Tzu Chi Medical Journal 2024; 36 (1): 23-29

Review Article

# Restraint stress-associated gastrointestinal injury and implications from the Evans blue-fed restraint stress mouse model

*Der-Shan Sun, Te-Sheng Lien, Hsin-Hou Chang**

Department of Molecular Biology and Human Genetics, Tzu Chi University, Hualien, Taiwan

**Submission** : 26-Apr-2023
**Revision** : 15-May-2023
**Acceptance** : 27-Jun-2023
**Web Publication** : 07-Sep-2023

## Abstract

The association between stress and gastrointestinal (GI) tract diseases is well established, while the exact mechanism remains elusive. As a result, it is urgent to establish mouse models to investigate restraint stress-associated GI leakage, but current models have their limitations. A new Evans blue-fed restraint mouse model has recently been developed that allows researchers to study restraint stress-associated GI leakage in live animals. This review article will focus on this model, including its mechanisms, clinical implications, and applications for studying restraint stress-associated GI injury. Recent findings from studies using this model will also be highlighted, along with their potential for diagnosis and treatment. The article aims to discuss about current research and provide recommendations for further study, ultimately improving our understanding of the link between stress and GI injury and improving patient outcomes.

**Keywords:** *Apoptosis, Gastrointestinal injury, Inflammation, Restraint stresses, Tight junction*

## Restraint stress and gastrointestinal injury

Restraint stress is the emotional and physiological response to perceive a situation as demanding or overwhelming, impacting various aspects of well-being [1]. Restraint stress-associated gastrointestinal (GI) injury refers to the physical damage that can occur in the digestive system due to psychological and physiological stresses. Psychological stress can activate the body's "fight or flight" response, which can cause changes in the digestive system, such as decreased blood flow and increased inflammation [2]. These changes can lead to damage to the gut lining, disruption of gut motility and secretion, and alterations in gut microbiota [3-5]. The gut and brain maintain a bidirectional communication system that involves multiple pathways, including immune pathways, endocrine pathways, neural pathways, the autonomic nervous system, enteric nervous system (ENS), hypothalamic–pituitary–adrenal (HPA) axis, and the vagus nerve [Figure 1]. This intricate network allows for extensive interactions and information exchange between the gut and the brain, facilitating the regulation of physiological processes [6-9]. Understanding the mechanisms of restraint stress-associated GI injury and developing effective management strategies is crucial for maintaining digestive health and overall well-being.

Access this article online

Quick Response Code:

Website: www.tcmjmed.com

DOI: 10.4103/tcmj.tcmj_101_23

## Stress-associated gastrointestinal injury in clinical settings

The risk of GI tract diseases can be increased by psychological stress [10-12]. For example, an increased risk of GI tract diseases has been linked to neurocognitive and psychiatric disorders [13], such as autism [14], dementia [15,16], schizophrenia [17], bipolar disorder [18], and depression and anxiety [11]. Psychological stress-associated GI injury can lead to increased gut permeability, dysbiosis, inflammation, and immune response, all of which have been linked to the development of GI disorders [4,19-21]. Studies have shown that individuals who experience chronic stress are more likely to develop irritable bowel syndrome, inflammatory bowel disease, and other gut-related disorders [20,21].

In addition to the cross-talk between psychological stress and GI disorders, psychological stress may also lead to various adverse effects, which may as lead to GI injuries. For example, stress-associated GI injury has been implicated in impaired nutrient absorption [22,23]. Meanwhile, psychological stress has been involved in poor immune

**Address for correspondence:** Prof. Hsin-Hou Chang, Department of Molecular Biology and Human Genetics, Tzu Chi University, 701, Section 3, Chung-Yang Road, Hualien, Taiwan.
E-mail: hhchang@mail.tcu.edu.tw



**How to cite this article:** Sun DS, Lien TS, Chang HH. Restraint stress-associated gastrointestinal injury and implications from the Evans blue-fed restraint stress mouse model. Tzu Chi Med J 2024;36(1):23-9.

