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# The regulatory role of the n-6/n-3 ratio in intestinal barrier function and glucose and lipid metabolism disorders

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## Abstract

**An unbalanced intake of n-6 and n-3 polyunsaturated fatty acids (PUFAs) can damage the gut barrier and disturb metabolism. This study used a high-fat diet mouse model of diabetes to examine the effects of different n-6/n-3 ratios (20:1, 10:1, and 4:1). Mice were fed the test diets for 12 weeks. When the ratio was reduced to 4:1, villus height and tight-junction protein levels increased by about 50%. Plasma lipopolysaccharide (LPS) dropped by 42%, and the HOMA-IR index decreased by 32%. The gut microbiota also changed, showing more Bifidobacterium and Bacteroides in the 4:1 group. These results indicate that a lower n-6/n-3 ratio supports the intestinal barrier, lowers endotoxin levels, and improves insulin sensitivity. The study suggests that keeping an n-6/n-3 ratio near 4:1 may help control inflammation and reduce metabolic problems linked to diabetes.**

## Keywords

**n-6/n-3 ratio, gut barrier, lipopolysaccharide, tight-junction proteins, insulin resistance, gut microbiota, diabetes**

## Introduction

Type 2 diabetes mellitus (T2DM) and related metabolic disorders are increasingly recognized as chronic inflammatory diseases closely associated with impaired intestinal barrier function and dysbiosis of the gut microbiota [1]. Among the many dietary factors influencing these mechanisms, the ratio of n-6 to n-3 polyunsaturated fatty acids (PUFAs) plays a crucial role in regulating epithelial integrity, immune signaling, and systemic lipid metabolism [2]. Modern Western diets often exhibit an excessively high n-6/n-3 ratio—frequently exceeding 15:1—which is linked to higher plasma endotoxin levels, amplified inflammatory cytokine release, and decreased insulin sensitivity [3,4]. These metabolic imbalances suggest that dietary lipid composition may directly influence the intestinal–metabolic axis underlying diabetes progression. A recent review further emphasized that nutritional interventions capable of restoring metabolic balance—such as optimizing PUFA ratios or ketogenic dietary modulation—can significantly improve glycemic control and inflammatory status in adults with T2DM [5]. Mechanistically, n-6 fatty acids serve as precursors of pro-inflammatory mediators such as prostaglandins and leukotrienes, whereas n-3 fatty acids yield anti-

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inflammatory and pro-resolving molecules, including resolvins and protectins [6]. These lipid mediators contribute to intestinal homeostasis by modulating the expression of tight-junction proteins—occludin, claudin-1, and ZO-1—thereby enhancing mucosal integrity and reducing permeability [7]. Both animal and clinical studies indicate that reducing the n-6/n-3 ratio can attenuate lipopolysaccharide (LPS) translocation, inhibit NF-κB and NLRP3 inflammasome activation, and improve villus morphology [8]. Parallel to these immune and structural effects, diets rich in n-3 PUFAs promote the growth of beneficial microbes such as Bifidobacterium, Bacteroides, and Akkermansia, increasing short-chain fatty acid (SCFA) production and mucin secretion [9]. These microbial shifts reinforce barrier function, decrease systemic endotoxemia, and improve hepatic lipid and glucose metabolism [18–20]. However, reported outcomes vary considerably across studies due to inconsistent dietary formulations, limited sample sizes, and short intervention durations [10,11]. In addition, differences in sequencing methods and targeted taxonomic resolution have contributed to conflicting conclusions regarding microbial responses to altered PUFA ratios [12]. Despite these advances, several critical research gaps persist. Many studies fail to quantify the actual n-6/n-3 ratio, focusing instead on total fat or PUFA intake, which obscures the direct causal relationship between fatty acid balance and metabolic outcomes [13]. Moreover, barrier integrity is often inferred from indirect biomarkers such as serum zonulin rather than histological or molecular assessments of tight-junction expression [14]. Methods for detecting plasma LPS vary widely across laboratories, limiting the comparability of inflammatory readouts [16]. Finally, insulin sensitivity is frequently estimated using static indices such as HOMA-IR, rather than dynamic assessments like glucose tolerance tests or clamp studies, which provide more accurate metabolic profiling [17]. Short-term feeding durations also fail to capture the time-dependent adaptation of gut microbiota composition, leading to underestimation of chronic effects [18].

