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The Gastrointestinal Exertional Heat Stroke Paradigm: Pathophysiology, Assessment, Severity, Aetiology and Nutritional Countermeasures

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Abstract

Exertional heat stroke (EHS) is a life-threatening medical condition involving thermoregulatory failure and is the most severe condition along a continuum of heat related illnesses. Current EHS policy guidance principally advocates a thermoregulatory management approach, despite growing recognition that gastrointestinal (GI) microbial translocation contributes to the pathophysiology. Contemporary research has focussed on understanding the relevance of GI barrier integrity and strategies to maintain it during periods of exertional-heat stress. GI barrier integrity can be assessed non-invasively using a variety of *in vivo* techniques, including *active* inert mixed-weight molecular probe recovery tests and *passive* biomarkers indicative of GI structural integrity loss or microbial translocation. Strenuous exercise is well-characterised to disrupt GI barrier integrity, and aspects of this response correlate with the corresponding magnitude of thermal strain. The aetiology of GI barrier integrity loss following exertional-heat stress is poorly understood, though may directly relate to localised hyperthermia, splanchnic hypoperfusion mediated ischemic injury, and alternations in several neuroendocrine-immune responses. Nutritional countermeasures to maintain GI barrier integrity following exertional-heat stress provide a promising approach to mitigate EHS. The focus of this review is to evaluate: (1) the GI paradigm of exertional heat stroke; (2) techniques to assess GI barrier integrity; (3) typical GI barrier integrity responses to exertional-heat stress; (4) the aetiology of GI barrier integrity loss following exertional-heat stress; and (5) nutritional countermeasures to maintain GI barrier integrity in response to exertional-heat stress.

Abbreviations

BC	Bovine Colostrum
Caco-2	Human Colonic Carcinoma Cell Line
CFU	Colony Forming Units
CHO	Carbohydrate
CHS	Classic Heat Stroke
DSAT	Dual-Sugar Absorption Test
EHS	Exertional Heat Stroke
GI	Gastrointestinal
I-BABP	Ileal Bile-Acid Binding Protein
I-FABP	Intestinal Fatty Acid Binding Protein
IFN	Interferon
IGF-1	Insulin-Like Growth Factor-1
I-HSP	Intracellular Heat Shock Protein
IL	Interleukin
kDa	Kilodalton
LBP	Lipopolysaccharide Binding Protein
LPS	Lipopolysaccharide
L/R	Lactulose-to-Rhamnose Ratio
MOF	Multiple Organ Failure
MSAT	Multi-Sugar Absorption Test
MT	Microbial Translocation
NO	Nitric Oxide
NO^3	Nitrate
NO^2	Nitrite
NOS	Nitric Oxide Synthase
NSAIDs	Non-Steroidal Anti-Inflammatory Drugs
PAMPs	Pathogen Associated-Molecular Patterns
PCT	Procalcitonin
RDA	Recommended Daily Allowance
RES	Reticuloendothelial system

76	RH	Relative Humidity
77	SAPS	Simplified Acute Physiology Score
78	sCD14	Soluble Cluster of Differentiation 14
79	SIRS	Systemic Inflammatory Response Syndrome
80	S/E	Sucralose-to-Erythritol Ratio
81	S/R	Sucrose-to-Rhamnose Ratio
82	T_{core}	Core Body Temperature
83	TJ	Tight Junction
84	TLR	Toll-Like Receptor
85	TNF	Tumour Necrosis Factor
86	VO_{2max}	Maximal Oxygen Uptake
87	$Watt_{max}$	Maximal Power (wattage) Output
88	ZnC	Zinc Carnosine
89		

Introduction

Exertional heat stroke (EHS) is a life-threatening medical condition involving thermoregulatory failure, which is the most severe condition along a continuum of heat-related illnesses [1]. Although anecdotal records from biblical times have documented mortality from EHS [2-3], the condition still has no universal medical definition [4]. Instead, the most popular definitions broadly outline characteristic patient symptoms at time of clinical admission [5]. These principally include: (1) a core body temperature (T_{core}) above 40°C; (2) severe central nervous system disturbance (e.g. delirium, seizures, coma); and (3) multiple organ injury. Whilst classic heat stroke (CHS) primarily impacts incapacitated individuals (e.g. elderly, infants, chronic illness) whose thermoregulatory responses are unable to compensate for increased ambient temperatures [6], EHS sporadically impacts individuals (e.g. athletes, military personnel, firefighters) engaged in arduous physical activity [7]. Indeed, the primary cause of EHS is prolonged metabolic heat production, whilst exposure to high ambient temperature is less important than in CHS cases, despite further compromising thermoregulation [8].

The incidence of EHS has been frequently surveyed within high-risk populations since the beginning of the 20th century [3], though issues surrounding misdiagnosis (e.g. with less severe heat illness events) has generally limited accurate classification [9-10]. Over the last two-decades, the annual incidence of EHS has remained relatively stable within both athletic [11] and military [12] settings. Indeed, prevailing EHS incidence rates are reported to be *circa*: 0.1-1.5 cases per 10,000 US high school athletes per season [13-14]; 0.5-20 cases per 10,000 entrants during warm weather endurance races [15-17]; and 2-8 cases per 10,000 person years in both the United Kingdom [18] and United States [12, 19] armed forces. Given global predications of increased ambient surface temperature, coupled with a greater frequency, duration and intensity of extreme weather events, the risk of EHS is likely to increase [20]. Whilst timely medical intervention (e.g. whole-body cooling) can help prevent direct mortality from EHS [21], many affected individuals still experience long-term health complications because of residual organ damage. These health-complications include: heat intolerance [22], neurological impairment [23], chronic kidney disease [24] and cardiovascular disease [25]. The burden of EHS not only relates to the health of the impacted patients, but can also result in reduced occupational effectiveness [26-27], significant medical/legal expenses [28-29], and in

some instances high-profile media criticism [30-31] to the patients governing body or employer. In consideration of these issues, numerous published consensus documents have provided occupational guidance on effective management of EHS (e.g. [32-35]). However, these documents predominately focus on a thermoregulatory approach to disease management (e.g. cooling, heat acclimation). A gastrointestinal paradigm of EHS pathophysiology (also known as "endotoxemia" or "heat sepsis") is starting to receive more extensive recognition as a secondary pathway for EHS management [36-37], though consensus documents are present unavaliable.

The gastrointestinal (GI) tract, is an organ extending between the stomach to the colon. It is the human body's longest mucosal interface (250-400 m^2) forming a selectively permeable barrier to the external environment. The GI microbiota is a collection of microorganisms that colonise the GI tract and have co-evolved inside humans to provide various mutually beneficial functions [38]. The GI microbiota has an estimated size *circa* 10^{14} cells, between 1- to 10-fold greater than the total number of cells within the human body [39]. Alongside a predominant role in the absorption of dietary nutrients, a second vital function of the GI tract is to prevent the translocation of immunomodulatory GI microbial products (e.g. endotoxin, flagellin, bacterial DNA) into the systemic circulation [40]. To achieve this role, the structure of the GI tracts forms a multi-layered physical and immunological barrier. The physical barrier comprises a monolayer of epithelial cells interconnected by tight junction (TJ) protein complexes, and is reinforced by a mucosal lining secreted by goblet cells. The immunological barrier comprises crypt paneth cells within the epithelial monolayer that secrete antimicrobial proteins, and gut associated lymphoid tissue within the *lamina propria* that stimulate multiple effector immune responses. In healthy individuals, the GI tract is largely effective in preventing GI microbial translocation (MT) into the systemic circulation [40], though several reports have hypothesised a fundamental role of GI MT within the pathophysiology of EHS [36-37]. The focus of this review is to evaluate: (1) the GI paradigm of EHS; (2) GI barrier integrity assessment techniques; (3) typical GI barrier integrity responses to exertional-heat stress; (4) the aetiology of GI barrier integrity loss; and (5) nutritional countermeasures to support GI barrier integrity during exertional-heat stress.

The GI Exertional Heat Stroke Paradigm

The GI EHS paradigm was first introduced as a novel pathophysiology concept in the early 1990s [41] and was integrated into conventional EHS medical classifications in 2002 [5]. The broad scientific basis of the GI EHS paradigm centres on the notion that sustained exertional-heat strain initiates damage to multiple layers of the GI barrier, which consequently permits GI MT into the systemic circulation. To counter this response, the liver's reticuloendothelial system (RES) provides the first line of GI microbial detoxification (e.g. kupffer cells and hepatocytes) through the portal circulation. However, this confers only a limited capacity for microbial neuralization before microbial leakage into the systemic circulation occurs [42]. Alternatively, GI MT might bypass the RES altogether, instead translocating directly through the mesenteric lymph nodes into the systemic circulation [43]. In the systemic circulation MT products are neutralized through multiple host-binding pathways, including: natural antibodies (e.g. immunoglobin G and M), leukocyte granular proteins (e.g. bactericidal permeability increasing protein, lactoferrin, lysozyme) and high-density lipoproteins [42]. In EHS patients, it appears that microbial detoxification capabilities might also be reduced, via the combined effects of RES dysfunction at T_{core} above 41-42°C [44] and immune antibody suppression, as demonstrated following strenuous exercise [45]. Failure of GI microbial detoxification mechanisms permits binding of unique GI pathogen associated-molecular patterns (PAMP) to toll-like receptors (TLR) located on cell surface membranes [46]. TLR activation initiates a cascade of intracellular events that culminate in the production of pro-inflammatory cytokines (e.g. interleukin [IL] 1-β, IL-2, IL-6, IL-8, tumour-necrosis factor [TNF]-α), which are counteregulated by the production of anti-inflammatory cytokines (e.g. IL-1ra, IL-4, sIL-6r, IL-10, sTNFr). Downstream of this systemic inflammatory response syndrome (SIRS), a complex interplay of responses can culminate in haemorrhagic shock, disseminated intravascular coagulation (DIC), multiple organ failure (MOF) and possibly death [47]. The GI EHS paradigm is considered to be the primary cause of EHS in cases where T_{core} remains below the threshold (~42-44°C) of heat cytotoxicity [48]. A simplified schematic of the GI EHS paradigm is shown in Figure 1. Interested readers are referred to several detailed reviews on this topic [1, 36-37].

[Insert Figure 1 Here]

To date, direct pathophysiological investigation into the GI EHS paradigm has been limited, which is surprising given the substantial morbidity/mortality associated with the disease. The best available evidence is reliant on animal experimental models of CHS or opportunistic monitoring of human EHS patients. In a pioneering study, prior antibiotic administration in a canine CHS model (peak T_{core}= ~43.5°C) both suppressed GI microbial stool concentration and increased survival rate (71% versus 20%), indirectly suggesting the importance of inhibited GI MT [49]. In a seminal series of studies using a primate CHS model (peak T_{core}= ~43.5°C), plasma endotoxin concentrations were found to increase in parallel with T_{core} (50-52), but prior antibiotic [50-51] or corticosteroid [52] treatment attenuated this effect. Importantly, 100% of prior- treated animals survived, in comparison with less than 30% of control animals. However, once hyperthermia was above the intensity to evoke heat cytotoxicity (peak T_{core}= ~44.5°C), mortality rates were 100% irrespective of pharmaceutical intervention. This suggests that the GI EHS paradigm is probably most relevant in cases when T_{core} remains below ~42-43°C [48]. Several studies have confirmed these findings in similar rodent CHS models (peak T_{core}= ~43.5°C), whereby prior corticosteroid injection inhibited GI MT and increased survival rate [53-55], whilst indomethacin injection enhanced gross morphological GI haemorrhage and suppressed survival rate [56].

Direct endotoxin injection into rodents before sub-lethal CHS (peak T_{core} = ~42-43°C) unexpected killed 40% of animals (versus 0% in controls; [57]) and/or increased multiple-organ injury [58]. In the only animal models of EHS (peak T_{core}= 40.5-42.5°C), significant histopathological damage to all GI segments [59], in addition to GI epithelial injury [59-60] and systemic inflammation [61], were observed. However, in comparison to CHS models with a similar clinical endpoint (peak T_{core}= ~42-42.5°C), the magnitude of GI barrier integrity loss was reduced during EHS, though this was likely attributable to a ~50% lower thermal area [60]. No published animal EHS research has yet evaluated the role of GI MT on EHS pathophysiology. However, recent data show the pattern of cytokine response during EHS is largely inconsistent with typical GI microbial PAMP recognition (e.g. minimal TNF-α/IL-1β response; [60]. With this is mind, it is plausible intracellular cytokine production initiated following multiple organ injury (e.g. skeletal muscle; [61]) performs a greater role in EHS pathophysiology than

previously proposed in GI EHS consensus documents [37, 47].

In humans, the role of GI barrier integrity in the pathophysiology of EHS is a relatively recent area of research; which has been established on historical evidence of severe GI symptoms, ulceration and haemorrhage in military EHS fatalities [62-64]. Evidence supporting the present GI EHS model was first reported by Graber et al. [65], who observed endotoxin translocation into the systemic circulation and symptomology of experimental endotoxin shock in a single EHS case report. More substantial evidence was collated in the 1990s, from EHS patients (peak T_{core} = ~42°C) who had been on religious pilgrimage to Mecca [66]. The plasma endotoxin concentration increased ~1000-fold more than in healthy controls (8.6 $ng \cdot ml^{-1}$ vs 9 $pg \cdot ml^{-1}$). In this study, weak correlations were reported between endotoxin and SIRS responses (e.g. TNF-α *r*= 0.46; IL-1β *r*= 0.47), whilst in a follow-up study that did not monitor endotoxin responses, IL-6 concentration weakly correlated (*r*= 0.52) with the disease Simplified Acute Physiology Score (SAPS; [67]). In support, IL-2 (*r*= 0.56), IL-6 (*r*= 0.57) and IFN-γ (*r*= 0.63) concentrations weakly correlated with the SAPS in a cohort of military EHS (peak T_{core} = ~41.5°C) patients, though the SAPS did not correlate with the time-course of any other cytokine monitored (IL-1β, IL-2ra IL-4, IL-8, IL-10; TNF-α) [68]. Likewise, IL-6 and sTNFR, but not IL-1ra and C reactive protein, predicted survival in a later cohort of EHS patients (peak T_{core} = ~41.5°C) on Mecca pilgrimage [69]. Whilst none of these studies directly monitored GI MT responses, sub-clinical exertional-heat stress (T_{core} = < 40°C) experiments have reported similar patterns of endotoxin translocation and SIRS kinetics in some [70-71], but not all cases [72-73].

A key limitation of previous research has been the exclusive reliance of endotoxin to assess GI MT. There is evidence blood samples may be cross-contaminated during collection or analysis, for example one EHS case study reported the presence of β-glucan (a fungal cell wall component) in blood which was unlikely to be of GI origin [74]. Variations in sample contamination might explain EHS induced endotoxemia independent of GI MT [74]. Future research should focus on determining the sensitivity/specificity of GI barrier/MT biomarkers on EHS outcome. One potentially relevant novel biomarker is procalcitonin (PCT), a pro-inflammatory acute phase reactant, which offers strong sensitivity/specificity in diagnosing acute bacterial infections [75]. In EHS patients, PCT measured 2 hours following intensive care

unit admission was able to predict Acute Physiology and Chronic Health Evaluation (APACHE) II score (r = 0.59) and had an odds-ratio of 2.98 for predicting disease mortality [76]. Furthermore, in CHS patients, PCT concentrations were significantly greater in fatal versus non-fatal cases [77-78].

Assessment of GI Barrier Integrity

Various techniques are available for the *in vivo* assessment of GI barrier integrity. These techniques can be broadly categorised as either: (1) *active* tests involving the oral ingestion and extracellular recovery of water-soluble non-metabolizable inert molecular probes; (2) *passive* tests involving monitoring blood biomarkers indicative of GI barrier integrity; and (3) *microbial translocation* (MT) tests involving monitoring blood biomarkers indicative of the passage of GI microbial products across the GI barrier secondary to integrity loss (Table 1 [40]).

[Insert Table 1 Here]

The Dual Sugar Absorption Test (DSAT) is presently promoted as the gold-standard *active* GI function test [79], which has received almost exclusive application with the field of exercise science [80-82]. This test involves co-ingestion of both a large disaccharide (e.g. lactulose [342 kDa] or cellobiose [342 kDa] ~5 grams) that only transverses the GI tract paracellularly upon barrier integrity loss, and a small monosaccharide ($_{L}$-rhamnose [164 kDa] or $_{D}$-mannitol [182 kDa] ~1-2 grams) that freely transverses the GI tract transcellularly independent of barrier integrity [83]. In the five hour period post-ingestion the excretion of both sugars are measured in urine and are believed to be equally affected by non-mucosal factors, such as gastric emptying and renal clearance [84]. The urinary ratio of lactulose-to-rhamnose (L/R) relative to the ingested dose is the clinical endpoint of this test. Recently, the DSAT has been validated in serum/plasma with improved sensitivity over a time-courses ranging between 60-150 minutes [85-88], and with comparable reliability to traditional urinary assessment [89]. Unfortunately, the DSAT has several practical limitations, most

notably: a requirement to perform basal/exercise tests on separate days and a lack of universal test standardisation (e.g. pre-trial controls, sugar dose, ingestion timing, biofluid timing) [84]. Furthermore, based on the degradation of lactulose in the large intestine, the test only provides information regarding small GI barrier function, with further sugar probes (i.e. multi-sugar absorption test; MSAT) required to assess gastroduodenal (e.g. sucrose/rhamnose; S/R) and large intestinal (e.g. sucralose/erythritol; S/E) barrier function [82]. Whilst routine implementation of the MSAT would be desirable, hyperosmolar stress utilising recommended sugar dosages will cofound the test result. In attempt to overcome this issue, validation of a low dose (1 gram lactulose, sucrose, sucralose; 0.5 grams $_{L}$-rhamnose, erythritol) MSAT protocol has recently been favourable evaluated against the traditional dose (5 grams lactulose, 2 grams $_{L}$-rhamnose) DSAT protocol [87,90]. Polyethylene glycols (PEG; 100-4000 kDa) are a less-common, though a validated alternative to the MSAT for whole-GI barrier integrity assessment [91]. An advantage of PEG assessment is the ability to provide information on the size based permeability of molecules able to transverse the GI barrier. However, this method does require additional lifestyle controls, as PEGs can be found in various commercial/dietary products (e.g. toothpaste, soft drinks) [82]. The application of single molecular probes tests (e.g. non-metabolizable sugars, ^{51}Cr-EDTA, Iohexol, Blue #1 Dye) cannot be recommended in exercise-settings given the confounding influence of non-mucosal factors [84].

Several *passive* blood-based biomarkers of GI barrier integrity are available, which can assess epithelial injury to specific regions of GI tract, TJ breakdown and MT [40]. Epithelial injury to the duodenum and jejunum can be evaluated via intestinal fatty-acid binding protein (I-FABP); and to the ilium via ileal bile-acid binding protein (I-BABP). These cytosolic proteins are involved in lipid metabolism, though offer strong diagnostic specificity/sensitivity in detecting GI barrier integrity loss [92], given their tissue specificity and transient 11 minute half-life [93]. Alternative biomarkers of GI epithelial/transmural injury include: alpha-glutathione s-transferase (α-GST), diamine oxidase (DAO) and smooth muscle protein 22 (SM22); however a lack of tissue specificity limits their application in settings (e.g. exercise) where multiple-organ injury is commonplace [40, 94]. There is presently no available biomarker of large intestinal epithelial injury. To assess TJ breakdown, zonulin, a pre-curser protein to haptoglobin, has received most widespread attention, given its recognised role in

disassembling GI TJs [95]. However, the two commercial assays presently available for this biomarker are susceptible to cross-reactivity (e.g. for complement protein C3). Consequently data collected with this technique should be interpreted with caution until the methods have been validated [96]. Claudin-3, is a non-tissue specific, highly expressed GI TJ protein, which is an emerging biomarker for TJ breakdown. Preliminary data has shown claudin-3 concentrations are elevated in clinical conditions where GI TJ damage has been confirmed histologically [97]. The test-retest reliability of I-FABP and claudin-3 was recently considered acceptable when assessed both at rest and following exertional-heat stress [89]. All GI epithelial injury/TJ breakdown biomarkers can be assayed in plasma/serum by ELISA, whilst future developments in auto-analysers and validation of capillary blood and urine samples have potential to make assessment simpler in the future.

The definition of MT was traditionally founded on the transloaction of live bacteria from the GI lumen into the mesenteric lymph. However, given practical constraints of mesenteric lymph biopsy in healthy humans, this definition has been extended to include the detection of microbial products/fragments in blood [98]. To determine GI MT, measurement of endotoxin, a form of lipopolysaccharide (LPS) located on the outer membrane of gram-negative bacteria, has been widespread [80]. Endotoxin is detectable within the portal/systemic circulations following bacterial cleavage during both cell lysis and division, with assessment widely undertaken using the chromogenic limulus amoebocyte lysate (LAL) assay. Whilst popular, there are major flaws to endotoxin assessment, as it is prone to false-positive (e.g. from exogenous contamination, cross-reactivity) and false-negative (e.g. from hepatic clearance, immune neutralization) results [99]. Two indirect surrogate biomarkers for endotoxin exposure that can be quantified by ELISA are the acute phase proteins: lipopolysaccharide binding-protein (LBP; [100]) and soluble-CD14 (sCD14-ST; [100]). Whilst the roles of these biomarkers have been characterised during life-threatening septic shock [101], evidence regarding their time-course, sensitivity and specificity in predicting transient GI MT following exertional-heat stress is sparse [80]. D-lactate is a secondary enantiomer of L-lactate, hypothesised as a biomarker of GI MT given that the enzyme D-lactate dehydrogenase is specific to bacteria [102]. That said, human cells do produce small-quantities of D-lactate through secondary methylglyoxal metabolism [102]. Whilst D-lactate has been shown to predict GI MT in animal models of gut trauma [103-104], its low-molecular weight

(0.09 kDa) might permit false-positive results through transcellular translocation following production within the GI tract. Bacterial DNA (bactDNA) is a stable bacterial component, which through targeting phyla with high GI specificity offers potential as an improved MT biomarker [105]. Whilst a universal analytical procedure is currently lacking (e.g. target primers, positive/negative controls), one major advantage of bactDNA over endotoxin assessment, is an apparent lack of rapid hepatic clearance [46]. As the GI microbiota is dominated (≥ 90%) by two bacterial phlya *Firmicutes* and *Bacteroidetes*, which comprise only a minor proportion (0-10%) of the whole blood/plasma microbiota [106], developing methodologies that target these specific gene regions are likely to provide high GI specificity. Pioneering studies have shown total 16S DNA to offer good reliability at rest and post exertional-heat stress, however *Bacteroides* DNA (the dominant *Bacteroidetes* bacterial genus) offered poor reliability at both time points [89].

Severity of GI Barrier Integrity loss following Exertional-Heat Stress

Numerous research models have characterised the influence of exertional-heat stress on GI barrier integrity. This research has primarily monitored small intestinal integrity using the DSAT, though attempts have been made to quantify gastroduodenal and large intestinal integrity using the MSAT [80]. Over the last decade, several passive GI integrity and/or MT biomarkers have become commonplace as an alternative to, or for use in combination with the DSAT. Generally, I-FABP has been monitored to assess GI epithelial integrity, and endotoxin to assess GI MT. The exercise models assessed are disparate, ranging from 45 minutes brisk walking [107] to a 230-km ultramarathon [71]. That said, most studies comprise 1-2 hours of continuous, submaximal (60-70% VO_{2max}) running or cycling. Given the hypothesised relevance of GI barrier integrity within the pathophysiology of EHS, the impact of exercise-induced thermal strain (e.g. T_{core}) on GI barrier integrity has been a specific topic of investigation [81]. In comparison to acute exercise-interventions, few studies have attempted to evaluate the effect of either chronic exercise training or multi-day occupational performance (e.g. sports competition, military/firefightining operation) on GI barrier integrity. Such exercise models would appear particualaly relevant to EHS incidence, given that many documented EHS risk factors (e.g. prior heat exposure, skeletal muscle injury) relate to multi-day exercise [37]. Review tables are provided to summarise the effects of acute exercise on: DSAT (Table 2); I-FABP (Table 3); and MT (Table 4).

