitial layers of the intestinal wall. In this model, we observed elongation of intestinal villi, deepening of the Lieberkühn crypts, and a significant reduction in the numerical areal density of c-Kit–positive interstitial cells of Cajal (ICC) within the muscular layer. This combination of adaptive epithelial and degenerative interstitial changes indicates that the jejunum is a highly responsive segment of the small intestine to metabolic stress in early diabetes and aligns with the contemporary concept of diabetic enteropathy as a pan-alimentary complication (1,4,5,22). It should be noted that the present study did not aim to establish a direct correlation between epithelial remodeling and ICC reduction, but rather to document their concurrent appearance in the early diabetic setting.

In our model, a statistically significant elongation of villi and an increase in the depth of the Lieberkühn crypts were observed, while the overall architecture of the jejunal wall remained preserved. A similar pattern of early mucosal remodeling has been described in several experimental studies, particularly in STZ-induced diabetes, where increased villus height, deepened crypts, and alterations in villous microvascular architecture have been reported (8–10). Studies in Goto–Kakizaki rats further indicate that type 2 diabetes leads to segment-specific remodeling of the small intestine, accompanied by marked structural and functional changes within the mucosa (13,23). Our findings are consistent with these observations and confirm that early type 2 diabetes does not result in mucosal atrophy but rather in a hyperplastic epithelial response in the jejunum. The increase in crypt depth, together with villus elongation, suggests enhanced proliferative activity within the intestinal stem cell zone, as previously demonstrated in studies quantifying epithelial cell number and proliferative markers along the crypt–villus axis (9,21). More recent work in animal models and human enteroids shows that a hyperglycemic environment can directly compromise epithelial barrier function, alter the balance between proliferation and apoptosis, and disrupt the structural and functional stability of tight junctions (24–26). In this context, villus elongation and crypt deepening in our model may represent a morphological

manifestation of an attempt to maintain absorptive capacity under conditions of early epithelial functional instability.

It is important to note that recent advances in three-dimensional analysis of the intestinal mucosa and enteroendocrine cells have demonstrated that jejunal segments are among the most dynamic regions in terms of remodeling under metabolic disturbances (11,12,27). These findings support selecting the jejunum as the target segment in our study and indicate that changes in villus height and crypt depth should not be viewed in isolation but rather within the broader context of the intestinal mucosa's global adaptive responses to hyperglycemic stress.

The reduction in ICC number in the jejunum's muscular layer is a key interstitial finding of this study. The role of ICC as pacemaker cells and as mediators of signal transmission between enteric neurons and smooth muscle cells has been thoroughly documented in foundational studies (14,28,29). Loss of ICC, or disruption of their network, has been identified as a central morphological substrate underlying various gastrointestinal motility disorders (15,30). In diabetes, multiple studies have shown that ICC are highly sensitive to disturbances in insulin signaling, microcirculation, and oxidative stress. In animal models of diabetes mellitus, reduced ICC density, alterations in their morphological arrangement, and impaired functional connectivity with enteric neurons have been reported (16,20).

Our results, which show an approximately 27% reduction in ICC numerical density in the early stage of diabetes, are quantitatively consistent with the range of values reported in these models, although appropriate caution is required in interpretation due to differences in diabetes type, disease duration, and the intestinal segments examined. Particularly significant are studies investigating the potential reversibility of ICC alterations. Antioxidant interventions, such as quercetin supplementation, have been shown to partially restore ICC density and improve jejunal neuromuscular function in diabetic rats (31,32). A similar protective effect on motility and the neuromuscular apparatus has also been described following certain nutritional interventions and bioactive compounds (33,34). These findings support the concept that early ICC loss may represent a potentially reversible phase of diabetic injury, which is especially relevant for interpreting our results in the six-week model.

Furthermore, recent studies indicate that ICC should not be viewed in isolation but rather in interaction with other interstitial populations, particularly PDGFRα-positive cells and enteric neurons (17,29,35). This concept of an “interstitial unit” suggests that the reduction in ICC observed in our model may reflect earlier disturbances within the broader neuromuscular network, although we did not directly quantify these alterations.

