

Stressed Mucosa

Geoffrey Davidson, Stamatiki Kritas, Ross Butler

Centre for Paediatric and Adolescent Gastroenterology, Women's and Children's Hospital, North Adelaide, Australia

Abstract

Stress has been defined as an acute threat to the homeostasis of the organism. The mucosal lining of the gastrointestinal tract, a single layer of epithelial cells held together by tight junctions, provides a barrier between the external environment and the body's internal milieu. Any mechanism that breaches the tight junction exposes the body to foreign material be it protein, microorganisms or toxins. Stresses include physiological (exercise), psychological, disease-related or drug-induced factors. Stress associated gastrointestinal disorders include functional dyspepsia irritable bowel syndrome (IBS), gastroesophageal reflux disease peptic ulcer disease, and inflammatory bowel disease (IBD). Some disease states disrupt gastrointestinal barrier function, e.g. infectious diarrhea, IBD, or celiac disease, whilst in others such as eczema it can be indirectly related to antigenic disruption of the barrier. Drugs, e.g. chemotherapy agents and nonsteroidal anti-inflammatory drugs, also disrupt barrier function. Malnutrition and nutritional deficiencies (zinc, folic acid, vitamin A) also predispose to mucosal damage. Assessment of gastrointestinal mucosal health has proved problematic as invasive techniques, whilst useful, provide limited data and no functional assessment. Noninvasive tests particularly breath tests do provide functional assessment and many can be used together as biomarkers to improve our ability to define a stressed mucosa. Therapeutic options include pharmacotherapies, immunomodulation or immunotherapy.

Copyright © 2007 Nestec Ltd., Vevey/S. Karger AG, Basel

Introduction

Stress can be defined as any threat to an organism's homeostasis. The function of the stress response is to maintain homeostasis [1]. The mucosal lining is a single layer of epithelial cells providing a barrier between the external environment, the luminal contents and the body's internal milieu. The epithelial cells are joined by tight junctions which if breached become permeable

Table 1. Factors contributing to a stressed mucosa

Stress	Conditions
Physiological stress	Elite athletes
Psychological stress	IBS
Disease states	IBD Infectious diarrhea Atopic diseases CD
Nutrient deficiencies	Malnutrition Micronutrient deficiency, e.g. Zn, folic acid, vitamin A
Drugs	Nonsteroidal anti-inflammatory drugs Chemotherapy agents

allowing the passage of undesirable luminal solutes, microorganisms, antigens or foreign proteins to enter the circulation [2].

There are many factors that contribute to barrier function including the mucous layer, mucosal enzymes, microflora as well as the tight junctions. The tight junctions do not only have a barrier function but also regulate paracellular transport, acting as a fence by maintaining separate apical and basolateral domains and have a signaling function during stress. The body has a number of mechanisms that allow it to protect this barrier from stress. For example the acidification of the stomach provides a barrier against infectious agents passing into the small intestine. However, if this acid is refluxed into the esophagus then it can act as a stressor causing esophageal mucosal damage. Gastrointestinal motility and the mucosal immune system are other mechanisms that provide protection for the gastrointestinal mucosa. The gut mucosa faces many potential stressors and the aim of this dissertation is to discuss some of these and where possible highlight the mechanisms and consequences of this stress.

One of the limitations to our understanding the effect of stress on the gastrointestinal mucosa is the lack of simple, safe, noninvasive diagnostic techniques. The ideal would be the ability to sample tissue at various levels of the gut but even then the biopsy may not inform on the whole of the intestine. Alternative safe, noninvasive tests that inform on the total gut function or regions of the gut individually such as stomach, small intestine or colon function, in health and disease, are being developed and will also be discussed.

Table 1 highlights possible contributors to a stressed mucosa and some of these factors including physiological and psychological stress, inflammation, infection, nutrient deficiencies, and drugs will be discussed below in more detail.

Physiological Stress

The gastrointestinal tract can be stressed by physical exertion such as long distance running producing symptoms of abdominal pain, diarrhea and flatulence [3]. The symptoms do not appear to correlate with either the intensity or duration of the exercise. Studies of the gastrointestinal tract have shown a high incidence of mucosal erosions with hemorrhagic and ischemic colitis [4, 5]. Oktedalen et al. [3] have also shown increased intestinal permeability in symptomatic athletes whereas others have shown similar changes but without gastrointestinal symptoms [6, 7]. There is also a correlation between the intensity of running and loss of barrier function expressed as permeability.

