

# Protective nutrients and functional foods for the gastrointestinal tract<sup>1-3</sup>

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**ABSTRACT** Epithelial and other cells of the gastrointestinal mucosa rely on both luminal and bloodstream sources for their nutrition. The term *functional food* is used to describe nutrients that have an effect on physiologic processes that is separate from their established nutritional function, and some of these nutrients are proposed to promote gastrointestinal mucosal integrity. We review the recent in vitro, animal, and clinical experiments that evaluated the role of several types of gastrointestinal functional foods, including the amino acids glutamine and arginine, the essential micronutrients vitamin A and zinc, and 2 classes of food additives, prebiotics and probiotics. Many of the data from preclinical studies support a strong role for enteral nutrients in gastrointestinal health; in comparison, the data from human studies are limited. In some cases, impressive data from in vitro and animal studies have not been replicated in human trials. Other clinical trials have shown positive health benefits, but some of those studies were plagued by flaws in study design or analysis. The methods available to detect important changes in human gastrointestinal function and structure are still limited, but with the development of more sensitive measures of gastrointestinal function, the effects of specific nutrients may be more easily detected. This may facilitate the development of phase 3 clinical trials designed to more rigorously evaluate the effects of a particular nutrient by focusing on valid and reliable outcome measures. Regulatory changes in the way in which health claims can be made for dietary supplements should also be encouraged. *Am J Clin Nutr* 2002;75:789–808.

## INTRODUCTION

It is often stated that the human gastrointestinal (GI) tract serves 2 main purposes: acting as a barrier to the external environment and as the main portal of entry for nutrients. The duality of this paradigm, however, may obscure an important feature of some nutrients, namely, to preserve the integrity and function of the GI mucosa itself. Because GI epithelial cells are the primary interface between ingested nutrients and the blood and lymph streams, it is not surprising that these cells are dependent on both luminal and bloodstream sources for their nutrition. Other cells of the GI tract, including M cells, enteroendocrine cells, intraepithelial lymphocytes, and the multiple cell types of the lamina propria are all affected by intra- and extraluminal nutrient intake.

The term *functional food* was coined to describe foods or nutrients whose ingestion leads to important physiologic

changes in the body that are separate and distinct from those associated with their role as nutrients (1, 2). We review several such nutrients that play an important and increasingly recognized role in human health, namely, the maintenance of normal GI mucosal function. By reviewing the laboratory and clinical evidence surrounding these nutrients, we hope to suggest further paths of investigation that will help confirm or refute the clinical importance of these nutrients.

## GLUTAMINE

Glutamine is a precursor for nucleotide synthesis, serves as a substrate for hepatic gluconeogenesis, and is an important nutrient for the renal handling of ammonia. It is also an important fuel source for cells that rapidly turn over, including GI epithelia, lymphocytes, fibroblasts, and reticulocytes (3).

## In vitro and animal experimental data

Many experimental data support the importance of glutamine in GI function. Rats fed via parenteral nutrition show less mucosal atrophy when supplemented with glutamine (4), as do piglets (5). Animal data also show decreased intestinal permeability, as measured by the ratio of lactulose to mannitol in urine, with glutamine treatment (6). Rats subjected to gram-negative sepsis show less GI mucosal damage and higher mucosal rates of protein synthesis with glutamine supplementation (7). Isolated enterocytes were also shown to have increased rates of protein synthesis with glutamine supplementation (8). When the intestinal tracts of rats were subjected to ischemia and reperfusion, treatment with glutamine helped to preserve mucosal glutathione concentrations and decreased markers of lipid peroxidation (9).

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Tumor-bearing rats show decreased whole-body protein breakdown and increased muscle protein synthesis with glutamine-supplemented parenteral nutrition, although no effect on the rate of protein synthesis in GI mucosa was observed (10). The specific role of glutamine as a possible mediator of intestinal adaptation in short-bowel syndrome has been indicated in some (11, 12) but not all (13) animal models.

Glutathione is a crucial antioxidant found in high concentrations in the GI mucosa (14), and inhibition of its synthesis leads to degeneration of the mucosa, diarrhea, and growth failure (15). Intestinal synthesis of glutathione depends on the presence of its precursor amino acids glutamate, cysteine, and glycine. Dietary glutamine probably contributes significantly to intestinal glutathione synthesis, both through the deamination of glutamine to form glutamate in the arterial blood supply of mucosal cells and through the ubiquitous presence of glutaminase among intestinal cells. Animal data suggest that glutamine-supplemented intravenous solutions improve mucosal (16) and plasma (17) glutathione concentrations.

### Human studies

Under normal conditions, glutamine is a nonessential amino acid. However, in catabolic patients, glutamine has been postulated to be a conditionally essential nutrient (18). Humans under catabolic stress show an increased efflux of glutamine from skeletal muscle (19). Severely ill patients whose nutrition is supported solely by the parenteral route are at risk of receiving inadequate dietary glutamine because of the relative instability of this amino acid in standard protein solutions (20). Because of the combination of reduced dietary intake and increased metabolic demands, patients under catabolic stress may be in a state of glutamine deficiency.

Numerous human studies showed that supplemental glutamine results in improved GI function. These studies documented a role for glutamine in ameliorating the mucosal atrophy seen in prolonged states of parenteral nutrition (4, 21, 22), in the healing of GI mucosa after damage from either radio- or chemotherapy (23, 24), in improving gut and systemic immune function (25–27), in attaining nitrogen balance and in weaning from parenteral nutrition (28), and in reducing episodes of bacterial translocation (29, 30) and clinical sepsis (31, 32). Oral rehydration solutions with added glutamine have not been proven to be more effective than standard solutions in correcting dehydration due to acute diarrhea (33), although some trials used solutions whose osmolarity may have masked an effect (34). In 6 patients with short-bowel syndrome, the use of a glutamine-containing isotonic rehydration solution resulted in lower sodium absorption than was observed with the use of a glucose-containing solution (35).

In the past 10 y, many clinical trials of glutamine supplementation have been published (Table 1). Although the results of several of these studies appear quite convincing, methodologic problems have been noted with some. These problems include inadequate sample size, incomplete description of blinding and randomization procedures, lack of a control group, and lack of an isonitrogenous control. Some studies also did not detail their inclusion criteria or did not follow intent-to-treat data analysis, and others reported only subgroup analyses. In addition, many of these studies did not present data on dietary intake during the trial, an omission that may lead to doubts about the comparability of the nitrogen and nutrient intakes of the study groups. A

recent meta-analysis of 14 randomized trials of glutamine supplementation in surgical and critically ill patients showed a lower rate of infection (relative risk: 0.91; 95% CI: 0.64, 1.00) and a shorter hospital stay ( $\bar{x}$ :  $-2.6$  d; 95% CI:  $-4.5$ ,  $-0.7$ ) with glutamine supplementation. In other studies, high-dose glutamine and parenteral glutamine were also associated with reductions in mortality (66). In addition, a large randomized trial of parenteral glutamine among low-birth-weight infants in the United States is anticipated to be complete soon.

Although many in vitro and in vivo data point to an important role for glutamine in the maintenance and repair of GI mucosa, larger, well-designed randomized trials are needed. Important research issues include the selection of valid and reliable outcome variables that have biological plausibility and can be routinely measured in study subjects. Examples would include measures of intestinal permeability, bacterial translocation, and small-bowel histology.

### ARGININE

Arginine is an amino acid with important roles in the transport, storage, and excretion of nitrogen; in polyamine synthesis; and in the disposition of ammonia via the urea cycle (67). Much dietary arginine is removed by first-pass metabolism by the splanchnic bed, indicating that the small bowel is an important site of arginine metabolism (68). On the basis of experiments performed in the 1950s, arginine is considered to be a nonessential amino acid because positive nitrogen balance in healthy adult humans can be attained in its absence (69). However, like glutamine, arginine has been hypothesized to be a conditionally essential amino acid because alterations in its metabolism during catabolic states such as trauma and sepsis indicate that it may be essential under these conditions (70, 71).

