HIGHLIGHTED TOPIC  |  Molecular Adaptations to Exercise, Heat Acclimation, and Thermotolerance

Intestinal epithelial barrier function and tight junction proteins with heat and exercise

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1Department of Internal Medicine, Health Sciences Center, Health Exercise & Sports Science of University of New Mexico, Albuquerque, New Mexico; 2School of Health Sciences, Central Michigan University, Mount Pleasant, Michigan; and 3The Novo Nordisk Foundation Center for Protein Research, Faculty of Health and Medical Sciences, University of Copenhagen, Denmark

Submitted 25 June 2015; accepted in final form 8 September 2015

Dokladny K, Zuhl MN, Moseley PL. Intestinal epithelial barrier function and tight junction proteins with heat and exercise. J Appl Physiol 120: 692–701, 2016. First published September 10, 2015; doi:10.1152/japplphysiol.00536.2015.—A single layer of enterocytes and tight junctions (intercellular multiprotein complexes) form the intestinal epithelial barrier that controls transport of molecules through transcellular and paracellular pathways. A dysfunctional or “leaky” intestinal tight junction barrier allows augmented permeation of luminal antigens, endotoxins, and bacteria into the blood stream. Various substances and conditions have been shown to affect the maintenance of the intestinal epithelial tight junction barrier. The primary focus of the present review is to analyze the effects of exertional or nonexertional (passive hyperthermia) heat stress on tight junction barrier function in in vitro and in vivo (animals and humans) models. Our secondary focus is to review changes in tight junction proteins in response to exercise or hyperthermic conditions. Finally, we discuss some pharmacological or nutritional interventions that may affect the cellular mechanisms involved in maintaining homeostasis of the intestinal epithelial tight junction barrier during heat stress or exercise.

intestinal epithelial barrier; heat shock response; HSP70; exercise

IN MULTICELLULAR ORGANISMS, absorption, secretion, and transport across the epithelium of water, ions, and organic molecules is tightly regulated by the intestinal epithelial barrier, which consists of the apical plasma membrane of the enterocytes and the intercellular tight junctions (TJs) (8). Transcellular transport through the apical plasma membrane is highly regulated by specific membrane pumps and channels. On the other hand, paracellular transport is guarded by the TJs that form a continuous, embracing belt between adjacent epithelial cells (8). TJs are the most apical multiprotein complexes that regulate epithelial permeability and paracellular diffusion. Two main pathways (leak and pore) regulate transport across the TJs and influence transepithelial electrical resistance, a frequently used parameter in assessing transepithelial permeability (83). The leak pathway controls paracellular transport of noncharged large solutes (proteins and bacterial LPS, but not bacterial cells). On the other hand, the pore pathway is associated with claudins of the TJs and restricts transport of charged and large (>4 Å) molecules. Permeability of the pore pathway is regulated by myosin light-chain kinase (MLCK), which is directly modulated by TNF-α, IFN-γ, IL-1β, and PKC (6, 84-86, 91).

TJs are dynamic structures with complex architecture. They are composed of transmembrane barrier proteins (e.g., claudins, junctional adhesion molecules, coxsackie adenovirus receptor, marvelD3, occludin, and tricellulin) and cytoplasmic scaffolding proteins (e.g., the ZO family, cingulin, and afadin), which are directly connected with the intracellular cytoskeleton (actin and microtubules) and linked to regulatory proteins (e.g., aPKC, G proteins, Rab13, and Rab3B) (8, 88). The transmembrane proteins facilitate cell adhesion and form the paracellular barrier. The main role of the scaffolding proteins is to modulate the strand formation and TJ localization of transmembrane proteins (87). They also serve as adaptors by providing a direct connection between transmembrane proteins via their PSD95/DLG/ZO-1 (PDZ) domains and the cytoskeleton through their C-termini (26, 32, 39, 93). The regulatory proteins are critical for TJ formation and modulation of cell polarization (18, 78, 79). They also modulate TJ permeability through phosphorylation of TJ proteins (24, 67, 80) or their expression (3, 5, 33). A “leaky” TJ barrier characterized by increased TJ permeabil-
ity of toxic luminal substances is a hallmark of pathological conditions including exertional stress (9) and heat stroke (10). The purpose of this review is to summarize the current literature on the regulation of TJ barrier function and changes in TJ protein expressions in in vitro and in vivo models (animals and humans) under conditions of exertional and nonexertional (passive hyperthermia) heat stress. We also discuss cellular mechanisms that are directly responsible for maintaining the integrity of the intestinal epithelial TJ barrier during heat stress or exercise.

