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Biomarkers for warfighter safety and performance in hot and cold environments

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ABSTRACT

Exposure to extreme environmental heat or cold during military activities can impose severe thermal strain, leading to impairments in task performance and increasing the risk of exertional heat (including heat stroke) and cold injuries that can be life-threatening. Substantial individual variability in physiological tolerance to thermal stress necessitates an individualized approach to mitigate the deleterious effects of thermal stress, such as physiological monitoring of individual thermal strain. During heat exposure, measurements of deep-body (T_c) and skin temperatures and heart rate can provide some indication of thermal strain. Combining these physiological variables with biomechanical markers of gait (in)stability may provide further insight on central nervous system dysfunction – the key criterion of exertional heat stroke (EHS). Thermal strain in cold environments can be monitored with skin temperature (peripheral and proximal), shivering thermogenesis and T_c. Non-invasive methods for real-time estimation of T_c have been developed and some appear to be promising but require further validation. Decision-support tools provide useful information for planning activities and biomarkers can be used to improve their predictions, thus maximizing safety and performance during hot- and cold-weather operations. With better understanding on the etiology and pathophysiology of EHS, the microbiome and markers of the inflammatory responses have been identified as novel biomarkers of heat intolerance. This review aims to (i) discuss selected physiological and biomechanical markers of heat or cold strain, (ii) how biomarkers may be used to ensure operational readiness in hot and cold environments, and (iii) present novel molecular biomarkers (e.g., microbiome, inflammatory cytokines) for preventing EHS.

Practical implications

- An individualized approach (i.e. real-time physiological monitoring) is needed to effectively minimize the health and performance detriments of heat and cold exposure, thereby optimizing performance of the weakest link to maximize squad performance.
- Biomarkers coupled with artificial intelligence (AI) can be used to make informed decisions during pre-activity planning and be monitored in real-time to detect potential casualties during training and operations.
- Continued technological advances (e.g., textile-integrated sensors, low-power communication methods) will further augment warfighters' effectiveness in hot and cold environments.

1. Introduction

Warfighters train and operate in harsh thermal environments of heat and cold that can negatively impact performance, health and safety, and ultimately the mission. Excessive heat or cold stress arising from weather conditions (e.g., high or low ambient temperatures, wind speed, humidity, solar radiation) and mission-related factors (e.g., physical work intensity, exposure duration, clothing/equipment) can impose significant thermal strain that diminishes work capacity, impairs task performance and increases the risk of exertional heat

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stroke (EHS) and cold injuries (e.g., frostbite, non-freezing cold injuries, hypothermia) that can cause morbidity or even death.[1,2] Such injuries that compromise individual health will ultimately also impose a reduction in combat effectiveness and military readiness. Although several heat- and cold-risk mitigation strategies, guidelines and policies have been implemented, EHS[3] and cold injuries[4] remain a significant challenge to the military. With the rising intensity and frequency of extreme weather events (e.g., heat waves, cold spells) due to climate change,[5] warfighters will face increasingly higher levels of thermal stress and risk of injury.

Physiological tolerance to thermal stress varies markedly between and within individuals due to a variety of factors (e.g., age, body mass, physique and composition, aerobic capacity, illness, medication, nutritional and hydration status)[6,7] such that under the same conditions, less tolerant individuals may experience greater thermal strain, performance losses and/or negative health outcomes compared to their more tolerant counterparts. This variability emphasizes the need for an individualized risk-management approach. One potential strategy is individual physiological monitoring,[8,9] and when combined with artificial intelligence (AI), will enable early identification of individuals with excessive thermal strain or signs of impending injury so that countermeasures (e.g., reducing work intensity, finding shelter) can be applied quickly to prevent further injury progression. Physiological monitoring can also guide self-regulation of work rate, redistribution of mission workloads among unit members and allow better management of training activities, leading to improved performance, training and mission outcomes.[9,10]

Thus, we aimed to review (i) selected physiological and biomechanical markers of heat or cold strain, (ii) how biomarkers may be used to ensure operational readiness in hot and cold environments, and (iii) present microbiome and proteins as novel molecular biomarkers for preventing EHS.

2. Biomarkers of thermal strain

2.1. Hot environments

2.1.1. Physiological biomarkers

The combination of physical exertion, environmental heat exposure, impermeable clothing and carried loads increase whole-body physiological strain, which is commonly (albeit incompletely) characterized by elevations in deep-body temperature (T_c), skin temperature (T_{sk}) and heart rate. Measuring these variables simultaneously and integrating them into a composite heat-strain index can provide a general assessment of heat strain.[8,9] An example is the Physiological Strain Index (PSI) that can be calculated from T_c and heart rate only,[11] or with T_{sk} as well (i.e. adaptive PSI).[9] A modified PSI that uses a higher upper limit of T_c and age-predicted maximal heart rate was also proposed for trained individuals.[12]

While heart rate and T_{sk} are easily obtained, a practical and accurate method for routine monitoring of T_c in field settings is currently unavailable. Valid measures of T_c are invasive (e.g., rectal and esophageal probes) or expensive (telemetric pills) and are mostly used for research purposes.[13] Recent work has thus focused on estimating T_c from various non-invasive physiological parameters (e.g., heart rate, T_{sk}, skin heat flux).[14,15] For example, an algorithm that uses only heart rate to estimate T_c in real time was presented previously.[16] Validation was performed using several independent datasets involving a range of exertional-heat stress conditions, achieving a root mean square error (RMSE) of 0.30 ± 0.13 °C (range: 0.19–0.44 °C) and 95% limits of agreement (LoA) of ±0.63 (range: ±0.40 to 0.71 °C). Although generally acceptable for predicting group responses, these errors are too large for effective individual monitoring, especially in high-risk environments. Assessing model performance using validation metrics based on *individual* instead of *group* prediction errors, such as the percentage of target attainment (PTA — percentage of prediction errors within a predetermined range),[17,18] would be more appropriate. More recently, Nazarin et al.[18] introduced an algorithm that estimates T_c using a combination of heart rate, T_{sk} and air temperature data obtained through a smartwatch and reported an impressive 95% PTA within ±0.27 °C; however, only 15 participants were included in the study.

