The harmful impact, mechanism and potential treatment of exertional and non-exertional hyperthermia

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List of abbreviations

Abbreviation	Meaning
AKI	Acute kidney injury
BAT	Brown adipose tissue
BBB	Blood–brain barrier
CHS	Classical heatstroke
CI	Confidence interval
Da	Dalton
DIC	Disseminated intravascular coagulopathy
DNA	Deoxyribonucleic acid
EAC	Exercise-associated collapse
EHI	Exertional heat illness
EHS	Exertional heatstroke
EU	European Union
Ex	Exercise
Ex-Heat	Exercise in heat
GI	Gastrointestinal
HR	Heart rate
HWI	Hot water immersion
I-FABP	Intestinal fatty acid-binding protein
IL	Interleukin
INFγ	Interferon gamma
IQR	Interquartile range
KDIGO	Kidney Disease: Improving Global Outcomes
LPS	Lipopolysaccharides
L:R	Lactulose:rhamnose concentration ratio
MH	Malignant hyperthermia
MRI	Magnetic resonance imaging
NMS	Neuroleptic malignant syndrome
OVLT	Organum vasculosum of the lamina terminalis
PCT	Procalcitonin
PG	Prostaglandin
POA	Pre-optic area
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses

Abbreviation	Meaning
RNA	Ribonucleic acid
RoB	Risk of bias
RR	Relative risk
SCr	Serum creatinine
TNFα	Tumour necrosis factor alpha
TREK	TWIK-related potassium channel
TRP	Transient receptor potential
$\dot{V}O_{2max}$	Maximum oxygen consumption rate
WBGT	Wet-bulb globe temperature
WBSL	Whole-body sweat loss

List of definitions

Term	Definition
Classical heatstroke	Heatstroke occurring in individuals at rest or minimal exertion.
Exertional heatstroke	Heatstroke occurring in individuals undergoing exercise or exertion.
Heat illness	Spectrum of medical conditions that may occur as a result of hyperthermia. Heatstroke is the most severe form.
Heat stress	The pathophysiological mechanisms underlying the development of heat illness.
Heatstroke	Core temperature above 40.0°C with central nervous system dysfunction [1].
Hyperthermia	Core temperature above normal. Often taken as greater than 38.2°C.

NB: *Many of these terms have no universally agreed definition but are defined here for consistency below.*

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Primary publications

1. Hodgson LE, Walter E, Venn RM, Galloway R, Pitsiladis Y, Sardat F, Forni L. Acute kidney injury associated with endurance events – is it a cause for concern? A systematic review. BMJ Open Sport Exerc Med. 2017; 3: e000093.

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Abstract

The harmful impact of exertional and non-exertional hyperthermia

This publication-based thesis aims to add to the existing knowledge surrounding three consecutive aspects of hyperthermia: (1) the threats to human health from hyperthermia, (2) the putative mechanisms underlying the systemic damage, and (3) potential treatments. The six primary research papers forming the basis of the thesis have been published in peer-reviewed journals, using a variety of methodologies, including field studies, lab-based studies and systematic reviews.

Prolonged exertion, especially if causing hyperthermia, and passive heat illness, for example in heat waves or due to adverse effects of certain medications, are common causes of multi-organ failure and death. Similarities between the various hyperthermic states suggest that the raised temperature rather than the underlying cause in itself is predominant. Two of these effects, acute kidney injury (AKI) and electrolyte disturbances in exertional heat illness (EHI), are examined further in papers one and two.

The deleterious effects of hyperthermia may include the heat rendering the intestinal tract more permeable, allowing bacteria and toxins from the intestinal tract to enter the systemic circulation. Endurance exercise also appears to stimulate a systemic inflammatory response and increase intestinal permeability. Hyperthermia, especially when associated with exertion, is further characterised in papers three and four, which found that the intestinal permeability was higher after exercise-induced hyperthermia than passive hyperthermia or exercise, respectively, alone, suggesting synergistic effects.

There may therefore be the possibility of reducing the systemic damage caused by the exertional and non-exertional hyperthermia by reducing the inflammatory response or the bacterial load in the circulation. Papers five and six are systematic reviews suggesting that in animals both drugs appear to be beneficial in reducing the harm and risk of death from hyperthermia.

The work presented here allows the possibility for development of future work on reducing the inflammatory response and intestinal toxin absorption, for example with steroids or antibiotics, to reduce the risk of death and disability after heatstroke and hyperthermia.

Aims and objectives

This publication-based thesis aims to add to the existing knowledge surrounding three consecutive aspects of hyperthermia: (1) the threats to human health from hyperthermia, (2) the putative mechanisms underlying the systemic damage, and (3) potential treatments.

After a discussion of the physiology of thermoregulation, the first section describes the common causes of hyperthermia and what is known about the effects of hyperthermia on organ function and outcomes. Papers one and two, summarised in this section, further characterise the risk of AKI associated with endurance events, specifically in athletes who develop EHI, and the changes that occur in electrolyte balance and renal function in runners who collapse and develop exertional heal illness.

The second section discusses what is known about the mechanisms underlying the cellular and organ damage associated with heat illness, which include direct cellular damage from thermal injury and loss of gastrointestinal (GI) integrity, which may allow translocation of intestinal toxins and bacteria into the systemic circulation. This section includes discussion of publications three and four, which further characterise the effect of exercise-induced hyperthermia on GI permeability compared with exercise or passive hyperthermia alone.

The third section explores what is currently known about the proposed pathway of endotoxaemia from intestinal bacteria entering the systemic circulation, and the possibility of reducing systemic damage from hyperthermia by reducing the inflammatory response or the intestinal bacterial load in the circulation. This section includes discussion of publications five and six, which are systematic reviews on the effect of administration of steroids and antibiotics on the outcomes of heat illness.

It is hoped that the research presented in this thesis will add to what is currently known, to allow further research into potential treatments to improve outcomes from these common conditions.

Personal biography and project motivation

I currently work as a consultant in Intensive Care Medicine and Anaesthesia in the UK and have provided medical cover for large national and international sporting and public events since 2005. At the time, a number of patients with hyperpyrexia from heat illness were presenting to the medical facilities, who were cooled and then discharged with little or no follow-up. A proportion of these patients re-presented with complications or ongoing illness, and it was appearing that this group of patients were potentially more unwell and for longer than we had appreciated, and warranted special attention.

The initial work was to identify the number of patients with heatstroke and characterise the range of complications with which the heatstroke patients may present.

The intestinal tract is increasingly known to be associated with development of a number of conditions, such as chronic neurological, systemic metabolic and GI diseases, and there was at this time interesting reports of a similar role that the intestinal tract may have in the development of complications after heat illness. Using this analogous information, the next stage of the project was to further describe the role of intestinal permeability in heat illness.

Some patients with hyperpyrexia, for example, those with exertional heat illness, have concurrent circumstances, such as exercise, that in themselves may cause physiological derangement. This led to a number of experiments, some in collaboration with Sports Medicine students undertaking Masters' projects, to characterise further the role of hyperthermia in itself in the inflammatory and intestinal disturbances reported.

With my clinical background, my focus has always been on treatments that would have applications to improve patient outcomes. Animal studies have reported the beneficial role of administration of steroids and antibiotics against intestinal bacteria. We believe there is now enough circumstantial data to suggest that these treatments may also be beneficial in humans; future work is planned to investigate the effect of these drugs in humans on the inflammatory response after heat stress, with the hope that this will lead on to clinical trials and evidence-based treatments for use in the field, emergency departments and intensive care units in the future.

While most patients with hyperpyrexia develop the condition through exercise or exposure to hot conditions, other conditions, such as ingestion of medications and illicit drugs, may also be responsible. If manipulation of intestinal permeability is indeed possible to improve heatstroke

outcomes, then future work may investigate application of anti-inflammatory and anti-microbial medications in all cases of non-septic pyrexias, irrespective of the cause.

In conjunction with this work, I have been keen to raise awareness of the dangers of heat illness and the need for urgent recognition and treatment. I have spoken in the UK and abroad to a number of sporting and occupational groups, and written about the condition in several non-medical journals, including New Scientist, and the Guardian, a national newspaper. I have written several clinical articles for medical groups, including the Royal College of General Practitioners, and been involved in producing clinical practice guidelines for a wide range of sporting events and the Faculty of Sport and Exercise Medicine. I have peer-reviewed a large number of articles on heat illness for international journals, and advised an international working group on the development of an international standard on thermal damage in MRI scanning.

Section 1

Introductory section

1.1 Introduction

Normal human body core temperature varies between around 36.5 and 37.8°C [2], maintained by a number of sensory and effector mechanisms, described in Section 1.2: Pathophysiology of thermoregulation, page 28. A core temperature greater than this has a variety of causes, which may be divided into two broad categories: in some conditions, such as infections and inflammatory states, the hypothalamic thermoregulatory set-point is increased, primarily as a result of circulating pyrogenic mediators, which invokes a number of physiological mechanisms to maintain the body temperature at a higher level, described in Section 1.2.2.1: Fever associated with sepsis, page 33. A raised temperature due to sepsis is often defined as *fever*. The precise temperature defining a fever varies. The American College of Critical Care Medicine and the Infectious Diseases Society of America define 'fever' as a temperature of greater than 38.2°C [3], based partly on 37.7°C being the upper limit of normal human temperature [4]. In sepsis, defined as organ dysfunction as a result of an infection [5], a mild pyrexia appears to improve survival [6, 7]. However, the mortality increases as the temperature exceeds 40°C [6, 7], suggesting that at this stage the deleterious effects of hyperthermia on organ and cellular function outweigh any benefit conferred from the pyrexia in acute sepsis.

In other conditions, such as classical heat illness, often seen in meteorological heatwaves, EHI, occurring in those undergoing strenuous activity, and due to ingestion of certain drugs and medications, the thermoregulatory set-point is not increased, but instead, heat generation is greater than heat loss, resulting in a rise in the core temperature to above normal. These conditions are often termed *hyperthermia.* There appears to be no teleological benefit to a pyrexia in this group, and a raised temperature is associated with significant morbidity and mortality. A temperature of 38.5°C or greater at any point during a critical illness is associated with a worse outcome, independent of the cause of the illness [7].

Most patients fully recover after a period of hyperthermia, but patients exposed to higher temperatures and for longer periods of time are more at risk of systemic damage and other complications, which may lead to multi-organ dysfunction, which may be long-term, and death in extreme cases [8]. The similarities in clinical features and presentation suggest that it is the development of the hyperthermia itself rather than the underlying cause that is primarily responsible for the morbidity and mortality.

A proportion of the systemic damage is due to direct thermal damage, but there is growing evidence of increased GI permeability in hyperthermic states, which may allow translocation of toxins and bacteria from within the intestinal tract to enter the systemic circulation, termed *endotoxaemia*, stimulating a pro-inflammatory response, with detrimental consequences.

Current guidelines on the management of hyperthermia highlight the importance of immediate cooling [9–11], which remains the mainstay of treatment; a delay in a reduction in the core temperature is associated with increased mortality [12]. However, in addition, recent evidence, including the work presented here, suggests that there may be benefit in reducing the inflammation and bacterial translocation in hyperthermia to reduce morbidity and mortality.

This thesis describes the risks of non-septic hyperthermia in general, and specifically in endurance athletes. It then describes characterisation of GI permeability after hyperthermia and exercise, and finally discusses early work on the potential to reduce the deleterious effects by pharmaceutical modification of the intestinal translocation and inflammatory response.

1.2 Pathophysiology of thermoregulation

Thermoregulation concerns the homeostatic mechanisms, described further in this section (Section 1.2.1: Normal physiology, page 28), that maintain a relatively constant core body temperature, between around 36.5 and 37.8°C [2]. Upper and lower critical temperatures define ambient temperatures between which an unclothed human is able to maintain a normal core temperature by thermoregulatory mechanisms. The lower critical temperature is around 1°C in adults [13]; the upper depends on both temperature and humidity but appears to be a wet-bulb globe temperature (WBGT) of around 35°C [14] (see Section 1.2.2.3.1: Wet-bulb globe temperature estimation, page 36). Disruption to the homeostasis that may occur in febrile and hyperthermic states is described in Section 1.2.2: Disruption in hyperthermic states (page 33).

1.2.1 Normal physiology

Thermoregulation involves a variety of sensors (thermoreceptors) and control and effector mechanisms, which control the balance of heat generation and heat loss, via negative feedback.

Thermal equilibrium is the balance between the rate of heat production and the rate of heat loss. Heat production is a by-product of normal metabolic activity. At rest, the basal metabolic rate (BMR) is roughly 40 kcal/m^2/h, around 2000 kcal/d (100 W) for a normal adult, around 5–10% lower in females than males [15]. Around two-thirds of energy extracted from metabolism of glucose, amino acids and fat is dissipated as heat, with metabolism of fat providing more than twice the energy extracted from glucose for the same mass of substrate [13].

Heat loss occurs via a number of mechanisms:

- thermal radiation (heat transfer from the body via electromagnetic waves, related to the fourth power of the temperature gradient between the skin and surrounding area). It is generally the major method of heat loss at rest, and may account for up to 60% [13].
- convective heat loss, due to movement of cooler air across the skin.
- evaporation of water to vapour from the skin surface, for example, from sweating.
- the warming of subsequently exhaled air.
- conduction to surfaces in direct contact with skin or clothing.
- storage of heat within the body.

In order to maintain a constant core temperature, heat production (the difference between the total rate of energy production and the rate at which external work is being performed) must equate to the rate of heat loss, described in equation 1:

$$M - W = R + C + E + L + K + S$$

[equation 1]

where:

M = total rate of energy production

W = rate of external work performed

R = net heat loss via radiation

C = net heat loss via convection

E = net heat loss via evaporation.

L = warming of subsequently exhaled air.

K = net heat loss via conduction.

S = rate of storage of heat in the body.

1.2.1.1 Thermoreceptors

A thermoreceptor is the receptive portion of a sensory neuron that detects and transduces absolute and relative changes in temperature. Around 20% of thermosensory inputs to the control centre arise from cutaneous thermoreceptors [13]; the remainder come from thermoreceptors found elsewhere, including in internal viscera [16], the spinal cord [17] and the hypothalamus itself [18], where they are predominantly warm-sensing [19].

Cutaneous thermoreceptors are structurally of several types. Different types appear to co-exist in thermoreceptor neurones rather than in isolation to provide co-ordinated transduction over a wide temperature range [20].

Transduction of temperature in cutaneous thermoreceptors in part involves activation of thermally gated ion channels of the transient receptor potential (TRP) family. Each TRP subtype appears to be activated by a narrow range of environmental temperatures, classified by some authors into four thermal sensations (cold −10 to 15°C, cool 16–30°C, warm 31–42°C and hot 43–60°C), where the categories cold and hot are defined as noxious or painful [21]. Some, for example, the subtype TRPM8, is activated by cold, whereas TRPV3 and TRPV1 and 2 are activated by warm and hot external temperatures, respectively [21, 22]. Activation of the TRP channels allows influx of calcium. Afferent neurones of warm sensations are thought to be unmyelinated C-fibres [23], cold sensations are primarily but not exclusively myelinated A-delta fibres [24].

The TWIK-related potassium channel (TREK) subfamily is a more recently discovered cutaneous thermoreceptor, comprised of three members: TREK1, TREK2 and TRAAK. Activation of the ion channel allows intracellular influx of potassium. These receptors respond to a variety of physical and chemical stimuli as well as heat (TREK1, TREK2) [25, 26] and cold sensation (TREK2) [26].

Cutaneous temperature sensation is carried in the contralateral lateral spinothalamic tract in the spinal cord to the hypothalamus for integration with other thermosensory inputs.

1.2.1.2 Control centre

The hypothalamus, primarily the pre-optic area (POA), is the predominant site for integration of deep and peripheral thermosensing afferent neurones [27, 28]. It also receives and integrates endocrine inputs, such as from thyroid hormones [29] and information concerning metabolic activity [18]. Outputs from the hypothalamus control thermo-effector mechanisms, predominantly via neural and neuroendocrine pathways.

Various sites within the cerebral cortex also receive thermo-afferent and other inputs and also have a role in the control of thermo-effector mechanisms [30].

1.2.1.3 Thermo-effector mechanisms

Effector mechanisms to maintain core body temperature can be broadly considered as behavioural (under conscious control) and physiological (under involuntary control).

The former includes the wearing of appropriate clothing, the degree of physical activity undertaken, moving towards or away from a source of heat or cold, and the consumption of appropriate food and drink. Maximal intensity of exercise can increase heat production by up to 20-fold [13].

Physiological mechanisms include the control of heat loss, primarily through the control of vasomotor tone of cutaneous blood vessels, piloerector function and sweating, and thermogenesis, primarily through shivering and metabolism of brown adipose tissue (BAT).

1.2.1.3.1 Heat loss

Under normal ambient conditions, heat loss is primarily via radiation [13], via various cutaneous mechanisms, described in this section.

Control of vasomotor tone of cutaneous blood vessels

In hot conditions, vasodilation of cutaneous blood vessels occurs, to increase heat loss by thermal radiation, convection and conduction. Thermoregulatory vasodilation appears to be primarily under cholinergic sympathetic neuronal control with contributions from other local vasodilatory mediators, such as nitric oxide [31, 32]. Resting skin blood flow is around 250 ml/min, which allows for heat dissipation of 80–90 kcal/h, roughly that of basal metabolic heat production [31], but can increase significantly, to up to 6–8 L/min [31].

Piloerector function

In cold conditions, arrector pili muscles contract to cause piloerection, to lift the attached hair follicle upright to reduce air movement and reduce convective heat loss, thought to be under sympathetic neuronal control, with noradrenaline as the neurotransmitter at the α1 adrenergic receptor [33]. In hot conditions, hairs on the skin lie flat, preventing heat from being trapped by the layer of still air between the hairs.