Much of the interest in arginine relates to its role as the precursor for nitric oxide (NO), a molecule with a wide range of functions (72). Arginine is converted to citrulline by the action of nitric-oxide synthase (NOS), which combines the terminal guanidino nitrogen atom of arginine with oxygen to form NO. The constitutive form of NOS (cNOS) produces the small amounts of NO that are necessary for certain cell functions in the nonpathologic state, such as neurotransmission and vascular relaxation. An inducible form of NOS (iNOS) is found in a wide variety of mammalian cells (macrophages, neutrophils, mast cells, fibroblasts, hepatocytes, vascular endothelial cells, smooth muscle cells, and cardiac myocytes). iNOS is induced by a variety of events including the presence of inflammatory cytokines and bacterial endotoxin and results in the production of larger amounts of NO. NO may have both anti- and proinflammatory effects; homeostatic, antiinflammatory effects are observed with small amounts of NO produced by cNOS, whereas the proinflammatory effects are seen with the iNOS production of excessive amounts of NO. Excessive NO production in the setting of endotoxemia, septic shock, and increased intestinal vascular permeability has been studied in a variety of animal and human experiments.

### In vitro and animal experimental data

Many animal studies have examined the role of arginine and NO in immunity and inflammation. Arginine supplementation leads to higher thymic weight, higher thymic lymphocyte content, and an

**TABLE 1**Summary of clinical studies of glutamine in patients at risk of gastrointestinal mucosal disease<sup>1</sup>

Reference	Study type	Subjects	Glutamine dose and route of administration	Comparison group	Outcomes of glutamine group
Ziegler et al (31) Scheltinga et al (36) MacBurney et al (37) Ziegler et al (38)	Randomized, blinded clinical trial	45 adult allogeneic BMT patients	0.57 g · kg <sup>-1</sup> · d <sup>-1</sup> IV	Isonitrogenous and isoenergetic PN	Better nitrogen balance, fewer clinical infections, shorter LOS, lower hospital costs, less ECF expansion, increased total lymphocyte count, increased CD4 <sup>+</sup> and CD8 <sup>+</sup> counts
Schloerb and Amare (39)	Randomized, blinded clinical trial	29 adult allogeneic and autologous BMT patients	0.57 g · kg <sup>-1</sup> · d <sup>-1</sup> IV	Isonitrogenous and isoenergetic PN	Shorter LOS, less ECF expansion, no decrease in clinical infections
Van der Hulst et al (21)	Randomized, blinded clinical trial	20 adults requiring inpatient PN for 2 wk	0.23 g · kg <sup>-1</sup> · d <sup>-1</sup> IV via dipeptides	Isonitrogenous and isoenergetic PN	Preserved villus height, lactulose-mannitol ratio preserved
Hornsby-Lewis et al (40)	Case series	7 adults receiving home PN	0.285 g · kg <sup>-1</sup> · d <sup>-1</sup> IV	None	Increased hepatic transaminases?
Tremel et al (22)	Randomized, blinded clinical trial	12 adults requiring PN in intensive care	0.30 g · kg <sup>-1</sup> · d <sup>-1</sup> IV L-Ala-L-Gln	Isonitrogenous and isoenergetic PN	Higher area under the D-xylose × time curve
Nattakom et al (41) Goringe et al (42)	Case series	3 patients with VOD treated with Gln and vitamin E (400 mg/d)	20 g PO QD	None	Recovery from VOD?
Jensen et al (43)	Randomized, blinded clinical trial	28 adults in ICU with APACHE scores > 10 who received enteral nutrition by 48 h of admission	EN with 25% Gln	Isonitrogenous and isoenergetic EN	Reduced Phe-Tyr ratio, transient rise in CD4 <sup>+</sup> -CD8 <sup>+</sup> ratio
Skubitz and Anderson (44)	Case series	14 adults who previously had stomatitis with chemotherapy	4 g swish and swallow BID	None	Less mucositis?
Bozzetti et al (45)	Randomized, blinded clinical trial	67 adult women with advanced breast cancer	30 g PO QD	Maltodextrin	No differences in diarrhea incidence
Griffiths et al (46)	Randomized, blinded clinical trial	84 adults in ICU with APACHE II scores > 10	PN with 2.5% Gln	Isonitrogenous PN	Reduced mortality at 6 mo
Neu et al (32)	Randomized, blinded clinical trial	68 premature infants	≤0.31 g · kg <sup>-1</sup> · d <sup>-1</sup> in enteral formula	Unsupplemented formula	Fewer infections, better tolerance of enteral feeds
Anderson et al (47)	Randomized, blinded clinical trial	193 BMT patients	1 g/m <sup>2</sup> body surface area swish and swallow QID	Same dose of Gly	In autologous BMT, Gln patients had less mouth pain and used opiates for fewer days (5 compared with 10 d); no differences in GVHD, antibiotic use, or LOS noted
Brown et al (48)	Randomized, blinded clinical trial	34 adult BMT patients	50 g glycyl-Gln IV	Isonitrogenous PN	Protein C and serum albumin concentrations better maintained; markers of thrombin and plasmin generation not different
de Beaux et al (49)	Randomized, blinded clinical trial	14 adults with severe acute pancreatitis	0.22 g · kg <sup>-1</sup> · d <sup>-1</sup> IV	Isonitrogenous PN	Lowered IL-8 release by mononuclear cells; no changes in tumor necrosis factor or IL-6
Houdijk et al (50)	Randomized, blinded clinical trial	72 adults with trauma and injury severity scores > 20	30.5 g Gln/100 g protein	Isonitrogenous EN	Fewer episodes of bacteremia, pneumonia, and sepsis
Morlion et al (51)	Randomized, blinded clinical trial	28 adults with colon carcinoma or other reason for colon surgery	0.3 g · kg <sup>-1</sup> · d <sup>-1</sup> Ala-Gln IV	Isonitrogenous PN	Higher nitrogen balance, shorter LOS

(Continued)

TABLE 1 (Continued)

Reference	Study type	Subjects	Glutamine dose and route of administration	Comparison group	Outcomes of glutamine group
Noyer et al (52)	Randomized, blinded clinical trial	24 adults with AIDS and abnormal lactulose-mannitol tests	4 ( $n = 8$ ) or 8 ( $n = 8$ ) g Gln PO/d $\times$ 28 d	6 g sucrose	No changes in intestinal histology or lactulose-mannitol ratios
Rubio et al (53)	Case series	9 adult women with breast cancer receiving methotrexate	0.5 g $\cdot$ kg <sup>-1</sup> $\cdot$ d <sup>-1</sup>	None	Less toxicity from methotrexate?
Yoshida et al (54)	Randomized clinical trial	13 patients with advanced esophageal cancer	30 g/d PO	None	Less reduction in lymphocyte count with chemotherapy
Barbosa et al (55)	Randomized, blinded clinical trial	9 infants in ICU with sepsis or respiratory failure	0.3 g $\cdot$ kg <sup>-1</sup> $\cdot$ d <sup>-1</sup> EN	Casein	No differences
Decker-Baumann et al (56)	Randomized, blinded clinical trial	24 adults with metastatic colon cancer receiving chemotherapy	0.4 g/kg glycyl-L-Gln IV	Nonsupplemented group	Less small bowel and gastric inflammation by esophagogastroduodenoscopy, higher villus-to-crypt ratio
Den Hond et al (57)	Randomized, blinded clinical trial	14 patients with Crohn disease and increased intestinal permeability	7 g PO TID $\times$ 4 wk	Gly	No change in intestinal permeability or Crohn disease activity
Jones et al (58)	Randomized, blinded clinical trial	50 adults in ICU with APACHE scores $> 10$ who tolerated enteral nutrition by 48 h after admission	11–21 g/d in Gln-supplemented elemental formula	Isonitrogenous EN (Gly)	No differences in mortality, morbidity, ICU stay or LOS; hospital costs lower
Okuno et al (59)	Multicenter, randomized, blinded clinical trial	134 adults receiving 5-fluorouracil chemotherapy for the first time	4 g BID swish and swallow $\times$ 14 d	Identically appearing placebo	No differences in oral mucositis scores
Pietsch et al (60)	Case series	17 children (aged 2–19 y) receiving chemotherapy ( $n = 14$ ) or BMT ( $n = 3$ )	Gln-supplemented EN	None	Tube feedings were well tolerated; hospital charges for enteral support lower than for PN
Powell-Tuck et al (61)	Randomized, blinded clinical trial	168 adults requiring PN	Gln-supplemented PN	Isonitrogenous PN	Shorter LOS in surgical patients only
Schloerb and Skikne (62)	Randomized, blinded clinical trial	66 adults undergoing allogeneic or autologous BMT	10 g PO TID or 0.57 g/kg IV	10 g Gly PO TID or isonitrogenous PN	No significant differences in mortality, LOS, engraftment, incidence of infections, or GVHD
Shabert et al (63)	Randomized, blinded clinical trial	26 HIV-infected adults with $> 5\%$ weight loss	40 g/d $\times$ 12 wk plus additional antioxidants	40 g Gly/d	Increased body weight, body cell mass, and intracellular water
Akobeng et al (64)	Randomized, blinded clinical trial	18 children with active Crohn disease	Polymeric EN with 7.9–8.3 g Gln/100 g formula	Isonitrogenous and isoenergetic EN	Less improvement in Crohn disease activity score
Coghlin Dickson et al (65)	Randomized, blinded clinical trial	58 adult BMT patients	30 g Gln PO QD	30 g sucrose PO QD	No significant differences in mortality, LOS, engraftment, mucositis, or diarrhea

