UNIVERSITY OF BANJA LUKA
FACULTY: Faculty of Medicine

Form 3

REPORT
on the Evaluation of the Completed Doctoral Dissertation / Doctoral Art Project[1]

1. DATA ON THE COMMITTEE

Body that appointed the Committee: Scientific-Teaching Council of the Faculty of Medicine and the Senate of the University of Banja Luka.

Date of appointment of the Committee: 12.5.2026.

Decision number: 18/3.349/26

Members of the Committee:

1.	Đurić dr Dragan	MD, PhD, Full Professor	
	Surname and name	Academic title	
	Physiology		
	Scientific field and a narrower scientific/artistic field		
	Faculty of Medicine, University of Belgrade	Chair of the Defense Committee	
	Institution of employment	Function within the Committee	
2.	Golić dr Darko	MD, PhD, Full Professor	
	Surname and name	Academic title	
	Pathophysiology		
	Scientific field and a narrower scientific/artistic field		
	Faculty of Medicine, University of Banja Luka	Member of the Defense Committee	
	Institution of employment	Function within the Committee	
3.	Milivojac dr Tatjana	MD, PhD, Assistant Professor	
	Surname and name	Academic title	
	Pathophysiology		
	Scientific field and a narrower scientific/artistic field		
	Faculty of Medicine, University of Banja Luka	Member of the Defense Committee	
	Institution of employment	Function within the Committee	

[1] Hereinafter: „dissertation / artistic work".

2. DATA ON THE STUDENT

First name, parent's name, last name: Uglješa, Sretko, Maličević

Date of birth: 10.11.1994.

Place and country of birth: Banja Luka, Bosnia and Herzegovina

2.1. First-cycle studies / undergraduate studies / integrated studies

Year of enrolment:	2013.	Year of completion:	2021.	Average mark during studies:	9.21

University: University of Banja Luka

Faculty/Academy: Faculty of Medicine

Study program: Medicine

Degree obtained: Doctor of Medicine (MD)

2.2. Second-cycle studies / Master's studies

Year of enrolment:		Year of completion:		Average mark during studies:	

University:

Faculty/Academy:

Study program:

Title of Master's thesis / final thesis and date of defense:

Narrower scientific/artistic field of the Master's thesis:

Degree obtained:

2.3. Third-cycle studies

Year of enrolment:	2022.	Number of ECTS credits earned to date:	120	Average mark during studies:	9.35

Faculty/Academy: Faculty of Medicine

Study program: Biomedical Sciences

2.4. Overview of the student's scientific and professional or artistic works[2]

List individual publications, including DOI numbers, or concerts / recorded works. Add rows as necessary. Use the same citation style for all references in section 2.4.

No.	Basic information on the scientific paper	Citation database

[2] In line with Article 34 of the Rules of studying at the 3rd cycle as of September 2022.

1.	**Maličević U**, Rai V, Skrbic R, Agrawal DK. Hyperglycemia impairs the expression of inflammatory mediators in rat intestine: an implication for intestinal inflammation and inflammatory bowel disease. Mol Cell Biochem. 2026 Mar;481(3):1325-1337. doi: 10.1007/s11010-025-05474-x. Epub 2026 Jan 9. PMID: 41511724; PMCID: PMC12995948.	Web of Science Core Collection
2.	**Malicevic U**, Rai V, Skrbic R, Agrawal DK. Intricate interplay between ORMDL3, ER stress, and autophagy in the diabetic intestine. Mol Cell Biochem. 2026 Feb;481(2):791-807. doi: 10.1007/s11010-025-05424-7. Epub 2025 Nov 3. PMID: 41182648; PMCID: PMC12963212.	Web of Science Core Collection
3.	Matković Z, Gajić Bojić M, **Maličević U**, Krivokuća A, Mandić-Kovačević N, Uletilović S, et al. Levosimendan Pretreatment Attenuates Mesenteric Artery Ischemia/Reperfusion Injury and Multi-Organ Damage in Rats. Int J Mol Sci. 2025 Sep 18;26(18):9131. doi: 10.3390/ijms26189131. PMID: 41009694; PMCID: PMC12470194.	Web of Science Core Collection
4.	**Ugljesa Malicevic**, Smith J, Agrawal DK, Rai V. Sex-based differences in streptozotocin-induced type 2 diabetes rat models. Journal of Clinical and Translational Research. 2025 Oct 28;0(0):025170020–0. Available from: https://www.accscience.com/journal/JCTR/articles/online_first/5767	Other Sources
5.	Milivojac T, Grabež M, Amidžić Lj, Prtina A, Krivokuća A, **Malicevic U**, et al. Ursodeoxycholic and chenodeoxycholic bile acids alleviate endotoxininduced acute lung injury in rats by modulating aquaporin expression and pathways associated with apoptosis and inflammation. Front Pharmacol. 2025 Mar 6;16:1484292. doi: 10.3389/fphar.2025.1484292. PMID: 40115259; PMCID: PMC11922783.	Web of Science Core Collection
6.	Bajic Z, Sobot T, Uletilovic S, Mandić-Kovačević N, **Malicevic U**, Skrbic R, et al. Liraglutide Treatment Restores Cardiac Function After Isoprenaline-Induced Myocardial Injury and Prevents Heart Failure in Rats. Life (Basel). 2025 Mar 12;15(3):443. doi: 10.3390/life15030443. PMID: 40141787; PMCID: PMC11943469.	Web of Science Core Collection
7.	Matković Z, **Maličević U**, Gajić-Bojić M, Krivokuća A, Đukanović Đ, Đekić-Matković N, et al. Clinical aspects of acute mesenteric ischaemia. Scripta Medica.2024;55(5):623–35. Available from: https://scindeks.ceon.rs/Article.aspx?artid=2490-33292405617M	SCOPUS
8.	**Malicevic U**, Rai V, Skrbic R, Agrawal DK. Modulation of Orosomucoid-like Protein 3 Activity in the Management of Inflammatory Bowel Disease. J Biotechnol Biomed. 2024;7(4):433-444. doi: 10.26502/jbb.2642-91280167. Epub 2024 Oct 18. PMID: 39619146; PMCID: PMC11606571.	Other Sources
9.	**Malicevic U**, Rai V, Skrbic R, Agrawal DK. NLRP3 Inflammasome and Gut Dysbiosis Linking Diabetes Mellitus and Inflammatory Bowel Disease. Arch Intern Med Res. 2024;7(3):200-218. doi: 10.26502/aimr.0178. Epub 2024 Aug 31. PMID: 39328924; PMCID: PMC11426418.	Other Sources
10.	Milivojac T, Grabež M, Krivokuća A, **Maličević U**, Gajić Bojić M, Škrbić R, et al. Ursodeoxycholic and chenodeoxycholic bile acids attenuate systemic and liver inflammation induced by lipopolysaccharide in rats. Mol Cell Biochem. 2025 Jan;480(1):563-576. doi: 10.1007/s11010-024-04994-2. Epub 2024 Apr 5. PMID: 38578526; PMCID: PMC11695453.	Web of Science Core Collection
11.	Sobot T, Bajic Z, Skrbic R, Uletilovic S, Mandic-Kovacevic N, **Malicevic U**, et al. Effect of folic acid on isoprenaline-induced myocardial injury in rats. Physiol Int. 2024 Jan 22;111(1):80-96. doi: 10.1556/2060.2023.00291. PMID: 38261080.	Web of Science Core Collection