HEAT EXPOSURE AND REGULATION OF TIGHT JUNCTION PERMEABILITY—CELL CULTURE STUDIES

Over the past three decades, it has become apparent that hyperthermia leads to increased TJ permeability in cell culture models. In our early studies, we have shown in Madin-Darby canine kidney epithelial (MDCK) cells that even a relatively small elevation (1.3°C) in environmental temperature resulted in rapid and reversible changes in epithelial barrier integrity (56). Similarly, in porcine renal epithelial cells (LLC-PK1) (38), human colon adenocarcinoma cells (Caco-2) (20), and MDCK cells (21, 22) heat exposure produces a decrease in transepithelial resistance that returns to basal values within the first 24 h after exposure. It has also been shown that the effect of heat stress on TJ permeability is dose dependent (i.e., the more severe heat stress, the greater TJ permeability) (20, 94, 95).

Less is known about the effect of heat stress on TJ protein expression in cell culture models (Fig. 1). Our studies were among the first to show that continuous exposure to heat stress (39 or 41°C for 24 h) resulted in an upregulation of occludin protein expression, downregulation of ZO-1, and no apparent effect on claudin-3 levels in Caco-2 cells (20, 23). Those studies were recently confirmed in Caco-2 cells exposed to a brief heat stress (39 or 41°C for 1 h) that resulted in an increase in occludin and decrease in ZO-1 protein expression. In studies similar to ours, protein expression of claudin-2 was not affected by short heat exposure (94). These changes in protein expressions paralleled the changes in mRNA levels, suggesting that heat stress affects TJ protein expression at the transcriptional levels, but not through posttranslational modifications (23). In porcine renal cells, severe heat exposure (42°C for 3 h) did not affect total ZO-1 protein expression but it did influence cellular redistribution from the TJ to the cytosolic compartment (38). On the basis of the cell culture studies, it is apparent that heat stress causes an increase in TJ permeability that is associated with some changes in TJ protein expression, mainly an increase in occludin and a decrease in ZO-1.

HYPERThERMIA AND INTESTINAL PERMEABILITY—ANIMAL STUDIES

Numerous studies have shown that severe heat stress produces a rapid increase in intestinal permeability as evidenced by splanchnic endotoxemia in rats (35) and portal (31) or peripheral venous endotoxemia in primates (27, 29, 31). Increased intestinal permeability due to heat exposure was also documented in rodents in vivo as measured by plasma dextran (48, 60, 61) or radiolabeled probes (65). In ex vivo studies, in isolated intestinal segments, heat stress resulted in increased permeability to endotoxin (72) or dextran (48).

In early studies in primates, acute and severe heat exposure produced no significant change in intestinal permeability until rectal temperature reached approximately 42–43°C. At a core body temperature of 44.2°C, a dramatic increase (approximately fivefold) in plasma LPS concentrations, a marker of intestinal permeability, was observed (27). These results were confirmed in numerous studies showing an average fivefold increase in plasma LPS concentration at a body temperature of ~43.5°C compared with preheated values (28–31). In more recent studies in which rats and mice were predominantly used as experimental models, a 2- to 3-fold increase in intestinal

Fig. 1. A summary of exercise- or passive heat-induced changes in tight-junction (TJ) barrier, TJ protein expression or appearance, and macroscopic changes in gastrointestinal tract in vitro and in vivo.
permeability was documented at a core body temperature of ~42°C (35, 48, 61, 65).