<sup>1</sup>BMT, bone marrow transplantation; IV, intravenous; PN, parenteral nutrition; LOS, length of stay; ECF, extracellular fluid; VOD, venoocclusive disease; PO, orally (by mouth); QD, every day; ICU, intensive care unit; APACHE, acute physiology and chronic health evaluation; EN, enteral nutrition; BID, twice a day; QID, 4 times a day; GVHD, graft-versus-host disease; IL, interleukin; TID, 3 times a day.

increased response of T lymphocytes to mitogen stimulation (73). In addition, rats receiving arginine-supplemented parenteral nutrition show an increased ability to synthesize acute-phase proteins when challenged with sepsis (74). Tumor-bearing rats treated with parenteral nutrition high in arginine and branched-chain amino acids have lower rates of tumor protein synthesis and higher rates of whole-body protein synthesis than do those that receive standard nutrition (75). Similar findings were reported for arginine-enriched nutrition in another rat model of cancer (76);

higher muscle concentrations of glutamine, arginine, and other amino acids were also reported with arginine-supplemented parenteral nutrition (77). In a mouse model of graft-versus-host disease, suppression of NO production by nitro-L-arginine methyl ester, a selective NOS inhibitor, was associated with splenic atrophy, decreased extramedullary hematopoiesis, a reduction in bone marrow cellularity, enhanced weight loss, and decreased overall survival (78). Arginine is also important in the synthesis of connective tissue and arginine-rich proteins. Ani-

mals subjected to wounds or fractures have improved rates of wound healing, nitrogen retention, and growth when supplemented with dietary arginine (79, 80).

The role of arginine has been examined indirectly as well through the study of NO. Several studies suggest that NO production by cNOS plays a role in the maintenance of the normal intestinal mucosal barrier. Alican and Kubes (81) reviewed animal data suggesting that inhibition of cNOS by nitro-L-arginine methyl ester increases the permeability of the small intestine to smaller molecules but does not appear to increase the permeability to larger molecules or to result in mucosal damage. One suggested mechanism of the action of NO is an indirect one, through the inhibition of mast cell reactivity. In one study, the intraluminal administration of nitro-L-arginine methyl ester resulted in an increase in markers of mast cell degranulation as well as an increase in intestinal permeability (82).

Although iNOS was previously thought to play only a detrimental role in mucosal inflammation, more recent evidence in models of chemically induced colitis in iNOS-deficient mice suggests otherwise. The presence of iNOS expression in leukocytes decreases early granulocyte mucosal infiltration, and a lack of iNOS expression is correlated with the presence of greater macroscopic mucosal damage (83, 84). The timing and duration of these effects varies depending on the type of model used. In addition, mice in which iNOS is expressed have lower leukocyte recruitment to various tissues in response to administration of lipopolysaccharide than do iNOS-deficient control mice, suggesting that iNOS may have a role in regulating the immune response via an influence on leukocyte migration (85).

In vivo rat perfusion studies showed that arginine and NO are both intestinal secretagogues and that inhibition of NOS can result in intestinal ischemia as well as increased fluid secretion (86). Animals subjected to intestinal ischemia have better outcomes when pretreated with arginine (87). Animal models of intestinal transplantation also showed that arginine supplementation results in less disruption of the basement membrane when reperfusion occurs (88) and in improved morphology (89). Rats subjected to massive small-bowel resection that were treated with arginine had better preserved intestinal barrier function (as measured by the ratio of lactulose to rhamnose recovered in the urine) than did those that were not treated with arginine, although intestinal protein synthesis was lower in the arginine-treated group (90). Recovery from radiation enteritis was also shown to improve with arginine supplementation, as measured by increased mucosal thickness, villous height, and number of villi per centimeter of small bowel (91). Quantitative bacterial cultures of mesenteric lymph nodes, a measure of bacterial translocation, were also reported to decrease in this rat model (92).

### Human studies

In healthy subjects, NO formation can be increased by L-arginine administration as the result of enhanced NOS activity (93). Dietary supplementation of healthy adults and postoperative patients with arginine also increases the mitotic response of their peripheral lymphocytes to standard stimuli (94, 95). NO produced by human intestinal epithelial cells inhibits the growth, encystation, and excystation of the pathogen *Giardia lamblia*, and *G. lamblia* inhibits NO production by these cells by competing for the arginine substrate (96).

Neonatal necrotizing enterocolitis (NEC) is marked by increased mucosal permeability and inflammation. In studies of premature infants who developed NEC, serum concentrations of arginine and glutamine decreased both before and during an episode of NEC (97, 98). A recent study of 152 premature, low-birth-weight infants compared the use of arginine-supplemented nutrition with that of standard nutrition. During the first 28 d of life, infants who received arginine had a significantly lower incidence and a later median age of onset of NEC. Plasma arginine concentrations were low in all infants who were diagnosed with NEC (99). Additional clinical trials evaluating the effects of arginine supplementation in infants at risk of NEC might help to further define the role of arginine in the maintenance of GI mucosal health.

Because inflammatory bowel disease is marked by an increased synthesis of cytokines and increased intestinal permeability, the role of NO in inflammatory bowel disease has been evaluated in many studies. Using intestinal specimens from surgical resection, one group of researchers showed that the mucosal iNOS activity of specimens from patients with ulcerative colitis was 8-fold higher than that of control specimens, but the mucosal iNOS activity of specimens from 4 patients with Crohn colitis did not differ significantly from that of control specimens (100). In contrast, mucosal biopsy specimens from patients with active ulcerative colitis or Crohn colitis showed significantly higher iNOS activity than did biopsies from healthy control subjects, and the addition of antiinflammatory drugs (eg, steroids) reduced iNOS activity (101). Similarly, another study compared iNOS expression in surgical specimens from patients with active ulcerative colitis with those of patients with inactive ulcerative colitis or nonulcerative colitis (102). iNOS was expressed in the active ulcerative colitis specimens but was virtually undetectable in control specimens. In addition, iNOS was localized to neutrophils and macrophages in ulcer bases. Patients with active ulcerative colitis also had higher serum concentrations of nitrates and nitrites, breakdown products of NO.

Many clinical trials evaluated arginine in patients at risk of intestinal disease because of trauma, critical illness, or cancer (Table 2). In several of these studies (107, 114, 120, 130), the researchers used the term *immune-enhancing diet* or other value-laden terms to describe the treatment received by the treatment group. Many clinical trials of arginine supplementation did not compare the amino acid in an isonitrogenous, isoenergetic manner, and intention-to-treat analysis was not uniformly followed. Many studies combined arginine with added n-3 fatty acids, branched-chain amino acids, and nucleotides and used a commercial product (Impact; Novartis Nutrition, Basel, Switzerland; or Immune-Aid; McGaw, Inc, Irvine, CA). As such, it is difficult to assess the effects of arginine apart from any effects of the other additives. Although some researchers suggest that arginine works best in conjunction with other nutrients, the justification for the precise formulation of these products is not well established.

