Stress-induced gastrointestinal barrier dysfunction and its inflammatory effects

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ABSTRACT: The intestinal barrier is formed by enterocyte membranes, tight junctions, secreted mucus, and immunologic factors, such as tissue macrophages. Dysfunction of this barrier can be caused by different types of stress (e.g., physiological, pathological, psychological, pharmacological) and can lead to increased intestinal permeability. Increased permeability to endotoxin, a component of the walls of gram-negative bacteria, causes local or systemic inflammatory reactions, or both. The immune response(s) can then promote more serious conditions. Exertional heat stroke is an example of such a condition. During severe exercise-heat stress, possibly combined with other stresses, reductions in intestinal blood flow, direct thermal damage to the intestinal mucosa, or both, can cause intestinal barrier disruption and endotoxemia. The resulting inflammatory response is believed to be involved in altered thermoregulation and multiple-organ dysfunction. Possible means for preventing or attenuating, or both, many stress-induced intestinal barrier problems include environmental, pharmaceutical, or nutritional approaches, or a combination of these.

Key words: endotoxin, heat, inflammation, intestine, nutrition, stress

INTRODUCTION

Several stresses affect the integrity of the intestinal barrier. Included among these are psychological stress (Soderholm and Perdue, 2001), prolonged strenuous exercise (Pals et al., 1997; Lambert et al., 1999), and heat stress (Lambert et al., 2002; Prosser et al., 2004; Singleton and Wischmeyer, 2006). Furthermore, certain drugs, such as nonsteroidal anti-inflammatory drugs, are well-known to damage the gastrointestinal (GI) mucosa (Bjarnason et al., 1986). The intestinal barrier is formed by the enterocyte membranes and tight junctions between enterocytes in the intestinal epithelium. In addition, factors such as mucus and tissue macrophages contribute to the barrier to restrict unwanted substances from entering the internal environment. Such substances include food antigens, bile, hydrolytic enzymes, and endotoxin (i.e., lipopolysaccharide, LPS).

Loss of intestinal barrier integrity (i.e., intestinal barrier dysfunction) leads to increased intestinal permeability. Intestinal permeability is defined as the nonmediated diffusion of large (i.e., molecular weight >150 Da), normally restricted molecules from the intestinal lumen to the blood. A low level of permeability is always present, but a properly functioning immune system normally is able to keep pathogens from causing harm. However, increased permeability can result in harmful local and systemic inflammatory reactions. In humans, this situation is commonly referred to as leaky gut syndrome. The intent of this brief review is to bring attention to the causes and consequences of stress-induced intestinal barrier dysfunction. Severe physical exertion and heat stress will be highlighted as a particularly damaging stresses to the intestinal barrier. Potential interventions that can possibly reduce intestinal permeability caused by such stresses will also be examined.

ASSESSING INTESTINAL BARRIER FUNCTION AND MEASURING INTESTINAL PERMEABILITY

The primary means of determining intestinal permeability in humans or animals is by measuring the passage of high molecular weight probes across the GI barrier. In humans, this involves ingestion of a solution containing nontoxic, nonmetabolizable substances and assessing their excretion in the urine. To measure gastric (i.e., stomach) permeability, sucrose (molecular weight = 342 Da) is a commonly used probe (Meddings et al., 1993). Because sucrase, the enzyme that hydrolyzes sucrose, is not present in the stomach, the appearance of sucrose in the urine indicates loss of barrier...
function in that organ. To determine small intestinal permeability, lactulose (molecular weight = 342 Da) is commonly utilized (Bjarnason et al., 1995). Lactulose is nondigestible in the small intestine and only begins to be degraded in the large intestine (i.e., colon) by colonic bacteria. Thus, the presence of lactulose in the urine provides an index of small intestine permeability. Because of its relatively large size, lactulose likely only enters the circulation via the paracellular pathway (i.e., through tight junctions) or damaged epithelium. Lactulose is normally co-ingested with a smaller passively absorbed probe, such as rhamnose or mannitol, to control for nonbarrier-related factors in urinary excretion between experimental conditions, such as tissue distribution (Bjarnason et al., 1995). Colonic permeability can be assessed by measuring the excretion of orally ingested sucralose (molecular weight = 397.6 Da; Meddings and Gibbons, 1998). This substance passes through the entire GI system without digestion and likely reaches the colon within a few hours. Thus, urinary excretion of this probe in the 24-h period after ingestion is primarily indicative of colonic permeability; however, it also indicates whole-gut permeability. Thus, examination of the sucralose:lactulose ratio can provide further information regarding the extent of colonic to small intestinal permeability (Meddings and Swain, 2000).