12.	Marinković ST, Đukanović Đ, Duran M, Bajic Z, Sobot T, **Maličević U**, et al. Pomegranate Peel Extract Attenuates Isoprenaline-Induced Takotsubo-like Myocardial Injury in Rats. Pharmaceutics. 2023 Jun 9;15(6):1697. doi: 10.3390/pharmaceutics15061697. PMID: 37376144; PMCID: PMC10302705.	Web of Science Core Collection
13.	Mandić-Kovačević N, Kukrić Z, Latinović S, Cvjetković T, **Maličević U**, Uletilović S, et al. Antioxidative potential of pomegranate peel extract: In vitro and in vivo studies. Scripta Medica. 2023 April 7;54(1), 9-18.	SCOPUS
14.	Bajic Z, Sobot T, Uletilovic S, Mandic-Kovacevic N, **Malicevic U**, Skrbić R, et al. Cardioprotective effects of liraglutide pretreatment on isoprenaline-induced myocardial injury in rats. Can J Physiol Pharmacol. 2023 May 1;101(5):258-267. doi: 10.1139/cjpp-2022-0534. Epub 2023 Feb 27. PMID: 36848640.	Web of Science Core Collection
Does the paper belong to the narrower scientific/artistic field of the doctoral dissertation research topic?		☐ YES ☐ NO

3. INTRODUCTORY PART OF THE EVALUATION OF THE DISSERTATION / ART PROJECT

Title of the Doctoral Dissertation: "The Association of *ORMDL3* Expression with Hyperglycemia in Intestinal Tissue: A Potential Role in the Pathogenesis of Inflammatory Bowel Disease (IBD)"

Scientific Field: B500 - Medical and Health Sciences
Specific Scientific Area: B510 - Basic Medicine - Pathophysiology
Date of Acceptance of the Doctoral Dissertation Topic and Decision Numbers of the Relevant Bodies: The Scientific-Teaching Council of the Faculty of Medicine, University of Banja Luka, at the session held on 12 May 2025, adopted the Decision on the Appointment of the Commission for the Assessment of Student Eligibility, Topic (Title), and Mentor, under Decision No. 18/3.361/25.
The Scientific-Teaching Council of the Faculty of Medicine, University of Banja Luka, accepted the Report of the Commission at the session held on 17 June 2025, under Decision No. 18/3.447/25.
The Senate of the University of Banja Luka, at the session held on 3 July 2025, gave its approval for the acceptance of the Report of the Commission of the Faculty of Medicine, University of Banja Luka, under Decision No. 02/04-3.1416-58/25.

Dissertation Format: The dissertation is written in English, in Latin script, Times New Roman font, size 12 pt, with 1.5 line spacing, comprising 101 pages in A4 format. The introductory section contains 11 unnumbered pages, consisting of the title page in English and Serbian, mentor information, abstracts in English and Serbian, acknowledgements, and a table of contents.

The content is organized into 8 chapters:

1. Introduction - 21 pages
2. Research Hypotheses - 2 pages
3. Research Aims - 1 page
4. Materials and methods - 8 pages
5. Results - 33 pages
6. Discussion - 17 pages
7. Conclusions - 1 page
8. References - 18 pages

At the end of the dissertation, comprising 8 unnumbered pages, the following are included:

- List of Abbreviations
- Biography of the Candidate

- Signed Statement of Authorship
- Signed Statement Authorizing the University of Banja Luka to Make the Doctoral Dissertation Publicly Available
- Signed Statement on the Authenticity of the Printed and Electronic Versions of the Doctoral Dissertation
- Addendum

The Addendum at the end of the dissertation contains scientific papers in this field in which the candidate is the first author.