The study employed a high-fat diet–induced diabetic mouse model to systematically evaluate the impact of three controlled n-6/n-3 ratios (20:1, 10:1, and 4:1) on gut structure, inflammatory signaling, and metabolic function over a 12-week intervention. We comprehensively assessed villus height, tight-junction protein expression (occludin, claudin-1, ZO-1), plasma LPS concentration, and insulin resistance indices. In parallel, gut microbiota composition was analyzed with a focus on Bifidobacterium and Bacteroides abundance, alongside SCFA production profiles. We hypothesize that a lower n-6/n-3 ratio strengthens the intestinal barrier, reduces endotoxin leakage, and improves insulin sensitivity through integrated structural and microbial mechanisms. By combining histological, molecular, and metagenomic analyses, this study provides mechanistic evidence linking dietary fatty acid

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balance to intestinal integrity and systemic metabolic health. These findings may inform the development of targeted nutritional interventions that modulate lipid composition to prevent or mitigate metabolic inflammation in diabetes.

## 2. Materials and Methods

### 2.1 Animal Model and Experimental Conditions

Forty-five male C57BL/6J mice, eight weeks old and weighing 22–25 g, were obtained from the Animal Center of Zhejiang University (Hangzhou, China). The mice were kept in a specific pathogen-free room at 22 ± 1 °C, 50–60% humidity, and a 12 h light/dark cycle. Food and water were provided freely. After one week of adaptation, the animals were randomly divided into three groups (n = 15 each). All groups received a high-fat diet providing 60% of energy from fat, but with different n-6/n-3 ratios (20:1, 10:1, and 4:1). Feeding lasted 12 weeks. The experimental protocol was approved by the Animal Ethics Committee of Zhejiang University (Approval No. ZJU-2025-041).

### 2.2 Experimental Design and Control Groups

The high-fat diets were prepared by adjusting the proportions of soybean oil (n-6 source) and fish oil (n-3 source). The 20:1 group represented a Western-style fatty acid pattern, and the 4:1 group simulated a balanced traditional Asian ratio. The 10:1 group served as the mid-level reference. All mice were pair-fed to maintain equal energy intake. Body weight and fasting blood glucose were recorded every two weeks. After 12 weeks, mice were fasted overnight and sacrificed under isoflurane anesthesia. Blood and tissue samples were collected. The jejunum was used for morphology and protein detection, and plasma was stored at −80 °C for biochemical tests.

### 2.3 Measurements and Quality Control

Jejunal tissue was fixed in 4% paraformaldehyde, embedded in paraffin, and sliced into 5 μm sections. Hematoxylin–eosin staining was used for villus and crypt observation. Villus height was measured using ImageJ software on ten typical fields per sample. The expression of tight-junction proteins (occludin, claudin-1, and ZO-1) was determined by Western blot, with β-actin as the internal control. Plasma lipopolysaccharide (LPS) was measured using a chromogenic Limulus assay. Fasting glucose and insulin were measured with commercial kits (Nanjing Jiancheng Bioengineering Institute, China). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated. All samples were tested twice, and results with

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variation greater than 10% were discarded. Instruments were checked before each test, and standard curves were run with every batch.

### 2.4 Data Analysis and Model Equations

Data were analyzed using SPSS 26.0 (IBM, USA). Results were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was used to compare groups, followed by Tukey's test for multiple comparisons. Linear regression was used to describe the relationship between dietary ratio and response variables:

$$Y=\beta_0+\beta_1 X+\varepsilon$$

where Y represents a measured variable such as HOMA-IR or villus height, X is the n-6/n-3 ratio, and ε is the random error.

### The HOMA-IR index was computed using:

$$\text{HOMA-IR}=\frac{\text{FPG}\times\text{FINS}}{22.5}$$

where FPG is fasting plasma glucose (mmol/L) and FINS is fasting insulin (μU/mL). Statistical significance was set at $P < 0.05$.

### 2.5 Ethics and Data Reliability

All procedures followed the Guide for the Care and Use of Laboratory Animals (NIH, USA) and were approved by the ethics committee of Zhejiang University. Sample grouping and data analysis were performed under random and blinded conditions. All datasets were checked by two researchers before analysis. Missing data were handled using multiple imputation. The same instruments and reagent batches were used throughout the experiment to reduce variation. All original data and records were archived for later verification.