Sweating

In hot conditions, cutaneous sweat glands secrete sweat via the sweat ducts, through the sweat pores and onto the surface of the skin, to cause heat loss by evaporative cooling. Sweat production may contribute to significant heat loss, around 580 kcal of heat per 1 kg of evaporated sweat [34], and is the predominant method of heat loss during exercise [35].

Sweat is composed primarily of water and sodium and chloride ions, and is initially roughly isotonic with plasma, although some electrolyte absorption occurs in the ducts. Eccrine glands are the most widely distributed and relevant for heat loss; apocrine and apoeccrine glands less widely distributed [36] and comprise only 10% of the four million sweat glands overall [37].

Sweating is induced in response to rises in core temperature [38] and in response to increases in local skin blood flow and temperature [39], predominantly mediated by the release of the neurotransmitter acetylcholine from sympathetic nerve terminals [37].

Water and sodium loss may be significant, over 3 L/h and 60 mmol/h [40], with inherent risk of hyponatraemia, potentially exacerbated by ingestion of hypotonic fluids, and hypovolaemia.

1.2.1.3.2 Thermogenesis

Shivering

Shivering is an involuntary somatic motor response occurring in skeletal muscles to produce heat during exposure to cold environments or during the development of a fever. Shivering increases heat production by up to six-fold [13]. Neural control appears to originate from the POA of the hypothalamus, with the efferent neurones stimulating cholinergic-activated muscle activity [41].

Metabolism of brown adipose tissue

Mammalian adipose tissue is classified broadly into two types, white adipose tissue (WAT) and thermogenic fat tissue (BAT and beige adipose tissue). In adult humans, BAT is predominantly located in the neck, supraclavicular region, chest and abdomen [42] and is the predominant source of non-shivering thermogenesis [43], but is more widely located and functionally more important for overall heat production in infants.

In most human mitochondria, the electron transport chain converts energy substrates into ATP, a ubiquitous intracellular energy source; in BAT, protons otherwise involved in conversion of ADP to ATP are released as heat energy. Thermogenesis by proton release is thought to be primarily due to activation of uncoupling protein 1 situated on the inner mitochondrial membrane [44].

Neuronal control appears to originate from the POA of the hypothalamus via a mechanism similar to that stimulating shivering, but instead the efferent pathway is via sympathetic rather than somatic neurones [41]. Proliferation of BAT is stimulated by agonism at the β1 adrenoceptor [45], metabolic activity in humans by agonism of β2 adrenoreceptors [46]. BAT activity also appears to be hormonally regulated, such as by thyroid and parathyroid hormones [43] and by metabolites and hormones originating from the GI tract [43], suggesting a pathway by which BAT is also important in influencing basal metabolic rate and body mass.

1.2.2 Disruption in hyperthermic states

1.2.2.1 Fever associated with sepsis

Pyrogenic fever is a common response to sepsis and other pro-inflammatory states. The febrile response is well preserved across the animal kingdom, with some experimental evidence suggesting it may be beneficial in infection. A raised temperature in patients with infection in the first 24 hours following admission to the intensive care unit (ICU) is associated with a better outcome compared with normothermia or hyperthermia above 40°C [6]. A temperature between 37.5°C and 39.4°C trends towards improved outcome compared with normothermia [7], and in elderly patients with community-acquired pneumonia, the mortality rate was significantly higher in patients who lacked fever (29%) compared with patients who developed a pyrexia (4%) [47]. A temperature greater than 38.2°C has also been found to have a protective role against invasive fungal infections in critically ill patients [48].

The raised temperature in sepsis may provide host protection by several mechanisms. Firstly, human infective pathogens often demonstrate optimal replication at temperatures below 37°C; an elevated host temperature therefore inhibits reproduction [49]. Secondly, increasing the temperature in vitro from 35°C to 41.5°C increases the antimicrobial activity of many classes of antibiotics [50]. Thirdly, a rise in temperature may also be associated with an increase in innate immunity associated with microbial destruction [51]. However, mortality increases again at temperatures above around 40°C [6, 7], suggesting that at this stage the deleterious effects of hyperthermia on organ and cellular function outweigh any benefit conferred from the fever in sepsis.

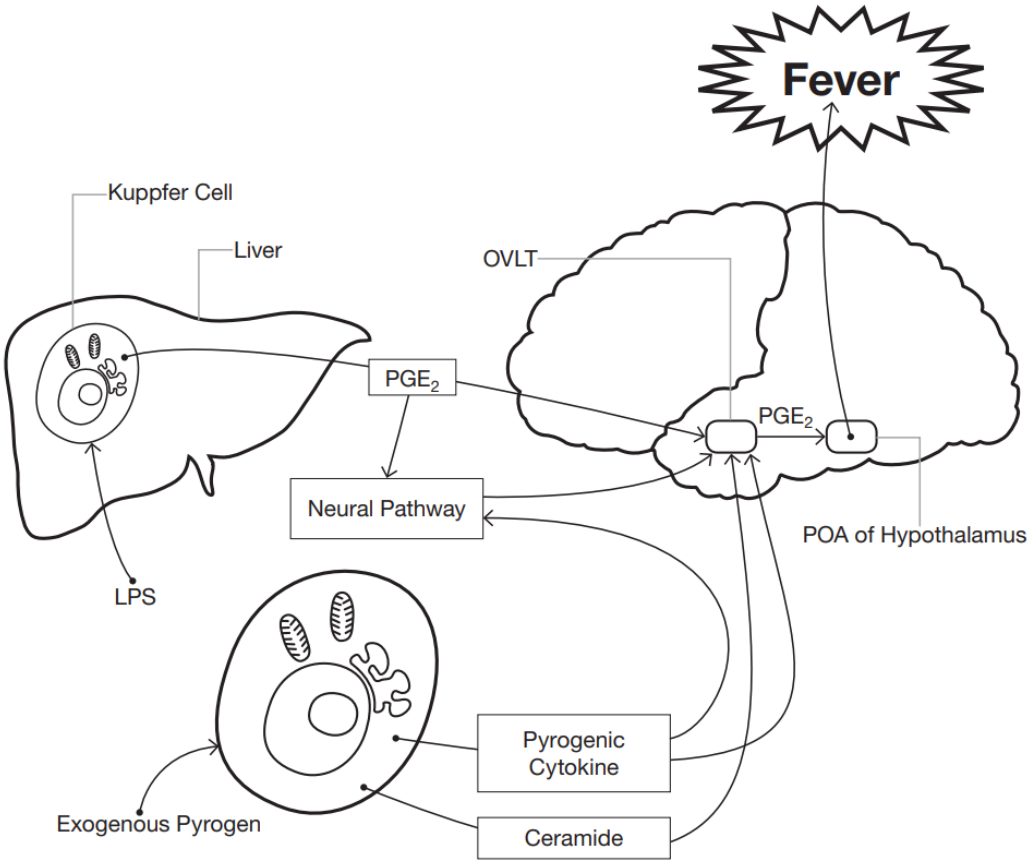
The generation of fever occurs through several mechanisms. The interaction of exogenous pyrogens (e.g., micro-organisms) or endogenous pyrogens (e.g., interleukin (IL)-1, IL-6, tumour necrosis factor alpha (TNFα)) with the organum vasculosum of the lamina terminalis (OVLT) leads to the production of fever. Exogenous pyrogens may stimulate cytokine production or may act directly on the OVLT. The OVLT is located in the anterior hypothalamus within the lamina terminalis, located in the optic recess at the anteroventral end of the third ventricle and adjacent to the POA of the hypothalamus. It is highly vascular and lacks a blood–brain barrier (BBB), allowing direct stimulation by pyrogenic substances. Its stimulation leads to increased synthesis of prostanoids including prostaglandin (PG)E2, which acts in the pre-optic nucleus of the hypothalamus slowing the firing rate of the warm sensitive neurons and resulting in an increase in body temperature. Prostaglandin E2 is synthesised from arachidonic acid, which is released from cell membrane lipid by phospholipase. Arachidonic acid is metabolised by two isoforms of the COX enzyme, which is inhibited by the anti-pyretic non-steroidal anti-inflammatory class of drugs [52].The precise

mechanism of action of the OVLT is unclear; dysfunction of the OVLT in an animal model resulted in marked and persistent fever but unresponsive to administration of the pyrogen IL-1β [53].

The bioactive lipid derivative ceramide, which has a pro-apoptotic as well as a cell signalling role, may act as a second messenger independent of PGE2, and may be of particular importance in the early stages of fever generation [54].

Lipopolysaccharides (LPS) from Gram-negative bacteria may stimulate peripheral production of PGE2 from hepatic Kupffer cells [55, 56]. Lipopolysaccharide-stimulated fever may also be neurally mediated [57]. Neural pathways may account for the rapid onset of fever, with cytokine production responsible for the maintenance, rather than the initiation, of fever [57]. Fever generation is also thought to occur by signalling via the Toll-like receptor cascade, which may be independent of the cytokine cascade [58] (see Figure 1, page 34).

Figure 1: *Proposed mechanisms for the generation of fever in sepsis. POA: pre-optic area of the hypothalamus; PGE2: prostaglandin E2; LPS: lipopolysaccharide; OVLT: organum vasculosum of the lamina terminalis. Taken from reference [8] and reproduced under a Creative Commons Attribution 4.0 International Licence.*



1.2.2.2 Fever associated with inflammation

Fever is one of the four cardinal features, along with pain, redness and swelling, of inflammation, first described by Celsus around 2000 years ago. Inflammation is commonly observed to aid repair after traumatic or infective insults.

Fever is a ubiquitous component of inflammation across the animal kingdom, and enhances the host response. A large number of both the cell-derived and plasma-derived inflammatory mediators are pyrogenic; fever associated with inflammation is probably also neural- and cytokine-mediated in a similar way to sepsis as described above [59] (see Section 1.2.2.1: Fever associated with sepsis, page 33). As in sepsis, PGE2 appears to be the final common mediator of fever generation [59].

1.2.2.3 Non-pyrogenic fever

When heat gain is greater than the thermoregulatory capacity for heat loss, the core temperature rises despite no change in the set-point of the hypothalamus. In contrast with a fever in sepsis, a non-pyrogenic fever is not of any perceived teleological benefit. A temperature of 37.5°C or greater during a critical illness trends towards a worse outcome, and becomes significant at temperatures greater than 38.5°C [7].

The risk of non-pyrogenic fever is higher with increased heat production or reduced capacity for heat loss.

Basal heat production is around 75 W, increasing to 1000–1500 W with intense exercise, significantly more than used as mechanical energy [60]. Aerobic training improves oxygen delivery and utilisation, and heat acclimation improves the efficiency of mechanisms of heat loss, primarily by a greater whole-body sweat rate, but also by increased cutaneous vasodilatory ability [60]. The rate of heat gain is therefore less in fit and in heat-acclimatised individuals; untrained individuals reached an average core temperature of 39.4°C during an 85-min treadmill walk in 40°C and 25% relative humidity, compared with trained and heat-acclimatised runners, whose core temperature only reached 38.2°C [61]. Conversely, older individuals, more at risk of non-exertional heat illness, have reduced sweating and cutaneous vasodilatory ability and reduced cutaneous blood flow in response to heat stress, attenuating heat loss mechanisms [60]. In one study, 65-year-old males dissipated 13% less heat than 21-year-old males, despite the same heat production [62].

Heat loss is greater in cool and dry conditions, increasing radiation and evaporative losses through a larger temperature and water vapour pressure gradient. Mechanisms for heat loss are therefore less effective in hot and humid conditions. If the temperature of the surroundings approaches 35°C or is greater than that of the skin, then heat loss by radiation and conduction is minimal or heat

transfer to the body occurs [14, 63, 64]. Heat loss can then only occur by evaporation. In humid conditions, evaporative loss is also reduced [65].

1.2.2.3.1 Wet-bulb globe temperature estimation

The WBGT estimation combines the effect of ambient temperature, humidity, wind speed (wind chill), and visible and infrared radiation. It is used by industry, the military and athletes as an objective measure of the risk of heat stress due to the ability to lose heat. The WBGT is derived from the formula shown in equation 2:

$$WBGT = 0.7T_w + 0.2T_g + 0.1T_d$$

[equation 2]

where:

T_w = natural wet-bulb temperature (combined with dry-bulb temperature indicates humidity)

T_g = globe thermometer temperature (indicates radiant heat transfer)

T_d = dry-bulb temperature (indicates actual air temperature).

Due to the combined high humidity and ambient temperature, a WBGT of 35°C has been estimated as the point at which all heat loss mechanisms become ineffective and has therefore been suggested as the upper critical temperature in humans [14].

1.3 The problem of hyperthermia

This section discusses the diverse causes (Section 1.3.1: Causes of hyperthermia, page 37) and adverse effects (Section 1.3.2: Adverse effects of hyperthermia, page 44) of hyperthermia, and the conditions that predispose to its development and complications (Section 1.3.3: Genetics and other conditions predisposing to hyperthermia, page 51), to demonstrate its frequency and risks to human health.

Publications one (Paper 1: Acute kidney injury associated with endurance events – is it a cause for concern? A systematic review) and two (Paper 2: Association between collapse and serum creatinine and electrolyte concentrations in marathon runners: a 9-year retrospective study) are discussed in this section, which further characterise the risk of renal failure and electrolyte disturbance in endurance events, especially in association with heat illness.

1.3.1 Causes of hyperthermia

There are a variety of causes of hyperthermia in humans, many of which are relatively common and associated with significant risks to health (see

Table **1**, page 39, taken from reference [66]). The commoner causes are described further in this section. *Heat illness* is defined for the purposes of this thesis as hyperthermia associated with systemic damage. *Heatstroke* represents the most severe form of the heat illness spectrum, associated with a significant risk of organ failure and death.

The similarities in clinical features (see Section 1.3.2: Adverse effects of hyperthermia, page 44), high mortality and the relationship between outcome and temperature (see Section 1.3.6: Current treatments for hyperthermia and heat illness, page 56) between the different hyperthermic aetiologies suggest that the pathological features are at least partly due to hyperthermia itself, irrespective of the cause.

Table 1: *table of causes of non-septic hyperthermia. Taken from reference [66], reproduced with the publisher's permission.*

Category
Heat illness (e.g., exertional heatstroke, classical heatstroke)
Drug reactions
Immunological and inflammatory diseases
Malignancy
Metabolic disorders (e.g., gout, porphyria)
Reaction to incompatible blood products
Thromboembolic disease
Tissue destruction (e.g., haemolysis, surgery, infarction, rhabdomyolysis)

1.3.1.1 Exertional heatstroke

Exertional heatstroke (EHS), usually defined as a core temperature above 40°C with neurological dysfunction [1], represents the most severe form of EHI, and occurs in those undergoing strenuous activity, especially in warmer conditions. It is becoming increasingly common in endurance athletes [67, 68], and is among the leading causes of deaths in athletes [67], described more fully in Section 1.3.2.1: Functional outcomes (page 44) below. It is also seen in a number of occupations, including the fire brigade, the military and riot police. It was first described in 24 BC, in a Roman account during an expedition to Egypt, when it was noticed that 'the desert, the sun and the water, which had some peculiar nature, all caused his men great distress so that the larger part of the army perished' [69].

1.3.1.2 Classical heatstroke

Classical heatstroke (CHS) occurs in warm ambient conditions, especially in heatwaves, and is thought to be responsible for tens of thousands of deaths each year. It is becoming increasingly common, due to climate change [70, 71].

1.3.1.3 Drug-induced hyperthermia

1.3.1.3.1 Malignant hyperthermia

Malignant hyperthermia (MH) is a life-threatening condition usually triggered by exposure to volatile anaesthetic agents or the depolarising neuromuscular blocking drug succinylcholine. It is reported to affect up to 1:5000 patients [72]. It is reported twice as commonly in males than in females, and frequently in young people. However, all ages groups, including neonates, are at risk [72]. It has also been observed in other species, such as dogs, cats, horses and pigs. When skeletal muscle is exposed to the causative agent, abnormalities in the skeletal muscle response generate excessive calcium, which generate a hypermetabolic state. Heat is generated during the processing of the excess calcium. The increased energy demands deplete adenosine triphosphate (ATP); myocytes are damaged by the loss of ATP and the hyperthermia. Intracellular constituents, such as myoglobin, creatine, phosphate, potassium and creatine kinase, leak into the circulation. In the acute phase, patients therefore develop an oxygen debt, hyperthermia, hypercarbia and tachycardia due to hypermetabolism, and hyperkalaemia and rhabdomyolysis due to cell breakdown. Collapse, multi-organ failure and death follow if it is not rapidly treated. Untreated, the mortality rate may be around 80% [73].

The first documented survivor of MH was a young man in Australia in 1961, requiring surgery for a fractured tibia. Ten of his family members had previously developed uncontrolled hyperthermia and had died during general anaesthesia with ether. Halothane, a volatile anaesthetic agent, had been recently introduced and was therefore used instead, but after 10 minutes the patient developed MH. The halothane was discontinued; the patient was cooled with ice and subsequently recovered uneventfully [74].

1.3.1.3.2 Neuroleptic malignant syndrome

Neuroleptic malignant syndrome (NMS) is most often caused by an adverse reaction to neuroleptic or antipsychotic drugs. It is probably related to central dopaminergic (DA2) receptor blockade; other dopaminergic antagonists, including metoclopramide, have been implicated. The incidence of NMS may be up to 2% [75] in patients receiving causative drugs, although other studies with newer drugs or using different diagnostic criteria report a lower incidence [76]. The mortality rate has previously been reported at 76% [75].