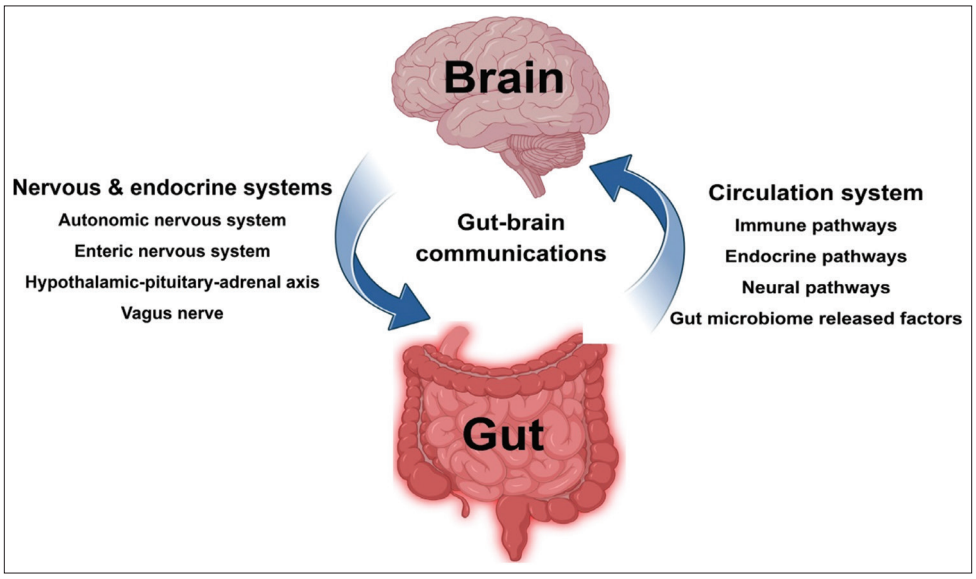


**Figure 1:** Bidirectional communication between the gut and the brain. The diagram illustrates the bidirectional communication between the gut and the brain, which is influenced by various pathways such as the immune pathways, endocrine pathways, neural pathways, autonomic nervous system, enteric nervous system, hypothalamic–pituitary–adrenal axis, and vagus nerve

function, making individuals more susceptible to infections and other illnesses [24,25]. In addition, psychological stress has been implicated in delayed wound healing [26,27], which may delay the repair of GI injury, leading to complications in surgical and other medical procedures [26,27]. All these effects may cause GI disorders.

## Mechanisms of psychological stress-associated gastrointestinal injury

The mechanism that leads to psychological stress-associated GI injury is still not fully understood.

The communication between the brain and the digestive system involves intricate pathways partly mediated by the vagus nerve. Through sensory fibers, the vagus nerve transfers information about the state of the digestive system to the brain. This bidirectional communication occurs through various mechanisms, such as the direct vagus nerve-to-brain connection and interactions with the ENS and enteroendocrine cells with neuropods. By conveying neuronal, endocrine, and immune messages, the vagus nerve influences the gut microbiota and ultimately impacts brain health [9,28,29].

In this communication process, GI bioactive molecules, including neurotransmitters, hormones, cytokines, and microbial metabolites play a significant role [9,28,29]. Evidence suggested a bidirectional microbiota–gut–brain axis that psychological stress can cause changes in the gut microbiota, alter gut permeability, and promote inflammation, leading to GI injury and brain damage [30-33]. Furthermore, the immune cells, including dendritic cells, macrophages, neutrophils and T-cells are also involved in the inflammatory response to psychological stress. These cells may produce pro-inflammatory cytokines, such as interleukin-1β, interleukin-6, and tumor necrosis factor-α, which are released in response to stress-associated inflammation in the gut [34-36]. These cytokines and inflammatory responses can cause the suppression of epithelial tight junction and damage to the intestinal epithelium, leading to further increases in GI permeability and promoting the entry of more bacteria and toxins into the bloodstream [34-39]. These results suggested that epithelial tight junctions, regulated cell death, and inflammatory pathways of the GI system are potential research directions for studying restraint stress-associated GI injury. These bioactive molecules are produced within the GI system under the influence of external factors such as prebiotics, psychobiotics, drugs, and lifestyle habits. They can traverse the blood–brain barrier, which consists of endothelial cells lining the brain capillary wall, astrocyte end-feet surrounding the capillary, and pericytes embedded in the capillary basement membrane. Thus, these bioactive molecules can directly reach brain tissue and exert their effects [9,28,29].

The activation of the HPA axis is another important aspect of brain–gut communication [9,28,29]. The HPA axis is characterized by releasing corticotropin-releasing hormone from the hypothalamus upon psychological stress induction, which stimulates the anterior pituitary gland to produce adrenocorticotropic hormone (ACTH). ACTH, in turn, acts on the adrenal gland, producing and releasing cortisol, a stress hormone [40,41]. Cortisol modulates the intestinal epithelial barrier and immune responses, contributing to the overall interplay between the brain and the gut [9,28,29].

Within the gut, enteroendocrine cells play a crucial role in bidirectional communication with the brain. The innervation induced by the vagus nerve stimulates signaling between enterochromaffin cells and neuronal circuits. This signaling influences various aspects, such as pain, background emotions, immune responses, neurogenesis, and neurodevelopment. Furthermore, the vagus nerve exhibits immunomodulatory properties and has a significant impact on GI and psychiatric

disorders, highlighting its essential role in maintaining homeostasis between the gut and the brain [9,28,29].