Another promising method for non-invasive T_c estimation is technologies based on skin heat flux. A unique device based on this approach was developed and has been tested under various exertional-heat stress conditions in the laboratory.[19–21] The device was also adapted for use in firefighters by inserting it under their helmet.[19] As the device was intended for clinical use, a recent study constructed new T_c-estimation algorithms to improve its accuracy under exertional-heat stress, subsequently obtaining a PTA of 78% within ±0.3 °C when the device was placed on the forehead, whereas measurements from the wrist were less accurate (PTA: 64% within ±0.3 °C).[21] Previous studies also reported variable results between different measurement sites when using heat flux-based methods to estimate T_c.[22,23] Furthermore, the heat flux measures do not account for the influence of blood flow on heat flux from the underlying tissues, likely leading to errors in the estimates of T_c; this issue may be overcome by combining heat flux techniques with photoplethysmography to correct for tissue perfusion. Overall, the aforementioned techniques for estimating T_c non-invasively are promising, but they require more rigorous validation by independent laboratories at higher T_c values (≥38.5 °C), under real-world scenarios and across more diverse populations (e.g., women, middle-aged individuals) and environmental conditions (e.g., cold environments).[14]

Additional parameters that may help characterize heat strain and can be monitored with recently emerged wearables include respiratory rate and blood pressure.[24,25] Respiratory rate increases in response to exertional-heat stress, leading to cerebral hypoperfusion and potentially syncope.[26,27] Similarly, a progressive decline in blood pressure during exertional-heat stress is suggestive of cardiac insufficiency and often precedes collapse/syncope.[28,29] The magnitude of hyperthermia-induced hypotension was also positively associated with heat stroke severity in a rat model.[30] Furthermore, the use of cardiovascular reserve index – derived from blood pressure and heart rate – was suggested to differentiate between heat-tolerant and intolerant individuals[31]; it may also be used to identify (and thus prevent) excessive dehydration and over-hydration.[32–34] Nevertheless, the blood-pressure and respiratory-rate signals obtained from the novel wearables require further validation.

2.1.2. Biomechanical markers

EHS is the most severe manifestation of exertional heat injuries and is a life-threatening illness.[1] The key diagnostic criterion for EHS is central nervous system dysfunction which often presents as ataxia or wobbly gait.[35] This suggests that a decrease in gait stability during exertional-heat stress may indicate central nervous system dysfunction associated with EHS. Gait monitoring may therefore provide a means for early identification and prevention of EHS.

The effects of exertional-heat stress on gait characteristics have been investigated in a limited number of studies. One study observed an increase in the number of crossover steps (i.e. feet overlapping) during 90 min of loaded treadmill walking under moderate heat stress.[36] In another study, an increase in the variability of double-stance time was seen during more severe exertional-heat stress conditions (peak T_c = 38.9 °C).[37] However, these studies used gait analysis techniques (e.g., high-speed video cameras, force plates) that are expensive, require technical expertise and mostly restricted to laboratory environments.

The utility of chest-worn triaxial accelerometery for detecting gait instability, and subsequently predicting EHS, during a range of field-based military training activities was recently examined.[38] A measure of gait instability was developed based on changes in wobble magnitude and variability in movement patterns relative to the individual's baseline. In a post-hoc analysis of collected data, the gait instability score was able to identify all six EHS victims upon incapacitation with 92

(2.7%) false positives. Prediction accuracy improved (31 or 0.9% false positives) when gait instability was combined with heart rate-based estimation of T_c to derive an overall EHS-risk score, underscoring the importance of a multivariable approach for assessing thermal strain. Given the importance of rapid cooling in prehospital care of EHS,[1] the authors further analyzed how much earlier EHS could be predicted with the combined measures of gait instability and estimated-T_c and reported a time window of 3.5 min with 209 (6.1%) false positives; however, this may be insufficient for reactionary measures. The false-negative rate of the predictions also needs to be determined. Future work can assess whether other gait parameters (e.g., plantar pressure distribution via pressure-sensing insoles) may enable earlier or more accurate predictions, or help elucidate mechanisms contributing to gait disturbances in EHS.

