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

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Abstract

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. Dysfunctional or “leaky” intestinal tight junction barrier allows augmented permeation of luminal antigens, endotoxins, and bacteria to 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 the exertional or non-exertional (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 providing direct insights into the cellular mechanisms involved in maintaining homeostasis of the intestinal epithelial tight junction barrier during heat stress or exercise.
Introduction

In multicellular organisms, absorption, secretion, as well as transport across the epithelium of water, ions, and organic molecules is tightly regulated by the intestinal epithelial barrier that consists of the apical plasma membrane of the enterocytes and the intercellular tight junctions (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 tight junctions that form a continuous, embracing belt between adjacent epithelial cells (8).

Tight junctions are the most apical multiprotein complexes regulating epithelial permeability and paracellular diffusion. There are two main pathways (leak and pore) regulating transport across the tight junctions and influencing transepithelial electrical resistance, a frequently used parameter in assessing transepithelial permeability (83). The leak pathway controls paracellular transport of non-charged large solutes (proteins and bacterial lipopolysaccharides, but not bacterial cells). On the other hand, the pore pathway is associated with claudins of the tight junctions and restricts transport of charged and large (> 4 Å) molecules. Permeability of the pore pathway is regulated by myosin light-chain kinase, which activity is directly modulated by tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), interleukin-1 beta (IL-1β), and protein kinase C (PKC) (6, 84-86, 91).

Tight junctions 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. αPKC, 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 tight junction localization of the transmembrane proteins (87). They also serve as adaptors providing a direct connection between transmembrane proteins via their PDZ (PSD95/DLG/ZO-1) domains and the cytoskeleton through their C-terminus (26, 32, 39, 93). The regulatory proteins are critical for the TJ formation and modulation of cell polarization (18, 78, 79). They also modulate the tight junction permeability through phosphorylation of the tight junction proteins (24, 67, 80) or their expression (3, 5, 33). A “leaky” TJ barrier characterized by increased TJ permeability 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 tight junction barrier function and changes in tight junction protein expressions in in-vitro and in-vivo (animals and humans) models under conditions of exertional and non-exertional (passive hyperthermia) heat stress. We also discuss cellular mechanisms that are directly responsible for maintaining the integrity of the intestinal epithelial tight junction 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 tight junction permeability in cell culture models. In our early studies, we have shown in MDCK cells (Madin-Darby Canine Kidney Epithelial) 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), or MDCK cells (21, 22) heat exposure produces a decrease in trans-epithelial resistance that returns to the basal values within the first 24 h post 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 tight junction protein expression in cell culture models (Fig. 1). Our studies were among the first to show that continuous exposure to heat stress (39°C or 41°C for 24 h) resulted in an upregulation of occludin protein expression, downregulation in ZO-1, with no apparent effect on claudin-3 levels in Caco-2 cells (20, 23). These 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. Similarly to our studies, 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 tight junction protein expression at the transcriptional levels, but not through post translational modifications (23). In porcine renal cells, severe heat exposure (42°C for 3 h) did not affect total ZO-1 protein expression but influenced its cellular redistribution from the TJ to the cytosolic compartment (38). Based on 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 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). In *in-vivo* studies, increased intestinal permeability due to heat exposure was also documented in rodents 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 of ~42-43°C. At core body temperature
of 44.2°C, a dramatic increase (~ 5-fold) in plasma LPS concentrations, a marker of intestinal
permeability, was observed (27). These results were confirmed in numerous studies showing on
average a 5-fold increase in plasma LPS concentration at body temperature of ~43.5°C as
compared to the pre-heated values (28-31). In more recent studies, in which rats and mice were
predominantly used as an experimental model, a 2- to 31-fold increase in intestinal permeability
was documented at 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 massive (~22-fold) increase in plasma endotoxin concentration at 24-h post 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 the
control levels at 18-24 h post exposure (73, 77). The effect of long-term hyperthermic conditions
(7 days) were studied recently in pigs (63, 64). In these studies, animals were exposed to heat
stress of 35°C that resulted in rectal temperature of 41°C and produced a moderate increase
(~50%) in intestinal permeability over the 7-day period. Together, these findings demonstrate
that exposure to severe heat stress results in an increase in intestinal permeability in animals.
Hyperthermia (brief, extended, or repeated) has been consistently shown to cause prominent changes in the intestinal tract (Fig. 1). In early studies, in 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 resulted in cell death and subsequent shedding of the epithelium from the intestinal surface, accumulation of vacuoles, loss of microvilli, and the 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) rising rectal temperature 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 exposures to heat stress severely damaged an epithelial layer characterized by shortening of the villi height, sloughing of the epithelium exposing the underlining 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 tight junction proteins (occludin, ZO-1, and claudin) (64). Lastly, in rats, repeated exposures (40°C for 2 h daily for 3
days) to heat stress damaged the tight junction structure leading to bacterial translocation (51).

