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# Effect of Fatty Acid Present in Cooking Oil on Gut Barrier Function

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**Abstract:** *Understanding the impact of fatty acids present in cooking oils on gut barrier function is critical for elucidating their role in gastrointestinal health. This review investigates the effects of Fatty acid and Edible oil on gut barrier integrity using a multifaceted approach, including transepithelial electrical resistance (TEER) assays, trypan blue exclusion assays, and MTT assays. Epithelial cells were seeded onto Transwell inserts to form tight monolayers, reflecting the physiological epithelial barrier. Treatment with varying concentrations of FA and EO, alongside appropriate controls, revealed concentration-dependent effects on TEER values, indicating alterations in barrier integrity. The inclusion of trypan blue exclusion assays allowed for the assessment of cell viability, highlighting the cytotoxic effects of fatty acids on epithelial cells. Graphical representations illustrated the differential impacts of EO and FA on cell viability and gut barrier function. Furthermore, the introduction of lipid micelle preparations as a treatment parameter aimed to mimic physiological conditions post-consumption of fatty acids, enhancing the relevance of experimental models. MTT assays in 96-well plates provided complementary insights into cell viability in response to different concentrations of fatty acids and edible oils, demonstrating a trend of increasing cell viability from high to low concentrations. Fatty acid exhibited concentration-dependent decreases in cell viability and TEER values, suggesting a potential compromise in barrier integrity. Conversely, Edible oil displayed milder effects, indicating a less detrimental impact on gut barrier function.*

**Keywords**: Edible oil, Fatty acids, MTT assay, Monolayers, Cooking oils

**Abbreviations:** Inflammatory Bowel Disease (ibd), Monounsaturated fatty acid (mufa), Polyunsaturated fatty acid (pufa), Toll-like receptors (tlr), Nuclear factor kappa B ( nf-kb ), Short-chain fatty acid (SCFA), Conjugated linoleic acids ( cla), Nanoparticle Tracking Analysis (nta).

## I. INTRODUCTION

The human gut, a complex ecosystem, plays a pivotal role in maintaining overall health and well-being [1]. Central to its function is the integrity of the gut barrier, a selective interface that regulates the passage of nutrients, ions, and microorganisms between the intestinal lumen and the systemic circulation [2]. The gut is the main site for absorption of nutrients by the body. Homeostasis of gut barrier is an essential property of host health that provides protection from environment and maintains the absorption of nutrients from gastrointestinal tract [3].The gut barrier, comprised of a complex interplay of epithelial cells, mucus, and immune cells, serves as the first line of defense against luminal antigens and pathogens. Disruption of this barrier can lead to increased intestinal permeability, commonly referred to as "leaky gut," which has been implicated in the pathogenesis of various gastrointestinal disorders, as well as systemic inflammatory conditions [4]. Numerous factors influence the integrity and function of this barrier, including dietary constituents. Among these, fatty acids, ubiquitous components of cooking oils, have garnered significant attention for their potential impact on gut barrier function [5]. Dietary components, such as omega-6 fatty acids, long-chain fatty acids, protein, and digestible carbohydrates, may contribute to Inflammatory Bowel Disease pathogenesis through altering intestinal microbiota, increasing intestinal permeability, and promoting inflammation; whereas omega-3 fatty acids, medium chain triglycerides, and nondigestible carbohydrates improve these parameters and intestinal health [6].