A recent study has shown that in recreational runners small intestinal permeability increased before and after an 8-week exercise program. The severity of the disturbance to barrier function in recreational runners was similar to that seen in patients with IBD in remission [8].

It has been suggested that gastrointestinal mucosal ischemia is the major stress leading to gastrointestinal damage [8]. Other factors such as dehydration and changes in body temperature may also contribute. It is also possible that the increased susceptibility of individual athletes to illness may be related to changes in intestinal permeability.

Attempts have been made to reduce the mucosal damage in athletes during training. Bovine colostrum has been shown to improve athletic performance, reduce infections and reduce intestinal permeability caused by administration of nonsteroidal anti-inflammatory drugs. A recent study in athletes during running training showed that bovine colostrum increased intestinal permeability [10]. It was postulated that this may have allowed absorption of bioactive components from the colostrum that enhanced athletic performance and immunity.

Psychological Stress

Several recent reviews [10, 11] have discussed the role of psychological stress in the pathophysiology of gastrointestinal disease. The stress response is complex and involves a number of regions in the brain and brain stem together with somatic and visceral afferents and the endocrine system (fig. 1).

The release of corticotrophin-releasing factor (CRF), an important mediator of the central stress response, triggers a cascade of events via the anterior pituitary gland, the adrenal gland and the autonomic nervous system reaching the enteric nervous system which contains a network of approximately 100 million neurons. The enteric nervous system in turn influences gut motility, exocrine and endocrine functions, and the microcirculation. Together with this there also appears to be an effect of chronic stress leading

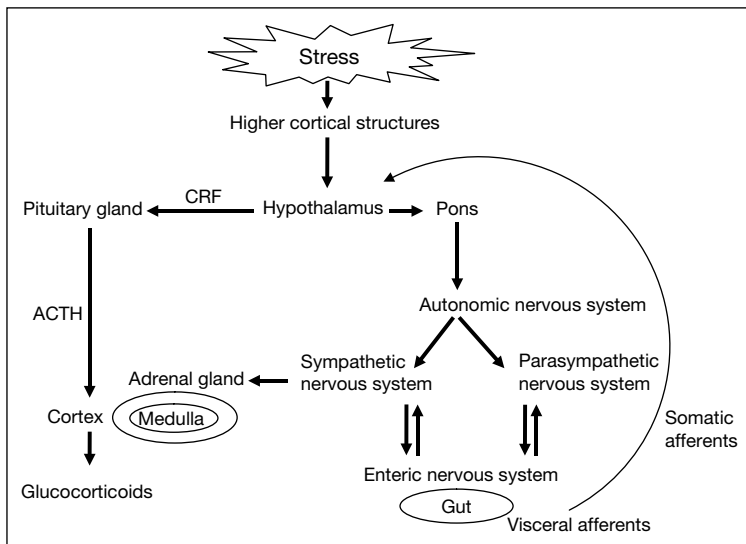


Fig. 1. Stress-induced pathways affecting the gastrointestinal tract. ACTH = Adrenocorticotrophic hormone.

Table 2. Stress-associated gastrointestinal disorders

Gastroesophageal reflux disease
Functional dyspepsia
Peptic ulcer disease
IBD
IBS

to increased CRF synthesis affecting both the immune system and causing a prolonged inflammatory response. In a recent study of patients with irritable bowel syndrome (IBS), the use of a nonselective CRF inhibitor significantly improved gastrointestinal motility, visceral perception and negative mood in response to gut stimulation [12].

Stress has been associated with a number of gastrointestinal disorders (table 2) and can be associated with increased colonic motility, increased water, electrolyte and colonic mucous secretions and increased mast cell mediators. There are few studies in humans demonstrating the above changes in gut function but quite strong evidence in IBD that stress associated with adverse life events is associated with a higher rate of relapse [13].

IBS is a common condition affecting up to 15% of the adult population. Major stressful life events, bacterial gastroenteritis and sexual abuse seem to be important trigger factors. Recent studies suggest that a low grade inflammation characterizes this syndrome. This supraphysiological inflammatory

condition may predispose to an increased intestinal permeability and potentially contribute to altered motility in different regions of the gastrointestinal tract. Surrogate markers including fecal calprotectin (a neutrophil-specific protein) and intestinal permeability have been suggested as discriminators of organic versus nonorganic disease [14]. Carbohydrate malabsorption, particularly fructose and lactose, are also prominent in IBS and may contribute to symptoms [15].