A key contribution of this study is the simultaneous evaluation of mucosal morphometric parameters and ICC numerical density within the same jejunal segment, in the same early stage of experimental type 2 diabetes. To our knowledge, no studies within a single experimental model—focused on the same intestinal segment and a clearly defined early stage of type 2 diabetes—have simultaneously quantified and interpreted changes in villus height, Lieberkühn crypt depth, and ICC numerical density in the jejunum. Existing studies tend to focus either on mucosal remodeling or on the neuromuscular component, but not on their integrated assessment within a unified pattern of early intestinal remodeling. Our findings, therefore, fill an important gap in the literature and suggest that early diabetic processes in the jejunum may be regarded as parallel streams of adaptive epithelial changes and degenerative alterations within the interstitial neuromuscular network.

Diabetic enteropathy is increasingly recognized as a systemic disorder that affects motility, secretion, absorption, and the integrity of the intestinal barrier, with concurrent involvement of both the autonomic and enteric nervous systems (22,36). Our findings align with this concept, showing that early diabetes is characterized by concurrent adaptive epithelial hyperplasia (villus elongation and crypt deepening) and initial ICC degeneration. This combined pattern provides a morphological basis for later motility and absorptive disturbances, even though clinical symptoms are not yet evident at this stage.

An increasing body of research highlights the importance of the gut microbiota and its interactions with the enteric nervous system and ICC in the pathogenesis of diabetic gastroenteropathy (37–40). Alterations in microbial composition, elevated pro-inflammatory signaling, and increased intestinal permeability may further exacerbate oxidative stress and contribute to progressive dysfunction of the neuromuscular network (25,35). Although we did not

directly assess the microbiota in this study, the morphological pattern observed—mucosal hyperplasia alongside ICC loss—fits within a multifactorial model in which metabolic, inflammatory, and neural factors intersect.

At the translational level, our findings support the view that early recognition of diabetic enteropathy requires expanding diagnostic focus beyond clinical symptoms and functional testing to include early histological biomarkers across different gastrointestinal segments. An integrated evaluation of mucosal morphometric parameters and the interstitial neuromuscular component may, in the future, provide a basis for combined therapeutic strategies aimed at preserving intestinal barrier integrity, reducing oxidative stress, and maintaining ICC and related interstitial cell populations (31,33,34,41).

In summary, experimentally induced diabetes mellitus resulted in pronounced morphological and histoarchitectural alterations in the jejunal wall. These changes were characterized by increased villus height, deepened Lieberkühn crypts, and a marked reduction in the number of interstitial cells of Cajal in the muscular layer. This pattern suggests the presence of early functional disturbances in enteric neuromuscular regulation and likely reflects the contribution of diabetic neuropathy to the remodeling of the intestinal wall.

Limitations of the study

This study has several limitations that should be acknowledged. First, the six-week observation period after diabetes induction is relatively short and does not allow evaluation of later, chronic structural changes in the intestinal wall. Second, the study was conducted exclusively on male Wistar rats, and the findings may not fully reflect possible sex-related differences in mucosal or interstitial responses. Additionally, ICC quantification was performed collectively across the entire muscular layer without sublayer analysis, which may obscure subtle regional differences in the degree of injury or functional load. A more refined, layer-specific evaluation would provide deeper insight into the neuromuscular network's sensitivity. The analysis focused on morphological and immunohistochemical parameters without direct functional assessment of motility, making conclusions regarding neuromuscular consequences indirect. Measurements were not fully blinded,

introducing a minor degree of observer bias, although without a meaningful impact on the consistency of the results. Moreover, oxidative stress markers and inflammatory parameters were not evaluated; incorporating these in future studies would allow more comprehensive integration of morphological and biochemical findings. Despite these limitations, the results provide reliable and consistent indicators of early mucosal and interstitial remodeling and may serve as a foundation for future longitudinal and functional investigations.

CONCLUSION

In the early stage of experimentally induced type 2 diabetes mellitus, the jejunal wall undergoes parallel but divergent alterations. Villus elongation and crypt deepening represent an adaptive epithelial response to impaired glycemic homeostasis, whereas the reduction in c-Kit–positive interstitial cells of Cajal reflects initial degeneration of the interstitial neuromuscular network. The combined morphometric and immunohistochemical approach proved to be a reliable method for early detection of these changes and provides deeper insight into the initial mechanisms of diabetic enteropathy. These findings highlight the importance of early recognition of histological indicators of injury and may contribute to the development of preventive and therapeutic strategies aimed at preserving the integrity of the intestinal wall in diabetes.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding this study or the publication of its results.

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