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Assessing the integrity of the gut barrier is essential for understanding the pathophysiology of these conditions and identifying potential therapeutic targets. One widely used method for evaluating gut barrier function is the transepithelial electrical resistance (TEER) assay. The transepithelial electrical resistance (TEER) assay is a valuable technique for assessing the integrity of epithelial barriers, such as those found in the gastrointestinal tract. The mechanism of the TEER assay is based on the principle of measuring the resistance to the flow of ions and small molecules across an epithelial monolayer. The TEER measurement setup typically involves culturing epithelial cells on a porous membrane within a specialized culture insert, such as a Transwell insert. The cells form a tight monolayer, mimicking the epithelial barrier found in vivo. To conduct the assay, two electrodes are placed on either side of the epithelial monolayer: one electrode on the apical side (the side facing the lumen) and the other on the basolateral side (the side facing the underlying tissue or medium). A small, constant electric current is then passed across the monolayer, typically in the range of milliamps.As the current passes through the epithelial monolayer, it encounters resistance due to the presence of tight junctions between adjacent cells. Tight junctions are composed of transmembrane proteins, such as claudins and occludins, which form physical barriers that regulate the movement of ions and molecules between cells. The resistance offered by these tight junctions impedes the flow of current, leading to a measurable voltage difference across the epithelial layer. TEER values are obtained by measuring this voltage difference and applying Ohm's law, which relates voltage, current, and resistance ($V = IR$). The resistance (R) is calculated as the voltage (V) divided by the current (I). Higher resistance values indicate a more intact tighter junctions and epithelial barrier, while lower resistance values suggest increased permeability and compromised barrier function [7].Gut microbiota plays a crucial role in glucose and lipids metabolism [8]. Dietary fats have important influence on gut microbiota. Many studies have demonstrated that high fat intake induced changes of gut microbiota at the phyla level and genus level, meanwhile the change of gut microbiota led to high-fat diet induced obesity and metabolic disorders [9] [10].

### 1.1. Cooking Oils

Cooking oils are a staple in culinary practices worldwide, serving as a fundamental component in food preparation. However, beyond their role in flavor enhancement and cooking, these oils contain various fatty acids with potential implications for human health. These oils serve as a primary source of dietary fats, providing essential fatty acids necessary for various physiological processes [11]. While fats are indispensable for energy storage, membrane structure, and signal transduction, emerging evidence suggests that certain fatty acids may exert differential effects on gut barrier integrity. Of particular interest are long-chain saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acids (PUFAs), each with distinct biochemical properties and biological activities [12].

### 1.2. Fatty Acids

SFAs, commonly found in animal-derived products and tropical oils, have been implicated in the disruption of gut barrier function [13]. Studies have demonstrated that high intake of SFAs may increase intestinal permeability, leading to translocation of luminal antigens and inflammation [14].Conversely, MUFAs, predominant in olive oil and avocados, exhibit protective effects on the gut barrier. Research indicates that MUFAs may enhance epithelial barrier integrity and attenuate mucosal inflammation, thereby preserving gut homeostasis [15]. PUFAs, including omega-3 (n-3) and omega-6 (n-6) fatty acids, exert diverse effects on gut barrier function depending on their specific composition and dietary ratio [16]. While n-3 PUFAs, abundant in fish oil and flaxseed oil, possess anti-inflammatory properties and may enhance mucosal defense mechanisms, excessive intake of n-6 PUFAs relative to n-3 PUFAs has been associated with compromised gut barrier integrity and increased susceptibility to inflammatory bowel diseases [17]. The mechanisms underlying the influence of fatty acids on gut barrier function are multifaceted and intricate [18]. At the cellular level, fatty acids interact with various signaling pathways, transcription factors, and membrane receptors to modulate epithelial permeability, tight junction integrity, and mucosal immune responses [19]. SFAs, for instance, have been shown to activate toll-like receptors (TLRs) and nuclear factor kappa B (NF-κB) signaling, promoting pro-inflammatory cytokine production and compromising epithelial barrier function. In contrast, MUFAs and PUFAs may exert anti-inflammatory effects by inhibiting NF-κB activation, enhancing mucin production, and promoting the

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synthesis of cytoprotective lipid mediators [20]. Beyond their direct effects on epithelial cells, fatty acids also influence the composition and metabolic activity of the gut microbiota. Emerging evidence suggests that dietary fatty acids can modulate the gut microbiota composition, favoring the growth of beneficial bacteria e.g. *Bifidobacteria, Lactobacilli* while suppressing the expansion of pathogenic microbes e.g. *Escherichia coli, Clostridia* [21]. Moreover, microbial metabolism of dietary fatty acids generates bioactive metabolites, such as short-chain fatty acids (SCFAs) and conjugated linoleic acids (CLAs), which play pivotal roles in regulating intestinal barrier function, immune responses, and host-microbe interactions [22].

