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Exercising in Environmental Extremes

A Greater Threat to Immune Function?

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Abstract

Athletes, military personnel, fire fighters, mountaineers and astronauts may be required to perform in environmental extremes (e.g. heat, cold, high altitude and microgravity). Exercising in hot versus thermoneutral conditions (where core temperature is $\geq 1^{\circ}\text{C}$ higher in hot conditions) augments circulating stress hormones, catecholamines and cytokines with associated increases in circulating leukocytes. Studies that have clamped the rise in core temperature during exercise (by exercising in cool water) demonstrate a large contribution of the rise in core

temperature in the leukocytosis and cytokinaemia of exercise. However, with the exception of lowered stimulated lymphocyte responses after exercise in the heat, and in exertional heat illness patients (core temperature $>40^{\circ}\text{C}$), recent laboratory studies show a limited effect of exercise in the heat on neutrophil function, monocyte function, natural killer cell activity and mucosal immunity. Therefore, most of the available evidence does not support the contention that exercising in the heat poses a greater threat to immune function (vs thermoneutral conditions).

From a critical standpoint, due to ethical committee restrictions, most laboratory studies have evoked modest core temperature responses ($<39^{\circ}\text{C}$). Given that core temperature during exercise in the field often exceeds levels associated with fever and hyperthermia ($\sim 39.5^{\circ}\text{C}$) field studies may provide an opportunity to determine the effects of severe heat stress on immunity. Field studies may also provide insight into the possible involvement of immune modulation in the aetiology of exertional heat stroke (core temperature $>40.6^{\circ}\text{C}$) and identify the effects of acclimatisation on neuroendocrine and immune responses to exercise-heat stress. Laboratory studies can provide useful information by, for example, applying the thermal clamp model to examine the involvement of the rise in core temperature in the functional immune modifications associated with prolonged exercise.

Studies investigating the effects of cold, high altitude and microgravity on immunity and infection incidence are often hindered by extraneous stressors (e.g. isolation). Nevertheless, the available evidence does not support the popular belief that short- or long-term cold exposure, with or without exercise, suppresses immunity and increases infection incidence. In fact, controlled laboratory studies indicate immuno-stimulatory effects of cold exposure.

Although some evidence shows that ascent to high altitude increases infection incidence, clear conclusions are difficult to make because of some overlap with the symptoms of acute mountain sickness. Studies have reported suppressed cell-mediated immunity in mountaineers at high altitude and in astronauts after re-entering the normal gravity environment; however, the impact of this finding on resistance to infection remains unclear.

Athletes engaged in heavy training programmes, particularly those involved in endurance events, appear to be more susceptible to infection.^[1-3] Laboratory and field-based experiments have implicated immune depression as being at least partly responsible for the reported increased incidence of infection in athletes.^[4-9] Many factors are known to influence the immune response to exercise, including: the intensity and duration of exercise,^[8,10,11] nutritional status,^[12,13] and the psychological stress of training and competition.^[14] Unfortunately, relatively little is known about the effects of exercising in environmental extremes on immune responses. This is

somewhat surprising given the widely acknowledged neuroendocrine role in the exercise-induced changes in immune function^[15] and the potential for exaggerated neuroendocrine responses during exercise in a stressful environment.^[16] Athletes, military personnel, fire fighters and mountaineers are often required to perform vigorous physical activity in adverse environmental conditions. These adverse environmental conditions may present themselves as extremes of heat and humidity, cold, high altitude and in a small number of cases as the microgravity that accompanies spaceflight. Even in staged world sporting events such as the Olympic Games, an

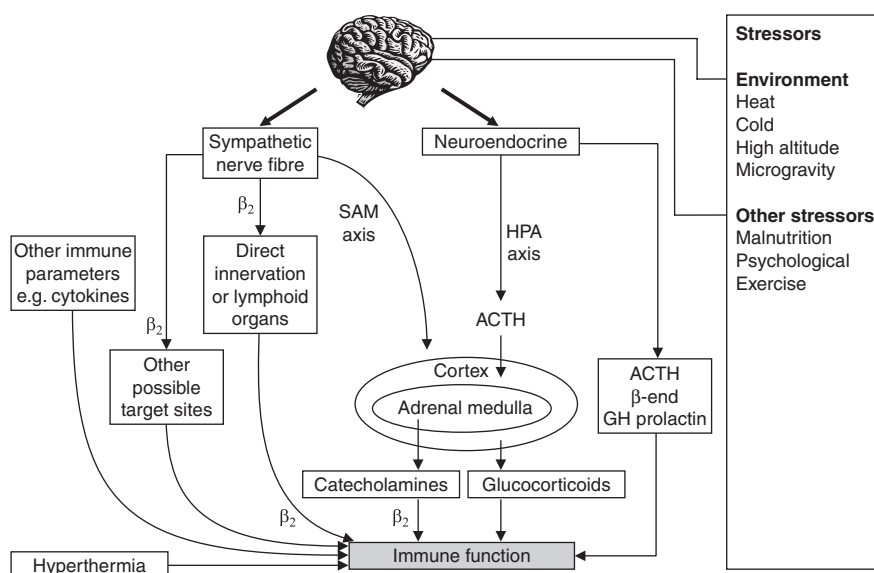


Fig. 1. Overview of the potential modulators of immune function under stress. Environmental stressors such as heat, cold, high altitude or microgravity may indirectly influence immune function through the initiation of a stress hormone response involving the hypothalamic-pituitary-adrenal (HPA) axis and sympatheticoadrenal-medullary (SAM) axis. Hyperthermia may also have a direct effect on immune function (reproduced from Jonsdottir,^[17] with permission). **ACTH** = adrenocorticotrophic hormone; **β-end** = β-endorphin; **GH** = growth hormone.

athlete can be required to compete in adverse environmental conditions to which they are neither native nor resident, such as the altitude of Mexico City in 1968 or the extreme heat and humidity of Athens in 2004. Despite appropriate preparation, exercise in environmental extremes can induce a stereotypical stress hormone response over and above that seen during exercise in more favourable conditions.^[16] Following a brief overview of stress-induced mediators of immune function, this article will critically discuss the research evidence to date from studies examining the effects of environmental extremes (heat, cold, high altitude and spaceflight) on immune responses at rest and after exercise to determine if exercising in environmental extremes poses a greater threat to immune function than exercising in more favourable conditions.

1. Overview of Stress-Induced Mediators of Immunity

Stressors such as exercise, heat or hypoxia, are characteristically met by a series of coordinated hormonal responses controlled by the CNS (figure

1).^[17] Specifically, the central control stations of the stress system reside within the hypothalamus and the brain stem. The hypothalamic-pituitary-adrenal (HPA) axis and sympatheticoadrenal-medullary (SAM) axis provide the effector limbs by which the brain influences the body's response to stress principally by controlling the production of adrenal hormones.^[18] Stimulated by the hypothalamic corticotropin-releasing hormone,^[19] the HPA axis regulates the release of adrenocorticotrophic hormone from the anterior pituitary, which, in turn, regulates the pulsatile release of cortisol by the adrenal cortex. Sympathetic innervation of peripheral organs also mediate the body's response to stress with further catecholamine (adrenaline [epinephrine] and noradrenaline [norepinephrine]) contributions from the SAM axis.^[20] Aside from these dominant axes, anterior pituitary hormones known to modulate immune function (e.g. growth hormone and prolactin) may also be released during stressful situations.^[21,22]

Evidence supports an interaction between neuroendocrine and immune responses to exercise.^[15] For example, sympathetic nerve innervation

of organs of the immune system (e.g. primary lymphoid tissue) indicates an autonomic nervous system involvement in immune modulation under stress.^[23] The expression of β -adrenergic receptors on immune cells is well documented and since these receptors are the targets for catecholamine signalling it is generally considered that catecholamines have significant effects on immune cell function during stress.^[16] Besides the widely recognised influence of stress hormones on leukocyte trafficking,^[24,25] stress hormones are also thought to influence immune status through the differentiation and stimulation of type I (cell-mediated) and type II (humoral) T lymphocytes through modulation of the cytokines produced by innate immune cells.^[26] For example, glucocorticoids and catecholamines suppress aspects of type I (cell-mediated) immunity through direct effects on cytokine production (e.g. tumour necrosis factor- α [TNF α], interferon- γ [IFN γ], interleukin [IL]-2 and IL-6) by type I T-helper cells,^[27-30] and down-regulation of IL-12 receptors on T and natural killer (NK) cells.^[31] Given that these cytokines stimulate functional activity of T cytotoxic cells, NK cells and activated macrophages, a reduction in the activity of the cell-mediated (type I) arm of immunity may result. Furthermore, type I and type II responses are thought to be mutually inhibitory. In line with this, type II T-helper cells treated with glucocorticoids increase the production of IL-10, which is known to inhibit type I immunity (e.g. by decreasing macrophage function).^[32,33] Therefore, stress hormone responses may influence cell-mediated and humoral arms of immunity by altering the local cytokine milieu.

In addition to the better established stress hormone/immune interactions, additional stress-induced mediators of immune function are thought to be involved. Heat shock proteins (HSPs) are cytosolic intracellular proteins present in all cells to maintain protein homeostasis. An up-regulation of intracellular HSPs occurs in response to a wide number of stressors such as heat, ischaemia, oxidative stress and microbial infections.^[34] Of relevance, HSP70 ((Author: Hsp70 for consistency?)) is thought to protect the host by negatively regulating

lipopolysaccharide (LPS)-induced production of pro-inflammatory cytokines in macrophages during bacterial infection.^[35,36] Aside from the well characterised intracellular roles, there is emerging evidence that HSPs have extracellular roles in immunity.^[37] For example, it has been demonstrated that Hsp72 binds specifically to antigen-presenting cells, resulting in an up-regulation of pro-inflammatory cytokines, co-stimulatory and antigen presenting molecules, which have crucial roles in adaptive immunity.^[38,39] Furthermore, it has been demonstrated that exercise causes an increase in circulating Hsp72,^[40-43] leading many authors to posit a role for HSPs in the stress-induced alteration in immune function.^[37,38,44,45] In addition, several immune functions also appear to be mediated by the actions of reactive oxygen and nitrogen species.^[46]

Given this information, compared with exercise in more favourable environmental conditions, we might hypothesise that exercise in adverse environmental conditions, with increased stress responses, will cause greater disruption to immune function and host defence.

2. Heat Stress and Immune Function

It is important to distinguish between the increase in body temperature that accompanies a fever (core temperature maintained at $>37.2^{\circ}\text{C}$) and the increase in body temperature that accompanies passive heat exposure and vigorous physical activity.^[16] An increase in endogenous pyrogens such as IL-1, IL-6, IFN and TNF α raise body temperature during a fever through an increase in the hypothalamic temperature set point. During passive heat exposure or vigorous physical activity, the hypothalamic temperature set point remains the same but problems with heat dissipation cause body temperature to rise.^[16] It is widely accepted that the rise in body temperature during a fever activates the immune system, which improves survival during an infection and shortens disease duration.^[47-49] In addition, the rise in body temperature during a fever is known to inhibit bacterial growth rate.^[47] Immunologists have examined the effects of a range of incubation temperatures on immune function *in vitro*^[50-53] and the

efficacy of inducing artificial fever by whole-body heating as a treatment to enhance suppressed *in vivo* immune function in cancer patients.^[54-56] Animal and clinical data support preclinical observations that whole-body hyperthermia can enhance cytotoxic ionising irradiation and chemotherapy without a commensurate increase in bone marrow suppression and morbidity.^[57-59] Also, whole-body hyperthermia has been shown to result in early engraftment of donor marrow in the setting of allogeneic bone marrow transplantation.^[60] Empirical studies have also shown that regular sauna bathing decreases the incidence and duration of viral infections.^[61,62]

Recently, exercise immunologists have investigated the role that the rise in core temperature may play in the neuroendocrine and immune alterations associated with heavy exercise.^[63-65] Such a putative role for the rise in core temperature in the immune alterations associated with vigorous exercise might not be surprising given the temperature dependency of a wide number of immune functions shown in *in vitro* studies. During vigorous exercise, particularly in warm weather, core temperature frequently exceeds levels associated with fever and hyperthermia ($\sim 39.5^{\circ}\text{C}$) and after races, core temperatures of $>40.6^{\circ}\text{C}$ have been reported in conscious runners^[66] and $42\text{--}43^{\circ}\text{C}$ in collapsed runners.^[67]

Section 2.1 will focus on the effects of passive heat stress on leukocyte and cytokine responses in studies that have involved sauna bathing, exposure to a climate chamber, water immersion and hyperthermic limb perfusion. Section 2.4 will focus on the effects of exercise in the heat on leukocyte and cytokine responses and a possible role for immune activation in the aetiology of exertional heat illness (also see reviews by Shephard^[16,68] and Lim and Mackinnon^[69]).

2.1 Passive Heating and Leukocyte Counts

It has been known for some time that exposure to heat stress resulting in elevated core temperature evokes an increase in circulating leukocytes.^[70] Artificial fever (core temperature $\sim 39.5^{\circ}\text{C}$), evoked by approximately 2 hours of immersion in $41\text{--}45^{\circ}\text{C}$ water, has been shown to increase circulating neu-

trophils, lymphocytes, NK cells and eosinophils and decrease monocyte numbers (table I).^[71,72] In contrast, more recent studies have shown limited changes in circulating leukocyte, neutrophil and lymphocyte numbers when core temperature was increased to $\sim 38^{\circ}\text{C}$ by immersing men to mid-chest in 39°C water for 80 minutes or by exposing men to 3 hours in a climate chamber at 40°C (table I).^[63,73]

The discrepant findings between the more recent studies and those previously are probably due to the relatively modest increase in core temperature ($\sim 1^{\circ}\text{C}$) in the recent studies compared with the larger increase in core temperature ($2\text{--}2.5^{\circ}\text{C}$) in the older studies. In support of this, immersion in hot water (39.5°C) to mid-chest for 2 hours, during which time core temperature rose to 39.5°C , resulted in a greater number of circulating neutrophils, NK cells and monocytes after immersion compared with immersion in warm water (34.5°C) [table I].^[74] Similarly, whole-body hyperthermia (2°C core temperature rise) has been shown to have beneficial effects on immune function in cancer patients,^[54-56] which include amongst others, an increase in circulating lymphocyte counts in the hours after heating.^[56] Hyperthermia has been shown to decrease circulating T-lymphocyte (CD3+) numbers after hot water immersion mainly due to a decrease in the number of circulating T-helper (CD4+) lymphocytes (table I).^[74] The helper : suppressor (CD4+/CD8+) lymphocyte ratio fell from 1.8 to 0.9 during artificial hyperthermia in another study^[75] and, given that a CD4+/CD8+ ratio of >1.2 is thought to be important for immune competence, this fall might impair host defence.^[76,77] Likewise, in patients with heatstroke (mean core temperature 41.4°C), a decrease in T-lymphocyte (CD3+), T-helper (CD4+) lymphocytes and the CD4+/CD8+ ratio has been reported (table I).^[78] Conversely, the increase in CD14+ monocyte count observed 2 hours after hot water immersion (table I)^[74] might represent improved immune function and possibly host defence as the important role that monocytes serve in phagocytosis, antigen presentation and cytokine production is well recognised.^[77]

Table 1. Effects of whole-body passive heating on circulating leukocyte, differential leukocyte and lymphocyte subset counts

Study	Participants	Method	T _{re} (°C)	% Change circulating leukocytes ^a
Kappel et al. ^[21]	8 M RA	2h whole-body immersion in ~39.5°C water	39.5–39.8	Leukocytes ↑7% (↑72% at 2h) Lymphocytes ↔ (↓17%) Neutrophils ↑32% (↑139%) Monocytes ↔ (↑125%) CD3+ ↔ (↓27%)
Cross et al. ^[63]	9 M RA	80 min whole-body immersion in 39°C water	~38	Leukocytes ↑22% Lymphocytes ↑13% Granulocytes ↑13% Monocyte ↑75%
		80 min whole-body immersion in 23°C water	36.4	Leukocytes ↔ Lymphocytes ↔ Granulocytes ↔ Monocytes ↔
Downing et al. ^[72]	3	Immersion in 41–45°C water	39–39.5	Leukocytes ↑27% Lymphocytes ↑10% Neutrophils ↑39% Monocytes ↔
Severs et al. ^[73]	11 M RA	3h exposure to 40°C 30% RH air	↑0.7	Leukocytes ↔ Lymphocytes ↔ Granulocytes ↔ Monocytes ↔
		3h exposure to 23°C air	↓0.3	Leukocytes ↔ Lymphocytes ↔ Granulocytes ↔ Monocytes ↔
Kappel et al. ^[74]	8 M RA	2h whole-body immersion in 39.5°C water	39.5	Leukocytes ↑38%* (↑140%* at 2h) Lymphocytes ↑31% (↓20%)* Neutrophils ↑38% (↑254%)* Monocytes ↔ (↑56%)* %CD3+ ↓16% (↓19%) %CD16+ ↑67% (↔)
		2h whole-body immersion in 34.5°C water	36.7	Leukocytes ↔ (↔) Lymphocytes ↔ (↔) Neutrophils ↔ (↔) Monocytes ↔ (↔) %CD3+ ↔ (↔) %CD16+ ↔ (↔)
Bouchama et al. ^[78]	11 M and F heat-stroke patients	Patients presented to hospital	41.4	Versus healthy controls Leukocytes ↑133% Lymphocytes ↑73% % CD3+ ↓14% % CD4+ ↓60% % CD8+ ↓78% % CD16+/CD56+ ↑100% % CD19+ ↓29%

^a Data are presented as approximate percentage change from baseline or vs healthy controls. Data in parentheses indicate 2h post-immersion vs baseline.