2.2. Cold environments

Compared to heat exposure, humans are extremely maladapted to cold environments. In the initial stages of cold exposure, decreases in T_{sk} stimulate peripheral vasoconstriction to reduce heat loss. The ensuing decrease in peripheral blood flow results in decreasing hand and foot temperatures, which are the first signs of cold stress. As cooling progresses and the rate of heat loss increases, additional cold-protective mechanisms are activated to maintain T_c at ~37 °C. A decrease in T_c will occur when body heat storage decreases as heat loss exceeds the rate of heat production. Consequently, the rate of heat loss is the main driving force in the cold-stress response and is determined by the environmental conditions and individual physiological and morphological characteristics.[39,40] In most daily conditions, peripheral vasoconstriction is adequate to remain in thermal balance or in a thermoneutral zone.[41] When cold stress becomes more severe and T_{sk} decreases further, metabolic cold effectors including non-shivering thermogenesis in brown adipose tissue and shivering thermogenesis in muscles are activated to stimulate heat production. Cold exposure is deemed *compensable* when increases in metabolic heat production are sufficient to fully compensate for increases in heat loss and can be sustained, and/or when heat loss can be stabilized or reduced through behavioral responses (e.g., using isolative clothing and/or shelter, acquiring an external heat source). To date, however, it is still unclear what could impede heat production, but it does not seem to be related to availability of metabolic fuels for sustaining shivering.[42] Even at the maximal reported heat production of 5 times the resting metabolic rate,[43,44] heat production can be generated using multiple fuel combinations even when glycogen availability is reduced.[45,46] However, the limited capacity to increase heat production also means that, depending on the severity of the environmental conditions, heat loss can quickly surpass heat production. Cold exposure subsequently becomes *uncompensable* where T_c inevitably decreases. The rate at which T_c decreases will be determined by the duration of cold exposure and the difference between heat production and heat loss. If left untreated, the progressive fall in T_c may result in loss of cognitive and metabolic functions, eventually leading to multiple organ and systems failure.[47] In this context, identifying biomarkers to detect the progression of cold strain is essential to prevent cold injuries, maintain performance and ensure survival in cold environments.

Recognizing the stages and severity of cold strain can be achieved by assessing three main biomarkers: T_{sk} (peripheral and proximal), shivering thermogenesis and T_c. In contrast to heat exposure, heart rate varies little in the cold and does not represent an adequate biomarker for determining cold strain. Changes in T_{sk} are determined using sensors placed at various key sites on the body. To allow comparison between groups and studies, cold experiments generally standardize the placement of thermal sensors based on Dubois and Dubois[48] to estimate average T_{sk}, which includes finger but not foot temperature. Due to the initial increase in peripheral vasoconstriction, both hands and feet would display the first reductions in T_{sk}. Cold injuries can develop even when individuals are in compensable cold conditions where heat production matches heat loss. Exposed skin is particularly vulnerable to freezing cold injuries which generally occurs when T_{sk} falls below −5 °C[49]. As cold exposure continues or intensifies, peripheral blood flow is further reduced leading to greater reductions in hand and foot temperatures. Shivering activity will also increase progressively and become extremely intense (15% of maximal voluntary contraction) during moderate cold exposure (>3.5 times resting metabolic rate).[50] Shivering thermogenesis can be assessed by placing electromyography and/or mechanomyography[51] sensors on the belly of specific muscles, typically on large muscles to account for the effects on the whole body as shivering mostly occurs in muscles located on the torso and upper leg, whereas arm muscles do not contribute significantly to overall shivering thermogenesis.[46,52] Expected shivering intensities range from 2 to 20% of maximal voluntary contractions.[46,51] Shivering is extremely debilitating and can affect motor skills.[53] Cold acclimation[54,55] and the ingestion of specific thermogenic compounds (e.g., capsaicin, fish oil)[56] have been shown to increase non-shivering thermogenesis and reduce shivering. During compensable cold exposure, measurements of both T_{sk} (peripheral and proximal) and shivering thermogenesis are generally sufficient to assess the severity of cold strain. However, in uncompensable cold conditions, close monitoring of T_c is essential as decreasing T_c results in a progressive loss of cognitive capacity, shivering capacity, and may lead to death. Also, as T_c decreases, more energy is dedicated to protect T_c at the expense of the peripheries, increasing the risk of peripheral cold injuries. Compared to cold-air exposure, cold-water immersion, especially at water temperatures <18 °C, can cause a rapid decrease in T_c and severe hypothermia if the individual remains in the water. In cold air, cooling rates are greatly reduced in comparison with cold-water immersion and thus, hypothermia should only occur accidentally, following unforeseen events or a series of inadequate decisions.

In addition to physiological parameters, dynamic balance may also be useful for assessing cold strain. Dynamic balance was shown to worsen during lower-limb cooling and may indicate degradation in neuromuscular function or risk of injury.[57] Moreover, commercial wearables (e.g., inertial measurement units, pressure-sensing insoles) are available for acquiring real-time balance data. Potential areas of future research include the development of non-invasive methods for measuring deep-muscle or joint temperatures, or characteristics of the synovial fluid, which may provide additional insight into cold-induced impairments in manual function and performance.[58]

3. Decision-support tools for hot and cold environments

Performance degradation or injury in adverse weather conditions may be prevented by accurate prediction and/or early diagnosis of at-risk individuals and providing mitigation measures.[59] That is, harsh environments require appropriate behavioral actions and use of protective clothing and equipment, otherwise *"performing a mission can quickly turn into fighting for survival.*[60]*"*

Physiological monitoring of thermal strain, along with the methods that are available for monitoring the specific biomarkers (Section 3), at least in research contexts, are relevant for predicting or diagnosing the onset of EHS or whole-body and peripheral cold injuries in real time. They support the decision to remove a person from an activity before a negative health outcome occurs; however, they can only be used *during* the activity itself. During the planning process *before* the activity, other tools (e.g., thermoregulatory models, indices) can be used to guide decision making by estimating safe exposure durations, selecting appropriate clothing and equipment, or predicting the potential risk of EHS or cold injuries (Table 1). Potter et al.[61] recently demonstrated the capability of such tools in predicting the risk of cold injuries in solders during a real-world situation.

The most commonly used military operational decision-support tools are environmental-based indices such as the windchill index

(WCI) or wet-bulb globe temperature (WBGT).[59,62] The WCI gives an indication of cold stress based on air temperature and wind and can inform the risk of freezing cold injury of an exposed cheek. The WBGT indicates the combined heat stress provided by humidity, air temperature and mean radiant temperature – and they are influenced by wind speed. These environmental indices are extremely practical in use and can be combined with charts to interpret the risk of EHS or cold injuries; however, they do not adequately consider individual characteristics that modify personal vulnerability to thermal stress (Table 1). For example, the military WBGT thresholds recommended by the North Atlantic Treaty Organization (NATO) can be adjusted for activity level and body armor or personal protective equipment, but not for heat-acclimatization status[59]. This contrasts with occupational standards and specific national military guidelines that have separate WBGT thresholds for heat-acclimatized and unacclimatized workers.[63] The WCI, on the other hand, does not account for individual factors.