Seminal research using the DSAT, investigated the effects of one hour's treadmill running in temperate conditions on GI barrier integrity [108]. These authors found the DSAT ratio increased relative to both the magnitude of metabolic (60, 80 and 100% VO_{2max}) and thermal (38.0, 38.7 and 39.6°C T_{core} peak) strain [108]. Later studies monitoring GI barrier integrity following exercise in temperate conditions corroborated this seminal finding, with low-to-moderate intensity (~40-60% VO_{2max}) exercise having little influence on DSAT results compared with rest [e.g. 109-111]; whereas moderate-to-high intensity (~70-120% VO_{2max}) exercise of durations ≥20 minutes increase permeability by 100-250% [e.g. 86, 88, 112-116]. Unfortunately, the present data does not allow more specific conclusions to be drawn, given large intra-study variability in absolute DSAT ratios, which can be attributed to modifications in the DSAT procedure (e.g. sugar probe type/dose/timing, analytical protocol) and/or a frequent lack of basal GI permeability correction (Table 2). That said, individual studies highlight the importance of particular aspects of the exercise stimulus on GI barrier integrity, with increased DSAT ratios after matched interventions comparing: running and cycling [117]; permissive dehydration versus rehydration [118-119]; and following ingestion of non-steroidal anti-inflammatory drugs (NSAID) [120-124]. To date, only two published studies have directly compared the influence of ambient temperature on GI barrier permeability [115, 125]. In conflict with *a priori* hypotheses, the first of these studies found two hours of moderate intensity (60% VO_{2max}) treadmill running in temperate (22°C/44% relative humidity [RH]) versus mild hyperthermic (30°C/35% RH) conditions resulted in comparable DSAT responses (0.025 ± 0.010 vs. 0.026 ± 0.008 [125]). However, these results were perhaps not entirely surprising given that Tcore responses showed minimal divergence between the two environmental conditions (e.g. peak T_{core} = 38.1°C vs. 38.4°C [125]). A follow-up trial on the same subjects compared the results of the temperate exercise condition (22°C/44% RH) with a third trial conduced in a more severe hyperthermic (35°C/26% RH) environment [115]. The DSAT data (0.032 ± 0.010) remained statistically indifferent to the temperate condition, despite greater T_{core} elevations (e.g. peak T_{core} = 39.6°C [115]). These null findings might be interpreted with caution, as there was poor analytical reproducibility of sugar concentrations (duplicate sample coefficient of variation = 13.8%) and no basal DSAT correction.

In comparison with the extensive literature examining the acute effect of exercise on small GI integrity using the DSAT, few studies have assessed the influence of exercise or

393 exertional-heat stress on either gastroduodenal or large GI barrier integrity utilising the MSAT
394 [80]. In the only published evidence where the MSAT was applied with reference probe co-
395 administration [82], both gastroduodenal (S/R; [124]) and large intestinal (S/E; [86]) integrity
396 were unaltered following one hour of moderate intensity cycling (70% $watt_{max}$) in temperate
397 conditions (~22°C), which was sufficiently intense to induce detectable small intestinal barrier
398 integrity loss using the DSAT. Similarly, gastroduodenal integrity, measured using a single
399 sugar-probe (sucrose) has been shown to be unaltered following one hour of moderate
400 intensity treadmill running (40-80% VO_{2max}) in temperate conditions [108, 119, 122], 18
401 repeated 400 metre supramaximal track sprints (120% VO2max) in temperate conditions [88]
402 and a ~33 minute exercise capacity trial at 80% ventilatory threshold in the heat (35°C/40%
403 RH [126]). No further studies have measured large intestinal integrity following acute exercise
404 using a single sugar-probe (sucralose). There is a clear gap in the literature regarding the
405 influence of exertional-heat stress on large intestinal integrity, which warrants future
406 investigation given the greater microbiota concentration in this segment of the GI tract (e.g.
407 duodenum = $<10^3$, ilium 10^3-10^7, colon= 10^{12}- 10^{14}) [166].

408

409 [Insert Table 2 Here]

410

411 Application of I-FABP as a biomarker of small-intestinal (duodenal and jejeneal)
412 epithelial injury was first applied in exercise settings during a series of studies conducted in
413 the Netherlands, which demonstrated peak concentrations (~50-100% increase) immediately
414 following termination of a one-hour moderate-intensity (70% $Watt_{max}$) cycle [86, 124, 127]. I-
415 FABP responses showed weak correlations with I-BABP (i.e. ilieum injury) and the DSAT [86],
416 suggestive of inconsistant injury across the small intestine. Since then, low intensity exercise
417 (~50% VO_{2max}) in temperate environments has typically shown little effect on I-FABP
418 concentrations [128-130], but moderate-to-high intensity exercise (60-120% VO_{2max}) elevates
419 concentrations by 50-250% [88, 125, 131-133]. Where measured, I-FABP responses quickly
420 recover within 1-2 hours of exercise termination, irrespective of the intensity/duration of the
421 protocol [125, 131]. Like DSAT results, I-FABP responses are elevated in otherwise matched

exercise-interventions comparing: hypoxic (F_iO_2 = 0.14) versus normoxic environments [128, 134]; permissive dehydration versus rehydration [135]; and post NSAID ingestion [124]. In comparison, since initial investigation [86], no studies have monitored the magnitude and time-course of I-BABP responses following exericse. Several studies have attempted to elucidate the influence of ambient temperature on GI epithelial injury [115, 125, 133, 136-137]. Compared with modest increases in I-FABP (127%) following two hours of moderate intensity cycling (60% VO_{2max}) in temperate (22°C/44% RH) conditions (peak T_{core} 38.1°C), performance of matched exercise in both mild (30°C/35% RH [115]) and severe heat stress conditions (35°C/26% RH; [125]) vastly enhanced peak T_{core} (38.4°C and 39.6°C) and percentage change in I-FABP (184% and 432%) responses, respectively. Furthermore, a moderate correlation (r = 0.63) was shown between peak T_{core} and I-FABP concentration in these studies. Ingestion of cold (7°C) relative to temperate (22°C) water during two hours moderate intensity cycling (60% VO_{2max}) in the heat, blunted the rise in both T_{core} (38.4 vs 38.8°C) and I-FABP (~400% vs 500%) concentration [137], though whether these responses are directly related is questionable. These conclusions were recently substantiated following one hour of low intensity (50-70% $watt_{max}$) cycling, where I-FABP concentration increased following performance in a hot (35°C/53 % RH; 140%), but not temperate (20°C/55% RH; 29%) ambient environment [133]. Importantly, these observations have been directly attributed to the influence of ambient temperature on whole-body thermal strain, given that when relative exercise-intensity is matched (VO_{2max}, T_{core}, heart rate), the influence of ambient heat stress (20 vs. 30C°) on I-FABP responses is abolished [136]. One study reported GI TJ breakdown (claudin-3) to increase to a similar extent following one hour of running in a temperate (22°C/62% RH) versus hot (33°C/50% RH) ambient environment [138], suggestive that TJ breakdown is insensitive to thermal stress. Alternatively, I-FABP and claudin-3 responses positively correlated (r = 0.41) following an 80-minute brisk walk (6 $km \cdot h^{-1}$/7% incline) in the heat (35°C/30% RH) [89].

[Insert Table 3 Here]

Endotoxin is a traditionally popular biomarker of GI MT and was the first technique utilised to assess GI barrier integrity in exercise settings. Seminal research monitoring endotoxin concentrations following exercise, found concentrations to increase transiently to magnitudes comparable to clinical sepsis patients (~50-500 pg·ml^{-1}) when measured following competitive ultra-endurance events [80]. These included: an ultra-triathlon [139], a 90 km ultra-marathon [140], a 100-mile cycle race [141] and a 42.2 km marathon [142]. More recently, only minor increases in endotoxin concentrations have been shown following comparable duration competitive ultra-endurance races [71, 144-145], whilst moderate intensity exercise (≤2 hours; 50-70% VO_{2max}) performed in a temperate environment generally does not influence circulating endotoxin concentrations [132-133, 138, 143]. These discrepant results may be due to cross-contamination from β-glucan during early research, which following development of more robust endotoxin assays is now less of an issue [144]. It appears a presently undefined threshold of GI barrier integrity loss is required to induce endotoxemia following exercise, given that endotoxin concentrations are often unchanged from rest irrespective despite concurrent rises in DSAT or I-FABP concentrations [116, 125, 132]. When endotoxin is assessed from systemic blood samples, hepatic/immune detoxification might lead to false-negative results, and in exercise settings access to portal blood is rarely feasible. Given the large range in absolute endotoxin concentrations reported between studies (Table 4), several recent attempts have been made to measure MT with alternative biomarkers, though results are equally inconsistent [131, 146-148]. Thermal stress appears to enhance endotoxin translocation above matched exercise performed in temperate conditions. In an early study, endotoxin concentrations increased linearly above 38.5°C when (measured at 0.5°C T_{core} increments), during uncompensable (40°C/30% RH) treadmill walking (4 km·h^{-1}) [146]. Likewise, a follow-up study found one hour of moderate intensity treadmill running (70% VO_{2max}) only increased endotoxin concentrations in hot (33°C/50% RH; 54%), but not temperate (22°C/62% RH) conditions [138]. In a series of studies monitoring endotoxin concentrations following two hours moderate intensity treadmill running (60% VO_{2max}), concentrations were found to increase by 4-10 pg·ml^{-1} irrespective of the thermal environment (22-35°C; [115, 125, 149]. Numerous other studies have measured endotoxin concentrations following exertional-heat stress, though large intra-study variability in absolute concentration make it impossible to make precise recommendations regarding the typical magnitude of response (Table 4). In studies where endotoxin concentrations do

483 increase following exertional-heat stress, responses peak immediately upon trial termination
484 [138, 150].

485 [Insert Table 4 Here]

486 Whilst many studies have monitored GI barrier integrity responses following acute
487 exertional-heat stress, relatively few studies have monitored GI barrier integrity following
488 chronic (multi-day) exertional-heat stress. Where chronic exercise studies have been
489 undertaken, they predominately focus on the influence of structured heat acclimation on GI
490 barrier integrity. In an early study, involving seven days fixed-intensity heat acclimation (100
491 minutes walking at 6.3 $km \cdot h^{-1}$ in 46.5°C/20% RH), endotoxin concentrations remained stable
492 both at rest and following exertional-heat stress, despite T_{core} peak above 39.0°C [143].
493 Utilising a variation of this experimental design, five consecutive days treadmill running at
494 lactate threshold pace in the heat (40°C/40% RH) until T_{core} had risen 2°C above rest, evoked
495 comparable post-exericse I-FABP and endotoxin responses compared to day-one [72].
496 Likewise, 10 days of fixed-intensity heat acclimation (one hour running at 50% VO_{2max} in
497 40°C/25% RH), had no influence on post-exericse I-FABP concentration compared to day one
498 [128]. In a recent study, neither seven nor thirteen days isothermic heat-acclimation (90
499 minutes to sustain T_{core} ~38.5°C) blunted the rise in endotoxin concentration following 45
500 minutes low intensity (40% $watt_{max}$) cycling in the heat (40°C/ 50% RH), despite large
501 reductions in thermal strain [151]. In a non-heat acclimation study, 14 days of 20% increased
502 training versus standard load, led to a reduction in resting endotoxin concentration (35%), but
503 did not influence peak concentrations following a 70% VO_{2max} treadmill run (35°C/40% RH)
504 until a T_{core} of 39.5°C was attained [150]. The influence of aerobic fitness has been shown to
505 both increase (I-FABP; [152]) and reduce (endotoxin; [146]) GI barrier integrity loss following
506 exertional heat stress that evoked comprable thermal strain between groups. Future research,
507 using well-designed and adequately powered studies coupled with sensitive biomarkers, is
508 required to determine the influence of heat acclimation on GI barrier integrity. As well as
509 ensuring an appropriate sample size, an exertional-heat stress protocol that evokes high
510 physiological strain should be used, using study participants that posse the same physiological
511 characteristics as the target population.

512

Aetiology of GI Barrier Integrity Loss following Exertional-Heat Stress

The aetiology of exertional-heat stroke induced GI barrier loss appears multifactorial and is incompletely understood. The best supported explanations relate to: hyperthermia-mediated dysregulation of GI TJs [153]; splanchnic hypoperfusion-mediated ischemia-reperfusion injury [82, 155]; and alternations in several complex neuroendocrine-immune related interactions [156].

Increased tissue metabolic rate during strenuous exercise, and/or environmental heat stress, can evoke uncompensable heat strain on the body as thermoregulatory cooling responses (e.g. sweating and increased skin perfusion) become overwhelmed [157]. Within the GI tract, exertional-heat stress results in a relatively uniform rise in tissue temperature across both the small and large intestinal segments (though this rise is lower in the stomach), which can be predicted from T_{core} assessement in the distal colon [158]. This will weaken the GI barrier by morphologically disrupting the enterocyte structure and opening TJ complexes [153]. Cell culture models have consistently shown temperature elevations from 1.3°C to rapidly disrupt the GI barrier in a dose/duration dependant manner [159]. Rodent studies support these conclusions, with evidence of both histopathological GI damage and increased GI permeability following passive heating >40°C [154]. Nevertheless, the mechanistic pathways directly linking hyperthermia to GI barrier integrity loss have been poorly characterised. The available evidence suggests that heat stress positively regulates the GI barrier through sodium-dependant glucose cotransporter/tyrosine kinase pathways [160] and negatively through the myosin light-chain kinase/protein kinase-c pathways [161]. Ethical constraints have prevented laboratory GI barrier integrity assessment following severe hyperthermia (>40°C) in humans. However, a systematic review including available data up until September 2016 reported strong correlations (r= 0.79) between peak T_{core} and GI barrier integrity loss (5-hr urine DSAT only) when all available T_{core} assessment techniques were included [81]. Data presented in tables 2-4 show a weak correlation between peak post-exercise T_{core} (rectal, gastrointestinal or oesophageal) with peak I-FABP (Δ; r= 0.52; p = <0.001), but not the DSAT (5-hr urine only; r= 0.30; p= 0.19), or endotoxin (Δ; r= 0.14; p= 0.56) concentration (note: studies without T_{core} assessment were excluded).

Splanchnic vascular beds receive ~20% of total resting cardiac output but consume only 10-20% of the available oxygen [162]. Consequently, blood flow during strenuous exercise can be safely redistributed from splanchnic organs to skeletal muscle to maintain aerobic metabolism, and to skin to assist thermoregulation [157]. Hypoperfusion of splanchnic vascular beds, measured using doppler ultrasonography, appears to be proportional to exercise intensity and duration [162]. Specifically, splanchnic blood flow declines by 30-60% following both 30 minutes of moderate-intensity (60-70% VO_{2max}) and 1-2 hours of low-intensity exercise (40-50% VO_{2max}) [163]. These responses appear amplified when exercise is performed in a warm environment [164]. A key downstream event following GI hypoperfusion is GI ischemia measured using gastric tonometry, which is also known to be suppressed following exercise in an intensity dependant manner [86, 165]. Localised GI hypoperfusion is considered to evoke secondary adenosine triphosphate depletion, acidosis, altered membrane ion pump activity and oxidative stress, all physiological responses that damage the GI barrier [154, 159, 167]. One limitation of this research is the inability of tonometry to measure large intestinal ischemia in exercising humans, especially as the largest microbial biomass is located in the distal GI segments [166]. The partial pressure of oxygen across the GI tract displays a proximal-to-distance gradient [168], which might have clinical manifestations on MT given that the integrity of the large intestine is considered less susceptible to ischemic injury [82]. Contrary to previous beliefs, the influence of splanchnic reperfusion following exertional-heat stress appears to be an unlikely mechanism of GI barrier integrity loss [82]. Indeed, one study found plasma I-FABP concentrations correlated with splanchnic (stomach) hypoperfusion during moderate intensity exercise (r= 0.59), though following post-exercise intestinal reperfusion, I-FABP concentrations began to recover within the first 10 minutes [86].

Inflammatory cytokines comprise a large family of intercellular pleiotropic signalling molecules that perform many regulatory functions, and are primarily involved in innate immunity [169]. Strenuous exercise induces strong pro-inflammatory (TNF-α, IL-1β, IL-6, IFN-γ), followed by anti-inflammatory (IL-1ra, IL-4, IL-10) responses throughout numerous cells and tissues across the body [170]. The specific biological roles of individual cytokines are incompletely understood and are likely context dependant. That said, several pro-inflammatory cytokines released post-exercise (e.g. TNF-α) appear to disrupt GI barrier

integrity [153]. Potential regulatory mechanisms might include: direct modulation of several cell signalling pathways that regulate TJ protein complex stability [171-173]; and the indirect pyrogenic modulation of body temperature where local hyperthermia damages the GI barrier [174-175]. With EHS cases, pro-inflammatory cytokines are produced upon immune activation (e.g. nuclear factor kappa-β transcription) following binding between MT products and toll-like receptors located on cell surface membranes [156]. This response appears to operate through a positive feedback loop that may further promote GI MT, cytokine production, and potentially culminate in fatal septic shock [176].

Nutritional Countermeasures

Nutritional countermeasures could modulate key cellular pathways involved in mitigating exertional-heat stress induced GI barrier integrity loss. Diet regimens and nutrition supplements with evidence they can influence GI barrier integrity following exercise and/or exertional-heat stress will be reviewed. The mechanistic basis of each nutritional intervention, evidence of improved GI barrier function following exercise and practical recommendations are presented.

Carbohydrate

Carbohydrates (CHO) are the main macronutrient of western diets and are an essential energy substrate in susustained moderate and high intensity exercise. The physiological response to CHO ingestion is highly dependant upon its biochemical formula, where high glycaemic index CHO (e.g. glucose, maltose) have rapid bioavailability, and low glycaemic index CHO (e.g. fructose, galactose) have delayed bioavailability. The volume, tonicity and osmolality of CHO is equally influential. In healthy resting humans, ingestion of a single CHO-rich meal (55-70% of total kilo-calories) evokes equivocal (endotoxin [177-179] or slightly improved (I-FABP; [180-181]) GI barrier integrity postprandially. However, rodent experimental models of acute GI distress indicate that oral ingestion of maltodextrin [182] or sucrose [183] favourably influence GI barrier integrity. Mechanisms of action at the whole-body level are likely multifactorial, including regulation of the GI microbiota [184] and an elevation of splanchnic perfusion [185]. Nevertheless, *in vivo* and *in vitro* studies indicate that

high glucose exposure might reduce GI TJ stability through an abnormal redistribution of several TJ proteins [186]. Compared with ingestion of a single CHO-rich meal, ingestion of a single fat-rich meal results in acute GI MT [178-179, 187].

The ingestion of CHO pre-, during and post-exercise in athletic populations is widely recommended to improve exercise performance [188], accelerate recovery [189] and maintain immune function [190]. In comparison, the influence of CHO on GI barrier integrity has received less attention, despite being associated with the onset of GI complaints [191] and increased splanchnic perfusion [192]. Contrary to proposed hypotheses, preliminary research found no influence of CHO beverage ingestion (30-60 g·hour^{-1} glucose), compared with water, on GI barrier integrity (utilising the DSAT) during 60-90 minutes of moderate intensity exercise (70% VO_{2max}) [111, 122]. However, follow-up studies reported attenuated GI barrier integrity loss (I-FABP and DSAT) with glucose ingestion (60 g·hour^{-1}) during two-hours moderate intensity running (60% VO_{2max}) in the heat (35°C and 25% relative humidity (RH); [193]), and with sucrose ingestion (40 g·hour^{-1}) prior/during a one-hour moderate intensity cycle (70% $watt_{max}$) [131]. However, neither intervention ameliorated the severity of GI MT. Formulations of single- and multi-transportable CHO mixtures (i.e. 1.8 g·min^{-1} glucose; 1.2 and 0.6 g·min^{-1} glucose plus fructose; 0.6 and 1.2 g·min^{-1} glucose plus sucrose) all tended to (interaction effect p = 0.10) reduce I-FABP concentrations (area under the curve at 30 minute intervals) to a similar extent relative to water during three hours of low-intensity cycling (50% $Watt_{max}$) [130]. Similarly, ingestion of 60 g·hour^{-1} of either potato flesh puree or carbohydrate gel (2:1 maltodextrin/fructose) were able to completely attenuate the rise in I-FABP observed throughout a 2.5 hour mixed-intensity cycle (2 hours 60% VO_{2max} then a 20 km time trial in temperate conditions) [181]. To date, only one study has reported an adverse effect of CHO ingestion during exercise (1 hour 70% VO_{2max} running in 35°C and 12-20% RH) on GI barrier integrity, with ingestion of a multi-transportable CHO gel (18 g maltodextrin and 9 g fructose) 20-minutes into exercise shown to increase GI barrier integrity (I-FABP and endotoxin) loss relative to a placebo [194]. Surprisingly, in the placebo condition exertional-heat stress had no influence on GI barrier integrity, whilst in the CHO condition the magnitude of GI integrity loss was minimal. Currently little is known about the influence of pre-exercise CHO availability on GI barrier integrity. One study reported that 48-hour low (20% CHO, 65% fat) versus high (60% CHO, 25% fat) CHO-diet had no influence on GI MT after a laboratory duathlon [195];

whilst a similar study reported no influence of a 24 hour low or high FODMAP diet on GI barrier integrity (I-FABP, LBP, sCD14-ST) following 2 hours of exertional-heat stress [147].

Practical recommendations for CHO ingestion on GI barrier integrity are unable to be established at present, given the large variation in findings from seemingly comparable studies. This lack of consistency cannot be attributed to differences in prandial state, exercise intensity, CHO type/dose or participant demographic. In general, the application of traditional sports nutrition guidelines for CHO ingestion do not appear to adversely influence GI barrier integrity, and more likely would appear to offer favourable benefits. Future work is required to determine the most effective CHO formulations for fueling exercise and maintraining GI barrier integrity. Factors that may be important include: the carbohydrate source (e.g. potato, maize), dextrose equivalence, osmolarity, sugar profile and delivery format (e.g. drink, gel, energy chew, or bar). The impact of pre-exercise CHO status (e.g. low carbohydrate training, or fasted training) may also influence the GI barrier response to feeding. The strategy of gut-training (i.e. multiple exercise sessions with high [90 $g \cdot hour^{-1}$] CHO intake) to improve CHO tolerance during exercise does not appear to strengthen the GI barrier [191].

Glutamine

Glutamine is the most abundant amino acid in human tissue and plasma, where it performs numerous important regulatory functions. It is a *conditionally essential* nutrient during states of catabolic stress (e.g. starvation, trauma and severe infection), and is the major energy substrate of GI enterocytes. The use of $_{L}$-glutamine supplementation to support GI barrier function has received extensive examination [196]. Benefits have repeatedly been shown in humans following large intravenous $_{L}$-glutamine infusions (~0.2-0.5 $g \cdot kg \cdot day^{-1}$) in patients with critical illness indicative of glutamine deficiency, including severe burns [197-198], post-infectious irritable bowel syndrome [199], and major abdominal trauma [200]. In comparison, benefits are less prominent with low dose oral ingestion (<0.2 $g \cdot kg \cdot day^{-1}$) in chronic GI diseases patients, whom are unlikely to be glutamine deficient and/or exposed to acute stress [201-202]. Mechanisms of action appear multifactorial including: increased epithelial cell proliferation [203]; upregulation of cytoprotective intracellular heat shock protein (I-HSP) expression [204]; modulation of inflammatory signalling pathways [205];

663 increased vasodilating factors (e.g. nitric oxide); GI microbiota regulation [206]; enhanced GI
664 glutathione status [207]; and improvement in TJ stability through increased expression of
665 multiple TJ proteins [208-209].