F = female; **M** = male; **RA** = recreationally active; **RH** = relative humidity; **T_{re}** = rectal temperature; ↑ indicates significant increase ($p < 0.05$); ↓ indicates significant decrease ($p < 0.05$); ↔ indicates no change; * indicates significant difference between conditions ($p < 0.05$).

The proposed mechanisms responsible for the leukocytosis associated with hyperthermia include increases in cardiac output^[16] and plasma catecholamines,^[4] which cause leukocytes to demarginate

from the blood vessel walls and enter the circulation. In addition, the secretion of cortisol, which induces migration of neutrophils from the bone marrow into the circulation,^[25] has also been proposed to account

for the hyperthermia-induced leukocytosis.^[74] As the leukocytosis induced by 30 minutes of exposure to an 80°C sauna was 2-fold greater in rehydrated compared with dehydrated subjects, a dominant role for the increase in cardiac output in the hyperthermia-induced leukocytosis has been suggested.^[16]

To further elucidate the mechanisms responsible for the leukocytosis associated with hyperthermia, Kappel et al.^[21] had subjects perform 2 hours of hot water immersion (where core temperature reached 39.5°C) on four occasions with prior infusion of saline (control), propranolol, somatostatin or naloxone to block β -adrenergic receptors, growth hormone release and β -endorphin receptors, respectively. Only somatostatin (growth hormone blocker) decreased the leukocytosis 2 hours after hot water immersion compared with saline infusion due to a blunted neutrophilia at this time. There was no influence of propranolol (β -adrenergic receptor blocker) or naloxone (β -endorphin receptor blocker) on the circulating number of leukocytes or leukocyte subsets. Hormone receptor blockade had no influence on the numbers of T-helper (CD4+) and suppressor (CD8+) lymphocytes or the numbers of NK cell (CD16+ and CD56+), B-lymphocytes (CD19+) and monocytes (CD14+), although β -adrenergic blockade with propranolol slightly increased T-cell (CD3+) numbers during hyperthermia. As β -adrenergic and β -endorphin receptor blockade had limited influence on circulating leukocyte, lymphocyte and monocyte counts in response to hyperthermia the authors suggested that circulating concentration of catecholamines and β -endorphins have limited involvement in the circulating leukocyte response to hyperthermia. These findings also suggest that growth hormone is a powerful mediator of neutrophil recruitment into the peripheral circulation in response to hyperthermia. Although the authors did not block the cortisol response in this study (e.g. by infusion of metyrapone, mifepristone or etomidate) others have shown that cortisol administration induces a neutrophilia.^[25] Taken together, the findings of these studies suggest that growth hormone and possibly cortisol may both mediate the

hyperthermia-induced neutrophilia. However, a recent article has shown that neutralisation of granulocyte colony stimulating factor (G-CSF), with intraperitoneally administered monoclonal antibody to G-CSF, blocked the hyperthermia-induced peripheral neutrophilia in mice housed for 24–36 hours at 34.5°C (core temperature 39.5–40°C).^[79] Interestingly, blockade of the glucocorticoid receptor with mifepristone failed to modify hyperthermia-induced neutrophilia.^[79] These very recent data, in an animal model of hyperthermia, suggest that hyperthermia-induced expression of G-CSF drives the sustained peripheral neutrophilia that occurs during sustained (24–36 hours) hyperthermia, whereas glucocorticoid generation plays a limited role in this response.

In summary, passive heat stress that results in a core temperature >39°C is associated with an increase in circulating leukocyte numbers primarily due to a large increase in circulating neutrophils and also to smaller increases in circulating lymphocytes, NK cells and monocytes. In contrast, circulating T-lymphocyte (CD3+) numbers fall during hyperthermia; this is due to a decrease in circulating T-helper (CD4+) lymphocytes. The leukocytosis associated with hyperthermia may be partly accounted for by an increase in the demargination of leukocytes from the blood vessel walls as a result of the increase in cardiac output (increase in shear stress). Studies using hormonal blockade and hormone infusion do not support a role for catecholamines and β -endorphin in the circulating leukocyte response to hyperthermia. Recent evidence indicates a more limited role for cortisol than previously thought and a larger role for growth hormone and G-CSF as powerful mediators of the leukocyte response to hyperthermia.

2.2 Passive Heating, Thermal Conditioning and Cytokine Responses

The cellular responses to heat insults or 'heat shock' are highly conserved and predominantly mediated by HSPs. Interestingly, it seems apparent that once subjected to excessive heat, cells are able to survive subsequent endotoxin challenges that would normally result in death.^[80] This acquired tolerance

may be mediated by the direct effect of heat on immune cells,^[81] or more likely by an indirect effect of HSP accumulation. HSP accumulation is associated with a decreased expression of the pro-inflammatory mediators of endotoxin shock, TNF α and IL-1 β .^[35,36,49] While tightly regulated expression of these cytokines is required for host defence, dysregulated expression of these cytokines has been shown to cause multiorgan injury, sepsis, shock and death.^[82] For example, mice lacking a functional TNF α gene not only show the expected susceptibility to lethal infections with intracellular pathogens but surprisingly mount a progressive lethal inflammatory response to stimuli that are not normally lethal.^[82,83] It has also been suggested that dysregulated expression of pro-inflammatory cytokines is involved in the aetiology of heat illness (see section 2.8).^[84] Indeed, a growing body of literature suggests that elevated temperatures alter the expression of IL-1, TNF α and IFNs. This appears to be dependent upon the magnitude and timing of the temperature change, whether there is a bacterial challenge and the cellular source of the cytokines.^[85] For example, housing mice at 35.5°C rather than 23°C raised core temperature from 36.5–37.5°C to 39.2–39.7°C, improved survival and reduced the bacterial load in mice infected with *Klebsiella pneumoniae* peritonitis.^[82] This effect was associated with activation of heat shock protein gene (*HSF-1*) and a suppression of TNF α . Since the growth rate of *Klebsiella pneumoniae* on agar plates at 39.5°C and 37°C was not different, the authors indicated that the reduced bacterial load in the mice housed at 35.5°C resulted from alterations in host-pathogen interaction rather than a direct toxic effect of the warmer temperature on bacteria.^[82]

Katschinski et al.^[57] showed that whole-body hyperthermia (1 hour of exposure to 41.8°C) induces peripheral cytokines (~6-fold increase in G-CSF, IL-1 β , IL-6, IL-8 and TNF α), which have both direct stimulatory effects on haematopoietic tissue and indirect effects by stimulating the production of haematopoietic growth factors (IL-3 and granulocyte macrophage-colony stimulating factor [GM-CSF]) in the bone marrow (figure 2).^[57,86,87] In sup-

port of this, the induction of G-CSF, IL-1 β and IL-6 is known to have a direct stimulatory effect on stem cells and/or progenitor cells related to leukocyte or platelet production (figure 2).^[88] Alongside the more widely acknowledged role for stress hormones, the hyperthermia-induced increase in cytokines in peripheral blood (G-CSF, IL-1 β , IL-6, IL-8 and TNF α) and the bone marrow (IL-3 and GM-CSF) might also mediate the widely reported leukocytosis with hyperthermia.^[70-72] In accordance with this hypothesis, a recent study indicates a substantial role for raised plasma G-CSF in the hyperthermia-induced neutrophilia.^[79]

Katschinski et al.^[57] suggested that the hypothesis (figure 2) provides a basis for the observation that specific anticancer therapies, such as chemotherapy and radiotherapy for which myeloid suppression is the major toxicity, can have their beneficial effects markedly enhanced by whole-body hyperthermia without a concomitant increase in morbidity.^[59,89] In summary, the hyperthermia-induced increase in pro-inflammatory cytokines in peripheral blood might account, at least in part, for the hyperthermia-induced leukocytosis and the improved immune function and reduced morbidity in cancer patients and bacterially challenged mice exposed to hyperthermia.

2.3 Passive Heating and Leukocyte Function

2.3.1 Neutrophils

Incubation of neutrophils at 40°C for 1 hour has been reported to increase bactericidal capacity compared with incubation at 37°C.^[53] Increasing incubation temperature from 37 to 39°C has also been shown to enhance the rate of neutrophil migration.^[90] Total body hyperthermia treatment in cancer patients using isolated limb perfusion (heating perfusate to 41.5°C) for up to 6 hours transiently raised neutrophil bactericidal capacity to within healthy control values.^[54] However, the temperature dependence of neutrophil function appears to be more complicated than previously thought. For example, a recent study showed that increasing the *in vitro* incubation temperature between 33 and 41°C decreased the up-regulation of neutrophil CD11b

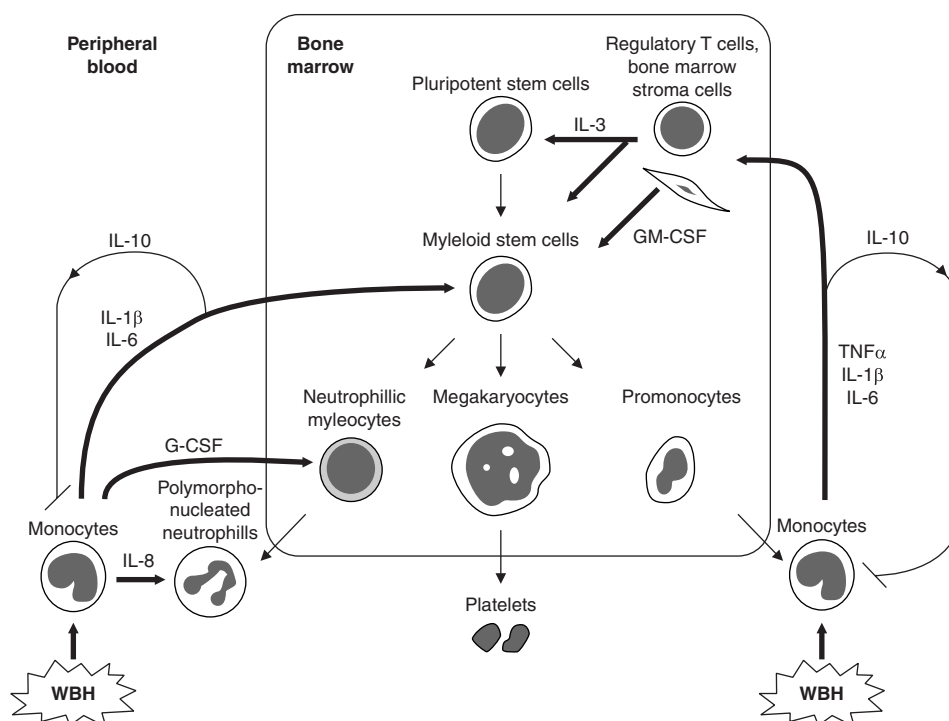


Fig. 2. Schematic description of 41.8°C whole-body hyperthermia (WBH) × 60 minutes WBH-induced haematopoietic effects. Peripheral monocytes produce interleukin (IL)-1 β , IL-6, IL-8, IL-10, granulocyte colony stimulating factor (G-CSF) and tumour necrosis factor- α (TNF α) in response to WBH. Several positive and negative feedback loops are shown: IL-1 β , IL-6 and G-CSF have direct stimulatory effects on maturation and differentiation of haematopoietic cells. Additionally, IL-1 β , IL-6 and TNF α have secondary effects, i.e. induction of granulocyte macrophage-colony stimulating factor (GM-CSF) and IL-3 production by bone marrow stroma cells and/or regulatory T cells, which lead to stimulation of pluripotent and/or myeloid stem cells. IL-8 acts as a neutrophil activator; IL-10 serves as a negative feedback control by inhibiting cytokine synthesis by monocytes. Thin lines with arrow heads represent differentiation, maturation; thick lines with arrow heads represent stimulation and production, thin lines originating from thick lines represent a negative feedback loop. The area within the oval represents the marrow space and the area outside the oval represents the peripheral blood (reproduced from Katschinski et al.^[57] with permission from Elsevier).

expression with TNF α but increased phorbol-myristate acetate-stimulated neutrophil oxidative burst linearly with incubation temperature.^[52] In contrast, others^[91] have shown little change in neutrophil oxidative burst when core temperature was increased to 39.5°C during 2 hours of hot water immersion in healthy young men.

2.3.2 Lymphocytes

A 3-hour period seated in a climate chamber (40°C), which evoked a modest 0.7°C rise in core temperature (~38°C) did not alter lymphocyte proliferative responses to a number of mitogens or the *in vitro* production of immunoglobulins.^[73] However, IL-2 production in phytohaemagglutinin

(PHA)-stimulated mononuclear cells is reported to be elevated in hyperthermic (core temperature 39°C) compared with normothermic (core temperature 37°C) individuals after water immersion.^[71] Similarly, whole-body heating in cancer patients (2°C rise in core temperature) increased mitogen-stimulated mononuclear cell IL-1, IL-2 and IFN production *in vitro*. Mitogen responses were increased 2- to 3-fold when the temperature was raised.^[56] The increase in mononuclear cell IL-1 and IL-2 production may represent improved immune function as these cytokines are known to stimulate NK cell activity,^[92,93] monocyte phagocytosis^[94] and monocyte cytotoxic activity.^[95,96] IFN- γ production from lymphocytes stimulated with PHA increased

4- to 16-fold in hyperthermic monkeys^[71] and humans^[72] after a 2°C rise in core temperature. IFN γ production is critical to antiviral defence and it has been suggested that the suppression of IFN γ production may be implicated in the increased risk of infection after prolonged bouts of exercise.^[97] Hyperthermic isolated limb perfusion (heating perfusate to 42°C) for 1 hour enhanced PHA-stimulated T-lymphocyte function 24 hours after and 1 week after treatment in patients with malignant melanoma.^[55] These authors,^[55] and others^[54,56,98,99] have acknowledged a potential role for immunological activation using hyperthermia for the treatment of malignant melanomas.

2.3.3 Natural Killer Cell Activity

Natural killer cell activity (NKCA) was increased when core temperature was raised from 37 to 39°C by hot water immersion.^[71] Both NK cell (CD16+) number and IL-2 stimulated NKCA were increased after hot water immersion that evoked a core temperature of 39.5°C.^[74] Hyperthermic isolated limb perfusion in melanoma patients has also been shown to increase NKCA.^[55] Clearly the magnitude of the rise in core temperature is important as a more modest 0.7°C rise in core temperature during 3 hours of seated rest in a climate chamber at 40°C did not alter either NK cell number (CD16+ and CD56+) or cytolytic activity compared with seated rest in thermoneutral (23°C) conditions.^[73] Although a role for stress hormones is often put forward, the increase in plasma catecholamines, growth hormone and β -endorphin with hyperthermia are unlikely to account for the increase in NKCA because blocking the effects of these hormones had no effect on NKCA after 2 hours of hot water immersion that resulted in a core temperature of 39.5°C.^[21] Downing and Taylor^[71] have proposed that the increase in NKCA with elevated core temperature is most likely due to increased plasma concentration of IL-1, IL-2 and IFN γ known to enhance NKCA per cell.^[92,93,100]

2.3.4 Monocytes

Monocyte bactericidal capacity for a range of organisms (including *Escherichia coli*, *Salmonella*

typhimurium and *Listeria monocytogenes*) was unaltered when *in vitro* incubation temperature was increased from 37 to 40°C.^[53] Furthermore, another *in vitro* study has shown that IL-1 production by macrophages did not change appreciably after increasing the incubation temperature from 38 to 40°C.^[101] In an *in vivo* study, raising core temperature from 37 to 39°C with hot water immersion did not alter LPS-stimulated monocyte TNF α release.^[71] However, the duration of exposure to febrile range temperature may be important. For example, during *in vitro* incubation at 40°C the initial rate of LPS-stimulated monocyte cytokine release (IL-1 β , IL-6 and TNF α) was preserved but more prolonged incubation (24 hours) decreased cytokine production.^[102] The authors speculated that the suppressed cytokine profile after 24 hours at 40°C might prevent prolonged exposure to potentially cytotoxic cytokines.^[102] Taken together, the *in vitro* and *in vivo* work to date suggests that monocyte bactericidal capacity and cytokine production are unaffected by a modest increase in temperature, but prolonged exposure to febrile range temperatures may decrease cytokine production.