The doctoral dissertation contains 5 tables and 31 figures, with 231 references cited.

1. **Title of the dissertation / art project**
2. **Scientific field and narrower scientific/artistic field**
3. **Date of approval of the dissertation / art project topic and decision numbers of the competent bodies of the faculty and the University**
4. **Date of approval of the report of the Committee for the evaluation of the eligibility of the candidate, topic, and mentor for the preparation of the dissertation / art project, and decision numbers of the competent bodies of the faculty and the University**
5. **Structure of the dissertation / art project, including number of pages**
6. **Provide key information on the dissertation / art project, including scope, number and titles of chapters, number of tables, figures, diagrams, graphs, and references.**

4. INTRODUCTION AND LITERATURE REVIEW

The doctoral dissertation entitled "The Association of *ORMDL3* Expression with Hyperglycemia in Intestinal Tissue: A Potential Role in the Pathogenesis of Inflammatory Bowel Disease (IBD)" addresses an important and currently underexplored intersection between metabolic disorders and chronic intestinal inflammation. The study was undertaken in response to increasing evidence suggesting mechanistic overlap between diabetes mellitus and inflammatory bowel disease, particularly through pathways involving endoplasmic reticulum (ER) stress, autophagy dysregulation, and innate immune activation.
The main problem addressed in this dissertation is the lack of mechanistic understanding of how chronic hyperglycemia contributes to intestinal dysfunction and whether ORMDL3 may act as a molecular link between metabolic stress and intestinal inflammatory processes. The subject of the research is the role of ORMDL3 and associated ER stress, autophagy, and inflammatory pathways in the development of intestinal alterations under hyperglycemic conditions.
The introduction systematically addresses the epidemiology and pathophysiology of both diabetes mellitus and IBD, exploring their shared mechanisms including TLR signaling, NLRP3 inflammasome activation, gut dysbiosis, and bile acid dysregulation. A dedicated section introduces ORMDL3, its biological function as an ER-resident protein, and its emerging relevance as a potential molecular link between these two conditions.

Diabetes mellitus (DM) represents one of the most significant global public health challenges of the 21st century, characterized by persistent hyperglycemia and progressive multisystem complications [1,2]. Beyond its classical metabolic manifestations, accumulating evidence indicates that chronic hyperglycemia is associated with sustained low-grade inflammation, driven by key pro-inflammatory mediators including TNF-α, IL-6, and IL-1β [3,4]. Notably, these same cytokines are centrally implicated in the pathogenesis of inflammatory bowel disease (IBD), a chronic immune-mediated condition encompassing Crohn's disease and ulcerative colitis [5,6]. The epidemiological and molecular convergence of these two conditions has prompted growing scientific interest in their shared pathogenic mechanisms [7-9].

The relationship between DM and IBD extends beyond clinical co-occurrence, reflecting a deeper convergence of genetic, immunological, and metabolic pathways. Approximately ten susceptibility loci

have been associated with both conditions, including ORMDL3, suggesting a shared genetic architecture [10]. At the molecular level, innate immune signaling through Toll-like receptors (TLRs), NLRP3 inflammasome activation, gut microbiota dysbiosis, and bile acid dysregulation represent key interconnected mechanisms that are dysregulated in both diseases [11-16]. Hyperglycemia has been shown to directly compromise intestinal barrier integrity, facilitating microbial translocation and further amplifying systemic and mucosal inflammation [17].

Within this framework, endoplasmic reticulum (ER) stress has emerged as a critical mechanistic link between metabolic dysfunction and intestinal inflammation. The unfolded protein response (UPR), activated under conditions of cellular stress, engages multiple signaling branches, including ATF6, IRE1, and PERK, that intersect with inflammatory and autophagic pathways [18-21]. Dysregulation of autophagy, a fundamental cellular homeostatic mechanism, has been independently implicated in both DM and IBD pathogenesis, with key regulators including NOD2, ULK1, and ATG4 shown to modulate intestinal immune responses and epithelial integrity [22,23].

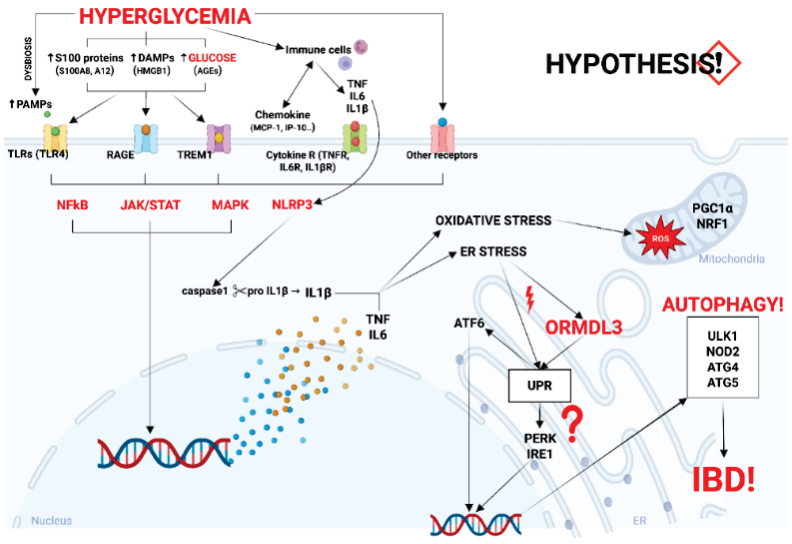
ORMDL3, an ER-resident transmembrane protein, has emerged as a particularly relevant molecular candidate in this context. Originally identified as a susceptibility gene for childhood-onset asthma, ORMDL3 regulates ER calcium homeostasis through modulation of the SERCA pump, influences sphingolipid biosynthesis, and activates UPR signaling [24-27]. Recent transcriptomic studies have reported altered ORMDL3 expression in both T1DM and IBD, suggesting its involvement in shared pathogenic pathways [22,28,29]. However, existing data remain largely associative, and functional experimental evidence directly linking ORMDL3 to hyperglycemia-induced intestinal dysfunction is limited. The precise role of ORMDL3 in modulating ER stress, autophagy, and inflammatory signaling within the intestinal microenvironment under diabetic conditions has not been systematically investigated [18,20,24,30].