More complex decision-support tools for thermal-strain assessments are based on models that include the physics of heat transfer to determine the thermal state of the human body and the resulting thermophysiological responses (e.g., sweating, skin blood flow); they therefore require detailed input on the operational context, including activity level, clothing and equipment, weather, terrain and individual characteristics.[64,65] Information on the operational context allows more accurate simulation of heat exchange between the body and environment as well as the thermophysiological responses. The outcomes of such simulations either show the steady-state equilibrium or the evolution of body heat content, T_c and T_{sk} over time. However, these contextual data are often highly variable (e.g., activity level) or not always available (e.g., clothing level), which can lead to prediction errors. Although certain thermoregulatory models incorporate predictive biomarkers (e.g., body composition and size, aerobic fitness, age) to simulate the changes in T_c, none of the available decision-support tools, to our knowledge, predict the effects of these biomarkers on performance or health outcomes for a given thermal state. An example is the potential influence of hand and finger morphology on the impairment in manual dexterity for a given reduction in extremity temperature,[66] or the impact of aerobic fitness on cardiovascular strain and risk of EHS for a given magnitude of hyperthermia.[67] The potential influence of motivation – an important risk factor for injuries among military populations – should also be considered in the thermoregulatory simulations.[68] Nonetheless, further research is needed to quantify the impact of such individual risk factors on the prediction accuracy of the decision-support tools. Monitoring data (see Section 3) obtained from human studies can also be used to update and refine the model outputs, leading to more accurate predictions and better decision making.

4. Novel molecular biomarkers for EHS prevention

The tolerance to sustain heat stress exists within a wide range of inter-individual variability. Under the same conditions, individuals who experience an earlier and higher rise in T_c than others are regarded as “heat intolerant” and are prone to EHS. Thus far, commonly used clinical markers have primarily predicted the severity of occurring EHS rather than the individual heat tolerance/intolerance per se. Therefore, a reliable scale of correlative biomarkers, beyond the traditional systemic phenotypic physiological responses, is indispensable to understanding the level of tolerance to heat and the extent of EHS pathology. Such markers should be applied at critical points during active service (e.g., prior to deployment or sustained missions) for assessing possible hazards of impaired systemic homeostasis or in monitoring recovery from EHS. Our current understanding of the systemic inflammatory response syndrome (SIRS), the microbiome, cell-free molecular markers (namely small DNA fragments released from cells) and proteomics potentially expand on available predictive markers.

4.1. Acute heat stress and acclimatization to heat

Acute exposure to heat stress differs significantly from that of long-term exposure to moderate temperatures (i.e. heat acclimatization) (Fig. 1); the former results in acute activation of physiological heat stress-induced reflexes, while the latter results in physiological and cellular adaptations, depending on the duration and magnitude of the stress applied.[69] It follows that blood levels of various heat-shock

Table 1
Shortlist of decision-support tools or indices for hot and/or cold environments, personal input and output variables or predictions. *Outcome variables that are biomarkers and can be monitored. For an overview of thermoregulatory models that may support decision-support tools by simulation of body temperature distribution, interested readers are referred to Havenith and Fiala[64] and Xu and Tikuisis.[65]

Decision-support tool	Type	Input variables of individual characteristics	Output variables or predictions
Heat Strain Decision Aid (HSDA)[91]	Heat	Body mass and height Initial T_{sk} and T_c Dehydration level Heat-acclimatization status Metabolic rate/activity level	T_c* Sweat loss*
Predicted Heat Strain (PHS)[92]	Heat	Body mass and height Heat-acclimatization status Metabolic rate/activity level	T_c* Sweat loss* Duration limit of heat exposure
Wet-bulb Globe Temperature (WBGT)[59,63]	Heat index	Heat-acclimatization status Metabolic rate/activity level	Hydration requirement Duration limit of heat exposure
Universal Thermal Climate Index (UTCI)[93]	Heat & cold index	—	General heat or cold strain
ClimApp[94]	Heat & cold	Age Biological sex Body mass and height Heat-acclimatization status Metabolic rate/activity level	T_c* Sweat loss* Risk of freezing for exposed skin Required intrinsic clothing insulation Duration limit of heat or cold exposure
Windchill Index[62,95]	Cold index	—	Risk of freezing for exposed skin*
Cold Exposure Survival Model (CESM)[96,97]	Cold	Age Biological sex Body mass and height Body fat percentage	Risk of freezing for exposed skin* Survival time for cold-dry or cold-wet exposure
Cold Weather Decision Aid (CoWEDA)[98]	Cold	—	Endurance time for hands and feet* Endurance time for hypothermia* Comfort time
Insulation Required (IREQ)[95]	Cold	Metabolic rate/activity level	Required intrinsic clothing insulation Duration limit of cold exposure for whole body

T_{sk}, skin temperature. T_c, deep-body temperature.

proteins[70] and histones,[71] for example, can serve as putative biomarkers to reflect the level of heat strain and cellular adaptation to heat load.