In summary, with the exception of monocyte function, which does not appear to be affected by a brief increase in temperature within the range 37–39°C, an increase in *in vitro* or *in vivo* temperature of ~2°C is acknowledged to enhance neutrophil, lymphocyte and NK cell function. The magnitude of the change in leukocyte cell counts and function with heat stress appears to be dependent upon the magnitude of the rise in *in vitro* or *in vivo* temperature and possibly the duration of exposure. For example, leukocyte counts and function remained unaltered with a ~0.7°C rise in core temperature but were enhanced with ~2°C rise in core temperature.^[73,74] Furthermore, a brief increase in incubation temperature from 37 to 40°C did not alter LPS-stimulated monocyte cytokine release but more prolonged incubation at 40°C (24 hours) decreased cytokine production.^[102]

2.4 Exercise in the Heat and Immune Function

Compared with exercise in thermoneutral conditions, exercise in hot conditions is associated with increased core temperature, cardiovascular drift, circulating stress hormones and catecholamines and an increased reliance on carbohydrate as a fuel source.^[103-105] Unsurprisingly, compared with thermoneutral conditions, endurance performance in the heat is impaired.^[105] Evidence supports an interaction between neuroendocrine and immune responses to exercise.^[15] Therefore, performing exercise in hot conditions with associated elevated circulating stress hormones and catecholamines would be expected to cause greater immune disturbance compared with exercise in thermoneutral conditions. Athletes, military personnel and fire fighters regularly undertake vigorous activity in hot conditions, often when wearing protective clothing that does not facilitate evaporative heat loss, resulting in core temperature often $>40^{\circ}\text{C}$.^[66,67] It is therefore somewhat surprising that to date the number of studies that have examined the effects of exercise in the heat and exertional heat illness on immune function just break into double figures. Comparing neuroendocrine responses to exercise in hot with responses to exercise in thermoneutral conditions also presents an attractive experimental model to investigate the effects of stress on immune function and the possible role that neuroendocrine modulation plays in the altered immune response with exercise. Exercise-induced immune disturbances in thermoneutral conditions^[106,107] are often attributed to neuroendocrine responses and the rise in core temperature associated with exercise.^[16] However, it is difficult to identify whether the observed effects of exercise on immune function are due to: (i) a direct effect of elevated core temperature on immune responses; (ii) elevated core temperature exerting its influence on immune function indirectly through increased neuroendocrine activation; (iii) increased neuroendocrine activation as a result of exercise *per se* and not as a result of the rise in core temperature; (iv) an as yet unknown mechanism; or (v) a combination of one or more of the above.

An elegant experimental model used by one research team,^[63-65] which involves both exercise with a rise in core temperature (exercise in hot water) and exercise without a rise in core temperature (exercise in cold water: thermal clamp), has provided an interesting insight into the contribution of the exercise-induced rise in core temperature to the neuroendocrine and immune alterations with exercise. Section 2.5 will present and critically discuss the findings from the small number of studies investigating the effect of fixed-duration exercise in hot compared with thermoneutral conditions on leukocyte and cytokine responses. Potential mechanisms underlying the immune responses to exercise in the heat will be discussed with a particular emphasis on the results from the thermal clamping studies. Finally, a possible role for immune activation in the aetiology of exertional heat illness (section 2.8) will also be discussed.^[68,69]

2.5 Exercise in the Heat and Leukocyte Counts

Severs et al.^[73] had recreationally active subjects randomly perform two 30-minute bouts of cycling at 50% maximal oxygen uptake ($\dot{V}\text{O}_{2\text{max}}$) with 45 minutes of rest between in thermoneutral (23°C) and hot (40°C) conditions. Core temperature increased by 0.9 and 1.6°C after the two bouts of exercise performed in thermoneutral and hot conditions, respectively (table II). Both bouts of exercise produced a significant leukocytosis with typical increases in circulating neutrophil, lymphocyte and monocyte numbers. Under thermoneutral conditions, the second bout of exercise evoked a similar leukocytosis to the first bout. However, in hot conditions, the core temperature rise was larger after the second bout of exercise with larger increases in circulating neutrophil, monocyte, lymphocyte and lymphocyte subsets (CD3+, CD4+ and CD8+). The authors suggested some synergism between heat and exercise exposure, possibly due to large increments in circulating levels of catecholamines under hot conditions.

In an attempt to identify the contribution of hyperthermia to the differential leukocytosis of ex-

Table II. Effects of environmental temperature on the circulating leukocyte, differential leukocyte and lymphocyte subset count response to exercise

Study	Participants	Method	Final T _{re} (°C)	% Change circulating leukocytes ^a
Cross et al. ^[63]	9 M RA	40 min cycling 65% $\dot{V}O_{2max}$ immersed to mid-chest in 39°C water	39.1	Leukocytes ↑50%* Lymphocytes ↑61%* Granulocytes ↑40% Monocytes ↑100%
		40 min cycling 65% $\dot{V}O_{2max}$ immersed to mid-chest in 23°C water	37.8	Leukocytes ↑33% Lymphocytes ↑30% Granulocytes ↑38% Monocytes ↑94%
Rhind et al. ^[64]	10 M RA	40 min cycling 65% $\dot{V}O_{2peak}$ immersed to mid-chest in 39°C water	39.2	Leukocytes ↑29%* (50% at 2h post*) Lymphocytes ↑38%* (↔) Granulocytes ↑29%* (88%*) Monocytes ↔ (↔) CD3+ ↑24%* (↓14%*) CD4+ ↑10%* (↔*) CD8+ ↑35%* (↓12%) CD19+ ↔ (↑26%) CD16+/CD56+ ↑157%* (↔)
		40 min cycling 65% $\dot{V}O_{2peak}$ immersed to mid-chest in 18°C water	37.5	Leukocytes ↔ (↑40) Lymphocytes ↑31% (↔) Granulocytes ↔ (↑50%) Monocytes ↔ (↔) CD3+ ↑7% (↔) CD4+ ↓6% (↔) CD8+ ↔ (↔) CD19+ ↔ (↑44%) CD16+/CD56+ ↑86% (↔)
Severs et al. ^[73]	11 M RA	2 × 30 min exercise at 50% $\dot{V}O_{2max}$, separated by 45 min sitting in 40°C, 30% RH	↑1.6	Bout 1, bout 2 Leukocytes ↑50%*, ↑80%** Lymphocytes ↑70%, ↑110%* Granulocytes ↑30%, ↑100%* Monocytes ↑75%, ↑125% CD3+ ↑60%, ↑95%* CD19+ ↑60%, ↑80% CD4+ ↑40%, ↑120%* CD8+ ↑70%, ↑90%*
		2 × 30 min exercise at 50% $\dot{V}O_{2max}$, separated by 45 min sitting in 23°C, 30% RH	↑0.9	Bout 1, bout 2 Leukocytes ↑30%, ↑40% Lymphocytes ↑30%, ↑60% Granulocytes ↑30%, ↑75% Monocytes ↑30%, ↑75% CD3+ ↑35%, ↑50% CD19+ ↑35%, ↑50% CD4+ ↑25%, ↑30% CD8+ ↑30%, ↑55%
Mitchell et al. ^[108]	10 M RA	75 min cycling 55% $\dot{V}O_{2peak}$ in 38°C, 45% RH (euhydrated)	~38.8	Leukocytes ↑14%* (↑12% at 2h post*) Lymphocytes ↑11%* (↓32%) Neutrophils ↑23% (↑45%*) CD3+ ↑5%* (↓30%*) CD4+ ↔* (↓33%) CD8+ ↑12%* (↓24%) CD16+/CD56+ ↑119%* (↔)

Continued next page

Table II. Contd

Study	Participants	Method	Final T_{re} (°C)	% Change circulating leukocytes ^a
		75 min cycling 55% $\dot{V}O_{2peak}$ in 22°C, 30% RH (euhydrated)	~37.6	Leukocytes ↔ (↔) Lymphocytes ↔ (↓22%) Neutrophils ↔ (↔) CD3+ ↓9% (↓21%) CD4+ ↓17% (↓25%) CD8+ ↓6%* (↓17%) CD16+/CD56+ ↑35% (↓29%)
Niess et al. ^[109]	7 M ET	60 min running 90% V_{IAT} in 28°C and 50% RH	39.8†	Lymphocytes ↑50% (↔ at 3h post) Neutrophils ↑90% (↑250%)* Monocytes ↔ (↑63%)*
		60 min running 90% V_{IAT} in 18°C and 50% RH	38.7	Lymphocytes ↑47% (↔) Neutrophils ↑83% (↑170%) Monocytes ↔ (↑63%)
Laing et al. ^[110]	13 M ET	2h cycling 60% $\dot{V}O_{2max}$ in 30°C, 76% RH	38.7	Leukocytes ↑184% (↑129%) Lymphocytes ↑37% (↔) Neutrophils ↑285% (↑236%) Monocytes ↑125%** (↑75%**)
		2h cycling 60% $\dot{V}O_{2max}$ in 20°C, 60% RH	38.1	Leukocytes ↑153% (↑94%) Lymphocytes ↑47% (↔) Neutrophils ↑238% (↑179%) Monocytes ↑100% (↑25%)
McFarlin and Mitchell ^[111]	10 M RA	60 min cycling 60% $\dot{V}O_{2peak}$ in 38°C, 45% RH air	~38.7	Leukocytes ↑24% (↑77% at 2h post) Lymphocytes ↑60%* (↔*) Neutrophils ↑50% (↑165%)
		60 min cycling 60% $\dot{V}O_{2peak}$ in 8°C, 50% RH air	~37.7	Leukocytes ↑24% (↑53%) Lymphocytes ↔ (↔) Neutrophils ↑31% (↑87%)
Brenner et al. ^[112]	7 M military recruits	60 min cycling 55% $\dot{V}O_{2peak}$ immersed to mid-chest in 35°C water	38.3	Leukocytes ↑40% Lymphocytes ↑40% Granulocytes ↑38%* Monocytes ↔ CD3+ ↔ CD4+ ↔ CD8+ 66% CD19+ ↔ CD16+/CD56+ ↑218%
		60 min cycling 55% $\dot{V}O_{2peak}$ immersed to mid-chest in 18°C water	37.2	Leukocytes ↑35% Lymphocytes ↑38% Granulocytes ↑35% Monocytes ↔ CD3+ ↔ CD4+ ↔ CD8+ ↑50% CD19+ ↔ CD16+/CD56+ ↑113%

^a Data are presented as approximate percentage change from baseline. Data in parentheses indicate sample collected later in recovery vs baseline.

ET = endurance trained; **M** = male; **post** = post-exercise; **RA** = recreationally active; **RH** = relative humidity; T_{re} = rectal temperature; V_{IAT} = velocity at individual anaerobic threshold; $\dot{V}O_{2max}$ = maximum oxygen uptake; $\dot{V}O_{2peak}$ = peak oxygen uptake; ↑ indicates significant increase ($p < 0.05$); ↓ indicates significant decrease; ↔ indicates no change; * indicates significant difference between conditions ($p < 0.05$); ** indicates significant difference between conditions ($p < 0.01$); † indicates T_{re} determined by pill telemetry.

ercise two studies had subjects perform 40 minutes of cycling at 65% peak oxygen uptake ($\dot{V}O_{2peak}$) on

one occasion with a rise in core temperature (cycling immersed to mid-chest in 39°C water) and on another

Table III. Plasma adrenaline (epinephrine), noradrenaline (norepinephrine) and cortisol responses to 40 minutes of cycling at 65% peak oxygen uptake in hot and cold water (reproduced from Rhind et al.,^[64] with permission)

Hormone	Condition	Sample time ^a		Recovery	
		rest (0 min)	exercise (40 min)	70 min	160 min
Adrenaline (pmol/L)	Hot	284 ± 33	1 776 ± 2034	1 072 ± 236*	468 ± 71
	Cold	242 ± 29	716 ± 127*‡	311 ± 47	259 ± 41
Noradrenaline (pmol/L)	Hot	2 528 ± 243	11 535 ± 1718†	5 054 ± 2458*	2 663 ± 262
	Cold	2 291 ± 196	4 491 ± 540†‡	3 684 ± 513*	2 822 ± 272
Cortisol (nmol/L)	Hot	470 ± 44	608 ± 60	768 ± 68*	373 ± 35
	Cold	535 ± 67	551 ± 63	384 ± 47‡	295 ± 37

a Values are mean ± SEM, n = 10 males. Significantly different from resting: * p < 0.05 and † p < 0.001. Significant difference between trials: ‡ p < 0.05.

er occasion without a significant rise in core temperature (thermal clamp condition involved cycling immersed to mid-chest in 18 or 23°C water [figure 3]).^[63,64] Exercising in the thermal clamp condition substantially reduced the rise in circulating leukocytes, neutrophils, lymphocytes (table II),^[63,64] lymphocyte subsets (CD3+, CD4+ and CD8+) and NK cell (CD16+ and CD56+) numbers (table II)^[64] but not the increase in circulating monocyte numbers.^[63,64] The thermal clamp reduced exercise-induced increments of plasma adrenaline, noradrenaline and growth hormone and abolished the increase in plasma cortisol concentration (table III).^[63,64] Multiple regression analysis showed that core temperature had no direct association with lymphocyte subsets but was significantly correlated

with hormone levels. The authors stated that hyperthermia mediates exercise-induced leukocyte redistribution to the extent that it causes sympathoadrenal activation, with alterations in circulating adrenaline, noradrenaline and cortisol.^[64]

More recently, 75 minutes of cycling at 55% $\dot{V}O_{2\text{peak}}$ in recreationally active males evoked a larger increase in leukocyte and neutrophil numbers post-exercise and 2 hours post-exercise and a larger increase in lymphocytes, lymphocyte subsets (CD3+, CD4+ and CD8+) and NK cell (CD16+ and CD56+) numbers post-exercise after the exercise was performed in hot (38°C) compared with thermoneutral (22°C) conditions where final core temperatures were ~38.7 and 37.7°C, respectively (table II).^[108] Interestingly, the authors had subjects perform the exercise bouts in both hot and thermoneutral conditions on one occasion with sufficient fluids to match sweat losses (euhydrated) and on another occasion without fluids (dehydrated). The results showed that hydration status had little impact on leukocyte counts after exercise suggesting a much greater impact of heat stress than hydration status on leukocyte trafficking after exercise.

In highly trained runners, 1 hour of treadmill running at 75% $\dot{V}O_{2\text{max}}$ in hot (28°C) compared with thermoneutral (18°C) conditions, where final core temperatures were 39.8 and 38.7°C, respectively, resulted in significantly higher circulating neutrophils and monocyte counts 3 hours after exercise (table II).^[109] The authors postulated that the larger increase in neutrophils after exercise in the heat was due to the larger increase in plasma cortisol and growth hormone immediately after exercise on this

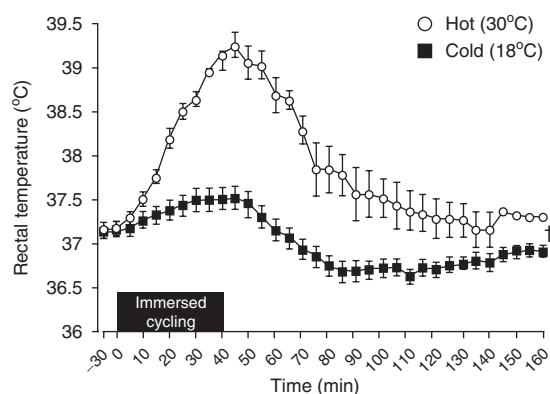


Fig. 3. Rectal temperature during and after 40 minutes of cycling at 65% peak oxygen uptake in hot and cold water. Values are mean ± standard error of the mean, n = 10 males, p < 0.01 (reproduced from Rhind et al.,^[64] with permission). † indicates significant difference over time for the entire session.

trial: both cortisol^[25] and growth hormone have been shown to mobilise neutrophils into the peripheral circulation during and after exercise.^[21,113] Unfortunately, the authors^[109] did not acknowledge a possible role for raised plasma G-CSF in the augmented neutrophilia with exercise and heat stress: recent evidence points towards G-CSF, rather than cortisol, driving the peripheral neutrophilia associated with exercise^[114] and hyperthermia.^[79] The authors^[109] suggested that the lack of an additional effect of exercise in hot conditions on lymphocyte counts might be due to the similar adrenaline response to exercise in hot and thermoneutral conditions: plasma adrenaline is known to be an important determinant of circulating lymphocyte counts.^[64] It is noteworthy that studies involving prolonged exercise in hot compared with thermoneutral conditions, with *ad libitum* fluid intake, where only a modest augmentation of core temperature was reported under hot conditions (< 1°C), have shown increased physiological stress but failed to show a significant effect of additional heat stress on leukocyte counts.^[110,111]

In summary, in comparison with exercise in thermoneutral conditions, exercise of the same duration in hot conditions that evokes a larger increment in core temperature ($\geq 1^\circ\text{C}$ vs thermoneutral conditions) is associated with larger numbers of circulating leukocytes during recovery. The effects of prolonged exercise performed to fatigue in hot compared with thermoneutral conditions on leukocyte trafficking has not been determined. Given that subjects would not be expected to exercise for as long in hot conditions, we might anticipate similar circulating stress hormone and leukocyte responses to fatiguing exercise in hot and thermoneutral conditions. The findings of studies that have clamped the rise in core temperature during exercise (by having subjects exercise in cold water) show that as much as half of the leukocytosis observed with exercise is attributable indirectly to the rise in core temperature through hyperthermia-induced sympathoadrenal activation and the known effects of elevated plasma catecholamines and cortisol on leukocyte trafficking. More recently, a putative role for G-CSF in the recruitment of neutrophils into the circulation after

maximal exercise in thermoneutral conditions in humans^[114] and sustained hyperthermia in mice^[79] has been proposed. It remains to be shown if larger increases in plasma G-CSF are also responsible for the larger recruitment of neutrophils into the circulation after exercise in the heat.