In summary, both in normal and psychological stress conditions, the complex interaction between the brain and the gut incorporates several key elements. These include the vagus nerve, GI bioactive molecules, the HPA axis, the gut microbiome, and enteroendocrine cells. However, the precise mechanisms underlying gut–brain communication remain largely elusive. To provide a visual representation of these intricate connections, we have summarized the gut–brain communication pathways in a concise diagram [Figure 1].

## Restraint stress mouse model for studying stress-associated gastrointestinal injury

### The restraint stress mouse model is useful for exploring stress-associated pathophysiological changes

The restraint stress mouse model is a widely accepted approach for examining the physiological, behavioral, and biochemical alterations linked to psychological stress in mice [42-46]. By studying the effects of restraint and immobilization stress, researchers can uncover the pathophysiological changes that contribute to anxiety and stressed behavior in experimental animals [42]. As a result, the restraint stress mouse model is a valuable tool for exploring stress-associated GI injuries [47,48].

### Comparisons between traditional restraint stress models versus Evans blue-fed restraint stress mouse model

There are certain drawbacks to current animal models used to measure GI leakage. For instance, some models may be time-consuming (e.g., lactulose/mannitol test), require specialized equipment (e.g., liquid chromatography and mass spectrometry), or involve isotope labeling (e.g., 51Cr-labeling) [49]. Although endoscopy is commonly used for evaluating GI injuries in patients [50,51], it is not feasible for mice due to their small size [47]. Therefore, we developed a relatively simple method that involves feeding mice with GI nonabsorbable Evans blue dye, enabling us to measure plasma Evans blue levels to observe the timely changes of GI leakage [47,48]. The traditional use of intravenous injection of Evans blue, which binds to serum albumin and only leaks into peripheral tissues, when there is increased vascular permeability (dye leaks from blood to tissue) [52-56]. Unlike the traditional use, in this oral-fed model, Evans blue leaks into the bloodstream from the GI system when GI injury occurs (dye leaks from tissue to blood) [Figure 2, experiment outline] [47,48]. The restraint stress can be applied for a specific or extended period of time, and the mice are euthanized for examination of their GI tissues for signs of GI injury, such as inflammation, suppressed tight junction, and increased epithelial cell death [47,48]. This model enables researchers to investigate the mechanisms of GI injury induced by restraint stress, such as molecular regulations of gut epithelial tight junctions and cell death. The Evans blue-fed restraint stress mouse model is advantageous in that it allows for real-time examination of GI injury, enabling researchers to explore the temporal relationship between stress exposure and GI injury. In addition, this non-invasive model is ethical and practical, making it a useful tool for preclinical studies to evaluate potential therapies for stress-related gut disorders. It is a convenient and useful animal model. From a practical standpoint, it is important to note that the experimental results can be significantly influenced by the circadian rhythm. In order to obtain meaningful results, it is recommended to perform the restraint stress during the dark cycle (active period) of the experimental mice.

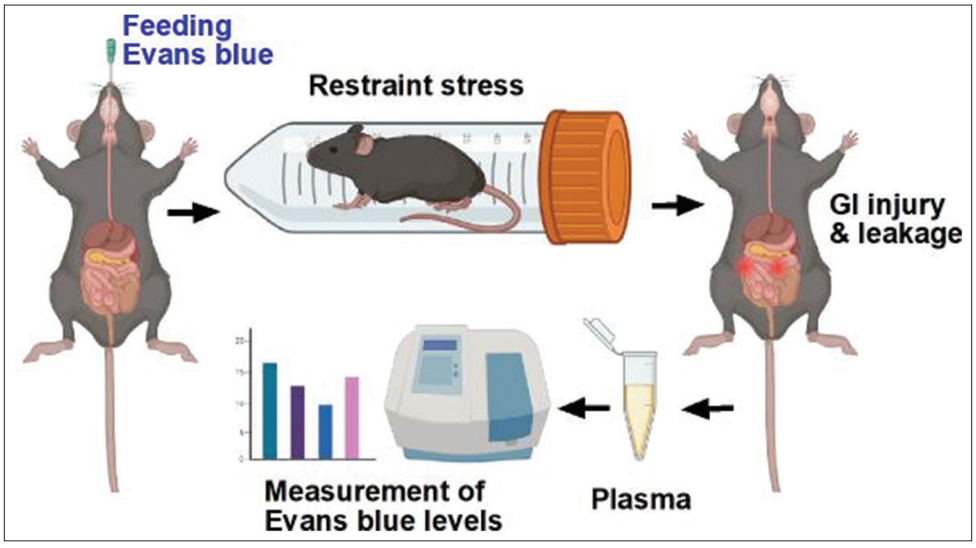


**Figure 2:** Experiment outline of the Evans blue-fed restraint stress mouse model. The experimental protocol for the Evans blue-fed restraint stress mouse model was adopted from previous studies [47,48]. Mice were subjected to 9 h of restraint stress in a 50-mL plastic falcon tube with air holes. Blood samples were collected at 0, 5, 7, and 9 h, and Evans blue (1.2 g/kg) was fed to the mice before stress. Their blood plasma was isolated by collecting blood in an Eppendorf tube and mixing it with an equal proportion of anticoagulant citrate dextrose solution to prevent coagulation [54,57,58]. The concentration of Evans blue in the plasma was measured using a spectrum analyzer

