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Nutritional Modulation of Gut Inflammation

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Introduction

Chronic inflammation of the intestinal tract remains a common cause of morbidity, reduced quality of life and, in certain cases, may be fatal. Despite intensified research efforts and significant advancements, our understanding of the mechanisms underlying many conditions such as ischemia reperfusion injury of the gut, inflammatory bowel disease (IBD) and sepsis of gut origin remains unclear. Crohn's disease and ulcerative colitis are phenotypically distinct forms of IBD whose pathogenesis is believed to involve aberrant mucosal immunoregulation, leading to intestinal epithelial cell injury mediated by activated T cells, mononuclear cells and macrophages. Despite the recent identification of disease susceptibility genes, the etiologies of these disorders remain unclear. Although the environmental trigger(s) responsible for disease onset and exacerbations remain unknown, an increasing body of evidence points to an important role of the gut flora and aberrant mucosal permeability. Corticosteroids and immunomodulatory drugs, the mainstays of therapy, often offer less than ideal results.

There is a growing scientific rationale for the use of dietary factors, or nutriceuticals, to modulate the inflammatory response in the management of various chronic inflammatory disorders. Increased attention has focused interest on the importance of nutritional factors in the pathogenesis and treatment of IBD. Elemental diets have been employed to control disease activity in Crohn's disease for almost three decades. Nevertheless, we have a limited understanding of their underlying mechanisms of action. The benefits of dietary therapy may be derived by removing luminal factors that induce or maintain intestinal inflammation. Alternatively, nutriceuticals in such diets are capable of modulating the gut immune response, downregulating the uncontrolled inflammation. More recently, experimental studies and clinical trials have supported the use of antioxidants, supplements of marine oils, probiotics, as well as defined 'designer' formula diets to improve the clinical course in Crohn's disease

and ulcerative colitis. The ability of nutrients to exert profound effects on immune system function is well established. Enteral nutrients are also capable of enhancing mucosal barrier function, an important protective function against noxious compounds, toxins and microbial pathogens omnipresent in the gut lumen.

The generation of reactive oxygen species (free radicals) is an important factor in the development and maintenance of inflammation in IBD. The gastrointestinal tract has the highest concentration of xanthine oxidase in any tissue. Furthermore, the vast number of activated leukocytes that normally reside in the bowel mucosa are greatly multiplied in IBD, producing high concentration of free radical species. The teeming number of catalase-negative bacteria in the distal small bowel and colon also produce enormous quantities of O_2^-. The gastrointestinal tract has thus been described as a veritable 'free radical time bomb' and it would appear as though IBD is the setting where the fuse has been lit [1]. Nutrients serve as a key source of antioxidants, and there is a growing scientific rationale for their use. Polyunsaturated fatty acids (PUFAs) of the n-3 family have been claimed to be beneficial in IBD, possibly by suppressing the gut immune system's proinflammatory cytokine repertoire, as well as modulating eicosanoid production. They induce a shift in synthesis from leukotriene (LT) B_4 to the much less bioactive LTB_5. Data from animal models as well as clinical trials suggest that probiotics represent promising therapeutic advances for certain infectious, inflammatory as well as allergic bowel disorders. This chapter focuses on the specific nutritional components that modulate gut inflammation. The experimental and clinical evidence supporting the role of specific nutrients in modulating gut inflammation are presented, using chronic IBD as a disease model. The purpose of this review is to provide a rationale for the use of nutriceuticals in order to control inflammatory conditions of the bowel, as well as to highlight areas worthy of further research in this promising field of clinical nutrition.

Diet as Primary Therapy of Crohn's Disease: A Model

Since the first reports on the use of elemental diet therapy to induce remission in active Crohn's disease almost 3 decades ago, many advances have been achieved in our understanding of the mechanisms underlying the beneficial results [2] Controlled trials in both adults and children established the efficacy of elemental diets to induce remission effectively. Meta-analyses have shown an overall statistical advantage for corticosteroids over defined formula diets in terms of remission rate in Crohn's disease [3,4]. Nevertheless, there are certain clinical situations where nutrition as primary therapy warrants consideration [2,5]. The use of defined formula nutrition exclusive to other food intake, when administered intermittently (4 of every 16-week period), led to fewer relapses and improved growth in children and adolescents with Crohn's disease [6]. Cyclical home elemental diet therapy has also been employed with success to maintain remission in adult patients. The mechanisms of action of diet therapy to control disease activity in IBD are summarized in Table 1. The potential role of certain nutrients in modulating gut immune response and inflammation are discussed below.

Table 1. Potential mechanismsunderlying the beneficial effectsof defined formula diets as primary therapy in inflammatory bowel disease

- Removal of luminal antigens
- Modulation of gut immune response
- Improved antioxidant status
- Altered PUFA intake (n-6/n-3 fatty acids)
- Enhancing gut barrier function/ repair
- Altered gut flora
- Altered motility
- Altered pancreaticobiliary secretions
- Removal of dietary particles

Oxidative Stress and the Gut

Oxygen free radicals may be defined as oxygen molecules or molecular fragments that have an unpaired electron on their outer orbit. They are formed during physiologic and pathophysiological metabolism, and potentially cause cell and tissue damage due to their high chemical reactivity. Low concentration of reactive oxygen species may be beneficial, or even indispensable in processes such as intracellular signalling and defense against micro-organisms. Nevertheless, higher amounts play a role in the pathogenesis of a number of inflammatory disorder [7]. To neutralize their electric charge, free radicals try to steal an electron from, or donate an electron to neighboring molecules, setting off an oxidative chain reaction that damages scores of molecules. Antioxidants neutralize free radicals by stabilizing them, preventing oxidative damage. Oxidant-antioxidant balance is critical for immune cell function because it maintains cell membrane integrity and functionality, cellular proteins and nucleic acids, as well as controlling signal transduction and gene expression [8]. There is compelling evidence that oxidative stress, defined as an imbalance between the overabundance of oxidants and insufficient antioxidants, leads to many biochemical changes that contribute to numerous chronic disorders, including IBD [9]. Oxidants can be generated in numerous ways, from both endogenous and exogenous sources. Important cellular sources of oxidative stress include the formation of reactive oxygen species by incomplete reduction of oxygen in the respiratory chain of mitochondria, and the 'oxidative burst' mediated by NADPH oxidase in host defense systems, leading to superoxide radical, and myeloperoxidase, forming hypochlorous acid. Nitric oxide, another molecule produced during inflammatory responses in the presence of oxygen or superoxide, is also converted to more reactive species, such as nitrogen dioxide and the more toxic radical peroxynitrite. Several of the proinflammatory cytokines that are overproduced in IBD, including interleukin (IL) -1β and tumor necrosis factor (TNF)-α, are involved in the formation of free radicals. The administration of iron supplements can potentially increase inflammation in IBD through the production of the hydroxyl radical via its catalytic activity in the Fenton reaction. In this regard, Fe supplementation was recently shown to increase the production of pro-inflammatory cytokines in IL-10-deficient mice [10].

