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Review

Alterations of gut barrier and gut microbiota in food restriction, food deprivation and protein-energy wasting

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SUMMARY

Increasing evidence shows that gut microbiota composition is related to changes of gut barrier function including gut permeability and immune function. Gut microbiota is different in obese compared to lean subjects, suggesting that gut microbes are also involved in energy metabolism and subsequent nutritional state. While research on gut microbiota and gut barrier has presently mostly focused on intestinal inflammatory bowel diseases and more recently on obesity and type 2 diabetes, this review aims at summarizing the present knowledge regarding the impact, *in vivo,* of depleted nutritional states on structure and function of the gut epithelium, the gut-associated lymphoid tissue (GALT), the gut microbiota and the enteric nervous system. It highlights the complex interactions between the components of gut barrier in depleted states due to food deprivation, food restriction and protein energy wasting and shows that these interactions are multidirectional, implying the existence of feedbacks.

1. Introduction

Protein energy wasting (PEW), also termed protein energy malnutrition, occurs in 20 to 50% of hospitalized patients [1]. It refers to a “state of decreased body stores of protein and energy fuels (body protein and fat masses)”, which is generally accompanied by decreased functional capacity [2]. PEW is associated with chronic diseases, its pathophysiological mechanism involves not only anorexia and the subsequent decrease of energy intakes, but also inflammation, insulin resistance and hypogonadism, and its hallmarks are body weight loss in adults and growth failure in children [2]. It should be differentiated from depleted states due solely to food restriction or food deprivation.

With the emergence of techniques measuring gut microbiota composition and function, such as 16S rDNA high-throughput sequencing and shotgun sequencing, there is a growing interest in understanding the relationship between gut microbiota and nutritional state. Nowadays, it is accepted that the gut microbiota composition differs between obese and lean subjects [3] and varies with weight changes [4]. Furthermore, several studies have highlighted that gut microbiota composition is associated with gut barrier function [5]. These findings suggest that gut microbiota is involved in energy metabolism and subsequent nutritional state and it is likely that, just as obesity, PEW is associated with changes of the gut barrier including gut microbiota.

This review aims at summarizing the present knowledge regarding the impact, *in vivo,* of depleted nutritional states due to food restriction, food deprivation and PEW, on structure and function of the gut epithelium, gut-associated lymphoid tissue (GALT), gut microbiota and enteric nervous system (ENS). Figure 1 summarizes the speculated links of PEW with gut barrier, which will be discussed in this article.

2. Structure and function of the gut barrier

The gut barrier is secured by the epithelium, the tight junction proteins, which include mainly occludin, claudins, zonula-occludens 1 and the junctional adhesion molecule, the overlying mucus, the GALT, the gut microbiota, and very likely the ENS. The gut barrier modulates the transfer of molecules as nutrients, electrolytes, water, toxins, microbes and microbial byproducts, from the intestinal lumen to the mucosa. These molecules can use either the transcellular pathway and cross the apical and basolateral membranes of enterocytes, or the paracellular pathway sealed by tight

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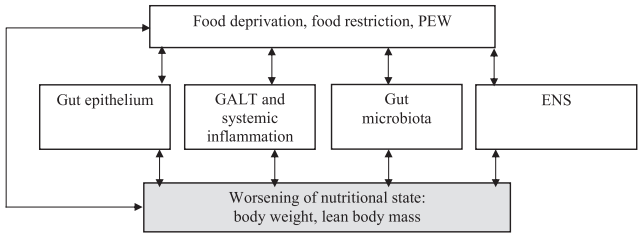

Fig. 1. This figure highlights the speculated mechanisms underlying the worsening of nutritional state at the gut barrier level. We speculate that food deprivation or restriction are associated with alterations at the level of epithelial gut barrier, GALT, gut microbiota and ENS, which closely interact with each other. These alterations in turn may contribute to worsening of nutritional state.

junction proteins. As a consequence, the gut barrier may affect energy balance, water homeostasis, tolerance to food antigens and mucosal inflammation.

