# Diet, nutrition and intestinal permeability: A mini review

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**Running title:** Nutrition and intestinal permeability

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**ABSTRACT**

**Background and Objectives:** Intestinal permeability (IP) is known to contribute to the immune system activation and inflammation; thus, it is proposed to have a role in the pathogenesis and exacerbation of many chronic diseases. Several studies have indicated that diet and nutritional status are risk factors for increased IP. In this mini review, we discussed the recent evidence on the association of diet, nutritional status, and intestinal permeability assessed by zonulin concentrations in serum and feces. **Methods and Study Design:** Literature searching was conducted in Pubmed, ProQuest and Google Scholar using the keywords "diet quality", "intestinal permeability", "nutritional status", and "zonulin" combined with Boolean operators "AND" and "OR". **Results:** Some studies indicated that intake of proper nutrition and good diet such as low total calorie intake, high intakes of omega-3 polyunsaturated fatty acids, fiber, vitamins, minerals, probiotics, and polyphenol-rich diet have significant impact on improvement of intestinal permeability marked by lower zonulin concentrations. Higher zonulin concentrations are found in those with overweight and obesity indicating that these population have increased IP. Most studies were conducted in adults and there are limited studies in children and adolescents. In addition, no studies have assessed diet quality to obtain a comprehensive picture on the complexities of diet in the population in relation to intestinal permeability. **Conclusions:** Diet and nutritional status are linked to zonulin concentrations, indicating a role in intestinal permeability. Further research should be conducted to investigate the relationship between diet quality, as measured by appropriate diet quality indices, and intestinal permeability in children, adolescents, and adults.

**Key Words: diet quality, nutrient intake, nutritional status, zonulin**

## INTRODUCTION

Dietary quality is associated with nutritional status, biological markers of inflammation, various health outcomes, well-being, and health-related quality of life in children and adolescents.[1-4] However, studies found that adolescents have poor diet quality. A study in Brazil found that diet quality among adolescents was the worst compared to diet quality of adults and elderly. Adolescents had low scores in total grains, dark-green and orange vegetables and legumes, oils and solid fat, alcohol and added sugar.[5] A study in West Java also showed poor diet quality and diversity in adolescent girls, especially among anemic girls.[6]

Zonulin is regarded as a prospective biomarker for intestinal permeability. It is known as the only physiological protein reversibly controlling intestinal permeability by modulating intercellular tight junctions.[7,8] Higher concentration of zonulin indicated more permeable tight junctions, which enable microbial translocation.[9] Mörkl et al[10] found that zonulin concentration is positively associated with total calorie, protein, carbohydrate, sodium, and vitamin B12 intakes in women but no association was found between zonulin concentration with fiber and fat intake. Other study showed that omega-3 polyunsaturated fatty acids (PUFAs), fiber, and a range of vitamins and minerals intakes were higher in overweight pregnant women with low zonulin concentration compared to those with high zonulin concentration.[11] This indicate that better diet quality might be associated with lower zonulin concentration, thus, lower intestinal permeability.

Studies exploring the relation between nutrition and health outcomes commonly focus on specific macro- or micronutrients.[12-15] This approach is important to investigate specific biologic mechanism, but humans eat diverse foods, not single nutrient. Besides, single nutrient studies did not account for their interaction with other nutrients that will affect the overall health outcomes. As studies on diet quality and intestinal permeability are limited, we would like to review several studies regarding dietary intakes and nutritional status in association with intestinal permeability assessed by zonulin.

## MATERIALS AND METHODS

Literature searching was conducted in Pubmed, ProQuest and Google Scholar using the keywords “diet quality”, “intestinal permeability”, “nutrient intake”, “nutritional status”, and “zonulin” combined with Boolean operators “AND” and “OR”. Original, peer-reviewed human studies published in English that assessed the association between dietary intakes, nutritional status, and zonulin were included in this review.

## RESULTS AND DISCUSSION

### *Intestinal permeability*

Recent findings have signified the crucial role of intestinal barrier and intestinal permeability in health and disease. Intestinal barrier is a functional body dividing the intestinal lumen from the inner host, which involves mechanical components (mucus, epithelial layer), humoral components (defensins, immunoglobulin A), immunological components (lymphocytes, innate immune cells), muscular and neurological components.[16] The intestinal barrier protects an area of approximately 400 $m^2$ and needs about 40% of energy expenditure of the body. It

inhibits water and electrolytes loss as well as antigen and microorganism invasion into the body whilst permitting molecules exchange between host and environment and nutrient absorptions from food.[16,17] Meanwhile, the definitions of intestinal permeability, normal and impaired intestinal permeability is described in Table 1.

Several factors may modify intestinal permeability, such as change in gut microbiota and mucus layer, and damage of epithelial layer that cause translocation of luminal content to the inner layers of the intestinal wall. In addition, lifestyle changes and dietary factors, for instance alcohol and energy dense-food or high fat diet (as in Western lifestyle and diet), can raise intestinal permeability.[18-20] A systematic review reported that the strongest risk factors for altered intestinal permeability were Western style diet, anthropometric measurement related to obesity, hyperglycemia, dyslipidemia, inflammation, advanced disease severity, and comorbidity.[21] These findings suggest that nutrition and infection factors could modify the intestinal permeability.