## Major findings of Evans blue-fed restraint stress mouse model

The Evans blue-fed restraint stress mouse model [Figure 2, experiment outline] has revealed several significant findings, including (1) the ability to assess the dynamic changes of GI leakage in live animals and (2) timely evaluation of tight junctions, inflammation, and epithelial cell death at a cellular level [47,48].

### Increased gut permeability

Research has shown that restraint stress can result in an increase in gut permeability. To measure gut permeability and monitor its changes over time in live animals with good reproducibility, the Evans blue-fed restraint stress mouse model has been utilized [47,48]. This model enables researchers to determine the appropriate time points for peak restraint stress, facilitating further mechanism studies and functional analyses.

### Tight junction, inflammation, and epithelial cell death

The advantage of the Evans blue-fed mouse model is its ability to assess the dynamic changes in gut permeability over time *in vivo*. This time course information allows for the collection of gut tissue at appropriate time points to evaluate tight junction integrity, inflammation, and epithelial cell death accurately. For example, exposure to restraint stress has been shown to increase inflammation and cellular stress in the gut, leading to GI damage and dysfunction [47,48]. The restraint stress-associated abnormal suppression on the gene and protein expressions of tight junctions (e.g., zonula occludens-1, claudin-3; and junctional adhesion molecule 3) could be revealed in proper time courses [47,48]. In addition, restraint stress-associated regulated cell death pathways [54,57,58], such as apoptosis, in the GI epithelial cells could be revealed [47,48]. As a result, when using the Evans blue-fed restraint stress mouse model in combination with various other analysis methods, such as RNA (e.g., quantitative real-time reverse transcription polymerase chain reaction assay) and protein identification (e.g., Western blotting, flow cytometry, enzyme-linked immunosorbent assay, and immunohistochemistry) methods [47,48], it becomes a powerful preclinical model for mechanism studies.

### Effects of interventions for preclinical studies

In previous studies, the restraint stress mouse model has been employed to evaluate the impact of different interventions on GI injury caused by psychological stress [59-61]. Platelet-rich plasma is a well-established therapeutic agent to facilitate tissue repair and anti-inflammation [62-64]. Utilizing platelet-rich-plasma and platelet transfer have been tested in Evans blue-fed restraint stress mouse model [48], revealing potential alternative treatment strategies for stress-associated GI injury. Although the precise molecular pathways involved in restraint stress-associated gut injury remain unknown, previous studies utilizing the Evans blue-fed restraint stress mouse model have shed some light on the topic. Specifically, these studies have demonstrated the importance of activating transcription factor 3 (ATF3) and P-selectin in repairing restraint stress-associated gut injury. ATF3 is a member of the ATF/CREB family of transcription factors involved in the regulation of cellular processes, including anti-stress, anti-inflammation, and pro-survival responses [65,66]. P-selectin, also known as CD62P, is a transmembrane protein found on activated endothelial cells and platelets, which plays a critical role in mediating leukocyte adhesion during inflammation and participates in the regulation of thrombosis, angiogenesis, and cell signaling [67]. In comparison to wild-type control mice, mutant mice lacking the ATF3 and P-selectin gene expressions exhibited significantly higher levels of GI leakage and suppressed epithelial tight junction following restraint stress [47,48]. These findings indicate that both the ATF3 and P-selectin pathways play a role in protecting against stress-associated inflammation and epithelial damage, though further researches are needed to fully elucidate the underlying mechanisms and roles of ATF3 and P-selectin pathways on the amelioration of restraint stress-associated gut injury.