Reactive oxygen species cause oxidative stress, resulting in cellular damage as a result of chain reactions leading to disruption of macromolecular structure. Lipid peroxidation reactions

are the results of free radical-driven chain reactions, in which one radical can induce the oxidation of a large number of substrate molecules, such as PUFAs [11]. The peroxidaton of membrane lipids can lead to functional changes in epithelial cells [9]. Reactive oxygen species can damage nucleic acid structure, compromising cell survival and modifying gene expression, leading to disordered cell turnover. The oxidation of thiols and the formation of carbonyl groups on proteins can deteriorate cell viability, with loss of receptor, enzyme and transporter function, Chemotactic by-products of lipid peroxidation provide positive feedback to accelerate and perpetuate the inflammatory process, of potential importance in the pathogenesis of IBD. Modifications of DNA bases that can occur under conditions of oxidative stress can lead to activation of oncogenes or to mutations which, if not repaired, may lead to an increased risk of cancer [12]. It is well recognized that patients with chronic IBD, particularly ulcerative colitis, are at high risk of developing adenocarcinoma of the colon. Oxidant stress has been one of the factors contributing to an increased risk of gut malignancy in IBD. In addition to repairing oxidatively damaged biomolecules, the host mucosal defense must scavenge free radicals and toxic nitrogen species such as nitric oxide that are produced in the bowel in the course of the inflammatory response, preventing excessive tissue damage. Host defenses include antioxidant enzymes, such as intracellular superoxide dismutases and catalase, glutathione peroxidase and reductase. These enzymes are completed by antioxidants, which include molecules derived exclusively from the diet. The list of small molecule antioxidants include vitamins C and E, and glutathione. Inasmuch as the generation of reactive oxygen species appears to correlate with tumor promotion, scavenging the radicals with antioxidants would be expected to antagonize tumorigenesis [12].

Of particular interest with respect to immune-mediated bowel disorders is the role of free radicals in modulating immune function. The ability of reactive oxygen species to affect signal transduction pathways and cytokine production is highly relevant to the understanding of the pathogenesis of chronic inflammation in the gut. A large number of genes, enzymes and proteins, as well as intracellular signaling elements and molecules are regulated by oxidative stress and the redox state of the cell [for review see 13]. The ability of antioxidants to modulate DNA damage and gene expression in the context of bowel disorders is an emerging field of nutrition research.

Another model of oxidative stress-induced disease is represented by reperfusion injury. This form of tissue damage is defined by the results of resumption of aerobic respiration after a period of oxygen deprivation. There is substantial experimental evidence that reactive oxygen species are generated during reperfusion, and that antioxidant therapy may be beneficial. In experimental models of ischemia-reperfusion or necrotizing entercolitis, pre-treatment with antioxidants, deferoxamine, catalase and superoxide dismutase reduced intestinal inflammation [14].

Role of Dietary Antioxidants in Gut Disorders

Clinical trials for the prevention of cancer using dietary supplementation with β-carotene or a combination of antioxidants have been met with disappointing results. Despite the lack of clinical benefit, there is clear biochemical evidence of the effect of antioxidants on cellular signaling and gene expression [13].

Glutathione

Glutathione is a key intracellular peptide with multiple physiological functions, including antioxidant defense and modulating critical cellular processes [15, 16]. Among its vital functions are detoxifying electrophils, maintaining the essential thiol status of proteins by preventing oxidation of –SH groups or by reducing disulfide bonds induced by oxidant stress, scavenging free radicals, providing a reservoir for cysteine, and modulating DNA synthesis and immune function [15]. Numerous studies have shown that oxidative stress can induce cellular apoptosis, and that antioxidants such as thiol-reduced glutathione (GSH) can be protective [16].

GSH is synthesized in the cytosol of all mammalian cells, in a highly regulated manner. The rate-limiting enzyme is γ-glutamylcysteine synthetase. Glutathione exists in a thiol-reduced (GSH) and disulfide-oxidized forms (GSSG). The unusual structure of this molecule renders it resistant to intracellular degradation. It is only subject to extracellular hydrolysis by γ-glutamyltranspeptidase, an enzyme on the external surface of certain cell types [15].

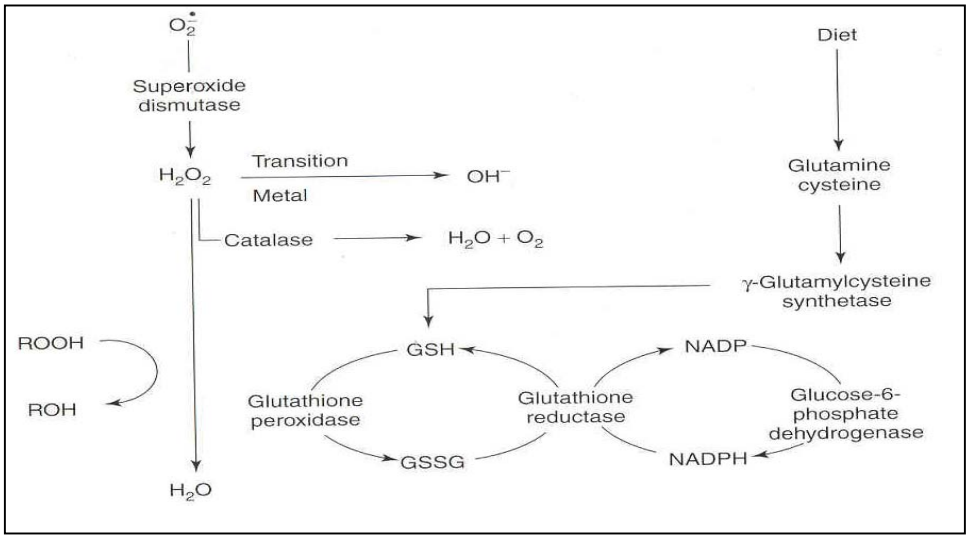

Fig. 1. Schematic diagram of the redox cycle. Removal of hydrogen peroxide (H_2O_2) is essential for protection against damage inflicted by toxic reactive oxygen species. Glutathione peroxidase and catalase metabolize H_2O_2 to innocuous products. Glutathione (GSH) levels are regulated by the dietary intake of cysteine.

In the course of oxidative stress, intermediates are formed such as superoxide (O_2^-) and hydrogen peroxide, which lead to the further production of toxic oxygen radicals that can lead to lipid peroxidation and cell injury. Endogenously produced hydrogen peroxide is reduced by GSH in the presence of selenium-dependent GSH peroxidase (Fig. 1). Consequently, GSH is oxidized to GSSG, which can then be reduced back to GSH by GSSG reductase at the expense of NADPH, forming a redox cycle. Although hydrogen peroxide can also be reduced by catalase, this enzyme is only present in the peroxisome (Fig. 1). Thus, in the mitochondria, GSH is vitally important to defend against physiologically as well as pathologically-generated oxidative stress. Severe oxidative stress thus depletes cellular GSH [15]. The GSH/GSSG

ratio may be employed to quantify oxidative stress in tissue, using N-ethylmaleimide to trap GSH in a state in which it cannot oxidize to GSSG [17].
One of the major determinants of GSH synthesis is the availability of cysteine in the diet. Starvation has been shown experimentally to substantially reduce GSH levels, while refeeding results in restoration of normal content within hours. Supplementation with selenium has been shown to enhance GSH levels in renal transplant patients [18].
Several clinical conditions are associated with reduced GSH levels which, as a consequence, can result in a lowered cellular redox potential. GSH and the redox potential of the cell are components of the cell-signaling system, influencing the translocation of the transcription factor nuclear factor κB (NFκB), which subsequently regulates the synthesis of cytokines and adhesion molecules. Therefore, one potential route to protect cells from reactive oxygen species-induced damage is to restore intracellular glutathione levels. This can be accomplished by administration of its precursors such as glutamine or cysteine.