2.6 Exercise in the Heat and Cytokine Responses

It is widely recognised that hyperthermia is associated with an increase in plasma cytokines (e.g. IL-1 β and TNF α)^[71,115] and altered *in vitro* and *in vivo* cytokine production.^[102,116,117] It is also widely recognised that heavy exercise in thermoneutral conditions evokes sequential increases in the plasma concentration of several pro-inflammatory cytokines (e.g. TNF α , IL-1 β and IL-12) and subsequently several anti-inflammatory cytokines (IL-1ra and IL-10).^[65,118] Given the importance of well regulated cytokine responses for optimal immune function and host defence^[82,83,119,120] and the potential for exercise in the heat to demonstrate greater immune disturbances,^[73,108,109] it is somewhat surprising that the effects of combined exercise and heat stress on plasma cytokine responses and intracellular cytokine production have received little attention. Plasma IL-6 concentration has been shown to increase after prolonged exercise with additional heat stress but remain unchanged when the exercise was performed in thermoneutral^[112,121] or cold conditions.^[65] However, confusion exists over plasma IL-6 responses to exercise with additional heat stress because others^[109,122,123] have shown similar plasma IL-6 responses to prolonged exercise with and without additional heat stress. In addition, the contribution of the rise in core temperature to the stereotypical increases in plasma cytokines with exercise in thermoneutral conditions has until recently remained unknown.^[65] To investigate this, Rhind et al.^[65] recently had ten men perform 40 minutes of cycling at 65% $\dot{V}O_{2\text{peak}}$ on one occasion immersed in hot water (39°C) and on another occasion in cold water (18°C), where core temperature after exercise in hot water rose from 37.2 to 39.1°C (+1.9°C) and where core temperature was successfully clamped

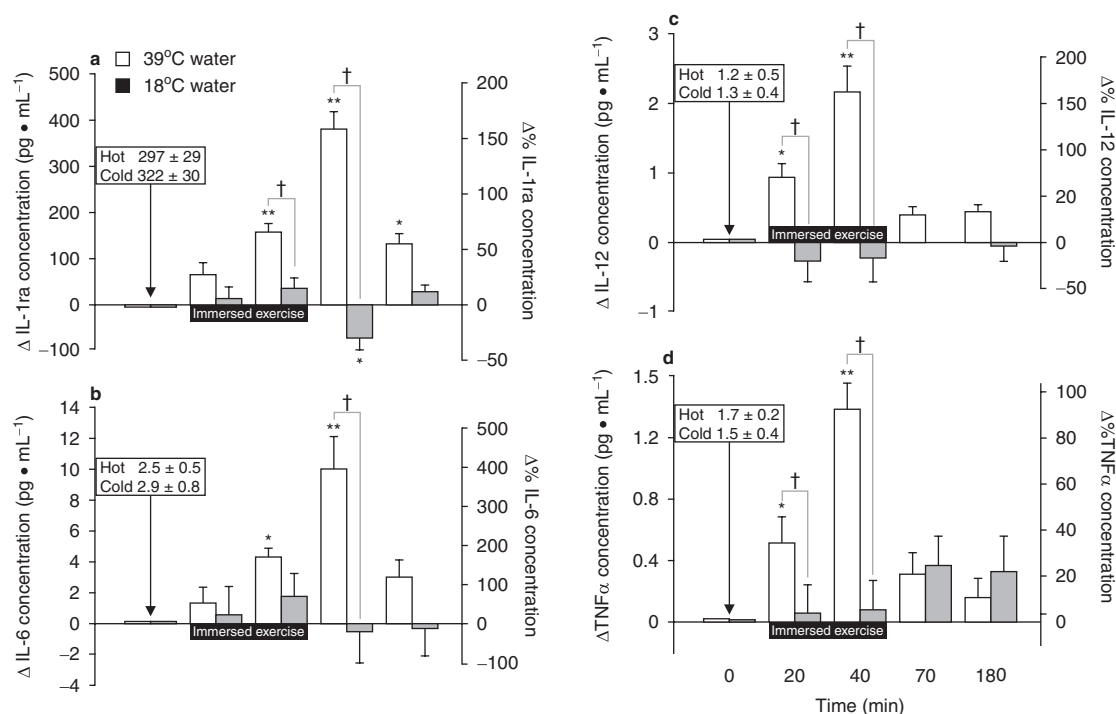


Fig. 4. Changes in circulating concentrations of (a) interleukin (IL)-1ra receptor antagonist, (b) IL-6, (c) IL-12 and (d) tumour necrosis factor- α (TNF α) responses to cycle exercise during hot (39°C) and cold (18°C) water immersions. Mean values ($\pm$ standard error) are expressed as both change (Δ) relative to the respective baseline (time 0) values (as shown in insets) and as percentage change ($\Delta\%$) from baseline ($n = 10$) [reproduced from Rhind et al.,^[65] with permission from Taylor & Francis Ltd, <http://www.tandf.co.uk/journals>]. * indicates significant ($p < 0.01$) within-condition differences vs pre-exercise; ** indicates significant ($p < 0.0001$) within-condition differences vs pre-exercise; † indicates significant ($p < 0.01$) difference between hot and cold conditions.

(+0.4°C) during exercise in cold water. Exercising under the thermal clamp conditions blunted increases in circulating catecholamines, cortisol and growth hormone. The stereotypical exercise-induced elevations in plasma IL-1ra, IL-6, IL-12 and TNF α were abolished when exercise was performed with a thermal clamp (figure 4). The authors noted that given the close interrelationship between neuroendocrine hormones and cytokines^[124] that it was likely that the significant increases in circulating cytokines with heat stress were induced at least partly by the elevation in circulating stress hormones.^[65] Indeed, stimulation of α_2 -adrenoceptors by noradrenaline has been shown to reduce cyclic adenosine monophosphate (cAMP) and enhance the synthesis of the pro-inflammatory cytokines TNF α and IL-12.^[26] Conversely, stimulation of β_2 -adrenoceptors by adrenaline infusion has been shown to

decrease synthesis of the pro-inflammatory cytokines and increase synthesis of the anti-inflammatory cytokines IL-6, IL-1ra and IL-10 via increases in cAMP.^[26]

A recent study^[121] examined intracellular cytokine responses to 90 minutes of cycling at 70% $\dot{V}O_{2peak}$ on one occasion at 35°C and on another occasion at 15°C where core temperature after exercise in the heat was 39.1°C (+1 vs 15°C trial). LPS-stimulated monocytes produced less IL-6 and TNF α during recovery from exercise, possibly due to elevated stress hormones, but additional heat stress did not alter monocyte cytokine production. These findings agree with another study showing no significant difference between exercising in hot and thermoneutral conditions on LPS-stimulated monocyte TNF α release.^[125] Despite the lack of an effect of additional heat stress on monocyte cytokine produc-

tion after exercise, plasma IL-6 and TNF α were shown to be ~4- and ~2-fold higher after exercise in the heat.^[121] This suggests that IL-6 and TNF α were being produced by tissues and organs other than mononuclear cells. The most likely source of the additional IL-6 after exercise and heat stress is skeletal muscle^[126-128] particularly as contracting skeletal muscle IL-6 production is raised when intramuscular glycogen stores are reduced^[128] and the reduction in intramuscular glycogen stores is exacerbated with raised muscle temperature^[129] and after exercise with additional heat stress.^[130] The glucoregulatory role for skeletal muscle IL-6 release has recently been reviewed elsewhere.^[131] A similar explanation for the raised plasma TNF α with exercise and heat stress is unlikely given that skeletal muscle does not produce or release TNF α .^[132] Indeed, the authors^[121] were unable to determine the source of the raised plasma TNF α with exercise and heat stress.

In summary, with the exception of IL-6, there is limited information on the effects of exercise with additional heat stress on plasma cytokine responses. Using an elegant thermal clamp model, one research team^[65] have shown that exercise (albeit only 40 minutes at 65% $\dot{V}O_{2peak}$) does not provide a sufficient stimulus for cytokine release unless the exercise is accompanied by a rise in core temperature. The evidence to date points towards elevated circulating stress hormones as being at least partly responsible for altered plasma cytokine responses (e.g. TNF α , IL-1ra, IL-12) to exercise with additional heat stress.^[65] A role for intramuscular glycogen depletion and associated skeletal muscle IL-6 production most likely accounts for the augmented plasma IL-6 response to exercise with additional heat stress.^[121]

2.7 Exercise in the Heat and Leukocyte Function

2.7.1 Neutrophils

In highly trained runners, 1 hour of treadmill running at 75% $\dot{V}O_{2max}$ in hot (28°C) compared with thermoneutral (18°C) conditions evoked a greater neutrophilia 3 hours into recovery.^[109] How-

ever, similar increases in plasma myeloperoxidase (MPO) concentration were observed after exercise on hot and thermoneutral trials. The authors noted that augmented release of neutrophil granule constituents, such as MPO and elastase, in response to exercise reflect direct neutrophil activation *in vitro*. As the greater neutrophilia after exercise in hot conditions was not paralleled by a larger increase in plasma MPO it was speculated that this might reflect a suppressive effect of severe heat stress on neutrophil activation. We recently had trained cyclists perform 2 hours of cycling at 60% $\dot{V}O_{2max}$ in a randomised crossover design, once in hot (30°C) and on another occasion in thermoneutral (20°C) conditions where final core temperatures were 38.7 and 38.1°C, respectively.^[110] Prolonged exercise in hot conditions resulted in significantly higher plasma cortisol concentration post-exercise (~20%) and 2 hours post-exercise (~30%) compared with thermoneutral conditions. Circulating neutrophil numbers were elevated post-exercise and 2 hours post-exercise but were not different between trials. LPS-stimulated neutrophil degranulation (elastase release per neutrophil) decreased after prolonged exercise but once again was not significantly different between trials. These results suggest that compared with thermoneutral conditions, a modest augmentation in the core temperature response (0.6°C) and plasma cortisol response to prolonged exercise in hot conditions does not influence neutrophil trafficking or neutrophil degranulation responses.

2.7.2 Lymphocytes

Performing two 30-minute bouts of cycling at 50% $\dot{V}O_{2max}$ with 45 minutes of rest between thermoneutral (23°C) and hot (40°C) conditions resulted in decreased PHA-stimulated lymphocyte proliferation that was exacerbated when the exercise was performed in hot conditions.^[73] The authors suggested that the larger decrease (56%) in PHA-stimulated lymphocyte proliferation after the second exercise bout under hot conditions most likely reflected altered proportions of the various lymphocyte subsets (e.g. increased NK cells or B lymphocytes). In the same study, pokeweed mitogen stimulated immunoglobulin (Ig) production (particularly

IgM) was unaffected by exercise in thermoneutral conditions but elevated when exercise was performed in the heat. Given that Ig production depends on B- and T-lymphocyte counts (T-helper lymphocytes aid and T-suppressor lymphocytes inhibit the process), the authors suggested that increases in the proportion of B lymphocytes and T-helper lymphocytes could account for the increase in Ig production when exercise was performed in the heat. More recently, we have shown that 2 hours of cycling at 60% $\dot{V}O_{2\max}$ in either hot (30°C) or thermoneutral (20°C) conditions evokes a similar reduction in saliva IgA (s-IgA) secretion rate in trained cyclists.^[133]

2.7.3 Natural Killer Cells

Using the same experimental protocol, involving two 30-minute bouts of cycling at 50% $\dot{V}O_{2\max}$, another study found no effect of exercise either in thermoneutral or hot conditions on NKCA.^[73] Studies involving prolonged continuous exercise at ~60% $\dot{V}O_{2\max}$ show elevated NKCA immediately after exercise that is unaffected by performing the exercise in hot conditions.^[108,111]

2.8 Exertional Heat Illness (EHI) and Immune Function

2.8.1 A Role for Immune Activation in the Aetiology of EHI

Exertional heat stroke (EHS), the most severe form of exertional heat illness (EHI), is a life threatening acute heat illness characterised by hyperthermia (core temperature >40.6°C) and neurological abnormalities that occur after exposure to high ambient temperature and humidity.^[134] Although the causes appear to be multifactorial, a systemic inflammatory response appears to be involved in the pathogenesis of EHI and EHS.^[69,84,135,136] During exercise-heat stress, the redistribution of cardiac output from internal organs to cutaneous circulation produces hypoxia, oxidative stress and nitrosative stress in the gastrointestinal (GI) epithelium resulting in GI dysfunction (figure 5). The GI ischaemia causes damage to the intestinal mucosal membrane including enterocyte necrosis and opening of tight junctions, which results in increased permeability and leakage of endotoxin. A direct effect of

hyperthermia on the opening of tight junctions has also been shown with large molecules being able to penetrate the barrier at temperatures >41.5°C (figure 5).^[137] Hyperthermia also induces nitric oxide production in the splanchnic region and IFN γ production from activated T cells and NK cells, both of which have been shown to open tight junctions and reduce tight junction integrity.^[138,139]

Endotoxaemia appears to be the major contributing factor to the multiple organ failure in EHS.^[139] In support of this, monkeys treated with oral antibiotics to reduce gut bacteria do not become endotoxaemic during experimental heat stroke^[140] and those treated with anti-LPS plasma have increased survival rates when exposed to heat stroke.^[141] HSP expression has been shown to protect against heat-induced loss of tight junction integrity and ischaemia and reperfusion injury of the gut.^[137] Collectively, the research evidence suggests that inhibiting endotoxin exposure or its internal effects improves EHS survival.^[139]

Increases in plasma endotoxin concentration have been reported after prolonged heavy exercise in race horses,^[142] ultra-marathon runners^[143] and triathletes.^[144,145] At the completion of an ultradistance triathlon, mean plasma LPS concentration was 294 pg/mL,^[143] which is comparable with levels seen in florid sepsis patients.^[146] The increase in GI permeability and resulting endotoxaemia with severe exercise-heat stress stimulates pyrogenic cytokine release (TNF α , IL-1 and IL-6) from activated tissue macrophages and plasma monocytes.^[139] These pyrogenic cytokines may raise the hypothalamic thermoregulatory set point contributing to the development of EHS (figure 5).^[139] In support of this contention are results from studies showing that animals receiving IL-1 receptor antagonist demonstrate enhanced survival after experimentally induced heatstroke.^[141] In addition, the production of pyrogenic cytokines in skeletal muscle, particularly damaged muscle (e.g. rhabdomyolysis), may also play a role in the onset of EHS again through pyrogenic cytokine-induced increases in the hypothalamic thermoregulatory set point (figure 5).^[134,147] Interestingly, the skeletal muscle relaxant

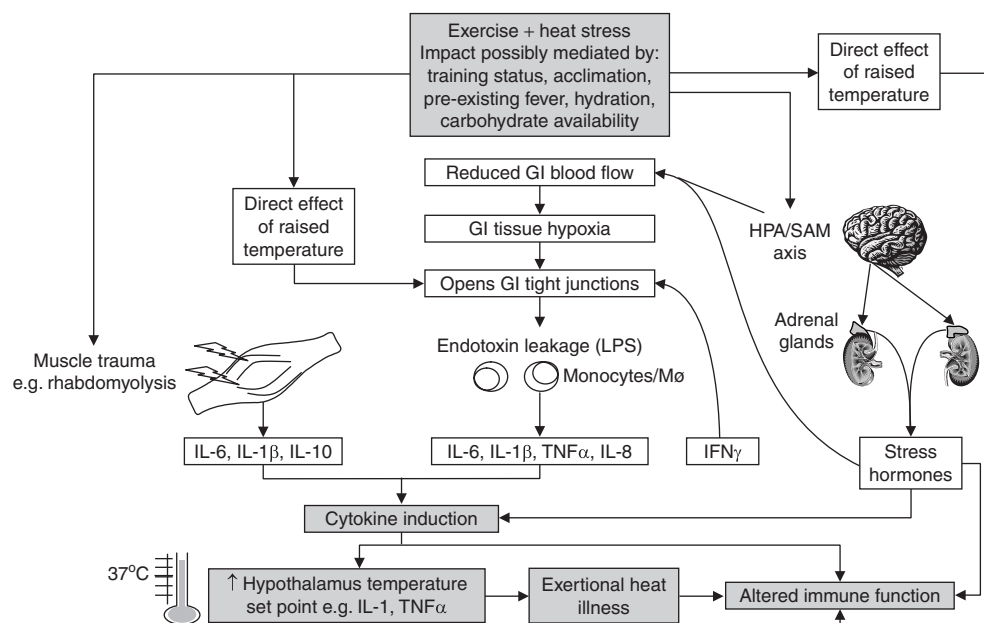


Fig. 5. Possible links and mechanisms involved in the alteration of immune function with exercise-heat stress. A direct effect of raised temperature and indirect effect of augmented circulating stress hormones may alter immune function during exercise-heat stress. Reduced gastrointestinal (GI) blood flow during exercise-heat stress may cause GI membrane damage and endotoxin leakage. Endotoxin activates circulating monocytes/tissue macrophages increasing the release of cytokines known to modify immune function. Augmented circulating cytokines during exercise-heat stress may raise the hypothalamic-thermoregulatory temperature set point, possibly contributing to the development of exertional heat illness. Accordingly, immune function has been reported to be suppressed in patients with exertional heat illness and heat stroke. **HPA** = hypothalamic-pituitary-adrenal axis; **IFN** = interferon; **IL** = interleukin; **LPS** = lipopolysaccharide; **Mφ** = macrophage; **SAM** = sympatheticoadrenal-medullary axis; **TNFα** = tumour necrosis factor-α.

dantrolene, which has received recent attention as the drug of choice for treating malignant hyperthermia, has been shown to decrease tissue and plasma pyrogenic cytokines and improve survival in an animal model of septic shock.^[134] These results might suggest a significant contribution of muscle-derived pyrogenic cytokines in EHS aetiology, but this requires substantiation in a human model of EHS alongside further research into the potential therapeutic use of dantrolene for treatment of EHS.