***Zonulin as a biomarker for intestinal permeability***

Intestinal permeability controls molecular exchange between the intestinal lumen and the submucosa, resulting in tolerance or immunity to non-self-antigens.[22,23] The intercellular tight junctions controls this paracellular antigen exchange strictly. The tight junctions are recognized as dynamic structures of the intestinal epithelium with a number of key functions in physiological and pathological conditions.[24]

Zonulin is known as a physiological mediator that controls intestinal permeability by modifying intercellular tight junctions.[7,25] Zonulin was first identified as a human zonula occludens toxin (Zot) homologue. Zot was discovered as a toxin that destroyed tight junction mechanisms produced by Vibrio cholera.[26] Zonulin is a 47-kDa protein, classified as pre-haptoglobin-2 (precursor of haptoglobin-2), which raises intestinal permeability in small intestine and involves in innate immunity of the intestine. Zonulin attached to the epidermal growth factor receptor (EGFR) and protease-activated receptor 2 (PAR2) in the epithelium of intestine. This complex prompt zonula occludens proteins phosphorylation, which resulted in derangement of the tight junctions in the small intestine.[27] The tight junctions opening stimulated by zonulin, cause water to be secreted into the intestinal lumen according to hydrostatic pressure gradients, which could subsequently flushed out bacteria and other microorganisms. It is a host defense mechanism against the presence of microorganisms or their cell wall components on the mucose of proximal small intestine.[28]

Serum zonulin is regarded as a potential marker of intestinal permeability.[7] It has been validated to be strongly associated with lactulose/mannitol ratio, a test used to examine intestinal permeability in several gastrointestinal diseases and malnutrition.[29,30] The main secretion of zonulin is from the liver, but it is also secreted by other organs and tissues such as brain, heart, lungs, kidney, skins, enterocytes, adipose tissue, and immune cells.[7,31] Zonulin secretion is stimulated by gluten and bacteria.[32]

***Association of zonulin with inflammatory markers, nutritional status and dietary intakes***

Zak-Golab et al.[33] revealed that plasma zonulin could indicate systemic microinflammation related to gut microbiota in obese subjects as it was positively correlated with total bacteria count. Moreover, plasma zonulin is also positively correlated with soluble tumor necrosis factor receptors 1 (sTNFR1 concentration), which is a sensitive marker of low-grade inflammation in obese subjects.[34] This finding verify plasma zonulin as an inflammatory marker, like its precursor, haptoglobin.[27] The study also found that plasma zonulin concentration was higher in obese subjects than their normal counterpart. In addition, plasma zonulin concentration was positively correlated with age, body mass index (BMI), fat mass and fat percentage, daily energy intake, and serum glucose concentration, and negatively correlated with percentage of dietary protein.[33]

A study in Caucasian men, also found the positive correlation between circulating zonulin and BMI. The study also found that circulating zonulin was proportionately correlated with waist-to-hip ratio (WHR), fasting insulin, fasting triglycerides, uric acid, and interleukin-6 (IL-6). However, circulating zonulin was inversely correlated with HDL-cholesterol and insulin sensitivity.[24]

Similar results also found in children. Küme et al[35] investigated correlation between serum zonulin concentrations and parameters related to childhood obesity in obese and healthy children aged approximately 11 years old. They discovered that obese children had higher serum zonulin concentration than healthy children. They also found that serum zonulin concentration was inversely correlated with HDL-cholesterol and positively correlated with serum leptin concentrations, after adjustment of age and BMI.

A study in Korea, also in young adolescents (overweight or obese vs normal weight), with mean age 12.8 years old also found that serum zonulin concentration was higher in overweight/obese children compared to their normal counterpart. Serum zonulin concentration was positively correlated with BMI z-score, triglyceride, fasting insulin and insulin resistance. In addition, multivariate regression analysis revealed that alanine

aminotransferase was positively correlated with serum zonulin concentration in young adolescents with overweight or obesity, after adjustment for potential confounding factors. This finding suggest a potential pathophysiological link between zonulin, as biomarker of intestinal permeability, with hepatic metabolism in young adolescents.[8] Similar study in Korea, involving older adolescents (mean age 15.2 years old), also found similar results. In addition, the study found that serum zonulin concentrations were more strongly correlated with WHR instead of weight-height ratio.[36]

Mörkl et al[10] found that zonulin concentrations were positively correlated to the intake of total calories, protein, carbohydrates, sodium, and vitamin B12 in adult women, but there was no relationship between zonulin concentrations and fiber and fat intake. Another study showed that intake of omega-3 PUFA, fiber, and some vitamins and minerals was higher in overweight pregnant women with low zonulin concentrations than pregnant women with high zonulin concentrations.[11]

In addition, we also found several studies with randomized controlled trial designs that investigated the effect of dietary supplements or interventions on intestinal permeability. Interventions using probiotic supplements with or without fiber (prebiotics) in overweight and obese adults can control body fat mass and this is associated with low zonulin concentrations.[37] However, a study in patients with depression who were given probiotics and vitamin B7 did not find a significant difference in zonulin concentrations between the intervention and placebo groups.[38]