Little is known about the long-term effect of acute hyperthermia or long-term heat exposure on intestinal permeability. In anesthetized rats, severe whole body hyperthermia resulted in a massive (approximately 22-fold) increase in plasma endotoxin concentration at 24 h after heat exposure (75). In rats, intraperitoneal hyperthermic perfusion (43°C) induced an increase in bacterial translocation that lasted up to 7 days after exposure (11). In contrast, in rabbits and mice, heat stress produced a transient increase in intestinal permeability that returned to control levels at 18–24 h after exposure (73, 77). The effect of long-term hyperthermic conditions (7 days) were studied recently in pigs (63, 64). In those studies, animals were exposed to heat stress of 35°C that resulted in a rectal temperature of 41°C and produced a moderate increase (~50%) in intestinal permeability over a 7-day period. These findings taken together demonstrate that exposure to severe heat stress results in an increase in intestinal permeability in animals.

HYPERTERMIA AND TIGHT JUNCTIONS—ANIMAL STUDIES

Hyperthermia (brief, extended, or repeated) has been consistently shown to cause prominent changes in the intestinal tract (Fig. 1). In early studies using dogs and monkeys, prolonged exposure (60 min) to hyperthermic conditions (core temperature of 42°C) resulted in delayed signs of edema and massive bleeding from the stomach and rectum to the peritoneal cavity, eventually leading to hemorrhagic and hypovolemic shock (25). In rats (48) and mice (61), brief whole body hyperthermia has resulted in cell death and subsequent shedding of the epithelium from the intestinal surface, accumulation of vacuoles, loss of microvilli, and reduction of the microvilli height with no apparent changes in crypt depth or villi width (60, 61). Similarly in pigs, a progressive reduction in villus height was observed in response to long-term hyperthermic conditions (35°C for 24 h) with rectal temperature rising to 40.9°C (64). Consistently, brief but severe hyperthermic conditions (44°C for 20 min) in eventrated rat intestinal segments developed a full range of symptoms from mild (accumulation of subnuclear vacuoles and villi edema); through moderate (cleft formation and villi shedding); to fully developed, characterized by villi destruction and hemorrhage (81). In rats, repeated exposure to heat stress has severely damaged an epithelial layer characterized by shortening of the villi height and sloughing of the epithelium thereby exposing the underlying lamina propria (51).

In early studies in rats, whole body hyperthermia (42.5°C) caused no apparent changes in TJ appearance (48). However, recent studies utilizing extended or repeated hyperthermic conditions have shown significant changes in TJ protein expression. In growing pigs, 24-h heat exposure produced an increase in occludin and claudin 3, but not claudin 1 (63). These changes in TJ protein expressions were preceded by an increase in mRNA levels of TJ proteins (occludin, ZO-1, and claudin) (64). Finally, in rats, repeated exposure (40°C for 2 h daily for 3 days) to heat stress damaged the TJ structure leading to bacterial translocation (51). These results taken together indicate that intense heat stress damages the intestinal wall. However, microscopic changes to TJ structure and expression of TJ proteins still remain largely unknown.

EXERCISE AND INTESTINAL PERMEABILITY—ANIMAL STUDIES

In racing dogs, sustained strenuous exercise has produced a moderate increase in intestinal permeability accompanied by erosions and ulcerations in the stomach mucosa (0% prerace vs. 61% postrace) (17). Similarly in mice, acute strenuous exercise (80% VO_{2,max} until exhaustion) resulted in a moderate increase in gut permeability lasting up to 5 h after exercise (34). The authors of that study proposed formation of lamellipodia as a protective mechanism against exercised-induced apoptosis of the intestinal epithelial cells. Recently, it has been shown that mice running in a hot environment (body temperature ~39.5°C) had elevated intestinal permeability compared with control animals at a 4-h time point (15). Summing up, a limited number of studies have shown that exercise produces an increase in gut permeability. More research that includes different animal models is needed to support the previous findings and focus on specific cellular mechanisms involved in exercise-induced intestinal permeability.