Taken together, intense heat stress damages the intestinal wall. However, microscopic changes of TJ structure and expression of TJ proteins still remain largely unknown.

**Exercise and intestinal permeability – animal studies**

In racing dogs, sustained strenuous exercise produced a moderate increase in intestinal permeability that was accompanied by erosions and ulcerations in the stomach mucosa (0% prerace versus 61% postrace) (17). Similarly in mice, acute strenuous exercise (80% VO2max till exhaustion) resulted in a moderate increase in gut permeability lasting up to 5 h post exercise (34). The authors proposed the formation of lamellipodia as a protective mechanism explaining lack of damage in exercising animals when compared with apoptosis and epithelium damage in heat-exposed animals. Recently, it has been shown that mice running in a hot environment (body temperature ~39.5°C) had elevated intestinal permeability when compared with control animals at 4 h time point (15). Summing up, a limited number of studies have shown that exercise produces an increase in gut permeability. More research including different animal models is needed to support the previous findings and focus on specific cellular mechanisms involved in exercise-induced intestinal permeability.

**Hyperthermia and intestinal permeability – human studies**

While 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 in heat stroke or burn patients.
Among heat stroke victims with average core temperatures of 42°C, plasma endotoxin, which is used as an indirect measure of intestinal permeability, has been elevated (10). The levels of plasma endotoxin decreased after cooling (~65 min), but remained higher than thermal neutral controls. Coltart et al. reported elevated levels of intestinal permeability using a radioisotope labeled EDTA method among colorectal cancer patients exposed to localized tissue hyperthermia (3-4 treatments of 43°C for 23 minutes combined with radiation therapy across 4 weeks) (14).

Recently, the cultures of *Pseudomonas aeruginosa* and *Escherichia coli* were isolated from blood of a heat stroke patient with 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 fatal heatstroke victims (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 conditions, luminal LPS and bacteria have no effect on the intestinal wall barrier integrity. Upon hyperthermic stress, 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 hemodynamics and respiratory rates, production of inflammatory cytokines (50), and endotoxemia (7, 16). As previously discussed, the intestinal endotoxins contribute to the intestinal barrier breakdown, but also activate innate immune (circulating monocytes and tissue macrophages such as Kupffer cells) production of pro-inflammatory cytokines such as TNF-α, IL-1β, and INF-γ. These plasma proteins have all been shown to disrupt the intestinal epithelial barrier and contribute to the increase in intestinal permeability (2-4, 19). In summary, hyperthermia triggers a cascade of events that begins with physical stress to the intestinal wall causing TJ breakdown and an increase in permeability of luminal endotoxins. The endotoxin heightens intestinal permeability in this condition by two possible mechanisms: (1) acting on the basolateral membrane of the epithelial wall; and (2) activation of pro-inflammatory cytokine release from immune cells.