Dietary intervention research that we found provides an intervention in the form of an Okinawan-based Nordic diet that is low in carbohydrates but high in fiber, fat, and protein. In this study, it was found that after 12 weeks of diet, zonulin concentrations in serum and feces increased. Serum zonulin concentrations remained high at 28 weeks. The zonulin concentration is associated with the percentage of energy from protein.[39,40] Another study providing dietary and synbiotic interventions was conducted by Janczy et al.[41] in adults who are overweight. After administering a reduction diet based on the recommendations of the Polish Society of Dietetics with or without synbiotics for three months, there was a significant decrease in fecal zonulin concentrations in the group receiving synbiotics, while in the placebo group there was no significant decrease.[41]

A randomized, controlled, cross-over trial in elderly found that a polyphenol-rich diet could decrease serum zonulin concentrations after eight weeks intervention. In a stratification analysis by gender, this significant decrease was found only in women but not in men.[42] This study indicated that polyphenol-rich diet could improve intestinal permeability in the elderly.

A systematic review identified risk factors for intestinal permeability in adults. The study shows that consumption of a Western-style diet is one of the strongest risk factors for changes in gut integrity, in addition to other factors, namely increased pro-inflammatory markers, dyslipidemia, hyperglycemia, insulin resistance, obesity, and comorbidities. Most of these risk factors are related to metabolic conditions that can also be influenced by diet.[21]

### *Conclusion*

Although there are several studies found regarding diet, nutritional status, and intestinal permeability assessed by zonulin, most studies were conducted in adults and there are limited studies in children and adolescents. Some studies indicated that intake of proper nutrition and good diet such as low total calorie intake, high intakes of omega-3 PUFA, fiber, vitamins, minerals, probiotics, and polyphenol-rich diet have significant impact on improvement of intestinal permeability marked by lower zonulin concentrations. However, studies investigating associations of diet quality using proper diet quality indices and intestinal permeability have not been found in children, adolescents, and adults. Therefore, further studies should explore these associations to obtain a comprehensive picture of diet complexities in the population and its correlation with intestinal permeability.

## CONFLICT OF INTEREST AND FUNDING DISCLOSURE

The authors declare no conflict of interest.

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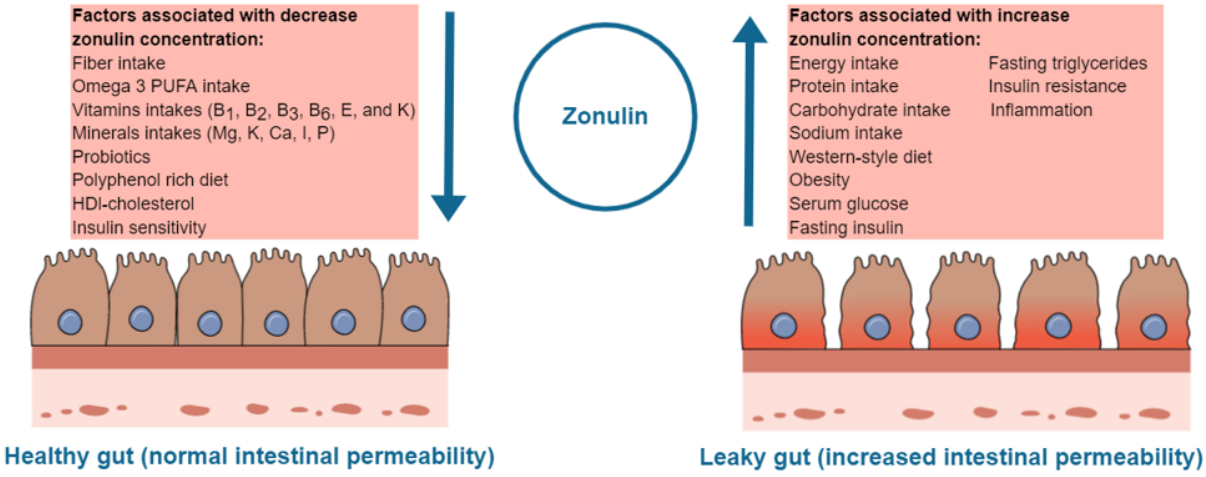
**Table 1.** Definitions of intestinal permeability

| Term | Definition |
|---|---|
| Intestinal permeability | is defined as a functional feature of the intestinal barrier at given sites, measurable by analyzing flux rates across the intestinal wall as a whole or across wall components of defined molecules that are largely inert during the process and that can be adequately measured in these settings |
| Normal intestinal permeability | is defined as a stable permeability found in healthy individuals with no signs of intoxication, inflammation or impaired intestinal functions |
| Impaired intestinal permeability | is defined as a disturbed permeability being non-transiently changed compared to the normal permeability leading to a loss of intestinal homeostasis, functional impairments and disease |

Source: Bischoff et al, 2014.[16]




**Graphical abstract.** The association of diet, nutritional status and intestinal permeability assessed by zonulin concentrations in serum and feces