HYPERTERMIA AND INTESTINAL PERMEABILITY—HUMAN STUDIES

Although the sequence of events that induce intestinal permeability during heat stress/stroke have been hypothesized and diagrammed, little empirical evidence exists in humans exposed to classic (i.e., nonexertional) hyperthermic conditions. Due to ethical reasons, no measures of intestinal permeability have been performed on passively heat-stressed human subjects in controlled environments. Most data are available from studies of patients being treated for heat stroke or burn. Among patients treated for heat stroke with average core temperatures of 42°C, plasma endotoxin, which is used as an indirect measure of intestinal permeability, has been elevated (10). Plasma endotoxin levels decreased after cooling (~65 min), but remained higher than thermal neutral controls. Coltart et al. (14) reported elevated levels of intestinal permeability using a radioisotope-labeled EDTA method among patients being treated for colorectal cancer and exposed to localized tissue hyperthermia (3–4 treatments of 43°C for 23 min combined with radiation therapy across 4 wk) (14). Recently, the cultures of Pseudomonas aeruginosa and Escherichia coli were isolated from the blood of a patient being treated for heat stroke with an axillary temperature of 42°C. Although coincidence of heat stroke and bacteremia was not completely ruled out, it was suggested that bacteremia was secondary to intestinal translocation in response to heat stress (66). Interestingly, no significant macroscopic abnormalities were found in thoracic and abdominopelvic cavities. In contrast, peritoneal hemorrhage was reported among 82 of 125 fatalities from heat stroke (54), which provides indirect evidence of heat-induced intestinal damage.

It is likely that a complex interplay of acute physiological events that begins with a rising core temperature as a result of compromised cardiovascular function leads to tissue damage and endotoxin translocation (Fig. 2). The cause and effect relationship between defective intestinal barrier and penetration of luminal LPS has been established (7, 45, 92); however, recent work demonstrates that LPS in the basolateral, not luminal, membrane compartment contributes to TJ disruption (33). Based on this data, under normal physiological condi-
tions, luminal LPS and bacteria have no effect on the intestinal wall barrier integrity. At the onset of hyperthermia, physical damage to the intestinal barrier occurs, causing an increase in intestinal permeability resulting in luminal LPS leakage (48). The LPS further acts on the basolateral membrane of the epithelial cells causing additional intestinal barrier dysfunction and permeability in a feed-forward fashion. Intestinal barrier permeability plays a major role in systemic inflammatory response syndrome (SIRS), which is characterized by hyperthermia, increased hemodynamic and respiratory rates, production of inflammatory cytokines (50), and endotoxemia (7, 16). As previously discussed, the intestinal endotoxins contribute to intestinal barrier breakdown, but they also activate innate immune (circulating monocytes and tissue macrophages) and Kupffer cells (not shown) causing the release of proinflammatory cytokines (TNF-α, IFN-γ, IL-1β), which also contribute to TJ barrier breakdown. In heat preconditioning, inactive cytosolic heat shock factor-1 (HSF-1) monomers (1a) trimerize (1b) and translocate to the nucleus. The active HSF-1 trimer binds to the heat shock element of the occludin and HSP70 promoters, upregulating occludin and HSP70 mRNA (1c) and proteins (2). Compensatory overexpression of the occludin protein is thought to be directly involved in tightening of the epithelial TJ barrier (3). In addition, heat-induced HSP70 overexpression may protect the epithelial TJ barrier through several mechanisms: direct interaction with TJ proteins occludin and ZO-1 (4); inhibition of cytokine release from immune cells (5); and inhibition of myosin light-chain kinase (MLCK), nuclear factor-κB, or JNK pathways shown to be involved in disruption of the epithelial TJ barrier (6).