Intestinal permeability refers to the property of unmediated passive diffusion across the intestinal wall. It refers to the paracellular pathway, which is mostly regulated by tight junction proteins and allows the passage of molecules smaller than 600 Da [6]. Intestinal permeability is higher in the small bowel than in the colon [7]. It can be evaluated *in vivo* by the flux of fluorescein isothiocyanate-dextran (FITC-dextran, molecular weight $\geq$ 1000 Da) across the gut epithelium and recovery in the blood. However, this method cannot be applied to humans due to potential serious side effects of dextran. In humans, intestinal permeability is generally assessed by ingesting oral probes such as sugars, Cr-labeled EDTA, polyethylene glycols and water-soluble contrast medium, and by measuring their urinary recovery [8]. The most commonly used probes are sugars. Small bowel permeability is classically expressed as the ratio of the fractional urinary excretion of a large-size sugar like lactulose (342 Da) to a small-size sugar like mannitol (182 Da) or L-rhamnose (164 Da) [9]. The higher this ratio is, the higher is the small bowel permeability. However, it should be noted that permeability depends not only on the intrinsic characteristics of gut barrier but also on the concentration gradients across the gut epithelium, the surface area of the epithelium and the transit time [9]. Interestingly, the lactulose/mannitol (L:M) ratio does not change with aging because the urinary excretion of both sugars decreases [10].

The transcellular pathway ensures the transport of molecules from the apical to the basolateral membrane of enterocytes through transcellular diffusion (e.g. water), transcytosis (e.g. food antigens) and carrier-mediated transport (e.g. glucose), and the transport of microbes through M cells or dendritic cells. The mechanisms of transcellular transport of bacteria and food antigens are described in details elsewhere [11]. In animals, the transcellular pathway can be tested with enteral administration of large molecules as ovalbumin (45 kDa), horseradish peroxidase (40 kDa) and β-lactoglobulin (18 kDa), and measuring their recovery in the mesenteric or portal blood [11]. In humans, this pathway can be evaluated by oral intake of D-xylose or by the D-xylose/3-O-methyl-D-glucose ratio and measuring their recovery in the urine or venous blood.

Surrogate *in vivo* markers of intestinal permeability include plasma levels of lipopolysaccharides (LPS) and D-Lactate, and urinary levels of claudin-3. The often associated intestinal inflammation can be tested with neutrophil-derived proteins as fecal levels of calprotectin, lactoferrin, elastase, while enterocytic damage may be evaluated through plasma fatty acid binding proteins [12].

3. Structural alterations of gut barrier in food deprivation, food restriction and PEW

The structural alterations occurring with food restriction and deprivation and subsequent weight loss have been characterized mostly in rodents and are summarized in Table 1 [13–28].

A few animal studies focused specifically on the effects of protein restriction, as compared to calorie restriction. Belmonte et al. fed rats with a control diet containing 23% of protein or an isocaloric protein-free diet for 2 weeks. The protein-free diet decreased body weight, jejunal villi and lamina propria heights, and hepatic and jejunal levels of glutathione compared to the control diet, and led to some positive cultures in lymph nodes homogenates, defined as over 100 colony forming units/g tissue after 48 h [29]. Protein restriction also results in decreased secretory IgA levels [30] as well as absolute and relative amounts of intraepithelial lymphocytes

Table 1
Structural intestinal changes occurring with food deprivation (D) and restriction (R), in animals.

Structure	Condition	Impact	References
Whole gut	D + R	↓ DNA, ↓ total protein content	13–16
	D	↓ Intestinal weight	13
	D	↓ RNA	15
	D	↓ Proteins involved in glycolysis and energy metabolism	17
	D	↓ Proteins involved in protein synthesis and amino acid metabolism	17
	D	↑ Oxidized glutathion	16
Gut mucosa	D + R	↓ Villous volumes, number of villi, villous height and crypt depth, villous height-to crypt depth ratio	15, 17, 19, 22, 23
	D + R	↓ Crypt cell production rate, proliferation of small intestine epithelial cells	20, 21
	D + R	↑ Apoptosis of small intestine epithelial cells	22,26
	D	↓ Mucosal weight, mucosal surface areas and villous volumes	13, 18
	D	↑ Microvilli	27
	D	↓ Microvilli area	24
	D	No difference in small intestine morphology, villus length and crypt depth	20
	D	↑ Goblet cells	27
	D	↓ Total protein content	16
	D	↓ mRNA expression of paneth cells antimicrobials	20
	R	No difference in number of goblet cells	23
	R	↓ n-3 and n-6 fatty acid concentrations of gut mucosa	28
	R	↑ Claudin-3 expression in jejunal crypts, no difference in ZO-1, occludin, claudin-1 tight junction proteins	22
Mucus	R	↓ Mucin in small bowel	14
GALT	R	↓ CD4+cells, CD8+ cells, dendritic cells, macrophages, lymphocytes	23
	R	↓ Cells producing IL-2, IL-12, TNF-alpha, IFN-gamma, IL-6, IL-4 and IL-10 in the lamina propria	23
	R	↓ Phagocytic activity of spleen and peritoneal macrophages	23
	R	No difference in number of IgA+cells	23