666 Supplementation with L-glutamine is not presently endorsed by sports nutrition
667 guidelines, on the basis of weak evidence demonstrating improved immune function [190] or
668 exercise-performance [210]. Early research investigating the effect of L-glutamine
669 supplementation on exercise-induced GI permeability (assessed with DSAT), found no
670 additional benefit of co-administering L-glutamine (0.018 g·kg^{-1} BM) with CHO (0.18 g·kg^{-1}BM)
671 every 10 minutes during a one-hour moderate-intensity run (70% VO_{2max}), in comparison to
672 CHO alone [122]. Unfortunately, L-Glutamine was not assessed in isolation and the total dose
673 consumed was only *circa* 8-12 g. Since then, researchers have changed their focus from low
674 dose L-glutamine supplementation to maintain circulating concentrations, to provision of large
675 oral doses to saturate the GI tissue prior to exercise. Both chronic (3x 0.3 g·kg·FFM^{-1} for seven
676 days; [211]) and acute (0.9 g·kg·FFM^{-1} two-hours pre-exercise [116]) L-glutamine ingestion
677 raised circulating concentrations by ~2.5-fold (suggestive of GI saturation) and attenuated the
678 rise in the GI permeability (DSAT ratio) from basal conditions following a one-hour moderate-
679 intensity run (70% VO_{2max}) in the heat (30°C/12-20% RH). Using an identical experimental-
680 design, it was subsequently shown that L-glutamine doses of 0.25, 0.5 and 0.9 g·kg·FFM^{-1}
681 supressed the post exertional-heat stress rise in serum I-FABP concentration (~0-20%) and
682 DSAT ratio (~25-40%). Although the authors reported a dose-dependent effect on GI barrier
683 integrity [212], statistical significance testing was not undertaken, with these conclusions
684 drawn from magnitude based inference analysis. Recently, ingestion of 0.9 g·kg·FFM^{-1} of L-
685 glutamine one hour prior to a 20 km cycling time trial in the heat (35°C, 50% RH) blunted the
686 rise in circulating post-exercise I-FABP, although this studies conclusions were drawn from a
687 linear mixed methods Bayesian statistical approach [213].

688 Practical recommendations support the use of a single L-glutamine dose (0.90
689 g·kg·FFM^{-1}) two-hours pre-exercise to protect GI barrier integrity. Given the requirement to
690 only ingest a single acute-dose in the hours prior to exertional-heat stress, the
691 supplementation protocol has clear real-world application in terms of both implementation
692 logistics and expense. Further work is required to confirm these findings following more

severe exertional-heat stress protocols and extending analysis to include secondary markers of GI MT. The oral tolerance and safety of such large L-glutamine doses requires clinical assessment as it is above general guidelines (5-10 g) for sports supplements [214]. Likewise, a limitation of all previous research has been the performance of trials in the fasted state, whereby positive findings are potentially attributable to improvement in post-prandial splanchnic perfusion, rather than any benefits directly related to L-glutamine. Indeed, ingesting 15 g·20 min^{-1} of whey protein hydrolysate during a 2-hour moderate-intensity (60% VO_{2max}) run in the heat (35°C/30% RH) has also been shown to be highly effective in maintaining GI barrier integrity [193]. Future research should focus on determining if specific amino acid mixtures are as effective, or can even outperform L-glutamine alone, for maintaining GI barrier integrity.

Bovine Colostrum

Bovine colostrum (BC) is the milk produced by cows during the first 24-48 hours post-partum, and its composition markedly differs from milk produced later in lactation [215]. In humans, colostrum provides many health benefits to the neonate, including rapid tissue development and immune defence [216]. BC contains a variety of growth factors (e.g. insulin-like growth factor-1; IGF-1) and immunomodulatory components (e.g. immunoglobulins, cytokines) at higher concentrations than human colostrum [217]. The use of a BC nutritional supplement (liquid and powder) to maintain GI barrier function in healthy adults has been shown to reduce GI permeability post NSAID administration [218], and can blunt systemic elevations in endotoxin following critical illness [219]. These findings are supported by *in vitro* studies on Caco-2 cells, where BC blunted GI cell apoptosis and increased epithelial resistance during heat exposure [113, 220]. Mechanisms of action include: increased epithelial cell proliferation [113, 221], upregulation of cytoprotective I-HSP expression [114] and improved TJ stability through a reduction in phosphorylated tyrosine concentrations of occludin and claudin-1 [114].

Supplementation with BC has increased in athletic populations in response to recent evidence of enhanced muscle growth rates [222], blunted exercise-associated immunosuppression [223] and improved exercise performance [224]. More recent investigations have assessed the influence of BC on exercise-induced GI damage. In a series

of experiments, 14 days of BC (20 g·day^{-1}) halved the 3-fold rise in urinary DSAT ratio and circulating I-FABP concentrations following short-duration (20 minutes) high-intensity running (80% VO_{2max}) [113, 114, 225]. Whilst these results show promise, such benefits appear attenuated by more demanding exercise protocols. Two comparable studies reported no effect of either a moderate (14 days at 20 g·day^{-1}; [226] or high (7 days at 1.7 g·kg·day^{-1} (circa ~120-150g); [152]) BC dosing on I-FABP concentrations following a fatiguing run in the heat (35-40°C; 50% RH). Likewise, March et al. [105], using their earlier BC supplementation protocol [225], found only minor (~10%) suppression of I-FABP concentration and a non-significant blunting of circulating bacteroides DNA following a 1-hour run (70% VO_{2max}) in the heat (30°C/60% RH).

Practical recommendations support a BC dose of 20 g.day^{-1} for 14 days to protect the GI tract during moderately demanding exercise, though little-to-no benefits appear likely during more intense exercise. Two days of BC supplementation with the same daily dose offered no protective benefits [144]. Chronic low dose (500 mg·day^{-1}) BC ingestion improved resting GI permeability (DSAT ratio) in athletes during heavy training [227], but chronic high dose (60 g·day^{-1}) BC ingestion appeared to increase GI permeability [228]. Further work is required to determine the optimal time-course and BC dose to support GI barrier function. As there are large inter-manufacturer variations in BC formulations, future research should include accurate characterisation of the bioactive components in intervention trials, as these components are likely to have a significant bearing on study findings [229]. No studies have successfully measured the influence of BC on secondary GI MT post-exercise. BC appears to be well-tolerated in healthy individuals in doses up to 60 g·day^{-1} over several weeks, and although IGF-1 is on the World Anti-Doping Agency banned substance list, it is unlikely BC can result a positive doping control [230].

Nitric Oxide

The free radicle gas, Nitric Oxide (NO), performs multiple signalling roles in the body. Synthesis occurs through two complementary pathways: the NO synthase (NOS) dependant $_{L}$-arginine pathway; and the NOS independent nitrate (NO3), nitrite (NO2), NO serial reduction pathway [231]. Supplementation with NO precursors, including $_{L}$-arginine [232], $_{L}$-citrulline and inorganic NO3 [233], are all capable of upregulating NO bioavailability across the

splanchnic organs. Rodent models show this increase in NO blunts GI histopathological damage and subsequent MT following NSAID ingestion [234], small bowel obstruction [235] and experimentally induced ischemic-reperfusion injury [236-237]. The vasodilatory role of NO in maintaining GI microcirculation appears to be one of the main mechanisms [82], with enhanced antioxidant scavenging [238], constrained neutrophil activation [239] and increased GI TJ protein expression [240] as complementary pathways.

No guidelines exist for L-arginine or L-citrulline supplementation in athletic populations [241], and consensus documents do not support its use to improve oxygen uptake kinetics or exercise performance [242]. Only two studies have investigated the influence of nitric oxide precursurs on exercise-induced GI barrier integrity loss. A rodent study found addition of 2% L-arginine to the standard diet (over seven days) prevented a rise in GI barrier loss relative to the control following ~1-hour forced running to fatigue in the heat (34°C) [243]. Similarly in humans, Van Wijck et al. [127] found acute L-citrulline supplementation (10g given 30 minutes pre-exercise) successfully maintained splanchnic perfusion and blunted the rise in systemic I-FABP during one hour of moderate intensity cycling (70% $watt_{max}$). However, this intervention did not reduce peak post-exercise I-FABP concentrations, or the urinary DSAT ratio.

Inorganic NO3 supplementation has increased in athletic populations over the last decade [241]. Its popularity is founded upon evidence showing NO3 supplementation (~ 8 mmol, acutely and chronically) reduces the oxygen cost of exercise, enhances muscle efficiency and improves prolonged aerobic performance (10-40 minutes) [244]. There is limited evidence addressing NO3 supplementation and exercise-induced GI barrier integrity loss. One placebo controlled study found acute sodium NO3 (800 mg given 2.5 hours pre-exercise), did not attenuate the rise in ciculating I-FABP or LBP concentration concentration following 1-hour of moderate intensity cycling (70% $watt_{max}$) [131].

Practical recommendations regarding the use of L-arginine, L-citrulline or inorganic NO3 to protect the GI tract during exercise are inconclusive. Further work is required to substantiate present findings and to verify any benefits over a range of exercise protocols. Likewise, evidence is required to confirm whether benefits are observed in highly-trained populations (who tend not to respond to NO supplementation), and to determine which NO

precursors provide the most effective GI protection. A further practical consideration is the apparent impaired thermoregulation associated with reduced cutaneous vasodilation, which might disrupt the GI barrier especially when exercising in the heat [245-246].

Probiotics

Probiotics are live microorganisms considered to regulate the GI microbiota, which might confer health benefits when consumed in adequate quantities [247]. They are found in low concentrations across various food sources (e.g. non-pasteurised dairy products), and regular consumption has been recommended in patients with GI conditions since the early 1900s [247]. More recently, probiotic supplementation to support GI barrier function has received extensive examination. Whilst positive barrier effects are reported in ~50% of human studies, these are not universal, and may refect the large variations in dose and strains administered [248-249]. Inconclusive effects are also reported *in vitro* on GI cellular apoptosis and epithelial integrity when Caco-2 cells are cultured with probiotics prior to insult [250-251]. Mechanisms of action are incompletely understood, but are believed to include: inhibition of pathogenic bacterial overgrowth; competition with pathogenic bacteria for binding sites on mucins and/or epithelial cells; increased mucosal immunoglobulin and antimicrobial proteins secretion; increased epithelial cell proliferation; upregulated I-HSP concentrations; suppressed local GI inflammation; and increased TJ stability through upregulation of GI TJ protein expression (for review see: [252]).

Probiotic supplementation is increasingly popular in athletic populations, despite inconsistent effects of their use for either maintaining immune health or improving exercise performance [253]. With respect to GI barrier integrity, four weeks daily consumption of a multi-strain probiotic (45×10^9 colony forming units [CFU]; from three strains) blunted DSAT ratios (8%) and circulating endotoxin concentrations (~12%) following a ~35-minute fatiguing run (80% ventilatory threshold) in the heat (35°C/40% RH) [261]. A follow-up study reported daily ingestion of a similar multi-strain probiotic (3×10^9 CFU; from nine strains) for a period of twelve weeks approximately halved basal endotoxin concentrations immediately prior to and 6-days following an ultra-triathlon [254]. In contrast, seven days high-dose single strain probiotic supplementation (45×10^{11} CFU.day^{-1} *Lactobacillus Casei*) was associated with an increased rise in endotoxin concentrations, compared with placebo,

following two hours moderate-intensity running (60% VO_{2max}) in the heat (34°C/32% RH) [149]. Similarly, the daily ingestion of another single strain probiotic (35 x 10^9 CFU *Bifidobacterium longum*) had no effect on resting endotoxin concentrations following six weeks of pre-season training in collegiate swimmers [255]. Likewise, four weeks daily supplementation with a multi-strain probiotic (25 x 10^9 CFU; from five strains) had no influence on either DSAT, I-FABP or sCD14 responses following a simulated 42.2 km marathon in temperate conditions [148]. Finally, four weeks supplementation with a single strain probiotic (2 x 10^8 CFU *Lactobacillus Salivarius*) had no influence on DSAT responses, (or faecal microbial composition), following two hours of moderate intensity running (60% VO_{2max}) in temperate conditions [256]. It is unlikely the final two studies were sufficiently powered to detect any influence of probiotic supplementation of GI barrier integrity.

The present data indicate that probiotic supplementation has little for supporting GI barrier integrity in response to exericse. It is not possible to elucidate whether incosistant responses are attributable to the specific probiotic strain, duration of supplementation or another factor. Future research is required to develop probiotic supplementation regimes and will need to address factors such as strain(s), timing and dose. It will alos be necessary to verify potential efficacy using relevant exercise (heat stress) protocols. Global metabolomics approaches have linked exercise-induced GI barrier function loss with alterations in GI microbiota composition during a four-day military arctic training exercise (51 km ski march; [257]), and such methodologies should be applied when developing probiotic supplements to support GI barrier integrity. Probiotic use is considered safe in healthy populations, when consumed acutely and chronically [253].

Polyphenols

Polyphenols are natural compounds that defend plants against damage from radiation and pathogens. Over 8000 polyphenols have been identified, which are classified into four major groups: flavonoids; phenolic acids; stilbenes; and lignans. Quercetin is the most abundant dietary flavonoid polyphenol [258], and in rodents' supplementation has been shown to maintain GI barrier integrity [259]. However, *in vitro* evidence from human Caco-2 cells is less conclusive, with quercetin shown to both improve [260-261] and impair [262-263] GI barrier integrity in response to heat stress. Proposed mechanisms in favourable studies

include modulation of vasodilatory factors (e.g. NO [263]), elevated antioxidant scavenging [265] and improved TJ stability through upregulation of several TJ proteins [266]. Proposed mechanisms in non-favourable studies relate to reduced cytoprotective I-HSP expression [267] and TJ stability through disruption in occludin TJ protein localisation [262]. Both positive and negative responses have been comparatively reported when Caco-2 cells are supplemented *in vitro* with additional polyphenols [264, 266]. Human studies assessing polyphenol supplementation efficacy on GI barrier integrity are lacking [264], and where *in vitro* studies administerphysiologically relevant polyphenol doses the effects have been negligible [268].

Polyphenol supplementation is increasingly popular in athletic populations [269]. This is founded upon moderate evidence of enhanced skeletal muscle recovery from micro-damage [270], blunted exercise-associated immunosuppression [271] and in some cases improved (1-3%) endurance exercise performance [272]. With respect to polyphenol supplementation and exercise-induced GI barrier integrity, the effect of daily quercetin supplementation (2 $g \cdot day^{-1}$ one hour pre-exercise) on GI permeability following the first and seventh days of a standardised isothermic walking (100 minutes; 1.8 $m \cdot s^{-1}$ in 46°C/20% RH) heat acclimation regime was assessed [143]. On both days, quercetin ingestion stimulated a ~two-fold rise in urinary lactulose and plasma endotoxin compared with a placebo condition. More promisingly, supplementation with curcumin (3 days of 0.5 $g \cdot day^{-1}$), a constituent of turmeric, blunted circulating I-FABP concentrations by ~30% after one-hour moderate intensity running (65% VO_{2max}) in the heat (37°C/25% RH; [273]).

There are no practical recommendations supporting polyphenol use to protect the GI tract during strenuous exercise. Despite promising *in vitro* observations, more work is required to determine the optimal formulation, time-course and polyphenol dose to support GI barrier function across different exercise-modalities. No studies have successfully measured the effect of polyphenols on secondary GI MT post-exercise and clearly future studies should attempt to control for dietary polyphenol intake.

Zinc-Carnosine

Zinc-Carnosine (ZnC) is a pharmaceutical chelate of zinc and L-carnosine [274]. It is widely used in Japan to treat gastric ulcers [275], and more recently has been marketed in Europe to support GI health [276]. Zinc is an essential trace element and a co-factor in numerous tissue regenerative and immunomodulatory enzymatic reactions [277], whilst L-carnosine is a cytoplasmic dipeptide of beta-alanine and L-histidine [278]. Daily ZnC ingestion improves GI barrier integrity in healthy humans following chronic GI barrier damaging NSAID ingestion [276, 279]. These protective benefits are reported to be synergistic compared with consuming either ingredient individually [280]. *In vitro* studies of rat intestinal and human Caco-2 cells support these reports, where ZnC blunts GI cellular apoptosis [281-282] and increases epithelial electrical resistance [114] upon damage, in a dose-dependent fashion. Mechanisms of action appear multifactorial, including increased: epithelial cell proliferation [276]; I-HSP concentrations [114]; antioxidant activity [283]; and stability of TJs through blunting phosphorylated occludin and claudin-1 expression [114].

No guidelines exist concerning ZnC supplementation in athletic populations. Athletes are recommended to ensure sufficient dietary zinc ingestion (EU RDA = 10 mg·day^{-1}) to prevent deficiencies, and to supplement with large oral doses (~75 mg·day^{-1}), when suffering from acute upper respiratory tract infection to accelerate recovery [190]. Though L-Carnosine supplementation is uncommon, supplementing β-alanine (~65 mg·kg·day^{-1}) the rate-limiting precursor for muscle L-carnosine synthesis, has been shown to increase muscle carnosine stores [283]. To date, only one study has investigated the influence of ZnC on exercise-induced GI damage. Fourteen days of ZnC (75 mg·day^{-1}) attenuated a 3-fold rise is DSAT ratio by 70% after short-duration (20 minutes) high-intensity running (80% VO_{2max}) [114]. This effect was comparable to that observed with BC (20 g·day^{-1} for 14 days) in the same study, and when the two-treatments were combined the benefits appeared synergistic (85% reduction DSAT ratio). Furthermore, the combination of ZnC and BC blunted the exercise-induced increase in DSAT ratio by 30% after only two-days, whilst no protection was offered by either ingredient alone at this point [114].

Practical recommendations support ZnC use at a dose of 75 mg·day^{-1} for 14 days to protect the GI tract during moderately demanding exercise. Further work is needed to substantiate existing findings and verify the potential benefits of ZnC during more strenuous

exercise. No studies have successfully measured the influence of ZnC on secondary GI MT post-exercise. Research is required to determine the optimal time-course and dose of ZnC to support GI barrier function with chronic and acute supplementation. Larger doses of ZnC (150 mg·day^{-1}) appear well-tolerated in GI disease patients in the short-term [285], and dose-dependent *in vitro* evidence suggests this might offer greater protection [280]. Co-ingestion of copper with zinc (1:10 ratio or 2 mg·day^{-1}) appears to prevent zinc inhibiting copper absorption [190].

Limitations and Future Directions

Investigation of nutritional countermeasures that support GI barrier integrity during strenuous exercise is an important and expanding area of research. Preliminary observations indicate some diet regimens and dietary supplements could benefit exercising populations. Optimal supplementation strategies should be safe, well-tolerated, practical (e.g. affordable/low mass), fast acting and effective in a wide range of scenarios (e.g. exercise intensity/duration, population). It is also important that they are without secondary adverse responses, especially thouse relating to skeletal muscle adaptation, thermoregulation, immune function, bone health etc. Whilst there are numerous examples of well-conducted studies reporting beneficial effects from diet regimens and individual supplements on GI barrier integrity, it is currently not possible to provide definitive guidance. In part this is due to limitations and variations in study designs and in some instance's incomplete characterisation of the bioactive nutrients.

Future research should address diet regimens/nutritional supplements that satisfy the above requirements when tested in the most demanding scenarios (e.g. high intensity/prolonged exertional-heat stress). It would appear very worthwhile to assess the synergy between ingredients that maintain GI integrity, especially if they are though to act via different biochemical pathways. Further supplements that warrant future exploration include: omega-3 polyunsaturated fatty acids [286]; vitamin C [287]; vitamin E [287]; vitamin D [288] and prebiotics [289]. Research should target specific populations (e.g. gender, training status, heat-acclimated, GI disease), exercise modalities (especially prolonged duration), supplementation timings (e.g. repeat dosing, delayed/post-exercise ingestion) and monitor the continued efficacy of supplementation following chronic application. Of note, future

research is warranted to determine the most damaging exercise protocol on GI barrier, which possibly involves a combination of prolonged/intense exercise performed in the heat.

From a methodological perspective, it is recommended that future studies assess a battery of relevant GI barrier integrity markers (e.g. DSAT, plus I-FABP/I-BABP/claudin-3, plus endotoxin/LBP/sCD14/bactDNA) and monitor alterations in the proposed mechanistic pathways (e.g. splanchnic perfusion, I-HSPs) underpinning any functional benefits. Key extraneous variables should be controlled, including: prandial state [180]; hydration status [135]; beverage temperature [137]; prior NSAID ingestion [121]; habitual diet and supplement use.

Conclusions

EHS is a life-threatening disease involving thermoregulatory failure, which sporadically arises in otherwise healthy individuals following performance of strenuous exercise or occupationally arduous tasks. Current EHS management policy primarily takes a thermoregulatory management approach despite evidence of MT following loss of GI barrier integrity being an important process in the disease pathophysiology. A range of techniques are available to assess GI barrier integrity *in vivo*, and a battery approach monitoring multiple measures in both field and research settings is recommended. The severity of GI barrier integrity loss following exertional-heat stress appears to be intensity and duration-dependant, with thermoregulatory strain being an additional risk factor. Considerations for the specific GI barrier integrity assessment technique must be made when interpreting individual studies conclusions, whereby I-FABP responses typically provided the greatest sensitivity. The specific aetiology of exertional-heat stress induced GI barrier integrity loss is poorly defined, but likely relates to the direct effects of localised hyperthermia, ischemia-reperfusion injury and neuroendocrine-immune alterations.

A range of nutritional countermeasures have been shown to positively affect GI barrier integrity following strenuous exercise and exercise-heat stress. However, despite rapid advancements in this field, definitive recommendations cannot be provided due to the heterogeneity of experimental designs. Nevertheless, promising effects have been associated with following general sports nutrition CHO supplementation guidelines during

exercise (30-100 $g \cdot h^{-1}$ liquid multi-transportable CHO), and acute $_{L}$-glutamine ingestion two hours pre-exercise (0.25-0.9 $g \cdot kg \cdot FFM^{-1}$). Benefits from BC, and probiotics likely relate to the specific supplement formulation, and hence require further investigation. Despite a sound rationale for the use of NO precursors and polyphenols to limit exercise-induced GI barrier integrity loss, substantive supporting evidence is currently absent. ZnC requires further verification, where short-term (1-3 days) high-dose supplementation appears an attractive consideration. Further well-controlled research in nascent areas could elucidate potential treatment options for exercise-induced GI barrier integrity loss.

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Not Applicable

Consent for Publication

Not Applicable

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Not Applicable

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The authors declare that they have no competing interests

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REFERENCES

1. Leon, L.R. and Helwig, B.G., 2010. Heat stroke: role of the systemic inflammatory response. *Journal of applied physiology*, *109*(6), pp.1980-1988.

2. Casa, D.J., Armstrong, L.E., Carter, R., Lopez, R., Mcdermott, B. and Scriber, K., 2010. Historical perspectives on medical care for heat stroke, part 1: ancient times through the nineteenth century: a review of the literature. *Athletic training and sports health care*, *2*(3), pp.132-138.

3. Casa, D.J., Armstrong, L.E., Carter, R., Lopez, R., Mcdermott, B. and Scriber, K., 2010. Historical perspectives on medical care for heat stroke, part 2: 1850 through the present: a review of the literature. *Athletic Training and Sports Health Care*, *2*(4), pp.178-190.

4. Laitano, O., Leon, L.R., Roberts, W.O. and Sawka, M.N., 2019. Controversies in exertional heat stroke diagnosis, prevention, and treatment. *Journal of Applied Physiology*, *127*(5), pp.1338-1348.

5. Bouchama, A. and Knochel, J.P., 2002. Heat stroke. *New England journal of medicine*, *346*(25), pp.1978-1988.

6. Kravchenko, J., Abernethy, A.P., Fawzy, M. and Lyerly, H.K., 2013. Minimization of heatwave morbidity and mortality. *American journal of preventive medicine*, *44*(3), pp.274-282.

7. Epstein, Y. and Yanovich, R., 2019. Heatstroke. *New England Journal of Medicine*, *380*(25), pp.2449-2459.

8. Cramer, M.N. and Jay, O., 2016. Biophysical aspects of human thermoregulation during heat stress. *Autonomic Neuroscience*, *196*, pp.3-13.

9. DeGroot, D.W., Mok, G. and Hathaway, N.E., 2017. International Classification of Disease coding of exertional heat illness in US Army Soldiers. *Military medicine*, *182*(9-10), pp.e1946-e1950.

10. Stacey, M.J., Parsons, I.T., Woods, D.R., Taylor, P.N., Ross, D. and Brett, S.J., 2015. Susceptibility to exertional heat illness and hospitalisation risk in UK military personnel. *BMJ open sport & exercise medicine*, *1*(1), p.e000055.

11. Kerr, Z.Y., Casa, D.J., Marshall, S.W. and Comstock, R.D., 2013. Epidemiology of exertional heat illness among US high school athletes. *American journal of preventive medicine*, *44*(1), pp.8-14.