Diagnostic criteria vary, but the features are often grouped into four: hyperthermia, changes in the level of consciousness, instability of the autonomic nervous system (for example, unstable blood pressure, tachycardia, sweating, incontinence) and muscle rigidity [77]. Development of

hyperthermia in NMS is incompletely understood but is thought to be related to the blockade of heat loss pathways in the hypothalamus and heat production from the muscle rigidity. The muscle rigidity is due to exaggerated calcium release from the sarcoplasmic reticulum, in a similar manner to MH. Dantrolene or bromocriptine (a dopamine agonist) may be used, but evidence of their efficacy is limited [78].

1.3.1.3.3 Serotonin syndrome

The neurotransmitter serotonin is found widely in the peripheral and central nervous systems (CNS). Serotonin syndrome is caused by an increased level of serotonin in the CNS and is therefore a predictable consequence of therapeutic or recreational drugs moderating serotonin pathways, rather than an idiopathic drug reaction. Agonism at the 5-HT1A and 5-HT2A receptors is thought to be responsible [79, 80].

The symptoms can be grouped into three: autonomic effects (hyperthermia, sweating, tachycardia, nausea), neuromuscular hyperactivity (tremor, myoclonus, ataxia, hyper-reflexia) and neurocognitive symptoms (agitation, confusion, hallucinations, coma).

Patients may become hyperthermic in life-threatening cases. Complications are similar to other hyperpyrexic states, and include metabolic acidosis, rhabdomyolysis, seizures, renal failure and disseminated intravascular coagulopathy (DIC). The mortality rate is up to 12% [81].

A large number of medications either alone in high dose or in combination can produce serotonin syndrome (see Table 2: examples of causes of serotonin syndrome. Taken from reference [66].page 42), taken from reference [66]).

Table 2: *examples of causes of serotonin syndrome. Taken from reference [66].*

	Drugs
Antidepressants	Monoamine oxidase inhibitors, tricyclic antidepressants, selective serotonin reuptake inhibitors, serotonin-noradrenaline reuptake inhibitors, bupropion
Opioids	Tramadol, pethidine, fentanyl, pentazocine, buprenorphine oxycodone, hydrocodone
CNS stimulants	MDMA, amphetamines, sibutramine, methylphenidate, methamphetamine, cocaine
Psychedelics	5-Methoxy-diisopropyltryptamine, lysergide
Herbs	St John's Wort, Syrian rue, Panax ginseng, nutmeg, yohimbine
Others	Tryptophan, L-Dopa, valproate, buspirone, lithium, linezolid, chlorpheniramine, risperidone, olanzapine, antiemetics (ondansetron, granisetron, metoclopramide), ritonavir, sumatriptan

Management is based primarily on stopping administration of the precipitating drugs and treating complications. Serotonin receptor antagonists, for example, cyproheptadine, are sometimes suggested, but evidence for their use is poor. Supportive care, including controlling agitation, autonomic instability and hyperthermia, is advocated.

1.3.1.3.4 Propofol infusion syndrome

Propofol infusion syndrome (PRIS) [82] is a rare syndrome, originally described in children in the 1990s who were sedated with propofol, a commonly used anaesthetic agent. Thirty-eight percent of patients receiving propofol develop hyperthermia although the criteria for PRIS may not otherwise be met [83]. The mortality from PRIS is high, reported to be 30% overall, but as high as 83% dependent upon the clinical manifestations [84]. The mortality is higher in males compared with females, younger patients and those with more severe clinical features [84]. The mechanism of the development of hyperthermia in PRIS is unclear, but is thought to be caused by the effect of propofol inhibiting the mitochondrial respiratory chain or impairing mitochondrial fatty acid metabolism, with the energy otherwise dissipated as heat [85].

1.3.1.3.5 Sympathimometic syndrome

Drugs that mimic or enhance actions of the sympathetic nervous system (sympathomimetic agents) are widely used in prescription medications, for example in those used to treat asthma. They are also commonly found in non-prescription drugs such as cold remedies (containing ephedrine),

illegal street drugs (e.g., cocaine, amphetamines, methamphetamine ('ecstasy', MDMA), mephedrone) and dietary supplements (e.g., ephedra alkaloids).

3,4-Methylenedioxy-methamphetamine (MDMA) is one of the commonest sympathimometic agents to cause hyperthermia, and is reported to cause around 70–90 deaths in England and Wales each year [86]. It was originally produced in 1912 as a precursor to a haemostatic drug in development [87]. It was not until the 1920s that its similarities to adrenaline were noted. There was some interest in the 1950s and 1960s in whether it could be used as a stimulant or as an interrogation tool, and in the late 1960s it was discovered to have psychoactive properties. A number of psychiatrists noted that it helped patients overcome emotional barriers, and it gained some use in psychotherapy as a result. It earned the nickname 'penicillin for the soul' until the 1980s, when its illicit street use became more widespread, and it became subject to class A restrictions.

The development of hyperthermia with MDMA appears to have several mechanisms. The drug probably partly acts as an indirect serotonergic agonist, acting on the serotonin transporter and increasing the amount of serotonin available to be released into the synapse. It also enhances the release of dopamine and noradrenaline, probably in a similar manner to serotonin, has effects on monoamine reuptake, and may act as an agonist on various receptors, including 5HT2, α2 adrenergic, M1 muscarinic cholinergic, and D1 and D2 dopamine receptors.

1.3.2 Adverse effects of hyperthermia

Hyperthermic states, such as those described above (Section 1.3.1: Causes of hyperthermia, page 37), are associated with systemic damage, including multi-organ failure and death [8], explored further in this section. The similarities in clinical features, high mortality and relationship with outcome and temperature (see Section 1.3.6: Current treatments for hyperthermia and heat illness, page 56) between the different hyperthermic aetiologies suggest that the pathological features are at least partly due to hyperthermia itself, irrespective of the cause.

Hyperthermia has effects at the cellular, local and organ level [8]. Most organs appear to be at risk of damage from hyperthermia, with putative mechanisms described in Section 1.4: The mechanisms of systematic damage from heat stress (page 57). Organ dysfunction and functional outcomes after an episode of hyperthermia are described below.

1.3.2.1 Functional outcomes

Many of the hyperthermic states are associated with a high risk of death and poor long-term outcomes.

The risk of developing classical heat illness is significant and may become more so with climate change. The World Health Organization and others estimate that heatwaves are responsible for tens of thousands of direct and indirect excess deaths each year [70, 71], with some predictions suggesting that the mortality may rise by over 2.5-fold in the next 30 years [88].

The risk from hyperthermia to the individual may also be significant; CHS is reported to have a mortality rate of up to 64% [12]. Even in survivors of the acute episode, hyperthermia reduces life expectancy and worsens functional outcome. In CHS, the 28-day mortality is 58%, increasing to 71% at two years after the initial event [89]. Classical heatstroke is reported to cause moderate to severe functional impairment in 33% of survivors at one year [90]. There may be little or no improvement even after discharge from hospital [90].

Exertional heatstroke is among the leading causes of sudden death in athletes, with the number of sports-related EHS deaths in the USA estimated to have doubled since 1975 [67]. The National Center for Catastrophic Sports Injury Research database has identified heat illness as the third-most common cause of sports-related fatalities in US high school and college football players over the 20 years between 1990 and 2010, accounting for 15.6% of reported deaths [67]. An episode of EHS is associated with an increased risk of mortality of 40% even after recovery from the initial episode [91].

Mortality rates from drug-induced hyperthermic states are similarly high. In MH, untreated mortality may be over 80% [73]. Administration of dantrolene, a skeletal muscle relaxant, reduces the associated muscle rigidity, hypermetabolism and hyperthermia, which, along with supportive care, has significantly reduced the morbidity and mortality from that first reported; however, mortality may still be as high as 11% [92]. Non-fatal complications are reported in 35% of patients, and include cardiac, renal or hepatic dysfunction; coma or change in consciousness level; pulmonary oedema; and DIC [93]. Complications are associated with the extent of the high temperature. In one study, the likelihood of any complication increased 2.9 times per 2°C increase in maximum temperature and 1.6 times per 30-minute delay in administration of dantrolene [93].

In NMS, mortality is currently around 11% [94] but has previously been significantly higher, at up to 76%, due to a number of factors including early clinical recognition, prompt withdrawal of the neuroleptic agent and more effective cooling and supportive care [75]. Development of myoglobinaemia and renal failure are strong predictors of mortality, associated with a mortality rate of 50% [75]; similarly, if NMS is associated with organic brain disease, mortality remains high (38.5%) [94].

1.3.2.2 Gastrointestinal dysfunction

In the intestinal tract, systemic hyperthermia causes cellular damage, reduces blood flow and increases permeability of the tract, which may increase the rate of intestinal bacterial translocation, discussed further in Section 1.4: The mechanisms of systemic damage from heat stress (page 57) below. Blood flow to the GI tract is reduced at temperatures above 40°C [95]. Hyperthermia causes direct damage to cell membranes, denatures cellular proteins and may increase oxidative stress. This leads to loss of the GI barrier integrity and increases the potential for endotoxaemia, which initiates release of pro-inflammatory cytokines, leading to a systemic inflammatory cascade [96], described further in Section 1.4.2: Gastrointestinal permeability and endotoxin translocation (page 60). Gastrointestinal oedema and petechial haemorrhage are also observed and have been described [97].

1.3.2.3 Renal dysfunction

Renal function is also affected by hyperthermia, described more fully in Section 1.3.4: Further characterisation of renal dysfunction with exertional heat illness (page 53), below. Classical heatstroke is associated with the development of AKI; of 58 patients hospitalised with CHS during the 1995 Chicago heatwave, 53% had at least moderate renal impairment [90]. Renal failure

sufficient to require renal replacement therapy has also been described after hyperthermia due to other causes, including NMS [98], MH [93, 99] and recreational drug use [100], suggesting that the raised temperature rather than the cause of the hyperthermia is the predominant cause of the renal failure.

1.3.2.4 Cognitive dysfunction

Cognitive and neurological manifestations of hyperthermia have been reviewed recently [101].

Cognition refers to mental abilities and processes, and includes memory, knowledge, attention, reasoning, problem solving, and comprehension. Hyperthermia has been shown to adversely affect attention [102], memory [103] and processing of information [104] acutely. Some of the cognitive processes appear to be affected by hyperthermia more than others. Short-term memory processing, for example, may be more affected than attentional processes [105].

Even mild and short-lived hyperthermia may cause cognitive impairment, which in a few cases may be permanent. One study of induced hyperthermia in healthy volunteers showed that memory was impaired at a core temperature of only 38.8°C compared with normothermia [106]. Artificially induced hyperthermia may induce cognitive impairment after only one to two hours of temperature elevation [104, 107]. Cognitive changes may not occur immediately at the time of hyperthermic insult, but instead develop a short time (60–120 min) after the cessation of the insult [107].

Functional neuroimaging supports the presence of a large number of pathways and connections in cognitive pathways, with many of these being affected acutely in hyperthermia. In general, connections appear to be increased around the limbic system [108], consistent with the observed changes in memory and learning ability. The dorsolateral prefrontal cortex (involved in executive functions, for example, memory, cognition and reasoning), and the intraparietal sulcus (involved in processing and memory) also show increased activity in acute cerebral hyperthermia [109]. Conversely, connections in other parts of the brain, including the temporal, frontal and occipital lobes, appear to be reduced in acute hyperthermia [108].

Hyperthermia-induced changes in short-term memory formation can also be detected using electroencephalography. The electrical response of the brain to a specific cognitive or sensory event is termed an ‘event-related potential’ (ERP). If the brain is subjected to a repeated identical sound, and subsequently an alternate sound is introduced, the ERP is altered, termed ‘mismatch negativity’ (MMN). Mismatch negativity has validity in studies into auditory memory formation. Individuals

exposed to hyperthermia for as little as one hour show significant decline in MMN compared with a control group [104], consistent with clinical observations of a decline in short-term memory.

In most cases, patients recover fully from the acute cognitive dysfunction. Some, however, are left with persistent changes in attention, memory or personality [110]. These may be mild, or severe, up to and including severe global dementia. Cognitive changes have been reported after CHS [111], EHS [112] and drug-induced hyperthermia [113, 114].

1.3.2.5 Neurological dysfunction

1.3.2.5.1 Acute neurological effects

Acute neurological deficits have been described after hyperthermia from a number of causes, including heat illness and drug ingestion. Deficits are well recognised after CHS; in the acute phase, many patients have disturbance of consciousness of severity up to and including deep coma. Of 87 patients with CHS after a Mecca pilgrimage, all had a reduced level of consciousness, and 25 (29%) were deeply comatose [115]. Constricted pupils were reported in all cases in this series, with 17 (20%) showing automatisms, including chewing, swallowing and lip smacking. Delirium, lethargy, disorientation, seizures, hypertonia and hypotonia are also described [90].

Acute neurological damage after drug-induced hyperthermia has been reported to result from MH [116] and NMS [113]. However, most survivors of NMS recover completely, with a mean recovery time of 7–11 days [113]; the incidence of long-term sequelae has been reported at 3.33% [114].

Development of neurocognitive dysfunction may be related to disruption of the protective BBB, which appears to be related to hyperthermia (see Section 1.4.4.2: Blood–brain barrier disruption and neurocognitive dysfunction, page 65) or to the presence of LPS in the circulation [117], itself thought to be related to the effects of thermal damage on the integrity of the intestinal barrier (see Section 1.4.2: Gastrointestinal permeability and endotoxin translocation, page 60).

1.3.2.5.2 Persisting neurological deficits

Neurological deficits that persist after the acute phase are well described. Persistent neurological deficits have been reported after CHS (the majority of cases) and EHS. Cases after drug-induced hyperthermia are also reported but are much rarer. The incidence of persisting neurological deficits is difficult to discern; of 87 patients who developed CHS during the same Mecca pilgrimage, 75 (87%) made a full recovery, 10 patients (11%) died, and two (3% of the survivors) recovered but developed pancerebellar syndrome [115]. In a clinical observational report from Tel-Aviv University

Medical School of 36 male patients with EHS aged 17–24 years seen during 1956–1966, eight (22%) died, two (7% of the survivors) had cognitive impairment, and a further two (7%) had neurological signs, one with paraplegia, and one with cerebellar disturbance [110].

However, the mortality and neurological impairment after a severe episode of heat illness may be more significant than these data suggest; if patients require admission to an ICU for management of hyperthermia, the mortality may approach 50% to 65% [12, 118], and persistent neurological effects may affect 50% of survivors [118]. Early deaths are usually from multi-organ failure but, in one study, 50% of the deaths were in patients who were comatose or tetraplegic [12]. In one series of patients admitted to ICU with CHS after a heatwave, 33% had significant neurological impairment, and a further 33% of patients had mild impairment at discharge. Only 24% of patients had no neurological impairment [90].

Drugs known to cause hyperthermia and persistent neurological deficits include those responsible for NMS [113, 119–122] and the serotonin syndrome [123], and Chinese herbal medications [124]. Neurological manifestations are reported in up to one-fifth of patients with MH [93].

Cerebellar dysfunction is by far the predominant clinical picture in cases of persistent neurological dysfunction [110, 111, 115, 118, 125–128]. Ataxia, dysarthria, and co-ordination problems are common; nystagmus is more rarely reported [125]. Of the five reported cases of persistent neurological dysfunction after NMS, all showed cerebellar signs [113, 119–122]. Reported less commonly is damage to the cerebral cortex [127, 129], brainstem [130], spinal cord [110], and the peripheral nervous system [118]. Frontal cortical dysfunction is rare but has been reported. Basal ganglia dysfunction is reported after heatstroke [127], and is well recognised after NMS [114, 131]; the latter however may represent the effects of treatment with neuroleptic medication in some patients rather than damage from the hyperthermia [132]. Clinical features are usually bilateral. Neurological dysfunction may be profound; a persistent vegetative state has been reported [133]. Patients may show signs of improvement over weeks or months [129], but, in some cases, the vegetative state may persist for many months or years [111]. Recovery may be minimal or absent [126]. In the vast majority of these reported cases, an initial core body temperature of 40°C or higher is recorded.

1.3.2.5.3 Neuroradiological and neuropathological findings

Various radiological findings have been described on magnetic resonance imaging (MRI) after heatstroke, including haemorrhage, oedematous changes, ischaemia, encephalitis and atrophic changes [130, 134], suggesting that a number of pathological processes are responsible. Lesions have been observed throughout the central nervous system (CNS), including the brainstem,

cerebellum, hippocampus, external capsule and cerebrum [130, 134, 135]. Haemorrhage on MRI may represent a poorer prognosis – of eight patients in one follow-up study, three patients had evidence of haemorrhage on MRI imaging, and all three died. The remaining five showed no haemorrhage, and all survived [134].

Neuropathological studies of humans with hyperthermic damage are rare, but in the existing studies, cerebellar damage is frequently observed, in keeping with the clinical syndrome. In one reported study of patients with CHS, the Purkinje cells displayed the most marked thermal damage [135]. Purkinje cells are found predominantly in the cerebellum and regulate motor function, in keeping with cerebellar dysfunction as a common feature after a hyperthermic episode (see Section 1.3.2.5.2: Persisting neurological deficits, page 47). Almost complete loss of the Purkinje cells may be seen when death occurs after more than 24 hours [136]. Neuronal loss may be seen in other parts of the brain, where they are replaced by glial cells. Oedematous and haemorrhagic change is also reported [136]. In one case series of eight deaths after EHS, reports of six autopsies showed petechial haemorrhages in a number of locations in five brains, including the meninges, ventricles, cerebellum and hypothalamus, an intracranial bleed in two, and venous congestion in three. Four of the brains had cerebral oedema, and Purkinje cell degeneration was found in four specimens [110].