Inflammatory Bowel Disease

IBD including Crohn's disease and ulcerative colitis is usually diagnosed in children, adolescents or young adults and has a prevalence of about 20/100,000 people. Clinical assessment of disease activity has proved to be problematic particularly in children. Conventional laboratory markers of disease activity such as erythrocyte sedimentation rate and C-reactive protein may reflect disease activity but do not reflect intestinal function.

Increased intestinal permeability may play a significant role in the pathogenesis of IBD and can be measured using nonmetabolized sugar probes such as L-rhamnose, mannitol and lactulose. The urinary excretion ratios of these probes such as lactulose/L-rhamnose or lactulose/mannitol provide a simple, noninvasive and reproducible index of intestinal permeability [16]. Intestinal permeability can be increased not only in active disease but also in noninflamed mucosa and in first degree relatives of patients with IBD [17]. Increased intestinal permeability has also been noted in children with active ulcerative colitis but not in inactive disease [16].

Alkanes are major volatile hydrocarbons and indicate lipid peroxidation which may be an important pathogenic factor in IBD and a marker of a stressed mucosa. They can be measured in breath and have been used as markers of IBD activity [18]. Other tests such as fecal calprotectin have also been used to noninvasively determine intestinal inflammation correlating with clinical disease activity [14].

Infectious Diarrhea

There are two major mechanisms leading to diarrhea secondary to infection. The most common may be a secretory diarrhea, e.g. enterotoxigenic *Escherichia coli* or *Vibrio cholerae* where a bacterial toxin stimulates secretion. The other mechanism is mucosal injury leading to an osmotic or malabsorptive diarrhea, e.g. rotavirus or *Campylobacter jejuni*.

The stressors for secretory diarrhea are generally toxins whilst the stressor in infectious diarrhea is cell penetration and lysis leading to rapid cell turnover with blunting of villi and replacement of mature enterocytes by immature enterocytes [19].

In a recent review, Liu et al. [2] discussed the mechanisms whereby pathogenic, enteric microorganisms interact with tight junctions. This interaction and disruption are related to the effect of various toxins on zonula occlusion proteins altering occludin distribution [20]. There is also a suggestion that rotavirus infection may act in a similar way through its nonstructural NSP4 protein.

In communities in the developing world rotavirus is often the first gut infection occurring as early as at 3–4 months of age. This is a very damaging illness, where both a secretory and osmotic diarrhea may be present concurrently. Acute infectious diarrhea is often a precursor to recurrent diarrhea and malnutrition. This occurs because of the recurrent infections both gastrointestinal and extraintestinal that children in developing countries are exposed to due to environmental contamination aggravated by undernutrition. It is likely that all children living in developing communities, particularly in the tropics, have an abnormal small intestinal mucosal structure and function with villous blunting, increased cellular infiltrate in the lamina propria, increased intestinal permeability and disaccharidase deficiencies, mainly lactase. This setting has been labeled as tropical or environmental enteropathy [21].

Kukuruzovic and Brewster [22] have studied the enteropathy in rural and urban Aboriginal communities in the north of Australia presenting to hospital with diarrheal disease and malnutrition. Their aim was to show that the severity of the diarrheal disease was a consequence of small intestinal mucosal damage. They measured intestinal permeability using lactulose/L-rhamnose ratios in Aboriginal children with and without diarrhea and non-Aboriginal controls. Aboriginal children with and those without diarrhea had markedly elevated permeability compared to non-Aboriginal controls. In the same study, diarrheal complications such as acidosis, hypokalemia and osmotic diarrhea were associated with high lactulose/L-rhamnose ratios reflecting greater small intestinal damage.

Atopic Diseases

There is evidence that intestinal barrier function particularly intestinal permeability is altered in patients with atopic eczema and gastrointestinal allergic symptoms [22]. The permeable mucosa allows food antigens to reach the submucosal immune system and stimulate the production of cytokines and inflammatory mediators which in turn further disrupt the mucosal barrier.

Celiac Disease

Celiac disease (CD) can be considered as an autoimmune-like systemic disorder in genetically susceptible persons perpetuated by gluten-containing cereals, wheat, rye, barley and possibly oats. Intestinal mucosal damage in

CD is dependent on dietary exposure to prolamins. There is a genetic predisposition to CD related to the HLA-DQ2 and HLA-DQ8 molecules. These molecules stimulate proliferation of T cells and excretion of inflammatory cytokines. There is evidence to show an increase in intestinal permeability in CD, which seems to relate to gluten intake. There is also evidence of increased intestinal permeability in unaffected relatives of celiac patients compared to nonrelated controls. Cummins et al. [24] have shown that improvement in intestinal permeability precedes by many months the recovery in intestinal morphology.