Excessive elevation in T_c facilitates two pathways leading to systemic inflammatory dysfunction and ultimately to heat stroke.[72] The first pathway ("heat sepsis") is a consequence of splanchnic ischemia, which results in gastrointestinal (GI) dysfunction. The vulnerability of the intestinal barrier integrity leads to the presentation of endotoxin in the circulating blood, triggering the innate inflammatory response and ultimately the progression into systematic inflammation.[73] "Heat sepsis" is believed to precede the second pathway, a direct cytotoxic effect of hyperthermia ("heat toxicity"), under which elevated T_c is associated with acidosis, energy depletion, derangements in intracellular calcium, and upregulation of reactive oxygen species-production, eliciting oxidative cell damage which triggers cell necrosis and apoptosis.[74]

4.2. The microbiome, gastrointestinal integrity, and inflammation

The dual-pathway model of heat stroke hints to a pivotal role of the microbiome and GI integrity. A small level of GI permeability is normal and a healthy immune system prevents its adverse effects. Consequently, low levels of endotoxin (used synonymously with Lipopolysaccharide) that leak into the blood stream are rapidly detoxified and removed by monocytes and particularly by the Kupffer cells that reside in liver tissues.[75] However, in extensive GI tight-junction dysfunction, endotoxin leakage overwhelms detoxification capacity. Under this condition, endotoxin becomes a contributing factor in the pathophysiology of heat stroke, as supported by direct experimental evidence on the impairment of intestinal tight-junction integrity under heat stress and by clinical observations.[76] Thus, a common indicator of GI barrier disruption is the presence of endotoxin in systemic circulation and liver portal system, which differs in individuals exposed to various levels of heat stress. Changes in tight-junction proteins (e.g. increased claudin and reduced occludin and zonula occludens-1 (ZO-1) expression) might also reflect thermal strain.[77]

GI barrier destruction might reflect on the role of the microbiome in thermal balance and modulation in the gut microbiome may provide a compensatory response to elevated body temperatures. Studies describing the effects of gut microbiota on human health are relatively abundant, but the effect of heat stress on gut microbiota is scarce and mostly derived from animal studies. Short exposure of pigs to heat stress induced dysbiosis in the gut microbial community, i.e., reduced diversity and changes in composition.[78,79] Under acute heat stress, gut Firmicutes-to-Bacteroidetes ratio significantly increased with an increase of Firmicutes and the decrease of Bacteroidetes.[79] Extending the heat stress (29 °C) to 13 weeks led to noticeably less diverse gut microbiota.[79] It follows that a decline in microbiota diversity associated with repeated stimulatory heat stress (acclimatization) protects against the harmful effects of heat stress.[80] Longitudinal observations in soldiers revealed a concomitant relationship between dysbiosis in gut microbiota and increased intestinal permeability.[81,82] Hence, observations on the dynamic changes in peripheral microbiota markers in blood and/or GI/saliva can differentiate between heat-tolerant or intolerant individuals. Regarding the role of the microbiome in the cold, there is evidence that the gut microbiota undergoes changes (e.g., increase in Lachnospiraceae and short-chain fatty acids) in response to hypothermia and facilitates host thermogenesis during cold stress.[83] Moreover, findings from a recent study in piglets suggest that the gut microbiota-blood-liver and fat axis may regulate thermogenesis during cold acclimation.[84] However, a discussion of the research on microbiome and cold stress is beyond the scope of this review and interested readers are referred to excellent reviews on this topic for more information.[83,85].

The severity of EHS is associated with a protracted SIRS, including a progressive cytokine storm. Therefore, markers reflecting the inflammatory response are useful in assessing the severity of EHS at its early stage or recovery (thus supporting return to duty), or the state of heat acclimatization. Studies in patients with heat stroke show marked elevation in levels of pro- and anti-inflammatory cytokines and a rise in Th1:Th2 (T-helper cells' subsets) ratio, indicating higher proinflammatory cytokine levels. Conversely, during heat acclimation, anti-inflammatory cytokines predominate.[86,87]

An integration of the traditionally used systemic parameters together with accepted molecular markers may help to identify heat-intolerant individuals. These markers may also serve to monitor the progression of heat adaptation and the extent and severity of EHS at an early stage, thereby assisting the clinician in assessing the patient's condition and in determining the prognosis and proper choice of treatment. In military scenarios, this will help enhance the soldier's survivability and effectiveness.