Vitamins A, C and E

Vitamin E encompasses a group of tocopherols and tocotrienols, of which α-tocopherol has the highest biological activity. Vitamin E partitions into lipoproteins and cell membranes, where it functions as a potent antioxidant to protect polyunsaturated membrane lipids against free radical attack [19]. A potent free radical scavenger, vitamin E prevents the destructive chain reaction that typifies free radical injury. The impact of vitamin E in the prevention of oxidative stress has often been studied, with beneficial effects demonstrated for a number of conditions [20]. Rather than function in isolation, vitamin E is part of an adjoined set of redox antioxidant cycles (Fig. 2), termed the 'antioxidant network'. Vitamin C can regenerate vitamin E directly, and thiol antioxidants, such as glutathione and lipoic acid, can indirectly regenerate it via vitamin C [20]. In addition to its key antioxidant capacity, vitamin E plays a role in cellular signaling, particularly via decreasing protein kinase C. It thus has been shown to possess biological effects on signal transduction, inhibiting tumor promotion [20]. The oxidant-antioxidant balance is an important determinant of immune cell function. Vitamin E, as an antioxidant, is thought to play an important role in the maintenance of the immune system. It also downregulates the expression of adhesion molecules, potentially affecting leukocyte adhesion and tissue infiltration. Studies have also pointed to a role for vitamin E in arachidonic acid metabolism, enhancing the release of prostacyclins via upregulating cytosolic phospholipase A2 and cyclooxygenase. Ascorbic acid is an essential micronutrient for normal metabolic functioning, as humans have lost the ability to synthesize vitamin C. Among its multiple bioactivities, vitamin C plays an important antioxidant role, scavenging reactive oxygen and nitrogen species [21]. Vitamin C can also act as a co-antioxidant, regenerating vitamin E and glutathione from the α-tocopherol radical, as part of the antioxidant network (Fig. 2). A recent study in critically ill patients demonstrated that supplementation of a formula with vitamins A, C and E improved antioxidant defenses [22]. On the other hand, the interaction of vitamin C with 'free' catalytically active metal ions could contribute to oxidative damage via the production of hydroxyl and alkoxyl radicals [23].

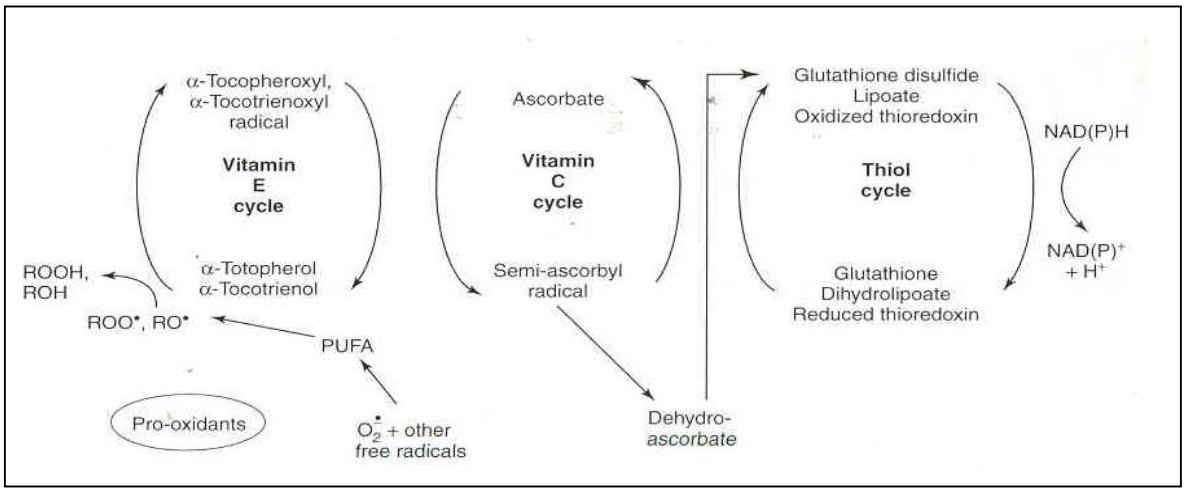

Fig. 2 The antioxidant network.

Antioxidant-Associated Free metals: Selenium, Copper, Zinc

Three primary antioxidant enzymes have been demonstrated in mammalian systems: superoxide dismutase, catalase, and glutathione peroxidase [24]. Superoxide dismutase utilizes Cu-Zn or Mn as co-factors in converting the superoxide radical into hydrogen peroxide, which is then converted to water by catalase and glutathione peroxidase (Fig. 1). Selenium is an essential trace element and an integral component at the catalytic sites of glutathione peroxidase. Selenium deficiency is associated with a profound reduction in the activity of glutathione peroxidase in several tissues, resulting in increased oxidative stress. Conditions involving oxidative stress and inflammation have shown the benefits of a higher selenium status [25].

Reactive Oxygen Species, Antioxidants and IBD

Baseline Measurements

Despite intense research efforts, the etiology of IBD remains poorly understood. The pathogenic factors implicated include microbial agents, immune dysregultion, genetic susceptibility, and environmental factors, such as diet [26]. A growing body of evidence suggests that uptake of luminal antigens across the mucosal epithelium elicits an aberrant immune response, leading to intestinal inflammation, local peroxidation, and systemic circulation of inflammatory cytokines and mediators. These disturbances are often associated with malnutrition, secondary to inadequate intake and malabsorption, impaired essential PUFA status and antioxidants status [27].

An increasing body of evidence supports the hypothesis that oxidative stress plays an important role in the pathogenesis of IBD (Fig. 3). The gastrointestinal mucosa in IBD is the target of intense chronic, if not incessant inflammation. An increased number of activated immune cells invades the epithelium and lamina propria, producing heightened levels of oxygen and nitrogen free radicals. Lipid peroxidation, as measured by breath pentane, ethane and F2-isoprostane levels, were all significantly higher in Crohn's disease patients [28]. We observed increased plasma malondialdehyde levels in Crohn's disease [27], consistent with oxidative stress-induced lipid peroxidation. It has been suggested that the gut may be

particularly sensitive to oxidative stress because of the relatively low concentrations of endogenous antioxidant enzymes. Further evidence has been provided by experimental studies showing a benefit of therapy with topical antioxidants in an animal model of colitis.