Two meta-analyses of the effects of these commercially available formulas containing arginine and other additives have been published. One analysis of 12 trials with a total of 1557 patients showed that patients fed these formulas had shorter hospital stays, had lower overall infection rates, and spent fewer days on ventilator support than did those who were not fed these formulas (135). However, differences in the time-dependent outcomes disappeared when deaths were censored, and the analysis did not show any effect on mortality. In another systematic review (136), 22 trials among 2419 subjects



**TABLE 2**Summary of clinical studies of arginine in patients at risk of gastrointestinal mucosal disease<sup>1</sup>

Reference	Study type	Subjects	Arginine dose and route of administration	Comparison group	Outcomes of arginine group
Cerra et al (103)	Randomized, double-blind, clinical trial	22 adult surgical ICU patients	12.5 g/L supplemented with nucleotides and n-3 FA	Isoenergetic formula	Greater in vitro stimulation of peripheral blood lymphocytes
Gottschlich et al (104)	Randomized, double-blind, clinical trial	50 adult and pediatric patients with >10% body surface area burn	5 g/L supplemented with n-3 FA	2 control groups; both isoenergetic, isonitrogenous formulas	Decreased LOS, controlled for percent burn; lower incidence of wound infections
Daly et al (105)	Randomized clinical trial	85 adult surgical patients with upper gastrointestinal cancer	12.5 g/L supplemented with nucleotides and n-3 FA	Isoenergetic formula	Fewer infectious and wound complications, decreased LOS
Sigal et al (106)	Randomized, double-blind, clinical trial	30 adult surgical patients with lower gastrointestinal cancer	20 g/d parenterally	Isoenergetic, isonitrogenous parenteral mixed amino acid solution	No difference in postoperative complications; lower lymphocyte response to phytohemagglutinin stimulation on postoperative day 4; higher percentage of CD3 <sup>+</sup> lymphocytes by postoperative day 7
Moore et al (107)	Randomized multicenter trial	114 adult ICU trauma patients with ISS $\geq 16$ or ATI $\geq 18$	15.4 g/L supplemented with nucleotides and n-3 FA	Isoenergetic formula	Lower incidence of intraabdominal abscesses, lower multiorgan failure scores, higher total lymphocyte and CD3 <sup>+</sup> and CD4 <sup>+</sup> concentrations
Bower et al (108)	Randomized, double-blind, multicenter trial	326 adult ICU patients with APACHE II scores $\geq 10$ or TISS $\geq 20$	12.5 g/L supplemented with nucleotides and n-3 FA	Isoenergetic formula containing a lower amount of nitrogen	Decreased mortality rate in analysis of successfully fed subgroup
Daly et al (109)	Randomized clinical trial	60 adult surgical patients with upper gastrointestinal tract cancer	12.5 g/L supplemented with nucleotides and n-3 FA	Formula with higher protein, fat, and energy	Shorter LOS, fewer infectious and wound complications, higher plasma concentrations of n-3 FA at postoperative day 7
Kemen et al (110)	Randomized, double-blind, clinical trial	44 adult surgical ICU patients with upper gastrointestinal tract cancer	12.5 g/L supplemented with nucleotides and n-3 FA	Isoenergetic, isonitrogenous formula	Higher overall T lymphocyte and CD4 <sup>+</sup> , CD3 <sup>+</sup> , and activated CD3 <sup>+</sup> subset concentrations by postoperative day 10; higher B lymphocyte concentrations by postoperative day 7
Senkal et al (111)	Randomized, double-blind, clinical trial	42 adult surgical patients with upper gastrointestinal tract cancer	12.5 g/L supplemented with nucleotides and n-3 FA	Isoenergetic, isonitrogenous formula	Faster decline of APACHE II scores, lower IL-6 concentrations on postoperative days 3 and 7
Braga et al (112)	Randomized, double-blind, clinical trial	44 adult patients with upper or lower gastrointestinal tract cancer	12.5 g/L supplemented with nucleotides and n-3 FA given from preoperative day 7 to postoperative day 7	Isoenergetic formula from preoperative day 7 to surgery, then isonitrogenous to postoperative day 7	Lower CRP postoperatively, higher serum NO concentrations postoperatively, higher mean Doppler intestinal blood flow, lower serum concentrations of intestinal alkaline phosphatase postoperatively, higher phagocytic and respiratory burst activity postoperatively
Braga et al (113)	Randomized clinical trial	60 adult surgical patients with upper gastrointestinal tract cancer	12.5 g/L supplemented with nucleotides and n-3 FA	1) Isoenergetic, isonitrogenous enteral formula or 2) PN	Shorter postoperative LOS compared with that of the PN group, lower postoperative sepsis score in patients with infectious complications, higher visceral protein concentrations at postoperative day 8, lower plasma IL-6 concentrations by postoperative day 8
Kudsk et al (114)	Randomized clinical trial	35 adult trauma patients with ATI $\geq 25$ or ISS $\geq 20$	14 g/L supplemented with nucleotides, n-3 FA, and Gln	1) Isonitrogenous, isoenergetic formula or 2) unfed trauma patients	Fewer infectious complications, shorter LOS

(Continued)

TABLE 2 (Continued)

Reference	Study type	Subjects	Arginine dose and route of administration	Comparison group	Outcomes of arginine group
Schilling et al (115)	Randomized clinical trial	41 adult surgical patients with upper or lower gastrointestinal cancer	12.5 g/L supplemented with nucleotides and n-3 FA	1) Isoenergetic enteral formula or 2) PN without protein	No overall differences in mortality, LOS, and infectious complications
Gianotti et al (116)	Randomized clinical trial	260 adult surgical patients with upper gastrointestinal tract cancer	12.5 g/L supplemented with nucleotides and n-3 FA	Isoenergetic, isonitrogenous 1) enteral formula, or 2) PN	Shorter hospital LOS, faster decline of plasma IL-6 concentration, higher plasma prealbumin concentration, and faster recovery of delayed hypersensitivity score and neutrophil phagocytic function by postoperative day 8
Heslin et al (117)	Randomized clinical trial	195 adult surgical patients with upper gastrointestinal tract cancer	12.5 g/L supplemented with nucleotides and n-3 FA	Intravenous crystalloid	No overall differences in mortality, LOS, and infectious complications
Heys et al (118)	Randomized clinical trial	18 adult surgical patients with lower gastrointestinal tract cancer	30 g/d for 3 d preoperatively in addition to standard hospital diet	Standard hospital diet	Higher percentage of cells expressing both CD16 <sup>+</sup> and CD56 <sup>+</sup> found in tumor-infiltrating lymphocyte population
Mendez et al (119)	Randomized, double-blind, clinical trial	59 adult ICU trauma patients with ISS >13	6.6 g/L supplemented with n-3 FA, selenium, chromium, molybdenum, L-carnitine, and taurine	Isoenergetic, isonitrogenous formula	Faster recovery of lipopolysaccharide-stimulated monocyte tumor necrosis factor and prostaglandin E <sub>2</sub> production by postoperative days 6 and 10, respectively; greater neutrophil oxidative burst activity by postoperative day 6
Saffle et al (120)	Randomized, double-blind, clinical trial	50 adult and pediatric burn patients	14 g/L supplemented with nucleotides and n-3 FA	Isoenergetic formula	No overall differences in mortality, LOS, and infectious complications
Senkal et al (121)	Randomized, double-blind, multicenter trial	164 adult surgical patients with upper gastrointestinal tract cancer	12.5 g/L supplemented with nucleotides and n-3 FA	Isoenergetic, isonitrogenous formula	Fewer patients with complications occurring after postoperative day 5
Atkinson et al (122)	Randomized, double-blind, clinical trial	398 adult ICU patients with APACHE II scores >10 and TISS >20	12.5 g/L supplemented with nucleotides and n-3 FA	Isoenergetic, isonitrogenous formula	Decreased need for mechanical ventilation and decreased LOS in a priori subgroup (intake >2.5 L by 24 h)
Braga et al (123)	Randomized clinical trial	166 adult surgical patients with upper gastrointestinal tract cancer	12.5 g/L supplemented with nucleotides and n-3 FA	Isoenergetic, isonitrogenous 1) enteral formula or 2) PN	No difference between the enteral formula groups; lower postoperative sepsis score than that for the PN group; lower LOS than that of the PN group in patients having <90% usual body weight
Braga et al (124)	Randomized clinical trial	30 adult surgical patients with gastric adenocarcinoma	12.5 g/L supplemented with nucleotides and n-3 FA given from preoperative day 7 to postoperative day 7	Same formula given for only 7 d during the postoperative period	Higher serum prealbumin concentration and lower plasma IL-6 concentration postoperatively
Wiemann et al (125)	Randomized, blinded clinical trial	32 adult ICU trauma patients with ISS >20	12.5 g/L supplemented with nucleotides and n-3 FA	Isoenergetic, isonitrogenous formula	No overall difference in mortality, LOS, and infectious complications; fewer systemic inflammatory response syndrome days per patient; lower multiple organ failure scores
Braga et al (126)	Randomized, double-blind, clinical trial	206 adult surgical patients with upper or lower gastrointestinal tract cancer	12.5 g/L supplemented with nucleotides and n-3 FA given from preoperative day 7 to postoperative day 7	Isoenergetic, isonitrogenous formula	Decreased LOS and infectious complications