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 indicating 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) five hours after a 60-minute treadmill run at 70% VO₂max in subjects with average core temperature of 39.4°C (96, 97). In contrast, Kuennnen et al. reported no significant increase in urinary lactulose in subjects (peak core temperature 39.3°C) who exercised for 45-minutes at 50% of VO₂max (43). The differences in the results may be due to intensity of exercise (50% vs. 70%) as 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 VO$_2$max) on intestinal permeability was performed by Pals et al. (62). Statistically significant increase in small intestinal permeability was only observed in subjects running at 80% VO$_2$max; however, there was a linear relationship between core temperature and the ratio of lactulose to rhamnose. Marchbank et al. demonstrated increased intestinal permeability (L/R ratio) among human subjects who exercised at 80% VO$_2$max for 20 min, but core temperature rose only modestly to 38.4°C (55). Additional studies that reported increases in exercise-induced intestinal permeability have used exercise protocols that elicited at least a 70% VO$_2$max with the average core temperatures ranging from 38.2°C to 39.6°C (46, 71, 74, 89). Based on these data, it is not clear what 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 tight junction protein breakdown (as discussed in the following section). Evidence suggests that the elevation in free radicals occurs during the reperfusion cycle after the 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. reported heightened intestinal permeability during running under a fluid restriction condition (49). Further, peripheral artery disease patients have shown elevated intestinal permeability after a 200 meter 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. While 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 under this condition as 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 rise in core temperature and dehydration.

**Mechanisms of heat-induced changes in tight junction permeability**

**Cell culture studies**

In cell culture studies, only a few cellular pathways have been shown to be involved in preventing or enhancing 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 an important role in protecting tight junction barrier during the recovery after heat stress. In contrast, pretreatment with eicosapentaenoic acid a member of the polyunsaturated fatty acids prevented heat stress-induced disruption of the epithelial barrier (94). Although direct effects of eicosapentaenoic acid in preventing TJ barrier against hyperthermia remain to determined, several possible cellular mechanisms have been proposed such as prevention of heat stress-
induced cellular disruption of tight junction proteins (occludin, ZO-1, claudin-2), direct effect on
TJ protein expression, and an anti-inflammatory effect of eicosapentaenoic acid inhibiting NF-
κB signaling pathway (94, 95). In contrast to the protective role of eicosapentaenoic acid on TJ
barrier, protein kinase C and myosin light-chain kinase are important negative regulators of TJ
barrier under heat stress (95).

*Interaction between TJ and HSP*

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
review on HSPs please see (69)). This expression of HSPs initiates a physiological adaptation
called tolerance described as the ability to withstand a subsequent and potentially lethal stress. A
conditioning heat stress sufficient to elevate 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. Besides, the important role
of HSP70 in protection 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 siRNA
knock-down of HSF1 (heat shock factor 1 – 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 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 co-immunoprecipitated 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 heat shock proteins, can interact through its ATPase domain with SH3 domain of ZO-1 and regulates transcriptional activity of ZO-1-associated nucleic acid binding protein that is involved in modulation of cell proliferation (82). Moreover, reduced Apg-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 heat shock proteins are important regulators of TJ assembly (1). Future studies are needed to determine whether other members of heat shock protein family are directly involved in TJ assembly and to determine cellular mechanisms responsible for this regulation.

Animal and human studies (ex-vivo and in-vivo experiments)

To date, the most prevailing hypothesis explaining hyperthermia-induced disruption of tight junction barrier is as follows: hyperthermia reduces splanchnic blood blow to accommodate heat dissipation in the periphery; reduced blood supply in the intestine and liver produces cellular hypoxia leading to disruption of Ca²⁺ homeostasis and subsequent oxidative stress, among others, resulting in damage of 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 uncontrolled leads
to deleterious consequences contributing to heat stroke. This suggests that depending on pre-
existing oxidative conditions, antioxidants may have no effect on intestinal permeability or 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 exact
mechanisms will require extensive future research, but a direct effect on tight junction structure
and upregulation of heat stress response was proposed. Recently, the effect of amino acids in
preventing heat- or exercise-induced disruption of intestinal barrier have also been studied.
Specifically, glutamine treatment prevented 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 pre-treatment with arginine (15). Several possible
mechanisms explaining the role of arginine in prevention of heat stress-induced destruction of TJ
barrier have been suggested including inhibition of oxidative stress, activation of immune
function, and direct effect on the tight junction structure. Lastly, in exercising humans, quercetin, a plant flavonoid or aspirin treatment produced a significant increase in intestinal permeability. The authors suggest some cellular mechanisms, 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 thirty years of research have enhanced our understanding with regards to the effects of severe hyperthermia and strenuous exercise on intestinal epithelial TJ permeability \textit{in-vitro} and \textit{in-vivo}. It is evident that heat stress and vigorous exercise increase intestinal epithelial TJ permeability. There are several culprits that are implicated in damaging TJ barrier. High temperature imposing physiological adaptations, including reduced blood flow to the gastrointestinal tract triggers hypoxic environment and protein denaturing conditions leading to intestinal epithelial cell damage. As a result, endotoxemia and subsequent pro-inflammatory cytokines release may also contribute to the TJ barrier breakdown under hyperthermic conditions or prolonged exercise.