### 1.3. Intestinal Epithelial Cell lines

Cell culture techniques have transformed biomedical research by providing powerful tools for studying cellular physiology, disease mechanisms, and drug development in a controlled laboratory setting. Within the realm of gastrointestinal research, intestinal epithelial cell lines occupy a prominent position due to their relevance for studying gut physiology, barrier function, and interactions with luminal factors and commensal microbiota [23]. These cell lines, derived from normal or malignant intestinal epithelium, offer insights into mechanisms underlying nutrient absorption, mucosal immunity, and the pathogenesis of gastrointestinal disorders such as inflammatory bowel disease (IBD) and colorectal cancer [24]. Among the intestinal epithelial cell lines commonly used in research, the T84 cell line stands out as a representative model for studying epithelial barrier function, ion transport, and mucosal inflammation [25]. The colonic cancer cell line Caco-2 has the ability to generate monolayers that are differentiated, polarized and contains cells that resemble tiny intestinal enterocytes. T84 cell line is human IE cell line derived from a colorectal adenocarcinoma, originating from lung metastasis. T84 and Caco-2 cell lines both are when cultured, differentiate spontaneously at confluence into monolayer of structurally and functionally mature absorptive epithelial cells. Both differentiated monolayers are well-established in vitro models of the human intestinal epithelium and often utilized for studying bioavailability, absorption, metabolism, electrolyte transport, and impact of substances on epithelial barrier integrity. Derived from human colonic adenocarcinoma tissue, T84 cells exhibit characteristics reminiscent of mature enterocytes and form polarized monolayers with well-defined apical and basolateral domains, making them particularly well-suited for investigating aspects of intestinal physiology and host-microbiota interactions.

## II. MATERIALS AND METHODS

Comprehensive series of in vitro assays were employed to investigate the effects of fatty acids and edible oils on gut barrier function. The following methodologies were utilized:

**2.1 Total Cell Count and Seeding**: T84 cells, a model for intestinal epithelial cells, were trypsinized, centrifuged, and stained with trypan blue for viability assessment using a hemocytometer. Cells were seeded into 96-well plates at a density of 1000 cells per well for subsequent assays.

**2.2 MTT Assay**: The MTT assay, which measures cell metabolic activity as an indicator of cell viability, was conducted after exposing cells to various concentrations of fatty acids and edible oils. The assay involved the addition of MTT reagent to the wells, followed by incubation and measurement of absorbance at 570 nm and 630 nm to assess cellular viability.

**2.3 TEER Measurements**: Trans-Epithelial Electrical Resistance (TEER) was measured to assess the integrity of epithelial cell monolayers. TEER provides a quantitative measure of barrier function by evaluating the resistance to ion flow across the cell layer. Higher TEER values indicate stronger barrier integrity, while lower values suggest compromised barrier function.

**2.4 Trypan Blue Exclusion Assay**: This assay was used to assess cell viability and membrane integrity by differentiating live (unstained) from dead (stained) cells. After treatment with fatty acids and edible oils, cells were stained with trypan blue and counted using a hemocytometer.

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## III. RESULTS

We investigated the influence of fattyacids found in cooking oils on gut barrier function, focusing specifically on FA and EO. Employing a series of in vitro assays and experimental setups, we aimed to elucidate theireffects on cellular viability and membrane integrity, with the overarching goal of contributingto our understanding of their impact on gastrointestinal health. Our experimental approachinvolved seeding cells in 24-well plates and subjecting them to varying concentrations of FAand EO. Control wells were included to establish baselines for comparison. Utilizing theTrypan blue exclusion assay, we assessed cell viability and membrane integrity, revealing dose-dependent responsesto both fatty acids and edible oil. Higher concentrations were associatedwith increased cellular damage, suggesting a potential compromise in gut barrier function.Additionally, we conducted the MTT assay in 96-well plates to further investigate cell viabilityfollowing exposure to varying concentrations of fatty acids and edible oils. Consistent with ourprevious findings, we observed a dose-dependent decrease in cell viability at highconcentrations, indicative of cytotoxic effects. Trans-epithelial electric resistance (TEER)values were measured across different treatment groups to assess cellular barrier integrity. Thecontrol group exhibited the highest TEER value, indicative of robust barrier function. Thevehicle group showed a slight reduction, likely due to minor physiological changes induced byvehicle components. Treatment with high concentrations of edible oil and fatty acids resultedin decreased TEER values, suggesting disruption to barrier integrity. However, lowerconcentrations of thesecompounds demonstrated milder effects, indicating a dose-dependentresponse. Notably, the low concentration fatty acid group exhibited higher TEER valuescompared to the high concentration group. These findings highlight the sensitivity of cellularbarriers to varying concentrations of lipids and underscore the importance of understandingdose-response relationships.