This dissertation addresses this critical gap in knowledge by providing integrated experimental evidence at the gene, protein, and tissue levels across two complementary animal models, Sprague Dawley rats and Yucatan mini pigs, thereby offering a comprehensive mechanistic framework for understanding the role of ORMDL3 in the diabetic intestine and its potential relevance to IBD pathogenesis.

HYPOTHESIS

The working hypothesis is clearly formulated and supported by a schematic representation of the proposed pathogenic mechanisms linking hyperglycemia to intestinal inflammation.

"Hyperglycemia increases the expression of ORMDL3, ATF6, and autophagy-related molecules in intestinal tissue. This could potentially contribute to molecular mechanisms relevant to the pathogenesis of inflammatory bowel disease (IBD)."



AIMS OF THE STUDY

The aims of the study are equally well-defined, encompassing three specific objectives: histopathological characterization of intestinal tissues for IBD-like changes under hyperglycemic conditions, investigation of ORMDL3 and ATF6 expression in the context of ER stress and UPR activation, and evaluation of key autophagy-related genes: NOD2, ULK1, and ATG4, and their relationship with ORMDL3 and ATF6 pathway activation.

1. To characterize intestinal tissues from nondiabetic and diabetic animal models for histopathological changes resembling those observed in inflammatory bowel disease (IBD), with a focus on inflammation, mucosal damage, and immune cell infiltration. This objective involves a detailed histopathological analysis of intestinal tissues from both diabetic and nondiabetic animal models, aiming to identify key structural and cellular alterations that may resemble those seen in IBD. By assessing features such as inflammatory cell infiltration, mucosal damage, and epithelial disruption, we intend to determine whether hyperglycemia could exacerbate or trigger IBD-like pathology in the intestines.

2. To investigate the effect of hyperglycemia on the expression of ORMDL3 and ATF6, key regulators of the unfolded protein response (UPR) and autophagy pathways, in the context of ER stress and cellular stress. Hyperglycemia, a hallmark of diabetes, can induce ER stress, disrupting cellular homeostasis. This study will examine whether hyperglycemia affects the expression of ORMDL3 and ATF6, crucial regulators of the UPR and autophagy pathways. By focusing on intestinal tissues, we aim to understand the molecular mechanisms that may link hyperglycemia-induced stress to the pathogenesis of diseases such as inflammatory bowel disease (IBD).

3. To evaluate the expression levels of key autophagy-related genes (NOD2, ULK1, ATG4) in nondiabetic and diabetic conditions and explore their relationship with ORMDL3 expression and ATF6 pathway activation. Expression levels of key autophagy-related genes (NOD2, ULK1, ATG4) will be evaluated in both nondiabetic and diabetic conditions to understand how hyperglycemia may influence autophagy pathways. The relationship between the expression of these genes and the activation of ORMDL3 and ATF6 will also be explored. This analysis aims to shed light on the interplay between autophagy, ER stress, and immune response, and their potential role in the development of inflammatory bowel disease (IBD) under diabetic conditions.

REFERENCES

1. Upamali S, Rathnayake S. Perspectives of older people with uncontrolled type 2 diabetes mellitus towards medication adherence: A qualitative study. PLoS One. 2023;18(8).
2. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, et al. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. Diabetes Res Clin Pract. 2022;183.
3. Donath MY, Shoelson SE. Type 2 diabetes as an inflammatory disease. Nat Rev Immunol. 2011;11(2):98–107.
4. Tsalamandris S, Antonopoulos AS, Oikonomou E, Papamikroulis GA, Vogiatzi G, Papaioannou S, et al. The Role of Inflammation in Diabetes: Current Concepts and Future Perspectives. Eur Cardiol. 2019;14(1):50–9.
5. Maloy KJ, Powrie F. Intestinal homeostasis and its breakdown in inflammatory bowel disease. Nature. 2011;474(7351):298–306.
6. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. Nature. 2007;448(7152):427–34.
7. Hyun CK. Molecular and Pathophysiological Links between Metabolic Disorders and Inflammatory Bowel Diseases. Int J Mol Sci. 2021;22(17).
8. Din H, Anderson AJ, Rivers CR, Proksell S, Koutroumpakis F, Salim T, et al. Disease Characteristics and Severity in Patients With Inflammatory Bowel Disease With Coexistent Diabetes Mellitus. Inflamm Bowel Dis. 2020;26(9):1436–42.