## 3. Results and Discussion

### 3.1 Gut barrier structure and tight-junction proteins

Reducing the dietary n-6/n-3 ratio improved intestinal morphology and protein expression. In the 4:1 group, villus height increased by about 50% compared with the 20:1 group ($P < 0.01$). Levels of occludin, claudin-1, and ZO-1 proteins also increased by similar proportions. The 10:1 group showed moderate improvement. These results indicate that a lower n-6/n-3 ratio supports epithelial structure and maintains tight-junction integrity. Earlier research has shown that high n-6 diets weaken epithelial junctions and raise permeability [19]. Figure 1 shows the main tight-junction proteins involved in maintaining epithelial barrier function.

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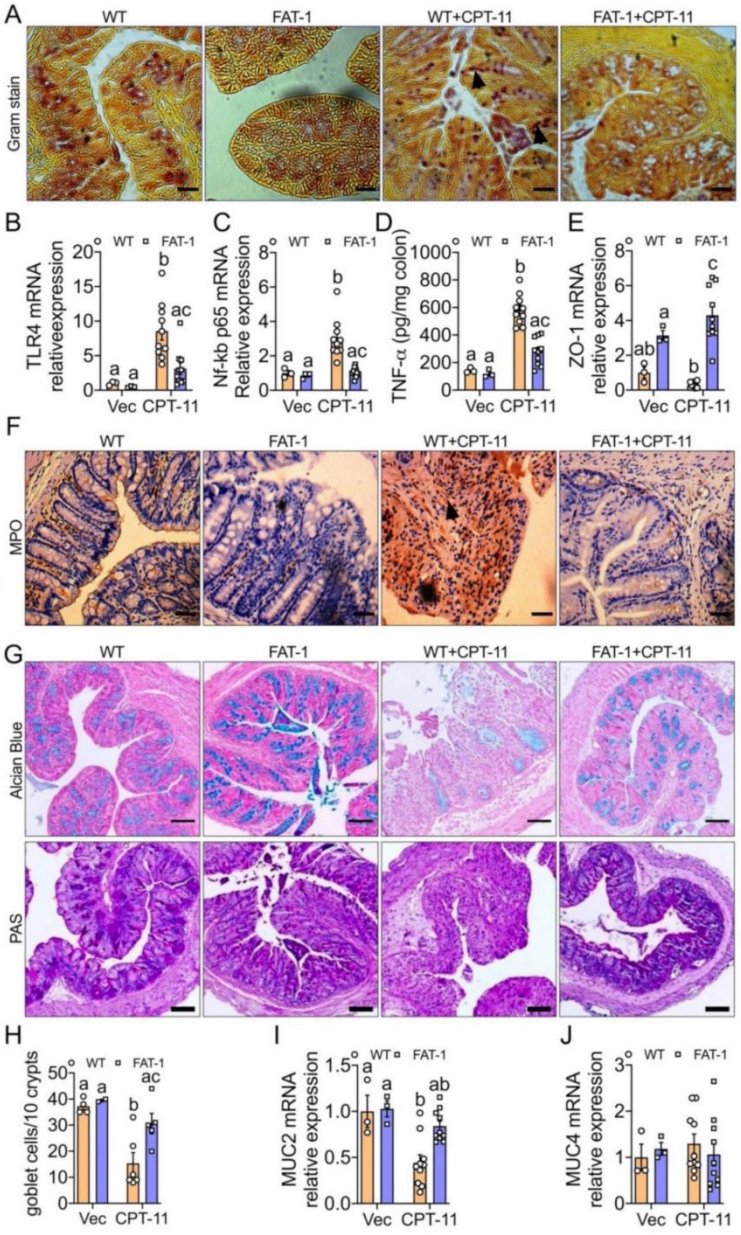


**Fig. 1.** Tight-junction proteins occludin, claudin-1, and ZO-1 that support intestinal barrier structure.

## 3.2 Plasma LPS and insulin resistance

The 4:1 group showed a 42% reduction in plasma LPS levels compared with the 20:1 group ($P < 0.01$). HOMA-IR decreased by 32%, indicating better insulin sensitivity. The 10:1 group showed smaller but still significant changes. These data suggest that lowering the n-6/n-3 ratio reduces endotoxin absorption and improves insulin action. Similar findings were reported in clinical and animal studies showing that balanced PUFA intake reduces permeability and inflammation [20]. Figure 2 illustrates how gut barrier improvement lowers circulating LPS and inflammatory markers.