2.8.2 Immune Responses in Patients with Heat Injury

During a pilgrimage to Mecca in 1990, one research group compared circulating leukocyte distribution in 11 heatstroke patients (core temperature 41.4°C) and 40 healthy controls resting at room temperature.^[78] Compared with the healthy controls, the number of circulating leukocytes and lymphocytes was significantly elevated in heatstroke pa-

tients (leukocytes: 13.1 in heatstroke vs 5.6 10⁹/L in controls [table I]). The lymphocytosis in heatstroke patients was mainly due to increases in suppressor T-lymphocytes (CD8+) and NK cells (CD16+ and CD56+). Decreased percentage of helper T-lymphocytes (CD4+) and increased percentage of suppressor T-lymphocytes (CD8+) resulted in a markedly suppressed helper: suppressor T-lymphocyte ratio in heatstroke patients (0.4 vs 1.8 in heatstroke and controls, respectively): possibly contributing to the increase in infections reported in heatstroke patients.^[78]

In a follow-up study that took place during the 1993 pilgrimage to Mecca,^[148] the same research group compared 14 heatstroke patients (core temperature 42.3°C) with 7 heat-stressed controls subjected to the same living conditions (core temperature not presented) and 11 controls rested at normal room temperature. In this study, blood samples were

also collected after cooling the heatstroke patients to $\leq 39.4^{\circ}\text{C}$ (range 25–255 minutes). To highlight the severity of these heatstroke cases, it is noteworthy that three patients had core temperature $>43^{\circ}\text{C}$, two patients were in shock (systolic blood pressure $<90\text{mm Hg}$) and three required intubation and mechanical ventilation. One patient died, and after cooling, four patients had persistent neurological deficits (stupor, coma) and one patient had GI bleeding.

In agreement with the previous investigation,^[78] lower T-lymphocytes (CD3+) and helper T-lymphocytes (CD4+) and higher percentages of suppressor T-lymphocytes (CD8+) and NK cells (CD16+ and CD56+) were observed in heatstroke compared with rested controls. More modest changes in lymphocyte subsets were observed in the heat-stressed controls (same direction as heatstroke patients) suggesting a continuum for the responses of lymphocyte subsets to heat stress. Cooling either partially or completely restored lymphocyte subsets to normal in heatstroke patients. Although a significant increase in circulating catecholamines was observed in heatstroke patients, there were no significant correlations between catecholamines and the percentage or absolute numbers of lymphocyte subsets. The authors suggested a possible role for elevated cytokines in the observed alterations in leukocyte and lymphocyte subsets.^[78,148] In support of this contention, the same authors have reported elevated circulating TNF α and IL-1^[135] and elevated circulating TNF receptors (TNFR 60 and 80) and IL-6 in heatstroke patients compared with rested controls.^[136] Indeed, it has been known for a while that the induction of a number of cytokines (e.g. G-CSF, IL-1 β and IL-6) has a direct stimulatory effect on leukocyte production (figure 2).^[88] Moreover, a very recent study has demonstrated a role for the hyperthermia-induced cytokinaemia in the altered leukocyte responses to hyperthermia in mice.^[79]

In another recent study, PHA-stimulated lymphocyte responses (lymphocyte CD69 expression, an early activation marker) were reduced in military recruits with suspected EHI (mean core temperature 40.4°C) for up to 24 hours compared with control

recruits (mean core temperature 38.6°C).^[149] Interesting is that these core temperature responses were observed after military recruits performed only a short duration run ($\sim 3.2\text{km}$) as part of their normal training in warm weather. This indicates that EHI can occur during relatively brief exercise in warm weather as well as more prolonged exercise in hot weather.

In summary, prolonged exercise of fixed duration in hot compared with thermoneutral conditions (where core temperature was $\geq 1^{\circ}\text{C}$ higher in hot conditions) augments circulating stress hormone, catecholamine and cytokine responses with associated increases in circulating leukocyte numbers. Studies comparing the effects of moderate heat stress and severe heat stress (heatstroke patients) suggest a continuum of the responses of circulating leukocytes, differential leukocytes, lymphocyte subsets and cytokine responses to heat stress. Accordingly, thermal clamping studies have demonstrated a large contribution of the rise in core temperature during exercise in the leukocytosis and cytokinaemia of exercise. With the exception of lowered PHA-stimulated lymphocyte responses after exercise in the heat, and in EHI patients, recent laboratory studies show a limited effect of exercise in the heat (vs thermoneutral conditions) on neutrophil function, monocyte function, NKCA and salivary immunity. Therefore, the results from these studies do not support the contention that exercising in the heat poses a greater threat to immune function (vs thermoneutral conditions). However, It is noteworthy that due to the tight restrictions enforced by ethical committees, most laboratory studies examining immune responses to exercise in the heat have to date evoked only modest increases in core temperature (final core temperature $<39^{\circ}\text{C}$). Given that core temperature during exercise in the field often exceeds 40°C in athletes, military personnel and fire fighters undertaking vigorous physical activity in hot conditions, field studies may provide an opportunity for researchers to determine the effects of severe heat stress on the immune response to exercise. From a more critical standpoint, laboratory studies investigating the effects of moderate heat

stress (core temperature $<39^{\circ}\text{C}$) on immune responses to exertion provide relatively limited information about the possible involvement of immune activation and systemic inflammation in the aetiology of EHS (core temperature $>40.6^{\circ}\text{C}$). Finally, the effects of heat acclimatisation on the immune response to exercise in hot conditions warrants enquiry.

3. Cold Stress and Immune Function

It is commonly believed that chilling of the body increases susceptibility to upper respiratory tract infection (URTI). Indeed, use of the term 'colds' may come from the common belief that cold exposure causes URTI.^[16,150] Exercise immunologists have a keen interest in the effects of cold exposure on immune function and URTI incidence because athletes and military personnel, particularly in the northern hemisphere, regularly perform in ambient conditions below 0°C (also see reviews by Shephard and Shek^[151] and Castellani et al.^[150]). Although evidence is scant, there are reports documenting increased incidence and severity of URTI at ski schools^[152] and during military patrols involving high levels of energy expenditure and exposure to cold conditions.^[153] It remains unclear if the reports showing increased URTI during prolonged cold exposure are due to: confined living conditions, negative energy balance, sleep deprivation, cold exposure *per se* or to a combination of these factors.

The most important factors that contribute to lowering of core temperature in cold conditions (hypothermia = core temperature $<36^{\circ}\text{C}$) appear to be inappropriate clothing and unanticipated wetting of clothing. The length of exposure to cold conditions and the reduction in metabolic heat production that accompanies fatigue are also important factors in the development of hypothermia.^[154] In an attempt to defend core temperature during cold exposure shivering thermogenesis and peripheral vasoconstriction are initiated. Shivering muscle contractions are the same as muscular contraction during exercise except that no useful work is performed.^[150] Peripheral vasoconstriction is under sympathetic nervous system (SNS) control and SNS activation is

known to mediate and modulate immune function. Therefore, it is plausible that any immune changes observed during cold exposure might be due indirectly to cold exposure evoked muscle contractions associated with shivering thermogenesis and/or to SNS activation rather than cold exposure directly.

The following sections will focus on the small number of studies that have investigated firstly the effects of passive cooling on immune responses and secondly the effects of a combination of cold exposure and exercise on immune responses. Studies that have provided URTI incidence in personnel stationed in Antarctica will also be critically discussed to establish whether the commonly held belief that cold exposure increases URTI incidence is credible and if so whether cold-induced suppression of immune function is responsible.

3.1 Passive Cooling and Leukocyte Counts

Immersion of healthy young men to mid-chest in cold water (14°C) for 1 hour evoked a leukocytosis.^[155] However, immersion in cold water (23°C) for 40 minutes, which resulted in $\sim 0.8^{\circ}\text{C}$ reduction in core temperature did not alter total leukocyte numbers.^[63] In another study, subjects sat for 2 hours in a climate chamber maintained at 5°C where core temperature decreased from 37.0 to 36.5°C .^[112] The authors observed a small but significant leukocytosis after cold exposure due to increases in circulating neutrophil and lymphocyte numbers. Cold exposure was associated with increased numbers of circulating T-lymphocytes (CD3+), B-lymphocytes (CD19+) and NK cells (CD16+ and CD56+). The authors suggested that the modest rise in circulating leukocyte numbers during cold exposure may be attributed to a noradrenaline mediated mobilisation of demarginated cells. Future studies may be able to confirm this by blocking the effects of noradrenaline during cold exposure. T-lymphocyte helper (CD4+) and suppressor (CD8+) numbers remained unaltered after acute cold water immersion in a previous study.^[155] Although an acute bout of cold exposure appears to have only a small impact on immune cell counts, there is some evidence that repeated cold exposure evokes improved immune responsiveness.

When Jansky et al.^[155] had subjects repeat cold water immersion (1 hour at 14°C) three times per week for 6 weeks they noted an increased T-lymphocyte (CD3+), helper (CD4+) and suppressor (CD8+) T-lymphocyte number and an increased proportion of activated T- and B-lymphocytes exhibited through increased expression of HLA-DR+.

In summary, short-term cold exposure may cause a small leukocytosis with an increase in circulating number of neutrophils, lymphocytes, T- and B-lymphocytes and NK cells that appears to be dependent on the interaction between the magnitude of the reduction in core temperature and the duration of exposure. Noradrenaline-mediated mobilisation of demarginated cells is most likely responsible for the small changes in circulating leukocyte numbers with cold exposure. In one study, immune responsiveness improved after repeated bouts of cold exposure over several weeks.^[155] Taken together, the results from studies involving an acute bout of cold exposure or repeated bouts of cold exposure over several weeks show if anything beneficial rather than detrimental effects of cold exposure on leukocyte counts.

3.2 Passive Cooling and Leukocyte Function

In ten patients undergoing surgery, intraoperative hypothermia, where core temperature fell by 4°C (core temperature 33°C) was associated with a decrease in neutrophil oxidative burst and neutrophil capacity to ingest *Escherichia coli*.^[156] However, lack of a control group undergoing surgery under normothermic conditions is a serious limitation in this study. It remains to be shown if a more modest reduction in core temperature (1–2°C) decreases neutrophil function in a tightly controlled laboratory study. In patients who experienced a modest 1°C drop in core temperature during surgery a reduction in lymphocyte proliferation and IL-2 production 24 and 48 hours after surgery has been reported compared with normothermic patients.^[157] It remains unclear if these findings translate into higher infection after surgery.

In tightly controlled laboratory studies, 30 minutes of exposure to a cold room (4°C) where core temperature decreased ~0.5°C^[158] and 2 hours in a

climate chamber (5°C) where core temperature decreased by ~0.6°C resulted in elevated NKCA.^[112] The authors noted, contrary to popular belief, that cold exposure had an immuno-stimulating effect possibly related to the enhanced noradrenaline response to cold exposure. Similarly, another study showed that monocytes incubated for 1 hour at 34°C killed a greater number of *E. coli* compared with incubation for 1 hour at 37°C.^[53] This finding might also be considered favourable for host defence.

In summary, there is limited evidence to support a suppression of leukocyte counts or function with either short- or long-term cold exposure. In fact, contrary to popular belief, tightly controlled laboratory studies indicate an immuno-stimulatory effect of acute and repeated bouts of cold exposure.

3.3 Exercise in Cold Conditions and Immune Function

Compared with steady-state exercise in thermoneutral conditions, exercise in cold air conditions is associated with similar or slightly lower core temperature, muscle temperature and cardiac output and with increased respiratory heat loss, ventilation,^[159] oxygen uptake^[160] and carbohydrate oxidation.^[154,161] Substrate depletion due to the higher rate of carbohydrate oxidation whilst cycling at 70% $\dot{V}O_{2max}$ in very cold (4°C) compared with thermoneutral conditions (21°C) most likely accounts for the decreased time to exhaustion whilst exercising in very cold conditions.^[105]

Few studies have examined immune responses to exercise in cold conditions in humans. These studies have mostly involved short-duration exercise protocols lasting <1 hour and have compared immune responses following exercise in cold conditions with immune responses to exercise in hot rather than thermoneutral conditions. Nevertheless, although limited, the evidence to date does not support the popular belief that exercising in cold conditions suppresses immune function. Two studies had healthy young men immersed to mid-chest in water perform 40 minutes of cycling at 65% $\dot{V}O_{2max}$ in cold (18–23°C) and hot (39°C) water where core temperature increased by ~0.5°C in cold and by

~2.0°C in hot conditions (figure 3).^[63,64] Exercising in cold water attenuated the leukocytosis observed after exercise in hot water with smaller increases in circulating neutrophils and lymphocytes (table II). The authors suggested that the significantly reduced (>50%) increment in plasma catecholamines and completely abolished plasma cortisol response to exercise in cold conditions (table III) was most likely responsible for the attenuated leukocytosis after exercising in the cold. Given that increases in plasma catecholamines and cortisol are thought to be partly responsible for the reported immune perturbations with prolonged high-intensity exercise,^[15] blunting these responses by exercising in cold water might be favourable for immune function and host defence.

In another study, the same group had subjects perform a 1-hour bout of cycling at 55% $\dot{V}O_{2\text{peak}}$ immersed in cold (18°C) and thermoneutral (35°C) water where a more modest ~1°C rise in core temperature occurred after exercise in thermoneutral water compared with ~0.2°C rise in core temperature after exercise in cold water.^[112] Total and differential leukocyte counts were similar after exercise in cold and thermoneutral water. In the same study, NKCA increased similarly after exercise in cold and thermoneutral conditions, which agrees with a more recent study involving 1 hour of cycling at 60% $\dot{V}O_{2\text{peak}}$ in an environmental chamber in cold (8°C) and hot (38°C) conditions.^[111]

Tomasi et al.^[9] suggested that the decrease in s-IgA concentration after a 50km cross-country ski race might have been partly due to the large inflow of cold air lowering the temperature of the mucous membranes. In an attempt to identify the effects of exercising in cold conditions on s-IgA, another group has shown similar s-IgA concentration after healthy males performed a 30-minute treadmill run at 80% $\dot{V}O_{2\text{max}}$ in an environmental chamber set at 6°C, 19°C and 34°C.^[162] From a critical standpoint, s-IgA was reported as a concentration and not as a secretion rate, thus ignoring any drying effect of oral breathing whilst exercising.^[163] Furthermore, only a short 30-minute exercise bout was used along with an ambient temperature above freezing and a high

relative humidity (85%). In addition to any cooling effect of breathing cold air on the oral cavity and upper airways, there might also be a drying effect because cold air is dry. In light of these criticisms, we had trained cyclists ride for 2 hours at 70% $\dot{V}O_{2\text{max}}$ in an environmental chamber in very cold conditions (-6.4°C) and thermoneutral conditions (19.8°C and 66% relative humidity).^[164] Interestingly, s-IgA secretion rate decreased to a similar extent after prolonged exercise in cold-dry conditions and thermoneutral conditions.^[164]

3.4 Immune Responses and Upper Respiratory Tract Infection in Personnel Stationed in Antarctica

s-IgA and IgM concentrations have been reported to be lower during the first 4 months of a 1-year Antarctica expedition.^[165,166] Unfortunately, the lack of a control group and data for URTI incidence are limitations in these studies. The authors suggested that the pattern of change in mucosal immunity during the expedition was consistent with the responses to the psychological stresses associated with prolonged Antarctic isolation.^[165,166] Indeed, psychological stress^[167] and changes in mood state^[168] have been shown to suppress mucosal immunity. Moreover, depressed cell-mediated immunity has been shown to correlate with psychological stress^[169] and perceived anxiety^[170] during Antarctic expeditions.

To date, there is still no conclusive evidence to support a direct effect of prolonged cold exposure on URTI incidence. Reports from a number of Antarctic research studies have shown little evidence of URTI among personnel except immediately after the visit of supply ships, when new strains of virus are imported into the community.^[151,171,172] The increase in the susceptibility to newly imported viruses in personnel stationed in Antarctica may be partly due to enhanced survival of viruses in the cold climate^[151] or to crowded living conditions.^[173,174] In addition, prolonged cold exposure may cause drying of the mucosal surfaces, slowing of tracheal cilia^[175] or a deterioration of the normal barrier function of the skin,^[176] all of which may decrease host de-

fence.^[150,151] It appears that once immunity has developed to the new strains of virus that the incidence of URTI is similar in inhabitants of cold and temperate climates.

In summary, the evidence to date does not support the popular belief that short- or long-term cold exposure, with or without exercise, suppresses immune function and increases URTI incidence.