There are several unanswered questions that require future investigation. First, direct interaction or binding between HSPs and some tight junction proteins have been documented, but no thorough investigation on specific role of HSP70 or other HSPs has been presented. Second, on cellular level, out of more than 40 tight junction 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
of pharmacological and nutritional compounds that can be safely and efficaciously used in tightening of 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 cellular and molecular mechanisms controlling classic hyperthermia or exertional stress is also needed. Detailed analysis of these mechanisms will not only have significant clinical and therapeutic implications, but will advance our knowledge and understanding at basic level of this still fascinating area of science.
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Figure legends

Figure 1. A summary of exercise- or passive heat-induced changes in TJ barrier, TJ protein expression or appearance, and macroscopic changes in GI tract in vitro and in vivo.

Figure 2. Figure 2. Schematic representation of hyperthermia-induced intestinal permeability and protective mechanisms of heat stress-induced HSP70 on tightening of the epithelial TJ barrier. (A) At thermal neutral core temperature the tight junction barrier is stable. Luminal LPS has no effect on TJ barrier. (B) As hyperthermia sets in a cascade of events occurs: (1) The physical stress of heat exposure causes initial tight junction breakdown through downregulation in ZO-1 protein expression and ZO-1 cellular redistribution from the membrane to the cytosolic fraction leading to LPS leakage. (2) LPS acts on the basolateral membrane in the interstitial space further enhancing TJ barrier disruption, and (3) LPS acts on innate immune cells (monocytes and macrophages) and Kupffer cells (not shown) causing the release of pro-inflammatory cytokines (TNF-α, IFN-γ, IL-1β), which also contribute to TJ barrier breakdown. (C) In heat preconditioning, inactive cytosolic 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 promotors, 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 tight junction barrier through several mechanisms: direct interaction with tight junction proteins occludin and ZO-1 (4); inhibition of the cytokine release from the immune cells (5); and inhibition of MLCK, NF-κB, or JNK pathways shown to be involved in disruption of the epithelial TJ barrier (6).
Figure 3. Schematic demonstrating some experimentally-examined treatments that increase (green, solid arrows) or decrease (red, dash arrows) function of intestinal epithelial TJ barrier in response to exercise or passive heat. MLCK (Myosin Light-Chain Kinase), PKCi (Protein Kinase Ci).
Heat stress

**Cell culture studies**

**Significant changes in TJ protein expression or appearance:**
- upregulation of occludin protein expression
- downregulation in ZO-1 protein expression
- no changes in claudin-3 and 2
- ZO-1 redistribution to the cytosolic compartment

**Animal or human studies**

**Major macroscopic changes in GI tract:**
- edema in GI tract
- bleeding from GI tract
- epithelial cell death
- shedding of epithelium
- vacuolization
- loss of microvilli
- shortening of microvilli
- peritoneal hemorrhage

**Significant changes in TJ protein expression or appearance:**
- damaged TJ structure
- increase in occludin and claudin 3 protein expression
- no changes in claudin 1 protein expression