### Involvements of psychological stress

Restraint stress can induce both physiological and psychological stresses in experimental mice. In order to explore the potential involvement of psychological stress in the Evans blue-fed restraint stress mouse model, we conducted the elevated plus maze mouse behavior test following previously reported methods [68]. These results demonstrate that mice subjected to Evans blue-fed restraint stress treatment exhibited

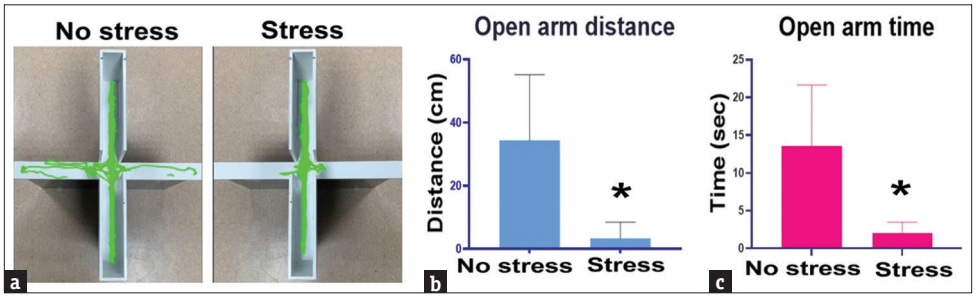


**Figure 3:** Restraint stress resulted in the development of anxiety-like behaviors in C57Bl/6J mice. Throughout the 20-h stress procedure, both the no stress and stress groups were deprived of access to food and water. After the termination of restraint stress, both the unstressed and stressed groups of mice were given access to food and water for a period of 2 h to restore their resources. Subsequently, the elevated plus maze (EPM) was conducted. Video recordings were captured using an iPhone Xs Max and later analyzed using ToxTrac_v2.98 software. Representative video tracking images captured during a 5-minute EPM are presented (a). A comparison was made between the control group (no stress) and the restraint stress group (stress) for open-arm traveled distance (b), and open-arm staying time (c). The number of samples used for analysis was 3 ($n = 3$). The statistical significance of the obtained results was examined using student's t-test. and *indicates statistical significance at $P < 0.05$, when compared to their respective no stress groups. All protocols for examining the experimental animals were approved by the Animal Care and Use Committee of Tzu Chi University, Hualien, Taiwan (approval ID: 111052)

anxiety-related behavior, providing further evidence of the involvement of psychological stress in this model [Figure 3]. Such data align with numerous previous reports identify psychological stress as one of the primary stressors associated with restraint stress animal models [42-46].

## Future directions

### Clinical applications

The Evans blue-fed restraint stress mouse model has clinical implications for the study of stress-associated GI injury. This model allows researchers to investigate GI leakage in live animals and study the mechanisms, diagnosis, and treatment of stress-related GI disorders. It provides a real-time examination of GI injury, enabling the exploration of the temporal relationship between stress exposure and GI injury. The model has been used to assess gut permeability and evaluate tight junction integrity, inflammation, and epithelial cell death. It has also been utilized to study the effects of interventions on GI injury caused by stress, and is likely helpful for the development of new therapeutic agents against stress-associated GI diseases [69].

### Challenges and future perspectives

Despite its advantages, the model also presents challenges. For instance, the variability in the stress response of individual mice can affect the reproducibility of study results. In addition, the sole use of restraint stress may not fully recapitulate the range of stressors that humans experience in daily life. Furthermore, the long-term effects of stress-associated GI injury in humans remain unclear, and the extent to which the mouse model translates to human physiology is limited. Future perspectives involve standardized stress protocols, measuring GI leakage, studying effects of stressors on GI injury, and exploring therapeutic interventions for stress-associated GI damage. Further validation of the model's relevance to human GI injury and the exploration of potential biomarkers for the early detection and diagnosis of stress-associated GI injury in humans are also needed.

## Conclusion

Stress-associated GI injury is a complex phenomenon that involves various mechanisms, including inflammation, gut microbiota dysbiosis, and cellular stress. The Evans blue-fed restraint stress mouse model has emerged as a valuable tool for studying stress-associated GI injury in live animals. The model has provided evidence for increased gut permeability in response to stress and has implications for the diagnosis and treatment of GI disorders associated with stress. However, the model has limitations and further studies are needed to validate its findings and overcome its limitations. The Evans blue-fed restraint stress mouse model has the potential to advance our understanding of the link between stress and GI injury and ultimately improve patient outcomes.

### Data availability statement

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

### Acknowledgments

The authors would like to express their gratitude to the Experimental Animal Center and Core Facility Center at Tzu Chi University for their invaluable assistance with animal care, confocal microscopy, and flow cytometry experiments.

### Financial support and sponsorship

This work is supported by research funding from National Science and Technology Council, Taiwan (105-2514-S-320-001-MY3, 105-2923-B-320-001-MY3, 107-2311-B-320-002 -MY3, 111-2320-B-320-006-MY3), and Tzu-Chi Medical Foundation (TCMMP108-04; TCMMP 111-01 and TCAS-112-02; TCAS-111-02). The funders have no role in the study design, in data collection, analysis, and interpretation, in writing the report, and in the decision to submit the article for publication.

### Conflicts of interest

Dr. Hsin-Hou Chang, an editorial board member at Tzu Chi Medical Journal, played no role in the peer review process or decision to publish this article. The other authors declared no conflicts of interest in writing this paper.