(Continued)

TABLE 2 (Continued)

Reference	Study type	Subjects	Arginine dose and route of administration	Comparison group	Outcomes of arginine group
Buchman et al (127)	Randomized, double-blind, clinical trial	23 adult marathon runners	10 g 3 times 3 times a day 14 d before marathon	10 g Gly 3 times a day for 14 d before marathon	No overall difference
Gianotti et al (128)	Randomized, double-blind, clinical trial	58 adult surgical patients with upper or lower gastrointestinal tract cancer	12.5 g/L supplemented with nucleotides and n-3 FA from preoperative day 7 to postoperative day 7	Isoenergetic formula with 1) less protein for 7 d before operation and 2) equivalent amount of protein from surgery to postoperative day 7	Faster decline of plasma IL-6 concentration and higher serum visceral protein concentrations by postoperative day 1
Senkal et al (129)	Randomized, double-blind, multicenter trial	178 adult patients with upper gastrointestinal tract cancer	14.8 g/L for 5 d before operation and 12.5 g/L after operation supplemented with nucleotides and n-3 FA	Isoenergetic formula for 5 d before operation and isoenergetic, isonitrogenous formula after operation	Fewer infectious complications
Galban et al (130)	Randomized multicenter trial	181 adult ICU patients with sepsis and APACHE II scores $\geq 10$	12.5 g/L supplemented with nucleotides and n-3 FA	Nearly isonitrogenous, higher energy formula	Lower overall mortality rate, especially in patients with lower APACHE II scores; fewer episodes of bacteremia; fewer recurrent nosocomial infections
Berard et al (131)	Randomized, single-blind, 2-center trial	15 adult surgical ICU patients	22.5 g/L parenterally	Isoenergetic, isonitrogenous parenteral mixed amino acid solution	Higher plasma arginine, ornithine, and glutamine concentrations during infusion; decreased muscle protein catabolism
Gianotti et al (132)	Randomized clinical trial	212 adult patients undergoing pancreaticoduodenectomy	12.5 g/L supplemented with nucleotides and n-3 FA	Isoenergetic, isonitrogenous 1) enteral formula or 2) PN	Lower sepsis score; fewer complications than those of the PN group; lower CRP, higher visceral protein stores, and better neutrophil phagocytic ability by postoperative day 8
Riso et al (133)	Randomized clinical trial	44 adult head and neck cancer surgical patients	6.25 g/L	Isoenergetic, isonitrogenous formula	Lower incidence of complications and LOS in malnourished subgroup
Amin et al (99)	Randomized, double-blind, clinical trial	152 premature infants (gestational age <32 wk and weight <1250 g) in neonatal ICU	0.261 g · kg <sup>-1</sup> · d <sup>-1</sup> in PN or formula	Isoenergetic, isonitrogenous PN or formula	Lower incidence of NEC, later onset of NEC
van Bokhorst-De Van Der Schueren et al (134)	Randomized clinical trial	56 malnourished (>10% of body weight loss over preceding 6 mo) head and neck cancer surgical patients	12.5 g/L	2 groups: 1) Isoenergetic, isonitrogenous for 9 d before operation and postoperatively 2) No nutrition therapy preoperatively and isoenergetic, isonitrogenous postoperatively	No difference

<sup>1</sup>ICU, intensive care unit; FA, fatty acids; LOS, length of stay; ISS, injury severity score; ATI, abdominal trauma index; APACHE, acute physiology and chronic health evaluation; TISS, therapeutic intervention severity score; CRP, C-reactive protein; PN, parenteral nutrition; NEC, necrotizing enterocolitis; IL, interleukin.

were evaluated. The use of immunologically active formulas was associated with fewer infectious complications and reduced lengths of hospital stay. In planned subgroup analysis, subjects who were undergoing elective surgery benefited more from these formulas than did critically ill patients. Trials with a higher quality score actually showed a higher mortality rate among patients treated with the experimental formulas (relative

risk = 1.19; 95% CI: 0.99, 1.43), although these studies also showed fewer infectious complications among those patients (relative risk = 0.53; 95% CI: 0.42, 0.68). Overall, it appears that arginine-containing formulas may reduce infectious complications in certain patient groups, particularly surgical patients. Further studies are needed to justify the use of arginine supplementation in other patient groups.



## ZINC

Zinc is a trace element that is a central component of hundreds of metalloenzymes, including alkaline phosphatase, carboxypeptidases, thymidine kinase, and DNA and RNA polymerases. Zinc is an important component in cell membrane structure and function, functions as an antioxidant, and protects against lipid peroxidation (137). The importance of zinc in protein synthesis and on transcription proteins, in which zinc fingers are important in regulating gene expression, points to its importance among cells with a high rate of turnover, such as GI epithelia and cells of the immune system. Zinc deficiency has also been associated with important changes in immune function, including reduced B and T cell function, decreased delayed cutaneous hypersensitivity reactions, decreased phagocytosis, and reduced cytokine production (138).

### In vitro and animal experimental data

Early studies in rats made zinc deficient showed relatively well-preserved intestinal structure as noted by histologic examination (139) or electron microscopy (140). Intestinal mucosal protein content and disaccharidase activities were also reported to be unchanged in zinc deficiency (141, 142). Other authors, in contrast, noted significant histologic evidence of intestinal damage in rat models of zinc deficiency, including ulcerations, inflammatory infiltration, and edema of the jejunum (143, 144). The reason for the disparate findings is not clear because all the animals in these studies were convincingly made zinc deficient by dietary means over 4–6 wk.

Functional changes in the GI mucosa have been shown in many animal models. In zinc-deficient rats, poor absorption of dietary fats, with accumulation of lipids within the enterocyte, was noted, presumably because of inadequate chylomicron synthesis (145). Decreased intestinal absorption of cholesterol was also reported in zinc-deficient rats (146). With the use of intestinal perfusion techniques, zinc-deficient rats were found to have significantly negative sodium and water balance compared with that of pair-fed control and ad libitum-fed rats (147). It has also been hypothesized that zinc deficiency may predispose the intestinal tract to damage by free radicals (143) and increased NO activity (148). In a rat model of short-bowel syndrome, zinc deficiency was associated with decreased mucosal protein and DNA amounts and decreased alkaline phosphatase activity (149).

Reverse transcriptase–polymerase chain reaction was used to identify intestinal genes regulated by zinc deficiency (150). The investigators reported that expression of the gene for cholecystokinin is increased with zinc deficiency in rats, calling into question whether increased cholecystokinin expression contributes to the anorexia of zinc deficiency. Human intestinal cell-line models were also used to evaluate the effect of zinc deficiency on epithelial cell death. Recent work has shown that intracellular zinc depletion leads to activation of the proapoptotic protein caspase 3, DNA fragmentation, and formation of apoptotic bodies in the human colonic epithelial line LIM1215 (151).

### Human studies

The first report of human zinc deficiency noted anorexia, hypogeusia, poor growth, alopecia, and delayed sexual maturation (152). In acrodermatitis enteropathica, or congenital zinc deficiency, severe diarrhea and perianal skin breakdown are seen (153). Few studies have been performed to systematically assess the structure of the GI tract in humans with zinc deficiency. Subtle changes in Paneth cell architecture (vacuolization of the

rough endoplasmic reticulum and the presence of lysosomal inclusion bodies) were reported in a study from 2 children with zinc deficiency (154).

Many reports have linked diarrhea and abnormal zinc status (155), including an elevated loss of zinc in stool (156), negative zinc balance (157), and low tissue zinc concentrations. Plasma concentrations of zinc are often used as measures of zinc nutritional status, although the limitations of this approach are well known. Nonetheless, a significant correlation ( $r = 0.506$ ,  $P < 0.01$ ) was noted between plasma zinc and enterocyte zinc concentrations among 25 subjects undergoing intestinal resection for inflammatory or oncologic conditions of the GI tract (158).