12. Military Health System. Available online: https://health.mil/News/Articles/2019/04/01/Update-Heat-Illness (Accessed on: 20/01/2020)

13. Yeargin, S.W., Kerr, Z.Y., Casa, D.J., Djoko, A., Hayden, R., Parsons, J.T. and Dompier, T.P., 2016. Epidemiology of Exertional Heat Illnesses in Youth, High School, and College Football. *Medicine and science in sports and exercise*, *48*(8), pp.1523-1529.

14. Yeargin, S.W., Dompier, T.P., Casa, D.J., Hirschhorn, R.M. and Kerr, Z.Y., 2019. Epidemiology of Exertional Heat Illnesses in National Collegiate Athletic Association Athletes During the 2009–2010 Through 2014–2015 Academic Years. *Journal of athletic training*, *54*(1), pp.55-63.

15. DeMartini, J.K., Casa, D.J., Belval, L.N., Crago, A., Davis, R.J., Jardine, J.J. and Stearns, R.L., 2014. Environmental conditions and the occurrence of exertional heat illnesses and exertional heat stroke at the Falmouth Road Race. *Journal of athletic training*, *49*(4), pp.478-485.

16. Divine, J.G., Daggy, M.W., Dixon, E.E., LeBlanc, D.P., Okragly, R.A. and Hasselfeld, K.A., 2018. Case Series of Exertional Heat Stroke in Runners During Early Spring: 2014 to 2016 Cincinnati Flying Pig Marathon. *Current sports medicine reports*, *17*(5), pp.151-158.

17. Hosokawa, Y., Adams, W.M., Belval, L.N., Davis, R.J., Huggins, R.A., Jardine, J.F., Katch, R.K., Stearns, R.L. and Casa, D.J., 2018. Exertional heat illness incidence and on-site medical team preparedness in warm weather. *International journal of biometeorology*, *62*(7), pp.1147-1153.

18. Stacey, M.J., Brett, S., Woods, D., Jackson, S. and Ross, D., 2016. Case ascertainment of heat illness in the British Army: evidence of under-reporting from analysis of Medical and Command notifications, 2009–2013. *Journal of the Royal Army Medical Corps*, *162*(6), pp.428-433.

19. Nelson, D.A., Deuster, P.A., O'Connor, F.G. and Kurina, L.M., 2018. Timing and predictors of mild and severe heat illness among new military enlistees. *Medicine and science in sports and exercise*, *50*(8), p.1603.

20. Lucas, R.A., Epstein, Y. and Kjellstrom, T., 2014. Excessive occupational heat exposure: a significant ergonomic challenge and health risk for current and future workers. *Extreme physiology and medicine*, *3*, pp.14-14.

21. Belval, L.N., Casa, D.J., Adams, W.M., Chiampas, G.T., Holschen, J.C., Hosokawa, Y., Jardine, J., Kane, S.F., Labotz, M., Lemieux, R.S. and McClaine, K.B., 2018. Consensus statement-prehospital care of exertional heat stroke. *Prehospital Emergency Care*, *22*(3), pp.392-397.

22. Hosokawa, Y., Stearns, R.L. and Casa, D.J., 2019. Is Heat Intolerance State or Trait?. *Sports Medicine*, *49*(3), pp.365-370.

23. Royburt, M., Epstein, Y., Solomon, Z. and Shemer, J., 1993. Long-term psychological and physiological effects of heat stroke. *Physiology & behavior*, *54*(2), pp.265-267.

24. Wang, J.C., Chien, W.C., Chu, P., Chung, C.H., Lin, C.Y. and Tsai, S.H., 2019. The association between heat stroke and subsequent cardiovascular diseases. *PloS one*, *14*(2), p.e0211386.

25. Wallace, R.F., Kriebel, D., Punnett, L., Wegman, D.H. and Amoroso, P.J., 2007. Prior heat illness hospitalization and risk of early death. *Environmental research*, *104*(2), pp.290-295.

1070 26. Epstein, Y., Druyan, A. and Heled, Y., 2012. Heat injury prevention—a military
1071 perspective. *The Journal of Strength & Conditioning Research*, *26*, pp.S82-S86.

1072 27. Mitchell, K.M., Cheuvront, S.N., King, M.A., Mayer, T.A., Leon, L.R. and Kenefick, R.W.,
1073 2019. Use of the heat tolerance test to assess recovery from exertional heat
1074 stroke. *Temperature*, *6*(2), pp.106-119.

1075 28. Bonauto, D., Anderson, R., Rauser, E. and Burke, B., 2007. Occupational heat illness in
1076 Washington State, 1995–2005. *American journal of industrial medicine*, *50*(12), pp.940-950.

1077 29. DeGroot, D.W., Kenefick, R.W. and Sawka, M.N., 2015. Impact of arm immersion cooling
1078 during ranger training on exertional heat illness and treatment costs. *Military*
1079 *medicine*, *180*(11), pp.1178-1183.

1080 30. Porter, A.M., 2000. The death of a British officer-cadet from heat illness. *The*
1081 *Lancet*, *355*(9203), pp.569-571.

1082 31. Stacey, M., Woods, D., Ross, D. and Wilson, D., 2014. Heat illness in military populations:
1083 asking the right questions for research. *Journal of the Royal Army Medical Corps*, *160*(2),
1084 pp.121-124.

1085 32. Armstrong, L.E., Casa, D.J., Millard-Stafford, M., Moran, D.S., Pyne, S.W. and Roberts,
1086 W.O., 2007. American College of Sports Medicine position stand. Exertional heat illness
1087 during training and competition. *Medicine and science in sports and exercise*, *39*(3), pp.556-
1088 572.

1089 33. Casa, D.J., DeMartini, J.K., Bergeron, M.F., Csillan, D., Eichner, E.R., Lopez, R.M., Ferrara,
1090 M.S., Miller, K.C., O'Connor, F., Sawka, M.N. and Yeargin, S.W., 2015. National Athletic
1091 Trainers' Association position statement: exertional heat illnesses. *Journal of athletic*
1092 *training*, *50*(9), pp.986-1000.

1093 34. Altman, J., Stern, E., Stern, M., Prine, B., Smith, K.B. and Smith, M.S., 2019. Current
1094 paradigms in the prehospital care of exertional heat illness: A review. *Current Orthopaedic*
1095 *Practice*.

1096 35. Ministry of Defence. Available online:
1097 https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file
1098 /793094/JSP_539_Part_2_V3.1__Updated_04-19_.pdf (Accessed 20/01/2020)

1099 36. Armstrong, L.E., Lee, E.C. and Armstrong, E.M., 2018. Interactions of Gut Microbiota,
1100 Endotoxemia, Immune Function, and Diet in Exertional Heatstroke. *Journal of Sports*
1101 *Medicine*, *2018*.

1102 37. Lim, C.L., 2018. Heat sepsis precedes heat toxicity in the pathophysiology of heat
1103 stroke—a new paradigm on an ancient disease. *Antioxidants*, *7*(11), p.149.

1104 38. Thursby, E. and Juge, N., 2017. Introduction to the human gut microbiota. *Biochemical*
1105 *Journal*, *474*(11), pp.1823-1836.

39. Sender, R., Fuchs, S. and Milo, R., 2016. Revised estimates for the number of human and bacteria cells in the body. *PLoS biology*, *14*(8), p.e1002533.

40. Wells, J.M., Brummer, R.J., Derrien, M., MacDonald, T.T., Troost, F., Cani, P.D., Theodorou, V., Dekker, J., Méheust, A., De Vos, W.M. and Mercenier, A., 2016. Homeostasis of the gut barrier and potential biomarkers. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *312*(3), pp.G171-G193.

41. Moseley, P.L. and Gisolfi, C.V., 1993. New frontiers in thermoregulation and exercise. *Sports medicine*, *16*(3), pp.163-167.

42. Munford, R.S., 2005. Invited review: detoxifying endotoxin: time, place and person. *Journal of endotoxin research*, *11*(2), pp.69-84.

43. Deitch, E.A., 2012. Gut-origin sepsis: evolution of a concept. *The Surgeon*, *10*(6), pp.350-356.

44. Gathiram, P., Gaffin, S.L., Brock-Utne, J.G. and Wells, M.T., 1987. Time course of endotoxemia and cardiovascular changes in heat-stressed primates. *Aviation, space, and environmental medicine*, *58*(11), pp.1071-1074.

45. Nieman, D.C. and Nehlsen-Cannarella, S.L., 1991. The effects of acute and chronic exercise on immunoglobulins. *Sports Medicine*, *11*(3), pp.183-201.

46. Fukui, H., 2016. Endotoxin and other microbial translocation markers in the blood: A clue to understand leaky gut syndrome. *Cell Mol Med*, *2*, p.3.

47. Heled, Y., Fleischmann, C. and Epstein, Y., 2013. Cytokines and their role in hyperthermia and heat stroke. *Journal of basic and clinical physiology and pharmacology*, *24*(2), pp.85-96.

48. Lim, C.L. and Mackinnon, L.T., 2006. The roles of exercise-induced immune system disturbances in the pathology of heat stroke. *Sports Medicine*, *36*(1), pp.39-64.

49. Bynum, G., Brown, J., Dubose, D., Marsili, M., Leav, I., Pistole, T.G., Hamlet, M., LeMaire, M. and Caleb, B., 1979. Increased survival in experimental dog heatstroke after reduction of gut flora. *Aviation, space, and environmental medicine*, *50*(8), pp.816-819.

50. Gathiram, P., Wells, M.T., Brock-Utne, J.G., Wessels, B.C. and Gaffin, S.L., 1987. Prevention of endotoxaemia by non-absorbable antibiotics in heat stress. *Journal of clinical pathology*, *40*(11), pp.1364-1368.

51. Gathiram, P., Wells, M.T., Brock-Utne, J.G. and Gaffin, S.L., 1987. Antilipopolysaccharide improves survival in primates subjected to heat stroke. *Circulatory shock*, *23*(3), pp.157-164.

52. Gathiram, P., Wells, M.T., Brock-Utne, J.G. and Gaffin, S.L., 1988. Prophylactic corticosteroid increases survival in experimental heat stroke in primates. *Aviation, space, and environmental medicine*, *59*(4), pp.352-355.

53. Lim, C.L., Wilson, G., Brown, L., Coombes, J.S. and Mackinnon, L.T., 2007. Pre-existing inflammatory state compromises heat tolerance in rats exposed to heat stress. *American*

Journal of Physiology-Regulatory, Integrative and Comparative Physiology, *292*(1), pp.R186-R194.

54. Yang, T.H., Shih, M.F., Wen, Y.S., Ho, W.Y., Leu, K.L., Wang, M.Y. and Liu, C.C., 2010. Attenuation of circulatory shock and cerebral ischemia injury in heat stroke by combination treatment with dexamethasone and hydroxyethyl starch. *Experimental & translational stroke medicine*, *2*(1), p.19.

55. Liu, C.C., Shih, M.F., Wen, Y.S., Lai, Y.H. and Yang, T.H., 2014. Dexamethasone improves heat stroke-induced multiorgan dysfunction and damage in rats. *International journal of molecular sciences*, *15*(11), pp.21299-21313.

56. Audet, G.N., Dineen, S.M., Stewart, D.A., Plamper, M.L., Pathmasiri, W.W., McRitchie, S.L., Sumner, S.J. and Leon, L.R., 2017. Pretreatment with indomethacin results in increased heat stroke severity during recovery in a rodent model of heat stroke. *Journal of Applied Physiology*, *123*(3), pp.544-557.

57. Caputa, M., Dokladny, K. and Kurowicka, B., 2000. Endotoxaemia does not limit heat tolerance in rats: the role of plasma lipoproteins. *European journal of applied physiology*, *82*(1-2), pp.142-150.

58. Lin, X.J., Li, Y.J., Li, Z.L., Zou, F. and Lin, M.T., 2009. Pre-existing lipopolysaccharide may increase the risk of heatstroke in rats. *The American journal of the medical sciences*, *337*(4), pp.265-270.

59. King, M.A., Leon, L.R., Mustico, D.L., Haines, J.M. and Clanton, T.L., 2015. Biomarkers of multiorgan injury in a preclinical model of exertional heat stroke. *Journal of Applied Physiology*, *118*(10), pp.1207-1220.

60. Lin, Y. and Zhang, Y., 2019. Renoprotective effect of oral rehydration solution III in exertional heatstroke rats. *Renal failure*, *41*(1), pp.190-196.

61. King, M.A., Leon, L.R., Morse, D.A. and Clanton, T.L., 2016. Unique cytokine and chemokine responses to exertional heat stroke in mice. *Journal of Applied Physiology*, *122*(2), pp.296-306.

62. Malamud, N., Haymaker, W. and Custer, R.P., 1946. Heat Stroke. A Clinico-Pathologic Study of 125 Fatal Cases. *Military surgeon*, *99*(5), pp.397-449.

63. Shibolet, S., Coll, R., Gilat, T. and Sohar, E., 1967. Heatstroke: its clinical picture and mechanism in 36 cases. *Quarterly Journal of Medicine*, *36*(144), pp.525-48.

64. Chao, T.C., Sinniah, R. and Pakiam, J.E., 1981. Acute heat stroke deaths. *Pathology*, *13*(1), pp.145-156.

65. Graber, C.D., Reinhold, R.B., Breman, J.G., Harley, R.A. and Hennigar, G.R., 1971. Fatal heat stroke: Circulating endotoxin and gram-negative sepsis as complications. *Jama*, *216*(7), pp.1195-1196.

66. Bouchama, A., Parhar, R.S., el-Yazigi, A., Sheth, K. and al-Sedairy, S., 1991. Endotoxemia and release of tumor necrosis factor and interleukin 1 alpha in acute heatstroke. *Journal of applied physiology (Bethesda, Md.: 1985)*, *70*(6), pp.2640-2644.

67. Bouchama, A., Al-Sedairy, S., Siddiqui, S., Shail, E. and Bezeig, M., 1993. Elevated pyrogenic cytokines in heatstroke. *Chest*, *104*(5), pp.1498-1502.

68. Lu, K.C., Wang, J.Y., Lin, S.H., Chu, P. and Lin, Y.F., 2004. Role of circulating cytokines and chemokines in exertional heatstroke. *Critical care medicine*, *32*(2), pp.399-403.

69. Hashim, I.A., Al-Zeer, A., Al-Shohaib, S., Al-Ahwal, M. and Shenkin, A., 1997. Cytokine changes in patients with heatstroke during pilgrimage to Makkah. *Mediators of inflammation*, *6*(2), pp.135-139.

70. Camus, G., Nys, M., Poortmans, J.R., Venneman, I., Monfils, T., Deby-Dupont, G., Juchmes-Ferir, A., Deby, C., Lamy, M. and Duchateau, J., 1998. Endotoxaemia, production of tumour necrosis factor α and polymorphonuclear neutrophil activation following strenuous exercise in humans. *European journal of applied physiology and occupational physiology*, *79*(1), pp.62-68.

71. Gill, S.K., Teixeira, A., Rama, L., Prestes, J., Rosado, F., Hankey, J., Scheer, V., Hemmings, K., Ansley-Robson, P. and Costa, R.J., 2015. Circulatory endotoxin concentration and cytokine profile in response to exertional-heat stress during a multi-stage ultra-marathon competition. *Exercise immunology review*, *21*, p.114.

72. Barberio, M.D., Elmer, D.J., Laird, R.H., Lee, K.A., Gladden, B. and Pascoe, D.D., 2015. Systemic LPS and inflammatory response during consecutive days of exercise in heat. *International journal of sports medicine*, *36*(03), pp.262-270.

73. Ng, Q.Y., Lee, K.W., Byrne, C., Ho, T.F. and Lim, C.L., 2008. Plasma Endotoxin and Immune Responses During a 21-km Road Race Under a Warm and Humid Environment. *Ann Acad Med Singapore*, *37*, pp.307-14.

74. Aibiki, M., Ohtsubo, S., Nishiyama, T., Maekawa, S., Oka, H., Dote, K. and Shirakawa, Y., 2005. Elevated serum beta-D-glucan level and depressed neutrophil phagocytosis in a heatstroke patient. *Resuscitation*, *65*(1), pp.115-117.

75. Wacker, C., Prkno, A., Brunkhorst, F.M. and Schlattmann, P., 2013. Procalcitonin as a diagnostic marker for sepsis: a systematic review and meta-analysis. *The Lancet infectious diseases*, *13*(5), pp.426-435.

76. Tong, H.S., Liu, Y.S., Wen, Q., Tang, Y.Q., Yuan, F.F. and Su, L., 2012. Serum procalcitonin predicting mortality in exertional heatstroke. *Emerg Med J*, *29*(2), pp.113-117.

77. Hausfater, P., Hurtado, M., Pease, S., Juillien, G., Lvovschi, V.E., Salehabadi, S., Lidove, O., Wolff, M., Bernard, M., Chollet-Martin, S. and Riou, B., 2008. Is procalcitonin a marker of critical illness in heatstroke?. *Intensive care medicine*, *34*(8), pp.1377-1383.

78. Yang, Q., Liu, W., Yu, J., Jiang, J., Xu, T. and Zhou, Y., 2019. Effect of prealbumin level on mortality in heatstroke patients. *Experimental and therapeutic medicine*, *17*(4), pp.3053-3060.

79. Galipeau, H.J. and Verdu, E.F., 2016. The complex task of measuring intestinal permeability in basic and clinical science. *Neurogastroenterology & Motility*, *28*(7), pp.957-965.

80. Costa, R.J.S., Snipe, R.M.J., Kitic, C.M. and Gibson, P.R., 2017. Systematic review: exercise-induced gastrointestinal syndrome—implications for health and intestinal disease. *Alimentary pharmacology & therapeutics*, *46*(3), pp.246-265.

81. Pires, W., Veneroso, C.E., Wanner, S.P., Pacheco, D.A.S., Vaz, G.C., Amorim, F.T., Tonoli, C., Soares, D.D. and Coimbra, C.C., 2017. Association Between Exercise-Induced Hyperthermia and Intestinal Permeability: A Systematic Review. *Sports medicine (Auckland, NZ)*, *47*(7), pp.1389-1403.

82. van Wijck, K., Lenaerts, K., Grootjans, J., Wijnands, K.A., Poeze, M., Van Loon, L.J., Dejong, C.H. and Buurman, W.A., 2012. Physiology and pathophysiology of splanchnic hypoperfusion and intestinal injury during exercise: strategies for evaluation and prevention. *American journal of physiology-gastrointestinal and liver physiology*, *303*(2), pp.G155-G168.

83. Menzies, I.S., Zuckerman, M.J., Nukajam, W.S., Somasundaram, S.G., Murphy, B., Jenkins, A.P., Crane, R.S. and Gregory, G.G., 1999. Geography of intestinal permeability and absorption. *Gut*, *44*(4), pp.483-489.

84. Bjarnason, I., Macpherson, A. and Hollander, D., 1995. Intestinal permeability: an overview. *Gastroenterology*, *108*(5), pp.1566-1581.

85. Fleming, S.C., Duncan, A., Russell, R.I. and Laker, M.F., 1996. Measurement of sugar probes in serum: an alternative to urine measurement in intestinal permeability testing. *Clinical chemistry*, *42*(3), pp.445-448.

86. van Wijck, K., Lenaerts, K., Van Loon, L.J., Peters, W.H., Buurman, W.A. and Dejong, C.H., 2011. Exercise-induced splanchnic hypoperfusion results in gut dysfunction in healthy men. *PloS one*, *6*(7), p.e22366.

87. van Wijck, K., van Eijk, H.M., Buurman, W.A., Dejong, C.H. and Lenaerts, K., 2011. Novel analytical approach to a multi-sugar whole gut permeability assay. *Journal of Chromatography B*, *879*(26), pp.2794-2801.

88. Pugh, J.N., Impey, S.G., Doran, D.A., Fleming, S.C., Morton, J.P. and Close, G.L., 2017. Acute high-intensity interval running increases markers of gastrointestinal damage and permeability but not gastrointestinal symptoms. *Applied Physiology, Nutrition, and Metabolism*, *42*(9), pp.941-947.

89. Ogden, H.B., Fallowfield, J.L., Child, R.B., Davison, G., Fleming, S.C., Edinburgh, R.M., Delves, S.K., Millyard, M., Westwood, C.S. and Layden, J.D., 2020. Reliability of

Gastrointestinal Barrier Integrity and Microbial Translocation Biomarkers at Rest and Following Exertional Heat Stress. *Physiological Reports*,

90. van Wijck, K., Verlinden, T.J., van Eijk, H.M., Dekker, J., Buurman, W.A., Dejong, C.H. and Lenaerts, K., 2013. Novel multi-sugar assay for site-specific gastrointestinal permeability analysis: a randomized controlled crossover trial. *Clinical nutrition*, *32*(2), pp.245-251

91. van Wijck, K., Bessems, B.A., van Eijk, H.M.H., Buurman, W.A., Dejong, C.H.C. and Lenaerts, K., 2012. Polyethylene glycol versus dual sugar assay for gastrointestinal permeability analysis: is it time to choose?. *Clinical and experimental gastroenterology*, *5*, pp.139-50.

92. Sun, D.L., Cen, Y.Y., Li, S.M., Li, W.M., Lu, Q.P. and Xu, P.Y., 2016. Accuracy of the serum intestinal fatty-acid-binding protein for diagnosis of acute intestinal ischemia: a meta-analysis. *Scientific reports*, *6*, pp.34371-34371.

93. van de Poll, M.C., Derikx, J.P., Buurman, W.A., Peters, W.H., Roelofs, H.M., Wigmore, S.J. and Dejong, C.H., 2007. Liver manipulation causes hepatocyte injury and precedes systemic inflammation in patients undergoing liver resection. *World journal of surgery*, *31*(10), pp.2033-2038.

94. Montagnana, M., Danese, E. and Lippi, G., 2018. Biochemical markers of acute intestinal ischemia: possibilities and limitations. *Annals of translational medicine*, *6*(17), p.341.

95. Sturgeon, C. and Fasano, A., 2016. Zonulin, a regulator of epithelial and endothelial barrier functions, and its involvement in chronic inflammatory diseases. *Tissue barriers*, *4*(4), p.e1251384.

96. Ajamian, M., Steer, D., Rosella, G. and Gibson, P.R., 2019. Serum zonulin as a marker of intestinal mucosal barrier function: May not be what it seems. *PloS one*, *14*(1), p.e0210728.

97. Thuijls, G., Derikx, J.P., de Haan, J.J., Grootjans, J., de Bruïne, A., Masclee, A.A., Heineman, E. and Buurman, W.A., 2010. Urine-based detection of intestinal tight junction loss. *Journal of clinical gastroenterology*, *44*(1), pp.e14-e19.

98. Nagpal, R. and Yadav, H., 2017. Bacterial translocation from the gut to the distant organs: an overview. *Annals of Nutrition and Metabolism*, *71*, pp.11-16.

99. Gnauck, A., Lentle, R.G. and Kruger, M.C., 2016. Chasing a ghost?–Issues with the determination of circulating levels of endotoxin in human blood. *Critical reviews in clinical laboratory sciences*, *53*(3), pp.197-215.

100. Mussap, M., Noto, A., Fravega, M. and Fanos, V., 2011. Soluble CD14 subtype presepsin (sCD14-ST) and lipopolysaccharide binding protein (LBP) in neonatal sepsis: new clinical and analytical perspectives for two old biomarkers. *The Journal of Maternal-Fetal & Neonatal Medicine*, *24*(sup2), pp.12-14.

101. Mierzchala, M., Krzystek-Korpacka, M., Gamian, A. and Durek, G., 2011. Quantitative indices of dynamics in concentrations of lipopolysaccharide-binding protein (LBP) as

prognostic factors in severe sepsis/septic shock patients—comparison with CRP and procalcitonin. *Clinical biochemistry*, *44*(5-6), pp.357-363.

102. Ewaschuk, J.B., Naylor, J.M. and Zello, G.A., 2005. D-lactate in human and ruminant metabolism. *The Journal of nutrition*, *135*(7), pp.1619-1625.

103. Sun, X.Q., Fu, X.B., Zhang, R., Lu, Y., Deng, Q., Jiang, X.G. and Sheng, Z.Y., 2001. Relationship between plasma D (-)-lactate and intestinal damage after severe injuries in rats. *World journal of gastroenterology*, *7*(4), pp.555-558.

104. Sobhian, B., Kröpfl, A., Hölzenbein, T., Khadem, A., Redl, H. and Bahrami, S., 2012. Increased circulating D-lactate levels predict risk of mortality after hemorrhage and surgical trauma in baboons. *Shock*, *37*(5), pp.473-477.