Malnutrition and Nutrient Deficiencies

Malnutrition is a major issue in the developing world with significant consequences of increased infections, growth retardation and diarrheal disease which creates a vicious cycle if malnutrition is present from early infancy. This cycle is often the basis for the tropical or environmental enteropathy seen in many developing countries [21]. This condition is aggravated by micronutrient deficiencies particularly zinc, folate and vitamin A. As previously noted there is a significant increase in this setting.

Permeability testing has been used in Malawi, a developing country, to show the superiority of a milk-based diet over local cereals in rehabilitating children with kwashiorkor [25]. Not only was there a significant improvement in intestinal permeability but also reduced mortality, sepsis and weight gains.

Zinc deficiency is now known to be a major contributor to the incidence severity and duration of diarrhea. Diarrhea results in a significant increase in fecal losses of zinc. There are a number of similarities between the features of zinc deficiency and those of an enteropathy including growth-faltering diarrhea, intestinal mucosal damage and inflammation. A recent study from Bangladesh suggests that zinc may be a factor in the etiology of the enteropathy with supplementation reportedly leading to improved gut function [26].

Drugs

There are obviously many drugs that can affect gastrointestinal function and stress the mucosa. Antibiotic therapy can cause diarrhea, alter gut flora and predispose to colitis. Nonsteroidal anti-inflammatory drugs have been implicated as a cause of gastrointestinal ulceration and bleeding. The pathogenesis of this damage is not established but the drugs do affect mitochondrial function and decrease prostaglandin production. Bovine colostrum has been shown to reduce the rise in intestinal permeability caused by nonsteroidal anti-inflammatory drugs and may provide a novel approach to prevention of this damage [27].

Table 3. Noninvasive techniques to assess the stressed mucosa

Function	Test		
	breath	urine	feces
Gut motility	¹³ C lactose ureide Lactulose		Marker dye
Gut inflammation	Ethane/pentane		
Pancreatic function	¹³ C-mixed triglyceride ^{13/14} C triolein		Elastase
Gastric function	¹³ C urea ¹³ C octanoate	Sucrose permeability	
Small intestinal function	Small bowel Bacterial overgrowth	Permeability (lactulose/ L-rhamnose/ mannitol)	Calprotectin
Large intestine	Carbohydrate malabsorption (H ₂ breath tests) Fermentation patterns (methane/hydrogen)	Sucralose permeability	Short-chain fatty acids

Chemotherapy drugs are also a major cause of gastrointestinal mucosal injury (mucositis) most commonly affecting the mouth, esophagus, stomach and small intestine. Small intestinal injury can often be difficult to detect but a recent animal study has shown that breath testing may be a useful tool [28].

Assessment of the Stressed Mucosa

Small intestinal health is pivotal to whole body health and the bioavailability of drugs but it is difficult to assess it in animals and humans. Current invasive techniques such as endoscopy and biopsy are painful, invasive, expensive and not necessarily representative of gastrointestinal function. The recent advance of wireless capsule endoscopy may change our ability to visualize the bowel but again not its functionality.

Accurate noninvasive repeatable tests involving breath, urine and feces, are safe and available for use in both health and disease. Table 3 shows some examples of how these tests can be used alone and together to describe function. Using these tests as biomarkers will improve our ability to define stressed mucosa and help to understand the causal mechanisms. Newer imaging techniques may also improve our ability to assess the stressed gut.

Therapeutic Interventions

Pharmacotherapies have obviously been used in disease states such as IBD to reduce inflammation and induce mucosal repair. Nonpharmacological therapies such as enteral nutrition with modular or polymeric formulas have also been shown to be effective in inducing remission and gut repair in IBD.

There may also be a role for immunomodulation in protecting or repairing a stressed mucosa. One oral approach has been the use of pre- and probiotics either separately or in combination. A recent study using this combined approach with a synbiotic (combined pre- and probiotic) in patients with active ulcerative colitis showed reductions in TNF- α and interleukin-1 α [29]. Probiotics have also been shown to have benefit in the treatment of viral diarrhea [30].

Immunotherapy using products such as human immunoglobulin, colostrum, and hyperimmune colostrum has been shown to provide passive protection against a number of viral and bacterial pathogens [31].