Many clinical trials of zinc supplementation showed improved outcomes in children with GI diseases. The most significant improvements were observed among patients whose diets were low in zinc or high in phytate. In patients with acute diarrhea and low rectal mucosal zinc concentrations, zinc supplements were associated with a reduced duration of acute diarrhea (159). Zinc supplements also improved markers of intestinal permeability in children with diarrheal diseases in Bangladesh (160). In a randomized, controlled trial among 937 Indian children, zinc supplementation was associated with a decrease in the mean number of watery stools per day and in the number of days with watery diarrhea (161). In Peru, children with persistent diarrhea had a substantial reduction in duration of illness after receiving zinc (162). A recent pooled analysis showed that zinc-supplemented children with acute diarrhea had a significant reduction of continuing diarrhea and that children with persistent diarrhea had a lower probability of continuing diarrhea, treatment failure, or death (163). A recent analysis of community-based trials of zinc interventions in infants and young children who received 5–10 mg Zn/d for 5 or 7 d/wk for 12–54 wk found that the pooled odds ratio for diarrhea incidence was 0.82 (95% CI: 0.72, 0.93) and that the odds ratio for pneumonia incidence was 0.59 (95% CI: 0.41, 0.83) (164).

In ongoing studies, researchers are examining the role of zinc in preventing childhood mortality in developing countries, and health officials are considering combining zinc supplementation with standard oral rehydration solutions in the treatment of acute diarrhea. Although clinical data linking higher zinc intake with the prevention and treatment of diarrhea are accumulating, further work is needed to identify the mechanisms behind this effect and to identify other patient populations whose dietary zinc needs are higher than expected.

## VITAMIN A

Vitamin A plays a central role in epithelial cell integrity, immune function, and retinal function. Vitamin A was discovered by McCollum and Davis in 1913 (165), and the unique clinical and pathologic features of its deficiency in infants were noted 20 y later (166).

### In vitro and animal experimental data

McCollum's landmark studies (165) proved that "fat-soluble A" is required for normal growth in rats. Early animal experiments in rats and guinea pigs also noted that vitamin A deficiency is associated with a widespread replacement of columnar epithelium with stratified, keratinizing epithelium. These changes are most prominent in the trachea, parotid glands, salivary glands, cornea, and bladder epithelium, but not in the GI epithelium (167). Vitamin A deficiency leads to reduced

intestinal cell division and differentiation and a reduced number of goblet cells in the crypt (168) and villus (169). In general, the histologic changes in the GI tract in vitamin A-deficient rats are mild, although with prolonged deficiency (>60 d), villus height decreases (169). Glucose transport, mucosal wet weights, and thymidine kinase activity are not altered by vitamin A deficiency.

When vitamin A deficiency is paired with another inflammatory or infectious insult, however, animal studies show significant histologic abnormalities. In a study of vitamin A-deficient rats, well-preserved intestinal villus and crypt architecture was noted at 77 d; however, when the rats were infected with rotavirus, the vitamin A-deficient rats had markedly greater damage to the villus tip than did pair-fed control animals (170). Similarly, vitamin A-deficient rats treated with methotrexate show significantly more small-intestinal injury and lower disaccharidase concentrations than do pair-fed rats also treated with methotrexate (171).

### Human studies

The observation that children with mild vitamin A deficiency are at increased risk of diarrhea, respiratory infections, and death (172, 173) led to a series of large, randomized clinical trials that resulted in impressive reductions in child mortality (174–177). A meta-analysis of these and other trials confirmed that an  $\approx 30\%$  reduction in infant and young child mortality was seen with vitamin A supplementation (178).

Although some of the early trials were not designed to evaluate cause-specific mortality and morbidity, subsequent studies determined that the prevalence and severity of diarrheal diseases are especially reduced with vitamin A supplementation. In Ghana (177), vitamin A resulted in a significant reduction in overall and diarrhea-specific mortality. In Bangladeshi children with acute shigella infection, vitamin A supplementation was associated with significant improvement in clinical recovery (179). In Brazil (180), vitamin A resulted in a significant reduction in the mean daily prevalence of loose stools and in the mean number of episodes of diarrhea, particularly severe episodes. In India, children older than 23 mo with acute diarrhea who were given a large dose of vitamin A had significantly fewer subsequent episodes of diarrhea and fever than did children who did not receive vitamin A, and children younger than 23 mo who received vitamin A had significantly fewer measles infections than did those who did not receive vitamin A (181). These data on the efficacy of vitamin A were especially notable in non-breast-fed infants (182). Finally, in Tanzania (183), children with pneumonia who were given vitamin A had a significantly reduced risk of developing severe watery diarrhea during the 1-y follow-up period. All-cause mortality and diarrhea-specific mortality were also reported to decrease after vitamin A supplementation, especially in HIV-infected children (184).

Poor vitamin A status has been associated with impaired barrier function of the GI tract (185). In placebo-controlled trials among infants from India, a large dose of vitamin A resulted in improvement of the barrier function of the gut as measured by the lactulose-mannitol dual-sugar intestinal permeability test (186).

It is unclear whether vitamin A is an important protective nutrient for the GI tract in the absence of widespread inadequate dietary intake of the nutrient. Although infectious diseases of even well-nourished (and presumably vitamin A-replete) children are

associated with low serum vitamin A concentrations, intervention studies have not been widely performed in these settings.

### PROBIOTICS

Probiotics represent the quintessential functional food and have been used for centuries for their health-promoting effects. The use of probiotics in the modern era evolved from a theory proposed by the Nobel Prize-winning scientist Elie Metchnikoff at the Pasteur Institute, who suggested that the prolonged life span of Bulgarian peasants was a result of their consumption of fermented milk products (187). Probiotics are defined as live microorganisms in fermented foods that promote good health through establishing an improved balance in intestinal microflora (188). Microorganisms that are principally used as probiotics include various species of lactobacilli or bifidobacteria used individually or in combination. A nonpathogenic yeast, *Saccharomyces boulardii*, has also been used in both animal studies and clinical trials. Because probiotics do not permanently colonize the intestine, they must be taken in sufficient quantities ( $> 1 \times 10^{10}/\text{d}$ ) to maintain adequate amounts in the colon. In addition, they must be of human origin and be able to adhere to intestinal enterocytes. Because many reviews (189, 190) and journal supplements (191–193) have been published on the topic of probiotics, only the more recent studies will be summarized here.

### In vitro and animal experimental data

The principal purported health-promoting effect of probiotics is their enhancement of mucosal immune defenses (194). Studies with gnotobiotic animals show that in the absence of intestinal colonization, the effector component of the mucosal immune system is underdeveloped, making the host more susceptible to pathologic bacterial infections (195). In addition, general mechanisms for probiotics have been ascribed to their protective effect against pathologic microbial colonization and translocation. These mechanisms include competition for receptor sites on the intestinal surface, production of antibiotic substances, enhancement of host immune defenses (adjuvant effect, increased polymeric immunoglobulin A production, and cytokine stimulus), and competition with pathogens for intraluminal nutrients (196, 197). Other studies suggest that probiotics can also affect other nonimmune intestinal host defenses, including strengthening intestinal tight junctions, increasing mucous secretion, enhancing motility, and producing metabolic products (amino acids such as arginine and glutamine and short-chain fatty acids) that secondarily function as protective nutrients (198–200).

Probiotics can be used to either prevent or reduce the severity of microbe-induced gut inflammation. Inflammatory bowel disease is a major clinical problem in the field of gastroenterology. Heretofore, antiinflammatory agents such as prednisone or antimetabolites such as azathioprine were used to control the chronic inflammatory response associated with this disease. More recently, animal studies suggested that probiotics may be used to prevent or reduce colitis (201, 202). In addition, the incidence of NEC was reduced in a rat model of NEC with bifidobacteria supplementation (203). Other animal studies suggest that *Helicobacter pylori* infection in gnotobiotic mice can be prevented with the use of lactobacilli to displace *H. pylori* (204) and that cell attachment and invasion by enteropathogenic *Escherichia coli* and other gram-negative bacteria can be inhibited with the use of *Lactobacillus acidophilus* (LA 1) probiotics (205).