## References

1. Schneiderman N, Ironson G, Siegel SD. Stress and health: Psychological, behavioral, and biological determinants. Annu Rev Clin Psychol 2005;1:607-28.
2. Godoy LD, Rossignoli MT, Delfino-Pereira P, Garcia-Cairasco N, de Lima Umeoka EH. A comprehensive overview on stress neurobiology: Basic concepts and clinical implications. Front Behav Neurosci 2018;12:127.
3. Madison A, Kiecolt-Glaser JK. Stress, depression, diet, and the gut microbiota: Human-bacteria interactions at the core of psychoneuroimmunology and nutrition. Curr Opin Behav Sci 2019;28:105-10.
4. Konturek PC, Brzozowski T, Konturek SJ. Stress and the gut: Pathophysiology, clinical consequences, diagnostic approach and treatment options. J Physiol Pharmacol 2011;62:591-9.
5. Pferschy-Wenzig EM, Pausan MR, Ardjomand-Woelkart K, Röck S, Ammar RM, Kelber O, et al. Medicinal plants and their impact on the gut microbiome in mental health: A systematic review. Nutrients 2022;14:2111.
6. Wachsmuth HR, Weninger SN, Duca FA. Role of the gut-brain axis in energy and glucose metabolism. Exp Mol Med 2022;54:377-92.
7. Foster JA, Rinaman L, Cryan JF. Stress and the gut-brain axis: Regulation by the microbiome. Neurobiol Stress 2017;7:124-36.
8. Dowling LR, Strazzari MR, Keely S, Kaiko GE. Enteric nervous system and intestinal epithelial regulation of the gut-brain axis. J Allergy Clin Immunol 2022;150:513-22.
9. Suganya K, Koo BS. Gut-brain axis: Role of gut microbiota on neurological disorders and how probiotics/prebiotics beneficially modulate microbial and immune pathways to improve brain functions. Int J Mol Sci 2020;21:7551.
10. Sapolsky RM. Why zebras don't get ulcers. 3rd ed. New York City, United States: W. H. Freeman; 2004.
11. Oligschlaeger Y, Yadati T, Houben T, Condello Oliván CM, Shiri-Sverdlov R. Inflammatory bowel disease: A stressed "gut/feeling". Cells 2019;8:659.
12. Rao M, Gershon MD. The bowel and beyond: The enteric nervous system in neurological disorders. Nat Rev Gastroenterol Hepatol 2016;13:517-28.
13. GBD 2017 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 354 diseases and injuries for 195 countries and territories,