Conclusion

Stress clearly plays a role in many gastrointestinal diseases but the extent of its contribution to the pathogenesis of many of these disorders is still unclear. Emerging functional diagnostic techniques are likely to provide a better understanding of the pathophysiology of the stressed gut and provide a simpler and more acceptable method to assess interventions.

References

- 1 Mawdsley JE, Rampton DS: Psychological stress in IBD: new insights into pathogenic and therapeutic implications. *Gut* 2005;54:1481–1491.
- 2 Liu Z, Li N, Neu J: Tight junctions, leaky intestines, and pediatric diseases. *Acta Paediatr* 2005;94:386–393.
- 3 Oktedalen O, Lunde OC, Opstad PK, et al: Changes in the gastrointestinal mucosa after long-distance running. *Scand J Gastroenterol* 1992;27:270–274.
- 4 Gaudin C, Zerath E, Guezennec CY: Gastric lesions secondary to long-distance running. *Dig Dis Sci* 1990;35:1239–1243.
- 5 Schwartz AE, Vanagunas A, Kamel PL: Endoscopy to evaluate gastrointestinal bleeding in marathon runners. *Ann Intern Med* 1990;113:632–633.
- 6 Pals KL, Chang RT, Ryan AJ, Gisolfi CV: Effect of running intensity on intestinal permeability. *J Appl Physiol* 1997;82:571–576.
- 7 Ryan AJ, Chang RT, Gisolfi CV: Gastrointestinal permeability following aspirin intake and prolonged running. *Med Sci Sports Exerc* 1996;28:698–705.
- 8 Southcott E: The Use of Probiotics in Intestinal Protection; PhD thesis, University of Adelaide, 2002.
- 9 Kolkman JJ, Groeneveld AB, van der Berg FG, Rauwerda JA, Meuwissen SG: Increased gastric PCO₂ during exercise is indicative of gastric ischaemia: a tonometric study. *Gut* 1999;44:163–167.

- 10 Buckley JD, Brinkworth GD, Southcott E, Butler RN: Bovine colostrum and whey protein supplementation during running training increase intestinal permeability. *Asia Pac J Clin Nutr* 2004;13(suppl):S81.
- 11 Bhatia V, Tandon RK: Stress and the gastrointestinal tract. *J Gastroenterol Hepatol* 2005;20:332–339.
- 12 Sagami Y, Shimada Y, Tayama J, et al: Effect of a corticotropin releasing hormone receptor antagonist on colonic sensory and motor function in patients with irritable bowel syndrome. *Gut* 2004;53:958–964.
- 13 Bitton A, Sewitch MJ, Peppercorn MA, et al: Psychosocial determinants of relapse in ulcerative colitis: a longitudinal study. *Am J Gastroenterol* 2003;98:2203–2208.
- 14 Tibble JA, Sigthorsson G, Foster R, et al: Use of surrogate markers of inflammation and Rome criteria to distinguish organic from nonorganic intestinal disease. *Gastroenterology* 2002;123:450–460.
- 15 Fernandez-Banares F, Rosinach M, Esteve M, et al: Sugar malabsorption in functional abdominal bloating: a pilot study on the long-term effect of dietary treatment. *Clin Nutr*, in press.
- 16 Miki K, Moore DJ, Butler RN, et al: The sugar permeability test reflects disease activity in children and adolescents with inflammatory bowel disease. *J Pediatr* 1998;133:750–754.
- 17 Peeters M, Geypens B, Claus D, et al: Clustering of increased small intestinal permeability in families with Crohn's disease. *Gastroenterology* 1997;113:802–807.
- 18 Pelli MA, Trovarelli G, Capodicasa E, et al: Breath alkanes determination in ulcerative colitis and Crohn's disease. *Dis Colon Rectum* 1999;42:71–76.
- 19 Davidson GP, Barnes GL: Structural and functional abnormalities of the small intestine in infants and young children with rotavirus enteritis. *Acta Paediatr Scand* 1979;68:181–186.
- 20 Nusrat A, von Eichel-Streiber C, Turner JR, et al: *Clostridium difficile* toxins disrupt epithelial barrier function by altering membrane microdomain localization of tight junction proteins. *Infect Immun* 2001;69:1329–1336.
- 21 Salazar-Lindo E, Allen S, Brewster DR, et al: Intestinal infections and environmental enteropathy: Working Group Report of the Second World Congress of Pediatric Gastroenterology, Hepatology, and Nutrition. *J Pediatr Gastroenterol Nutr* 2004;39(suppl 2):S662–S669.
- 22 Kukuruzovic RH, Brewster DR: Small bowel intestinal permeability in Australian aboriginal children. *J Pediatr Gastroenterol Nutr* 2002;35:206–212.
- 23 Jackson PG, Lessof MH, Baker RW, et al: Intestinal permeability in patients with eczema and food allergy. *Lancet* 1981;i:1285–1286.
- 24 Cummins AG, Thompson FM, Butler RN, et al: Improvement in intestinal permeability precedes morphometric recovery of the small intestine in coeliac disease. *Clin Sci (Lond)* 2001;100:379–386.
- 25 Brewster DR, Manary MJ, Menzies IS, et al: Comparison of milk and maize based diets in kwa-shiorkor. *Arch Dis Child* 1997;76:242–248.
- 26 Baqui AH, Black RE, El Arifeen S, et al: Effect of zinc supplementation started during diarrhoea on morbidity and mortality in Bangladeshi children: community randomised trial. *BMJ* 2002;325:1059.
- 27 Playford RJ, MacDonald CE, Calnan DP, et al: Co-administration of the health food supplement, bovine colostrum, reduces the acute non-steroidal anti-inflammatory drug-induced increase in intestinal permeability. *Clin Sci (Lond)* 2001;100:627–633.
- 28 Pelton NS, Tivey DR, Howarth GS, et al: A novel breath test for the non-invasive assessment of small intestinal mucosal injury following methotrexate administration in the rat. *Scand J Gastroenterol* 2004;39:1015–1016.
- 29 Furrie E, Macfarlane S, Kennedy A, et al: Synbiotic therapy (*Bifidobacterium longum*/Synergy1) initiates resolution of inflammation in patients with active ulcerative colitis: a randomized controlled pilot trial. *Gut* 2005;54:242–249.
- 30 Isolauri E, Salminen S: Probiotics, gut inflammation and barrier function. *Gastroenterol Clin North Am* 2005;31:437–450.
- 31 Davidson GP: Passive protection against diarrheal disease. *J Pediatr Gastroenterol Nutr* 1996;23:207–212.