**TABLE 3**  
Clinical conditions for which probiotic therapy has potential benefit

Reference	Clinical condition
Isolauri et al (217)	Rotavirus infections
Majamaa et al (218)	
Shornikova et al (219)	
Guandalini et al (220)	
Siitonen et al (213)	Antibiotic-associated diarrhea
Armuzzi et al (221)	
Vanderhoof et al (222)	
Armuzzi et al (221)	<i>Clostridium difficile</i> infections
Vanderhoof et al (222)	
Isolauri et al (223)	
Oksanen et al (212)	Infantile allergic dermatitis
Madsen et al (201)	Traveler's diarrhea
Steidler et al (202)	Inflammatory bowel disease
Gionchetti et al (224)	Necrotizing enterocolitis
Hoyos (225)	
Dai and Walker (226)	

Several in vitro and animal studies evaluated the effect of probiotics on the development of colon cancer (summarized in references 206 and 207). Colon cancer is associated with environmental risk factors that include diet and the nature of colonic flora. In animal studies, the use of probiotics reduces the occurrence of precancerous lesions (aberrant crypts) (206). However, these studies suggest but do not prove that probiotic supplementation can prevent malignant degeneration. In like manner, the use of probiotics to reduce hypertension and lower serum cholesterol is suggested by the results of animal and in vitro studies (208) but has not yet been confirmed in human studies.

### Human studies

Most of the clinical studies supporting the use of probiotics in the prevention or treatment of GI disease were carried out in pediatric patients (209), although increasing data are being reported from adult trials. Early studies that used a commercial preparation of dried *L. acidophilus* and *Lactobacillus bulgaricus* (Lactinex Lactobacillus; Becton Dickinson Consumer Products, Franklin Lakes, NJ) showed no significant effects in the prevention of traveler's diarrhea (210) or diarrhea due to enterotoxigenic *E. coli* (211). More recent preparations, especially *Lactobacillus casei* strain GG (212, 213) and combinations of probiotics, however, have proven more efficacious. The clinical conditions for which probiotic therapy has potential benefit (214–216) are listed in **Table 3**.

Probiotic-supplemented formula induces a bifidogenic stool pattern more similar to that of breast-fed infants (227). Formula supplemented with *Bifidobacterium bifidum* and *Streptococcus thermophilus* reduce the occurrence of diarrhea and rotavirus shedding among chronically hospitalized infants and young children (228). *Lactobacillus* GG treatment during acute rotavirus diarrhea has been associated with higher titers of polymeric immunoglobulin A to rotavirus (218). Among undernourished Peruvian infants with a high burden of diarrheal diseases, *Lactobacillus* GG was associated with fewer episodes of diarrhea, especially among non-breast-fed children (229). Recently, the incidence of nosocomial diarrhea among hospitalized children was shown to be dramatically lower in children who received *Lactobacillus* GG therapy (relative risk: 0.2; 95% CI:

0.06, 0.6) than in those who received a placebo (230). A large, placebo-controlled trial of *S. boulardii* failed to document a reduction in antibiotic-associated diarrhea in hospitalized elderly patients (231).

In a pediatric intensive care nursery with a high incidence of NEC, premature infants given probiotics had a much lower incidence of disease than did historical controls (225). However, prospective studies have not been published. Probiotics, principally *Lactobacillus* GG, have been used to reduce the incidence of traveler's diarrhea among adult subjects (208, 232). Probiotics were also shown to prevent microbe-induced gastroenteritis in young children attending daycare centers (233). This observation, if confirmed by similar observations from more extensive multicenter studies, could have a profound effect on the quality of life of working parents (234).

In addition to preventing GI disease, probiotics have been effective in lessening the severity and longevity of several GI conditions. Many trials, including a large, multicenter study in Europe (220), reported improved results when patients with acute diarrhea were treated with both oral rehydration solutions and probiotics (235, 236). A Finnish study reported that probiotics significantly decrease the shedding of rotavirus into the stool of infected patients (237). Several studies in both pediatric and adult populations with either *Lactobacillus* GG or *S. boulardii* showed a prevention of the recurrence of *Clostridium difficile* infection after initial antibiotic treatment (238–240). In addition, probiotics were used in conjunction with antibiotic therapy to prevent or lessen the severity of antibiotic-associated diarrhea in children (222), although a meta-analysis of this issue showed significant problems in study design in several studies (241).

A group of clinical investigators in Finland provided strong evidence that probiotics (*Lactobacillus* GG) in conjunction with protein hydrolysate (hypoallergenic formula) can lessen the objective assessment of clinical symptoms and reduce intestinal inflammation and mucosal barrier permeability in infants with allergic dermatitis (242). Recently, Kalliomaki et al (243) randomly assigned pregnant women with a family history of atopic disease to receive *Lactobacillus* GG or a placebo. Their infants received the same therapy for the first 6 mo of life. By age 2 y infants in the *Lactobacillus* group had a lower incidence of atopic dermatitis (relative risk: 0.51; 95% CI: 0.32, 0.84). These exciting clinical observations suggest an additional approach to managing this debilitating infantile condition. These studies suggest that probiotics alter allergic symptoms by bringing about a shift in the intestinal mucosal response from one predominated by the helper T cell 2 (T<sub>H</sub>2) subclass, as seen in infants with intestinal allergies, to one that is more balanced between T<sub>H</sub>1, T<sub>H</sub>2, and T<sub>H</sub>3, as seen in infants without allergies.

Other recent clinical studies suggest that probiotic therapy may be beneficial to patients with inflammatory bowel disease (244). In one placebo-controlled trial, patients with pouchitis (inflammation of the ileal pouch–anal anastomosis after colectomy) had fewer episodes of clinical relapse when treated with a combination of probiotics (245). In another trial, ulcerative colitis patients treated with nonpathogenic *E. coli* had clinical outcomes comparable with those of patients treated with the antiinflammatory agent mesalamine (246).

These clinical observations suggest that the use of probiotics in a variety of clinical settings may provide new approaches to

**TABLE 4**  
Proposed health effects of prebiotics

Reference	Health effect
Roberfroid (256)	Improvement of body functions
Salminen et al (259)	Lactose intolerance
Van Loo et al (260)	Immunostimulation
	Mineral bioavailability
	Hyperlipidemia
	Reduced risk of
	Constipation
	Diarrhea
	Osteoporosis
	Atherosclerosis
	Cancer

conventional therapy. However, the quality of some of the clinical studies has been questioned (224), and some of the studies suffered from poorly specified inclusion criteria and inadequate control of possible confounding factors. As noted for the experience with Lactinex, the precise species of probiotics under study may also be an important variable to consider. As with the other protective nutrients reviewed, larger, more rigorous clinical trials are needed.

**PREBIOTICS**

Prebiotics are defined as nondigestible food ingredients that beneficially affect the host by selectively stimulating the growth and activity of one species or a limited number of species of bacteria in the colon (188). Compared with probiotics, which introduce exogenous bacteria into the human colon, prebiotics stimulate the preferential growth of a limited number of health-promoting commensal flora already residing in the colon (247). The oligosaccharides in human breast milk are considered the prototypic prebiotic because they facilitate the preferential growth of bifidobacteria and lactobacilli in the colon of exclusively breast-fed neonates (248–250).

Inulin is a group of fructose polymers (or fructans) linked by  $\beta(2-1)$  bonds that limit their digestion by upper intestinal enzymes. Chain lengths of these fructans range from 2 to 60. Oligofructose is defined as any fructose oligosaccharide containing 2–10 monosaccharide residuals connected by glycosidic linkages (251). Both inulin and fructose oligosaccharides are found in many plant species, including wheat, onion, banana, and chicory. Adult Americans ingest on average 2.6 g inulin/d and 2.5 g fructose oligosaccharides/d (252). Commercial forms of fructose oligosaccharides are created either by extraction from natural food sources (with or without hydrolysis) or by synthesis from sucrose (by linking fructose monomers to sucrose via  $\beta$ -fructofuranosidase) (253).

Inulin and fructose oligosaccharides have multiple roles in food and nutrition. They are widely used to add fiber to food without adding additional viscosity. Inulin mixed with milk can form microcrystals that give foods such as table spreads and dairy products a creamy, fat-like feel in the mouth of the consumer. Both types of prebiotics have been used in yogurts in an effort to add a prebiotic effect to a food already containing probiotics. A term used to denote this combination is *synbiotic*. Because of their unique chemical structure, prebiotics are not

absorbed in the small intestine but are fermented in the colon to combustible gases, lactate, and short-chain fatty acids. They are therefore useful as a sugar replacement for diabetes patients and are classified as fibers. Of these various functions of inulin and fructose oligosaccharides, however, the prebiotic functions have been most widely studied.