1990-2017: A systematic analysis for the global burden of disease study 2017. Lancet 2018;392:1789-858.
14. Wasilewska J, Klukowski M. Gastrointestinal symptoms and autism spectrum disorder: Links and risks – A possible new overlap syndrome. Pediatric Health Med Ther 2015;6:153-66.
15. Zhang B, Wang HE, Bai YM, Tsai SJ, Su TP, Chen TJ, et al. Inflammatory bowel disease is associated with higher dementia risk: A nationwide longitudinal study. Gut 2021;70:85-91.
16. Alkasir R, Li J, Li X, Jin M, Zhu B. Human gut microbiota: The links with dementia development. Protein Cell 2017;8:90-102.
17. Severance EG, Prandovszky E, Castiglione J, Yolken RH. Gastroenterology issues in schizophrenia: Why the gut matters. Curr Psychiatry Rep 2015;17:27.
18. Karling P, Maripuu M, Wikgren M, Adolfsson R, Norrback KF. Association between gastrointestinal symptoms and affectivity in patients with bipolar disorder. World J Gastroenterol 2016;22:8540-8.
19. Lee SP, Sung IK, Kim JH, Lee SY, Park HS, Shim CS. The effect of emotional stress and depression on the prevalence of digestive diseases. J Neurogastroenterol Motil 2015;21:273-82.
20. Vasant DH, Ford AC. Functional gastrointestinal disorders in inflammatory bowel disease: Time for a paradigm shift? World J Gastroenterol 2020;26:3712-9.
21. Padhy SK, Sahoo S, Mahajan S, Sinha SK. Irritable bowel syndrome: Is it "irritable brain" or "irritable bowel"? J Neurosci Rural Pract 2015;6:568-77.
22. Lopresti AL. The effects of psychological and environmental stress on micronutrient concentrations in the body: A review of the evidence. Adv Nutr 2020;11:103-12.
23. Karl JP, Hatch AM, Arcidiacono SM, Pearce SC, Pantoja-Feliciano IG, Doherty LA, et al. Effects of psychological, environmental and physical stressors on the gut microbiota. Front Microbiol 2018;9:2013.
24. Segerstrom SC, Miller GE. Psychological stress and the human immune system: A meta-analytic study of 30 years of inquiry. Psychol Bull 2004;130:601-30.
25. Bae YS, Shin EC, Bae YS, van Eden W. Editorial: Stress and immunity. Front Immunol 2019;10:245.
26. Gouin JP, Kiecolt-Glaser JK. The impact of psychological stress on wound healing: Methods and mechanisms. Immunol Allergy Clin North Am 2011;31:81-93.
27. Walburn J, Vedhara K, Hankins M, Rixon L, Weinman J. Psychological stress and wound healing in humans: A systematic review and meta-analysis. J Psychosom Res 2009;67:253-71.
28. Mitrea L, Nemeş SA, Szabo K, Teleky BE, Vodnar DC. Guts imbalance imbalances the brain: A review of gut microbiota association with neurological and psychiatric disorders. Front Med (Lausanne) 2022;9:813204.
29. García-Cabrerizo R, Carbia C, Riordan KJ, Schellekens H, Cryan JF. Microbiota-gut-brain axis as a regulator of reward processes. J Neurochem 2021;157:1495-524.
30. Schächtle MA, Rosshart SP. The microbiota-gut-brain axis in health and disease and its implications for translational research. Front Cell Neurosci 2021;15:698172.
31. Chakrabarti A, Geurts L, Hoyles L, Iozzo P, Kraneveld AD, La Fata G, et al. The microbiota-gut-brain axis: Pathways to better brain health. Perspectives on what we know, what we need to investigate and how to put knowledge into practice. Cell Mol Life Sci 2022;79:80.
32. Long-Smith C, O'Riordan KJ, Clarke G, Stanton C, Dinan TG, Cryan JF. Microbiota-gut-brain axis: New therapeutic opportunities. Annu Rev Pharmacol Toxicol 2020;60:477-502.
33. Cryan JF, O'Riordan KJ, Cowan CS, Sandhu KV, Bastiaanssen TF, Boehme M, et al. The microbiota-gut-brain axis. Physiol Rev 2019;99:1877-2013.
34. Kaminsky LW, Al-Sadi R, Ma TY. IL-1β and the intestinal epithelial tight junction barrier. Front Immunol 2021;12:767456.
35. Al-Sadi R, Ye D, Boivin M, Guo S, Hashimi M, Ereifej L, et al. Interleukin-6 modulation of intestinal epithelial tight junction permeability is mediated by JNK pathway activation of claudin-2 gene. PLoS One 2014;9:e85345.
36. Stevens C, Walz G, Singaram C, Lipman ML, Zanker B, Muggia A, et al. Tumor necrosis factor-alpha, interleukin-1 beta, and interleukin-6 expression in inflammatory bowel disease. Dig Dis Sci 1992;37:818-26.
37. Kim HJ, Li H, Collins JJ, Ingber DE. Contributions of microbiome and mechanical deformation to intestinal bacterial overgrowth and inflammation in a human gut-on-a-chip. Proc Natl Acad Sci U S A 2016;113:E7-15.
38. Mohr AE, Crawford M, Jasbi P, Fessler S, Sweazea KL. Lipopolysaccharide and the gut microbiota: Considering structural variation. FEBS Lett 2022;596:849-75.
39. Slifer ZM, Blikslager AT. The integral role of tight junction proteins in the repair of injured intestinal epithelium. Int J Mol Sci 2020;21:972.
40. Sjörs Dahlman A, Jonsdottir IH, Hansson C. The hypothalamo-pituitary-adrenal axis and the autonomic nervous system in burnout. Handb Clin Neurol 2021;182:83-94.
41. Špiljak B, Vilibić M, Glavina A, Crnković M, Šešerko A, Lugović-Mihić L. A Review of psychological stress among students and its assessment using salivary biomarkers. Behav Sci (Basel) 2022;12:400.
42. Campos AC, Fogaça MV, Aguiar DC, Guimarães FS. Animal models of anxiety disorders and stress. Braz J Psychiatry 2013; 35(Suppl 2): S101-11.
43. Paré WP, Glavin GB. Restraint stress in biomedical research: A review. Neurosci Biobehav Rev 1986;10:339-70.
44. Glavin GB, Paré WP, Sandbak T, Bakke HK, Murison R. Restraint stress in biomedical research: An update. Neurosci Biobehav Rev 1994;18:223-49.
45. Shoji H, Miyakawa T. Differential effects of stress exposure via two types of restraint apparatuses on behavior and plasma corticosterone level in inbred male BALB/cAJcl mice. Neuropsychopharmacol Rep 2020;40:73-84.
46. Ma M, Chang X, Wu H. Animal models of stress and stress-related neurocircuits: A comprehensive review. Stress Brain 2021;1:108-27.
47. Chuang DJ, Pethaperumal S, Siwakoti B, Chien HJ, Cheng CF, Hung SC, et al. Activating transcription factor 3 protects against restraint stress-induced gastrointestinal injury in mice. Cells 2021;10:3530.
48. Pethaperumal S, Hung SC, Lien TS, Sun DS, Chang HH. P-selectin is a critical factor for platelet-mediated protection on restraint stress-induced gastrointestinal injury in mice. Int J Mol Sci 2022;23:11909.
49. Galipeau HJ, Verdu EF. The complex task of measuring intestinal permeability in basic and clinical science. Neurogastroenterol Motil 2016;28:957-65.
50. Delvaux M, Escourrou J. Endoscopy in peptic ulcer disease: Diagnosis, prognosis and management. Endoscopy 1992;24:41-4.
51. Dunlap JJ, Patterson S. Peptic ulcer disease. Gastroenterol Nurs 2019;42:451-4.
52. Sun DS, Chang YC, Lien TS, King CC, Shih YL, Huang HS, et al. Endothelial cell sensitization by death receptor fractions of an anti-dengue nonstructural protein 1 antibody induced plasma leakage, coagulopathy, and mortality in mice. J Immunol 2015;195:2743-53.
53. Chan H, Huang HS, Sun DS, Lee CJ, Lien TS, Chang HH. TRPM8 and RAAS-mediated hypertension is critical for cold-induced immunosuppression in mice. Oncotarget 2018;9:12781-95.
54. Lien TS, Sun DS, Wu CY, Chang HH. Exposure to dengue envelope protein domain III induces Nlrp3 inflammasome-dependent endothelial dysfunction and hemorrhage in mice. Front Immunol 2021;12:617251.
55. Chen PK, Chang HH, Lin GL, Wang TP, Lai YL, Lin TK, et al. Suppressive effects of anthrax lethal toxin on megakaryopoiesis. PLoS