4. High Altitude and Immune Function

It is commonly believed that travelling to regions of high altitude increases susceptibility to URTI. However, the evidence to support this is lacking and the problem is compounded by possible misdiagnosis due to some overlap in the symptoms of acute mountain sickness and URTI.^[177] However, one paper reports a higher prevalence of pneumonia in 20 000 soldiers stationed at an altitude of 3692m compared with >130 000 troops stationed at low altitude.^[178] Anecdotal reports and specific case studies also document an increase in URTI symptoms when ascending to altitudes >4000m.^[179] That heightened levels of stress encountered at altitude may suppress immune function and possibly raise URTI incidence has been investigated in both animals and humans. It is important to be cautious when interpreting the results from these studies as altered immune responses at high altitude might reflect the effects of hypoxia *per se* or the effects of other extraneous stressors associated with mountain ascents or residing in the unfamiliar environment of a decompression chamber.^[16] However, there is some evidence of reduced resistance to infection at altitude in animals.^[180]

Early studies adopted a model in which the immune system of mice or rats was challenged with pathogenic bacteria at simulated altitudes of 4000–8000m.^[181,182] Residing at altitude for periods of 14–100 days has been associated with reduced survival rate in exposed animals compared with non-exposed control animals.^[181,182] In humans, a handful of studies report alterations in immune function as a consequence of exposure to high altitude. Studies have investigated the effect of both short (~5 days)^[180,183] and longer (3 weeks) periods of expo-

sure to high altitude on lymphocyte trafficking and lymphocyte function.^[184] T-lymphocyte function has been reported to be suppressed following short periods (~5 days) of altitude exposure (>2500m).^[180] PHA-stimulated lymphocyte proliferation was also suppressed following a simulated 5-day stay at an altitude of 7620m in a decompression chamber.^[183]

More recent studies have extended these findings by showing that residing for longer (3-week) periods at altitudes of 2100–5050m also suppresses mitogen-stimulated lymphocyte proliferation and decreases the circulating helper : suppressor (CD4+/CD8+) T-lymphocyte ratio.^[184,185] In contrast, other aspects of immunity seem less affected by the stress of high altitude. Meehan et al.^[183] indicated no change in stimulated antibody production or mucosal immunity when humans were exposed to the hypoxic conditions of altitude. These findings are further supported by data indicating no change in T-cell dependent and T-cell independent antibody responses following stimulation by various antigens in humans and mice at altitudes >4000m.^[186] B-cell function, therefore, appears less affected by altitude and hypoxia. The functional consequences of this are implied in animal models, which despite indicating a reduced resistance to bacterial infection actually show decreased susceptibility to infection when inoculated with viruses such as influenza.^[180] Although the mechanisms underlying these findings are unclear, stress hormone-mediated maintenance of type II (humoral) immunity and suppression of type I (cell-mediated) immunity may be partly responsible.^[26,187,188] In line with this contention, a very recent study has shown decreased T-lymphocyte IFN γ expression (type I cytokine: cell-mediated response) and unchanged T-lymphocyte IL-4 expression (type II cytokine: humoral response) on ascent to and after 3 weeks at 5050m.^[184]

4.1 Exercise at High Altitude and Immune Function

Along with the well known decrement in physical performance, exercise at high altitude compared with exercise at sea level is associated with a num-

ber of responses that might further depress immune function. These include increased circulating stress hormone concentrations, increased reliance on glycogen and amino acids as fuels for exercise and tissue hypoxia that might enhance local inflammatory responses and facilitate the penetration of endotoxins through the gut wall.^[16,189] Given the acknowledged immune alteration with exercise performed at sea level^[106,107] and the additional stress responses to exercising at high altitude,^[190] we might expect a greater degree of immune depression when exercise is performed at high altitude.^[191,192]

Unfortunately, there are few tightly controlled studies that have assessed this using exercise protocols in hypoxic conditions. This may be due in part to the problems in prescribing comparable exercise intensities in normal and hypoxic environments, since $\dot{V}O_{2\max}$ is reduced in hypoxic conditions. However, one study has compared the effect of cycling for 20 minutes at 60% $\dot{V}O_{2\max}$ in normoxic and hypoxic conditions (11.5% oxygen) on circulating lymphocyte subpopulations.^[193] The combination of hypoxia and exercise increased the circulating number of lymphocytes over and above the response seen in normoxia, with the largest responses seen for NK cells. Circulating numbers of NK (CD16+ and CD56+) cells increased 2-fold during normoxic exercise but 5-fold during hypoxic exercise. Whilst the additional stress of hypoxia appeared to augment typical exercise responses in cell trafficking, NK cell activity following IL-2 or IFN α stimulation was unchanged in the hypoxic environment.^[193] Another group have noted that climbing from 1780 to 3198m suppresses the activation of circulating neutrophils normally seen during ascent without exercise.^[194] The authors speculated that down-regulation of neutrophil function whilst climbing to high altitude may serve to limit exercise-induced inflammatory tissue damage that might otherwise be exacerbated by cytotoxic neutrophils. It remains unclear whether down-regulation of neutrophil function whilst exercising at high altitude might alter host defence and risk of URTI.

While the handful of studies assessing the combined effects of exercise and altitude on immune

function have used acute exercise bouts, one recent training study has assessed the practice of 'living high, training low' (LHTL)^[195] on mucosal immunity.^[196] A group of cross-country skiers ($n = 11$) trained for 18 days at low altitude (1200m) and a sub-sample of six skiers slept in hypoxic rooms (11 hours/day) that progressively increased the simulated altitude from 2500 to 3500m over the course of the training camp (LHTL group). s-IgA concentration tended to decrease in both the LHTL group and control group ($n = 5$) who lived without altitude simulation (1200m) and this reduction reached significance in the LHTL group ($p < 0.01$) and approached significance ($p = 0.08$) in the control group versus pre-training. Whilst the authors interpreted this finding as a cumulative negative effect of both exercise and altitude on mucosal immunity, limitations in this study include the small number of subjects in each group, the lack of significant differences between the LHTL and control group for s-IgA concentration and the lack of a non-exercising control group sleeping in simulated altitude.^[196] In addition, the authors reported s-IgA only as a concentration and not as a secretion rate (saliva flow rate $\times$ s-IgA concentration). s-IgA secretion rate provides a better indication of mucosal immune status and protection against oral pathogens.^[10,163] both low saliva flow rate^[197] and low s-IgA concentration^[198-200] have been associated with raised URTI incidence.

In summary, although a small body of evidence supports the commonly held belief that high-altitude exposure increases URTI,^[178,179] clear conclusions are difficult to make as there appears to be some overlap in the symptoms of acute mountain sickness and URTI. Although high altitude exposure has limited effect on humoral immunity, a number of studies have shown suppression of cell-mediated immunity at high altitude.

5. Spaceflight and Immune Function

Extremes of heat,^[201] changes in pressure, vibration, sleep deprivation,^[202,203] impaired nutrition,^[204,205] weightlessness and psychological stress^[206] are examples of potentially stressful stim-

uli that occur during space flight. As one may expect, data from studies in these conditions are restricted to small sample sizes and the practicalities of assessing immune function in microgravity environments. Variations in uncontrolled stressors have also caused variation in findings between studies. However, alterations in immune function have been reported following spaceflight.^[207-212] Consistent with immune responses to other stressful environments, a marked leukocytosis is one of the most regularly reported findings in astronauts upon re-entry into the earth's atmosphere.^[213-216] For example, a meta-analysis of research into the effects of 8–57 days of spaceflight has confirmed a regularly reported leukocytosis predominantly consisting of raised circulating neutrophil numbers.^[213] Reported increases in circulating stress hormones and catecholamines, during the potentially lethal manoeuvres of re-entry and landing, most likely account for the observed leukocytosis upon return to the normal gravity environment.^[217]

Both animal and human studies have shown suppression of mitogen-stimulated lymphocyte proliferation as a result of spaceflight.^[209,218] Suppressed lymphocyte proliferation may prevail for up to 7 days after re-entry into the earth's atmosphere.^[208] Spaceflight may also alter cytokine production, following evidence suggesting a reduction in mitogen-stimulated IL-2 production following short- and long-term space flights;^[219,220] however, some studies report the contrary.^[221-223] There is also some disagreement between studies on the effects of spaceflight on IFN γ production, with some indication of both increased and decreased concentrations following short- and long-term flights.^[220,224,225] Research has, however, suggested a reduction in NK cell numbers and NKCA as a consequence of the conditions associated with space travel.^[214] There is evidence of a suppressed NK cell cytotoxicity index in cosmonauts following short- (7–14 days)^[220] and long-term (211–237 days)^[226] spaceflights.

Measures of cell-mediated immunity are possible through a skin inoculation test assessing the response to common recall antigens. The resulting delayed hypersensitivity response has been mea-

sured in astronauts, with some evidence of suppressed cell-mediated immunity by day 4 of spaceflight, with maximal suppression occurring after 5–10 days.^[227] Similar results have been found in cosmonauts^[228] during longer duration flights. Humoral immune responses to the microgravity environment have not been extensively studied, but the available data suggest little change in serum Ig concentrations after exposure to microgravity.^[214] Although the impact of the aforementioned immune changes on resistance to infection is unclear,^[208,229] there is some evidence of an increased virulence of pathogenic micro-organisms (e.g. *Salmonella*) with microgravity, which may lead to an increase in infections from micro-organisms during space missions.^[230,231]

In summary, re-entering the normal gravity environment after microgravity exposure is associated with a leukocytosis, suppressed cell-mediated immunity and unaltered humoral immunity. Less is known about the effects of spaceflight, particularly long-term spaceflight, on URTI incidence.

6. Conclusions

Exercising in hot compared with thermoneutral conditions (where core temperature is $\geq 1^{\circ}\text{C}$ higher in hot conditions) augments circulating stress hormone, catecholamine and cytokine responses with associated increases in circulating leukocytes. Studies that have clamped the rise in core temperature during exercise (40 minutes cycling in cool water) demonstrate a large contribution of the rise in core temperature in the leukocytosis and cytokinaemia of exercise. Interestingly, with the exception of lowered stimulated lymphocyte responses after exercise in the heat, and in exertional heat illness patients (core temperature $>40^{\circ}\text{C}$), recent laboratory studies show a limited effect of exercise in the heat on immune function. Therefore, most of the available evidence does not support the contention that exercising in the heat poses a greater threat to immune function (vs thermoneutral conditions). However, from a critical standpoint, because of ethical committee restrictions, most laboratory studies have evoked only modest core temperature responses

(<39°C). Given that core temperature during exercise in the field often exceeds levels associated with fever and hyperthermia (~39.5°C) field studies may provide an opportunity to determine the effects of severe heat stress on immune responses. Field studies may also provide insight into the possible involvement of immune modulation in the aetiology of exertional heat stroke (core temperature >40.6°C) and identify the effects of acclimatisation on neuroendocrine and immune responses to exercise in the heat. Laboratory studies can still provide useful information by, for example, applying the thermal clamp model to examine the involvement of the rise in core temperature in the functional immune modifications associated with more prolonged exercise.

Studies investigating the effects of cold, high altitude and microgravity on immunity and infection incidence are often hindered by extraneous stressors (e.g. isolation in an unfamiliar environment). Nevertheless, the available evidence does not support the popular belief that short- or long-term cold exposure, with or without exercise, suppresses immunity and increases infection. In fact, controlled laboratory studies indicate immuno-stimulatory effects of cold exposure. Although some evidence shows that ascent to high altitude increases infection, clear conclusions are difficult to make because of some overlap with the symptoms of acute mountain sickness. Studies have reported suppressed cell-mediated immunity in mountaineers at high altitude and in astronauts after re-entering the normal gravity environment but the impact of this finding on resistance to infection remains unclear. Given the US space agency's recently announced plans for long-term spaceflight (including visits to Mars), the effects of prolonged microgravity on immunity and infection warrants enquiry.

This article has highlighted some limitations in laboratory and field studies that have attempted to identify if residing or exercising in environmental extremes poses a greater threat to immune function (vs more comfortable conditions). Nevertheless, the weight of research evidence to date does not fully support the popular belief that residing or exercising

in environmental extremes poses a greater threat to immune function.

References

1. Brenner IK, Shek PN, Shephard RJ. Infection in athletes. *Sports Med* 1994; 17: 86-107
2. Nieman DC, Johanssen LM, Lee JW, et al. Infectious episodes in runners before and after the Los Angeles Marathon. *J Sports Med Phys Fitness* 1990; 30: 316-28
3. Peters EM, Bateman ED. Ultramarathon running and upper respiratory tract infections: an epidemiological survey. *S Afr Med J* 1983; 64: 582-4
4. Kappel M, Tvede N, Galbo H, et al. Evidence that the effect of physical exercise on NK cell activity is mediated by epinephrine. *J Appl Physiol* 1991; 70: 2530-4
5. Keast D, Cameron K, Morton AR. Exercise and the immune response. *Sports Med* 1988; 5: 248-67
6. Lewicki R, Tchorzewski H, Majewska E, et al. Effect of maximal physical exercise on T-lymphocyte subpopulations and on interleukin 1 (IL 1) and interleukin 2 (IL 2) production in vitro. *Int J Sports Med* 1988; 9: 114-7
7. Oshida Y, Yamanouchi K, Hayamizu S, et al. Effect of acute physical exercise on lymphocyte subpopulations in trained and untrained subjects. *Int J Sports Med* 1988; 9: 137-40
8. Robson PJ, Blannin AK, Walsh NP, et al. Effects of exercise intensity, duration and recovery on in vitro neutrophil function in male athletes. *Int J Sports Med* 1999; 20: 128-35
9. Tomasi TB, Trudeau FB, Czerwinski D, et al. Immune parameters in athletes before and after strenuous exercise. *J Clin Immunol* 1982; 2: 173-8
10. Blannin AK, Robson PJ, Walsh NP, et al. The effect of exercising to exhaustion at different intensities on saliva immunoglobulin A, protein and electrolyte secretion. *Int J Sports Med* 1998; 19: 547-52
11. Nieman DC, Miller AR, Henson DA, et al. Effects of high- vs moderate-intensity exercise on natural killer cell activity. *Med Sci Sports Exerc* 1993; 25: 1126-34
12. Bishop NC, Blannin AK, Walsh NP, et al. Nutritional aspects of immunosuppression in athletes. *Sports Med* 1999; 28: 151-76
13. Nieman DC. Exercise immunology: nutritional countermeasures. *Can J Appl Physiol* 2001; 26: S45-55
14. Clow A, Hucklebridge F. The impact of psychological stress on immune function in the athletic population. *Exerc Immunol Rev* 2001; 7: 5-17
15. Hoffman-Goetz L, Pedersen BK. Exercise and the immune system: a model of the stress response? *Immunol Today* 1994; 15: 382-7
16. Shephard RJ. Immune changes induced by exercise in an adverse environment. *Can J Physiol Pharmacol* 1998; 76: 539-46
17. Jonsdottir IH. Special feature for the Olympics: effects of exercise on the immune system: neuropeptides and their interaction with exercise and immune function. *Immunol Cell Biol* 2000; 78: 562-70
18. Brenner I, Shek PN, Zamecnik J, et al. Stress hormones and the immunological responses to heat and exercise. *Int J Sports Med* 1998; 19: 130-43
19. Vale W, Spiess J, Rivier C, et al. Characterization of a 41-residue ovine hypothalamic peptide that stimulates secretion of corticotropin and beta-endorphin. *Science* 1981; 213: 1394-7
20. Tsigos C, Chrousos GP. Physiology of the hypothalamic-pituitary-adrenal axis in health and dysregulation in psychiatric and