9. Jess T, Jensen BW, Andersson M, Villumsen M, Allin KH. Inflammatory Bowel Diseases Increase Risk of Type 2 Diabetes in a Nationwide Cohort Study. Clin Gastroenterol Hepatol. 2020;18(4):881–888.e1.
10. Imielinski M, Baldassano RN, Griffiths A, Russell RK, Annese V, Dubinsky M, et al. Common variants at five new loci associated with early-onset inflammatory bowel disease. Nat Genet. 2009;41(12):1335–40.
11. Esmaealzadeh N, Ram M, Abdolghaffari A, Marques AM, Bahramsoltani R. Toll-like receptors in inflammatory bowel disease: A review of the role of phytochemicals. Phytomedicine. 2024;123.
12. Kordjazy N, Haj-Mirzaian A, Haj-Mirzaian A, Rohani MM, Gelfand EW, Rezaei N, et al. Role of toll-like receptors in inflammatory bowel disease. Pharmacol Res. 2018;129:204–15.
13. Sun X, Pang H, Li J, Luo S, Huang G, Li X, et al. The NLRP3 Inflammasome and Its Role in T1DM. Front Immunol. 2020;11.
14. Huang Y, Xu W, Zhou R. NLRP3 inflammasome activation and cell death. Cell Mol Immunol. 2021;18(9):2114–27.
15. Nishida A, Inoue R, Inatomi O, Bamba S, Naito Y, Andoh A. Gut microbiota in the pathogenesis of inflammatory bowel disease. Clin J Gastroenterol. 2018;11(1).
16. Crudele L, Gadaleta RM, Cariello M, Moschetta A. Gut microbiota in the pathogenesis and therapeutic approaches of diabetes. EBioMedicine. 2023;97.
17. Thaiss CA, Levy M, Grosheva I, Zheng D, Soffer E, Blacher E, et al. Hyperglycemia drives intestinal barrier dysfunction and risk for enteric infection. Science. 2018;359(6382):1376–83.
18. Pathinayake PS, Hsu ACY, Waters DW, Hansbro PM, Wood LG, Wark PAB. Understanding the unfolded protein response in the pathogenesis of asthma. Front Immunol. 2018;9.
19. Zhang K, Kaufman RJ. From endoplasmic-reticulum stress to the inflammatory response. Nature. 2008;454(7203):455–62.
20. Wang M, Kaufman RJ. Protein misfolding in the endoplasmic reticulum as a conduit to human disease. Nature. 2016;529(7586):326–35.
21. Chipurupalli S, Samavedam U, Robinson N. Crosstalk Between ER Stress, Autophagy and Inflammation. Front Med. 2021;8.
22. Li J, Ullah MA, Jin H, Liang Y, Lin L, Wang J, et al. ORMDL3 Functions as a Negative Regulator of Antigen-Mediated Mast Cell Activation via an ATF6-UPR-Autophagy-Dependent Pathway. Front Immunol. 2021;12.
23. Gade P, Ramachandran G, Maachani UB, Rizzo MA, Okada T, Prywes R, et al. An IFN-γ-stimulated ATF6-C/EBP-β-signaling pathway critical for the expression of death associated protein kinase 1 and induction of autophagy. Proc Natl Acad Sci U S A. 2012;109(26):10316–21.
24. Hjelmqvist L, Tuson M, Marfany G, Herrero E, Balcells S, Gonzàlez-Duarte R. ORMDL proteins are a conserved new family of endoplasmic reticulum membrane proteins. Genome Biol. 2002;3(6).
25. Ma X, Long F, Yun Y, Dang J, Wei S, Zhang Q, et al. ORMDL3 and its implication in inflammatory disorders. Int J Rheum Dis. 2018;21(6):1154–62.
26. Davis D, Kannan M, Wattenberg B. Orm/ORMDL proteins: Gate guardians and master regulators. Adv Biol Regul. 2018;70:3–18.
27. Liu YP, Rajamanikham V, Baron M, Patel S, Mathur SK, Schwantes EA, et al. Association of ORMDL3 with rhinovirus-induced endoplasmic reticulum stress and type I Interferon responses in human leucocytes. Clin Exp Allergy. 2017;47(3):371–82.
28. Luthers CR, Dunn TM, Snow AL. ORMDL3 and Asthma: Linking Sphingolipid Regulation to Altered T Cell Function. Front Immunol. 2020;11.
29. Guo Q, Jin Y, Chen X, Ye X, Shen X, Lin M, et al. NF-κB in biology and targeted therapy: new insights and translational implications. Signal Transduct Target Ther. 2024;9(1).
30. Kaser A, Lee AH, Franke A, Glickman JN, Zeissig S, Tilg H, et al. XBP1 links ER stress to intestinal inflammation and confers genetic risk for human inflammatory bowel disease. Cell. 2008;134(5):743–56.

1. Briefly describe the reasons why the research was undertaken and present the problem, subject, objectives, and hypotheses[3].
2. Based on the literature review, provide a concise overview of previous research results related to the investigated problem (ensure inclusion of the most recent and most relevant findings nationally and internationally).
3. State the contribution of the thesis to addressing the research topic.
4. Indicate the expected scientific and practical or artistic contribution of the dissertation.

5. MATERIAL AND RESEARCH METHODOLOGY

In the materials and methods section of the dissertation, the research material is comprehensively described, encompassing experimental animals, biological tissues, reagents, and analytical tools, all selected on the basis of well-defined scientific criteria.