Exercise

**Animal or human studies**

**Major macroscopic changes:**
- mucosa erosions
- ulcerations
- formation of lamellipodia
- liver damage

**Figure 1.**
A. Thermal neutral

B. Hyperthermia

C. Heat preconditioning or HSP70 overexpression

Figure 2.
Heat stress or strenuous exercise

Disruption of tight junction barrier

- HSP70
- Tyrosine kinase
- Nitric oxide synthase
- Eicosapentaenoic acid
- Na-dependent glucose cotransporter
- Bovine colostrum
- Goat milk
- Arginine
- Glutamine
- MLCK
- PKCi
- Oxidative stress
- Aspirin
- Quercetin

Figure 3.
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>
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<tbody>
<tr>
<td>Camus et al., 1997</td>
<td>Marathon race (166-282 min).</td>
<td>Not measured</td>
<td>Increased blood endotoxin levels at 1 h post race.</td>
</tr>
<tr>
<td>Jeukendrup et al., 2000</td>
<td>The Ironman long-distance triathlon: swimming (3800 m) in 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 post exercise.</td>
</tr>
<tr>
<td>Kuennen et al., 2011</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 post exercise compared to resting values in quercetin, but not in placebo group.</td>
</tr>
<tr>
<td>Lambert et al., 2001</td>
<td>60 min running @ 70% VO₂max at 22.4°C combined with aspirin ingestion</td>
<td>38.3</td>
<td>Increased urinary L/R* ratio 4 h post exercise compared to placebo values</td>
</tr>
<tr>
<td>Lambert et al., 2007</td>
<td>60 min running @ 70% VO₂max at 23.2°C combined with aspirin ingestion</td>
<td>38.2</td>
<td>Increased urinary L/R* ratio 5 h post exercise compared to resting values.</td>
</tr>
<tr>
<td>Lambert et al., 2008</td>
<td>60 min running @ 70% VO₂max at 24.4°C with placebo, glucose, or no fluid ingestion</td>
<td>38.4 (placebo) 38.5 (glucose) 38.7 (no fluid)</td>
<td>Increased urinary L/R* ratio at 5 h post exercise compared to resting values in no fluid ingestion group only.</td>
</tr>
<tr>
<td>Marchbank et al., 2011</td>
<td>20 min running @ 80% VO₂max</td>
<td>Mean rise 1.4</td>
<td>Increased urinary L/R* ratio 5 h post exercise compared to resting values that was prevented by colostrum supplementation.</td>
</tr>
<tr>
<td>Pals et al., 1997</td>
<td>60 min running @ 40, 60, or 80% VO₂max at 22°C</td>
<td>38.0 (40% group) 38.7 (60% group) 39.6 (80% group)</td>
<td>40% group: no increase in urinary L/R* ratio at 6 h post exercise compared to rest. 60% group: no increase in urinary L/R* ratio at 6 h post exercise compared to rest and 40% group. 80% group: increased urinary L/R* ratio at 6 h post exercise compared to rest, 40 and 60% groups.</td>
</tr>
<tr>
<td>Shing et al., 2014</td>
<td>running to fatigue (33 min) at 80% of ventilator threshold at 35°C</td>
<td>39.4</td>
<td>Post exercise increased serum LPS concentration.</td>
</tr>
<tr>
<td>Smetanka et al., 1999</td>
<td>Chicago marathon (42.2 km) (120-240 min)</td>
<td>Not provided</td>
<td>Increased in 5-hour L/R* ratio in ibuprofen ingested group, but not in aspirin or control groups</td>
</tr>
<tr>
<td>van Wijck et al., 2011</td>
<td>60 min cycling @ 70% VO₂max</td>
<td>Not measured</td>
<td>Overall increased plasma L/R* ratio, but not at individual time points.</td>
</tr>
<tr>
<td>Zuhl et al., 2014</td>
<td>60 min running @ 70% VO₂max in a heated chamber (30°C)</td>
<td>39.4</td>
<td>Increased urinary L/R* ratio 5 h post exercise compared to rest</td>
</tr>
<tr>
<td>Zuhl et al., 2015</td>
<td>60 min running @ 70% VO₂max in a heated chamber (30°C)</td>
<td>39.51</td>
<td>Increased urinary L/R* ratio 5 h post exercise compared to rest</td>
</tr>
</tbody>
</table>

* lactulose to rhamnose