One 2013;8:e59512.

56. Yao L, Xue X, Yu P, Ni Y, Chen F. Evans blue dye: A revisit of its applications in biomedicine. Contrast Media Mol Imaging 2018;2018:7628037.
57. Lien TS, Chan H, Sun DS, Wu JC, Lin YY, Lin GL, et al. Exposure of platelets to dengue virus and envelope protein domain III induces Nlrp3 inflammasome-dependent platelet cell death and thrombocytopenia in mice. Front Immunol 2021;12:616394.
58. Lien TS, Sun DS, Hung SC, Wu WS, Chang HH. Dengue virus envelope protein domain III induces Nlrp3 inflammasome-dependent netosis-mediated inflammation in mice. Front Immunol 2021;12:618577.
59. Atrooz F, Alkadhi KA, Salim S. Understanding stress: Insights from rodent models. Curr Res Neurobiol 2021;2:100013.
60. Koh SJ, Kim JW, Kim BG, Lee KL, Kim JS. Restraint stress induces and exacerbates intestinal inflammation in interleukin-10 deficient mice. World J Gastroenterol 2015;21:8580-7.
61. Seewoo BJ, Chua EG, Arena-Foster Y, Hennessy LA, Gorecki AM, Anderton R, et al. Changes in the rodent gut microbiome following chronic restraint stress and low-intensity rTMS. Neurobiol Stress 2022;17:100430.
62. Thu AC. The use of platelet-rich plasma in management of musculoskeletal pain: a narrative review. J Yeungnam Med Sci 2022;39:206-15.
63. Liang Y, Li J, Wang Y, He J, Chen L, Chu J, et al. Platelet rich plasma in the repair of articular cartilage injury: A narrative review. Cartilage 2022;13:19476035221118419.
64. Cao Y, Zhu X, Zhou R, He Y, Wu Z, Chen Y. A narrative review of the research progress and clinical application of platelet-rich plasma. Ann Palliat Med 2021;10:4823-9.
65. Ku HC, Cheng CF. Master regulator activating transcription factor 3 (ATF3) in metabolic homeostasis and cancer. Front Endocrinol (Lausanne) 2020;11:556.
66. Sun DS, Chang HH. Emerging role of the itaconate-mediated rescue of cellular metabolic stress. Tzu Chi Med J 2022;34:134-8.
67. Geng JG, Chen M, Chou KC. P-selectin cell adhesion molecule in inflammation, thrombosis, cancer growth and metastasis. Curr Med Chem 2004;11:2153-60.
68. Walf AA, Frye CA. The use of the elevated plus maze as an assay of anxiety-related behavior in rodents. Nat Protoc 2007;2:322-8.
69. Siwakoti B, Lien TS, Lin YY, Pethaperumal S, Hung SC, Sun DS, et al. The role of activating transcription factor 3 in metformin's alleviation of gastrointestinal injury induced by restraint stress in mice. Int J Mol Sci 2023;24:10995.