**EXERCISE AND INTESTINAL PERMEABILITY—HUMAN STUDIES**

Exercise-induced, or exertional-intestinal permeability has been established in human subjects. Several studies have shown that aerobic exercise causes an increase in urinary markers that indicate augmented intestinal permeability (summarized in Table 1). Two separate studies from our laboratory have shown an increase in the urinary ratio of lactulose to rhamnose (L/R) 5 h after a 60-min treadmill run at 70% \( \text{VO}2\text{max} \) in subjects with an average core temperature of 39.4°C (96, 97). In contrast, Kuennen et al. (43) reported no significant increase in urinary lactulose in subjects who ran at 80% of \( \text{VO}2\text{max} \); however, there was only a significant increase in small intestinal permeability was observed in subjects who ran at 80% of \( \text{VO}2\text{max} \) because core temperature and training status were not different between the studies. Comprehensive analysis of the effect of 60-min treadmill running at varying intensities (40, 60, and 80% of \( \text{VO}2\text{max} \)) on intestinal permeability was performed by Pals et al. (62). A statistically significant increase in small intestinal permeability was observed only in subjects who ran at 80% of \( \text{VO}2\text{max} \); however, there was a linear relationship between core temperature and the L/R ratio. Marchbank et al. (55) demonstrated increased intestinal permeability (via the L/R ratio) among human subjects who
Table 1. A summary of selected studies showing the effect of exercise on changes in intestinal permeability in humans

<table>
<thead>
<tr>
<th>Reference</th>
<th>Exercise Protocol</th>
<th>Core Temperature, °C</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Camus et al., 1997 (12)</td>
<td>Marathon race (166–282 min)</td>
<td>Not measured</td>
<td>Increased blood endotoxin levels at 1 h after race</td>
</tr>
<tr>
<td>Jeukendrup et al., 2000 (41)</td>
<td>The Ironman long-distance triathlon: swimming (3,800 m) open water, cycling (185 km), and running (42.2 km)</td>
<td>Not measured</td>
<td>Increased blood LPS concentration at 1 and 16 h postexercise</td>
</tr>
<tr>
<td>Kuennnen et al., 2011 (43)</td>
<td>Two 50-min bouts at 46.5°C to maintain core temperature above 39°C with quercetin or placebo supplementation</td>
<td>39.33, placebo; 39.28, quercetin</td>
<td>Increased urinary lactulose at 8 h postexercise compared with resting values in quercetin, but not in placebo group</td>
</tr>
<tr>
<td>Lambert et al., 2001 (47)</td>
<td>60 min running at 70% VO$_2$max at 22.4°C combined with aspirin ingestion</td>
<td>38.3</td>
<td>Increased urinary L/R$^*$ ratio 4 h postexercise compared with placebo values</td>
</tr>
<tr>
<td>Lambert et al., 2007 (46)</td>
<td>60 min running at 70% VO$_2$max at 23.2°C combined with aspirin ingestion</td>
<td>38.2</td>
<td>Increased urinary L/R$^*$ ratio 5 h postexercise compared with resting values</td>
</tr>
<tr>
<td>Lambert et al., 2008 (49)</td>
<td>60 min running at 70% VO$_2$max at 24.4°C with placebo, glucose, or no fluid ingestion</td>
<td>38.4, placebo</td>
<td>Increased urinary L/R$^*$ ratio 5 h postexercise compared with resting values in no-fluid-ingestion group only</td>
</tr>
<tr>
<td>Marchbank et al., 2011 (55)</td>
<td>20 min running at 80% VO$_2$max</td>
<td>38.5, glucose</td>
<td>Increased urinary L/R$^*$ ratio 5 h postexercise compared with resting values that were prevented by colostrum supplementation</td>
</tr>
<tr>
<td>Pals et al., 1997 (62)</td>
<td>60 min running at 40, 60, or 80% VO$_2$max at 22°C</td>
<td>38.0 (40% group)</td>
<td>40% group: no increase in urinary L/R$^*$ ratio at 6 h postexercise compared with rest</td>
</tr>
<tr>
<td>Smetanka et al., 1999 (76)</td>
<td>Chicago marathon (42.2 km, 120–240 min)</td>
<td>Not provided</td>
<td>Increased in 5-h L/R$^*$ ratio in ibuprofen-ingested group, but not in aspirin or control groups</td>
</tr>
<tr>
<td>van Wijck et al., 2011 (89)</td>
<td>60 min cycling at 70% VO$_2$max</td>
<td>Not measured</td>
<td>Overall increased L/R$^*$ ratio, but not at individual time points</td>
</tr>
<tr>
<td>Zuhl et al., 2014 (97)</td>
<td>60 min running at 70% VO$_2$max in a heated chamber (30°C)</td>
<td>39.4</td>
<td>Increased urinary L/R$^*$ ratio 5 h postexercise compared with rest</td>
</tr>
<tr>
<td>Zuhl et al., 2015 (96)</td>
<td>60 min running at 70% VO$_2$max in a heated chamber (30°C)</td>
<td>39.51</td>
<td>Increased urinary L/R$^*$ ratio 5 h postexercise compared with rest</td>
</tr>
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*Lactulose to rhamnose.