In animal models, intestinal permeability is frequently determined by infusing fluorescent probes, such as fluorescein isothiocyanate (FITC)-dextran, into the intestinal area of interest and measuring plasma concentrations over time (Lambert et al., 2002). Such probes come in various molecular weights ranging from several hundred to several million. Thus, these probes provide not only an index of intestinal permeability, but also provide an idea of how large the opening in the intestinal epithelium may be. Other commonly used probes, used in a similar manner in animal models, are $[^{51}Cr]$-EDTA [molecular weight = 341 Da (Prosser et al., 2004)] and horseradish peroxidase [molecular weight = 44,000 Da (Cameron and Perdue, 2005)].

One other useful index of intestinal barrier dysfunction is plasma LPS concentrations. Lipopolysaccharide is a highly pathogenic component of the walls of gram-negative bacteria and is found in the intestinal tract in high concentrations. Its presence in the portal blood of animal models indicates passage from the intestinal lumen to the circulation (Hall et al., 2001). Increased LPS concentrations in the systemic circulation (Brock-Utne et al., 1988; Singleton and Wischmeyer, 2006) likely indicate severe intestinal barrier dysfunction, in that its high permeability has overwhelmed the ability of the liver to clear it from the blood.

**STRESSES KNOWN TO CAUSE INTESTINAL BARRIER DYSFUNCTION AND POTENTIAL MECHANISMS**

Life-threatening situations, such as hemorrhage shock (Russell et al., 1995) and severe burn injury (Carter et al., 1990), are known to produce increases in intestinal permeability. Interestingly, potentially less-threatening conditions such as psychological stress (Soderholm and Perdue, 2001) and physical stresses, like prolonged endurance exercise (Pals et al., 1997; Lambert et al., 1999) and heat stress (Lambert et al., 2002; Prosser et al., 2004; Singleton and Wischmeyer, 2006), can also significantly impair the intestinal barrier.

**Psychological Stress**

Meddings and Swain (2000) have shown that psychological stress in rats induced by restraint or a combination of psychological-physical stress induced by forced swimming results in GI barrier dysfunction. In addition, these authors observed that even the stress of transport and handling of animals during shipping results in greater GI permeability. It appears that a major mechanism involved is the release of glucocorticoids (i.e., corticosterone) because both adrenalectomy and corticosterone receptor blockade attenuated the increase in permeability. Other studies in this area have also shown that psychological stress-induced intestinal barrier dysfunction is related to release of acetylcholine (Saunders et al., 1997), corticotropin-releasing hormone (Saunders et al., 2002), and muscarinic receptor activation (Groot et al., 2000). Furthermore, the activation of intestinal mast cells, likely through parasympathetic involvement, appears to mediate stress-induced increases in intestinal permeability (Soderholm and Perdue, 2001). Another likely mechanism of GI barrier dysfunction with some stresses could be reduced intestinal blood flow due to sympathetically driven splanchnic vasoconstriction.

**Prolonged Exercise**

Initial indications that prolonged, strenuous physical exertion could result in intestinal barrier dysfunction were observed by Brock-Utne et al. (1988). In that study, it was found that runners completing an ultramarathon had significantly increased plasma LPS concentrations. Such findings were followed by a series of studies from the same laboratory indicating that gut-derived LPS is related to heat stroke and its effects. For example, survival from severe heat stress could be significantly increased in a primate model by cleansing the gut with antibiotics (Gathiram et al., 1987b) or by administration of anti-LPS (Gathiram et al., 1987a) before heat stress. Further evidence that intestinal permeability increases in humans performing prolonged, strenuous exercise was found by Lambert et al. (1999). These investigators studied athletes competing in the 1998 Ironman Triathlon in Hawaii. This event involved a 2.4-mile open-water swim, followed by a 112-mile bicycle ride, and finished with a 26.2-mile run. At the conclusion of this event, subjects ingested a solution containing lactulose and rhamnose to determine small intestinal permeability. The results showed a significant
increase in permeability in male and female subjects compared with resting control subjects. These findings are shown in Figure 1.