- autoimmune disorders. *Endocrinol Metab Clin North Am* 1994; 23: 451-66
21. Kappel M, Poulsen TD, Hansen MB, et al. Somatostatin attenuates the hyperthermia induced increase in neutrophil concentration. *Eur J Appl Physiol* 1998; 77: 149-56
 22. Woods J, Lu Q, Ceddia MA, et al. Special feature for the Olympics: effects of exercise on the immune system -exercise-induced modulation of macrophage function. *Immunol Cell Biol* 2000; 78: 545-53
 23. Madden KS, Felten DL. Experimental basis for neural-immune interactions. *Physiol Rev* 1995; 75: 77-106
 24. McCarthy DA, Dale MM. The leucocytosis of exercise: a review and model. *Sports Med* 1988; 6: 333-63
 25. Tonnesen E, Christensen NJ, Brinklov MM. Natural killer cell activity during cortisol and adrenaline infusion in healthy volunteers. *Eur J Clin Invest* 1987; 17: 497-503
 26. Elenkov IJ, Chrousos GP. Stress hormones, Th1/Th2 patterns, pro/anti-inflammatory cytokines and susceptibility to disease. *Trends Endocrinol Metab* 1999; 10: 359-68
 27. Breuninger LM, Dempsey WL, Uhl J, et al. Hydrocortisone regulation of interleukin-6 protein production by a purified population of human peripheral blood monocytes. *Clin Immunol Immunopathol* 1993; 69: 205-14
 28. Chrousos GP. The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. *N Engl J Med* 1995; 332: 1351-62
 29. Elenkov IJ, Papanicolaou DA, Wilder RL, et al. Modulatory effects of glucocorticoids and catecholamines on human interleukin-12 and interleukin-10 production: clinical implications. *Proc Assoc Am Physicians* 1996; 108: 374-81
 30. Waage A, Slupphaug G, Shalaby R. Glucocorticoids inhibit the production of IL6 from monocytes, endothelial cells and fibroblasts. *Eur J Immunol* 1990; 20: 2439-43
 31. Wu CY, Wang K, McDyer JF, et al. Prostaglandin E2 and dexamethasone inhibit IL-12 receptor expression and IL-12 responsiveness. *J Immunol* 1998; 161: 2723-30
 32. Morel PA, Oriss TB. Crossregulation between Th1 and Th2 cells. *Crit Rev Immunol* 1998; 18: 275-303
 33. Ramierz F, Fowell DJ, Puklavec M, et al. Glucocorticoids promote a TH2 cytokine response by CD4+ T cells in vitro. *J Immunol* 1996; 156: 2406-12
 34. Lindquist S. The heat-shock response. *Annu Rev Biochem* 1986; 55: 1151-91
 35. Ding XZ, Fernandez-Prada CM, Bhattacharjee AK, et al. Overexpression of hsp-70 inhibits bacterial lipopolysaccharide-induced production of cytokines in human monocyte-derived macrophages. *Cytokine* 2001; 16: 210-9
 36. Sun D, Chen D, Du B, et al. Heat shock response inhibits NF-kappaB activation and cytokine production in murine Kupffer cells. *J Surg Res* 2005; 129: 114-21
 37. Moseley PL. Exercise, stress, and the immune conversation. *Exerc Sport Sci Rev* 2000; 28: 128-32
 38. Asea A. Chaperone-induced signal transduction pathways. *Exerc Immunol Rev* 2003; 9: 25-33
 39. Vabulas RM, Ahmad-Nejad P, Ghose S, et al. HSP70 as endogenous stimulus of the toll/interleukin-1 receptor signal pathway. *J Biol Chem* 2002; 277: 15107-12
 40. Febbraio MA, Ott P, Nielsen HB, et al. Exercise induces hepatoplasmic release of heat shock protein 72 in humans. *J Physiol* 2002; 544: 957-62
 41. Fehrenbach E, Niess AM. Role of heat shock proteins in the exercise response. *Exerc Immunol Rev* 1999; 5: 57-77
 42. Lancaster GI, Moller K, Nielsen B, et al. Exercise induces the release of heat shock protein 72 from the human brain in vivo. *Cell Stress Chaperones* 2004; 9: 276-80
 43. Walsh RC, Koukoulas I, Garnham A, et al. Exercise increases serum Hsp72 in humans. *Cell Stress Chaperones* 2001; 6: 386-93
 44. Fleshner M, Campisi J, Johnson JD. Can exercise stress facilitate innate immunity? A functional role for stress-induced extracellular Hsp72. *Exerc Immunol Rev* 2003; 9: 6-24
 45. ((Author: please confirm second author's name))Fleshner M, Johnson JD. Endogenous extra-cellular heat shock protein 72: releasing signal(s) and function. *Int J Hyperthermia* 2005; 21: 457-71
 46. Niess AM, Dickhuth HH, Northoff H, et al. Free radicals and oxidative stress in exercise: immunological aspects. *Exerc Immunol Rev* 1999; 5: 22-56
 47. Bennett IL, Nicastrì A. Fever as a mechanism of resistance. *Bacteriol Rev* 1960; 24: 16-34
 48. Hanson DF. Fever, temperature, and the immune response. *Ann N Y Acad Sci* 1997; 813: 453-64
 49. Kluger MJ, Ringler DH, Anver MR. Fever and survival. *Science* 1975; 188: 166-8
 50. Callahan TE, Marins J, Welch WJ, et al. Heat shock attenuates oxidation and accelerates apoptosis in human neutrophils. *J Surg Res* 1999; 85: 317-22
 51. Fildes J, Fisher S, Sheaff CM, et al. Effects of short heat exposure on human red and white blood cells. *J Trauma* 1998; 45: 479-84
 52. Frohlich D, Wittmann S, Rothe G, et al. Mild hyperthermia down-regulates receptor-dependent neutrophil function. *Anesth Analg* 2004; 99: 284-92
 53. Roberts NJ, Steigbigel RT. Hyperthermia and human leukocyte functions: effects on response of lymphocytes to mitogen and antigen and bactericidal capacity of monocytes and neutrophils. *Infect Immun* 1977; 18: 673-9
 54. Grogan JB, Parks LC, Minaberry D. Polymorphonuclear leukocyte function in cancer patients treated with total body hyperthermia. *Cancer* 1980; 45: 2611-5
 55. Nakayama J, Nakao T, Mashino T, et al. Kinetics of immunological parameters in patients with malignant melanoma treated with hyperthermic isolated limb perfusion. *J Dermatol Sci* 1997; 15: 1-8
 56. Park MM, Hornback NB, Endres S, et al. The effect of whole body hyperthermia on the immune cell activity of cancer patients. *Lymphokine Res* 1990; 9: 213-23
 57. Katschinski DM, Wiedemann GJ, Longo W, et al. Whole body hyperthermia cytokine induction: a review, and unifying hypothesis for myeloprotection in the setting of cytotoxic therapy. *Cytokine Growth Factor Rev* 1999; 10: 93-7
 58. Robins HI, Cohen JD, Schmitt CL, et al. Phase I clinical trial of carboplatin and 41.8 degrees C whole-body hyperthermia in cancer patients. *J Clin Oncol* 1993; 11: 1787-94
 59. Robins HI, Longo WL, Lagoni RK, et al. Phase I trial of lonidamine with whole body hyperthermia in advanced cancer. *Cancer Res* 1988; 48: 6587-92
 60. Robins HI, Dennis WH, Steeves RA, et al. A proposal for the addition of hyperthermia to treatment regimens for acute and chronic leukemia. *J Clin Oncol* 1984; 2: 1050-6
 61. Ernst E. Sauna: a hobby or for health? *J R Soc Med* 1989; 82: 639
 62. Ernst E, Pecho E, Wirz P, et al. Regular sauna bathing and the incidence of common colds. *Ann Med* 1990; 22: 225-7

63. Cross MC, Radomski MW, VanHelder WP, et al. Endurance exercise with and without a thermal clamp: effects on leukocytes and leukocyte subsets. *J Appl Physiol* 1996; 81: 822-9
64. Rhind SG, Gannon GA, Shek PN, et al. Contribution of exertional hyperthermia to sympathoadrenal-mediated lymphocyte subset redistribution. *J Appl Physiol* 1999; 87: 1178-85
65. Rhind SG, Gannon GA, Shephard RJ, et al. Cytokine induction during exertional hyperthermia is abolished by core temperature clamping: neuroendocrine regulatory mechanisms. *Int J Hyperthermia* 2004; 20: 503-16
66. Pugh LG, Corbett JL, Johnson RH. Rectal temperatures, weight losses, and sweat rates in marathon running. *J Appl Physiol* 1967; 23: 347-52
67. Roberts WO. Exercise-associated collapse in endurance events: a classification system. *Physician Sportsmed* 1989; 17: 49-55
68. Shephard RJ, Shek PN. Immune dysfunction as a factor in heat illness. *Crit Rev Immunol* 1999; 19: 285-302
69. Lim CL, Mackinnon LT. The roles of exercise-induced immune system disturbances in the pathology of heat stroke: the dual pathway model of heat stroke. *Sports Med* 2006; 36: 39-64
70. Cohen P, Warren SL. A study of the leukocytosis produced in man by artificial fever. *J Clin Invest* 1935; 14: 423-33
71. Downing JF, Taylor MW. The effect of in vivo hyperthermia on selected lymphokines in man. *Lymphokine Res* 1987; 6: 103-9
72. Downing JF, Martinez-Valdez H, Elizondo RS, et al. Hyperthermia in humans enhances interferon-gamma synthesis and alters the peripheral lymphocyte population. *J Interferon Res* 1988; 8: 143-50
73. Severs Y, Brenner I, Shek PN, et al. Effects of heat and intermittent exercise on leukocyte and sub-population cell counts. *Eur J Appl Physiol* 1996; 74: 234-45
74. Kappel M, Staderager C, Tvede N, et al. Effects of in vivo hyperthermia on natural killer cell activity, in vitro proliferative responses and blood mononuclear cell subpopulations. *Clin Exp Immunol* 1991; 84: 175-80
75. Zanker KS, Lange J. Whole body hyperthermia and natural killer cell activity. *Lancet* 1982; i: 1079-80
76. Dahn MS, Whitcomb MP, Lange MP, et al. Altered T-lymphocyte subsets in severe sepsis. *Am Surg* 1988; 54: 450-5
77. Mackinnon LT. Advances in exercise immunology. Champaign (IL): Human Kinetics, 1999
78. Bouchama A, al Hussein K, Adra C, et al. Distribution of peripheral blood leukocytes in acute heatstroke. *J Appl Physiol* 1992; 73: 405-9
79. Ellis GS, Carlson DE, Hester L, et al. G-CSF, but not corticosterone, mediates circulating neutrophilia induced by febrile-range hyperthermia. *J Appl Physiol* 2005; 98: 1799-804
80. Ryan AJ, Flanagan SW, Moseley PL, et al. Acute heat stress protects rats against endotoxin shock. *J Appl Physiol* 1992; 73: 1517-22
81. DuBose DA, McCreary J, Sowders L, et al. Relationship between rat heat stress mortality and alterations in reticuloendothelial carbon clearance function. *Aviat Space Environ Med* 1983; 54: 1090-5
82. Jiang Q, Cross AS, Singh IS, et al. Febrile core temperature is essential for optimal host defense in bacterial peritonitis. *Infect Immun* 2000; 68: 1265-70
83. Marino MW, Dunn A, Grail D, et al. Characterization of tumor necrosis factor-deficient mice. *Proc Natl Acad Sci U S A* 1997; 94: 8093-8
84. Bouchama A, Knochel JP. Heat stroke. *N Engl J Med* 2002; 346: 1978-88
85. Hasday JD, Singh IS. Fever and the heat shock response: distinct, partially overlapping processes. *Cell Stress Chaperones* 2000; 5: 471-80
86. d'Oleire F, Schmitt CL, Robins HI, et al. Cytokine induction in humans by 41.8 degrees C whole-body hyperthermia. *J Natl Cancer Inst* 1993; 85: 833-4
87. Robins HI, Kutz M, Wiedemann GJ, et al. Cytokine induction by 41.8 degrees C whole body hyperthermia. *Cancer Lett* 1995; 97: 195-201
88. Demetri GD, Griffin JD. Granulocyte colony-stimulating factor and its receptor. *Blood* 1991; 78: 2791-808
89. Robins HI, Rushing D, Kutz M, et al. Phase I clinical trial of melphalan and 41.8 degrees C whole-body hyperthermia in cancer patients. *J Clin Oncol* 1997; 15: 158-64
90. Nahas GG, Tannieres ML, Lennon JF. Direct measurement of leukocyte motility: effects of pH and temperature. *Proc Soc Exp Biol Med* 1971; 138: 350-2
91. Kappel M, Kharazmi A, Nielsen H, et al. Modulation of the counts and functions of neutrophils and monocytes under in vivo hyperthermia conditions. *Int J Hyperthermia* 1994; 10: 165-73
92. Bozzetti F, Cozzaglio L, Villa ML, et al. Restorative effect of total parenteral nutrition on natural killer cell activity in malnourished cancer patients. *Eur J Cancer* 1995; 31A: 2023-7
93. Puente J, Carvajal T, Parra S, et al. In vitro studies of natural killer cell activity in septic shock patients: response to a challenge with alpha-interferon and interleukin-2. *Int J Clin Pharmacol Ther Toxicol* 1993; 31: 271-5
94. Simms HH, Gaither TA, Fries LF, et al. Monokines released during short-term Fc gamma receptor phagocytosis up-regulate polymorphonuclear leukocytes and monocyte-phagocytic function. *J Immunol* 1991; 147: 265-72
95. Onozaki K, Matsushima K, Kleiner ES, et al. Role of interleukin 1 in promoting human monocyte-mediated tumor cytotoxicity. *J Immunol* 1985; 135: 314-20
96. Philip R. Cytotoxicity of tumor necrosis factor (TNF)-resistant tumor targets: differential cytotoxicity of monocytes activated by the interferons, IL-2, and TNF. *J Immunol* 1988; 140: 1345-9
97. Northoff H, Berg A, Weinstock C. Similarities and differences of the immune response to exercise and trauma: the IFN-gamma concept. *Can J Physiol Pharmacol* 1998; 76: 497-504
98. Ghussen F, Kruger I, Groth W, et al. The role of regional hyperthermic cytostatic perfusion in the treatment of extremity melanoma. *Cancer* 1988; 61: 654-9
99. Skene AI, Bulman AS, Williams TR, et al. Hyperthermic isolated perfusion with melphalan in the treatment of advanced malignant melanoma of the lower limb. *Br J Surg* 1990; 77: 765-7
100. De Maeyer E, Maeyer-Guignard J. Immuno-modulating properties of interferons. *Philos Trans R Soc Lond B Biol Sci* 1982; 299: 77-90
101. Hanson DF, Murphy PA, Silicano R, et al. The effect of temperature on the activation of thymocytes by interleukins I and II. *J Immunol* 1983; 130: 216-21
102. Fairchild KD, Viscardi RM, Hester L, et al. Effects of hypothermia and hyperthermia on cytokine production by cultured human mononuclear phagocytes from adults and newborns. *J Interferon Cytokine Res* 2000; 20: 1049-55
103. Febbraio MA. Alterations in energy metabolism during exercise and heat stress. *Sports Med* 2001; 31: 47-59

104. Galbo H, Houston ME, Christensen NJ, et al. The effect of water temperature on the hormonal response to prolonged swimming. *Acta Physiol Scand* 1979; 105: 326-37
105. Galloway SD, Maughan RJ. Effects of ambient temperature on the capacity to perform prolonged cycle exercise in man. *Med Sci Sports Exerc* 1997; 29: 1240-9
106. Malm C. Exercise immunology: the current state of man and mouse. *Sports Med* 2004; 34: 555-66
107. Nieman DC. Current perspective on exercise immunology. *Curr Sports Med Rep* 2003; 2: 239-42
108. Mitchell JB, Dugas JP, McFarlin BK, et al. Effect of exercise, heat stress, and hydration on immune cell number and function. *Med Sci Sports Exerc* 2002; 34: 1941-50
109. Niess AM, Fehrenbach E, Lehmann R, et al. Impact of elevated ambient temperatures on the acute immune response to intensive endurance exercise. *Eur J Appl Physiol* 2003; 89: 344-51
110. Laing S, Blackwell J, Gwynne D, et al. Neutrophil degranulation response to 2h of exercise in a 30°C environment. *Aviat Space Environ Med* 2005; 76: 1068-73
111. McFarlin BK, Mitchell JB. Exercise in hot and cold environments: differential effects on leukocyte number and NK cell activity. *Aviat Space Environ Med* 2003; 74: 1231-6
112. Brenner IKM, Castellani JW, Gabaree C, et al. Immune changes in humans during cold exposure: effects of prior heating and exercise. *J Appl Physiol* 1999; 87: 699-710
113. Pedersen BK, Bruunsgaard H, Klokke M, et al. Exercise-induced immunomodulation: possible roles of neuroendocrine and metabolic factors. *Int J Sports Med* 1997; 18 Suppl. 1: S2-7
114. Yamada M, Suzuki K, Kudo S, et al. Raised plasma G-CSF and IL-6 after exercise may play a role in neutrophil mobilization into the circulation. *J Appl Physiol* 2002; 92: 1789-94
115. Neville AJ, Sauder DN. Whole body hyperthermia (41-42 degrees C) induces interleukin-1 in vivo. *Lymphokine Res* 1988; 7: 201-6
116. Jiang Q, Detolla L, Singh IS, et al. Exposure to febrile temperature upregulates expression of pyrogenic cytokines in endotoxin-challenged mice. *Am J Physiol* 1999; 276: R1653-60
117. Kappel M, Diamant M, Hansen MB, et al. Effects of in vitro hyperthermia on the proliferative response of blood mononuclear cell subsets, and detection of interleukins 1 and 6, tumour necrosis factor-alpha and interferon-gamma. *Immunology* 1991; 73: 304-8
118. Pedersen BK. Special feature for the Olympics: effects of exercise on the immune system -exercise and cytokines. *Immunol Cell Biol* 2000; 78: 532-5
119. Cross A, Asher L, Seguin M, et al. The importance of a lipopolysaccharide-initiated, cytokine-mediated host defense mechanism in mice against extraintestinally invasive *Escherichia coli*. *J Clin Invest* 1995; 96: 676-86
120. Cross AS, Sadoff JC, Kelly N, et al. Pretreatment with recombinant murine tumor necrosis factor alpha/cachectin and murine interleukin 1 alpha protects mice from lethal bacterial infection. *J Exp Med* 1989; 169: 2021-7
121. Starkie RL, Hargreaves M, Rolland J, et al. Heat stress, cytokines, and the immune response to exercise. *Brain Behav Immun* 2005; 19: 404-12
122. Montain SJ, Latzka WA, Sawka MN. Impact of muscle injury and accompanying inflammatory response on thermoregulation during exercise in the heat. *J Appl Physiol* 2000; 89: 1123-30
123. Niess AM, Passek F, Lorenz I, et al. Expression of the antioxidant stress protein heme oxygenase-1 (HO-1) in human leukocytes. *Free Rad Bio Med* 1999; 26: 184-92
124. Wilckens T, De Rijk R. Glucocorticoids and immune function: unknown dimensions and new frontiers. *Immunol Today* 1997; 18: 418-24
125. Laing SJ, Walsh NP, Walters R, et al. The effects of prolonged exercise in a hot environment on lipopolysaccharide (LPS)-stimulated monocyte TNF- α release in trained male cyclists. *J Physiol* 2003; 555P: PC88
126. Febbraio MA, Steensberg A, Keller C, et al. Glucose ingestion attenuates interleukin-6 release from contracting skeletal muscle in humans. *J Physiol* 2003; 549: 607-12
127. Hiscock N, Chan MH, Bisucci T, et al. Skeletal myocytes are a source of interleukin-6 mRNA expression and protein release during contraction: evidence of fiber type specificity. *FASEB J* 2004; 18: 992-4
128. Steensberg A, Febbraio MA, Osada T, et al. Interleukin-6 production in contracting human skeletal muscle is influenced by pre-exercise muscle glycogen content. *J Physiol* 2001; 537: 633-9
129. Starkie RL, Hargreaves M, Lambert DL, et al. Effect of temperature on muscle metabolism during submaximal exercise in humans. *Exp Physiol* 1999; 84: 775-84
130. Febbraio MA, Snow RJ, Hargreaves M, et al. Muscle metabolism during exercise and heat stress in trained men: effect of acclimation. *J Appl Physiol* 1994; 76: 589-97
131. Pedersen BK, Febbraio M. Muscle-derived interleukin-6: a possible link between skeletal muscle, adipose tissue, liver, and brain. *Brain Behav Immun* 2005; 19: 371-6
132. Febbraio MA, Steensberg A, Starkie RL, et al. Skeletal muscle interleukin-6 and tumor necrosis factor-alpha release in healthy subjects and patients with type 2 diabetes at rest and during exercise. *Metabolism* 2003; 52: 939-44
133. Laing SJ, Gwynne D, Blackwell J, et al. Salivary IgA response to prolonged exercise in a hot environment in trained cyclists. *Eur J Appl Physiol* 2005; 93: 665-71
134. Muldoon S, Deuster P, Brandom B, et al. Is there a link between malignant hyperthermia and exertional heat illness? *Exerc Sport Sci Rev* 2004; 32: 174-9
135. Bouchama A, Parhar RS, el Yazigi A, et al. Endotoxemia and release of tumor necrosis factor and interleukin 1 alpha in acute heatstroke. *J Appl Physiol* 1991; 70: 2640-4
136. Hammami MM, Bouchama A, al Sedairy S, et al. Concentrations of soluble tumor necrosis factor and interleukin-6 receptors in heatstroke and heatstress. *Crit Care Med* 1997; 25: 1314-9
137. Moseley PL, Gapen C, Wallen ES, et al. Thermal stress induces epithelial permeability. *Am J Physiol* 1994; 267: C425-34
138. Hall DM, Buettner GR, Matthes RD, et al. Hyperthermia stimulates nitric oxide formation: electron paramagnetic resonance detection of NO-heme in blood. *J Appl Physiol* 1994; 77: 548-53
139. Lambert GP. Role of gastrointestinal permeability in exertional heatstroke. *Exerc Sport Sci Rev* 2004; 32: 185-90
140. Gathiram P, Wells MT, Brock-Utne JG, et al. Prevention of endotoxaemia by non-absorbable antibiotics in heat stress. *J Clin Pathol* 1987; 40: 1364-8
141. Chiu WT, Kao TY, Lin MT. Increased survival in experimental rat heatstroke by continuous perfusion of interleukin-1 receptor antagonist. *Neurosci Res* 1996; 24: 159-63
142. Baker B, Gaffin SL, Wells M, et al. Endotoxaemia in racehorses following exertion. *J S Afr Vet Assoc* 1988; 59: 63-6