Experimental Animals and Groups

In this study, two complementary experimental animal models were employed. The first model comprised twenty-two Sprague Dawley rats (n=22), both male and female, aged 6–8 weeks and weighing approximately 180g. Animals were divided into four experimental groups: control female (CF), diabetic female (DF), control male (CM), and diabetic male (DM). The second model included ten female Yucatan Mini pigs (n=10), aged 5–7 months and weighing 30–35 kg, divided into two groups: control (n=5) and diabetic (n=5). The rat model was selected for its well-characterized physiology and suitability for mechanistic molecular analyses, while the porcine model was chosen due to its close anatomical and physiological similarity to humans. All procedures were conducted in accordance with IACUC guidelines at Western University of Health Sciences (Pomona, CA, USA).

Diabetes induction

Diabetes induction is described using a combined dietary and pharmacological protocol based on streptozotocin (STZ) administration, a well-established and widely validated approach in both rodent and large animal experimental models. In the rat model, animals received a high-fat diet (HFD) for six weeks, followed by two low-dose intraperitoneal (i.p.) STZ injections (25 mg/kg). Of particular note, a sex-dependent difference in the response to STZ was observed: while two injections were sufficient to achieve stable hyperglycemia in male rats, female rats required an additional third dose to reach comparable glycemic levels.
In the porcine model, animals received a high-fat, high-carbohydrate/fructose diet (HFHFD) for two months, followed by two intravenous (i.v.) STZ injections (50 mg/kg). Across both species, this protocol successfully reflected the key pathophysiological features of T2DM, including insulin resistance (IR) and sustained hyperglycemia, and is considered fully appropriate and scientifically justified for the aims of this dissertation.

Histopathological analysis

Intestinal tissue samples were collected from the terminal ileum and entire colon in rats, and from the small intestine (terminal ileum) and sigmoid colon in pigs. These regions were selected based on their distinct physiological and immunological roles, as well as their differential involvement in IBD pathogenesis - the terminal ileum being predominantly affected in Crohn's disease, and the colon in ulcerative colitis. Following collection, tissues were fixed in 10% neutral-buffered formalin for 48 hours and subsequently processed for histopathological analyses.
Histopathological analysis was performed on formalin-fixed, paraffin-embedded tissue sections stained with hematoxylin and eosin (H&E). These represent internationally recognized gold-standard technique for the morphological characterization of intestinal tissue, enabling systematic assessment of villous

3 Hypotheses are presented only for a scientific doctorate.

architecture, crypt morphology, mucosal integrity, goblet cell density, epithelial continuity, and the degree and nature of inflammatory cell infiltration. The application of this method is considered fully adequate, technically sound, and appropriate for the histopathological objectives of the study.

Gene expression analysis

Gene expression analysis was conducted using quantitative real-time polymerase chain reaction (RT-qPCR), currently regarded as the most sensitive, specific, and reproducible method for quantitative mRNA analysis in experimental research. A carefully selected and scientifically justified panel of target genes was analyzed, including *ORMDL3*, *ATF6*, *NOD2*, *ULK1*, *ATG4*, *CD68*, *iNOS*, *TNF-α*, and *IL-6* in rats, with the addition of *NF-κB*, *NLRP3*, *IL-1β*, *CD86*, and *CD163* in the porcine model, reflecting the broader inflammatory profiling capacity of this species. All primers were species-specific and validated, and *18S* ribosomal RNA was employed as the internal reference gene to ensure accurate and reliable normalization of expression data. The selection of target genes comprehensively covers the key molecular axes under investigation ER stress, autophagy, innate immune activation, and macrophage polarization, and is directly and appropriately aligned with the research objectives.

Immunohistochemistry (IHC)

Protein-level analysis was performed using immunohistochemistry (IHC) with validated primary antibodies against all target molecules and species-appropriate secondary antibodies. This approach enabled not only the quantification of protein expression but also its precise spatial localization within distinct intestinal tissue compartments, including the epithelium, lamina propria, and submucosa, thereby providing complementary, confirmatory, and spatially resolved data relative to the transcriptional findings. The integration of gene and protein-level analyses substantially enhances the methodological rigor and interpretive depth of the study.

Statistical analysis and data interpretation

Statistical analysis was conducted using GraphPad Prism 10 (version 10.1.1). One-way ANOVA followed by Tukey's *post hoc* test was applied for multi-group comparisons in the rat model, while an unpaired two-tailed Student's *t*-test was used for two-group comparisons in the porcine model. Data are presented as mean ± standard deviation, and a significance threshold of $p<0.05$ was consistently applied throughout. The statistical approach is considered appropriate, well-justified, and fully in line with current international standards for experimental biomedical research.

Correlation analysis was performed to systematically assess the quantitative relationships between the expression levels of key molecular markers and metabolic parameters, most notably fasting blood glucose levels. Pearson correlation coefficients (R^2) were calculated across both intestinal regions and both animal models, enabling a rigorous and biologically meaningful evaluation of the strength and directionality of these associations. This analytical approach provided critical mechanistic insight into the glucose-dependent regulation of the ORMDL3–ATF6–autophagy axis in the diabetic intestine, and represents an important added value of the study beyond simple group comparisons.

1. Describe and present the main characteristics of the material analyzed and the criteria used for selecting the material.
2. Provide a brief overview of the research methods applied, evaluating in particular:
   2.1. whether the applied research methods are adequate, sufficiently accurate and up to date in view of global developments in the field;
   2.2. justification of any modifications to the original research plan;
   2.3. whether the scope of research is sufficient for drawing reliable conclusions or whether expansion of existing or introduction of new methods is required;
   2.4. whether the statistical analysis of data is adequate, if applied in the processing of results.