**In vitro and animal experimental data**

In vitro studies showed that isolated specific bacteria (bifidobacteria and lactobacilli) will ferment selected prebiotics as defined by the production of short-chain fatty acids and the development of an acid milieu (50). The mechanism of this selectivity undoubtedly involves general factors including the lowering of colonic pH and the production of metabolites that both inhibit some bacterial growth and simultaneously stimulate the growth of probiotic bacteria and the production of antibiotic effects (247, 254). To establish the preferential nature of prebiotics for selected bacteria in the human colon, slurries of human feces are mixed with a selected prebiotic and then the bacteria are quantitated by established methods. These studies showed a preferential growth of bacteria such as bifidobacteria and lactobacilli (255–258).

The effect on specific bacterial proliferation is complex and not easily explained by the prebiotic acting as an exclusive substrate for a particular bacterium. The degree of stimulus by a prebiotic is in part dependent on the initial amount of the endogenous probiotic flora. If a colon fecal slurry contains a large number of a specific bacterium at the outset, the prebiotic has a lesser effect than if that bacterium is present in smaller quantities before supplementation. Another unknown factor is how long the prebiotic maintains its stimulus to the growth of a specific bacterium. Prokaryotes are infinitely adaptable, and over time other bacteria, including pathologic bacteria in the colon, may adapt their enzymatic pathways to utilize the prebiotic as a substrate.

Most of the evidence regarding the potential health benefits of prebiotics (Table 4) is derived from in vitro and animal model studies (261). The major direct effects of prebiotics that are characteristic of protective nutrients and functional foods include improved bowel function (eg, as treatment of irritable bowel syndrome and constipation), increased mineral absorption, altered lipid metabolism, and a reduced risk of colon cancer (255). Data from studies in experimental animals and humans show that prebiotics enhance the bioavailability and absorption of calcium (262–264) and may affect the metabolism of other minerals, including magnesium, iron, and zinc (264). In like manner, fructose oligosaccharides were shown to inhibit hepatic lipogenesis in rats and thereby cause a hypotriglyceride effect (257). Fructose oligosaccharides appear to have an antiinflammatory effect in experimental NEC (265).

Experimental evidence in rats suggests that inulin-type fructans can reduce the precancerous colonic lesion (aberrant crypt foci) after the ingestion of colon carcinogens such as azoxymethane or dimethylhydrazine (266, 267). In the latter study, a combination of pre- and probiotics (synbiotics) had an additive effect (267). In some instances, prebiotics stimulate the reduction of endogenous carcinogens (sialomucin) by commensal flora (268) and reduce the actual growth of implanted tumors in rodents (269). A recent study in rodents suggests that  $\beta$ -limited (2-1) fructans stimulate apoptosis of colonic epithelial cells, which can also be considered as an anticancer effect (270).



### Human studies

Many human studies confirm that fructose oligosaccharides have a bifidogenic effect on human colonic endogenous flora (188, 271, 272). Several clinical studies also showed a bifidogenic effect of inulin-type fructans in humans (256, 260, 273–275). In these studies, the growth of a bacterium, in this case bifidobacteria, was measured in the stool of human volunteers fed varying amounts of prebiotics. However, when stool slurries were incubated with a specific prebiotic and changes in bifidobacteria were quantitated, the results were mixed and a dose-response curve could not be obtained (256). These observations suggest that the bifidogenic effect is not simply that prebiotics are preferential substrates for bifidobacteria but that prebiotics interact with other bacteria and are associated with environmental changes, such as changes in luminal pH, and other factors to achieve the overall bifidogenic effect. No clinical studies have been done to determine how long the bifidogenic effect persists after supplementation and whether the effect is sustained after the prebiotic is discontinued.

These studies generally were small trials among healthy adult subjects, and there have been fewer studies among larger numbers of subjects. A study among young children attending day-care showed that prebiotic-supplemented cereal was associated with fewer episodes of diarrhea with fever than was control cereal, although the overall rate of diarrhea episodes was not different between the groups (276). A recent study in which Peruvian children were similarly supplemented did not show a reduction in diarrhea or other infections (277).

With regard to other functions of protective nutrients and functional foods in man, some studies suggest that prebiotics improve  $\text{Ca}^{2+}$  absorption from the human colon (278, 279). Other studies, however, had equivocal results (280). No definitive clinical studies support the animal studies to suggest that other minerals ( $\text{Mg}^{2+}$ ,  $\text{Fe}^{2+}$ , and  $\text{Zn}^{2+}$ ) are similarly affected.

Several clinical studies were conducted to determine the effect of prebiotics on lipid metabolism in humans. In a randomized, double-blind trial among adults with moderate hyperlipidemia, prebiotic supplementation was associated with reduction in fasting insulin and triacylglycerol concentrations. These effects did not persist after the supplement was stopped (281). Other studies showed no effect of prebiotics on lipids (282). These equivocal results were particularly true when healthy adults with normal lipid concentrations were studied (280).

As promising as prebiotics may be as protective nutrients, many clinical studies must be done before prebiotics can be recommended as a food additive (infant formula, yogurt, etc) or as a dietary supplement. (Note that prebiotics have already been introduced as food additives in Europe and Japan.) Studies to determine the longevity of the prebiotic effect are needed before these fructose oligosaccharides can be considered as a protective nutrient (283). In addition, the possible effect of combining prebiotics with probiotics should be evaluated. No human studies have been conducted to confirm the suggested *in vitro* animal study effect of prebiotics on carcinogenesis. Long-term trials with prebiotics, perhaps first among colon cancer-prone patients, would be required.


### CONCLUSION AND RESEARCH NEEDS

We have reviewed *in vitro*, animal, and human studies concerning several types of nutrients and dietary supplements that have relevance in maintaining GI mucosal health. Certainly other nutrients exist in this class that we did not review, including *n*–3

fatty acids (284) and nucleotides (285). Previous reviews concerning the importance of enteral nutrients have expressed skepticism about the role of enteral nutrition in human GI health, highlighting the contrast between the wealth of data in animal models and the limited human data (286). However, our ability to detect important changes in GI function and structure, eg, histologic changes, changes in digestive enzyme concentrations, and changes in absorptive capacity with balance studies, is still limited. With more sensitive measures of GI function, including measures of intestinal permeability (217, 287) [which may not correlate with intestinal structure (288)], measures of intestinal protein synthesis (289), molecular measures of intestinal gene expression, and better measures of nutrient absorption (290), the effects of specific nutrients on GI tract function may be more easily detected.

Nonetheless, it is striking that the quality of the clinical trials performed to evaluate nutrient interventions is poor in comparison with that of phase 3 trials of most medical therapies. Certainly the peculiarities of government oversight of dietary supplements and the lower standards required to market nutritional products contribute to this state of affairs. In the present review, we have noted significant shortcomings in trials of arginine, glutamine (291), and probiotics (292), whereas the quality of trials in the fields of vitamin A and zinc is significantly higher. Does the fact that commercial products with these newer nutrient supplements are being actively marketed contribute to this disparity? Others have noted similar shortcomings in the design and implementation of nutrition studies (293).

Several important issues should be addressed in future trials of these and other protective nutrients. First, the mechanism or mechanisms of action of these nutrients should be sought. Many trials could be improved by a design that allows insight into the hypothesized function of these functional foods. In addition, clinically important outcome measures, defined *a priori*, should be the focus of the analysis, performed on an intention-to-treat basis. Second, in the evaluation of pre- and probiotics, newer technologies (eg, oligonucleotide probes and microchip arrays for DNA) should be used to measure changes in fecal flora. Third, better measures of GI mucosal structure and function should be developed and used. Fourth, studies should be designed with adequate randomization, blinding, and selection of control arms. In addition, sample sizes need to be large enough to address the study's hypotheses with sufficient power. Finally, formula companies specifically and the food industry generally should be encouraged to support well-designed clinical trials of new food additives before advertising claims are made. The current loophole, in which companies may make broad-based health claims without adequate evidence of efficacy, allows the introduction onto the market of a bewildering array of enteral and other products. More oversight by federal or other governmental agencies may be required. Falk (294) and others have proposed a model for independent review of health claims for dietary supplements, which would be a welcome development.

Nutritional requirements in health and disease are subject to continuing evaluation, and it is likely that future studies will uncover important roles for a variety of GI-protective nutrients among all age groups. Rigorous clinical trials need to keep pace with the promise of exciting basic science discoveries in the field. 

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