exercised at 80% of VO$_2$max for 20 min, but whose core temperature rose only modestly to 38.4°C. Additional studies that reported increases in exercise-induced intestinal permeability have used exercise protocols that elicited at least 70% VO$_2$max with an average core temperature ranging from 38.2 to 39.6°C (46, 71, 74, 89). On the basis of these data, it is not clear which variable (heat or exercise intensity) plays a larger role in causing intestinal barrier dysfunction. During intense exercise, blood flow is directed away from the gastrointestinal tract to support skeletal muscle oxygen demand. Intestinal ischemia, measured through gastric tonometry, has been shown to occur within 10 min of high-intensity exercise (89). This ischemic environment causes a free radical milieu, which may lead to TJ protein breakdown (as discussed in the following section). Evidence suggests that an elevation in free radicals occurs during the reperfusion cycle after an ischemic event (70). The ischemic environment is established before core temperature rises, and intestinal permeability has been shown to occur within 20 min of a 60-min bout of treadmill running (89). In addition, dehydration may contribute to reduced intestinal blood flow, causing tissue hypoxia (36). Lambert et al. (49) reported heightened intestinal permeability during running under a fluid restriction condition. Furthermore, patients with peripheral artery disease have demonstrated elevated intestinal permeability after a 200-m walking protocol, which is relieved upon revascularization procedures (40). Although it was not reported, we can be confident that core temperature did not rise substantially among this subject population. Although the ischemic environment occurs in a relatively short period of time during high-intensity exercise, long-duration exercise has been shown to increase TJ permeability and plasma endotoxins (12, 41, 49, 76). During long-duration exercise, core temperature rises, along with susceptibility to dehydration, and both may contribute to TJ barrier breakdown. As discussed in the previous section, the rise in core temperature may cause physical damage to the intestinal wall.
In summary, it is well established that high-intensity aerobic exercise increases intestinal permeability among human subjects, and it occurs in as little as 20 min. Hypoperfusion may be the major factor underlying this condition because blood flow is diverted from the gut to the periphery, creating an ischemic-reperfusion scenario. During longer-duration exercise, additional factors may contribute to TJ dysfunction, including a rise in core temperature and dehydration.