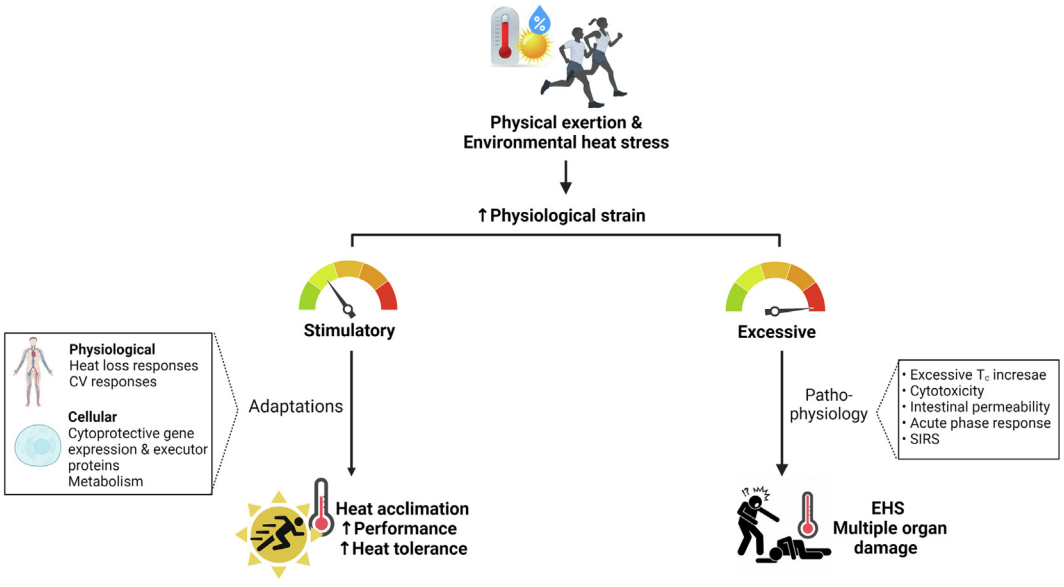

Fig. 1. The impact of heat stress on physiological/pathophysiological responses resulting in either adaptation or exertional heat stroke (EHS). Adaptive vs. acute (excessive) exposure to exertional-heat stress. The former will result in heat acclimatization, delayed onset of EHS, and enhanced performance, while the latter will result in EHS and organ failure. Different responses characterize the adaptive and excessive responses that can be mirrored by related biomarkers. CV, cardiovascular. T_c, deep-body temperature, SIRS, systemic inflammatory response syndrome.

5. Conclusions and future research

Heat or cold stress encountered during military training and operations can threaten the safety and performance of warfighters, subsequently reducing training and operational effectiveness. The large inter- and intra-individual variability in tolerance to heat or cold stress highlights the necessity for individualized risk-mitigation strategies. Real-time, physiological monitoring of thermal strain, coupled with AI, may be effective in this regard. Rapid advancements in wearable technology have made it possible to measure a range of biomarkers in the field. Moreover, continued technological advances will spur the development of wearable sensors and systems that can monitor a wider variety of biomarkers non-invasively, are more accurate and precise, cost-effective, unobtrusive and practical in various contexts.[88–90] Fig. 2 provides an example of this concept, where multiple textile-integrated sensors capable of measuring various parameters can be combined with wireless low-power communication technologies and machine learning algorithms to create a system that can provide valid and actionable information in real time.

In hot environments, the combination of T_c, T_{sk} and heart rate can provide some indication of thermal strain and when combined with biomechanical markers of gait (in)stability, EHS may be predicted in real time. Relevant biomarkers of thermal strain in the cold include T_{sk}, shivering thermogenesis and T_c. Novel solutions for non-invasive estimation of T_c have been developed, but their accuracy and reliability require more extensive validation, particularly in cold environments. Decision-support tools are useful for guiding activity