Neuroleptic malignant syndrome may produce similar neuropathological findings; cerebellar damage may be the most marked, with Purkinje cell loss and replacement by Bergmann's glia. Infiltration by macrophages, and axonal and myelin degeneration also occurs [119]. In a young patient who died after developing MH following an appendicectomy, cerebellar damage again predominated, with oedema and herniation [116]. The reason for this selective thermosensitivity is not clear.

1.3.2.6 Liver dysfunction and coagulopathy

Liver dysfunction is common; at temperatures above 40°C, elevations in plasma liver enzymes aspartate transaminase and alanine transaminase are observed [137]. In some cases, the hepatocellular damage has been sufficient to require hepatic transplantation [138, 139]. Coagulopathy has been reported to occur in 45–53% of individuals with CHS [90, 140]; derangement of coagulation is considered to reflect hepatic dysfunction, since coagulopathy is rare without liver derangement and is temporally related to alterations in liver function [141]. Coagulopathy has also been reported in NMS [142] and MH [93]; in the latter, development of coagulopathy is associated with a higher risk of death [143].

1.3.2.7 Cardiac dysfunction

Similar to many other organs, the cardiovascular system is also affected by hyperthermic states, including CHS [140, 144] and MH, where pulmonary oedema, a sign of cardiac failure, has been reported [93].

In one database of 3372 patients admitted after CHS over 12 years, circulatory failure occurred in 393 (12%) admissions. Circulatory failure was more commonly found in obese patients but was less common in older patients aged greater than 60 years. The need for mechanical ventilation, blood transfusion and renal replacement therapy were higher in patients with circulatory failure. Renal, respiratory, liver, neurological and haematological failure were associated with circulatory failure. The in-hospital mortality was 7.1 times higher in patients with circulatory failure, and the length of hospital stay and hospitalisation costs were higher when circulatory failure developed [144].

1.3.3 Genetics and other conditions predisposing to hyperthermia

A number of conditions are known to predispose to the development of heat illness, especially EHS (see Table 3, page 51). In one series, over 50% of patients with CHS had been taking at least one drug known to increase the risk of heat illness [140].

Table 3: *conditions and medications that may increase the risk of heatstroke. Taken from reference [68].*

Conditions associated with heat illness	**Medications increasing the risk of heat illness**
Conditions increasing heat production (e.g., thyrotoxicosis)	Alcohol
Current febrile illness	Amphetamines
Dehydration	Anticholinergic and sympathomimetic agents
Increasing age	Antihistamines
Lack of physical fitness	Benzodiazepines
Lack of sleep or food	Beta blockers
Obesity	Calcium channel blockers
Previous episode of heatstroke	Cocaine
Protective clothing	Diuretics
Skin diseases (e.g., psoriasis)	Laxatives
	Neuroleptic medications
	Phenothiazines
	Thyroid agonists
	Tricyclic antidepressants

1.3.3.1 Genetic abnormalities in hyperthermic states

In several hyperthermic states, a genotypic abnormality has been isolated which increases the risk of developing the disease. In some cases, there is a strong genetic basis. Malignant hyperthermia is often inherited as an autosomal dominant disorder. Mutation in the ryanodine receptor (RYR) accounts for 70–86% of cases [145, 146], with other genetic abnormalities having more recently been identified [145]. Ryanodine receptors form calcium channels and are the main mediators of calcium-induced calcium release in animal cells. The subtype RYR1 is found in the sarcoplasmic reticulum of skeletal muscle, and usually opens in response to increases in intracellular calcium mediated by L-type calcium channels, which results in a larger increase in intracellular calcium levels and muscle contraction. In MH, the RYR functions abnormally, so that calcium is released in a much higher amount, with hypermetabolism, hyperthermia and subsequent organ dysfunction.

Exertional heatstroke has clinical and biochemical similarities to MH [147], and there are case reports of patients with both conditions. While some patients with EHS display mutations in the RYR1 gene [148], the genetic basis is probably different to MH, and with more heterogenicity [149]; however, some authorities advise that patients with a prior episode of EHS should be considered for testing for susceptibility to develop MH [150]. Recently, there has been some interest in another similar sarcoplasmic skeletal muscle protein, named calsequestrin (CASQ1), which appears to modulate RYR1 function. Ablation of this protein in mice increases the risk of MH-like episodes when exposed to both heat and halothane, raising the possibility that there is also a genetic basis to EHS and a similarity to MH [151].

Neuroleptic malignant syndrome occurs with higher incidence in some families, suggesting the possibility of a genetic mechanism [152]. A1 and A2 alleles of the dopamine D2 receptor (DRD2) appear to affect DRD2 density and function. Postmortem studies have demonstrated that the presence of one or two A1 alleles are associated with low DRD2 density in the brain, especially in the corpus striatum on the caudate region. Individuals who are A1 allele carriers show a decrease in dopaminergic activity and glucose metabolism, and the risk of developing NMS is 10.5 times higher in A1 allele carriers than in non-carriers [153, 154].

Similarly, there may be a correlation between serotonin syndrome and genotype. Mutations in the receptor subtype 5-HT2A appear to be related to a higher rate of adverse drug reactions and tolerance of serotonergic drugs [155, 156]. Changes in the enzymes responsible for metabolism of serotonin-based drugs, the CYP enzymes, may also predispose to serotonin syndrome [157–159].

1.3.4 Further characterisation of renal dysfunction with exertional heat illness

There has so far been little work defining the extent of renal failure and electrolyte disturbance in the context of endurance heat illness. Primary papers one and two, summarised below, investigated renal function after endurance heat illness; renal failure and electrolyte derangement are likely to be worse if the exercise is associated with heat illness.

1.3.4.1 Renal dysfunction in exertional heat illness

Paper 1: Acute kidney injury associated with endurance events – is it a cause for concern? A systematic review.

This paper is a systematic review describing the risk and incidence of renal failure in those undergoing endurance events, including in association with heat illness [160]. The review included adult human studies of endurance or ultra-endurance events, written in English and published in peer-reviewed journals, which measured changes in markers of renal function such as serum creatinine. Full details of the search criteria and adherence to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (see Appendix 1, Table 5, page 98) [161] are described in the Methods section of the paper.

Four papers were identified investigating renal failure in EHI. The first paper presents military data that suggests that the incidence of EHS is increasing, from 2 cases per 100,000 to over 14 per 100,000 in the 20 years to 1999 [162], even though hospitalisations from heat illness in general decreased over the same time period. Of the heatstroke cases, 17% were associated with dehydration, 25% had rhabdomyolysis and 13% had acute renal failure, although this was not defined in this paper. Renal failure in EHS had an association with rhabdomyolysis: of 236 cases of heatstroke, 78 also had renal failure. However, dehydration was only rarely contributing to the renal dysfunction: of the heatstroke cases, only 6% were dehydrated. Heat illness appears to significantly increase the risk of renal failure in comparison with marathon runners generally; the Comrades marathon have reported an average of only one runner each year admitted with renal failure [163].

Three case reports describe renal failure requiring hospitalisation after EHS. In both, the renal failure improved spontaneously with supportive care, without the need for renal replacement therapy [164–166].

In a further case series, excluded from the systematic review as the extent of the exertion was not documented, describes the outcomes of 28 patients admitted to the ICU of a military hospital over a 12-year period [167]. Of the 28, 24 patients (86%) had acute renal failure, of whom seven (25%)

required dialysis. Renal failure recovered fully in 20 patients (83.3%) but two (7.1%) required long-term dialysis. In this series, the need for dialysis was lower if the core temperature of the patient was lower than 38°C within three hours (14.3%) compared with those in whom the core temperature was greater than 38°C at three hours (35.7%), although this did not reach statistical significance ($p = 0.351$).

In the largest series of renal failure in EHS found to date [168], and published after the systematic review was completed, of 187 patients admitted to hospital, 82 (43.9%) had at least AKI stage 1, according to the Kidney Disease: Improving Global Outcomes (KDIGO) criteria [169]. Patients with AKI had a significantly higher risk of dying before 90 days than those without AKI (26.8% vs 1.0%, $p < 0.001$). The risk of developing AKI was associated with other organ failure, including neurological dysfunction. Twenty-two patients (26.8%) died; the risk of dying was associated with worse liver, coagulation and overall organ dysfunction.

1.3.4.2 Electrolyte levels in exertional heat illness

Paper 2: Association between collapse and serum creatinine and electrolyte concentrations in marathon runners: a 9-year retrospective study.

This paper describes electrolyte changes in runners undergoing a full-distance marathon, who experience an exercise-associated collapse (EAC) [170]. Previous work has suggested that the majority of patients (60%) who experience EAC in a half-marathon have a raised temperature, of mean 38.2 ± 1.6°C and a maximum of 41.6°C [171]. In a 56-km ultramarathon, the average core temperature was 38.5 ± 1.3°C, to a maximum of 42.0°C [172]. Paper two reported electrolyte changes over a nine-year period from a large UK marathon in 224 EAC cases compared with 80 non-EAC (control) runners. The serum creatinine concentration, a marker of renal dysfunction, was raised in both collapsed (median (interquartile range (IQR)) 135 (107–168) μmol/L) and control (118.89 ± 24.2 μmol/L) compared with baseline (80.13 ± 12.4 μmol/L). The risk of the serum creatinine being above the normal range in collapsed runners showed a trend to being higher than the control group (relative risk (RR) 1.24, 95% confidence interval (CI) 0.99–1.54, $p = 0.05$). Similarly, the risk of the serum potassium being higher than the normal range was significantly higher in collapsed compared with control runners (RR 3.48, 95% CI 1.29–9.44, $p = 0.01$), consistent with worse renal dysfunction or rhabdomyolysis after hyperthermia. However, urea and sodium plasma levels were similar to baseline in both groups, suggesting that the latter may predominate.

1.3.5 Mechanisms of renal failure in heat illness

The reason for the increase in serum creatinine in heat illness is not clear but is likely to be multifactorial. Blood flow to the kidneys may be reduced, for example due to reduced blood volume [173], or microthrombi in renal afferent and efferent arteries [174]. However, even without significant changes to renal flow, intrinsic renal function also appears to be affected in hyperthermia. In one study on uptake of the radioisotope MAG3 in rabbits, there was delayed uptake (T_{max}) and clearance ($T_{1/2}$) of the isotope at 2, 3 and 4°C above normal core temperature uptake, despite no significant change in mean arterial pressure or renal blood flow [175] (see Table 4, page 55). There was a corresponding increase in serum creatinine and urea levels.

Table 4: *renal MAG3 uptake with different core temperatures (taken from reference [175]). T_{max}: time to peak activity; $T_{1/2}$: time from peak to 50% activity. N = 6, $p < 0.05$.*

Core temperature (°C)	T_{max} ± SD (min)	$T_{1/2}$ ± SD (min)
Normal	1.6 ± 0.1	2.77 ± 0.2
Normal + 2°C	2.8 ± 0.3	3 ± 0.4
Normal + 3°C	8.8 ± 1	8.9 ± 1.1
Normal + 4°C	15 ± 4	20 ± 3.4

Further possible reasons for this observed intrinsic renal dysfunction include blockage of the renal tubules by myoglobin from rhabdomyolysis, direct nephrotoxicity from thermal damage or renal interstitial cell inflammation [176]. The development of AKI is closely related to infiltration of macrophages [177, 178] and dendritic cells [179] in the renal interstitium; both these cell types are associated with inflammation, postulated to be a mechanism underlying organ failure and mortality in hyperthermia (see Section 1.4.2: Gastrointestinal permeability and endotoxin translocation, page 60).

Rhabdomyolysis is defined as muscle breakdown, with subsequent release of the myoglobin protein from the myocytes into the circulation. Passive hyperthermia [180–182], exercise [183] and exercise-induced hyperthermia [168] may cause rhabdomyolysis, of which 10–50% patients subsequently develop acute renal failure [184]. The myoglobin contains the heme protein, which adversely affects renal function through a variety of mechanisms, including renal vasoconstriction, activation of the cytokine cascade and direct cytotoxicity [182].

1.3.6 Current treatments for hyperthermia and heat illness

In the original description of EHS, treatment in the form of 'olive oil and wine, both taken as a drink and used as an ointment' was advocated [(69)].

Current guidelines for EHS, CHS and other hyperthermic states highlight the importance of immediate cooling as the mainstay of treatment, along with providing supportive care for the management of any organ dysfunction.

In one study of 28 patients admitted with EHS, rapid cooling improved mortality and reduced the risk of developing coagulopathy [167]. The median time overall to achieve a core body temperature of less than 38°C was 3.5 hours. In survivors, the median time to achieve a temperature of less than 38°C was three hours (IQR 1.75–6), compared with non-survivors, where the median time was 18 hours (IQR 12–24). Similarly, reducing the core temperature below 38°C within three hours was associated with a risk of developing DIC of 14.3%, compared with 50% if cooling time was longer.

In a systematic review of 521 patients investigating cooling rates in EHS, a cooling rate of less than 0.15 C/min was associated with a 4.57-fold higher risk of medical complications compared to patients who were cooled more quickly (95% CI 3.42–6.28) [185].

In CHS, a delay in a reduction in the temperature is also associated with increased mortality [12]. Cooling to below 38.9°C within 60 minutes is associated with a trend towards improved survival [186].

In MH, delay in cooling is associated with development of complications: the likelihood of any complication increases by 2.9 times per 2°C increase in maximum temperature and 1.6 times with every 30-minute delay in administration of dantrolene [93].

As described above, the reduction in mortality with cooling suggests that it is the hyperthermia that has a significant effect on outcome, rather than the precipitating cause per se.

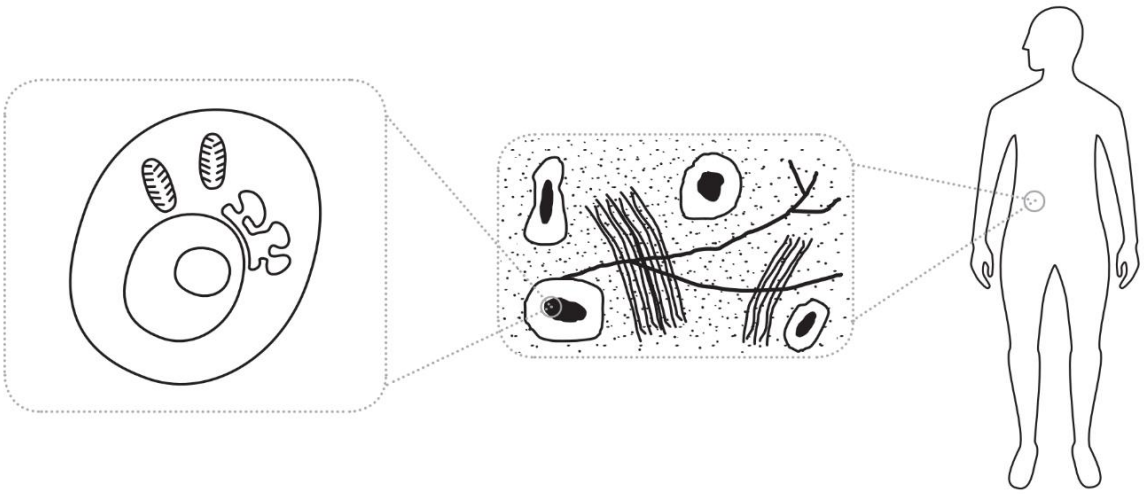
However, in addition to rapid cooling, there is emerging evidence that the morbidity and mortality is associated with systemic inflammation and bacterial translocation from the intestinal tract, and that reducing both may be of benefit. This is explored further in the next two sections.

1.4 The mechanisms of systemic damage from heat stress

The cellular and organ damage from hyperthermia is conventionally considered to be due to direct cellular damage from thermal injury (see Section 1.4.1: Direct thermal damage, page 59). However, there is increasing evidence that hyperthermia may increase GI permeability, which may allow translocation of GI toxins and bacteria to enter the systemic circulation, stimulating endotoxaemia and a pro-inflammatory response, described in more detail in Sections 1.4.2: Gastrointestinal permeability and endotoxin translocation (page 60) and 1.4.3: Lipopolysaccharide stimulation of inflammatory response and cytokine response (page 63), and shown in Figure 2: proposed mechanisms of systemic dysfunction in hyperthermia. Taken from reference [8] and reproduced under a Creative Commons Attribution 4.0 International Licence.(page 58), taken from reference [8].

This section also includes discussion of publications three (Paper 3: Changes in gastrointestinal cell integrity after marathon running and exercise-associated collapse) and four (Paper 4: Exercise hyperthermia induces greater changes in gastrointestinal permeability than equivalent passive hyperthermia), which further characterise the effect of exercise-induced hyperthermia on intestinal permeability compared with exercise and passive hyperthermia.

Figure 2: *proposed mechanisms of systemic dysfunction in hyperthermia. Taken from reference [8] and reproduced under a Creative Commons Attribution 4.0 International Licence.*



Cellular effects	**Local effects**	**Systemic effects**
• Membrane, mitochondrial and DNA damage • Stimulation of excitotoxic mechanisms • Protein denaturation • Cell death	• Ischaemia • Inflammatory changes • Oedema • Cytokine release • Vascular damage	• Endotoxaemia • Bacterial translocation through a dysfunctional gastrointestinal tract

1.4.1 Direct thermal damage

The cellular and surrounding microenvironment is susceptible to direct thermal damage. The microvasculature is affected rapidly; in a histological study, capillary dilatation, vascular stasis and extravasation into the interstitium was observed after only 30 minutes at 40.5°C [187].