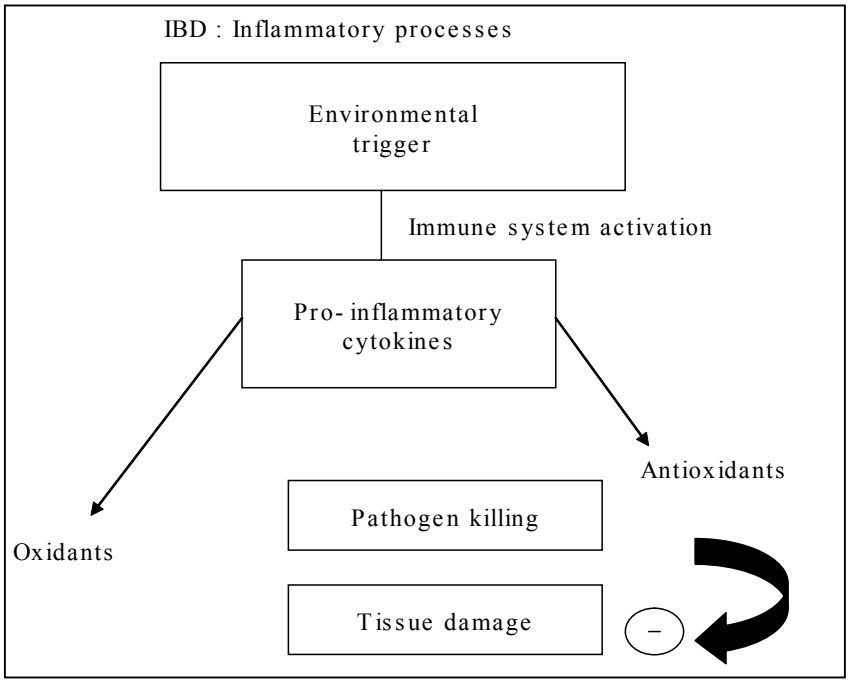

Fig. 3. The role of oxidative stress in gut inflammation. Excessive production of reactive oxygen species and inadequate antioxidant stores lead to intestinal epithelial cell dysfunction and tissue damage.

As summarized in Table 2, studies on antioxidant concentrations in IBD have revealed variable circulating concentrations of antioxidant vitamins and metals. Overall, antioxidant levels are decreased in active disease, especially in Crohn's disease and particularly among malnourished patients. The disparate results may reflect differences in methodology, age and gender, diet and medications administered, as well as disease type (Crohn's *vs.* ulcerative colitis; extent of bowel disease or of resections; disease activity) and location (small *vs.* large bowel, or both). Low selenium levels are more often encountered in patients with extensive small bowel resections. Smoking and other environmental factors may also influence antioxidant results. Several explanations may be put forth concerning low vitamin levels. Diminished antioxidant status has been correlated with disease activity by some studies [29], but not others. Low vitamin E levels in serum may be reflective of hypolipidemia, rather than a true deficiency. Decreased circulation ascorbic acid concentrations may simply reflect heightened utilization as an antioxidant in the gut. Patients may have consumed inadequate intakes of vitamin C. Another possibility is that ascorbic acid is consumed in order to replenish glutathione and vitamin E, as illustrated in Figure 2. In view of the potential pro-oxidant properties of ascorbic acid, caution has been advised in attempting to normalize levels [30]. Our experimental studies suggest that this is particularly an issue in patients receiving iron supplementation, as Fe-ascorbate adversely affects enterocyte function [31].

Table 2, Circulating antioxidant status in patients with inflammatory bowel disease

Studies	n	Adult/ Paeds	Vitamins			B-Carotene	GSH Peroxidase	GSH	Selenium	Zn	Cu
			A	C	E						
Bousvaros *et al* [58]	97	P	⇩		⇩						
Fernandez-Banares *et al* [3	23,29	A	⇩	⇩	⇩	⇩				⇩	⇧
Geerling *et al* [29]	32	A		⇩	⇩	⇩	⇩		⇩	⇩	
Genser *et al* [59]	24	A	N		N	⇩					
Hinks *et al* [60]	20	A							⇩	N	N
Hoffenberg *et al* [30]	24	P	N	⇩	⇧	N	⇧	⇧	N		
Kuroki *et al* [61]	24,13	A	⇩		⇩ / N[a]						
Levy *et al* [27[	22		⇩		N						
Rannem *et al* [62]	66,79	A					⇩		⇩		
Reimund *et al* [36]	26	A					⇩		⇩		
Ringstad *et al* [63]		A							⇩		
Schoelmerich *et al* [64]	54	A	⇩							⇩	
Thomas *et al* [65]	39	P					⇧ ⇩[b]		N		
Wendland *et al* [28]	37	A	⇩			⇩					

[a] Vitamin E status normal when corrected for hypolipidemia [b] Increased in plasma, reduced in erythrocytes

Another limitation in the interpretation of antioxidant status in IBD is the reliance on measures in the circulation, rather than tissue levels in the gut. Colonic mucosal glutathione was found to be markedly lower in active Crohn's disease [18, 32]. Tissue from uninvolved areas in Crohn's disease also had a significantly lower glutathione content. Malnutrition was observed to be associated with low levels. The investigators thus concluded that low mucosal glutathione levels in Crohn's disease could contribute to the prolonged inflammation and should serve as a target for nutriceutical therapy [32]. Substantial reductions in inflamed gut mucosa vitamin C levels have also been observed in IBD. Abnormally low plasma cysteine levels, a major precursor of glutathione, were associated with inflammation in IBD [18]. Furthermore, decreased activity of the key enzymes involved in glutathione synthesis was observed in IBD tissue [18]. In addition to increased levels of reactive oxygen species and lipid peroxidation in IBD tissue, diminished activity of superoxide dismutase as well as Zn and Cu were found in IBD mucosa [33]. Taken together, these findings suggest that decreased substrate availability as well as impaired synthetic capacity contribute to the low glutathione and other antioxidant defences in IBD.

Therapeutic Trials

Many raw foods contain natural antioxidants that are usually inactivated during processing. Furthermore, the bioavailability of nutriceutical compounds in different tissues such as the gut remains poorly understood. Thus, most trials have employed supplements with specific nutrients, or have employed defined formula diets to treat IBD. A phase-II trial provided evidence supporting a reduction in the severity of IBD using bovine Cu-Zn-Superoxide dismutase and deferoxamine [34]. The free radical scavengers, allopurinol and

dimethylsulfoxide, were also shown to be of benefit in treating ulcerative colitis [35]. *In vitro* studies suggested that antioxidants can inhibit the production of inflammatory cytokines by peripheral blood mononuclear cells from IBD patients [36]. However, mucosal biopsy cytokine secretion was less affected. A recent placebo-controlled study revealed that supplementation with antioxidants improved the serum levels of selenium, vitamin C and E, as well as the activity of superoxide dismutase in patients with quiescent Crohn's disease [37].