105. March, D.S., Jones, A.W., Thatcher, R. and Davison, G., 2019. The effect of bovine colostrum supplementation on intestinal injury and circulating intestinal bacterial DNA following exercise in the heat. *European journal of nutrition*, *58*(4), pp.1441-1451.

106. Païssé, S., Valle, C., Servant, F., Courtney, M., Burcelin, R., Amar, J. and Lelouvier, B., 2016. Comprehensive description of blood microbiome from healthy donors assessed by 16 S targeted metagenomic sequencing. *Transfusion*, *56*(5), pp.1138-1147

107. Nieman, D., Kay, C., Rathore, A., Grace, M., Strauch, R., Stephan, E., Sakaguchi, C. and Lila, M., 2018. Increased Plasma Levels of Gut-Derived Phenolics Linked to Walking and Running Following Two Weeks of Flavonoid Supplementation. *Nutrients*, *10*(11), p.1718.

108. Pals, K.L., Chang, R.T., Ryan, A.J. and Gisolfi, C.V., 1997. Effect of running intensity on intestinal permeability. *Journal of Applied Physiology*, *82*(2), pp.571-576.

109. JanssenDuijghuijsen, L.M., Keijer, J., Mensink, M., Lenaerts, K., Ridder, L., Nierkens, S., Kartaram, S.W., Verschuren, M.C., Pieters, R.H., Bas, R. and Witkamp, R.F., 2017. Adaptation of exercise-induced stress in well-trained healthy young men. *Experimental physiology*, *102*(1), pp.86-99.

110. van Nieuwenhoven, 1999. The effect of physical exercise on parameters of gastrointestinal function. *Neurogastroenterology & Motility*, *11*(6), pp.431-439.

111. van Nieuwenhoven, M.A., Brummer, R.J. and Brouns, F.J.P.H., 2000. Gastrointestinal function during exercise: comparison of water, sports drink, and sports drink with caffeine. *Journal of applied physiology*, *89*(3), pp.1079-1085.

112. van Nieuwenhoven, M.A., Brouns, F. and Brummer, R.J.M., 2004. Gastrointestinal profile of symptomatic athletes at rest and during physical exercise. *European journal of applied physiology*, *91*(4), pp.429-434.

113. Marchbank, T., Davison, G., Oakes, J.R., Ghatei, M.A., Patterson, M., Moyer, M.P. and Playford, R.J., 2010. The nutriceutical bovine colostrum truncates the increase in gut permeability caused by heavy exercise in athletes. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *300*(3), pp.G477-G484.

114. Davison, G., Marchbank, T., March, D.S., Thatcher, R. and Playford, R.J., 2016. Zinc carnosine works with bovine colostrum in truncating heavy exercise–induced increase in gut permeability in healthy volunteers. *The American journal of clinical nutrition*, *104*(2), pp.526-536.

115. Snipe, R.M., Khoo, A., Kitic, C.M., Gibson, P.R. and Costa, R.J., 2018. The impact of exertional-heat stress on gastrointestinal integrity, gastrointestinal symptoms, systemic endotoxin and cytokine profile. *European journal of applied physiology*, *118*(2), pp.389-400.

116. Zuhl, M., Dokladny, K., Mermier, C., Schneider, S., Salgado, R. and Moseley, P., 2015. The effects of acute oral glutamine supplementation on exercise-induced gastrointestinal permeability and heat shock protein expression in peripheral blood mononuclear cells. *Cell Stress and Chaperones*, *20*(1), pp.85-93.

117. van Nieuwenhoven, M.A., Brouns, F. and Brummer, R.J.M., 2004. Gastrointestinal profile of symptomatic athletes at rest and during physical exercise. *European journal of applied physiology*, *91*(4), pp.429-434.

118. van Nieuwenhoven, M.A., Vriens, B.E.P.J., Brummer, R.J. and Brouns, F.J.P.H., 2000. Effect of dehydration on gastrointestinal function at rest and during exercise in humans. *European journal of applied physiology*, *83*(6), pp.578-584.

119. Lambert, G.P., Lang, J., Bull, A., Pfeifer, P.C., Eckerson, J., Moore, G., Lanspa, S. and O'Brien, J., 2008. Fluid restriction during running increases GI permeability. *International journal of sports medicine*, *29*(03), pp.194-198.

120. Ryan, A.J., Chang, R.T. and Gisolfi, C.V., 1996. Gastrointestinal permeability following aspirin intake and prolonged running. *Medicine and science in sports and exercise*, *28*(6), pp.698-705.

121. Lambert, G.P., Boylan, M., Laventure, J.P., Bull, A. and Lanspa, S., 2007. Effect of aspirin and ibuprofen on GI permeability during exercise. *International journal of sports medicine*, *28*(09), pp.722-726.

122. Lambert, G.P., Broussard, L.J., Mason, B.L., Mauermann, W.J. and Gisolfi, C.V., 2001. Gastrointestinal permeability during exercise: effects of aspirin and energy-containing beverages. *Journal of Applied Physiology*, *90*(6), pp.2075-2080.

123. Smetanka, R.D., Lambert, C.P., Murray, R., Eddy, D., Horn, M. and Gisolfi, C.V., 1999. Intestinal permeability in runners in the 1996 Chicago marathon. *International Journal of Sport Nutrition and Exercise Metabolism*, *9*(4), pp.426-433.

124. van Wijck, K., Lenaerts, K., Van Bijnen, A.A., Boonen, B., Van Loon, L.J., Dejong, C.H. and Buurman, W.A., 2012. Aggravation of exercise-induced intestinal injury by Ibuprofen in athletes. *Med Sci Sports Exerc*, *44*(12), pp.2257-2262.

125. Snipe, R.M., Khoo, A., Kitic, C.M., Gibson, P.R. and Costa, R.J., 2018. The impact of mild heat stress during prolonged running on gastrointestinal integrity, gastrointestinal

symptoms, systemic endotoxin and cytokine profiles. *International journal of sports medicine*, *39*(04), pp.255-263.

126. Shing, C.M., Peake, J.M., Lim, C.L., Briskey, D., Walsh, N.P., Fortes, M.B., Ahuja, K.D. and Vitetta, L., 2014. Effects of probiotics supplementation on gastrointestinal permeability, inflammation and exercise performance in the heat. *European journal of applied physiology*, *114*(1), pp.93-103.

127. van Wijck, K., Wijnands, K.A., Meesters, D.M., Boonen, B., Van Loon, L.J., Buurman, W.A., Dejong, C.H., Lenaerts, K. and Poeze, M., 2014. L-citrulline improves splanchnic perfusion and reduces gut injury during exercise. *Medicine & Science in Sports & Exercise*, *46*(11), pp.2039-2046.

128. Lee, B.J. and Thake, C.D., 2017. Heat and Hypoxic Acclimation Increase Monocyte Heat Shock Protein 72 but Do Not Attenuate Inflammation following Hypoxic Exercise. *Frontiers in physiology*, *8*, pp.811-811.

129. Kartaram, S., Mensink, M., Teunis, M., Schoen, E., Witte, G., Duijghuijsen, L.J., Verschuren, M., Mohrmann, K., M'Rabet, L., Knipping, K. and Wittink, H., 2019. Plasma citrulline concentration, a marker for intestinal functionality, reflects exercise intensity in healthy young men. *Clinical Nutrition*, *38*(5), pp.2251-2258.

130. Trommelen, J., Fuchs, C., Beelen, M., Lenaerts, K., Jeukendrup, A., Cermak, N. and Van Loon, L., 2017. Fructose and sucrose intake increase exogenous carbohydrate oxidation during exercise. *Nutrients*, *9*(2), p.167.

131. Jonvik, K.L., Lenaerts, K., Smeets, J.S., Kolkman, J.J., Van Loon, L.J. and Verdijk, L.B., 2019. Sucrose but Not Nitrate Ingestion Reduces Strenuous Cycling-induced Intestinal Injury. *Med. Sci. Sports Exerc*, *51*, pp.436-444.

132. Karhu, E., Forsgård, R.A., Alanko, L., Alfthan, H., Pussinen, P., Hämäläinen, E. and Korpela, R., 2017. Exercise and gastrointestinal symptoms: running-induced changes in intestinal permeability and markers of gastrointestinal function in asymptomatic and symptomatic runners. *European journal of applied physiology*, *117*(12), pp.2519-2526.

133. Osborne, J.O., Stewart, I.B., Beagley, K.W. and Minett, G.M., 2019. The effect of cycling in the heat on gastrointestinal-induced damage and neuromuscular fatigue. *European journal of applied physiology*, pp.1-12.

134. Hill, G.W., Gillum, T.L., Lee, B.J., Romano, P.A., Schall, Z.J., Hamilton, A.M. and Kuennen, M.R., 2019. Prolonged treadmill running in normobaric hypoxia causes gastrointestinal barrier permeability and elevates circulating levels of pro-and anti-inflammatory cytokines. *Applied Physiology, Nutrition, and Metabolism*, (ja).

135. Costa, R.J., Camoes-Costa, V., Snipe, R.M., Dixon, D., Russo, I. and Huschtscha, Z., 2019. Impact of exercise-induced hypohydration on gastrointestinal integrity, function, symptoms, and systemic endotoxin and inflammatory profile. *Journal of Applied Physiology*, *126*(5), pp.1281-1291.

136. Sheahen, B.L., Fell, J.W., Zadow, E.K., Hartley, T.F. and Kitic, C.M., 2018. Intestinal damage following short-duration exercise at the same relative intensity is similar in temperate and hot environments. *Applied physiology, nutrition, and metabolism*, *43*(12), pp.1314-1320.

137. Snipe, R.M. and Costa, R.J., 2018. Does the temperature of water ingested during exertional-heat stress influence gastrointestinal injury, symptoms, and systemic inflammatory profile?. *Journal of science and medicine in sport*, *21*(8), pp.771-776.

138. Yeh, Y.J., Law, L.Y.L. and Lim, C.L., 2013. Gastrointestinal response and endotoxemia during intense exercise in hot and cool environments. *European journal of applied physiology*, *113*(6), pp.1575-1583.

139. Bosenberg, A.T., Brock-Utne, J.G., Gaffin, S.L., Wells, M.T. and Blake, G.T., 1988. Strenuous exercise causes systemic endotoxemia. *Journal of Applied Physiology*, *65*(1), pp.106-108.

140. Brock-Utne, J.G., Gaffin, S.L., Wells, M.T., Gathiram, P., Sohar, E., James, M.F., Morrell, D.F. and Norman, R.J., 1988. Endotoxaemia in exhausted runners after a long-distance race. *South African Medical Journal/Suid-Afrikaanse Mediese Tydskrift*, *73*(9), pp.533-536.

141. Moore, G.E., Holbein, M.E. and Knochel, J.P., 1995. Exercise-associated collapse in cyclists is unrelated to endotoxemia. *Medicine and science in sports and exercise*, *27*(9), pp.1238-1242.

142. Camus, G., Nys, M., Poortmans, J.R., Venneman, I., Monfils, T., Deby-Dupont, G., Juchmes-Ferir, A., Deby, C., Lamy, M. and Duchateau, J., 1998. Endotoxaemia, production of tumour necrosis factor α and polymorphonuclear neutrophil activation following strenuous exercise in humans. *European journal of applied physiology and occupational physiology*, *79*(1), pp.62-68.

143. Kuennen, M., Gillum, T., Dokladny, K., Bedrick, E., Schneider, S. and Moseley, P., 2011. Thermotolerance and heat acclimation may share a common mechanism in humans. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *301*(2), pp.R524-R533.

144. Jeukendrup, A.E., Vet-Joop, K., Sturk, A., Stegen, J.H.J.C., Senden, J., Saris, W.H.M. and Wagenmakers, A.J.M., 2000. Relationship between gastro-intestinal complaints and endotoxaemia, cytokine release and the acute-phase reaction during and after a long-distance triathlon in highly trained men. *Clinical Science*, *98*(1), pp.47-55.

145. Gill, S.K., Hankey, J., Wright, A., Marczak, S., Hemming, K., Allerton, D.M., Ansley-Robson, P. and Costa, R.J.S., 2015. The impact of a 24-h ultra-marathon on circulatory endotoxin and cytokine profile. *International journal of sports medicine*, *36*(08), pp.688-695.

146. Selkirk, G.A., McLellan, T.M., Wright, H.E. and Rhind, S.G., 2008. Mild endotoxemia, NF-κB translocation, and cytokine increase during exertional heat stress in trained and untrained individuals. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *295*(2), pp.R611-R623.

147. Gaskell, S.K., Taylor, B., Muir, J. and Costa, R.J., 2019. Impact of 24-hour high and low fermentable oligo-di-mono-saccharide polyol diets on markers of exercise-induced gastrointestinal syndrome in response to exertional-heat stress. *Applied Physiology, Nutrition, and Metabolism*, (ja).

148. Pugh, J.N., Sparks, A.S., Doran, D.A., Fleming, S.C., Langan-Evans, C., Kirk, B., Fearn, R., Morton, J.P. and Close, G.L., 2019. Four weeks of probiotic supplementation reduces GI symptoms during a marathon race. *European journal of applied physiology*, *119*(7), pp.1491-1501.

149. Gill, S.K., Allerton, D.M., Ansley-Robson, P., Hemmings, K., Cox, M. and Costa, R.J., 2016. Does short-term high dose probiotic supplementation containing lactobacillus casei attenuate exertional-heat stress induced endotoxaemia and cytokinaemia?. *International journal of sport nutrition and exercise metabolism*, *26*(3), pp.268-275.

150. Lim, C.L., Pyne, D., Horn, P., Kalz, A., Saunders, P., Peake, J., Suzuki, K., Wilson, G. and Mackinnon, L.T., 2009. The effects of increased endurance training load on biomarkers of heat intolerance during intense exercise in the heat. *Applied Physiology, Nutrition, and Metabolism*, *34*(4), pp.616-624.

151. Moss, J.N., Bayne, F.M., Castelli, F., Naughton, M.R., Reeve, T.C., Trangmar, S.J., Mackenzie, R.W. and Tyler, C.J., 2019. Short-term isothermic heat acclimation elicits beneficial adaptations but medium-term elicits a more complete adaptation. *European journal of applied physiology*, pp.1-12.

152. Morrison, S.A., Cheung, S.S. and Cotter, J.D., 2014. Bovine colostrum, training status, and gastrointestinal permeability during exercise in the heat: a placebo-controlled double-blind study. *Applied Physiology, Nutrition, and Metabolism*, *39*(9), pp.1070-1082.

153. Dokladny, K., Zuhl, M.N. and Moseley, P.L., 2015. Intestinal epithelial barrier function and tight junction proteins with heat and exercise. *Journal of Applied Physiology*, *120*(6), pp.692-701.

154. Oliver, S.R., Phillips, N.A., Novosad, V.L., Bakos, M.P., Talbert, E.E. and Clanton, T.L., 2012. Hyperthermia induces injury to the intestinal mucosa in the mouse: evidence for an oxidative stress mechanism. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *302*(7), pp.R845-R853.

155. Grootjans, J., Lenaerts, K., Buurman, W.A., Dejong, C.H. and Derikx, J.P., 2016. Life and death at the mucosal-luminal interface: New perspectives on human intestinal ischemia-reperfusion. *World journal of gastroenterology*, *22*(9), p.2760.

156. de Punder, K. and Pruimboom, L., 2015. Stress induces endotoxemia and low-grade inflammation by increasing barrier permeability. *Frontiers in immunology*, *6*, p.223.

157. Sawka, M.N., Leon, L.R., Montain, S.J. and Sonna, L.A., 2011. Integrated physiological mechanisms of exercise performance, adaptation, and maladaptation to heat stress. *Comprehensive Physiology*, *1*(4), pp.1883-1928.

158. Byrne, C. and Lim, C.L., 2007. The ingestible telemetric body core temperature sensor: a review of validity and exercise applications. *British journal of sports medicine*, *41*(3), pp.126-133.

159. Dokladny, K., Moseley, P.L. and Ma, T.Y., 2006. Physiologically relevant increase in temperature causes an increase in intestinal epithelial tight junction permeability. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *290*(2), pp.G204-G212.

160. Ikari, A., Nakano, M., Suketa, Y., Harada, H. and Takagi, K., 2005. Reorganization of ZO-1 by sodium-dependent glucose transporter activation after heat stress in LLC-PK1 cells. *Journal of cellular physiology*, *203*(3), pp.471-478.

161. Yang, P.C., He, S.H. and Zheng, P.Y., 2007. Investigation into the signal transduction pathway via which heat stress impairs intestinal epithelial barrier function. *Journal of gastroenterology and hepatology*, *22*(11), pp.1823-1831.

162. Ter Steege, R.W.F. and Kolkman, J.J., 2012. The pathophysiology and management of gastrointestinal symptoms during physical exercise, and the role of splanchnic blood flow. *Alimentary pharmacology & therapeutics*, *35*(5), pp.516-528.

163. Ahlborg, G.U.N.V.O.R., Weitzberg, E. and Lundberg, J., 1995. Metabolic and vascular effects of circulating endothelin-1 during moderately heavy prolonged exercise. *Journal of Applied Physiology*, *78*(6), pp.2294-2300.

164. Kenney, W.L. and Ho, C.W., 1995. Age alters regional distribution of blood flow during moderate-intensity exercise. *Journal of Applied Physiology*, *79*(4), pp.1112-1119.

165. Otte, J.A., Oostveen, E., Geelkerken, R.H., Groeneveld, A.J. and Kolkman, J.J., 2001. Exercise induces gastric ischemia in healthy volunteers: a tonometry study. *Journal of Applied Physiology*, *91*(2), pp.866-871.

166. Thursby, E. and Juge, N., 2017. Introduction to the human gut microbiota. *Biochemical Journal*, *474*(11), pp.1823-1836.

167. JanssenDuijghuijsen, L.M., Grefte, S., de Boer, V.C., Zeper, L., van Dartel, D.A., van der Stelt, I., Bekkenkamp-Grovenstein, M., van Norren, K., Wichers, H.J. and Keijer, J., 2017. Mitochondrial ATP depletion disrupts Caco-2 monolayer integrity and internalizes claudin 7. *Frontiers in physiology*, *8*, p.794.

168. Friedman, E.S., Bittinger, K., Esipova, T.V., Hou, L., Chau, L., Jiang, J., Mesaros, C., Lund, P.J., Liang, X., FitzGerald, G.A. and Goulian, M., 2018. Microbes vs. chemistry in the origin of the anaerobic gut lumen. *Proceedings of the National Academy of Sciences*, *115*(16), pp.4170-4175.

169. Turner, M.D., Nedjai, B., Hurst, T. and Pennington, D.J., 2014. Cytokines and chemokines: at the crossroads of cell signalling and inflammatory disease. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research*, *1843*(11), pp.2563-2582.

170. Suzuki, K., 2018. Cytokine response to exercise and its modulation. *Antioxidants*, *7*(1), p.17.

171. Al-Sadi, R., Guo, S., Dokladny, K., Smith, M.A., Ye, D., Kaza, A., Watterson, D.M. and Ma, T.Y., 2012. Mechanism of interleukin-1β induced-increase in mouse intestinal permeability in vivo. *Journal of Interferon & Cytokine Research*, *32*(10), pp.474-484.

172. Al-Sadi, R., Guo, S., Ye, D. and Ma, T.Y., 2013. TNF-α modulation of intestinal epithelial tight junction barrier is regulated by ERK1/2 activation of Elk-1. *The American journal of pathology*, *183*(6), pp.1871-1884.

173. Al-Sadi, R., Ye, D., Boivin, M., Guo, S., Hashimi, M., Ereifej, L. and Ma, T.Y., 2014. Interleukin-6 modulation of intestinal epithelial tight junction permeability is mediated by JNK pathway activation of claudin-2 gene. *PloS one*, *9*(3), p.e85345.

174. Fortes, M.B., Di, U.F., Dolci, A., Junglee, N.A., Crockford, M.J., West, L., Hillier-Smith, R., Macdonald, J.H. and Walsh, N.P., 2013. Muscle-damaging exercise increases heat strain during subsequent exercise heat stress. *Medicine and science in sports and exercise*, *45*(10), pp.1915-1924.

175. Dolci, A., Fortes, M.B., Walker, F.S., Haq, A., Riddle, T. and Walsh, N.P., 2015. Repeated muscle damage blunts the increase in heat strain during subsequent exercise heat stress. *European journal of applied physiology*, *115*(7), pp.1577-1588.

176. Cavaillon, J.M. and Annane, D., 2006. Invited review: Compartmentalization of the inflammatory response in sepsis and SIRS. *Journal of endotoxin research*, *12*(3), pp.151-170.

177. Ghanim, H., Sia, C.L., Upadhyay, M., Korzeniewski, K., Viswanathan, P., Abuaysheh, S., Mohanty, P. and Dandona, P., 2010. Orange juice neutralizes the proinflammatory effect of a high-fat, high-carbohydrate meal and prevents endotoxin increase and Toll-like receptor expression. *The American journal of clinical nutrition*, *91*(4), pp.940-949.

178. Deopurkar, R., Ghanim, H., Friedman, J., Abuaysheh, S., Sia, C.L., Mohanty, P., Viswanathan, P., Chaudhuri, A. and Dandona, P., 2010. Differential effects of cream, glucose, and orange juice on inflammation, endotoxin, and the expression of Toll-like receptor-4 and suppressor of cytokine signaling-3. *Diabetes care*, *33*(5), pp.991-997.

179. Ghanim, H., Abuaysheh, S., Sia, C.L., Korzeniewski, K., Chaudhuri, A., Fernandez-Real, J.M. and Dandona, P., 2009. Increase in plasma endotoxin concentrations and the expression of Toll-like receptors and suppressor of cytokine signaling-3 in mononuclear cells after a high-fat, high-carbohydrate meal: implications for insulin resistance. *Diabetes care*, *32*(12), pp.2281-2287.

180. Edinburgh, R.M., Hengist, A., Smith, H.A., Travers, R.L., Koumanov, F., Betts, J.A., Thompson, D., Walhin, J.P., Wallis, G.A., Hamilton, D.L. and Stevenson, E.J., 2018. Preexercise breakfast ingestion versus extended overnight fasting increases postprandial glucose flux after exercise in healthy men. *American Journal of Physiology-Endocrinology and Metabolism*, *315*(5), pp.E1062-E1074.

181. Salvador, A.F., McKenna, C.F., Alamilla, R.A., Cloud, R.M., Keeble, A.R., Miltko, A., Scaroni, S.E., Beals, J.W., Ulanov, A.V., Dilger, R.N. and Bauer, L.L., 2019. Potato ingestion is

as effective as carbohydrate gels to support prolonged cycling performance. *Journal of Applied Physiology*.

182. Deniz, T., Agalar, C., Ozdogan, M., Comu, F., Emirdogan, M., Taskin, S., Saygun, O. and Agalar, F., 2007. Oral carbohydrate solution ameliorates endotoxemia-induced splanchnic ischemia. *Digestive diseases and sciences*, *52*(1), pp.287-291.

183. Ramadass, B., Dokladny, K., Moseley, P.L., Patel, Y.R. and Lin, H.C., 2010. Sucrose co-administration reduces the toxic effect of lectin on gut permeability and intestinal bacterial colonization. *Digestive diseases and sciences*, *55*(10), pp.2778-2784.

184. David, L.A., Maurice, C.F., Carmody, R.N., Gootenberg, D.B., Button, J.E., Wolfe, B.E., Ling, A.V., Devlin, A.S., Varma, Y., Fischbach, M.A. and Biddinger, S.B., 2014. Diet rapidly and reproducibly alters the human gut microbiome. *Nature*, *505*(7484), p.559.

185. Gentilcore, D., Nair, N.S., Vanis, L., Rayner, C.K., Meyer, J.H., Hausken, T., Horowitz, M. and Jones, K.L., 2009. Comparative effects of oral and intraduodenal glucose on blood pressure, heart rate, and splanchnic blood flow in healthy older subjects. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, *297*(3), pp.R716-R722.

186. Lerner, A. and Matthias, T., 2015. Changes in intestinal tight junction permeability associated with industrial food additives explain the rising incidence of autoimmune disease. *Autoimmunity reviews*, *14*(6), pp.479-489.