6. RESULTS AND SCIENTIFIC / ARTISTIC CONTRIBUTION OF THE RESEARCH

In the Results section, the findings are clearly defined, systematically presented, and interpreted in a transparent manner, while in the Discussion section, they are critically analyzed in relation to previously published studies and placed within the broader context of metabolic and inflammatory intestinal disease.

Histopathological analysis revealed consistent structural and inflammatory alterations in the intestinal mucosa of diabetic animals across both experimental models, indicative of early intestinal barrier disruption under conditions of chronic hyperglycemia. In the Sprague Dawley rat model, diabetic animals exhibited villous atrophy, epithelial desquamation, goblet cell depletion, inflammatory cell infiltration, and thinning of the muscular layer in the small intestine, while the large intestine showed mucosal disruption, crypt architectural distortion, lamina propria hypercellularity, and mononuclear infiltration extending into the submucosa, accompanied by intracellular accumulation of lipofuscin-like pigment reflecting oxidative stress-induced cellular damage. Comparable histopathological findings were confirmed in the porcine model, where diabetic animals demonstrated villous degeneration, crypt depletion, goblet cell loss, and increased inflammatory cell infiltration, with relative preservation of the deeper intestinal layers. These structural alterations were further supported by quantitative histological injury scoring, which demonstrated significantly greater tissue injury in diabetic animals compared to controls across both models.

Molecular analysis demonstrated significant upregulation of ORMDL3 at both the gene and protein levels in all examined intestinal regions across both animal models. In the rat model, *ORMDL3* gene expression was significantly elevated in diabetic females and males in both the small intestine ($p < 0.0001$ and $p < 0.001$, respectively) and the large intestine ($p < 0.001$ for both sexes), with females consistently exhibiting a more pronounced response. Immunohistochemical analysis confirmed increased ORMDL3 protein expression in both sexes and both intestinal regions, with a significantly higher elevation in diabetic females compared to diabetic males in the small intestine ($p < 0.01$). In the porcine model, *ORMDL3* gene expression was significantly elevated in both the small and large intestine ($p < 0.001$), and protein expression was similarly increased in both intestinal segments ($p < 0.01$). Importantly, *ORMDL3* gene expression showed strong positive correlations with blood glucose levels across both intestinal regions and both models, with R^2 values reaching 0.92 in the small intestine and 0.95 in the large intestine of diabetic pigs, providing novel experimental evidence of a glucose-dependent regulation of ORMDL3 in intestinal tissue.

In parallel, activation of endoplasmic reticulum stress was evidenced by upregulation of *ATF6*, a key unfolded protein response sensor, in both models. In the rat model, *ATF6* expression was significantly elevated in both sexes in the small intestine ($p < 0.0001$), while in the large intestine a sex-specific pattern was observed, with significant upregulation in diabetic males ($p < 0.001$) but no significant change in diabetic females, likely reflecting sex-dependent modulation of unfolded protein response signaling in this region. In the porcine model, *ATF6* was transcriptionally upregulated in both intestinal segments, while protein-level increases were predominantly detected in the large intestine ($p < 0.05$). A strong positive correlation between *ORMDL3* and *ATF6* expression was consistently observed across both models and intestinal regions, with R^2 values reaching up to 0.96 in the rat model, supporting their coordinated upregulation under hyperglycemic conditions. Both markers also showed strong positive correlations with blood glucose levels in both species, further reinforcing the concept of a glucose-driven endoplasmic reticulum stress axis in the diabetic intestine.

Autophagy-related gene expression was consistently upregulated across both models. *NOD2*, a critical intracellular stress sensor linking microbial recognition to autophagy activation, was significantly elevated in both intestinal regions in both diabetic rats and pigs, with particularly pronounced increases in female rats. *ULK1* and *ATG4*, key regulators of autophagosome initiation and maturation, showed a strikingly sex-specific pattern in the rat model, with significant upregulation exclusively in diabetic females ($p < 0.0001$) and no significant changes in diabetic males. In the porcine model, *ULK1* demonstrated the most pronounced transcriptional upregulation among all autophagy-related genes examined ($p < 0.0001$ in the

small intestine), while *ATG4* was also significantly increased in both intestinal segments. However, a critical discrepancy was observed at the protein level in the porcine model, where ULK1 and ATG4a protein expression remained unchanged in both intestinal regions despite clear transcriptional upregulation, while NOD2 protein was increased only in the large intestine. This gene-protein disconnect suggests impairment of autophagic flux at the post-transcriptional or post-translational level, indicative of dysfunctional rather than absent autophagy under chronic hyperglycemic conditions. Strong positive correlations were consistently observed between endoplasmic reticulum stress markers and autophagy-related genes across both models and intestinal regions, with R^2 values reaching up to 0.98 in the porcine model, supporting the presence of a tightly coupled endoplasmic reticulum stress-autophagy axis in the diabetic intestine.