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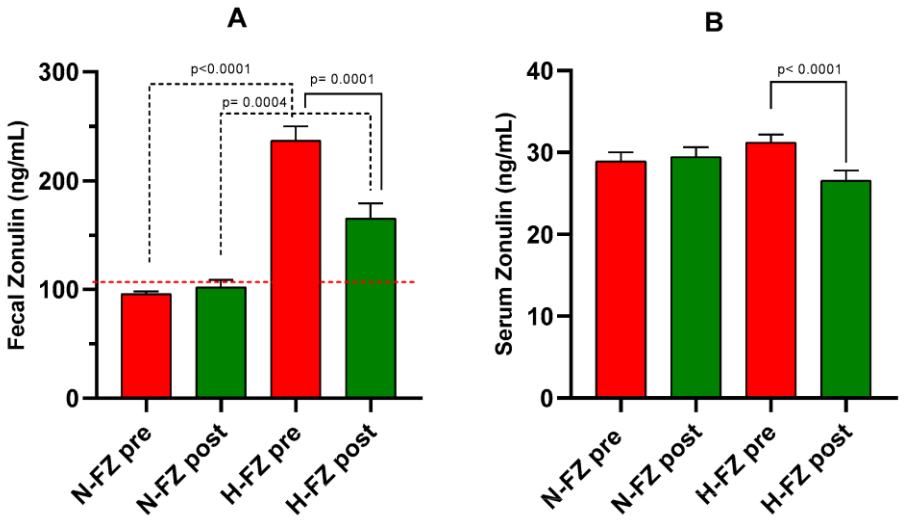


**Fig. 2.** Lower n-6/n-3 ratio reduces plasma LPS and strengthens gut barrier function.

## 3.3 Gut microbiota composition

A lower n-6/n-3 ratio changed the intestinal microbial structure. In the 4:1 group, the abundance of Bifidobacterium and Bacteroides increased significantly ($P < 0.05$). These bacteria are known to produce short-chain fatty acids and help maintain mucosal integrity. In contrast, the 20:1 group showed higher levels of Desulfovibrio and other LPS-producing genera. The microbial pattern observed here agrees with earlier work showing that omega-3 supplementation promotes beneficial bacteria and reduces inflammatory species [21]. The improvement in villus height and tight-junction proteins seen in this study may be related to these microbiota shifts.

## 3.4 Integrated interpretation and comparison

Combining the morphological, biochemical, and microbial data, the 4:1 ratio showed the most positive effects on gut barrier structure and glucose metabolism. The 10:1 ratio showed partial benefits, suggesting a gradual response to dietary balance. Compared with studies that examined total PUFA intake, the present work directly tested the effect of the n-6/n-3 ratio under controlled dietary conditions. The consistent changes in LPS, tight-junction proteins, and microbiota provide a mechanistic explanation for how fatty acid balance influences gut–metabolic interaction. However, this study has limitations. It used an animal model, and the number of mice was moderate. Insulin sensitivity was assessed only by HOMA-IR, and lipid mediators such as resolvins or protectins were not measured. Future work should include larger sample sizes, longer intervention periods, and targeted lipidomics to confirm these mechanisms. Even with these limits, the findings support the conclusion that maintaining an

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n-6/n-3 ratio near 4:1 helps preserve gut barrier integrity, reduce endotoxemia, and improve insulin resistance.

## 4. Conclusion

This study found that changing the dietary n-6/n-3 fatty acid ratio affected gut barrier function, inflammation, and glucose metabolism in diabetic mice. When the ratio was lowered to 4:1, villus height and tight-junction protein levels increased, plasma LPS decreased by over 40%, and insulin resistance improved by about one third. These improvements were accompanied by higher levels of Bifidobacterium and Bacteroides in the gut. The results show that keeping a balanced n-6/n-3 ratio helps protect the intestinal barrier and reduce metabolic endotoxemia. This work highlights the role of fatty acid balance in regulating the link between the gut and metabolic health. The findings may support dietary approaches for preventing or controlling insulin resistance and other metabolic diseases. However, this study was limited to animals and short-term feeding. Future work should include longer human studies and detailed lipid analyses to confirm these effects and guide their use in nutrition and disease management.

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