187. Erridge, C., Attina, T., Spickett, C.M. and Webb, D.J., 2007. A high-fat meal induces low-grade endotoxemia: evidence of a novel mechanism of postprandial inflammation. *The American journal of clinical nutrition*, *86*(5), pp.1286-1292.

188. Pöchmüller, M., Schwingshackl, L., Colombani, P.C. and Hoffmann, G., 2016. A systematic review and meta-analysis of carbohydrate benefits associated with randomized controlled competition-based performance trials. *Journal of the international society of sports nutrition*, *13*(1), p.27.

189. McCartney, D., Desbrow, B. and Irwin, C., 2018. Post-exercise ingestion of carbohydrate, protein and water: a systematic review and meta-analysis for effects on subsequent athletic performance. *Sports Medicine*, *48*(2), pp.379-408.

190. Bermon, S., Castell, L.M., Calder, P.C., Bishop, N.C., Blomstrand, E., Mooren, F.C., Krüger, K., Kavazis, A.N., Quindry, J.C., Senchina, D.S. and Nieman, D.C., 2017. Consensus Statement Immunonutrition and Exercise. *Exercise immunology review*, *23*, pp.8-50.

191. Costa, R.J., Miall, A., Khoo, A., Rauch, C., Snipe, R., Camões-Costa, V. and Gibson, P., 2017. Gut-training: the impact of two weeks repetitive gut-challenge during exercise on gastrointestinal status, glucose availability, fuel kinetics, and running performance. *Applied Physiology, Nutrition, and Metabolism*, *42*(5), pp.547-557.

192. Rehrer, N.J., Goes, E., DuGardeyn, C., Reynaert, H. and DeMeirleir, K., 2005. Effect of carbohydrate on portal vein blood flow during exercise. *International journal of sports medicine*, *26*(03), pp.171-176.

193. Snipe, R.M., Khoo, A., Kitic, C.M., Gibson, P.R. and Costa, R.J., 2017. Carbohydrate and protein intake during exertional heat stress ameliorates intestinal epithelial injury and small intestine permeability. *Applied physiology, nutrition, and metabolism*, *42*(12), pp.1283-1292.

194. Sessions, J., Bourbeau, K., Rosinski, M., Szczygiel, T., Nelson, R., Sharma, N. and Zuhl, M., 2016. Carbohydrate gel ingestion during running in the heat on markers of gastrointestinal distress. *European journal of sport science*, *16*(8), pp.1064-1072.

195. Moncada-Jiménez, J., Plaisance, E.P., Mestek, M.L., Ratcliff, L., Araya-Ramírez, F., Taylor, J.K., Grandjean, P.W. and AragónVargas, L.F., 2009. Duathlon performance unaltered by short-term changes in dietary fat and carbohydrates. *International journal of sport nutrition and exercise metabolism*, *19*(1), pp.47-60.

196. Kim, M.H. and Kim, H., 2017. The roles of glutamine in the intestine and its implication in intestinal diseases. *International journal of molecular sciences*, *18*(5), p.1051.

197. Zhou, Y.P., Jiang, Z.M., Sun, Y.H., Wang, X.R., Ma, E.L. and Wilmore, D., 2003. The effect of supplemental enteral glutamine on plasma levels, gut function, and outcome in severe burns: a randomized, double-blind, controlled clinical trial. *Journal of Parenteral and Enteral Nutrition*, *27*(4), pp.241-245.

198. Peng, X., Yan, H., You, Z., Wang, P. and Wang, S., 2004. Effects of enteral supplementation with glutamine granules on intestinal mucosal barrier function in severe burned patients. *Burns*, *30*(2), pp.135-139.

199. Zhou, Q., Verne, M.L., Fields, J.Z., Lefante, J.J., Basra, S., Salameh, H. and Verne, G.N., 2019. Randomised placebo-controlled trial of dietary glutamine supplements for postinfectious irritable bowel syndrome. *Gut*, *68*(6), pp.996-1002.

200. Shu, X.L., Yu, T.T., Kang, K. and Zhao, J., 2016. Effects of glutamine on markers of intestinal inflammatory response and mucosal permeability in abdominal surgery patients: A meta-analysis. *Experimental and therapeutic medicine*, *12*(6), pp.3499-3506.

201. Akobeng, A.K., Miller, V., Stanton, J., Elbadri, A.M. and Thomas, A.G., 2000. Double-blind randomized controlled trial of glutamine-enriched polymeric diet in the treatment of active Crohn's disease. *Journal of pediatric gastroenterology and nutrition*, *30*(1), pp.78-84.

202. Benjamin, J., Makharia, G., Ahuja, V., Rajan, K.A., Kalaivani, M., Gupta, S.D. and Joshi, Y.K., 2012. Glutamine and whey protein improve intestinal permeability and morphology in patients with Crohn's disease: a randomized controlled trial. *Digestive diseases and sciences*, *57*(4), pp.1000-1012.

203. Rhoads, J.M., Argenzio, R.A., Chen, W.U.N.I.A.N., Rippe, R.A., Westwick, J.K., Cox, A.D., Berschneider, H.M. and Brenner, D.A., 1997. L-glutamine stimulates intestinal cell proliferation and activates mitogen-activated protein kinases. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *272*(5), pp.G943-G953.

204. Singleton, K.D. and Wischmeyer, P.E., 2006. Oral glutamine enhances heat shock protein expression and improves survival following hyperthermia. *Shock*, *25*(3), pp.295-299.

205. Xue, H., Sufit, A.J. and Wischmeyer, P.E., 2011. Glutamine therapy improves outcome of in vitro and in vivo experimental colitis models. *Journal of Parenteral and Enteral Nutrition*, *35*(2), pp.188-197.

206. Dai, Z.L., Li, X.L., Xi, P.B., Zhang, J., Wu, G. and Zhu, W.Y., 2013. L-Glutamine regulates amino acid utilization by intestinal bacteria. *Amino Acids*, *45*(3), pp.501-512.

207. Harward, T.R., Coe, D., Souba, W.W., Klingman, N. and Seeger, J.M., 1994. Glutamine preserves gut glutathione levels during intestinal ischemia/reperfusion. *Journal of Surgical Research*, *56*(4), pp.351-355.

208. Li, N., Lewis, P., Samuelson, D., Liboni, K. and Neu, J., 2004. Glutamine regulates Caco-2 cell tight junction proteins. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *287*(3), pp.G726-G733.

209. Beutheu, S., Ghouzali, I., Galas, L., Déchelotte, P. and Coëffier, M., 2013. Glutamine and arginine improve permeability and tight junction protein expression in methotrexate-treated Caco-2 cells. *Clinical nutrition*, *32*(5), pp.863-869.

210. Ahmadi, A.R., Rayyani, E., Bahreini, M. and Mansoori, A., 2019. The effect of glutamine supplementation on athletic performance, body composition, and immune function: A systematic review and a meta-analysis of clinical trials. *Clinical Nutrition*, *38*(3), pp.1076-1091.

211. Zuhl, M.N., Lanphere, K.R., Kravitz, L., Mermier, C.M., Schneider, S., Dokladny, K. and Moseley, P.L., 2013. Effects of oral glutamine supplementation on exercise-induced gastrointestinal permeability and tight junction protein expression. *Journal of applied physiology*, *116*(2), pp.183-191.

212. Pugh, J.N., Sage, S., Hutson, M., Doran, D.A., Fleming, S.C., Highton, J., Morton, J.P. and Close, G.L., 2017. Glutamine supplementation reduces markers of intestinal permeability during running in the heat in a dose-dependent manner. *European journal of applied physiology*, *117*(12), pp.2569-2577.

213. Osborne, J.O., Stewart, I.B., Beagley, K.W., Borg, D.N. and Minett, G.M., 2019. Acute glutamine supplementation does not improve 20-km self-paced cycling performance in the heat. *European Journal of Applied Physiology*, *119*(11-12), pp.2567-2578.

214. Gleeson, M., 2008. Dosing and efficacy of glutamine supplementation in human exercise and sport training. *The Journal of nutrition*, *138*(10), pp.2045S-2049S.

215. Rathe, M., Müller, K., Sangild, P.T. and Husby, S., 2014. Clinical applications of bovine colostrum therapy: a systematic review. *Nutrition reviews*, *72*(4), pp.237-254.

216. Uruakpa, F.O., Ismond, M.A.H. and Akobundu, E.N.T., 2002. Colostrum and its benefits: a review. *Nutrition research*, *22*(6), pp.755-767.

217. Prosser, C., Stelwagen, K., Cummins, R., Guerin, P., Gill, N. and Milne, C., 2004. Reduction in heat-induced gastrointestinal hyperpermeability in rats by bovine colostrum and goat milk powders. *Journal of applied physiology*, *96*(2), pp.650-654.

218. Playford, R.J., Floyd, D.N., Macdonald, C.E., Calnan, D.P., Adenekan, R.O., Johnson, W., Goodlad, R.A. and Marchbank, T., 1999. Bovine colostrum is a health food supplement which prevents NSAID induced gut damage. *Gut*, *44*(5), pp.653-658.

219. Eslamian, G., Ardehali, S.H., Baghestani, A.R. and Shariatpanahi, Z.V., 2019. Effects of early enteral bovine colostrum supplementation on intestinal permeability in critically ill patients: A randomized, double-blind, placebo-controlled study. *Nutrition*, *60*, pp.106-111.

220. Bodammer, P., Kerkhoff, C., Maletzki, C. and Lamprecht, G., 2013. Bovine colostrum increases pore-forming claudin-2 protein expression but paradoxically not ion permeability possibly by a change of the intestinal cytokine milieu. *PloS one*, *8*(5), p.e64210.

221. Khan, Z., Macdonald, C., Wicks, A.C., Holt, M.P., Floyd, D., Ghosh, S., Wright, N.A. and Playford, R.J., 2002. Use of the 'nutriceutical', bovine colostrum, for the treatment of distal colitis: results from an initial study. *Alimentary pharmacology & therapeutics*, *16*(11), pp.1917-1922.

222. Antonio, J., Sanders, M.S. and Van Gammeren, D., 2001. The effects of bovine colostrum supplementation on body composition and exercise performance in active men and women. *Nutrition*, *17*(3), pp.243-247.

223. Jones, A.W., March, D.S., Curtis, F. and Bridle, C., 2016. Bovine colostrum supplementation and upper respiratory symptoms during exercise training: a systematic review and meta-analysis of randomised controlled trials. *BMC Sports Science, Medicine and Rehabilitation*, *8*(1), p.21.

224. Shing, C.M., Hunter, D.C. and Stevenson, L.M., 2009. Bovine colostrum supplementation and exercise performance. *Sports Medicine*, *39*(12), pp.1033-1054.

225. March, D.S., Marchbank, T., Playford, R.J., Jones, A.W., Thatcher, R. and Davison, G., 2017. Intestinal fatty acid-binding protein and gut permeability responses to exercise. *European journal of applied physiology*, *117*(5), pp.931-941.

226. McKenna, Z., Berkemeier, Q., Naylor, A., Kleint, A., Gorini, F., Ng, J., Kim, J.K., Sullivan, S. and Gillum, T., 2017. Bovine colostrum supplementation does not affect plasma I-FABP concentrations following exercise in a hot and humid environment. *European journal of applied physiology*, *117*(12), pp.2561-2567.

227. Hałasa, M., Maciejewska, D., Baśkiewicz-Hałasa, M., Machaliński, B., Safranow, K. and Stachowska, E., 2017. Oral supplementation with bovine colostrum decreases intestinal permeability and stool concentrations of zonulin in athletes. *Nutrients*, *9*(4), p.370.

228. Buckley, J., Butler, R., Southcott, E. and Brinkworth, G., 2009. Bovine colostrum supplementation during running training increases intestinal permeability. *Nutrients*, *1*(2), pp.224-234.

229. Jasion, V.S. and Burnett, B.P., 2015. Survival and digestibility of orally-administered immunoglobulin preparations containing IgG through the gastrointestinal tract in humans. *Nutrition journal*, *14*(1), p.22.

230. Davison, G., Jones, A.W., Marchbank, T. and Playford, R.J., 2019. Oral bovine colostrum supplementation does not increase circulating insulin-like growth factor-1 concentration in healthy adults: results from short-and long-term administration studies. *European journal of nutrition*, pp.1-7.

231. Lundberg, J.O., Carlström, M. and Weitzberg, E., 2018. Metabolic effects of dietary nitrate in health and disease. *Cell metabolism*, *28*(1), pp.9-22.

232. Castillo, L., DeRojas, T.C., Chapman, T.E., Vogt, J., Burke, J.F., Tannenbaum, S.R. and Young, V.R., 1993. Splanchnic metabolism of dietary arginine in relation to nitric oxide synthesis in normal adult man. *Proceedings of the National Academy of Sciences*, *90*(1), pp.193-197.

233. Petersson, J., Phillipson, M., Jansson, E.A., Patzak, A., Lundberg, J.O. and Holm, L., 2007. Dietary nitrate increases gastric mucosal blood flow and mucosal defense. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *292*(3), pp.G718-G724.

234. Tanaka, A., Araki, H., Komoike, Y., Hase, S. and Takeuchi, K., 2001. Inhibition of both COX-1 and COX-2 is required for development of gastric damage in response to nonsteroidal antiinflammatory drugs. *Journal of Physiology-Paris*, *95*(1-6), pp.21-27.

235. Batista, M.A., Nicoli, J.R., dos Santos Martins, F., Nogueira Machado, J.A., Esteves Arantes, R.M., Pacífico Quirino, I.E., Davisson Correia, M.I.T. and Cardoso, V.N., 2012. Pretreatment with citrulline improves gut barrier after intestinal obstruction in mice. *Journal of Parenteral and Enteral Nutrition*, *36*(1), pp.69-76.

236. Sukhotnik, I., Helou, H., Mogilner, J., Lurie, M., Bernsteyn, A., Coran, A.G. and Shiloni, E., 2005. Oral arginine improves intestinal recovery following ischemia-reperfusion injury in rat. *Pediatric surgery international*, *21*(3), pp.191-196.

237. Gou, L., Zhang, L., Yin, C., Jia, G., Yin, X., Zhuang, X., Xu, X. and Liu, Y., 2011. Protective effect of l-citrulline against acute gastric mucosal lesions induced by ischemia–reperfusion in rats. *Canadian journal of physiology and pharmacology*, *89*(5), pp.317-327.

238. Rubanyi, G.M., Ho, E.H., Cantor, E.H., Lumma, W.C. and Botelho, L.H.P., 1991. Cytoprotective function of nitric oxide: inactivation of superoxide radicals produced by human leukocytes. *Biochemical and biophysical research communications*, *181*(3), pp.1392-1397.

239. Kubes, P., Suzuki, M. and Granger, D.N., 1991. Nitric oxide: an endogenous modulator of leukocyte adhesion. *Proceedings of the National Academy of Sciences*, *88*(11), pp.4651-4655.

240. Beutheu, S., Ouelaa, W., Guérin, C., Belmonte, L., Aziz, M., Tennoune, N., Bôle-Feysot, C., Galas, L., Déchelotte, P. and Coëffier, M., 2014. Glutamine supplementation, but not combined glutamine and arginine supplementation, improves gut barrier function during chemotherapy-induced intestinal mucositis in rats. *Clinical nutrition*, *33*(4), pp.694-701.

241. Jones, A.M., Thompson, C., Wylie, L.J. and Vanhatalo, A., 2018. Dietary nitrate and physical performance. *Annual Review of Nutrition*, *38*, pp.303-328.

242. Bailey, S.J., Blackwell, J.R., Lord, T., Vanhatalo, A., Winyard, P.G. and Jones, A.M., 2015. L-citrulline supplementation improves O2 uptake kinetics and high-intensity exercise performance in humans. *Journal of Applied Physiology*, *119*(4), pp.385-395.

243. Costa, K.A., Soares, A.D.N., Wanner, S.P., Santos, R.D.G.C.D., Fernandes, S.O.A., Martins, F.D.S., Nicoli, J.R., Coimbra, C.C. and Cardoso, V.N., 2013. L-arginine supplementation prevents increases in intestinal permeability and bacterial translocation in male Swiss mice subjected to physical exercise under environmental heat stress. *The Journal of nutrition*, *144*(2), pp.218-223.

244. McMahon, N.F., Leveritt, M.D. and Pavey, T.G., 2017. The effect of dietary nitrate supplementation on endurance exercise performance in healthy adults: a systematic review and meta-analysis. *Sports Medicine*, *47*(4), pp.735-756.

245. Kuennen, M., Jansen, L., Gillum, T., Granados, J., Castillo, W., Nabiyar, A. and Christmas, K., 2015. Dietary nitrate reduces the O 2 cost of desert marching but elevates the rise in core temperature. *European journal of applied physiology*, *115*(12), pp.2557-2569.

246. McQuillan, J.A., Casadio, J.R., Dulson, D.K., Laursen, P.B. and Kilding, A.E., 2018. The effect of nitrate supplementation on cycling performance in the heat in well-trained cyclists. *International journal of sports physiology and performance*, *13*(1), pp.50-56.

247. Parvez, S., Malik, K.A., Ah Kang, S. and Kim, H.Y., 2006. Probiotics and their fermented food products are beneficial for health. *Journal of applied microbiology*, *100*(6), pp.1171-1185.

248. van Hemert, S., Verwer, J. and Schütz, B., 2013. Clinical studies evaluating effects of probiotics on parameters of intestinal barrier function. *Advances in Microbiology*, *3*(2), p.212.

249. Bron, P.A., Van Baarlen, P. and Kleerebezem, M., 2012. Emerging molecular insights into the interaction between probiotics and the host intestinal mucosa. *Nature Reviews Microbiology*, *10*(1), p.66.

250. Resta–Lenert, S. and Barrett, K.E., 2006. Probiotics and commensals reverse TNF-α–and IFN-γ–induced dysfunction in human intestinal epithelial cells. *Gastroenterology*, *130*(3), pp.731-746.

251. Hsieh, C.Y., Osaka, T., Moriyama, E., Date, Y., Kikuchi, J. and Tsuneda, S., 2015. Strengthening of the intestinal epithelial tight junction by Bifidobacterium bifidum. *Physiological reports*, *3*(3).

252. West, N.P., Pyne, D.B., Peake, J.M. and Cripps, A.W., 2009. Probiotics, immunity and exercise: a review. *Exerc Immunol Rev*, *15*(107), p.e26.

253. Pyne, D.B., West, N.P., Cox, A.J. and Cripps, A.W., 2015. Probiotics supplementation for athletes–clinical and physiological effects. *European journal of sport science*, *15*(1), pp.63-72.

254. Roberts, J., Suckling, C., Peedle, G., Murphy, J., Dawkins, T. and Roberts, M., 2016. An exploratory investigation of endotoxin levels in novice long distance triathletes, and the effects of a multi-strain probiotic/prebiotic, antioxidant intervention. *Nutrients*, *8*(11), p.733.

255. Carbuhn, A., Reynolds, S., Campbell, C., Bradford, L., Deckert, J., Kreutzer, A. and Fry, A., 2018. Effects of probiotic (Bifidobacterium longum 35624) supplementation on exercise performance, immune modulation, and cognitive outlook in Division I female swimmers. *Sports*, *6*(4), p.116.

256. Axelrod, C.L., Brennan, C.J., Cresci, G., Paul, D., Hull, M., Fealy, C.E. and Kirwan, J.P., 2019. UCC118 supplementation reduces exercise-induced gastrointestinal permeability and remodels the gut microbiome in healthy humans. *Physiological reports*, *7*(22).

257. Karl, J.P., Margolis, L.M., Madslien, E.H., Murphy, N.E., Castellani, J.W., Gundersen, Y., Hoke, A.V., Levangie, M.W., Kumar, R., Chakraborty, N. and Gautam, A., 2017. Changes in intestinal microbiota composition and metabolism coincide with increased intestinal permeability in young adults under prolonged physiological stress. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, *312*(6), pp.G559-G571.

258. De Vries, J.H., Hollman, P.C., Meyboom, S., Buysman, M.N., Zock, P.L., van Staveren, W.A. and Katan, M.B., 1998. Plasma concentrations and urinary excretion of the antioxidant flavonols quercetin and kaempferol as biomarkers for dietary intake. *The American journal of clinical nutrition*, *68*(1), pp.60-65.

259. Sukhotnik, I., Moati, D., Shaoul, R., Loberman, B., Pollak, Y. and Schwartz, B., 2018. Quercetin prevents small intestinal damage and enhances intestinal recovery during methotrexate-induced intestinal mucositis of rats. *Food & nutrition research*, *62*.

260. Amasheh, M., Schlichter, S., Amasheh, S., Mankertz, J., Zeitz, M., Fromm, M. and Schulzke, J.D., 2008. Quercetin enhances epithelial barrier function and increases claudin-4 expression in Caco-2 cells. *The Journal of nutrition*, *138*(6), pp.1067-1073.

261. Suzuki, T. and Hara, H., 2009. Quercetin enhances intestinal barrier function through the assembly of zonnula occludens-2, occludin, and claudin-1 and the expression of claudin-4 in Caco-2 cells. *The Journal of nutrition*, *139*(5), pp.965-974.

262. Dokladny, K., Ye, D., Kennedy, J.C., Moseley, P.L. and Ma, T.Y., 2008. Cellular and molecular mechanisms of heat stress-induced up-regulation of occludin protein expression: regulatory role of heat shock factor-1. *The American journal of pathology*, *172*(3), pp.659-670.

263. Freedman, J.E., Parker Iii, C., Li, L., Perlman, J.A., Frei, B., Ivanov, V., Deak, L.R., Iafrati, M.D. and Folts, J.D., 2001. Select flavonoids and whole juice from purple grapes inhibit platelet function and enhance nitric oxide release. *Circulation*, *103*(23), pp.2792-2798.

264. Oteiza, P.I., Fraga, C.G., Mills, D.A. and Taft, D.H., 2018. Flavonoids and the gastrointestinal tract: Local and systemic effects. *Molecular aspects of medicine*, *61*, pp.41-49.

265. Valenzano, M.C., DiGuilio, K., Mercado, J., Teter, M., To, J., Ferraro, B., Mixson, B., Manley, I., Baker, V., Moore, B.A. and Wertheimer, J., 2015. Remodeling of tight junctions and enhancement of barrier integrity of the CACO-2 intestinal epithelial cell layer by micronutrients. *PloS one*, *10*(7), p.e0133926.

266. Hosokawa, N., Hirayoshi, K., Kudo, H., Takechi, H., Aoike, A., Kawai, K. and Nagata, K., 1992. Inhibition of the activation of heat shock factor in vivo and in vitro by flavonoids. *Molecular and Cellular Biology*, *12*(8), pp.3490-3498.

267. Dokladny, K., Wharton, W., Lobb, R., Ma, T.Y. and Moseley, P.L., 2006. Induction of physiological thermotolerance in MDCK monolayers: contribution of heat shock protein 70. *Cell stress & chaperones*, *11*(3), p.268.

268. Sergent, T., Piront, N., Meurice, J., Toussaint, O. and Schneider, Y.J., 2010. Anti-inflammatory effects of dietary phenolic compounds in an in vitro model of inflamed human intestinal epithelium. *Chemico-Biological Interactions*, *188*(3), pp.659-667.

269. Knapik, J.J., Austin, K.G., Farina, E.K. and Lieberman, H.R., 2018. Dietary supplement use in a large, representative sample of the US armed forces. *Journal of the Academy of Nutrition and Dietetics*, *118*(8), pp.1370-1388.

270. Myburgh, K.H., 2014. Polyphenol supplementation: benefits for exercise performance or oxidative stress?. *Sports Medicine*, *44*(1), pp.57-70.

271. Nieman, D.C. and Mitmesser, S.H., 2017. Potential impact of nutrition on immune system recovery from heavy exertion: a metabolomics perspective. *Nutrients*, *9*(5), p.513.

272. Somerville, V., Bringans, C. and Braakhuis, A., 2017. Polyphenols and performance: A systematic review and meta-analysis. *Sports Medicine*, *47*(8), pp.1589-1599.

273. Szymanski, M.C., Gillum, T.L., Gould, L.M., Morin, D.S. and Kuennen, M.R., 2017. Short-term dietary curcumin supplementation reduces gastrointestinal barrier damage and physiological strain responses during exertional heat stress. *Journal of Applied Physiology*, *124*(2), pp.330-340.