Further studies in this area have shown that even shorter bouts of distance running can produce intestinal barrier dysfunction under certain circumstances. For example, Pals et al. (1997) found that 60 min of running at a fast pace \(\text{[i.e., } 80\% \text{ of maximal oxygen uptake (VO}_{2\max})\text{]}\) increased small intestinal permeability compared with running at slower paces that require only 40 to 60\% \(\text{VO}_{2\max}\). In addition, it has been found that running for 60 min at 70\% \(\text{VO}_{2\max}\) under conditions of fluid restriction during the run (Lambert et al., 2008) or after consuming aspirin or ibuprofen at therapeutic doses for 24 h leading up to the run (Lambert et al., 2007) results in significantly increased gastric small intestinal permeability.

**Heat Stress**

In rat models, heat stress increases intestinal permeability. For instance, Hall et al. (2001) observed a significant increase in portal LPS concentration in anesthetized rats heated to core temperatures of 41.5\(^\circ\)C, whereas Lambert et al. (2002) observed significant increases in intestinal permeability to a FITC-dextran (molecular weight = 4,000 Da) at core temperatures of 42.5\(^\circ\)C in anesthetized rats and at 41.5 to 42\(^\circ\)C in rat everted intestinal sacs. Dokladny et al. (2006) also recently showed Caco-2 cell monolayers maintained at 41\(^\circ\)C over 24 h have significantly increased paracellular permeability and reduced epithelial resistance. The mechanisms underlying the effects of heat stress on intestinal barrier function are highlighted in Figure 2. In this figure, it can be noted that the combination of reduced intestinal blood flow and hyperthermia caused loss of tight junction integrity and likely enterocyte membrane damage. Reduced intestinal blood flow occurs with exercise, heat stress, or both as blood is diverted away from the splanchnic region to provide adequate perfusion of the skin for heat dissipation (Rowell, 1974; Kregel et al., 1988; Sakurada and Hales, 1998). However, this can lead to intestinal hypoxia (Hall et al., 1999), which likely results in ATP depletion, acidosis, and altered ion pump activity. The result is reduced cellular viability and increased paracellular permeability. Reduced blood flow can also result in oxidative and nitrosative stress (Hall et al., 2001), which can damage cell membranes and open tight junctions. Furthermore, hyperthermia alone produces reactive oxygen and nitrogen species (Hall et al., 1994, 2001) leading to damaged cell membranes and tight junction opening. Taken together, the dual effect of reduced intestinal blood flow and tissue hyperthermia during heat stress likely promotes significant intestinal mucosal damage (see Figure 3) leading to the passage of substances such as LPS into the internal environment.

**The Immune Response**

Leakage of LPS to the internal environment stimulates an immune response involving the production of proinflammatory cytokines from cells such as monocytes and macrophages. This response likely causes further inflammatory damage to the intestinal epithelium and the initiation of a vicious cycle of events (Figure 2). The production of proinflammatory cytokines during severe heat stress has been shown in studies of human heat stroke victims. Increased concentrations of tumor necrosis factor-\(\alpha\) and IL-1\(\alpha\) (Bouchama et al., 1991) along with IL-6, IL-1\(\beta\), and interferon-\(\gamma\) (Bouchama et al., 1993) were observed and likely were associated with high concentrations of plasma LPS (Bouchama et al., 1991). Increased tumor necrosis factor-\(\alpha\) in the portal blood of anesthetized rats with core temperatures of 42.5\(^\circ\)C also was observed (Lambert, 2001; Figure 4) using the same model in which increased intestinal permeability to a 4,000-Da FITC-dextran was observed. The result of such an immune response could be a systemic inflammatory reaction ultimately leading to multiple-organ failure, such as is observed in sepsis. The flow of events associated with heat stress-induced intestinal barrier dysfunction, the immune response, and the possible consequences of such events are highlighted in Figure 5.

**POTENTIAL INTERVENTIONS**

Given the potentially serious consequences associated with intestinal barrier dysfunction during heat and
other stresses, it is important to attempt to identify potential interventions. The following is a summary of some interventions that may be beneficial based on recent studies.

**Reducing Oxidative Stress**

Hall et al. (2001) observed that administration of allopurinal was effective in reducing portal LPS concentrations in anesthetized rats heated to core temperatures of 41.5°C. Allopurinal inhibits the activity of xanthine oxidase, the enzyme that produces superoxide anion and hydrogen peroxide. Thus, its ability to reduce portal LPS concentrations during heat stress is likely through a reduction in oxidative stress-induced effects on tight junction integrity and enterocyte viability.