MECHANISMS OF HEAT-INDUCED CHANGES IN TJ PERMEABILITY

Cell Culture Studies

In cell culture studies, only a few cellular pathways have been shown to be involved in preventing or enhancing the TJ barrier under heat stress conditions (Fig. 3). Pretreatment with competitive inhibitors of sodium-dependent glucose cotransporter or tyrosine kinase (38) retarded the recovery of transepithelial resistance and ZO-1 cellular redistribution caused by heat exposure, suggesting that sodium-dependent glucose cotransporter and tyrosine kinase play important roles in protecting the TJ barrier during recovery after heat stress. In contrast, pretreatment with eicosapentaenoic acid, a polyunsaturated fatty acid, prevented heat stress-induced disruption of the epithelial barrier (94). Although direct effects of eicosapentaenoic acid in protecting the TJ barrier against hyperthermia remain to be determined, several possible cellular mechanisms have been proposed such as 1) that it prevents heat stress-induced cellular disruption of TJ proteins (occludin, ZO-1, claudin-2), 2) it has a direct effect on TJ protein expression, and 3) it has an anti-inflammatory effect by inhibiting the nuclear factor-κB signaling pathway (94, 95). In contrast to the protective role of eicosapentaenoic acid on the TJ barrier, PKC and MLCK are important negative regulators of the TJ barrier under heat stress (95).

Interaction Between the TJ and Heat Shock Proteins

Exposure to heavy metals (90), hyperthermia (20), or oxidative stress (44) triggers rapid expression of highly conserved proteins known as heat shock or stress proteins (HSPs) [for a review of HSPs see (69)]. This expression of HSPs initiates a physiological adaptation called tolerance, which is described as the ability to withstand a subsequent and potentially lethal stress. A conditioning heat stress sufficient to elevate a heat shock response renders resistance to a subsequent lethal heat exposure (22, 56), endotoxin shock (13, 37, 58, 68), serum starvation (53), or oxidative stress (58). The tolerance has been studied both at the level of single cells and whole organism as a measure of survival or maintaining organ homeostasis. In addition to the important role that HSP70 plays in protecting against different types of stresses, HSP70 plays a vital role in maintaining or protecting the intestinal epithelial TJ barrier. In cell culture studies, heat preconditioning associated with HSP70 protein expression significantly improved the recovery of epithelial barrier function (22, 95). Moreover, this recovery was significantly accelerated in HSP70-overexpressing cells (21, 95) and impaired in cells exhibiting low levels of HSP70 (20, 59). It was also demonstrated that the heat stress response elevated occludin protein expression and pretreatment with a commonly used heat shock response inhibitor, quercetin, or with small interfering RNA knock down of heat shock factor-1 (HSF1, the main regulator of heat-shock response) prevented the heat stress-induced increase in occludin protein expression and resulted in a significant disruption of occludin expression at the TJ (23). HSF1 has also been shown to directly bind to the occludin promoter, leading to its increased activity and subsequent upregulation in occludin mRNA and protein expression. Besides regulation of occludin protein expression by HSF-1, direct interaction between HSP70 and TJ proteins has also been reported. In the blood-brain barrier, exposure to whole body hyperthermia (41–42°C for 15 min) resulted in a significant increase in HSP70 that coimmunoprecipitated with ZO-1 and occludin, suggesting that HSP70 may play an important role in maintaining the function of TJ proteins through direct interaction by preventing the structure of TJ proteins under denaturing conditions (52). Similarly, in MDCK cells, Apg-2, a member of the HSP110 family of HSPs, can interact through its ATPase domain with the SH3 domain of ZO-1 and regulate the tran-
sorption activity of ZO-1-associated nucleic acid binding protein, which is involved in modulation of cell proliferation (82). Moreover, reduced Apo-2 protein expression delayed the junctional recruitment of ZO-1, ZO-2, ZO-3, and occludin and significantly delayed the recovery of barrier function as measured by transepithelial resistance after switching cells from low to normal calcium conditions, suggesting that HSPs are important regulators of TJ assembly (1). Future studies are needed to determine whether other members of the HSP family are directly involved in TJ assembly and to determine the cellular mechanisms responsible for this regulation.