274. Takei, M., 2012. Development of polaprezinc research. *Yakugaku zasshi: Journal of the Pharmaceutical Society of Japan*, *132*(3), pp.271-277.

275. Matsukura, T. and Tanaka, H., 2000. Applicability of Zinc Complex of L-Carnosine for Medical Use. *Biochemistry*, *65*(7), pp.817-823.

276. Mahmood, A., Fitzgerald, A.J., Marchbank, T., Ntatsaki, E., Murray, D., Ghosh, S. and Playford, R.J., 2007. Zinc carnosine, a health food supplement that stabilises small bowel integrity and stimulates gut repair processes. *Gut*, *56*(2), pp.168-175.

277. Roohani, N., Hurrell, R., Kelishadi, R. and Schulin, R., 2013. Zinc and its importance for human health: An integrative review. *Journal of Research in Medical Sciences*, *18*(2), p.144.

278. Sale, C., Artioli, G.G., Gualano, B., Saunders, B., Hobson, R.M. and Harris, R.C., 2013. Carnosine: from exercise performance to health. *Amino acids*, *44*(6), pp.1477-1491.

279. Watari, I., Oka, S., Tanaka, S., Aoyama, T., Imagawa, H., Shishido, T., Yoshida, S. and Chayama, K., 2013. Effectiveness of polaprezinc for low-dose aspirin-induced small-bowel mucosal injuries as evaluated by capsule endoscopy: a pilot randomized controlled study. *BMC gastroenterology*, *13*(1), p.108.

280. Omatsu, T., Naito, Y., Handa, O., Mizushima, K., Hayashi, N., Qin, Y., Harusato, A., Hirata, I., Kishimoto, E., Okada, H. and Uchiyama, K., 2010. Reactive oxygen species-quenching and anti-apoptotic effect of polaprezinc on indomethacin-induced small intestinal epithelial cell injury. *Journal of gastroenterology*, *45*(7), pp.692-702.

281. Fujii, Y., Matsura, T., Kai, M., Kawasaki, H. and Yamada, K., 2000. Protection by polaprezinc, an anti-ulcer drug, against indomethacin-induced apoptosis in rat gastric mucosal cells. *The Japanese Journal of Pharmacology*, *84*(1), pp.63-70.

282. Choi, H.S., Lim, J.Y., Chun, H.J., Lee, M., Kim, E.S., Keum, B., Seo, Y.S., Jeen, Y.T., Um, S.H., Lee, H.S. and Kim, C.D., 2013. The effect of polaprezinc on gastric mucosal protection in rats with ethanol-induced gastric mucosal damage: comparison study with rebamipide. *Life sciences*, *93*(2-3), pp.69-77.

283. Saunders, B., Elliott-Sale, K., Artioli, G.G., Swinton, P.A., Dolan, E., Roschel, H., Sale, C. and Gualano, B., 2017. β-alanine supplementation to improve exercise capacity and performance: a systematic review and meta-analysis. *Br J Sports Med*, *51*(8), pp.658-669.

284. Sakae, K. and Yanagisawa, H., 2014. Oral treatment of pressure ulcers with polaprezinc (zinc L-carnosine complex): 8-week open-label trial. *Biological trace element research*, *158*(3), pp.280-288.

285. Barbalho, S.M., Goulart, A.R., Quesada, K. and Bechara, M.D., 2016. Inflammatory bowel disease: can omega-3 fatty acids really help?. *Annals of gastroenterology*, *29*(1), pp.37-43.

286. Ashton, T., Young, I.S., Davison, G.W., Rowlands, C.C., McEneny, J., Van Blerk, C., Jones, E., Peters, J.R. and Jackson, S.K., 2003. Exercise-induced endotoxemia: the effect of ascorbic acid supplementation. *Free Radical Biology and Medicine*, *35*(3), pp.284-291.

287. Buchman, A.L., Killip, D., Ou, C.N., Rognerud, C.L., Pownall, H., Dennis, K. and Dunn, J.K., 1999. Short-term vitamin E supplementation before marathon running: a placebo-controlled trial. *Nutrition*, *15*(4), pp.278-283.

288. Raftery, T., Martineau, A.R., Greiller, C.L., Ghosh, S., McNamara, D., Bennett, K., Meddings, J. and O'Sullivan, M., 2015. Effects of vitamin D supplementation on intestinal permeability, cathelicidin and disease markers in Crohn's disease: Results from a randomised double-blind placebo-controlled study. *United European Gastroenterology Journal*, *3*(3), pp.294-302.

289. Carlson, J. and Slavin, J., 2016. Health benefits of fibre, prebiotics and probiotics: A review of intestinal health and related health claims. *Quality Assurance and Safety of Crops & Foods*, *8*(4), pp.539-554.

290. Buchman, A.L., O'Brien, W., Ou, C.N., Rognerud, C., Alvarez, M., Dennis, K. and Ahn, C., 1999. The effect of arginine or glycine supplementation on gastrointestinal function, muscle injury, serum amino acid concentrations and performance during a marathon run. *International journal of sports medicine*, *20*(05), pp.315-321.

291. Snipe, R.M. and Costa, R.J., 2018. Does biological sex impact intestinal epithelial injury, small intestine permeability, gastrointestinal symptoms and systemic cytokine profile in response to exertional-heat stress?. *Journal of sports sciences*, *36*(24), pp.2827-2835.

292. Lambert, G.P., Murray, R., Eddy, D., Scott, W., Laird, R. and Gisolfi, C.V., 1999. INTESTINAL PERMEABILITY FOLLOWING THE 1998 IRONMAN TRIATHLON. *Medicine & Science in Sports & Exercise*, *31*(5), p.S318.

293. JanssenDuijghuijsen, L., Van Norren, K., Grefte, S., Koppelman, S., Lenaerts, K., Keijer, J., Witkamp, R. and Wichers, H., 2017. Endurance exercise increases intestinal uptake of the peanut allergen Ara h 6 after peanut consumption in humans. *Nutrients*, *9*(1), p.84.

294. Nava, R.C., Zuhl, M.N., Moriarty, T.A., Amorim, F.T., Bourbeau, K.C., Welch, A.M., McCormick, J.J., King, K.E. and Mermier, C.M., 2019. The effect of acute glutamine supplementation on markers of inflammation and fatigue during consecutive days of simulated wildland firefighting. *Journal of occupational and environmental medicine*, *61*(2), pp.e33-e42.

295. Lis, D., Stellingwerff, T., Kitic, C.K., Ahuja, K.D. and Fell, J., 2015. No effects of a short-term gluten-free diet on performance in nonceliac athletes. *Medicine and science in sports and exercise*, *47*(12), pp.2563-2570.

296. Kashima, H., Harada, N., Miyamoto, K., Fujimoto, M., Fujita, C., Endo, M.Y., Kobayashi, T., Miura, A. and Fukuba, Y., 2017. Timing of postexercise carbohydrate-protein supplementation: roles of gastrointestinal blood flow and mucosal cell damage on gastric emptying in humans. *Journal of Applied Physiology*, *123*(3), pp.606-613.

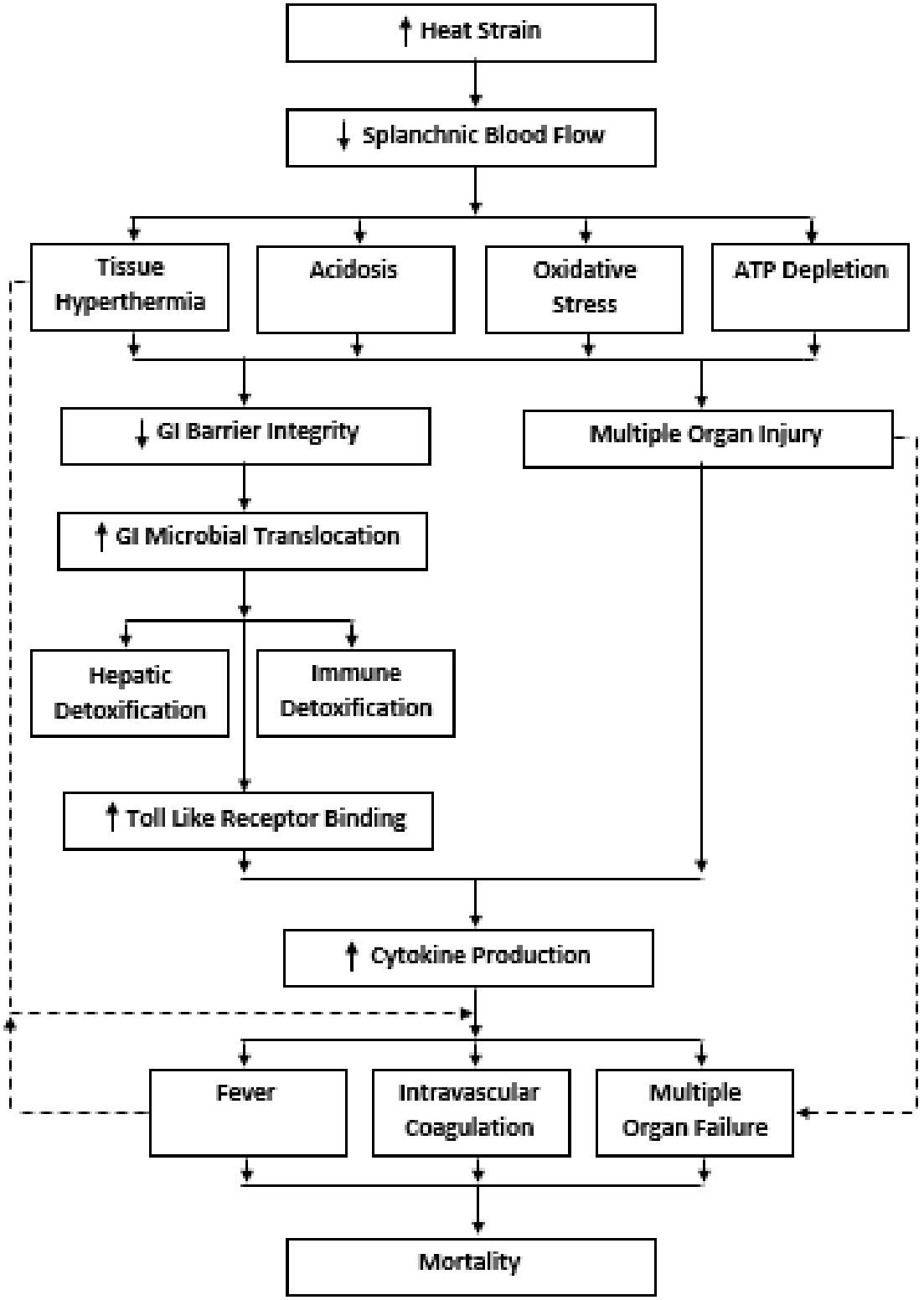
297. Antunes, B.M., Campos, E.Z., dos Santos, R.V.T., Rosa-Neto, J.C., Franchini, E., Bishop, N.C. and Lira, F.S., 2019. Anti-inflammatory response to acute exercise is related with intensity and physical fitness. *Journal of cellular biochemistry*, *120*(4), pp.5333-5342.

298. Guy, J.H., Edwards, A.M., Miller, C.M., Deakin, G.B. and Pyne, D.B., 2017. Short-term reliability of inflammatory mediators and response to exercise in the heat. *Journal of sports sciences*, *35*(16), pp.1622-1628.

299. Guy, J.H., Pyne, D.B., Deakin, G.B., Miller, C.M. and Edwards, A.M., 2016. Acclimation training improves endurance cycling performance in the heat without inducing endotoxemia. *Frontiers in physiology*, *7*, p.318.

300. Machado, P., Caris, A., Santos, S., Silva, E., Oyama, L., Tufik, S. and Santos, R., 2017. Moderate exercise increases endotoxin concentration in hypoxia but not in normoxia: A controlled clinical trial. *Medicine*, *96*(4), p.e5504.

301. Stuempfle, K.J., Valentino, T., Hew-Butler, T., Hecht, F.M. and Hoffman, M.D., 2016. Nausea is associated with endotoxemia during a 161-km ultramarathon. *Journal of sports sciences*, *34*(17), pp.1662-1668.



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1955 **Figure 1.** The gastrointestinal paradigm of exertional heat stroke

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Table 1. Overview of *In Vivo* techniques to assess GI Barrier Integrity

Technique	Sample	Method	Site	Limitations
Active Techniques				
Dual-Sugar Absorption Test (DSAT)	Urine or blood	HPLC (+) MS	Small GI Integrity	Gold-standard. High reliability. Time-consuming (5 hr urine, >2.5 hour blood). No standard protocol with exercise. Well-studied.
Multi-Sugar Absorption Test (MSAT)	Urine or blood	HLPC (+) MS	Entire GI Integrity	Gold-Standard. Segmental GI integrity. Time-consuming (5 hr urine, >2.5 hour blood). No standard protocol with exercise. Few studies.
Polyethylene Glycol (PEG) Absorption Test	Urine	HLPC (+) MS	Entire GI Integrity	Validated against MSAT. Can include multiple weight PEGs (e.g. 100, 400, 1000, 4000 kDa). Time-consuming (5 hr urine). Few studies.
Passive Techniques				
Intestinal Fatty Acid Binding Protein (I-FABP)	Urine or Blood	ELISA	Epithelial injury	Tissue specific (duodenum and jejunum). Short half-life (11 minutes). Weak correlations with DSAT. Well-studied.
Ileal Bile-Acid Binding Protein (I-BABP)	Urine or Blood	ELISA	Epithelial injury	Tissue specific (ileum). Few studies. Weak correlations with I-FABP. Few studies.
Diamine Oxidase (DAO), α-Glutathione s-Transferase (α-GST), Smooth Muscle 22 (SM22)	Blood	ELISA	Epithelial injury	Non-tissue specific. Few studies.
Claudin-3 (CLDN3)	Urine or Blood	ELISA	TJ Integrity	Non-tissue specific. Few studies.
Zonulin	Blood or Faeces	ELISA	TJ Integrity	Non-tissue specific. Assay cross-reactivity (complement C3). Moderate studies.
Endotoxin (LPS)	Blood	LAL assay	MT	Tissue specific. Sample contamination causes false-positives. Hepatic removal and receptor binding cause false-negatives. Well-studied.
LPS Binding Protein (LBP)	Blood	ELISA	MT	Tissue specific. Lower risk of false-positives than endotoxin. Indirect marker of endotoxin exposure. Influenced by hepatic production. Long half-life (12-14 hours). Few studies.
Soluable-CD14 (sCD14-ST)	Blood	ELISA	MT	Tissue specific. Lower risk of false positives than endotoxin. Influenced by hepatic production and monocytes shedding. Few studies.
D-lactate	Blood	ELISA	MT	Predominately tissue specific. Economical and time efficient assessment. Potentially influenced by methylglyoxal metabolism. Few studies.
16s Bacterial rDNA (bactDNA)	Blood	Real-time PCR assay	MT	Tissue specific. Novel. Lower risk of false-positives than endotoxin. Potential for regional integrity assessment. Few studies.

Abbreviations: HPLC, high performance liquid chromatography; MS, mass spectrometry; ELISA, enzyme-linked immunosorbent assay; LAL, limulus amoebocyte lysate assay; PCR, polymerase chain reaction

1961 **Table 2.** Influence of acute exercise-(heat) stress on small-intestine DSAT responses

Author	Subjects	Exercise Protocol	Peak T_{Core} (°C)	Mean HR (bpm)	Biofluid, DSAT L/R or L/M (timepoint)
van Nieuwenh-oven et al. [110]	10 male (MT)	90 minutes cycling at 70% $Watt_{max}$ (fasted) in T_{amb} 19°C (RH = N/A)	N/A	N/A	Urine L/R (5hr): 0.007 [s]
van Nieuwenh-oven et al. [118]	10 male (MT)	90 minutes cycling at 70% $Watt_{max}$ (fasted) in T_{amb} 19°C (RH = N/A)	38.8	N/A	Urine L/R (5hr): 0.008 [nb, c]
Nieman et al. [107]	20 male and female (UT)	45 minutes walking uphill (5% grade) at 60% VO2max (fasted) in T_{amb} not reported	N/A	132	Urine L/R (5hr): 0.009 [nb, c]
Smetanka et al. [123]	8 male (HT)	Chicago marathon (42.2 km) in T_{amb} (fed) 22°C (48% RH)	N/A	N/A	Urine L/R (5hr): 0.020 [ns]
Shing et al. [126]	10 male (HT)	~33 minutes running to fatigue at 80% VE (fed) in T_{amb} 35°C (40% RH)	39.4	172	Urine L/R (5hr): 0.022 [nb, c]
Janssen-Duijghuijsen et al. [109]	11 male (HT)	90 minutes cycling at 50% $watt_{max}$ (fed) in T_{amb} not reported following a *sleep-low* glycogen depletion regime	N/A	N/A	Urine L/R (5hr): ~0.022 [ns] Plasma L/R (1hr): ~0.110 [s]
Snipe et al. [115, 125]	6 male and 4 female (MT)	120 minutes running at 60% VO_{2max} (fed) in T_{amb} 22°C (44% RH)	38.5	~150	Urine L/R (5hr): 0.025 [nb]
Snipe et al. [125]	6 male and 4 female (MT)	120 minutes running at 60% VO_{2max} (fed) in T_{amb} 30°C (25% RH)	38.6	~155	Urine L/R (5hr): 0.026 [nb]
van Wijck et al. [127]	10 male (MT)	60 minutes cycling at 70% $watt_{max}$ (fasted) in T_{amb} not reported	N/A	N/A	Urine L/R (2hr): 0.027 [nb,c]
Snipe and Costa [291]	13 female (MT)	120 minutes running at 60% VO_{2max} (fed) in T_{amb} 35°C (25% RH)	38.8	~155	Urine L/R (5hr): 0.028 [nb]
Ryan et al. [120]	7 males (MT)	60 minutes running at 68% VO_{2max} (fasted) in T_{amb} not reported	N/A	N/A	Urine L/M (6hr): 0.029 [ns]
van Nieuwenh-oven et al. [112]	9 male and 1 female (MT)	90 minutes cycling at 70% $Watt_{max}$ (fasted) in T_{amb} 19°C (RH = N/A)	N/A	N/A	Urine L/R (5hr): 0.030 [ns]
van Wijck et al. [124]	9 male (MT)	60 minutes cycling at 70% $watt_{max}$ (fasted) in T_{amb} not reported	N/A	N/A	Urine L/R (2hr): 0.030 [s, c]
Pugh et al. [88]	11 male (MT-HT)	18x 400 metre sprint at 120% VO_{2max} (fed) in T_{amb} not reported	N/A	N/A	Urine L/R (2hr): 0.030 [ns] Serum L/R (2hr): ~0.051 [s]
Snipe and Costa [291]	11 male (MT)	120 minutes running at 60% VO_{2max} (fed) in T_{amb} 35°C (25% RH)	39.1	~150	Urine L/R (5hr): 0.030 [nb]
Buchman et al. [290]	17 male and 2 female	Competitive Marathon (fed) in T_{amb} 2°C with freezing rain	N/A	N/A	Urine L/R (6hr): 0.030 [ns, c]

Snipe et al. (Part B) [115]	6 male and 4 female (MT)	120 minutes running at 60% VO_{2max} (fed) in T_{amb} 35°C (26% RH)	39.6	~170	Urine L/R (5hr): 0.032 [nb]
Snipe et al. [193]	6 male and 5 female (MT)	120 minutes running at 60% VO_{2max} (fed) in T_{amb} 35°C (30% RH)	39.3	159	Urine L/R (5hr): 0.034 [nb, c]
March et al. [225]	9 male (MT)	20 minutes running at 80% VO_{2peak} (fasted) in T_{amb} 22°C (37% RH)	38.4	170	Urine L/R (5hr): 0.035 [s, c]
Pals et al. (Part A) [108]	5 male and 1 female (MT)	60 minutes running at 40% VO_{2peak} (fasted) in T_{amb} 22°C (50% RH)	38.0	N/A	Urine L/R (5hr): 0.036 [ns]
Marchbank et al. [113]	12 male (MT)	20 minutes running to fatigue at 80% VO_{2max} (fasted) in T_{amb} not reported	38.3	N/A	Urine L/R (5hr): 0.038 [s, c]
van Nieuwenh-oven et al. [111]	9 male and 1 female (MT)	90 minutes running at 70% VO_{2max} (fasted) in T_{amb} 19°C (RH = N/A)	N/A	N/A	Urine L/R (5hr): 0.040 [s]
van Wijck et al. [86]	6 male (HT)	60 minutes cycling at 70% $watt_{max}$ (fasted) in T_{amb} not reported	N/A	N/A	Urine L/R (5hr): 0.040 [ns] Plasma L/R (2.4hr): 0.060 [s]
Lambert et al. (Part A) [119]	11 male and 9 female (MT)	60 minutes running at 70% VO_{2max} (fasted) in T_{amb} 22°C (48% RH)	38.5	N/A	Urine L/R (5hr): 0.049 [ns, c]
Lambert et al. [122]	13 male and 4 female (HT)	60 minutes running at 70% VO_{2max} (fasted) in T_{amb} 22°C (48% RH)	38.3	N/A	Urine L/R (5hr): 0.050 [nb, c]
Zuhl et al. [211]	4 male and 3 female (LT/MT)	60 minutes running at 70% VO_{2max} (fasted) in T_{amb} 30°C (12-20% RH)	39.4	N/A	Urine L/R (5hr): 0.060 [nb, c]
Zuhl et al. [116]	2 male and 5 female (LT/MT)	60 minutes running at 70% VO_{2max} (fasted) in T_{amb} 30°C (12-20% RH)	39.5	N/A	Urine L/R (5hr): 0.060 [nb, c]
Lambert et al. (Part B) [119]	11 male and 9 female (MT)	60 minutes running at 70% VO_{2max} (fasted) in T_{amb} 22°C (48% RH) without fluid ingestion	38.5	N/A	Urine L/R (5hr): 0.063 [s, c]
Pals et al. (Part B) [108]	5 male and 1 female (MT)	60 minutes running at 40% VO_{2peak} (fasted) in T_{amb} 22°C (50% RH)	38.7	N/A	Urine L/R (5hr): 0.064 [ns]
Lambert et al. [121]	8 male (MT)	60 minutes running at 70% VO_{2max} (fasted) in T_{amb} 22°C (48% RH)	38.3	N/A	Urine L/R (5hr): 0.065 [nb, c]
Buchman et al. [287]	15 male and female (LT-HT)	Road marathon (42.2 km) (fed) in T_{amb} not reported	N/A	N/A	Urine L/M (6hr): 0.070 [ns, c]
Pugh et al. [212]	10 male (MT)	60 minutes at 70% VO_{2max} running (fasted) in T_{amb} 30°C (4-45% RH)	38.5	82.5% of max	Serum L/R (2hr): ~0.080 [s, c]
Pugh et al. [148]	10 male and 2 female (MT)	42.4 km track marathon (247 ± 47 minutes; fed) in T_{amb} 16-17°C (N/A RH)	N/A	~160	Serum L/R (1hr) 0.081 (37%) [s, c]

Lambert et al. [292]	12 female (LT-HT)	Hawaii Ironman (fed) in T_{amb} not reported	N/A	N/A	Urine L/R (5hr): 0.087 [nb]
Davison et al. [114]	8 male (MT/HT)	20 minutes running to fatigue at 80% VO_{2max} (fasted) in T_{amb} not reported	39.3	~170	Urine L/R (5hr): 0.098 [s, c]
Janssen-Duijghuijsen et al. [293]	4 male and 6 female (LT)	60 minutes cycling at 70% $watt_{max}$ (fed) in T_{amb} not reported	N/A	N/A	Plasma L/R (1hr): ~0.100 [s]
Lambert et al. [292]	29 male (LT-HT)	Hawaii Ironman (fed) in T_{amb} not reported	N/A	N/A	Urine L/R (5hr): 0.105 [nb]
Pals et al. (Part C) [108]	5 male and 1 female (MT)	60 minutes running at 40% VO_{2peak} (fasted) in T_{amb} 22°C (50% RH)	39.6	N/A	Urine L/R (5hr): 0.107 [s]