Dietary Lipids and Gut Function

A prevailing supposition underlying the use of marine oils in the treatment of autoimmune disorders is that 'Western diets' (high n-6, low n-3; ratio 25:1) are consumed in countries with a high incidence, whereas the diet in 'pre-industrialized countries' with a low incidence have a much lower n-6/n-3 ratio of about 2:1. Conceptually, n-3 PUFAs, taken up by immune cells in competition with n-6 fatty acids, decrease the induction of inflammatory eicosanoids such as LTB_4 and thromboxane A_2 and thus downregulate inflammation [38] (Fig. 4). In addition, n-3 PUFAs inhibit leukocyte function, proliferation, inflammatory cytokine synthesis (TNF, IL-1 etc.), natural killer cell activity, antibody production, as well as macrophage membrane surface molecule expression [38]. In general, dietary lipids rich in n-6 PUFA increase responsiveness to cytokines, whereas those enriched in n-3 have the opposite effect. Surprisingly, however, only half of subjects treated with fish oil supplements, were observed to have an inhibition of TNF-α production by peripheral blood mononuclear cells *in vitro* in response to lipopolysaccharide [39]. The mechanism by which PUFAs exert immunomodulatory effects remain poorly understood. Several hypotheses have been put forth, including changes in cell membrane phospholipids, alteration of eicosanoid production, formation of lipid peroxisomes, as well as regulation of gene expression. The sensitivity to the anti-inflammatory effects of fish oil is influenced by genotypic variations in lymphotoxin-α-promoter alleles [39]. Both the amount and the type of dietary fat modulate intestinal immune function. Thus, for example, the response of rat peritoneal macrophages to produce IL-1 and IL-6 in response to TNF-α is greatly influenced by the dietary intake of linoleic acid, within a range representing 1-4% of energy intake. The dose-response range in humans is not established. The absorption of long-chain fatty acids stimulates both lymphocyte blastogenesis and flux in intestinal lymphatics. It also enhances migration of T lymphocytes to Peyer's patches, possibly due to upregulation of adhesion molecules such as α_4-integrin and L-selectin. Lipoproteins may stimulate lymphocyte function by receptor-dependent and independent mechanisms [40].

There is increasing experimental evidence that changes in dietary lipid intake also alter the mechanisms involved in regulating physiological uptake of fatty acids by enterocyte. Patients with Crohn's disease have been shown to have PUFA deficiency, likely due to reduced intake as well as impaired fatty acid transport. Consumption of a high PUFA to saturated fatty acid diet was observed to improve the utilization of dietary C16:0 in Crohn's disease patients.

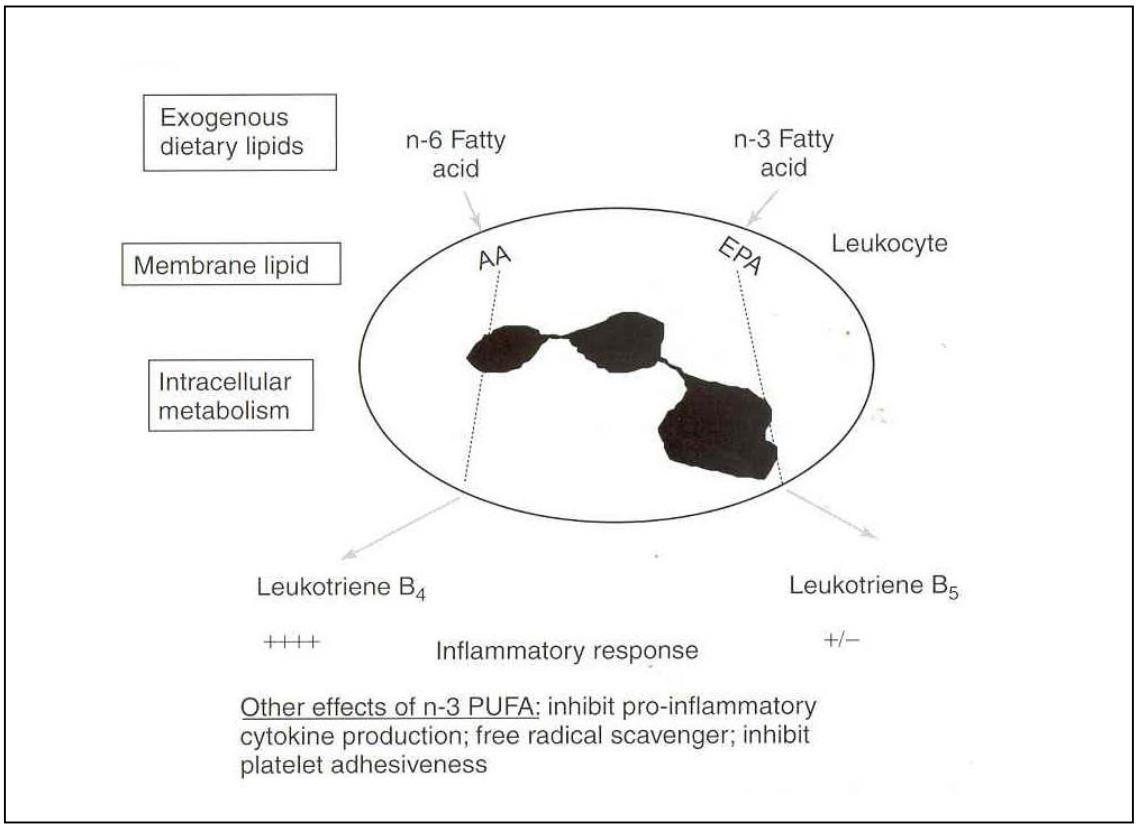

Fig.4 Beneficial effects of fish oil supplements in modulating gut inflammation.

Role of Dietary Lipids in the Management of IBD

One of the theoretical benefits of defined formula diets in the management of IBD may be derived by altering the type or quantity of dietary lipids (Table 1). It has been hypothesized that the effectiveness of enteral nutrition, as primary therapy in active Crohn's disease, may depend upon the content of PUFAs as precursors for arachidonate-derived eicosanoid synthesis [3]. Enternal nutrition as primary therapy in Crohn's disease has been shown to produce a reduction in Th-1 lymphokine secretion (IL-2, IFN-γ), similar to the results achieved with cyclosporine [41]. Altered dietary lipid intake was a potential explanation. Intravenous administration of eicosapentaenoic acid influenced the generation of LTs in active Crohn's disease, enhancing leukocyte LTB_5 levels [42]. Cholecystokinin has been considered to increase mononuclear cell intracellular calcium and is a lymphocyte co-mitogen. It can thus also be speculated that diets that increase cholecystokinin upregulate the gut immune response, whereas nutritional interventions that suppress cholecystokinin may decrease inflammatory mediator production.

The importance of n-3 fatty acids in the treatment and prevention of intestinal inflammation in IBD has been proposed by several investigators [43]. In addition to altering eicosanoid production, leukocyte function and cytokine synthesis, n-3 PUFAs are thought to serve as free radical scavengers (Fig. 4). Moreover, they inhibit platelet adhesion, an important function in view of the hypothesis that the pathogenesis of Crohn's disease involves multifocal bowel

infarcts. Epidemiological studies in Japan have revealed that the recently increased incidence of Crohn's disease correlated strongly with heightened intake of n-6 PUFA, animal protein, milk protein, and the ratio of n-6/n-3 PUFA consumption [44]. Nutritional supplementation with n-3 PUFAs has been shown to significantly reduce the relative proportion of arachidonic acid, while increasing eicosapentaenoic and docosahexaeroic acids in plasma phospholipids and adipose tissue of Crohn's disease patients in remission [37]. The combination of n-3 fatty acids and antioxidants would favour the production of eicosanoids with attenuated proinflammatory activity [37].