Hyperthermia is directly cytotoxic, affecting membrane stability and transmembrane transport protein function. Consequently, ionic transport is disrupted, leading to increased intracellular sodium and calcium with reduced intracellular potassium concentration. Protein and deoxyribonucleic acid (DNA) synthesis is disrupted at various stages in the pathway; while ribonucleic acid (RNA) and protein synthesis may recover quickly after cessation of hyperthermia, DNA synthesis remains disrupted for longer [188]. The nuclear matrix shows damage at lower temperatures than other parts of the cell, with significant endothermic changes observed at 40°C [189]. Direct cell death in humans occurs at temperatures of around 41°C, with the rate of cell death increasing markedly with even modest further increases in temperature [188, 190]. The thermal energy required for cell death is similar to that required for protein denaturation, suggesting that hyperthermic cell death may occur primarily through its effect on protein structure, although whether cell death occurs primarily through necrosis or from apoptosis depends on the cell line and the temperature [188]. Cells in mitosis are more thermosensitive than cells in other phases of replication. Given that organ dysfunction occurs at temperatures lower than that required for in vitro cell death, milder degrees of hyperthermia are also likely to affect cell structure and function with a degree of reversibility.

1.4.2 Gastrointestinal permeability and endotoxin translocation

In addition to direct thermal damage, organ dysfunction may be due to hyperthermic damage rendering the intestinal barrier more permeable, potentially allowing toxins to enter the systemic circulation.

The intestinal barrier is composed of physical factors such as enterocyte membranes and tight junctions between enterocytes, along with an immunological defence system. Its function is to prevent passage of potentially toxic substances from the intestinal lumen to the internal environment.

1.4.2.1 Increase in gastrointestinal permeability with heat stress

Increasing temperatures increase GI membrane permeability. Exposure to temperatures exceeding the critical thermal maximum of the enterocytes (41.6–42.0°C) even after only 60 mins induces a rapid sloughing of intestinal epithelial surface and an increase in intestinal permeability, including to large molecules, to a molecular weight of 4000 Da [191]. Other cell lines also display changes in permeability with heat stress. Heating in vitro canine kidney tubule cells to 41.3°C has been shown to increase paracellular permeability to larger molecules, up to a molecular weight of 180 Da [192]. Other cellular membranes, such as the BBB [193] and renal cellular membranes [194], also display increased permeability to systemic compounds; the BBB shows increased permeability above a brain temperature of 38.5°C [193].

Modest increases in temperature have also been shown in vitro to cause an increase in tight junction (paracellular) permeability of caco-2 cells at temperatures of 39 and 41°C. Caco-2 cells are a human colon epithelial cancer cell line used as a model of human intestinal absorption of drugs and other compounds [195, 196]. The changes to permeability happen early, within a few hours, but appear to be reversible, even after prolonged exposure to heat stress; paracellular permeability has been shown to return to normal even if the hyperthermia is maintained for 24 hours [197].

An increase in GI permeability and translocation of toxins, such as bacteria and LPS, may worsen hyperthermia-induced dysfunction and mortality, explored further below.

1.4.2.2 Translocation of lipopolysaccharides in heat stress and exercise

Lipopolysaccharides are large molecules that form part of the outer membrane of Gram-negative bacteria, and which stimulate production and release of pro-inflammatory mediators if they enter

the systemic circulation. The GI tract contains a large amount of Gram-negative bacteria and is therefore a major source of LPS. The LPS molecule contains a hydrophobic domain, known as *endotoxin* [198]. Administration of antibiotics against intestinal bacteria reduces circulating LPS levels [199], and endotoxaemia in heat stress [200], suggesting that endotoxaemia occurring with heat stress is derived from the GI tract. Lipopolysacharide is thought to be responsible for some of the deleterious effects of hyperthermia; attenuation of systemic LPS by corticosteroids [201] or by anti-LPS antibodies [202] improves survival after heat stress.

Further work confirms that heat stress produces endotoxaemia. In one study of admissions to hospital for heatstroke, LPS was elevated in all 17 patients, whose average admission rectal temperature was 42.1°C [203]. Significantly increased LPS levels have been found in the portal circulation of heat-stressed rats at a rectal temperature of 41.5°C [95]. Heat stress in primates increases portal and systemic LPS concentrations [204].

In humans, strenuous exercise also causes systemic endotoxaemia. In a study of 89 runners requiring admission to the medical tent after the Comrades Marathon (89.4 km), 81% were found to be clinically endotoxaemic, and 2% had concentrations above the level considered to be lethal [205]. In a study of 18 athletes competing in an ultradistance triathlon, the mean plasma LPS concentrations increased over three-fold, from 0.081 to 0.294 ng/mL, with a fall in the mean plasma anti-LPS immunoglobulin G (IgG) concentrations from 67.63 to 38.99 µg/mL [206]; a similar study of 29 ultra-endurance athletes showed endotoxaemia in 68% of the athletes [207]. In a further study in runners, exercising at 80% of maximum oxygen consumption rate ($\dot{V}O_{2max}$) produced a significant increase in intestinal permeability compared with running at 40 or 60% $\dot{V}O_{2max}$. Rectal temperatures at 80% $\dot{V}O_{2max}$ were 39.6°C [208], suggesting that the heat generated may also have been responsible for the changes in permeability. Repeated exercise and heat acclimation may lower the degree of endotoxaemia and may be protective against the development of heat injury [209]; heat acclimation is thought to occur through a number of short- and long-term mechanisms, including an increase in plasma volume, an increase in sweating rate, changes in endocrine responses to exercise [210] and a reduction in heat stress-induced GI permeability [211] with repeated heat exposure.

1.4.2.3 Translocation of other gastrointestinal compounds in heat stress

Heat stress may cause translocation of other GI compounds, including micro-organisms. In one study of patients admitted with CHS, over 50% of patients showed evidence of concomitant bacterial infections [90]. In a further study, procalcitonin (PCT), which has a high sensitivity and specificity for detecting bacteraemia, was elevated in 58% of patients with CHS, and was associated

with mortality [212]. Procalcitonin is produced from a number of tissues in response to bacterial infection, and is gaining use as a biomarker for the diagnosis of bacterial sepsis in clinical practice [213]. In this study, however, microbiological and clinical evidence of infection was not significantly higher in patients with a raised PCT, and it is therefore unclear whether this represents undiagnosed bacteraemia or PCT elevated in the absence of infection.

1.4.3 Lipopolysaccharide stimulation of inflammatory response and cytokine response

Endotoxaemia and raised LPS levels are thought to be responsible for the inflammatory response and stimulation of inflammatory mediators [214]. Administration of highly purified LPS creates diffuse endothelial injury, tissue hypoperfusion and refractory shock [214]; conversely, abolition of endotoxaemia significantly reduces cytokine production [215]. The cytokine profile of the two forms of heatstroke, classical and exertional, are similar, and mirrors that produced by exercise [215], and also shows similarities to that produced by endotoxaemia [215], further suggesting that heats stress is associated with endotoxin production and a pro-inflammatory response. Further clinical evidence for the link between intestinal permeability and an inflammatory response is suggested by a study of endurance runners, in which raising the core temperature during exercise by 2.4°C was associated with an increase in GI permeability as measured by serum concentrations of intestinal fatty acid-binding protein (I-FABP) (see Section 1.4.7.1: Intestinal fatty acid-binding protein, page 69) and stimulation of IL-10 production [216].

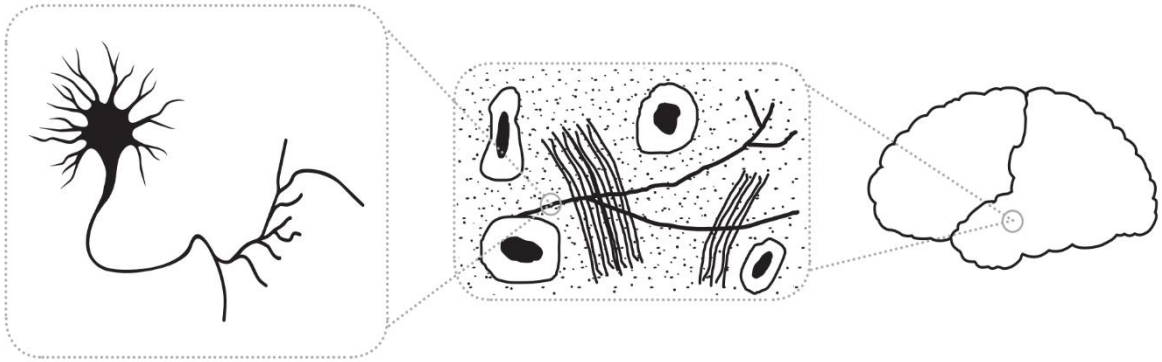
However, the precise role of cytokines in heat stress is unclear, showing an inconsistent response to thermal stress. The levels of a number of pro-inflammatory and anti-inflammatory cytokines are elevated at the time of hyperthermia from heatstroke. Acute phase reactants may also increase. Of these, some (for example, interferon gamma (INFγ), IL-1β) are raised in a proportion of patients, whereas IL-6 may be elevated in all patients [217]. Furthermore, there is some correlation with outcome; the rise in IL-6 and the duration of the increased expression is related to mortality, independent of the maximum core temperature obtained [218]. Mice pre-treated with IL-6 before exposure to heat take longer to reach 42.4°C, showing less organ damage and attenuation in the increase of other cytokines [219]. Conversely, antagonism of IL-1, a pro-inflammatory cytokine, also improves survival [220].

Development of other hyperthermic states may also be associated with inflammatory mediators. Neuroleptic malignant syndrome may be at least partly driven by an acute phase response; acute phase response mediators are reported to rise, and peak at 72 hours. Conversely, levels of anti-inflammatory agents such as serum iron and albumin initially decline then return to the normal range, coinciding with clinical improvement [221]. IL-6 and TNFα levels have also been found to be significantly increased in NMS [221], and IL-6 in MH [222].

1.4.4 Mechanisms of cerebral damage

The mechanism by which cellular and cerebral injury and death occurs as a result of hyperthermia is not yet fully defined but is probably multi-factorial. It may be grouped into three broad areas (Figure 3: proposed mechanisms of neurological dysfunction in hyperthermia. Taken from reference [101] and reproduced under a Creative Common Attribution 4.0 International Licence.page 64, taken from reference [101]); some of these are explored briefly below.

Figure 3: *proposed mechanisms of neurological dysfunction in hyperthermia. Taken from reference [101] and reproduced under a Creative Common Attribution 4.0 International Licence.*



Cellular effects	**Local effects**	**Systemic effects**
• Membrane, mitochondrial and DNA damage • Stimulation of excitotoxic mechanisms • Protein denaturation	• Ischaemia • Inflammatory changes • Oedema • Cytokine release • Vascular damage	• Changes in cerebral blood flow • Endotoxaemia • Bacterial translocation through a dysfunctional gastrointestinal tract

1.4.4.1 Cellular and local effects

The cellular and local neurological effects of heat stress are probably similar to the systemic effects described above, and include neuronal denaturation, neuronal mitochondrial damage, and stimulation and activation of cytotoxic mechanisms and cascades [223]. There are additional proposed mechanisms involved in the development of neurological dysfunction, described below.

1.4.4.2 Blood–brain barrier disruption and neurocognitive dysfunction

The BBB, similar to the GI membrane, is under normal conditions a very selective barrier of tight endothelial cells, which prevents the movement of large or hydrophilic molecules and toxic substances into the brain. The permeability of the BBB is highly temperature-dependent, allowing significantly increased transport of substances at temperatures above 38–39°C, increasing further at higher temperatures [193]. This increased permeability is considered by some to be the predominant factor in the development of cerebral oedema in hyperthermic states. The temperature at which cerebral oedema develops corresponds well to that at which disruption to the BBB occurs: cerebral oedema has been reported in patients dying of heat-related illness with a core temperature at death of 39°C [224]. Rats, with a similar core temperature to humans, develop cerebral oedema and a more permeable BBB at temperatures above 38.5–39°C [193].

Development of neurocognitive dysfunction in hyperthermia (see Section 1.3.2.5: Neurological dysfunction, page 47) may be related to disruption of the BBB and to the presence of LPS in the circulation [117], itself thought to be related to the effects of thermal damage on the integrity of the intestinal barrier (see Section 1.4.2: Gastrointestinal permeability and endotoxin translocation, page 60).

1.4.4.3 Cerebral blood flow and metabolism

Cerebral oxygen and glucose consumption generally increase in hyperthermic states, but the precise relationship with temperature is unclear, and there is considerable variability across different regions of the brain [225]. With modest increases in core temperature, cerebral metabolic rate increases in some areas, but decreases in others. In more extremes of hyperthermia, mitochondrial oxygen metabolism may not be increased beyond that occurring at normothermia; beyond 40°C, it may then decrease further [225]. This may be related either to impaired uptake of mitochondrial oxygen at hyperthermic temperatures, but in the absence of a raised lactate may indicate a reduction in cerebral metabolic activity at increased temperatures, and thereby account for the cognitive and neurological signs and symptoms.

Similarly, changes in cerebral blood flow (CBF) are incompletely understood. Animal studies suggest that CBF may increase by up to 24% for every 1°C increase in temperature [226], but at temperatures above 40–41°C, regional CBF may fall to baseline or below [225, 227]. At temperatures above 40°C in humans, coupling of CBF with systemic arterial blood pressure becomes deranged. In one study of patients undergoing therapeutic hyperthermia for hepatitis C infection, raising the core temperature to 41.8°C was associated with an increase in CBF velocity of 1.5- to 2-fold, independent of the arterial blood pressure [228]. This impairment of flow–pressure

coupling may aggravate vascular engorgement and intracranial hypertension, and therefore the development of cerebral oedema [225]. Disruption of autoregulation may further disrupt BBB integrity, rendering the brain more at risk from systemic insults.

1.4.5 Manipulation of gastrointestinal permeability

A number of nutritional components are known to affect intestinal permeability [229], raising the possibility of manipulating the risk of translocation and subsequent heat illness and other systemic diseases over the longer term. Vitamins A and D are involved in the regulation of intestinal barrier function by modifying the expression of tight junction molecules. Intestinal epithelial cell lines treated with vitamin A or D show increased transepithelial electrical resistance (TEER), a measure of transcellular and paracellular movement of ions through the epithelial layer, itself a measure of intestinal permeability, and upregulation of the tight junction molecules Zonula occludens-1 (ZO-1), occludins and several claudins [230, 231]. Deletion of genes coding for the vitamin D receptor in an animal model has been shown to increase susceptibility to colitis and increase paracellular permeability and death from intestinal dysfunction [232].

Similarly, there is emerging evidence of the role of amino acids in maintaining intestinal homeostasis and cellular integrity. Glutamine appears to have a number of roles in maintaining epithelial barrier function. It maintains the expression and function of tight junction proteins claudin-1, occludins and ZO-1 [233, 234]. Glutamine deficiency affects the expression of tight junction proteins and also appears to selectively suppress epithelial cell proliferation [235]. It also appears to have a role in maintaining GI immune function, by reducing production of pro-inflammatory IL-6 and IL-8 and enhancing production of anti-inflammatory IL-10 level in T and B lymphocytes and epithelial cells [236, 237]. Clinically, it may have benefit in intestinal disease, although evidence is currently lacking [238].

Arginine also appears to have a role in maintaining intestinal integrity and reducing permeability [239], probably via expression and function of the tight junction protein ZO-1 [240], although there are fewer data than for glutamine [229].

Glutamine supplementation appears to reduce intestinal permeability in exertional hyperthermia [241]. In one study, 0.25, 0.5 and 0.9 g/kg of glutamine were given to ten males two hours prior to exercising for 60 min at 30°C. Intestinal permeability was estimated by calculating the plasma lactulose:rhamnose ratio (L:R), as described in Section 1.4.7.2: Lactulose:rhamnose absorption during exercise-induced hyperthermia (page 70). The core temperature was raised to around 38.5°C in all cases. Compared with placebo, intestinal permeability was significantly reduced by supplementation with all three doses of glutamine, and by more with the largest of the three doses.

1.4.6 Clinical significance of gastrointestinal permeability

There is emerging evidence of the role of the GI tract in the development of a wide range of systemic diseases, supporting the hypothesis discussed above that change in GI permeability may also affect development of systemic dysfunction and outcome from heat illness. In addition to changes in GI permeability, development of systemic disease has also been suggested to be related to changes in intestinal microbiota and immune function [242].

A number of intestinal diseases, such as inflammatory bowel diseases, coeliac disease, food allergies, irritable bowel syndrome, and obesity and metabolic diseases [243] and colonic carcinoma [244] are associated with changes in intestinal permeability.

Similarly, there is evidence of increased intestinal permeability in a wide range of chronic neurological conditions, such as autism [245], Parkinson's disease [246], multiple sclerosis [247] and dementia [248]. How development of these diseases corelates with the proposed mechanisms outlined in Section 1.4.4: Mechanisms of cerebral damage (page 64) is not clear, but disrupted integrity of both the gut–blood and blood–brain barrier, allowing intestinal pathogenic material to affect cerebral function, has been suggested [249].

1.4.7 Further characterisation of the change in gastrointestinal permeability

What is not clear is whether exercise or the resulting hyperthermia is the predominant factor in increasing GI permeability [208]. Papers three and four sought to further characterise the change in GI permeability after heat, exercise at normothermia, and exertion-induced hyperthermia.

1.4.7.1 Intestinal fatty acid-binding protein

Paper 3: Changes in gastrointestinal cell integrity after marathon running and exercise-associated collapse.