Animal and Human Studies (Ex Vivo and In Vivo Experiments)

To date, the most prevailing hypothesis to explain hyperthermia-induced disruption of the TJ barrier is as follows: hyperthermia reduces splanchnic blood flow to accommodate heat dissipation in the periphery; reduced blood supply in the intestine and liver produces cellular hypoxia leading to disruption of Ca\(^{2+}\) homeostasis and subsequent oxidative stress, among others, resulting in damage to the intestinal barrier (35, 42, 48, 57). Several scientific reports have supported this hypothesis. In rats, heat stress-induced splanchnic endotoxemia was successfully reduced by treatment with xanthine oxidase inhibitor, an enzyme involved in purine metabolism and which is directly involved in production of reactive oxygen species and hydrogen peroxide (35). Moreover, inhibition of nitric oxide synthase (NOS), an enzyme involved in nitric oxide synthesis, resulted in even greater increase in plasma endotoxin levels suggesting that NOS is involved in the protection of gut tissue; however, when nitric oxide synthesis is uncontrolled it leads to deleterious consequences contributing to heat stroke. This suggests that depending on preexisting oxidative conditions, antioxidants may have no effect on intestinal permeability or they may significantly protect the barrier and the mucosa exposed to hyperthermic conditions (48, 61). Besides antioxidants, prior supplementation with either bovine colostrum or goat milk powder significantly reduced gastrointestinal permeability induced by heat stress or strenuous exercise in different experimental models including intestinal epithelia cells, animals, and athletes (55, 65). Due to a high level of heterogeneity of colostrum or milk powder containing a variety of compounds (amino acids, lipids, fatty acids, cytokines, and growth factors), the precise mechanisms how this supplementation works will require extensive future research, but a direct effect on TJ structure and upregulation of heat stress response has been proposed. Recently, the effect of amino acids in preventing heat- or exercise-induced disruption of the intestinal barrier have also been studied. Specifically, glutamine treatment prevented a heat stress-induced increase in intestinal permeability in mice (77), rats (75), and humans (97) probably due to increased heat stress response. Moreover, elevated intestinal permeability in mice exercising in a hot environment (body temperature 39.62°C) was inhibited by pretreatment with arginine (15). Several possible mechanisms to explain the role of arginine in preventing heat stress-induced destruction of the TJ barrier have been suggested including that it inhibits oxidative stress, activates immune function, and has direct effects on the TJ structure. Lastly, in exercising humans, quercetin, a plant flavonoid, or aspirin treatment produced a significant increase in intestinal permeability. The authors of that study suggest that some cellular mechanisms are responsible for this, including inhibition of heat stress response (quercetin) or direct damage of the epithelial tissue or inhibition of prostaglandin synthesis with regard to aspirin (43, 46, 47).

CONCLUSIONS AND FUTURE STUDIES

Hyperthermia and exercise represent a complex mesh of intertwining factors and processes. The last 30 yr of research have enhanced our understanding of the effects of severe hyperthermia and strenuous exercise on intestinal epithelial TJ permeability both in vitro and in vivo. It is evident that heat stress and vigorous exercise increase intestinal epithelial TJ permeability and that several culprits are implicated in damage to the TJ barrier. High temperature imposes physiological adaptations including reduced blood flow to the gastrointestinal tract, which triggers a hypoxic environment and protein denaturing conditions that lead to intestinal epithelial cell damage. As a result, endotoxemia and subsequent release of proinflammatory cytokines may also contribute to TJ barrier breakdown under hyperthermic conditions or prolonged exercise.

Several unanswered questions require future investigation. First, direct interaction or binding between HSPs and some TJ proteins have been documented, but no thorough investigation of a specific role for HSP70 or other HSPs has been presented. Second, at the cellular level, out of more than 40 TJ proteins already identified, only a few have been examined, and none has been shown to be directly responsible for TJ homeostasis under hyperthermia or strenuous exercise. Third, future research should also focus on the quest for pharmacological and nutritional compounds that can be safely and efficaciously used in tightening the intestinal TJ barrier in patients or athletes experiencing classic or exertional heat stroke. Finally, so far, only a few cellular mechanisms have been directly tested. Future research focusing on the cellular and molecular mechanisms that control classic hyperthermia or exertional stress is also needed. Detailed analysis of these mechanisms will have not only significant clinical and therapeutic implications, but it will advance our knowledge and understanding at basic level of this still-fascinating area of science.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES


Intestinal Epithelial Barrier with Heat and Exercise