A number of studies [for review see 43] have indicated that n-3 PUFAs are potentially effective in the treatment of active ulcerative colitis, reducing steroid requirements, as well as preventing relapses in both ulcerative colitis and disease. Dietary fish oil supplementation alleviated symptoms and reduced rectal LTB_4 concentrations in ulcerative colitis. In their landmark study, Beluzzi *et al*. [45] observed a significant decrease in relapse rate in disease patients randomised to receive n-3 fatty acid supplements. A group of 78 Crohn's disease patients with a high risk of relapse were randomised to receive 2.7 g of an enteric-coated fish oil supplement daily (2/3 eicosapentaenoic acid; 1/3 docosahezaenoic acid) or a placebo. After 1 year, 59% of patients in the fish oil group had maintained a remission, compared to only 26% in the placebo group [45]. This difference was highly significant ($\rho = 0.003$). Logistic regression analysis indicated that the fish oil was solely responsible for a decreased likelihood for relapse compared to the placebotreated group (odds ratio 4.2; 95% confidence limits 1.6-10.7). However, other studies on n-3 supplements did not confirm these findings [43]. The discrepancies between studies likely relates to the influence of concomitant therapies, type and dose of n-3 supplement as of the placebo, patient selection, baseline diet and adherence to therapy. Tsujikawa *et al.* [46] provided evidence from an open, pilot study to suggest that a diet rich in n-3 fatty acids (n-3/n-6 = 0.5), based on rice, cooked fish and soup, could be beneficial in terms of disease maintenance, without resorting to fish oil supplements. However, it is unlikely that such dietary interventions could be achieved in North America or Europe, in view of the high dietary intake of n-6 fatty acids. Further study in this area of nutritional research is certainly warranted.

Short-Chain Fatty Acids and IBD

Short-chain fatty acids (SCFAs) are produced in the colonic lumen by anaerobic fermentation of carbohydrates that have escaped absorption in the small bowel, particularly dietary fiber. SCFAs, especially butyrate, play a key role in the biology of the colonic epithelial cell, serving as their principal energy source. Indeed, a lack of luminal SCFAs is associated with epithelial atrophy and inflammation, as exemplified by diversion colitis. In ulcerative colitis, impaired butyrate oxidation by colonocytes has been implicated in disease pathogenesis, suggesting that this disease is due to a metabolic defect in epithelial cells. Butyrate enemas show some promise in treating this disorder when it affects the distal colon [47]. It would be of interest to develop a vehicle to release butyrate in the more proximal colon, other than depending on fermentation of unabsorbed carbohydrates. Butyrate is a potent inducer of colonic epithelial cell proliferation and differentiation, and has been shown to reduce paracellular permeability, possibly by promoting differentiation. Butyrate has also been shown to induce glutathione transferase activity by intestinal epithelial cells. SCFAs can also be beneficial in other

inflammatory bowel conditions. Histopathological assessment revealed less damage to the small and large bowel in response to the cytostatic drug Ara-C, when SCFAs were added to the diet of experimental mice [48]. This treatment represents a potential method to reduce the inflammation and lessening the mucositis caused by chemotherapy.

Modulation of Mucosal Immunity by Amino Acids

Among the hypotheses proposed for the beneficial effects of amino acid-based elemental diets is the removal of antigenic proteins which might stimulate gut immune responses (Table 1). Another consideration is the immunomodulatory capacity of certain peptides. For example, β-casein and its opioid peptides known as β-casomorphins have immunomodulatory activities, such as the promotion of antibody synthesis and phagocyte activity. Bovine β-casein was shown to enhance superoxide production by neutrophils as well as the proliferation of both T and B lymphocytes. It also increased IL-1 production by macrophages. Taken together, these results suggest that β-casein has selective modulating effects *in vitro,* mostly immunostimulatory, on both innate and adaptive immune responses in an experimental animal model. Thus, removal of this nutrient from the diet may also theoretically affect the gut immune response.

Another mechanism underlying the beneficial effects of defined formula diets in Crohn's disease is the capacity of glutamine to maintain gut barrier integrity and to enhance its repair in the face of inflammation [49]. Several roles have been ascribed to glutamine as an immunonutrient, including serving as the preferred energy source for intestinal epithelial cells, enhancing gut barrier function, as well as serving as an essential nutrient for immune cell function [39]. It is well established that gut disuse is associated with increased translocation of bacteria, their products such as endotoxin and even harmful pro-inflammatory cytokines such as TNF-α. Parenteral nutrition that is devoid of glutamine has detrimental effects on mucosal immune defenses, including decreased secretory IgA, IL-4 and IL-10 mRNA expression by intestinal lamina propria cells. Glutamine supplementation was observed to preserve normal levels for these gut-protective immune functions. Dietary glutamine supplementation modulated pro-inflammatory cytokine production (IL-8 and TNF-α) in the trinitrobenzene sulfonic acid rat colitis model [50]. Furthermore, glutamine is a substrate for glutathione synthesis [39], helping protect the gut mucosa against oxidant stress. The production of acute phase proteins and of GSH is greatly influenced by protein intake and especially of certain sulfur and related amino acids. The role of sulfur amino acids and polyamines as immunonutrients has recently been reviewed [51]. The level of cysteine and methionine in the diet directly influences GSH production, as discussed above.

Probiotics and Intestinal Immune Response

An increasing body of evidence points to the important role of the enteric microflora in inducing and sustaining chronic intestinal inflammation and arthritis. Probiotics are live microbial food ingredients that can alter the host intestinal microflora and which can have potential beneficial effects on health beyond their inherent basic nutrition. As an example,

Lactobacillus GG has been used successfully to prevent as well as treat refractory, relapsing colitis due to *Clostridium difficile*.

Whereas most nonantibiotic drugs modulate the immune response in IBD, probiotics have the ability to alter the luminal microflora, a major contributor to disease pathogenesis. Recent studies using probiotics to treat pouchitis [52] and ulcerative colitis [53] with the VSL#3 *Lactobacillus* have been very promising. However, because of strain-specific variability and therapeutic heterogeneity amongst patients, it cannot be assumed that beneficial results will be obtained in Crohn's disease. Nevertheless, based on our current understanding of disease pathogenesis, modification of enteric flora is a compelling potential avenue of biotherapeutics for the management of Crohn's disease. However, little data are yet available regarding controlled clinical trials with probiotics in Crohn's disease. An open label controlled trail using either 5-ASA alone (3 g/day) or 5-ASA with *Saccharomyces boulardii* (1 g/ day) in patients with Crohn's disease in remission was recently reported [54]. After 6 months, recurrence rates were 6/16 (38%) in the 5-ASA group, compared to 1/16 (7%) in the group treated with 5-ASA and the probiotic. Randomized, blinded trials are eagerly anticipated to confirm these promising results.

From the mechanistic point of view (Table 3), probiotics such as *Lactobacillus* species have the ability to prevent the attachment of pathogenic bacteria to the intestinal mucosa, and to restore abnormal intestinal permeability in animal models. They also have protective immunomodulatory ability, and have been shown to prevent the development of spontaneous colitis in the IL-10-deficient knockout mouse [55]. Using the same model, probiotic bacteria were found to enhance intestinal epithelial barrier function as well as reduce mucosal secretion of pro-inflammatory cytokines TNF-α and IFN-γ. Taken together, the results of these clinical trials with probiotics represent preliminary but encouraging therapeutic advances in IBD. They set the stage for large scale, randomized clinical trials.