143. Bosenberg AT, Brock-Utne JG, Gaffin SL, et al. Strenuous exercise causes systemic endotoxemia. *J Appl Physiol* 1988; 65: 106-8
144. Brock-Utne JG, Gaffin SL, Wells MT, et al. Endotoxaemia in exhausted runners after a long-distance race. *S Afr Med J* 1988; 73: 533-6
145. Jeukendrup AE, Vet-Joop K, Sturk A, et al. Relationship between gastro-intestinal complaints and endotoxaemia, cytokine release and the acute-phase reaction during and after a long-distance triathlon in highly trained men. *Clin Sci* 2000; 98: 47-55
146. Marshall JC, Christou NV, Meakins JL. Immunomodulation by altered gastrointestinal tract flora: the effects of orally administered, killed *Staphylococcus epidermidis*, *Candida*, and *Pseudomonas* on systemic immune responses. *Arch Surg* 1988; 123: 1465-9
147. Ducreux S, Zorzato F, Muller C, et al. Effect of ryanodine receptor mutations on interleukin-6 release and intracellular calcium homeostasis in human myotubes from malignant hyperthermia-susceptible individuals and patients affected by central core disease. *J Biol Chem* 2004; 279: 43838-46
148. Hammami MM, Bouchama A, Shail E, et al. Lymphocyte subsets and adhesion molecules expression in heatstroke and heat stress. *J Appl Physiol* 1998; 84: 1615-21
149. DuBose DA, Wenger CB, Flinn SD, et al. Distribution and mitogen response of peripheral blood lymphocytes following exertional heat injury. *J Appl Physiol* 2003; 95: 2381-9
150. Castellani JW, IK MB, Rhind SG. Cold exposure: human immune responses and intracellular cytokine expression. *Med Sci Sports Exerc* 2002; 34: 2013-20
151. Shephard RJ, Shek PN. Cold exposure and immune function. *Can J Physiol Pharmacol* 1998; 76: 828-36
152. Ritzel G. Critical evaluation of vitamin C as a prophylactic and therapeutic agent in colds. *Helv Med Acta* 1961; 28: 63-8
153. Sabiston BH, Livingstone SD. Investigation of health problems related to Canadian northern military operations. Toronto: Defence and Civil Institute of Environmental Medicine, 1973
154. Armstrong LE. Performing in extreme environments. Champaign (IL): Human Kinetics, 2000
155. Jansky L, Pospisilova D, Honzova S, et al. Immune system of cold-exposed and cold-adapted humans. *Eur J Appl Physiol* 1996; 72: 445-50
156. Wenisch C, Narzt E, Sessler DI, et al. Mild intraoperative hypothermia reduces production of reactive oxygen intermediates by polymorphonuclear leukocytes. *Anesth Analg* 1996; 82: 810-6
157. Beilin B, Shavit Y, Razumovsky J, et al. Effects of mild peri-operative hypothermia on cellular immune responses. *Anesthesiology* 1998; 89: 1133-40
158. Lackovic V, Borecky L, Vigas M, et al. Activation of NK cells in subjects exposed to mild hyper- or hypothermic load. *J Interferon Res* 1988; 8: 393-402
159. Doubt TJ. Physiology of exercise in the cold. *Sports Med* 1991; 11: 367-81
160. Timmons BA, Araujo J, Thomas TR. Fat utilization enhanced by exercise in a cold environment. *Med Sci Sports Exerc* 1985; 17: 673-8
161. Pitsiladis YP, Maughan RJ. The effects of exercise and diet manipulation on the capacity to perform prolonged exercise in the heat and in the cold in trained humans. *J Physiol* 1999; 517: 919-30
162. Housh TJ, Johnson GO, Housh DJ, et al. The effect of exercise at various temperatures on salivary levels of immunoglobulin A. *Int J Sports Med* 1991; 12: 498-500
163. Walsh NP, Blannin AK, Clark AM, et al. The effects of high-intensity intermittent exercise on saliva IgA, total protein and alpha-amylase. *J Sports Sci* 1999; 17: 129-34
164. Walsh NP, Bishop NC, Blackwell J, et al. Salivary IgA response to prolonged exercise in a cold environment in trained cyclists. *Med Sci Sports Exerc* 2002; 34: 1632-7
165. Francis JL, Gleeson M, Lugg DJ, et al. Trends in mucosal immunity in Antarctica during six Australian winter expeditions. *Immunol Cell Biol* 2002; 80: 382-90
166. Gleeson M, Francis JL, Lugg DJ, et al. One year in Antarctica: mucosal immunity at three Australian stations. *Immunol Cell Biol* 2000; 78: 616-22
167. Bosch JA, Ring C, de Geus EJ, et al. Stress and secretory immunity. *Int Rev Neurobiol* 2002; 52: 213-53
168. Stone AA, Cox DS, Valdimarsdottir H, et al. Evidence that secretory IgA antibody is associated with daily mood. *J Pers Soc Psychol* 1987; 52: 988-93
169. Williams DL, Climie A, Muller HK, et al. Cell-mediated immunity in healthy adults in Antarctica and the sub-Antarctic. *J Clin Lab Immunol* 1986; 20: 43-9
170. Muller HK, Lugg DJ, Quinn D. Cell mediated immunity in Antarctic wintering personnel; 1984-1992. *Immunol Cell Biol* 1995; 73: 316-20
171. Gard S. Respiratory virus infections other than influenza. *Arch Environ Health* 1968; 17: 543-6
172. Shephard RJ, Castellani JW, Shek PN. Immune deficits induced by strenuous exertion under adverse environmental conditions: manifestations and countermeasures. *Crit Rev Immunol* 1998; 18: 545-68
173. Clothier JG. Medical aspects of work in Arctic areas. *Practitioner* 1974; 213: 805-11
174. Edwards S, Hucklebridge F, Clow A, et al. Components of the diurnal cortisol cycle in relation to upper respiratory symptoms and perceived stress. *Psych Med* 2003; 65: 320-7
175. Giesbrecht GG. The respiratory system in a cold environment. *Aviat Space Environ Med* 1995; 66: 890-902
176. Halkier-Sorensen L, Menon GK, Elias PM, et al. Cutaneous barrier function after cold exposure in hairless mice: a model to demonstrate how cold interferes with barrier homeostasis among workers in the fish-processing industry. *Br J Dermatol* 1995; 132: 391-401
177. Bailey DM, Davies B, Castell LM, et al. Symptoms of infection and acute mountain sickness; associated metabolic sequelae and problems in differential diagnosis. *High Alt Med Biol* 2003; 4: 319-31
178. Singh I, Chohan IS, Lal M, et al. Effects of high altitude stay on the incidence of common diseases in man. *Int J Biometeorol* 1977; 21: 93-122
179. Basnyat B, Cumbo TA, Edelman R. Infections at high altitude. *Clin Infect Dis* 2001; 33: 1887-91
180. Meehan RT. Immune suppression at high altitude. *Ann Emerg Med* 1987; 16: 974-9
181. Ehrlich R, Mieszkuc BJ. Effects of space cabin environment on resistance to infection. I. Effect of 18,000-foot altitude on resistance to respiratory infection. *J Infect Dis* 1962; 110: 278-81
182. Highman B, Altland PD. A new method for the production of experimental bacterial endocarditis. *Proc Soc Exp Biol Med* 1950; 75: 573-7

183. Meehan R, Duncan U, Neale L, et al. Operation Everest II: alterations in the immune system at high altitudes. *J Clin Immunol* 1988; 8: 397-406
184. Facco M, Zilli C, Siviero M, et al. Modulation of immune response by the acute and chronic exposure to high altitude. *Med Sci Sports Exerc* 2005; 37: 768-74
185. Pyne DV, McDonald WA, Morton DS, et al. Inhibition of interferon, cytokine, and lymphocyte proliferative responses in elite swimmers with altitude exposure. *J Interferon Cytokine Res* 2000; 20: 411-8
186. Biselli R, Le Moli S, Matricardi PM, et al. The effects of hypobaric hypoxia on specific B cell responses following immunization in mice and humans. *Aviat Space Environ Med* 1991; 62: 870-4
187. Lancaster GI, Khan Q, Drysdale PT, et al. Effect of prolonged exercise and carbohydrate ingestion on type 1 and type 2 T lymphocyte distribution and intracellular cytokine production in humans. *J Appl Physiol* 2004; 98: 565-71
188. Steensberg A, Toft AD, Bruunsgaard H, et al. Strenuous exercise decreases the percentage of type 1 T cells in the circulation. *J Appl Physiol* 2001; 91: 1708-12
189. Kleessen B, Schroedl W, Stueck M, et al. Microbial and immunological responses relative to high-altitude exposure in mountaineers. *Med Sci Sports Exerc* 2005; 37: 1313-8
190. Bailey DM, Davies B. Physiological implications of altitude training for endurance performance at sea level: a review. *Br J Sports Med* 1997; 31: 183-90
191. Mazzeo RS. Altitude, exercise and immune function. *Exerc Immunol Rev* 2005; 11: 6-16
192. Pedersen BK, Steensberg A. Exercise and hypoxia: effects on leukocytes and interleukin-6-shared mechanisms? *Med Sci Sports Exerc* 2002; 34: 2004-13
193. Klokner M, Kjaer M, Secher NH, et al. Natural killer cell response to exercise in humans: effect of hypoxia and epidural anesthesia. *J Appl Physiol* 1995; 78: 709-16
194. Chouker A, Demetz F, Martignoni A, et al. Strenuous physical exercise inhibits granulocyte activation induced by high altitude. *J Appl Physiol* 2005; 98: 640-7
195. Levine BD, Stray-Gundersen J. 'Living high-training low': effect of moderate-altitude acclimatization with low-altitude training on performance. *J Appl Physiol* 1997; 83: 102-12
196. Tiollier E, Schmitt L, Burnat P, et al. Living high-training low altitude training: effects on mucosal immunity. *Eur J Appl Physiol* 2005; 94: 298-304
197. Fox PC, van der Ven PF, Sonies BC, et al. Xerostomia: evaluation of a symptom with increasing significance. *J Am Dent Assoc* 1985; 110: 519-25
198. Gleeson M, McDonald WA, Pyne DB, et al. Salivary IgA levels and infection risk in elite swimmers. *Med Sci Sports Exerc* 1999; 31: 67-73
199. Hanson LA, Bjorkander J, Qxelius VA. Selective IgA deficiency. In: Chandra RK, editor. Primary and secondary immunodeficiency disorders. Edinburgh: Churchill Livingstone, 1983: 62-4
200. Isaacs D, Webster AD, Valman HB. Immunoglobulin levels and function in pre-school children with recurrent respiratory infections. *Clin Exp Immunol* 1984; 58: 335-40
201. Jauchem JR. Environmental stressors during space flight: potential effects on body temperature. *Comp Biochem Physiol A* 1988; 91: 425-9
202. Dinges DF. Sleep in space flight: breath easy-sleep less? *Am J Respir Crit Care Med* 2001; 164: 337-8
203. Mallis MM, DeRoshia CW. Circadian rhythms, sleep, and performance in space. *Aviat Space Environ Med* 2005; 76: B94-107
204. Drummer C, Hesse C, Baisch F, et al. Water and sodium balances and their relation to body mass changes in microgravity. *Eur J Clin Invest* 2000; 30: 1066-75
205. Smith SM, Davis-Street JE, Rice BL, et al. Nutritional status assessment in semiclosed environments: ground-based and space flight studies in humans. *J Nutr* 2001; 131: 2053-61
206. Endler NS. The joint effects of person and situation factors on stress in spaceflight. *Aviat Space Environ Med* 2004; 75: C22-7
207. Durnova GN, Kaplansky AS, Portugalov VV. Effect of a 22-day space flight on the lymphoid organs of rats. *Aviat Space Environ Med* 1976; 47: 588-91
208. Sonnenfeld G. The immune system in space and microgravity. *Med Sci Sports Exerc* 2002; 34: 2021-7
209. Taylor GR. Overview of spaceflight immunology studies. *J Leukoc Biol* 1993; 54: 179-88
210. Taylor GR. Immune changes during short-duration missions. *J Leukoc Biol* 1993; 54: 202-8
211. Taylor GR, Dardano JR. Human cellular immune responsiveness following space flight. *Aviat Space Environ Med* 1983; 54: S55-9
212. Taylor GR, Neale LS, Dardano JR. Immunological analyses of U.S. Space Shuttle crewmembers. *Aviat Space Environ Med* 1986; 57: 213-7
213. Barger LK, Greenleaf JE, Baldini F, et al. Effects of space missions on the human immune system: a meta-analysis. *Sports Med Training Rehab* 1995; 5: 293-310
214. Borchers AT, Keen CL, Gershwin ME. Microgravity and immune responsiveness: implications for space travel. *Nutrition* 2002; 18: 889-98
215. Lange RD, Andrews RB, Gibson LA, et al. Hematological measurements in rats flown on Spacelab shuttle, SL-3. *Am J Physiol* 1987; 252: R216-21
216. Sams CF, Crucian BE, Clift VL, et al. Development of a whole blood staining device for use during space shuttle flights. *Cytometry* 1999; 37: 74-80
217. Macho L, Kvetnansky R, Fickova M, et al. Endocrine responses to space flights. *J Gravit Physiol* 2001; 8: 117-20
218. Tipton CM, Greenleaf JE, Jackson CG. Neuroendocrine and immune system responses with spaceflights. *Med Sci Sports Exerc* 1996; 28: 988-98
219. Cogoli A. Space flight and the immune system. *Vaccine* 1993; 11: 496-503
220. Konstantinova IV. Immune resistance of man in space flights. *Acta Astronaut* 1991; 23: 123-7
221. Chapes SK, Morrison DR, Guikema JA, et al. Production and action of cytokines in space. *Adv Space Res* 1994; 14: 5-9
222. Manie S, Konstantinova I, Breittmayer JP, et al. Effects of long duration spaceflight on human T lymphocyte and monocyte activity. *Aviat Space Environ Med* 1991; 62: 1153-8
223. Talas M, Batkai L, Stoger I, et al. Results of space experiment program 'Interferon'. I. Production of interferon in vitro by human lymphocytes aboard space laboratory Solyut-6 ('Interferon I') and influence of space flight on lymphocyte functions of cosmonauts ('Interferon III'). *Acta Microbiol Hung* 1983; 30: 53-61
224. Gould CL, Lyte M, Williams J, et al. Inhibited interferon-gamma but normal interleukin-3 production from rats flown on the space shuttle. *Aviat Space Environ Med* 1987; 58: 983-6

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225. Konstantinova IV, Rykova MP, Lesnyak AT, et al. Immune changes during long-duration missions. *J Leukoc Biol* 1993; 54: 189-201
226. Meshkov D, Rykova M. The natural cytotoxicity in cosmonauts on board space stations. *Acta Astronaut* 1995; 36: 719-26
227. Taylor GR, Janney RP. In vivo testing confirms a blunting of the human cell-mediated immune mechanism during space flight. *J Leukoc Biol* 1992; 51: 129-32
228. Gmunder FK, Konstantinova I, Cogoli A, et al. Cellular immunity in cosmonauts during long duration spaceflight on board the orbital MIR station. *Aviat Space Environ Med* 1994; 65: 419-23
229. Sonnenfeld G. Extreme environments and the immune system: effects of spaceflight on immune responses. *J Allergy Clin Immunol* 2001; 107: 19-20
230. Castellani JW. Symposium: immune function in environmental extremes -an introduction. *Med Sci Sports Exerc* 2002; 34: 2002-3
231. Nickerson CA, Ott CM, Mister SJ, et al. Microgravity as a novel environmental signal affecting *Salmonella enterica* serovar typhimurium virulence. *Infect Immun* 2000; 68: 3147-52
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