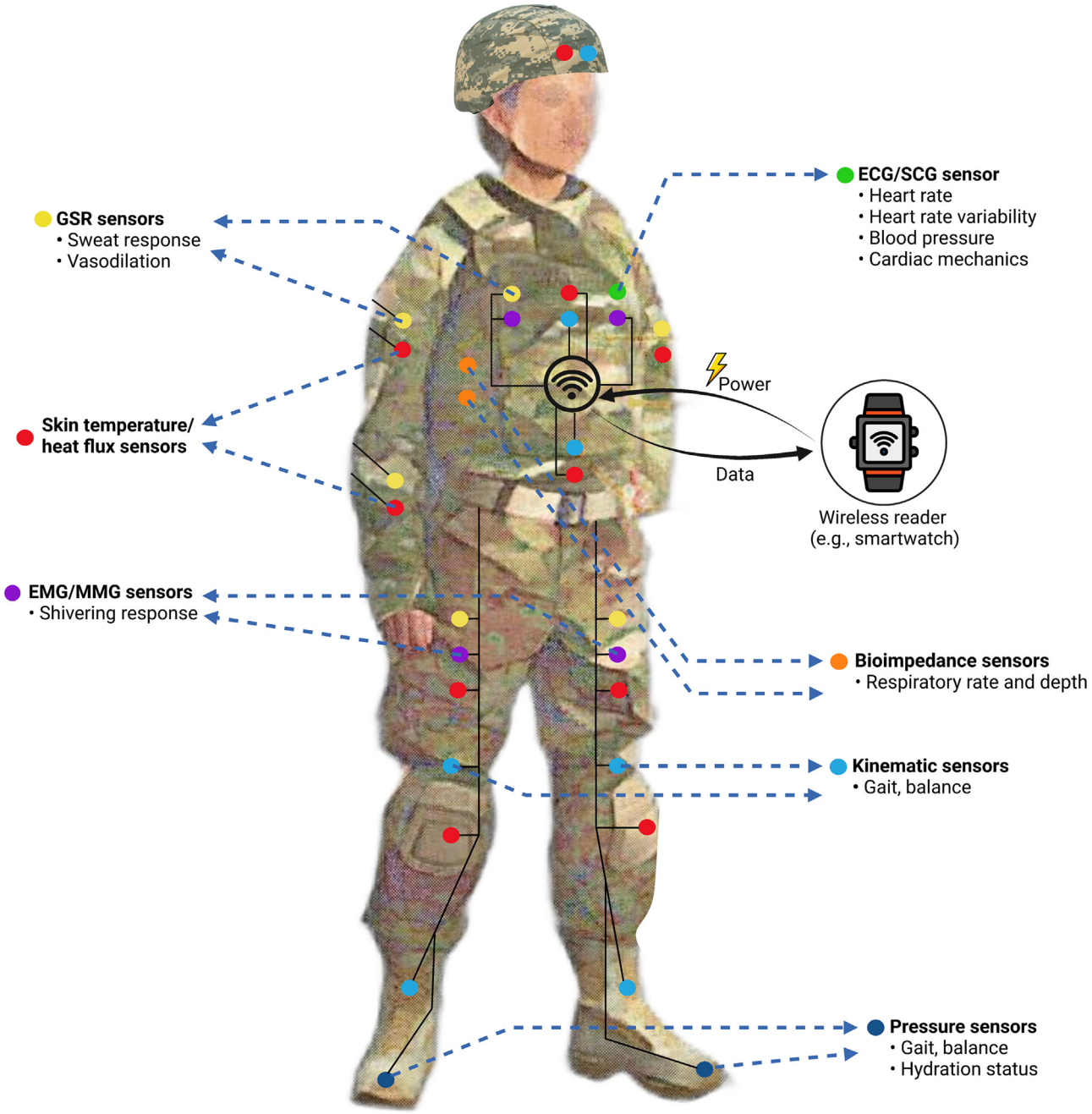

Fig. 2. Illustration of a warfighter equipped with a low-power, textile-integrated wearable system.[83,84] A variety of soft, wireless and battery-free sensors (colored circles) embedded within clothing and accessories (e.g., helmet, shoes) are interconnected via conductive threads (black lines) to a "hub," which serves as the interface for wireless data and power transmission between a reader (e.g., smartwatch) and the sensors. Data can be visualized in real time from the smartwatch. Predictive algorithms can also be incorporated within the smartwatch to provide real-time health alerts to the warfighter. Figure adapted with permission from Lin et al.[83,84] GSR, galvanic skin response. EMG, electromyography. MMG, mechanomyography. ECG, electrogradiography. SCG, seismocradiography.

planning; their predictions can be improved by incorporating biomarkers of individual susceptibility to thermal stress (e.g., aerobic fitness, motivation) in the thermoregulatory simulations, though they still do not provide the personalized predictions that can be achieved through individual physiological monitoring.[91] Lastly, novel molecular biomarkers of heat intolerance that may facilitate the prevention and clinical management of EHS were discussed; these include markers related to the microbiome, intestinal barrier integrity and inflammatory response.

Further research is required to establish the validity of the risk-prediction algorithms and tools, particularly in terms of their accuracy (false-positive and false-negative rates) in detecting or predicting EHS or cold injuries. Notably, the accuracy criteria may be dependent on each unit's risk tolerance and their capacity to respond to potential casualties. Validation methods based on group errors (e.g., RMSE, mean absolute error) are acceptable when predicting *group* responses for activity planning, but for real-time *individual* monitoring, individual prediction errors (e.g., PTA) are more appropriate. Given the complexity and multi-faceted nature of the overall response to thermal stress, traditional physiological biomarkers of thermal strain (e.g., T_c, T_{sk}, heart rate) are insufficient for depicting whole-body strain or eradicating injuries. Finally, rather than focusing on improving the accuracy of monitoring data or prediction tools, future research should seek to investigate and quantify the real-world impact of these strategies on injury rates, performance and operational effectiveness.