The third primary paper [250], presented in full in Paper 3: Changes in gastrointestinal cell integrity after marathon running and exercise-associated collapse, measured a GI protein that only appears in the circulation if the GI tract is more permeable than normal. Intestinal fatty acid-binding protein is present in the mature enterocytes of the small intestinal villi and is released when cell membrane integrity is compromised, subsequently appearing in the circulation following enterocyte injury [251]. It has a size of 15 kDa and is involved in the cellular uptake and metabolism of fatty acids. It appears to be a sensitive biomarker of intestinal injury [252] and may have a role in the detection of intestinal ischaemia [253], including during exercise [254], and mirrors the bacterial translocation through a permeable intestinal wall in pancreatitis [255]. Serum levels of I-FABP increase after exercise and are associated with changes in permeability [256], suggesting that intestinal cell damage is partly responsible for the increase in permeability. It is thought to have a short half-life in the circulation of a few minutes [254, 257], and is therefore a sensitive biomarker of time of cell injury and recovery.

In this study, 24 runners were recruited as controls prior to completing a standard UK marathon and had sequential I-FABP measurements before and on completion of the marathon, then at four and 24 hours later. Eight runners incapacitated with exertional heat illness (EHI) had I-FABP measured at the time of collapse and one hour later. Levels of I-FABP were increased immediately on completing the marathon (T0; 2593 ± 1373 ng/L) compared with baseline (1129 ± 493 ng/L; $p < 0.01$) in the controls, but there was no significant difference between baseline and the levels at four hours (1419 ± 1124 ng/L; $p = 0.7$), or at 24 hours (1086 ± 302 ng/L; $p = 0.5$). At the time of collapse or completing the marathon, EHI cases had a significantly higher I-FABP concentration (15,389 ± 8547 ng/L) compared with controls at T0 ($p < 0.01$) and remained higher at one hour after collapse (13,951 ± 10,476 ng/L) than the pre-race control baseline ($p < 0.05$). The study suggested that intestinal wall damage increases after marathon running and increases further if the endurance exercise is associated with EHI. After EHI, I-FABP remains high in the circulation for an extended

period, suggesting ongoing intestinal wall stress greater than that induced by exercise at normothermia.

1.4.7.2 Lactulose:rhamnose absorption during exercise-induced hyperthermia

Paper 4: Exercise hyperthermia induces greater changes in gastrointestinal permeability than equivalent passive hyperthermia.

However, primary paper four [258], presented in full in Paper 4: Exercise hyperthermia induces greater changes in gastrointestinal permeability than equivalent passive hyperthermia, used a different measure of GI permeability (absorption of ingested sugars) in a lab-based study, and found that exercise-induced hyperthermia appears to have much more effect on permeability than does passive hyperthermia.

A simple but well-validated measure of GI permeability involves the differential absorption of two orally administered sugars. Rhamnose is well absorbed from the GI tract, with around 10% of the oral dose excreted in urine within the first five hours [259]; lactulose is less well absorbed, unless permeability is increased. The ratio of lactulose to rhamnose in blood, or urine after renal excretion, therefore allows an assessment of GI permeability.

The aim of this pilot study was to determine whether different changes in GI permeability, characterised by an increased plasma (L:R), occurred in exercise hyperthermia in comparison to equivalent passive hyperthermia. Six healthy adult male participants underwent exercise in hot conditions (Ex-Heat) and passive heating during hot water immersion (HWI). Heart rate (HR) and rectal (core) temperature were recorded throughout the trials. The L:R ratio and peak and change in HR were higher in Ex-Heat than HWI, despite no differences in trial duration or peak core temperature. The L:R ratio was strongly correlated ($p < 0.05$) with HR peak ($r = 0.626$) and change in HR ($r = 0.615$) but no other variable. This study suggests that exercise exacerbates hyperthermia-induced GI permeability at the same absolute temperature. The increase in HR suggests that the increased cardiovascular strain occurring during exercise may cause the increase in GI permeability.

The data presented in paper four is part of a larger study. The six participants underwent four arms (passive hyperthermia and exercise hyperthermia as described above, exercise hyperthermia after precooling with ice ingestion, and exercise in a cool environment, to prevent hyperthermia), and had GI permeability measured by differential disugar absorption, as described above. The data are presented in Appendix 2, page 102. The core temperature in the third arm (exercise at normothermia) was significantly lower than the other three groups ($p < 0.01$ between exercise and all other groups, by pairwise *t* tests with Bonferroni correction applied for multiple comparisons)

(Figure 4**:** box-and-whisker plot to show core temperature changes during passive heating, exercise, exercise and heat, and precooling studies. Unpublished data.page 102 and Table 6, page 104). The L:R absorption was similar between exercise, passive hyperthermia and exercise hyperthermia, but there was higher absorption in the exercise hyperthermia group compared with the other three arms (Figure 5: box-and-whisker plot to show changes in lactulose:rhamnose absorption during passive heating, exercise, exercise and heat, and precooling studies. L:R: plasma lactulose:rhamnose ratio. Unpublished data. 103 and Table 6, page 104); however, with pairwise comparison with Bonferroni correction, no comparison reached statistical significance. The data suggest that both exercise and passive hyperthermia cause similar changes to permeability but are significantly higher after exercise and hyperthermia combined, suggesting synergistic effects (Appendix 2, page 102).

This study suggests that exercise-induced hyperthermia increases intestinal permeability more so than hyperthermia alone, yet it is unclear whether this is due to the aforementioned increased physiological strain, or by additional mechanisms. A reduction in intestinal blood flow may be responsible, with subsequent ischaemic cellular damage. Splanchnic blood flow falls by 20% at a core temperature of 38°C compared with 37°C, falling further with higher temperatures [260]. During exercise, blood flow has been shown to reduce in the superior mesenteric artery by 43% [261], and in the portal vein by 50% [262]; however, in neither of these studies was the effect of temperature assessed.

1.5 Potential novel treatments for hyperthermia and heat illness

The proposed pathway of endotoxaemia from intestinal Gram-negative bacteria entering the systemic circulation via a permeable intestinal tract wall allows several potential sites for modulating the pathway, explored further in this section.

This therefore raises the possibility of reducing the systemic damage caused by the exertional (and maybe non-exertional) hyperthermia by reducing the inflammatory response or the bacterial load in the systemic circulation.

This section includes discussion of publications five (see Paper 5: The efficacy of steroids in reducing morbidity and mortality from extreme hyperthermia and heatstroke – a systematic review) and six (see Paper 6: The efficacy of antibiotics in reducing morbidity and mortality from extreme hyperthermia and heatstroke – a systematic review), which are systematic reviews on the effect of administration of steroids and antibiotics on the outcomes of heat illness.

1.5.1 Administration of steroids

Paper 5: The efficacy of steroids in reducing morbidity and mortality from extreme hyperthermia and heatstroke – a systematic review.

This paper [263], presented in full in Paper 5: The efficacy of steroids in reducing morbidity and mortality from extreme hyperthermia and heatstroke – a systematic review, was a systematic review investigating whether anti-inflammatory steroids are beneficial in improving outcomes after heat illness.

Glucocorticoids inhibit many of the initial events in an inflammatory response, helping to promote the resolution of inflammation [264]. Acutely, glucocorticoids inhibit the vasodilation and increased vascular permeability that occurs following an inflammatory insult and decrease leukocyte migration to the site of injury [265]. Most of the anti-inflammatory and immunosuppressive actions of glucocorticoids are attributable to alterations in the genetic transcription in leukocytes [265]. While the precise role of the inflammatory response in heatstroke is unclear, reducing the inflammation appears to be of benefit. Corticosteroids reduce levels of the majority of cytokines [264] and the administration of prophylactic glucocorticoids prevents heatstroke-induced LPS rise in animal models [201, 266]. These data therefore suggest that administration of corticosteroids may have a beneficial role in the treatment of heatstroke.

The review was conducted according to the PRISMA guidelines (Appendix 1, Table 5, page 98) [161]. Five studies were found which met the criteria. Of the five studies, three used rats [267–269] and

two used primates [201, 270]. No human studies were found. Four of the studies used dexamethasone [267–270] and one methylprednisolone [201]. In three studies, the steroid was given after the onset of heat stress [267–269].

With the exception of one study [270], all studies reported improved survival, with three reaching statistical significance, and markers of organ dysfunction. Survival time was greatest when steroid administration preceded heat stress. In one study, a non-significant increase in mortality was seen. A dose response was observed, with higher doses extending survival time. While the numbers were small, and only animal studies have been reported so far, both drugs appear to be beneficial in reducing the harm and risk of death from hyperthermia.

One of the studies also investigated the effect of two anti-inflammatory agents in combination. A combination of mannitol and dexamethasone was found to improve cardiovascular instability, inflammatory mediators and survival time in heatstroke rats [269].

A further study, not included in the systematic review, showed that a dose of 30 mg/kg methylprednisolone prior to exposure of five primates to heat stress (43.5°C) significantly improved survival to 100%, compared with a survival of 33% in the control group [271]. The plasma LPS levels showed little change after heat stress, compared with non-surviving controls, where the LPS levels by almost three-fold (0.089 ± 0.007 to 0.257 ± 0.031 ng/mL), with a corresponding three-fold decrease in plasma anti-LPS IgG levels, further suggesting the role of LPS in heat-stress damage.

1.5.2 Administration of antibiotics

Paper 6: The efficacy of antibiotics in reducing morbidity and mortality from extreme hyperthermia and heatstroke – a systematic review.

This paper [272], presented in full in Paper 6: The efficacy of antibiotics in reducing morbidity and mortality from extreme hyperthermia and heatstroke – a systematic review, was a systematic review investigating whether antibiotics are beneficial in improving outcomes after heat illness. The review followed PRISMA guidelines (Appendix 1, Table 5, page 98) [161], to identify randomised controlled trials in animal or humans, where the effect of administration of antibiotic before or after exposure to hyperthermia or heat stress on organ dysfunction or death was compared with a control group.

Two papers were found that fit the criteria. In one [200], non-absorbable oral antibiotics were administered for five days prior to the onset of heat stress. There was a significant reduction in intestinal bacterial load in the group pre-treated with antibiotics, compared with the control group: the total bacterial count fell from $12.56 \pm 1.83 \times 10^9$ colony-forming units/g faeces to $0.183 \pm 0.04 \times 10^9$/g faeces between the two groups, primarily due to a large reduction in Gram-negative bacteria. There was an increase in the Gram-positive bacterial count. Administration of antibiotics reduced the cardiovascular and markers of hepatocellular dysfunction, but animals succumbed at a lower temperature. Administration of antibiotics also prevented a rise in endotoxaemia. Plasma LPS concentration increased seven-fold in the control group, whereas no significant increase in LPS from baseline was seen in the intervention group. Lipopolysaccharide levels when the rectal temperature reached 44–45°C were different between the intervention group (0.308 ± 0.038 ng/mL) and the control group (0.005 ± 0.002 ng/mL). The examined orally administered antibiotics, neomycin and kanamycin, are active against intestinal bacteria, but do not cross into systemic circulation in sufficient quantity to have systemic effect [273], suggesting that any effect on the endotoxaemia would be from alteration in the intestinal microbiome, and that the systemic LPS is likely to be derived from the intestinal tract. Higher LPS levels are associated with a poor outcome after heat stress [201, 214].

In the second study [274], 50 anaesthetised dogs in three intervention groups were heated by a water blanket until the rectal temperatures rose to 43.5°C. Animals were then cooled passively in room air at 28°C until death occurred or 18 hours had elapsed, and were euthanised. The first and second groups did not receive manipulation of bowel contents; the first group of 15 animals was heated to induce heatstroke, while the second group was not heated. The third group (12 animals) was heated in a similar manner to the first, but in addition received 1000 mg neomycin and 250 mg tetracycline four times per day for four days prior to the heat stress, a laxative and enema prior to the heat stress, and 1 million units of intravenous penicillin every four hours during the heating and

cooling periods. The fourth group (seven animals) were heated in the same way as the first and third, but only received the intravenous penicillin during and after heating, and not the prior antibiotics and laxatives that the third group received. The third group, who had intestine stool and bacterial contents reduced, showed an increase in 18-hour survival from 20.0% to 70.6%. In the group where antibiotics were administered after heatstroke, survival was 14.2% and not significantly different to survival in the heated but untreated group. The paper also reported average survival times of the animals who succumbed before the 18 hours had elapsed in the pre-heat antibiotic group (10.6 ± 2.7 h, n = 5), post-heat antibiotic group (3.2 ± 0.9 h, n = 6) and the heated control group (3.8 ± 2 h, n = 12). There was a significant improvement in survival time between the group receiving pre-insult antibiotics, but not between the group receiving post-insult antibiotics and the control group. The authors used a core temperature of 43.5°C, citing previous work suggesting that heatstroke occurs at this temperature.

This second study was the only study where antibiotics were given after heat trauma. However, intravenous penicillin was administered, which has limited efficacy against the predominant Gram-negative bacteria in the intestinal tract. This is likely to be due to penicillinases [275], enzymes produced by certain bacteria, which inactivate penicillin.

Given that only two animal studies met the search criteria of the effect of antibiotics on organ dysfunction or mortality, and that both studies primarily administered antibiotics before the onset of heat stress, application to humans during heatstroke is difficult.

1.6 Further work

It is likely therefore that hyperthermia exerts its deleterious effects partly by increasing GI permeability and allowing toxins to enter the systemic circulation, and by stimulating an inflammatory or sepsis-like response.

The next proposed step is to investigate the effects on markers of inflammation of steroids given to human volunteers after inducing hyperthermia. If steroids do reduce inflammation, then there is the possibility of including steroids in future management to reduce the risk of death and disability after heatstroke.

Published work on the effect of steroids has to date only involved animals. It is proposed now to induce hyperthermia in human subjects in the laboratory setting to further characterise the inflammatory response and its relationship to intestinal permeability, before and after the administration of systemic steroids. If an improvement is seen, then clinical trials in a larger group of patients, in clinical settings, for example, a marathon, will be proposed.

While most patients with hyperpyrexia develop the condition through exercise or exposure to hot conditions, other conditions, such as ingestion of medications and illicit drugs, may also be responsible. If manipulation of intestinal permeability is indeed possible to improve outcomes after heatstroke, then future work may investigate application of anti-inflammatory and anti-microbial medications in all cases of non-septic pyrexias, irrespective of the cause.

1.7 Conclusions and summary

Exertional and passive hyperthermia, especially if not associated with sepsis, is associated with significant morbidity and mortality. The publications described in this thesis add to existing knowledge around the risks to human health, specifically around the risk of renal injury and electrolyte imbalance. The publications also explore intestinal permeability, inflammation and endotoxaemia as additional putative mechanisms behind the morbidity, and the potential for modulating these mechanisms in animals with administration of steroids or antibiotics active against intestinal bacteria.

The work presented here allows the possibility for development of future work in humans on reducing the inflammatory response and intestinal toxin absorption to reduce the risk of death and disability after heatstroke and hyperthermia.

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1.9 Appendices

Appendix 1

PRISMA checklist

Table 5: *PRISMA checklist. Taken from reference [161].*

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g., for all	

Section and Topic	Item #	Checklist item	Location where item is reported
		measures, time points, analyses), and if not, the methods used to decide which results to collect.	
	10b	List and define all other variables for which data were sought (e.g., participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	
Effect measures	12	Specify for each outcome the effect measure(s) (e.g., risk ratio, mean difference) used in the synthesis or presentation of results.	
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g., tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g., subgroup analysis, meta-regression).	
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the	

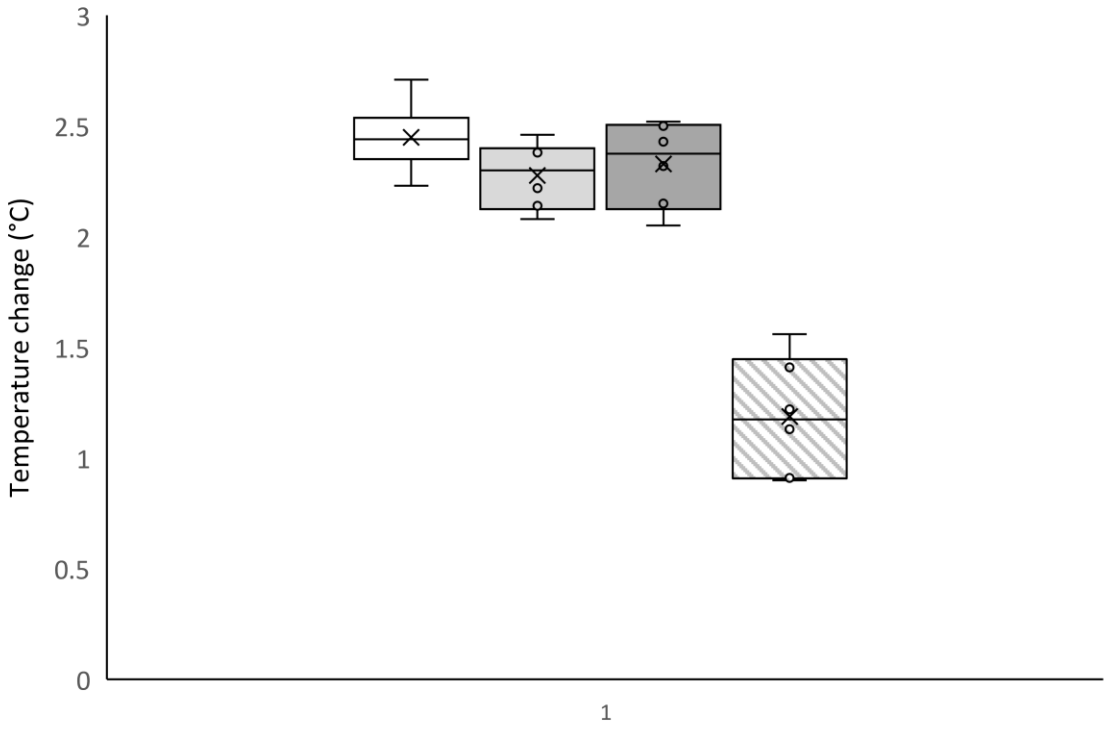
Section and Topic	Item #	Checklist item	Location where item is reported
		number of studies included in the review, ideally using a flow diagram.	
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	
Study characteristics	17	Cite each included study and present its characteristics.	
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g., confidence/credible interval), ideally using structured tables or plots.	
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g., confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	
	23b	Discuss any limitations of the evidence included in the review.	
	23c	Discuss any limitations of the review processes used.	
	23d	Discuss implications of the results for practice, policy, and future research.	
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	