Discussion

Dr. Aulestia: Can vitamins and iron produce damage in the mucosa of the normal child?

Dr. Davidson: I am not aware that vitamins and iron can damage the mucosa of normal children. The opposite can occur, however, where a deficiency such as folic acid leads to mucosal damage [1].

Dr. Puplampu: I found the use of folate in animals very interesting. What is the dosage and what have you used in humans?

Dr. Davidson: In the animal model we used calcium folinate, a metabolite and active form of folic acid at 10 mg/m² which approximates that used in humans for methotrexate 'rescue' [2]. In the study mentioned above the folic acid dose was 0.5 mg daily.

Dr. Puplampu: I have been using 5 mg/day for 10 days in the management of diarrhea, of course without zinc, and that is what I am very much interested in.

Dr. Davidson: Zinc has certainly been shown to have an important role in gut repair [3]. Two randomized controlled trials have however found no benefit from folic acid compared to placebo [4].

Dr. Nowak-Wegrzyn: Could you comment on the time course of the intestinal permeability change with exercise? How fast does it go back to baseline? There is a condition called food-dependent exercise-induced anaphylaxis that may be caused by increased absorption of food allergens following exercise. It would be very interesting to know what the time course of exercise-induced changes in intestinal permeability is.

Dr. Davidson: We haven't looked at that specifically. My assessment would be that if you look at those athletes who undergo moderate exercise their mucosal lining will be normal. The elite athletes we studied had an 8-week period of intensive exercise and whether their mucosa returned to normal or not would depend on whether they continued to exercise at a high level. During the time period when their training was less intensive they seemed predisposed to infections, but at that point in time we did not study their mucosa.

Dr. Bayhon: Can you comment on the role free radicals in the development or the pathophysiology of stress-induced enteropathies, and in terms of pharmaceutical therapy are prostaglandin E₂ inducers used at all in children as they are in other countries? The results are very promising especially in terms of prevention and quality of healing in induced enteropathies.

Dr. Davidson: I don't have any experience or expertise in that area. I am sure free radicals are produced, and this has certainly been documented, but we have not done any work on it.

Dr. Balanag: Obviously ventilated babies are very stressed. Are there any studies on the role of probiotics in babies who are ventilated?