Table 3. Probiotic requirements and the potential mechanisms underlying their beneficial effects as therapeutic agents in inflammatory bowel disease

Requirements

- Of human origin
- Nonpathogenic
- Resistant to gastric secretions, bile
- Adhere to gastrointestinal epithelium
- Persist in gastrointestinal tract
- Produce antimicrobial substances
- Modulate mucosal immune response

Mechanisms

- Competitive exclusion of bacterial adhesion and translocation
- Stimulation of protective mucosal immune response
  √Increase sIgA, IL-10
  √Decrease TNF-□, IFn-□,etc.
- Antimicrobial activity
  √Decrease luminal pH
  √Produce SCFAs, bacteriocins, H_2O_2
- Enhance gut barrier integrity
  √Prevents mucus degradation
  √Stimulates MUC gene expression

N-Acetyl Glucosamine

Glycosaminoglycan breakdown is an important consequence of inflammation at mucosal surfaces. Inhibition of metalloprotease activity might be effective in treating chronic inflammation. A recent pilot study by Salvatore *et al.* [56] examined the nutriceutical agent n-acetyl glucosamine (GlcNAc 3-6 g/day) an amino sugar directly incorporated into glycosaminoglycans and glycoproteins, as a substrate for tissue repair mechanisms in pediatric patients with Crohn's disease. Among the 12 patients given oral GlcNac, 8 showed clear improvement. Four others required resection. Among the 7 with symptomatic Crohn's strictures, 3 required surgery over a mean follow-up of over 2.5 years. Endoscopic or radiological improvement was detected in others. There was also evidence of histological improvement, as well as significant increases in epithelial and lamina propria content of glycosaminoglycans and intracellular GlcNAc. This pilot study provides preliminary evidence supporting this novel, nutriceutical approach.

Dietary particles

It is well established that abnormal intestinal permeability is associated with disease activity in IBD. The potential immune potentiation of ultrafine particles present in the diet has been the subject of recent investigations. It has been estimated that the typical 'Western' diet contains more than 10^{12} ultrafine particles that are ingested on a daily basis. Using the intestinal organ culture experimental model one study showed that the *in vitro* exposure of intestinal biopsies to titanium dioxide particles conjugated to LPS increased IL-1 production significantly. This study suggests that dietary particles are not immunologically inert and that they may be important adjuncts in overcoming normal gut cell hyporesponsiveness to endogenous luminal bacterial antigens. One might thus speculate that defined formula diets might exert their beneficial effect, in part, by virtue of a reduced content of ultrafine particles (Table 1). Microparticles accumulate in the phagocytes of intestinal lymphoid aggregates. In a recent double-blind study, patients with active Crohn's disease who received a low microparticle diet along with corticosteroids were more likely to achieve remission [57]. This might thus represent another potential benefit of defined formula diets.

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Discussion

Dr. Winter: There is a lot of interest in probiotics, but the problem seems to be the maintenance of colonization – as soon as you stop giving them they disappear. What do we do about that?

Dr. Seidman: Less than 10% of probiotics contain viable bacteria, and you need at least a million or more bacteria per gram in order to colonize the gut. So many of the so-called probiotics that are on the market and are reported to cure everything are ineffective. However, certain ones do seem to be effective and the problem is standardization and the conduct of properly controlled studies. I think there are some under way. The field of probiotics is broadening. For example, Erica Isolauri [1] in Finland has dome some very interesting experiments adding probiotics to cow's milk formula or alternate formulas, showing that she can prevent cow's milk allergy in young infants with a strong genetic predisposition to allergic disease. So adding probiotics to the diet can influence hypersensitivity reactions in the gut as well as immune reactions.

Dr Endres : The group of Erica Isolauri [1] has used Lactobacillus GG and Bifidobacterium lactis in two subsequent studies and showed that there was a positive effect on atopic eczema in infants.
Dr Grimble : How do the probiotics work? They are all bacterial, as I understand it. There were some studies indicating that bacteria contain receptors to cytokines. Do they act by that route or is there some other route?
Dr. Seidman : I think there are data suggesting that the bacteria influence the mucosal cytokine response themselves. So although they may have receptors for cytokines and presumably can consume some of the cytokines, they are producing an interleukin (IL) –10 response as opposed to a tumor necrosis factor (TNF) response, and they seem to be switching the profile of cytokines produced at the level of the mucosa as well. They also increase secretory IgA and return the gut mucosal inflammatory cells towards normal.
Dr Griffiths: Can you summarize the different theories behind the mechanism of action of probiotics? Is this related to bacterial exclusion, or to altering the existing bacterial flora to behave differently, or do they affect the damaging process in the mucosa?
Dr Seidman : I think it is likely to be a combination of all those factors. Various experimental models have been developed, using, say, T84 cells in culture with probiotics – lactobacilli or yeast organisms and so forth, which show that you can prevent the attachment of the enteropathogenic *Escherichia coli* that cause hemorrhagic colitis. This is a very big problem in North America, where too many hamburgers are not well cooked! You can also prevent the attachment of salmonellae, and you can prevent the translocation of bacteria. This presumably could have applications in the 'shocked gut' in people in intensive care units with multiple organ failure. There is strong evidence, both in humans and in animal models, that some probiotics can increase defense mechanisms – secretory IgA and IL-10 for example – and play a role in increase defense mechanisms – secretory IgA and IL-10 for example – and play a role in decreasing TNF. The theory here is that individuals with inflammatory bowel disorders have an abnormal hypersensitivity to their own flora but not to probiotic bacteria, so if we can cause some type of switch in the flora we can improve the cytokine profile. Probiotics do have antimicrobial activity and they are also able to enhance mucosal integrity. So I think the answer is that these agents have multiple effects.
Dr Docrat: I want to ask you about your butyrate enemas. It sounds like a wonderful way to treat a lot of the problems we surgeons have. Are they commercially available or is this purely experimental in your part of the world?
Dr Seidman: The butyrate enemas are more than experimental; they are clearly effective in managing diversion colitis, for example. I think that most clinicians – gastro-enterologists and surgeons – under-recognize the need for them. Part of the problem is that they are not commercially available, at least in North America, so you have to ask the pharmacy to make up a mixture of vinegars, butyrate, propionic acid, and so forth. But recipes are available in studies published in the *New England Journal of Medicine* [2]. Many patients have a rectal stump after a subtotal colectomy, and such patients are plagued with bleeding or persistent ulcerative colitis, and there could also be diversion proctitis in that situation, and those conditions respond well to butyrate enemas. Some researchers have suggested that ulcerative colitis might be a metabolic disorder related to aberrant butyrate metabolism. The problem is to arrange delivery of butyrate to the entire bowel. When you have distal colitis it is