Section and Topic	Item #	Checklist item	Location where item is reported
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	
Competing interests	26	Declare any competing interests of review authors.	
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	

Appendix 2

Gastrointestinal permeability during passive heating, exercise, exercise-induced heating and precooling

Figure 4: *box-and-whisker plot to show core temperature changes during passive heating, exercise, exercise and heat, and precooling studies. Unpublished data.*



<u>*Key*</u>

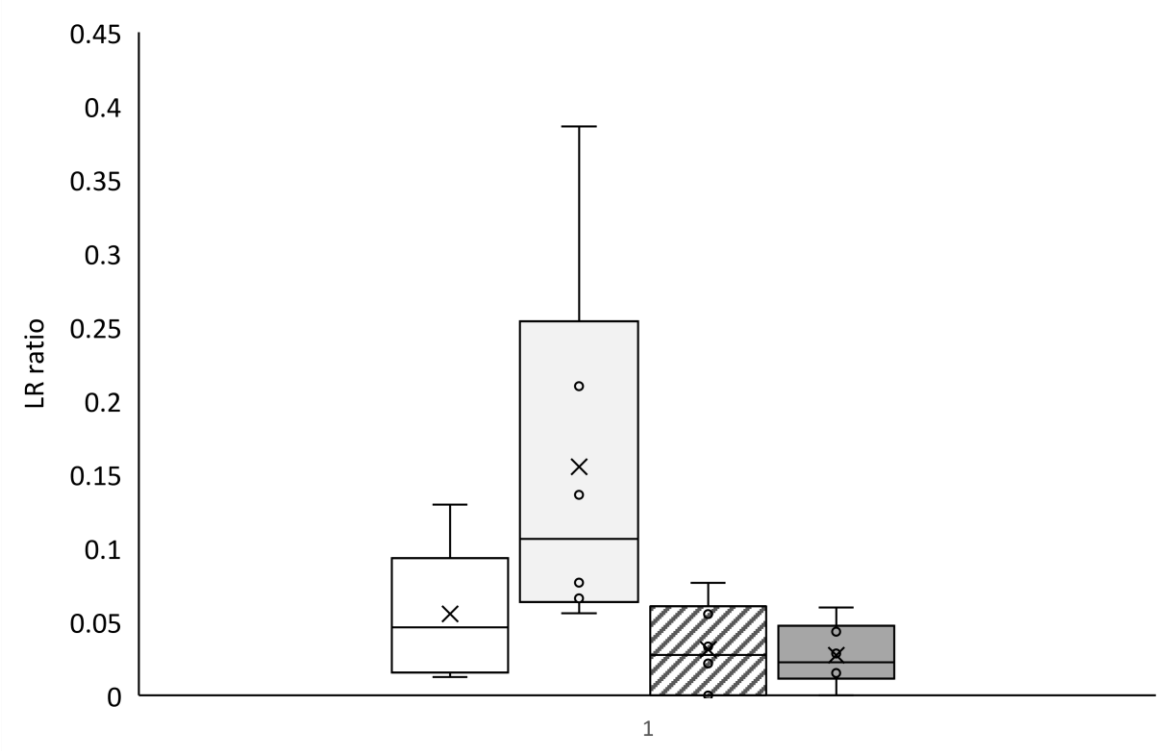
White: pre-cooling

Light grey: exercise and heat

Dark grey: hot-water immersion

Hatched: exercise

Figure 5: *box-and-whisker plot to show changes in lactulose:rhamnose absorption during passive heating, exercise, exercise and heat, and precooling studies. L:R: plasma lactulose:rhamnose ratio. Unpublished data.*



<u>Key</u>

White: pre-cooling

Light grey: exercise and heat

Hatched: exercise

Dark grey: hot-water immersion

Table 6: *temperature changes and lactulose:rhamnose ratios during passive heating, exercise, exercise and heat, and precooling studies. L:R: plasma lactulose:rhamnose ratio; PC: pre-cooling; Ex+H: exercise and heat: HWI: hot water immersion; Ex: exercise.*

Subject		1	2	3	4	5	6	Mean	SD
LR ratio	PC	0.016	0.081	0.044	0.047	0.129	0.012	0.055	0.040
	Ex+H	0.136	0.055	0.076	0.386	0.209	0.065	0.154	0.115
	Ex	0.033	0	0.076	0.055	0	0.021	0.031	0.027
	HWI	0.015	0	0.060	0.043	0.028	0.016	0.027	0.019
PC	Resting temperature	37.31	36.86	37.19	37.02	37.25	37.43	37.18	0.19
	Temp at end of ice, before study	36.94	36.58	36.95	36.79	36.73	37.06	36.84	0.16
	Max temperature	39.4	39.06	39.34	39.5	38.96	39.48	39.29	0.206
	Temp difference (pre ice to max)	2.09	2.2	2.15	2.48	1.71	2.05	2.113	0.227
	Temp difference (post-ice to max)	2.46	2.48	2.39	2.71	2.23	2.42	2.448	0.142
Ex + H	Resting temperature	37.2	37.22	36.7	37	37.09	37.04	37.042	0.172
	Max temperature	39.58	39.44	39.08	39.46	39.23	39.12	39.318	0.185
	Temp difference	2.38	2.22	2.38	2.46	2.14	2.08	2.277	0.138
HWI	Resting temperature	37.2	36.97	36.7	36.97	36.7	37.4	36.99	0.252
	Max temperature	39.63	39.29	39.2	39.12	39.22	39.45	39.318	0.172
	Temp difference	2.43	2.32	2.5	2.15	2.52	2.05	2.33	0.18
Ex	Resting temperature	36.76	36.36	36.88	36.99	37.42	37.2	36.94	0.33
	Max temperature	38.32	37.77	38.01	37.89	38.33	38.42	38.12	0.25
	Temp difference	1.56	1.41	1.13	0.9	0.91	1.22	1.18	0.24

Section 2

Theoretical framework and statistical analysis of the primary publications

2.1 Aims and objectives

The papers presented in section three aim to add to the existing knowledge surrounding three consecutive aspects of hyperthermia: (1) the threats to human health from hyperthermia, (2) the putative mechanisms underlying the systemic damage, and (3) potential treatments.

There are a number of common causes of hyperthermia, described in Section 1.3.1: Causes of hyperthermia (page 37). Emerging evidence suggests that hyperthermia adversely affects organ function and outcomes (see Section 1.3.2: Adverse effects of hyperthermia, page 44). Papers one and two, presented in full in section three, further characterise the risk of acute kidney injury associated with endurance events, specifically in athletes who develop EHI, and the changes that occur in electrolyte balance and renal function in runners who collapse and develop EHI.

The mechanisms underlying the cellular and organ damage associated with heat illness appear to include direct cellular damage from thermal injury (see Section 1.4.1: Direct thermal damage, page 59) and loss of GI integrity (see Section 1.4.2: Gastrointestinal permeability and endotoxin translocation, page 60), which may allow translocation of intestinal toxins and bacteria into the systemic circulation, with subsequent development of a cytokine-associated inflammatory response. Papers three and four, presented in full in section three, further characterise the effect of exercise-induced hyperthermia on GI permeability compared with exercise or passive hyperthermia alone.

The proposed pathway of endotoxaemia from intestinal bacteria entering the systemic circulation and a pro-inflammatory response may allow treatments or interventions to specific parts of the pathway to lessen the organ function and improve outcome. Publications five and six, presented in full in Section three, are systematic reviews to determine the effect of administration of steroids and antibiotics respectively on the outcomes of heat illness.

2.2 Hypothesis and research questions

This section summarises the rationale for the individual studies presented in section three and the hypotheses and research questions to be answered.

2.2.1 Hypothesis for Paper 1: Acute kidney injury associated with endurance events –is it a cause for concern? A systematic review

There are significant risks of short- and long-term cellular and organ dysfunction and mortality after an episode of hyperthermia and heat illness from all causes, which have been recently reviewed [(1)]. Separately, AKI and renal insult has been known for several decades to be a risk in endurance events [(2), (3)].

However, the extent of renal damage after endurance events with and without development of heat illness had not been systematically reviewed.

It was hypothesised that renal damage after an endurance event, especially if associated with heat illness, would be a significant problem, and which would warrant systematic examination and narration.

2.2.2 Hypothesis for Paper 2: Association between collapse and serum creatinine and electrolyte concentrations in marathon runners

The risk of hyponatraemia and other electrolyte derangements in endurance athletes is well documented [(4)], and despite current guidelines to drink according to thirst, the incidence of hyponatraemia in even asymptomatic runners has been reported at 12.5% [(5)]. Our initial systematic review (see Paper 1: Acute kidney injury associated with marathon events – is it a cause for concern? A systematic review) described an average increase in creatinine levels of 25.7 µmol/l after endurance running events [(6)], with 30% of marathon runners in a separate study having levels above the upper limit of normal [(4)]. Data on serum potassium concentrations are reported less often, but hyperkalaemia has been reported in 16% of runners after completing a marathon [(4)].

Data on the incidence of electrolyte derangement following the latest guidelines on fluid replacement and following emerging evidence on the factors to mitigate the risk of renal damage in endurance events had only been infrequently reported. The data presented in this study spans eight years and 224 collapsed runners, a larger series than many studies. In addition, it includes data from a control (non-collapsed) group of runners completing the marathon. It was therefore considered that publication of these data was warranted, to help inform the ongoing concern around the risks of biochemical abnormalities in EHI.

It was hypothesised that derangement of electrolyte and creatinine levels would be less frequent than previously reported, but would be higher in collapsed runners, especially those with heat illness, than in non-collapsed runners who complete the marathon.

2.2.3 Hypothesis for Paper 3: Changes in gastrointestinal cell integrity after marathon running and exercise-associated collapse

Following the publication of papers one and two highlighting the risks of renal dysfunction and biochemical abnormalities after EHI, we wanted to investigate further the proposed permeable GI tract model in heat illness as a mechanism for the development of the systemic abnormalities.

Heat strain [(7)] and endurance exercise [(8)] are both known to damage GI wall integrity and allow translocation of intestinal bacteria or endotoxins into the systemic circulation [(9)]. The combination of heat and exercise causes higher systemic levels of endotoxin than exercise of equivalent intensity with lower temperature gain [(10)], suggesting that GI permeability in EHI is multi-factorial and may not be entirely contingent on core temperature change alone [(11)].

Intestinal fatty acid-binding protein is a cytosolic protein involved in the uptake and metabolism of fatty acids in the GI tract. It is present on the luminal side of enterocytes of the small intestinal villi, and its presence in the systemic circulation suggests enterocyte injury or increased permeability in intestinal injury [(12)], ischaemia [(13)][(14)] and pancreatitis [(15)].

We sought to measure I-FABP serum levels to further characterise changes in GI wall integrity after endurance exercise with and without heat illness. The relative effects of exercise and heat illness on wall integrity were not well understood. In addition, little work had been published on I-FABP changes in exercise. Further characterisation of I-FABP levels in exercise and heat illness may allow validation of its use as a sensitive biomarker in further studies investigating GI wall integrity in heat illness.

It was hypothesised that I-FABP would increase following a marathon, and that greater increases would be observed in those incapacitated during or at the end of the event.

2.2.4 Hypothesis for Paper 4: Exercise hyperthermia induces greater changes in gastrointestinal permeability than equivalent passive hyperthermia

In comparison with paper three investigating GI wall integrity with endurance exercise and exertional hyperthermia, paper four investigates the relative effects of passive hyperthermia and exertional hyperthermia.

Hyperthermia increases GI wall integrity leading to increased permeability [(16)], although it is not known whether this is a result of the direct effect of temperature or local ischaemia [(17)] on the intestinal epithelium.

Exercise also damages GI wall integrity [(18), (19), (20)], although it is not fully known whether heat stress or exercise is the primary determinant of increased GI permeability in EHI. A recent systematic review suggests that the hyperthermia may have more association with increased permeability than does exercise [(21)].

Determining the independent and combined effects of heat stress and exercise on GI permeability may further understanding of the pathophysiology of EHI, in particular, the subsequent endotoxaemia and inflammatory response, which may support the development of preventative and/or therapeutic interventions. If exercise-induced hyperthermia is the predominant determinant of GI permeability, then further studies on hyperthermia may use exertion as a means to induce rises in core temperature since the exertion by itself would be known to have little effect on the GI permeability.

The aim of this pilot study was to determine whether GI permeability, characterised by plasma lactulose:rhamnose concentration ratio, differed between exercise hyperthermia compared with equivalent passive hyperthermia. It was hypothesised that exercise hyperthermia would increase GI permeability more than would passive hyperthermia, in association with a greater elevation in cardiovascular strain, despite an equivalent core temperature.

2.2.5 Hypothesis for Paper 5: The efficacy of steroids in reducing morbidity and mortality from extreme hyperthermia and heatstroke – a systematic review

Loss of the GI barrier integrity increases the potential for endotoxaemia and systemic LPS increases, which initiates the release of pro-inflammatory cytokines [(22), (23)]. Lipopolysaccharides are large molecules that form part of the outer membrane of gram-negative bacteria and contain a hydrophobic domain, known as endotoxin [(24)], which stimulates release of pro-inflammatory mediators if they enter the systemic circulation. A pro-inflammatory response, mediated by pro-inflammatory cytokines, for example, TNFα and IL-1, is a well-developed defence mechanism, triggered by infective pathogens and toxic insults, such as trauma, to remove injurious stimuli and initiate tissue repair.

Lipopolysaccharide is elevated in humans with heatstroke [(25)] and in animals with heat-stress [(17), (26)]. Lipopolysaccharide is thought to be responsible for some of the deleterious effects of hyperthermia, since administration of purified LPS produces diffuse endothelial injury, tissue hypoperfusion and refractory shock [(23)], and attenuation of systemic LPS by anti-LPS antibodies [(27)] improves survival after heat stress. Endotoxaemia occurring with heat stress appears to be GI tract derived, since administration of antibiotics against intestinal micro-organisms prevents endotoxaemia from occurring [(28)] and appears to improve mortality [(29)].

The role of cytokines in heat stress is unclear with an inconsistent response to thermal stress. A number of pro-inflammatory and anti-inflammatory cytokines , such as INFγ, IL-1β and IL-6 [(30)], are elevated at the time of hyperthermia from heat stroke. Acute phase reactants may also increase. The rise in cytokine production appears to be driven by LPS, since abolition of endotoxaemia significantly reduces cytokine production [(31)].

Glucocorticoids inhibit many of the initial events in an inflammatory response, promoting the resolution of inflammation [(32)]. Acutely, glucocorticoids inhibit the vasodilation and increased vascular permeability that occurs following an inflammatory insult and decrease leukocyte migration to the site of injury [(33)]. Most of the anti-inflammatory and immunosuppressive actions of glucocorticoids are attributable to alterations in the genetic transcription in leukocytes [(33)]. While the precise role of the inflammatory response in heatstroke is unclear, reduction of inflammation appears to be of benefit. Corticosteroids reduce levels of the majority of cytokines [(32)], and the administration of prophylactic glucocorticoids prevents heat-stroke-induced LPS rise in an animal model [(34), (35)].

These data therefore suggest that administration of corticosteroids may also have a beneficial role in the functional outcomes after heatstroke. We therefore undertook a systematic review of publications investigating the effect of administration of corticosteroids on survival or organ

dysfunction; the hypothesis was that administration of corticosteroids would improve markers of inflammation, organ dysfunction and mortality.

2.2.6 Hypothesis for Paper 6: The efficacy of antibiotics in reducing morbidity and mortality from extreme hyperthermia and heatstroke – a systematic review

One of the proposed mechanisms for poor functional outcomes after heat illness is that heat stress causes increase in GI permeability [(36), (37), (16)], which may allow translocation of intestinal bacteria into the systemic circulation [(38)] and subsequent bacterial stimulation of a pro-inflammatory response.

Lipopolysaccharides from intestinal gram-negative bacteria stimulate production of pro-inflammatory mediators if they enter the systemic circulation (see Section 1.4.3: Lipopolysaccharide stimulation of inflammatory response and cytokine response, page 63) [(23)]; a reduction in systemic LPS significantly reducing cytokine production [(31)]. Lipopolysaccharide or LPS-derived endotoxin appears to be responsible for some of the deleterious effects of hyperthermia, since administration of exogenous LPS can create diffuse endothelial injury, tissue hypoperfusion and refractory shock [(23)] and attenuation of systemic LPS by anti-LPS antibodies [(27)] improves survival after heat stress.

Reductions in intestinal bacterial load by non-absorbable antibiotics reduces circulating LPS levels [(39)], suggesting that the LPS is derived from GI bacteria.

The proposed pathway of endotoxaemia and subsequent pro-inflammatory response from GI gram-negative bacteria entering the systemic circulation via a permeable GI wall allows several potential sites for modulating the pathway and affecting the outcome from heat stress. We therefore undertook a systematic review of publications investigating the effect of administration of antibiotics on survival or organ dysfunction. It was proposed that antibiotics active against GI bacteria may reduce the bacterial translocation and endotoxaemia, and therefore improve the outcome from non-infective hyperthermia.