LT = Low-trained (35-49 ml·kg·min^{-1} VO_{2max}); MT = Moderate-trained (50-59 ml·kg·min^{-1} VO_{2max}); HT = High-trained (60+ ml·kg·min^{-1} VO_{2max}). s = significant change post-exercise ($p < 0.05$); ns = non-significant change post-exercise ($p > 0.05$); nb = no baseline resting data to compare against; c = control/placebo trial of study

1980 **Table 3.** Influence of acute exercise-(heat) stress on systemic I-FABP concentrations

Reference	Subjects	Exercise Protocol	Peak T_{Core} (°C)	Mean HR (bpm)	FABP2 (Δ pre-to-post exercise)
Janssen-Duijghuijsen et al. [109]	11 male (HT)	90 minutes cycling at 50% $watt_{max}$ (fed) in T_{amb} not reported following a "sleep-low" glycogen depletion regime	N/A	N/A	~-90 pg·ml^{-1} (~-65%) [c]
Kartaram et al. (Part A) [129]	15 male (MT)	60 minutes cycling at 50% $watt_{max}$ (fed) in T_{amb} not reported	N/A	N/A	~-50 pg·ml^{-1} (~-10%) [ns]
Lee and Thake (Part A) [128]	7 male (MT)	60 minutes cycling at 50% VO_{2max} (fed) in T_{amb} 18°C (35% RH) on day one of temperate acclimation	37.9	133	28 pg·ml^{-1} (8%) [ns,c]
Trommelen et al. [130]	10 male (HT)	180 minutes cycling at 50% $watt_{max}$ (fasted) in T_{amb} 18-22°C (55-65% RH)	N/A	N/A	N/A pg·ml^{-1} (20%) [ns,c]
Edinburgh et al. (Part A) [180]	12 male (MT)	60 minutes cycling at 50% VO_{2max} (fed) in T_{amb} 18°C (35% RH)	N/A	N/A	70 pg·ml^{-1} (34%) [s]
Edinburgh et al. (Part B) [180]	12 male (MT)	60 minutes cycling at 50% VO_{2max} (fasted) in T_{amb} 18°C (35% RH)	N/A	N/A	88 pg·ml^{-1} (20%) [s]
Osborne et al. (Part A) [133]	8 male (MT-HT)	30 minutes cycling at 50/70% $Watt_{max}$, then 30 minutes at 50% $watt_{max}$ (fasted) in T_{amb} 20°C (55% RH)	38.5	139	138 pg·ml^{-1} (29%) [ns]
Salvador et al. 2019 [181]	12 male (MT-HT)	120 minutes cycling at 60% VO_{2max} (fed) then 30-40 minutes (20 km) time trial in T_{amb} not reported	37.9	~168	N/A pg·ml^{-1} (~50%) [s, c]
van Wijck et al. [127]	10 male (MT)	60 minutes cycling at 70% $watt_{max}$ (fasted) in T_{amb} not reported	N/A	N/A	153 pg·ml^{-1} (72%) [s]
Nava et al. [294]	7 male and 4 female (LT-MT)	56 minutes mixed intensity (~55% VO_{2max}) discontinuous firefighting exercises (fed) in T_{amb} 38°C (35% RH) on day one of two	38.7	~161	~160 pg·ml^{-1} (23%) [ns, c]
Van Wijck et al. [124]	9 male (MT)	60 minutes cycling at 70% $watt_{max}$ (fasted) in T_{amb} not reported	N/A	N/A	179 pg·ml^{-1} (61%) [s]
Lee et al. (Part C) [128]	7 male (MT)	60 minutes cycling at 50% VO_{2max} (fed) in T_{amb} 18°C (35% RH) and F_iO_2 = 0.14% on day one of hypoxic acclimation	38.2	149	193 pg·ml^{-1} (43%) [s,c]
Lis et al. [295]	13 male and female (MT)	45 minutes cycling at 70% $watt_{max}$ and 15 min cycling time trial (fed) in 20°C (40% RH)	N/A	168	210 pg·ml^{-1} (223%) [s,c]
Pugh et al. [148]	10 male (MT)	60 minutes at 70% VO_{2max} running (fasted) in T_{amb} 30°C (4-45% RH)	38.5	82.5% of HR max	250 pg·ml^{-1} (71%) [s,c]
Snipe et al. (Part A) [115, 125]	6 male and 4 female (MT)	120 minutes running at 60% VO_{2max} (fed) in T_{amb} 22°C (44% RH)	38.5	~150	274 pg·ml^{-1} (127%) [s]
Sheahen et al. (Part A) [136]	12 male (MT)	45 minutes running at 70% VO_{2max} (fasted) in T_{amb} 20°C (40% RH)	38.2	165	281 pg·ml^{-1} (49%) [s]

Lee et al. (Part B) [128]	7 male (MT)	60 minutes cycling at 50% VO_{2max} (fed) in T_{amb} 40°C (25% RH) on day one of heat acclimation	38.7	151	282 pg·ml^{-1} (76%) ^{s,c}
Morrison et al. (Part B) [152]	8 male (UT)	30 minutes cycling at 50% heart rate reserve (HRR), 30 minutes jogging at 80% HRR and 30 minute running time trial (fed) in T_{amb} 30°C (50% RH)	38.6	N/A	283 pg·ml^{-1} (276%) ^{s,c}
Barberio et al. [72]	9 male (MT)	~24 minutes running at 78% VO_{2max} (fed) in T_{amb} 40°C (40% RH) prior to heat acclimation	39.0	N/A	297 pg·ml^{-1} (46%) ^{s,c}
Hill et al. [134]	10 male (MT)	60 minutes running at 65% VO_{2max} (fasted) in T_{amb} not reported	N/A	~170	300 pg·ml^{-1} (50%) ^{ns,c}
van Wijck et al. [86]	15 male (HT)	60 minutes cycling at 70% $watt_{max}$ (fasted) in T_{amb} not reported	N/A	N/A	306 pg·ml^{-1} (61%) ^{s}
Kashima et al. [296]	5 male and 3 female (MT)	30 intermittent 20 second cycle sprints at 120% $watt_{max}$, with 40 seconds recovery between each (fed) in 23°C (40% RH)	N/A	150	343 pg·ml^{-1} (266%) ^{s}
Pugh et al. [88]	11 male (MT-HT)	18x 400 metre sprint at 120% VO_{2max} (fed) in T_{amb} not reported	N/A	N/A	348 pg·ml^{-1} (72%) ^{s}
March et al. [225]	9 male (MT)	20 minutes running at 80% VO_{2peak} (fasted) in T_{amb} 22°C (37% RH)	38.4	170	350 pg·ml^{-1} (61%) ^{s,c}
Janssen-Duijghuijsen et al. [293]	4 male and 6 female (LT)	60 minutes cycling at 70% $watt_{max}$ (fed) in T_{amb} not reported	N/A	N/A	~350 pg·ml^{-1} (~77%) ^{s,c}
Sheahen et al. (Part B) [136]	12 male (MT)	45 minutes running at 70% VO_{2max} (fasted) in T_{amb} 30°C (40% RH)	38.3	163	369 pg·ml^{-1} (63%) ^{s}
Costa et al. [135]	11 male (MT-HT)	120 minutes running at 70% VO_{2max} (fed) in T_{amb} 25°C (35% RH)	N/A	148	371 pg·ml^{-1} (86%) ^{ns,c}
Osborne et al. [213]	12 male (MT-HT)	33 minutes (20 km) cycling time trial (fasted) in 35°C (50% RH)	39	167	441 pg·ml^{-1} (83%) ^{s,c}
Kartaram et al. (Part B) [129]	15 male (MT)	60 minutes cycling at 70% $watt_{max}$ (fed) in T_{amb} not reported	N/A	N/A	~500 pg·ml^{-1} (~66%) ^{s}
Kartaram et al. (Part C) [129]	15 male (MT)	60 minutes cycling at 85/55% $watt_{max}$ (fed) in T_{amb} not reported	N/A	N/A	~500 pg·ml^{-1} (~66%) ^{s}
McKenna et al. [226]	10 male (MT)	46 minutes running at 95% VE threshold (fasted) in T_{amb} 40°C (50% RH)	39.7	N/A	516 pg·ml^{-1} (52%) ^{s,c}
Karhu et al. [132]	17 male (MT-HT)	90 minutes running at 80% of best 10 km race time (fed) in T_{amb} not reported	N/A	N/A	531 pg·ml^{-1} (151%) ^{s}
Snipe and Costa [137]	6 male and 6 female (MT)	120 minutes running at 60% VO_{2max} (fed) in T_{amb} 30°C (35% RH)	38.8	160	573 pg·ml^{-1} (184%) ^{s,c}
Snipe et al. (Part B) [125]	6 male and 4 female (MT)	120 minutes running at 60% VO_{2max} (fed) in T_{amb} 30°C (25% RH)	38.6	~155	~ 580 pg·ml^{-1} (184%)

Hill et al. [134]	10 male (MT)	60 minutes running at 65% VO_{2max} (fasted) in T_{amb} not reported (F_iO_2 = 13.5%)	N/A	~170	700 pg·ml^{-1} (168%) ^{ns,c}
Osborne et al. (Part B) [133]	8 Male (MT-HT)	30 minutes cycling at 50/70% $Watt_{max}$, then 30 minutes at 50% $watt_{max}$ (fasted) in T_{amb} 35°C (53% RH)	39.5	159	608 pg·ml^{-1} (140%) ^{s}
Szymanski et al. [273]	6 male and 2 female (LT/MT)	60 minutes running at 68% VO_{2max} (fasted) in T_{amb} 37°C (25% RH)	39.0	174	800 pg·ml^{-1} (87%) ^{s,c}
Morrison et al. (Part A) [152]	7 male (HT)	30 minutes cycling at 50% heart rate reserve (HRR), 30 minutes jogging at 80% HRR and 30 minute running time trial (fed) in T_{amb} 30°C (50% RH)	38.6	N/A	806 pg·ml^{-1} (663%) ^{s,c}
Snipe et al. [193]	6 male and 5 female (MT)	120 minutes running at 60% VO_{2max} (fed) in T_{amb} 35°C (30% RH)	39.3	159	897 pg·ml^{-1} (288%) ^{s,c}
Snipe et al. (Part B) [115]	6 male and 4 female (MT)	120 minutes running at 60% VO_{2max} (fed) in T_{amb} 35°C (26% RH)	39.6	~170	1230 pg·ml^{-1} (432%) ^{s}
Pugh et al. [148]	10 male and 2 female (MT)	42.4 km track marathon (247 ± 47 minutes; fed) in T_{amb} 16-17°C (N/A RH)	N/A	~160	1246 pg·ml^{-1} (371%) $^{s, c}$
March et al. [105]	12 male (MT)	60 minutes running at 70% VO_{2max} (fasted) in T_{amb} 30°C (60% RH)	39.3	170	1263 pg·ml^{-1} (407%) $^{s, c}$
Snipe and Costa [291]	11 male (MT)	120 minutes running at 60% VO_{2max} (fed) in T_{amb} 35°C (25% RH)	39.1	~150	1389 pg·ml^{-1} (479%) ^{s}
Snipe et al. [291]	13 female (MT)	120 minutes running at 60% VO_{2max} (fed) in T_{amb} 35°C (25% RH)	38.8	~155	1445 pg·ml^{-1} (479%) ^{s}
Jonvik et al. [131]	16 male (HT)	60 minutes cycling at 70% $watt_{max}$ (fasted) in T_{amb} not reported	N/A	N/A	1745 pg·ml^{-1} (249%) ^{s}
Gaskell et al. [147]	10 male and 8 female (MT-HT)	120 minutes running at 60% VO_{2max} (fed) in T_{amb} 35°C (25% RH)	38.6	~151	1805 pg·ml^{-1} (710%) $^{s, c}$

LT = Low-trained (35-49 ml·kg·min^{-1} VO_{2max}); MT = Moderate-trained (50-59 ml·kg·min^{-1} VO_{2max}); HT = High-trained (60+ ml·kg·min^{-1} VO_{2max}). s = significant change post-exercise ($p < 0.05$); ns = non-significant change post-exercise ($p > 0.05$); c = control/placebo trial of study

1990 **Table 4.** Influence of acute exercise-(heat) stress on systemic gastrointestinal microbial
1991 translocation responses

Reference	Subjects	Exercise Protocol	Peak T_{core} (°C)	Mean HR (bpm)	Endotoxin (Δ pre-to-post exercise)
Antunes et al. [297]	19 male (MT)	56 ± 7 minutes cycling at 90% of first ventilatory threshold (fasted) in 22.1°C (55% RH)	N/A	[141]	-3 pg·ml^{-1} (-3%) [ns]
Yeh et al. (Part B) [138]	15 male and 1 female (LT)	60 minutes running at 70% VO_{2max} (fed) in T_{amb} 22°C (66% RH)	38.4	~145	-1.1 pg·ml^{-1} (-10%) [ns]
Zuhl et al. [116]	2 male and 5 female (LT/MT)	60 minutes running at 70% VO_{2max} (fasted) in T_{amb} 30°C (12-20% RH)	39.5	N/A	-0.2 pg·ml^{-1} (-7%) [ns, c]
Osborne et al. (Part A) [133]	8 Male (MT-HT)	30 minutes cycling at 50/70% $Watt_{max}$, then 30 minutes at 50% $watt_{max}$ (fasted) in T_{amb} 20°C (55% RH)	38.5	165	0.1 pg·ml^{-1} (1%) [ns, #]
Osborne et al. (Part B) [133]	8 Male (MT-HT)	30 minutes cycling at 50/70% $Watt_{max}$, then 30 minutes at 50% $watt_{max}$ (fasted) in T_{amb} 35°C (53% RH)	39.5	182	0.2 pg·ml^{-1} (1%) [s, #]
Karhu et al. [132]	17 males (MT-HT)	90 minutes running at 80% of best 10 km race time (fed) in T_{amb} not reported	N/A	N/A	0.3 pg·ml^{-1} (~ 1%) [ns, c]
Kuennen et al. [143]	8 male (MT)	100 minutes walking (6.3 km·h^{-1}) at 50% VO_{2max} (fasted) in T_{amb} 46.5°C (20% RH)	39.3	N/A	~0.5 pg·ml^{-1} (10%) [ns, c]
Ng et al. [73]	30 males (HT)	Half-marathon (fed) in T_{amb} 27°C (84% RH)	40.7	172	0.6 pg·ml^{-1} (32%) [s]
Jeukendrup et al. [144]	29 male and 1 female (HT)	Ironman (3.8 km swim; 185 km cycle; 42.2 km run) (fed) in T_{amb} 9-32°C	N/A	N/A	1.7 pg·ml^{-1} (666%) [s]
Guy et al. [298]	20 male (LT-MT)	10 minutes cycling at 50%, 60%, and 70% $watt_{max}$, then 5 km (fasted) in T_{amb} 35°C (70% RH)	38.9	160	2 pg·ml^{-1} (9%) [ns]
Selkirk et al. (Part B) [126]	12 male (HT)	To fatigue (~122 minutes) uphill walk at 4.5 km.h^{-1} (fasted) in T_{amb} 40°C (30% RH)	39.7	156	~3 pg·ml^{-1} (200%) [s]
Shing et al. [146]	10 male (HT)	~33 minutes running to fatigue at 80% VE (fed) in T_{amb} 35°C (40% RH)	39.4	172	4 pg·ml^{-1} (15%) [s]
Snipe et al. (Part A) [115, 125]	6 male and 4 female (MT)	120 minutes running at 60% VO_{2max} (fed) in T_{amb} 22°C (44% RH)	38.5	~150	4.1 pg·ml^{-1} (5%) [ns]
Yeh et al. (Part B) [138]	15 male and 1 female (LT)	60 minutes running at 70% VO_{2max} (fed) in T_{amb} 33°C (50% RH)	39.3	~145	5 pg·ml^{-1} (54%) [s]
Antunes et al. (Part B) [297]	19 male (MT)	45 ± 18 minutes cycling at midpoint between first and second ventilatory threshold (fasted) in 22.1°C (55% RH)	N/A	[162]	5 pg·ml^{-1} (7%) [ns]

Antunes et al. (Part C) [297]	19 male (MT)	10 ± 9 minutes cycling at midpoint between second ventilatory threshold and maximal aerobic power (fasted) in 22.1°C (55% RH)	N/A	[180]	6 pg·ml^{-1} (5%) [ns]
Ashton et al. [286]	10 males (LT)	VO_{2max} test (~15 minutes)- on cycle ergometer (fasted) in T_{amb} not reported	N/A	N/A	9.4 pg·ml^{-1} (72%) [s]
Snipe et al. (Part B) [115]	6 male and 4 female (MT)	120 minutes running at 60% VO_{2max} (fed) in T_{amb} 35°C (26% RH)	39.6	~170	9.8 pg·ml^{-1} (11%) [s]
Gill et al. [149]	8 male (MT-HT)	120 minutes running at 60% VO_{2max} (fed) in T_{amb} 32°C (34% RH)	38.6	165	10 pg·ml^{-1} (4%) [ns, c]
Snipe et al. [193]	6 male and 5 female (MT)	120 minutes running at 60% VO_{2max} (fed) in T_{amb} 35°C (30% RH)	39.3	159	10 pg·ml^{-1} (N/A %) [nb]
Selkirk et al. (Part A) [126]	11 male (LT-MT)	To fatigue (~106 minutes) uphill walk at 4.5 km.h^{-1} (fasted) in T_{amb} 40°C (30% RH)	39.1	164	~10 pg·ml^{-1} (300%) [s]
Lim et al. (Part B) [150]	9 male (HT)	To fatigue (time not given) at 70% VO_{2max} (fed) in T_{amb} 35°C (40% RH)	39.5	N/A	13 pg·ml^{-1} (92%) [s,c]
Guy et al. [299]	8 male (LT)	10 minutes cycling at 50%, 60%, and 70% $watt_{max}$, then 5 km (fasted) in T_{amb} 35°C (70% RH)	38.6	161	16 pg·ml^{-1} (9%) [ns, c, #]
Gill et al. [71]	13 male and 6 female (HT)	Multistage ultra-marathon stage 1 (37 km) (fed) in T_{amb} 32-40°C (32-40% RH)	N/A	N/A	40 pg·ml^{-1} (14%) [s]
Barberio et al. [72]	9 male (MT)	~24 minutes running at 78% VO_{2max} (fed) in T_{amb} 40°C (40% RH) prior to heat acclimation	39.0	N/A	40 pg·ml^{-1} (57%) [s,c]
Moss et al. [151]	9 male (HT)	45 minutes cycling at 40% PPO (unstated prandial state) in T_{amb} 40°C (50% RH) prior to heat acclimation	38.9	153	52 pg·ml^{-1} (27%) [s,c]
Costa et al. [135]	11 male (MT-HT)	120 minutes running at 70% VO_{2max} (fed) in T_{amb} 25°C (35% RH)	N/A	148	96 pg·ml^{-1} (46%) [ns, c, #]
Gill et al. [145]	14 male and 3 female (HT)	24 hour ultramarathon (fed) in T_{amb} 0-20°C (54-82% RH)	N/A	N/A	122 pg·ml^{-1} (37%) [s, #]
Machado et al. (Part A) [300]	9 male (MT)	60 minutes running at 50% VO_{2max} (fasted) in T_{amb} not reported	N/A	N/A	130 pg·ml^{-1} (33%) [ns, #]
Machado et al. (Part B) [300]	9 male (MT)	60 minutes running at 50% VO_{2max} (fasted) in T_{amb} not reported (FIO_2 = 13.5%)	N/A	N/A	250 pg·ml^{-1} (48%) [s, #]
Gaskell et al. [147]	10 male and 8 female (MT-HT)	120 minutes running at 60% VO_{2max} (fed) in T_{amb} 35°C (25% RH)	38.6	~151	LBP ~-2 µg·ml^{-1} (N/A%) [ns, c]
Selkirk et al. (Part A) [146]	11 male (HT)	To fatigue (~163 minutes) uphill walk at 4.5 km.h^{-1} (fasted) in T_{amb} 40°C (30% RH)	39.1	164	LBP ~0 µg·ml^{-1} (0%) [ns]

Moncada-Jiminez et al. [195]	11 male (MT-HT)	135-minute laboratory duathlon at 71% VO_{2max} (15km run and 30km cycle) (fasted) in T_{amb} not reported	38.5	N/A	LBP ~0.59 µg·ml^{-1} (22%) $^{s, c}$
Selkirk et al. (Part B) [146]	12 male (LT-MT)	To fatigue (~106 minutes) uphill walk at 4.5 km.h^{-1} (fasted) in T_{amb} 40°C (30% RH)	39.7	156	LBP ~1.5 µg·ml^{-1} (15%) ^{s}
Jonvik et al. [131]	16 male (HT)	60 minutes cycling at 70% $watt_{max}$ (fasted) in T_{amb} not reported	N/A	N/A	LBP 1.6 µg·ml^{-1} (13%) ^{s}
Costa et al. [135]	11 male (MT-HT)	120 minutes running at 70% VO_{2max} (fed) in T_{amb} 25°C (35% RH)	N/A	148	sCD14-ST 0.05 µg·ml^{-1} (N/A%) ^{ns,c}
Gaskell et al. [147]	10 male and 8 female (MT-HT)	120 minutes running at 60% VO_{2max} (fed) in T_{amb} 35°C (25% RH)	38.6	~151	sCD14-ST 0.1 µg·ml^{-1} (N/A%) $^{s, c}$
Stuempfle et al. [301]	15 male and 5 female (MT)	161-km ultramarathon (26.8 ± 2.4 hours; fed) in T_{amb} 0-30°C (N/A RH)	38.3	N/A	sCD14-ST 0.6 µg·ml^{-1} (63%) ^{s}
Pugh et al. [148]	10 male and 2 female (MT)	42.4 km track marathon (4.1 ± 0.8 hours; fed) in T_{amb} 16-17°C (N/A RH)	N/A	~160	sCD14-ST 5.4 µg·ml^{-1} (164%) $^{s, c}$

LT = Low-trained (35-49 ml·kg·min^{-1} VO_{2max}); MT = Moderate-trained (50-59 ml·kg·min^{-1} VO_{2max}); HT = High-trained (60+ ml·kg·min^{-1} VO_{2max}). s = significant change post-exercise ($p < 0.05$); ns = non-significant change post-exercise ($p > 0.05$); nb = no baseline resting data to compare with; c = control/placebo trial of study. # Where data have been converted from EU·ml^{-1} to pg·ml^{-1} through standard conversions (1 EU·ml^{-1} = 100 pg·ml^{-1})

2008 **Table 5.** Evidence basis of nutritional supplements to help protect exercise-induced GI barrier
2009 integrity loss

Nutrient	Evidence	Dosing	Consensus and Limitations
Carbohydrate	Cell: - - Clinical: + + - Exercise: + + +	30-108 g·kg·h^{-1} liquid multi-transportable CHO.	Effects of pre- exercise CHO status or solid CHO ingestion unknown. Greater exploration on CHO timing and types required.
L- Glutamine	Cell: + + + - Clinical: + + - Exercise: + + +	0.25-0.9 g·kg·FFM.$^{-1}$ given 1-2 hours pre-exercise.	Dose ≥ 0.25g·kg·FFM^{-1} appears favourable. High doses poorly tolerated in some individuals. No evidence during prolonged exercise or on MT.
Bovine Colostrum	Cell: + + + + Clinical: + + + Exercise: + +	20 g·day^{-1} for 14 days pre-exercise	Potentially useful following less demanding exercise. No effects with short-term supplementation. Certain formulations might be more beneficial.
Nitric Oxide	Cell: + + Clinical: + + Exercise: - -	More evidence required	No benefits of L-citrulline or sodium nitrate. Nitrate ingestion might compromise thermoregulation with exercise in the heat. Only two human exercise studies.
Probiotics	Cell: + - Clinical: + + - - Exercise: + - -	More evidence required	Contrasting results between formulations. Multi-strain probiotics seem favourable. Negative responses have been reported. Further evidence required.
Polyphenols	Cell: + + - - Clinical: + - Exercise: + -	3 days of 0.5 g·day^{-1} of curcumin. Quercetin not recommended	Contrasting results between formulations. Only two human exercise studies. Further evidence required.
Zinc Carnosine	Cell: + + + Clinical: + + Exercise: +	75 mg·day^{-1} for ≥ 2 days	Unknown effects in severe exercise situations. A 150 mg·day^{-1} dose warrants research. Only one human exercise study. Further evidence required.

2010