reasonable to use enemas, but when you have a pancolitis it is difficult to deliver butyrate to the whole bowel.
Dr Docrat: Xanthine oxidase seems to play an important role, but you discussed the use of allopurinol, a xanthine oxidase inhibitor, in one study and it had very limited benefit. Why should that be?
Dr Seidman: There was some benefit in that study. It just was never followed up, though I'm not sure why not. I think people have been treating oxidative stress disorders with antioxidants rather than with metabolic prevention of free radical formation. Xanthine oxidase is certainly an important enzyme, but is not the only one involved, in the generation of oxidative stress.
Dr Winter: There has been some concern about giving live bacteria to patients who may have compromised immunity or impaired barrier function of the gut. Do you think that is justified?
Dr Seidman : I do think it is justified, though to the best of my knowledge the only cases of sepsis have been in infants. Thus there is reluctance to use bacteria, even probiotic bacteria, in young infants who have impaired barrier function. I would certainly also be hesitant in other individuals who are immunocompromised. I don't think there is a high risk of problems in patients with inflammatory bowel disease.
Dr Waitzberg: What was the rationale for combining eight different probiotic bacteria in the Italian study [3]?
Dr Seidman: I have no idea what the rational was for using the eight bacteria, but I think it's called hedging your bets – perhaps only one or two of these eight bacteria are effective. This type of trial is difficult, and if only one or two bacterial are selected, the trial may fail.
Dr Waitzberg: Is there a special time to use probiotics in Cronh's disease?
Dr Seidman : If you look at the evidence base for using probiotics in inflammatory bowel disease you would have to say that at the moment the one indication that most people would agree on is recurring pouchitis. Other than that, the existing studies are very weak in that there has been an inadequate number of patients tested and an inadequate follow-up, so I don't want to go on record as saying that I recommend antioxidants, but I don't for the moment recommend probiotics. I think their use requires further study. One study in disease in remission appeared to show an advantage for probiotics over 5-ASA, but there were only 32 patients and the study only lasted 6 months [4]. Also the dose of 5-ASA was lower than usual for maintaining remission.
Dr Meier: In relation to the pouchitis study, the Italian group which did it [3] showed very nicely that the probiotics were only active as long as they were given. When they stopped treatment all of the patients who were in remission relapsed. So it appears that probiotics only work as long as we give them.
Dr Seidman: That is very true and it shows that the normal flora cause the pouchitis, so if the probiotic bacteria are no longer there and the normal flora replace them, the pouchitis recurs. What is interesting is that not everybody who has a pouch develops pouchitis. The tendency to develop inflammation in the pouch seems to be genetically controlled. It has been shown by several investigators now that patients who express perinuclear-antineutrophil cytoplasmic antibody, which is one of the autoimmune markers associated with ulcerative colitis and is only present in 55–60% of patients, are the ones who are at significantly higher risk to develop pouchitis.

Dr Segal: Could you comment on the factors in sub-Saharan Africa that are opposed to orthodox thinking? Crohn's disease and colorectal adenomatous polyps are rare there, and ulcerative colitis and colorectal cancer uncommon, despite undernutrition and a low intake of antioxidants. Our hypothesis is that African infants are exposed to qualitatively better bacteria in terms of bifidobacteria and lactobacilli compared with, say, European infants, and that the immunological response in infancy is different. In Africa, there is a degree of physiological malabsorption of the main dietary staple, which is maize, and there is substantial short-chain fatty acid production, which may be relevant. It seems that if the infant can survive for 2 or 3 years, you then have someone who is protected throughout life from these gut diseases despite suboptimal nutrition.

Dr Seidman: This is relevant to the theory that inflammatory bowel disease occurs in individuals, who not only have an abnormal intake of n-6 fatty acids, but who are also protected from enteric pathogens during infancy – much like the asthma story. The so-called 'hygiene' theory is that if you are exposed to bacteria and viruses early in life, and if you survive that, then you tolerate your flora, whereas if you are protected from these infections then later in life you can become hypersensitive to your flora. The point you raise is relevant to the question of how we administer short-chain fatty acids other than by giving fiber. Most clinicians recommend a low-residue diet, without any scientific reason. However, a study involving a very large number of disease patients showed that there was no advantage to a low-fiber diet, or a low refined sugar diet either [5]. So perhaps if people in sub-Saharan Africa are eating a high-fiber diet and have more short-chain fatty acids, that could be protective. However, the issue is complicated by genetic factors that effect the risk for IBD.

Dr Shenkin: I wanted to ask about zinc. There is good evidence that zinc is important in the management of children with chronic diarrhea in emerging and underdeveloped countries, especially in preventing relapse. Individuals with inflammatory bowel disease are obviously very much at risk of zinc depletion, partly from the diarrhea itself and partly because there is redistribution of zinc into the liver and elsewhere. Have there been any studies on this? I am not aware of any investigations specifically on the effects of supplementation with zinc in inflammatory bowel disease.

Dr Seidman: There are observational studies looking at zinc levels, but as you know the levels in the circulation do not necessarily reflect the body stores. There does appear to be a subset of patients who are zinc-deficient, particularly those with profuse diarrhea, but as far as I know zinc supplementation has not been used systematically. However, children with disease and growth failure who fail to respond to improved macronutrient intakes have been shown to grow when given zinc supplements. Zinc could be a two-edged sword: it is clearly be considered partially immunosuppressed, which might be an advantage in an immune-mediated disorder; on the other hand zinc deficiency would impair antioxidant generation by the enzymes that require zinc as a cofactor.

Dr Okada: The two types inflammatory bowel disease – Crohn's disease and ulcerative colitis – are quite different from a therapeutic point of view. In ulcerative colitis total colectomy is usually required, while disease is mostly now treated by elemental diets, so nutrition is a very important feature in that condition. How do you explain this difference?

Dr Seidman: I wish I knew the answer to that. I think that from a nonsurgical standpoint the response to treatment in ulcerative colitis and Crohn's disease is very similar. It is true that ulcerative colitis responds better to cyclosporine that colitis, but neither type of colitis

responds as readily to elemental or semi-elemental diets as Crohn's disease of the small bowel. I'm not sure why that should be, and we need to pursue this further.
Dr Meier: In relation to pouchitis, it has been shown that patients with a pouch and no inflammation have large numbers of bacteria in the mucosa, while those who develop pouchitis have a decrease in intramucosal bacteria. On the other hand, it has also been shown that in patients with active disease there are large numbers of bacteria in the mucosa. Why should patients with active disease have plentiful intramucosal bacteria while patients with pouchitis have decreased number of bacteria?
Dr Seidman: I think most people would accept that pouchitis is a form of bacterial overgrowth in the pouch. The bacteria are not necessarily visible or demonstrable within the mucosa, but they are probably adjacent to the epithelium in the lumen. I think it is the effect on the epithelium that are important, rather than invasion of the epithelium. Crohn's disease is a deeply ulcerating condition where the chances of seeing invasive bacteria are greater because the ulcers are deeper. Perhaps pouchitis, like ulcerative colitis, is a more superficial epithelial disease, which is probably why we don't see the bacteria in the epithelium.
Dr. Micskey: I am a pediatrician and I have a question about the use of probiotics. Have you ever seen or read about the motility side effects of probiotic use, which can worsen the original symptoms?
Dr. Seidman: I don't have any experience other than what I have read, and we haven't employed probiotics in our center. Can anyone else in the audience respond?
Dr. Endres: There is a study by Saavedra and co-workers [6] showing that the stool becomes looser during treatment with bifidobacteria. That may partly answer the question.

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