2.3 Statistical analysis

A variety of statistical analyses and techniques were used in the papers presented in Section 3; the use and rationale for these tests is described further in this section.

2.3.1 Statistical analysis for Paper 1: Acute kidney injury associated with endurance events – is it a cause for concern? A systematic review

2.3.1.1 Development of systematic review and meta-analysis

This paper (see Paper 1: Acute kidney injury associated with endurance events – is it a cause for concerns? A systematic review) was a systematic review. It was presented as a narrative systematic review, but consideration was given to whether the characteristics of the study population and interventions and the outcome measures of the individual studies were sufficiently similar to be combined with appropriate statistical analysis to produce a meta-analysis. A meta-analysis is often superior to individual studies or a narrative systematic review, since it is considered to improve precision compared with, answer questions not posed by and settle conflicting results arising from individual studies [(40)].

If a meta-analysis is deemed appropriate, a summary statistic, the type of which depends on the characteristics of the data, is calculated for each study and then combined to produce an overall outcome statistic, according to the weight assigned to each study. In addition, a measure of the degree of heterogeneity between the studies is often calculated, to allow assessment of the variability between studies, and therefore the validity of the combined results. The study populations, interventions and outcome measures in this paper were deemed too heterogeneous to allow combining into a meta-analysis.

To determine the risk of bias (RoB) and therefore the robustness of the individual papers, the Newcastle–Ottawa scale was used; it was originally designed for assessing the quality of non-randomised studies included in a systematic review or meta-analysis. Each study is judged on eight items, categorised into three groups: the selection of the study groups, whether the groups are comparable and how the outcomes were assessed. A maximum of nine points are available, the greater the number of points the more robust the study is deemed to be. It was designed following a Delphi process to include or eliminate assessments of bias that had been used for previously published papers [(41)].

2.3.1.2 Descriptive statistics

A variety of methods to describe the properties of the observed data were used in this and the other primary publications.

The changes in the creatinine levels after completing the endurance event were tested for normality prior to analysis. Since they were assumed to follow a normal distribution (see Section

2.3.1.2.1: Test of normality (page 117), the parametric descriptions mean and standard deviations were used to describe the average and spread of the creatinine changes.

2.3.1.2.1 Test of normality

Tests of normality are used to determine if a set of data set is well-modelled by a normal distribution and therefore likely it is for a random variable underlying the data set to be normally distributed. If so, parametric tests, instead of non-parametric tests, may be used for data analysis, which are more powerful but which rely on assumptions about a population's parameters.

Various tests for normality may be used. The Shapiro-Wilk Test of Normality assesses how closely the distribution of the sample matches a normal curve and therefore the likelihood that the sample is derived from a normal distribution, in which case, parametric tests may be used. An alternative test for normality is the Kolmogorov-Smirnov Goodness of Fit Test, which assesses the likelihood that the sample follows a defined distribution, which might include the normal distribution. The Kolmogorov-Smirnov Goodness of Fit Test is often used for larger sample sizes, the Shapiro-Wilk Test of Normality for smaller sample sizes. The Shapiro-Wilk Test of Normality was used in this study.

2.3.1.3 Personal involvement

I was involved with the collection and analysis of the data, as well as in the conception and design of the study and the write-up. I was involved in testing the data for normality and analysing the creatinine rises, with assistance and oversight from one of the co-authors. I was also involved with the RoB assessment of the individual papers.

2.3.2 Statistical analysis for Paper 2: Association between collapse and serum creatinine and electrolyte concentrations in marathon runners

2.3.2.1 Descriptive statistics

Prior to analysis of the data on electrolyte changes in the collapsed and non-collapsed runners (see Paper 2: Association between collapse and serum creatinine and electrolyte concentrations in marathon runners: a 9-year retrospective study), the data were tested for normality (i.e., whether the data could be assumed to follow a normal distribution; see Section 2.3.1.2.1: Test of normality, page 117) by the use of the Shapiro-Wilk Test of Normality.

The average and spread of the data on the changes in electrolyte values was therefore described using mean and standard deviation, or median and interquartile range, depending on whether the data were parametric or non-parametric, respectively.

In addition, the average and spread of the data were displayed graphically in a box and whisker plot; these plots were used to display the data in quartiles, with the box showing the median and second and third quartiles, and the whiskers showing the first and fourth quartiles. The plots also indicate the skewness in non-parametric data.

2.3.2.2 Inferential statistics

Inferential statistics, in comparison with descriptive statistics, concerns analysis of the observed data to draw conclusions and allow inferences about the population from which the sample is drawn. Data following a normal distribution may be analysed using parametric tests; non-normal data by non-parametric tests.

2.3.2.2.1 Statistical significance

The threshold for statistical significance (alpha level) describes the likelihood or the degree of confidence that a particular set of observations would occur if the null hypothesis, that there is no significant difference between the analysed groups, were true. In this paper, the alpha level was set at 0.05 The *p* value of a test describes the probability that the observed statistic occurred by chance alone. If the *p* value is lower than the alpha level, then the likelihood of an actual difference between the groups is high. For example, with an alpha level set at 0.05, the *p* value of the analysis of the difference in the pre- and post-marathon serum creatinine level was calculated less than 1%,

suggesting that the difference in the creatinine levels was highly unlikely to occur by chance alone and therefore suggesting the observed difference was real and therefore statistically significant.

2.3.2.2.2 Analysis of the independence of categorical data

Whether two groups of non-parametric categorical data are independent of each other may be analysed by a chi-squared test. Pearson's chi-squared test was used to assess whether the proportion of runners meeting the KDIGO criteria for AKI was significantly different immediately following the race compared with 24 hours later (Table 2). Pearson's chi-squared test assesses the difference between the observed data (in this case, the proportion of runners with AKI at 24 hours) compared with that expected (the proportion of runners with AKI immediately after the race if these groups were the same); the greater the difference between the observed and expected data, the more likely the groups are to be different. The number of degrees of freedom (i.e., the number of independent variables) and the defined alpha level determine whether the difference between the observed and expected data is significant.

2.3.2.2.3 Analysis of difference between the means of two groups

Assuming the data follows a normal distribution, whether there is a significant difference between the means of two groups was assessed by a Student *t* test. An unpaired (independent) *t* test was used if the populations were different, such as the electrolyte levels between collapsed and control marathon runners (see Results section), and a paired (dependent) *t* test was used if the subjects were the same but repeated measures were taken, for example, the serum creatinine levels at baseline, immediately after finishing the marathon, and at 24 hours following race completion (see Results section).

2.3.2.2.4 Relative risk

The RR and CI of the creatinine, urea and potassium results being above the normal range and the sodium results being above or below the normal range was calculated for the collapse and control groups in the study (Table 1 and Table 3). The RR describes the likelihood of an event occurring in one group (e.g., a serum potassium level being above the normal range in a collapsed runner, 153 out of 224 runners) compared to the probability of an event occurring in the other group (e.g., a serum potassium level being above the normal range in a runner in the control group, 44 out of 80 runners) as a ratio (1.24). The CI around the RR describes the range in which the true population

result may be based on the results of the sample used and based on the degree of certainty (alpha level) required (see Section 2.3.2.2.1: Statistical significance, page 118). A CI around a RR that does not cross one, such as in the RR of serum potassium levels in the collapsed and control runners, suggests a statistically significant difference.

2.3.2.3 Personal involvement

I led on defining the hypotheses to be tested, the determination of the most appropriate test and the statistical analysis throughout this paper, with collaboration from one of the co-authors and a professional statistician.

2.3.3 Statistical analysis for Paper 3: Changes in gastrointestinal cell integrity after marathon running and exercise-associated collapse

2.3.3.1 Descriptive statistics

Throughout this paper (see Paper 3: Changes in gastrointestinal cell integrity after marathon running and exercise-associated collapse), the average and spread of the participant demographic data (Table 1), physiological data (Table 2) and I-FABP concentrations (Table 3) were described using mean and standard deviation, or median and interquartile range, depending on whether the data were parametric or non-parametric, respectively. (see Section 2.3.2.1: Descriptive statistics, page 118).

2.3.3.2 Inferential statistics

Throughout this paper, the alpha level was set at 0.05 (see Section 2.3.2.2.1: Statistical significance, page 118).

2.3.3.2.1 Analysis of correlation

Correlation analysis estimates whether a change in one variable is associated with a change in another, without implying causation. A Spearman's rank correlation test, applicable for ordinal or continuous non-linear correlations, was used to assess for correlation between time to complete marathon and I-FABP levels, and between BMI and I-FABP levels (see Results section). In comparison, Pearson's correlations for continuous data were performed between the L:R ratio and dependent variables from the passive heating and exercise-induced heating trials in the lab-based study investigating changes in GI permeability after passive and exercise-induced hyperthermia (see Paper 2: Exercise hyperthermia induces greater changes in gastrointestinal permeability than equivalent passive hyperthermia). A Spearman's rank correlation test is more applicable than a Pearson's correlation test for non-linear data, where a change in one variable does not lead to a proportional change in the second variable, since in the former the rank rather than the absolute value of the variable is used.

2.3.3.2.2 Analysis of difference between the means of two groups

Following testing for normality (see Section 2.3.1.2.1: Test of normality, page 117), a Student *t* test was used to assess whether there was a significant difference between the means of two groups if the data were parametric, such as differences between physiological responses before and after the marathon in the control (non-collapsed) runners (see Results section). A paired (dependent) *t* test was used to assess differences in physiological data in runners before and after completing the marathon in the study on GI permeability since the subjects were the same.

A non-parametric equivalent of the dependent Student *t* test to assess for differences between two dependent groups is the Wilcoxon signed-rank test. This test was used to compare changes in I-FABP levels between baseline and T0, T4 and T24 in controls, and between physiological data at T0 and T1 in marathon runners with hyperthermia-induced collapse (see Results section).

A Mann-Whitney *U* test compares differences between independent non-parametric data. It was used for example to compare I-FABP levels between controls and marathon runners with heat illness (see Results section).It is a non-parametric equivalent to the independent samples *t* test.

2.3.2.3 Personal involvement

I led on defining the hypotheses to be tested. The determination of the most appropriate test and the statistical analysis throughout this paper were carried out by one of the co-authors and a professional statistician. I subsequently reviewed and interpreted the results of the statistical analyses in order to draw conclusions about the data.

2.3.4 Statistical analysis for Paper 4: Exercise hyperthermia induces greater changes in gastrointestinal permeability than equivalent passive hyperthermia

2.3.4.1 Descriptive statistics

Throughout this paper, the average and spread of the participant demographic data, physiological data perceptual measures and lactulose:rhamnose absorption (Table 1) were described using mean and standard deviation, or median and interquartile range, depending on whether the data were parametric or non-parametric, respectively (see Section 2.3.2.1: Descriptive statistics, page 118).

In addition, the L:R data were displayed graphically, including as a box and whisker plot; these plots are used to show the median and second and third quartiles, and the whiskers showing the first and fourth quartiles. The plots also indicate the skewness in the non-parametric data of the L:R ratios and temperature changes in subjects undergoing precooling, passive hyperthermia and exercise-induced hyperthermia (Appendix 2, page 102).

2.3.4.2 Inferential statistics

Throughout this paper, the alpha level was set at 0.05 (see Section 2.3.2.2.1: Statistical significance, page 118).

2.3.4.2.1 Analysis of difference between the means of two groups

After testing for normality (see Section 2.3.1.2.1: Test of normality, page 117), whether there was a significant difference between the means of two groups was assessed by a Student *t* test. An unpaired (independent) *t* test is used if the populations were different, such as the electrolyte levels between collapsed and control marathon runners (see Paper 2: Association between collapse and serum creatinine and electrolyte concentrations in marathon runners); in this study, since the same subjects were assessed before and after trials in a repeated measures design, a paired (dependent) *t* test was used in the analysis of parametric variables before and after the trial, such as L:R, trial duration, whole-body sweat loss (WBSL), T_{CORE} and HR (see Results section).

A non-parametric equivalent of the dependent Student *t* test to assess for differences between two dependent groups is the Wilcoxon signed-rank test, which was used to assess for differences before and after the trial in the non-parametric variables RPE, TS, TC and GPSS (see Results section).

2.3.4.2.2 Analysis of variance

Analysis of variance (ANOVA) is an extension of a *t* test that allows statistical differences between more than two parametric groups or characteristics to be identified. It was used to determine changes in the lactulose:rhamnose ratios before and after passive heating and exertion-induced heating in this study (see Results section). A one-way ANOVA is used to determine how one factor affects a response variable. A two-way ANOVA in comparison is used to determine how two factors affect a response variable, and to determine whether or not there is an interaction between the two factors on the response variable. A two-way analysis was used in this study since several physiological and perceptual variables were being tested simultaneously.

2.3.4.2.3 Sphericity

One of the conditions around the use of ANOVA testing is that the variances of outcomes in a repeated measures design, such as in this study (see Section 1.4.7.2: Lactulose:rhamnose absorption during exercise-induced hyperthermia, page 70), are equal. In the full study, each of the six subjects underwent measurement of lactulose and rhamnose absorption in four conditions: passive hyperthermia, exercise-induced hyperthermia, normothermic exercise and at rest (no exercise and at normothermia). One of the conditions of the use of the repeated-measures ANOVA in this study is that the variances in the L:R ratio between each of the arms of the study are equal. Mauchly's sphericity test was therefore used in this study to assess how similar were the variances between the arms of the study. Violation of sphericity invalidates the ANOVA *F* ratio; application of the Greenhouse–Geisser correction adjusts for lack of sphericity.

2.3.4.2.4 Multiple comparisons

Multiple statistical testing on the same data increases the probability of a false-possible (type I) error, which was considered a risk with the multiple analyses conducted on the L:R results between different subjects and different test conditions in this study (see Results section). For example, if 20 statistical analyses are carried out on the same data set, it would be expected that one of the tests would result in a *p* value of less than 0.05 (1/20) purely due to chance. A statistically significant difference may therefore be assumed when no difference actually exists. A Bonferroni correction was therefore applied to the statistical analyses of the measurement of GI permeability, which increases the threshold for statistical significance according to the number of analyses, to reduce

the risk of a type I error. In comparison, a type II (false negative) error occurs when a statistical test suggests no significant difference occurs when in reality a difference exists.

2.3.4.2.5 Analysis of correlation

Correlation analysis estimates whether a change in one variable is associated with a change in another, without implying causation. Pearson's correlations for continuous data were performed between the L:R ratio and all dependent variables pooled from the passive heating and exercise-induced heating trials in this study (see Results section). In comparison, Spearman's rank correlation test is more applicable for ordinal or continuous non-linear correlations, such as correlation between time to complete marathon and I-FABP levels, and between BMI and I-FABP levels in the study investigating GI permeability after heat illness (see Paper 3: Changes in gastrointestinal cell integrity after marathon running and exercise-associated collapse).

2.3.4.3 Personal involvement

I led on defining the hypotheses to be tested. The determination of the most appropriate test and the statistical analysis throughout this paper were carried out by one of the co-authors and a professional statistician. I subsequently reviewed and interpreted the results of the statistical analyses in order to draw conclusions about the data.

2.3.5 Statistical analysis for Paper 5: The efficacy of steroids in reducing morbidity and mortality from extreme hyperthermia and heatstroke – a systematic review

2.3.5.1 Development of systematic review and meta-analysis

This paper (see Paper 5: The efficacy of steroids in reducing morbidity and mortality from extreme hyperthermia and heatstroke – a systematic review) was a systematic review. Similar to paper one (see Paper 1: Acute kidney injury associated with endurance events –is it a cause for concern? A systematic review), it was presented as a narrative systematic review, as the study populations, interventions and outcome measures in this paper were deemed too heterogeneous to allow combining into a meta-analysis.

2.3.5.2 Risk of bias assessment

The RoB was assessed using the Syrcle tool. The Syrcle (Systematic Review Centre for Laboratory Animal Experimentation) RoB tool was originally designed specifically to assess the robustness of studies involving animals and requires the risk of selection, performance, detection, attrition and reporting biases to be assessed [(42)].

2.3.5.3 Personal involvement

I led on the additional significance testing required and on the choice and application of an appropriate method to assess the RoB.

2.3.6 Statistical analysis for Paper 6: The efficacy of antibiotics in reducing morbidity and mortality from extreme hyperthermia and heatstroke – a systematic review

2.3.6.1 Development of systematic review and meta-analysis

This paper was a systematic review. Similar to papers one and five (see Paper 1: Acute kidney injury associated with endurance events –is it a cause for concern? A systematic review; and Paper 5: The efficacy of steroids in reducing morbidity and mortality from extreme hyperthermia and heatstroke – a systematic review), it was presented as a narrative systematic review, as the study populations, interventions and outcome measures in this paper were deemed too heterogeneous to allow combining into a meta-analysis.

2.3.6.2 Risk of bias assessment

The RoB was assessed using the RoB2 system, for which I took the lead. The RoB2 tool was modified from the original RoB tool [(43)], which had been widely used to assess the robustness of Cochrane and other systematic reviews. The RoB2 system sought to improve on a number of areas in the original RoB tool; it assesses five areas of potential bias (RoB arising from the randomisation process, due to deviations from the intended interventions. measurement of the outcome or selection of the reported result, or from missing data), each given a rating of low RoB, some concerns or high RoB [(44)]. While it sought to improve on the original tool, some concern remains about the reproducibility between raters [(45)].

2.3.6.3 Personal involvement

I led on the choice and application of an appropriate method to assess the RoB.

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Section 3

Primary submitted papers (redacted)