Dr. Davidson: The role of probiotics in neonatology is attractive because of their safety record. There is some evidence to support their use in preventing necrotizing enterocolitis but I am not aware of studies in ventilated babies.

Dr. Rivera: You showed data on premature infants who are in the respirator. What was the response in terms of inflammation with carbon-13 and the other tests that you used? Were there reliable results with these particular tests. Have you any experience with probiotics in conditions like asphyxia and necrotizing enterocolitis in newborn babies?

Dr. Davidson: Breath can be collected from premature infants. We have in fact studied gastric emptying using the ¹³C-octanoic breath test in this age group. We have not studied the inflammatory response in neonates.

Dr. Rivera: Have you any experience or any knowledge on the use of probiotics in conditions like asphyxia and necrotizing enterocolitis in newborn babies [5]?

Dr. Davidson: No, I don't have any experience but as referred to above there is some supportive evidence at least for necrotizing enterocolitis.

Dr. B. Koletzko: I am fascinated by the methodology of the sucrose breath test and the results you obtained. You expressed them as a cumulative percentage of recovery over 90 min, so I assume you measured that in the postabsorptive state, in the fasted state? Is this not somewhat limiting in some patients, particularly at a young age or with certain diseases where it is difficult to perform the test in a fasted state? Have you ever considered applying the test in a nonfasting state?

Dr. Davidson: We have not looked at the nonfasting state. We have created a normal range looking at children and adults up to the age of 70 because we are also doing studies in irritable bowel syndrome. The normal range is relatively tight so it has enabled us to actually provide a cutoff but most of the children and adults we look at had a 6- to 8-hour fast.

Dr. B. Koletzko: My other question is related to the ethane breath test. I assume its results are more prone to variations in the patients' metabolic rate. Have you had a chance to look at its sensitivity and specificity in inflammatory states? One would assume that it is much less precise than a stable isotope breath test.

Dr. Davidson: I think it would be. We started a study on breath ethane in inflammatory bowel disease but unfortunately the machinery broke down. When you say that you are looking at the severe end of the spectrum in inflammatory bowel disease it still gives you a guide. I think it does have potential, not only in inflammatory bowel disease but in other conditions such as cystic fibrosis, rheumatoid arthritis and maybe chronic lung disease.

Dr. Milla: The gut is always exposed to lots of free radicals; bacteria in the lumen are constantly producing them. The most dominant antioxidant is vitamin E. It sits in the apical membrane of enterocytes very close to the lipid rafts that contain the functional proteins. We spent a long time trying to deplete enterocytes of vitamin E and to increase free radical attack on them but we failed miserably. We also looked at patients with a β - and hyper- β -lipoproteinemia that have incredibly low vitamin E levels. We could not detect any physiological abnormality in the membrane of their enterocytes. Trying to look at free radical attack on enterocytes is probably a nonstarter. Enterocytes have a beautiful protective mechanism with large amounts of vitamin E in the membranes. It mops up all the free radicals and has access to an enormous electron sink. I would not go down that avenue, I wasted a lot of time doing it. The question goes back to the ethane breath test. Many gastrointestinal inflammatory conditions are associated with respiratory inflammatory conditions, especially the allergic condition cystic fibrosis. What happens to the ethane breath test if there is an inflammatory response in the bronchial tree?

Dr. Davidson: I haven't seen any studies that have actually tried to differentiate where ethane or pentane may be coming from. Most of the studies have been done in inflammatory bowel disease. It would have an additive effect; therefore, you have a problem if you try to distinguish between the two sites, obviously it is easier if you just have one disease.

Dr. Fuchs: I would like to make a comment about folic acid and gastroenteritis. In your work did you use folate as a supplement in children who were receiving methotrexate?

Dr. Davidson: No, this was an animal model. Our oncologists use it in children who are receiving methotrexate, but we haven't actually studied this group of children.

Dr. Fuchs: One of my colleagues published a paper a few years ago. I was surprised at how little data there were, in fact none at that time, on folate supplementation

in acute watery diarrhea. He looked at all types of acute watery diarrhea irrespective of the pathogen and did not see a beneficial effect with folate, at least with the doses he was using. Why did you use sucrase as your marker as opposed to something else such as lactase?

Dr. Davidson: As you know the level of lactase tends to decrease over time and probably 90% of the world's population have lactase deficiency as adults but not necessarily malabsorption or intolerance. In children who are genetically predisposed to have lactase deficiency as adults the lactase level is decreased from about 3 years of age and 90% are deficient by the time they reach age 10 or 11. Sucrase activity however is maintained at a relatively constant level throughout life. Because sucrase like lactase is close to the villous tips it is affected by any event that damages the gastrointestinal mucosa, so it is a good marker.