**Bovine Colostrum and Goat Milk Powders**

Prosser et al. (2004) provided evidence that dietary supplementation with bovine colostrum or goat milk
powders was effective in reducing heat stress-induced GI permeability in rats. By measuring the accumulation of orally administered $^{51}$Cr-EDTA into the blood, these investigators found that when compared with a standard diet, a diet supplemented with 1.7 g/kg of bovine colostrum powder or 1.7 g/kg of goat milk powder significantly reduced GI permeability when core temperature was raised to 41.5°C. The mechanism for the beneficial effect of these substances appeared to be related to tight junction integrity because this was

*Figure 3.* Transmission electron micrographs of small intestinal epithelial cells from anesthetized control and heat-stressed rats. Control animals were maintained at a rectal temperature of approximately 37°C for 90 min. Heat-stressed rats achieved a peak rectal temperature of approximately 42.5°C over 90 min. Note damage to microvilli and cell membranes along with mitochondrial swelling and vacuolization in heat-stressed rats. Bar represents 1 μm. Reprinted from Lambert et al. (2002) with permission (copyright 2002, American Physiological Society).
enhanced in cell culture monolayers (i.e., Madin-Darby canine kidney cells, a kidney tubule cell line) stressed with ethylene glycol tetraacetic acid.

**Glutamine**

Glutamine is the preferred fuel source of enterocytes. Singleton and Wischmeyer (2006) have shown that rats orally administered glutamine (0.65 g/kg) twice daily for 5 d before a heat stress (i.e., a core temperature of 42°C) reduced gut permeability to FITC-dextran (molecular weight = 4,000 Da) at 6 and 24 h postheating. This effect was also related to decreased plasma LPS 24 h postheating, increased intestinal heat shock protein (HSP) 70 expression, increased heat shock factor-1 activation, and improved survival.

**Prior Heat Exposure**

Increasing evidence indicates that exposure to a prior heat stress can result in adaptations likely to improve intestinal barrier function upon exposure to a subsequent heat stress. For example, Ruell et al. (2004) observed that exposure of rats to a heat stress that produced rectal temperatures of 41°C for 60 min or 42°C for 15 min elicited significant increases in HSP 72 induction in gut tissue. Accumulation of HSP 72 was shown to be related to thermotolerance and potentially to better ability to withstand lethal levels of hyperthermia. Cell culture experiments by Dokladny et al. (2006) have shown increased HSP 70 expression during exposure of Caco-2 cells to a prolonged (i.e., 24 h) heat stress (i.e., 41°C) and that inhibition of such expression leads to increased paracellular permeability. This appears to be related to increased expression of the tight junction protein, occludin (Dokladny et al., 2008). In addition, Moseley et al. (1994) showed that epithelial cells (i.e., Madin-Darby canine kidney cells) exposed to a heat stress (42°C for 90 min) 48 h before a subsequent heat stress had a greater threshold for increased epithelial conductance. This effect was also related to increased HSP 70 expression in the cells. However, the HSP and conductance effects only lasted for 96 h. Thus, further investigations are warranted to determine if increased HSP concentrations can be maintained for prolonged periods, if intestinal barrier function can be enhanced, and if this can be accomplished using a heat stress protocol safe for humans and animals.

**Glucagon Like Peptide-2**

Glucagon like peptide-2 is an intestinotrophic growth hormone and recent findings indicate that it is effec-
tive at reducing chronic psychological stress-induced intestinal barrier dysfunction. Cameron and Perdue (2005) have shown that in mice subjected to 10 d of water avoidance stress, administration of glucagon-like peptide 2 four hours before each bout of water avoidance stress attenuated increased intestinal permeability to horseradish peroxidase (molecular weight = 44,000 Da) across jejunal, ileal, and colonic tissues mounted in Ussing chambers.

SUMMARY AND CONCLUSIONS

Intestinal barrier dysfunction can occur under several stresses. Evidence indicates that psychological stress-induced GI permeability may be mediated through parasympathetic nervous system activation, acetylcholine release, corticotropin-releasing hormone, glucocorticoids, and mast cell products. Physical stress, in the form of prolonged strenuous exercise, heat stress, or both, induces intestinal barrier dysfunction likely through reductions in GI blood flow that leads to tissue hypoxia, ATP depletion, acidosis, and oxidative stress. These effects can open tight junctions. Severe hyperthermia alone likely damages enterocyte membranes. Loss of tight junction integrity and enterocyte damage produces intestinal barrier dysfunction, increased permeability to unwanted molecules, and likely an inflammatory response. Because of the serious problems these effects can produce, means for attenuating intestinal barrier dysfunction are being investigated, including environmental, pharmaceutical, and nutritional approaches.

LITERATURE CITED