Inflammatory responses were consistently elevated across both models and intestinal regions. In the rat model, the macrophage marker CD68, the M1 effector iNOS, and the pro-inflammatory cytokines TNF-α and IL-6 were significantly upregulated at both the gene and protein levels in diabetic animals, with female rats exhibiting markedly stronger responses than males across both intestinal segments. IL-6 was notable in that it showed significant upregulation in both sexes at the protein level without sex-dependent differences, suggesting it may represent a more universal downstream effector of hyperglycemia-induced intestinal inflammation. In the porcine model, significant upregulation of *NF-κB*, *TNF-α*, *IL-6*, *NLRP3*, and *IL-1β* was observed in both intestinal segments, alongside pronounced macrophage activation evidenced by increased *CD68* and *CD86* expression, consistent with predominant M1 macrophage polarization. Concurrent upregulation of *CD163*, a marker of M2 macrophage polarization, suggested that anti-inflammatory and tissue repair mechanisms are simultaneously activated, reflecting a complex and dynamically regulated inflammatory microenvironment in the diabetic intestine.

In the rat model, female animals consistently demonstrated stronger molecular and inflammatory responses than males, a pattern that likely reflects the modulatory role of estrogen on endoplasmic reticulum stress, autophagy, and mucosal immune signaling pathways. The consistency of key findings across two animal species, two intestinal regions, and multiple levels of analysis strengthens the biological relevance of the observed alterations and supports their broader translational significance.

1. Briefly present the results obtained by the candidate.
2. Evaluate whether the results are clearly presented, properly, logically and clearly interpreted, compared with the results of other authors, and whether the candidate demonstrated an adequate level of critical analysis.
3. It is particularly important to highlight the new findings obtained through the research, their theoretical and practical contribution, and whether they indicate new directions for further research.

7. CONCLUSION AND PROPOSAL

The doctoral dissertation of candidate dr Uglješa Maličević, entitled "The Association of *ORMDL3* Expression with Hyperglycemia in Intestinal Tissue: A Potential Role in the Pathogenesis of Inflammatory Bowel Disease (IBD)", was prepared in accordance with the approved research proposal and the principles of scientific research, and represents an original and independent work of the candidate. The research methods described and applied in this study are appropriate, contemporary, and consistent with international standards in biomedical research, enabling reliable and scientifically relevant results.

Based on the obtained results and the conclusions derived from them, this dissertation represents an original scientific contribution in the field of biomedical sciences and pathophysiology. The findings demonstrate that chronic hyperglycemia is associated with consistent upregulation of ORMDL3 in intestinal tissue, at both the gene and protein levels, across two complementary animal models. In addition, the ORMDL3–ATF6 axis was identified as a hyperglycemia-associated component of endoplasmic reticulum stress in the diabetic intestine, while significant correlations were observed between blood glucose levels and the expression of key ER stress- and autophagy-related markers. The study further confirmed coordinated pro-inflammatory activation of the intestinal mucosa under conditions of chronic hyperglycemia, accompanied by pronounced sex-dependent differences in molecular responses.

Collectively, these findings support the potential role of ORMDL3 and associated cellular stress pathways as biomarkers and therapeutic targets linking metabolic dysfunction and inflammatory bowel disease, thereby contributing to a better understanding of their shared pathophysiological mechanisms.

Based on the overall evaluation of the dissertation, the members of the Dissertation Defense Committee unanimously give a positive assessment of the completed doctoral dissertation by candidate dr Uglješa Maličević. The Committee therefore proposes to the Scientific-Teaching Council of the Faculty of Medicine, University of Banja Luka, that this report be accepted and that the candidate be granted approval to proceed with the public defense before the Committee in the same composition.

1. State the most significant facts indicating the scientific/artistic contribution of the dissertation.
2. Based on the overall evaluation of the dissertation, the Committee proposes:
   - that the dissertation / art project be accepted and the candidate be approved to proceed to the defense,
   - that the dissertation / art project be returned to the candidate for revision (to be supplemented or amended), or
   - that the dissertation / art project be rejected.

Place and date:

Banja Luka and Belgrade, 25.5.2026.

Prof. dr Dragan Đurić, s.r, MD, PhD, Full Professor of *Physiology, Faculty of Medicine, University of Belgrade*

Chair of the Committee

Prof. dr Darko Golić, s.r, MD, PhD, Full Professor of *Pathophysiology, Faculty of Medicine, University of Banja Luka*

Committee member

Doc. dr Tatjana Milivojac, s.r, Assistant Professor of *Pathophysiology, Faculty of Medicine, University of Banja Luka*

Committee member

Name and surname, academic title and position

Committee member

Name and surname, academic title and position

Committee member

DISSENTING OPINION: A Committee member who does not wish to sign the report because they disagree with the opinion of the majority of the Committee members is required to include in the report an explanation, i.e., the reasons for refusing to sign the report.

Attach the following to the report:

1. Decision of the Artistic-Scientific-Teaching / Scientific-Teaching Council of the faculty on the appointment of the Committee for the evaluation of the completed doctoral dissertation / doctoral art project and public defense;
2. Decision of the Artistic-Scientific-Teaching / Scientific-Teaching Council of the faculty on the adoption of the Committee report on the evaluation of the completed doctoral dissertation / doctoral art project and public defense;
3. Committee Report on the evaluation of the completed doctoral dissertation / doctoral art project and public defense – Form 3;
4. Doctoral dissertation in PDF format;
5. Certificate issued by the Vice-Dean for Research and Development confirming originality check via official plagiarism-detection software;
6. Statement of authorship;
7. Statement authorizing the University of Banja Luka to make the doctoral dissertation / doctoral art project publicly available;
8. Statement confirming the identity of the printed and electronic versions of the doctoral dissertation / doctoral art project.