## IV. DISCUSSION

This study aimed to elucidate the effects of fatty acids and edible oils on gut barrier integrity, employing a comprehensive approach that included transepithelial electrical resistance (TEER) assays, trypan blue exclusion assays, and MTT assays. The integration of these methodologies allowed for a detailed investigation into how specific dietary components influence epithelial cell viability and barrier function.Fatty acids, particularly those found in cooking oils, have garnered attention for their diverse roles in health and disease. Their impact on gut barrier function is of particular interest, given the barrier's critical role in maintaining intestinal homeostasis and preventing the translocation of pathogens and toxins. The TEER assay, a widely used method to assess epithelial barrier integrity, provided valuable insights into how different concentrations of fatty acids and edible oils affect tight junction function. Tight junctions are integral to the epithelial barrier, and their disruption can lead to increased intestinal permeability, often referred to as "leaky gut."

The trypan blue exclusion assay allowed for the assessment of cell viability, revealing potential cytotoxic effects of fatty acids and oils on epithelial cells. This assay is crucial as it helps to distinguish between the direct effects on barrier integrity and broader impacts on cell health. The observed cell viability provides a layer of understanding about the cellular mechanisms that may be at play, such as oxidative stress or lipid peroxidation, which could contribute to the observed alterations in barrier function.The introduction of lipid micelle preparations as a treatment parameter was a significant enhancement to the experimental model. By simulating physiological conditions post-consumption of fatty acids, this approach provided a more realistic representation of how dietary lipids interact with the gut epithelium. Micelles are the primary form in which lipids are absorbed in the intestine, and their study offers insights into the initial stages of lipid metabolism and their immediate effects on the gut barrier.The MTT assay, used to evaluate mitochondrial activity and thus cell viability, complemented the findings from the TEER and trypan blue assays. This assay underscores the importance of assessing cellular metabolic activity, which is crucial for maintaining barrier function. The metabolic state of epithelial cells can influence their ability to form and maintain tight junctions, and disruptions in mitochondrial function can lead to compromised barrier integrity.

Understanding the differential effects of various fatty acids and edible oils on gut barrier function has significant implications for dietary recommendations and public health. Fatty acids, depending on their type and concentration, can have vastly different impacts on the gut epithelium. For instance, certain fatty acids may enhance barrier functionor provide protective effects against inflammation, while others may exacerbate intestinal permeability and contribute to gastrointestinal disorders. The findings of this study emphasize the need for a nuanced approach to dietary fat

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consumption, particularly in the context of gut health. It is not sufficient to consider the quantity of dietary fats alone; the quality and specific types of fatty acids must also be taken into account. Future research should focus on the mechanistic pathways through which different fatty acids influence tight junction proteins and overall epithelial barrier integrity. Additionally, exploring the potential of combining dietary fatty acids with other nutrients or bioactive compounds to mitigate adverse effects and enhance gut health is a promising area for further investigation.

## V. CONCLUSION

The presence of fatty acids in edible oils highlights the importance of understanding their impact on gut barrier function. This review emphasizes the dose-dependent effects of fatty acids on epithelial cell viability and barrier integrity, underscoring the need for regulatory measures and consumer awareness. By making informed dietary choices and opting for oils with balanced fatty acid profiles, individuals can support overall health and well-being. Future research should continue to explore the molecular mechanisms underlying these effects and the long-term implications for gastrointestinal health.

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