Dr. Vento: Could ethane or pentane be predictive of necrotizing enterocolitis since they are also predictive of bronchopulmonary dysplasia?

Dr. Davidson: I am not aware of any definitive study of breath ethane or pentane in necrotizing enterocolitis but it may be worth studying as I am sure lipid peroxidation would occur in the gut and alkanes produced.

Dr. Heine: I would like to come back to your data on eczema and permeability. I was surprised that you found no change in permeability in eczema patients. Was this perhaps due to patient selection in your study? What was the age mix in your group?

Dr. Davidson: We looked at older children assessing them with SCORAD. We studied the more severe end of the spectrum; they were a fairly standardized group of children, but the average age was about 7 or 8.

Dr. Heine: So it may be different in younger children?

Dr. Davidson: Yes, I think so.

Dr. Chubarova: Do you have any information about intestinal permeability in malnourished children or those with physical delay but without infection?

Dr. Davidson: There is a lot of information in developing communities where they have malnutrition and not necessarily infection. Studies carried out in Darwin by Kukuruzovic and Brewster [6] showed that Aboriginal children without symptoms at the time and who were failing to grow had enteropathy; they were able to show increased intestinal permeability compared to nonindigenous children.

Dr. S. Koletzko: I was impressed by the lactose-rhamnose ratio in your athletes and that it was comparable to levels in IBD patients. Are there any data on athletes after they stop excessive training? How long does it take until the levels normalize? Do athletes have any long-term consequences from this barrier dysfunction?

Dr. Davidson: We did not follow this group long term, so I can't answer that question, particularly if it does have any long-term effects in these athletes [7]. There have been several studies from Adelaide actually looking at the use of bovine colostrum in trying to prevent this problem. During the 2000 Olympic Games some athletes took colostrum on a regular basis because it had actually been shown to have a performance-enhancing effect. Why that is, is not really well understood but one of the things that it seems to do is to reduce the downtime so athletes are able to continue to train, repeat their training and even improve their training without actually becoming unwell. The performance enhancement really is that it allows athletes to train to a higher level than if they weren't taking it.

Dr. Milla: You concentrated on the epithelium and I was fascinated by these runners. How much changes in the epithelium, how much might be due to vascular changes, because I can imagine the blood shunting through the gut when there is a significant change in exercise, and how much might be neural?

Dr. Davidson: There have been studies that you are probably aware of in athletes showing damage related to mucosal ischemia. They have a significant transfer of blood

flow away from the splanchnic region to muscle during exercise. This may well be a contributing factor that interferes [5] with mucosal function and integrity and so they may be reflecting not only the stressed mucosa but also these other changes that occur. These were athletes who were training at a very high level.

References

- 1 Davidson GP, Townley RRW: Structural and functional abnormalities of the small intestine due to nutritional folic acid deficiency in infancy. *J Pediatr* 1977;90:590–594.
- 2 Clarke JM, Pelton NC, Bajka BH, et al: Use of the ¹³C-sucrose breath test to assess chemotherapy-induced small intestinal mucositis in the rat. *Cancer Biol Ther* 2006;5:34–38.
- 3 Bhutta ZA, Bird SM, Black RE, et al: Therapeutic effects of oral zinc in acute and persistent diarrhoea in children in developing countries: pooled analysis of randomised controlled trials. *Am J Clin Nutr* 2000;72:1516–1522.
- 4 Mahalanabis D, Bhan MK: Micronutrients as adjunct therapy of acute illness in children: impact on episode outcome and policy implications of current findings. *Br J Nutr* 2001;85 (suppl 2):5151–5158.
- 5 Bin-Nun A, Bromiker R, Wilschanski M, et al: Oral probiotics prevent necrotizing enterocolitis in very low birth weight neonates. *J Pediatr* 2005;147:192–196.
- 6 Kukuruzovic R, Brewster D: Small bowel intestinal permeability in Australian aboriginal children. *J Pediatr Gastroenterol Nutr* 2002;35:206–212.
- 7 Kolkman JJ, Groeneveld AB, van der Berg FG, Rauwerda JA, Meuwissen SG: Increased gastric PCO₂ during exercise is indicative of gastric ischaemia: a tonometric study. *Gut* 1999;44: 163–167.