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

1. Bouchama A, Abuyassin B, Lehe C et al. Classic and exertional heatstroke. *Nat Rev Dis Primers* 2022;8(1):8. doi:10.1038/s41572-021-00334-6.
2. US Army. *Prevention and Management of Cold-Weather Injuries, Technical Bulletin TB MED 508*. Washington DC, Headquarters Department of the Army and Air Force, 2005.
3. Alele FO, Malau-Aduli BS, Malau-Aduli AEO et al. Epidemiology of exertional heat illness in the military: a systematic review of observational studies. *Int J Environ Res Public Health* 2020;17(19):7037. doi:10.3390/ijerph17197037.
4. Armed Forces Health Surveillance Branch. Update: cold weather injuries, active and reserve components, U.S. Armed forces, July 2015-June 2020. *MSMR* 2020;27(11):15-24.
5. Intergovernmental Panel on Climate Change. Climate change 2021: the physical science basis. Contribution of Working Group I to the sixth assessment report of the Intergovernmental Panel on Climate Change. Available at: https://www.ipcc.ch/report/ar6/wg1/downloads/report/IPCC_AR6_WGI_Full_Report.pdf 2021. Accessed 6 April 2022.
6. Epstein Y. Heat intolerance: predisposing factor or residual injury? *Med Sci Sports Exerc* 1990;22(1):29-35.
7. O'Connor FG, Heled Y, Deuster PA. Exertional heat stroke, the return to play decision, and the role of heat tolerance testing: a Clinician's dilemma. *Curr Sports Med Rep* 2018;17(7):244-248. doi:10.1249/JSR.0000000000000502.
8. Notley SR, Flouris AD, Kenny GP. On the use of wearable physiological monitors to assess heat strain during occupational heat stress. *Appl Physiol Nutr Metab* 2018;43(9):869-881. doi:10.1139/apnm-2018-0173.
9. Buller MJ, Welles AP, Friedl KE. Wearable physiological monitoring for human thermal-work strain optimization. *J Appl Physiol* 2018;124(2):432-441. doi:10.1152/japplphysiol.00353.2017.
10. Tharion WJ, Potter AW, Duhamel CM et al. Real-time physiological monitoring while encapsulated in personal protective equipment. *J Sport Hum Perf* 2013;1(4):14-21.
11. Moran DS, Shitzer A, Pandolf KB. A physiological strain index to evaluate heat stress. *Am J Phys* 1998;275(1):R129-R134. doi:10.1152/ajpregu.1998.275.1.R129.
12. Byrne C, Lee JKW. The physiological strain index modified for trained heat-acclimatized individuals in outdoor heat. *Int J Sports Physiol Perform* 2019;14(6):805-813. doi:10.1123/ijspp.2018-0506.
13. Lim CL, Byrne C, Lee JK. Human thermoregulation and measurement of body temperature in exercise and clinical settings. *Ann Acad Med Singap* 2008;37(4):347-353.
14. Falcone T, Cordella F, Molinaro V et al. Real-time human core temperature estimation methods and their application in the occupational field: a systematic review. *Measurement* 2021;183:109776. doi:10.1016/j.measurement.2021.109776.
15. Nazarian N, Lee JK. Personal assessment of urban heat exposure: a systematic review. *Environ Res Lett* 2021;16(3):033005.
16. Buller MJ, Tharion WJ, Cheuvront SN et al. Estimation of human core temperature from sequential heart rate observations. *Physiol Meas* 2013;34(7):781-798. doi:10.1088/0967-3334/34/7/781.
17. Guo J, Chen Y, Fan PWP et al. Kalman filter models for the prediction of individualized thermal work strain, In: Serra GO, ed. *Kalman Filters, Chapter 6, Kalman Filters: Theory for Advanced Applications Theory for Advanced Applications*, IntechOpen, 2008. doi:10.5772/intechopen.71205.
18. Nazarian N, Liu S, Kohler M et al. Project Coolbit: can your watch predict heat stress and thermal comfort sensation? *Environ Res Lett* 2021;16(3):034031. doi:10.1088/1748-9326/abd130.
19. Gunga HC, Sandsund M, Reinersten RE et al. A non-invasive device to continuously determine heat strain in humans. *J Therm Biol* 2008;33(5):297-307. doi:10.1016/j.jtherbio.2008.03.004.
20. Mazgaoker S, Ketko I, Yanovich R et al. Measuring core body temperature with a non-invasive sensor. *J Therm Biol* 2017;66:17-20. doi:10.1016/j.jtherbio.2017.03.007.
21. Tsadok I, Scheinowitz M, Shpitzer SA et al. Assessing rectal temperature with a novel non-invasive sensor. *J Therm Biol* 2021;95:102788. doi:10.1016/j.jtherbio.2020.102788.
22. Xu X, Karis AJ, Buller MJ et al. Relationship between core temperature, skin temperature, and heat flux during exercise in heat. *Eur J Appl Physiol* 2013;113(9):2381-2389. doi:10.1007/s00421-013-2674-z.
23. Welles AP, Xu X, Santee WR et al. Estimation of core body temperature from skin temperature, heat flux, and heart rate using a Kalman filter. *Comput Biol Med* 2018;99:1-6. doi:10.1016/j.compbiomed.2018.05.021.
24. Dias D, Cunha JPS. Wearable health devices – vital sign monitoring, systems and technologies. *Sensors* 2018;18(8):2414. doi:10.3390/s18082414.
25. Nazari G, MacDermid JC. Reliability of Zephyr BioHarness respiratory rate at rest, during the modified Canadian aerobic fitness test and recovery. *J Strength Cond Res* 2020;34(1):264-269. doi:10.1519/JSC.0000000000003046.
26. Tsuji B, Hayashi K, Kondo N et al. Characteristics of hyperthermia-induced hyperventilation in humans. *Temperature (Austin)* 2016;3(1):146-160. doi:10.1080/23328940.201.
27. Nybo L, Nielsen B. Middle cerebral artery blood velocity is reduced with hyperthermia during prolonged exercise in humans. *J Physiol* 2001;534(Pt 1):279-286. doi:10.1111/j.1469-7793.2001.t01-1-00279.x.
28. Pearson J, Lucas RAI, Schlader ZJ et al. Active and passive heat stress similarly compromise tolerance to a simulated hemorrhagic challenge. *Am J Phys* 2014;307(7):R822-R827. doi:10.1152/ajpregu.00199.2014.
29. Schlader ZJ, Wilson TE, Crandall CG. Mechanisms of orthostatic intolerance during heat stress. *Auton Neurosci* 2016;196:37-46. doi:10.1016/j.autneu.2015.12.005.
30. Quinn CM, Duran RM, Audet GN et al. Cardiovascular and thermoregulatory biomarkers of heat stroke severity in a conscious rat model. *J Appl Physiol* 2014;117(9):971-978. doi:10.1152/japplphysiol.00365.2014.
31. Schiffmann N, Schermann H, Mazgaoker S et al. The cardiovascular reserve index-a noninvasive clinical insight into heat intolerance. *Clin J Sport Med* 2021;31(3):232-236. doi:10.1097/JSM.0000000000000712.
32. Luippold AJ, Charkoudian N, Kenefick RW et al. Update: efficacy of military fluid intake guidance. *Mil Med* 2018;183(9–10):e338-e342. doi:10.1093/milmed/usy066.
33. Tan XR, Low ICC, Stephenson MC et al. Altered brain structure with preserved cortical motor activity after exertional hypohydration: a MRI study. *J Appl Physiol* 2019;127(1):157-167. doi:10.1152/japplphysiol.00081.2019.
34. Poon BH, Prakaash S, Teo YS et al. Thermal strain and fluid balance during a 72-km military route march in a field setting. *Singap Med J* 2021. doi:10.11622/smedj.2021053.
35. Abriat A, Brosset C, Brégigeon M et al. Report of 182 cases of exertional heatstroke in the French Armed Forces. *Mil Med* 2014;179(3):309-314.
36. Tay CS, Lee JK, Teo YS et al. Using gait parameters to detect fatigue and responses to ice slurry during prolonged load carriage. *Gait Posture* 2016;43:17-23. doi:10.1016/j.gaitpost.2015.10.010.
37. Kong PW, Beauchamp G, Suyama J et al. Effect of fatigue and hypohydration on gait characteristics during treadmill exercise in the heat while wearing firefighter thermal protective clothing. *Gait Posture* 2010;31(2):284-288. doi:10.1016/j.gaitpost.2009.11.006.

38. Buller M, Fellin R, Bursey M et al. Gait instability and estimated core temperature predict exertional heat stroke. *Br J Sports Med* 2022;56(8):446-451. doi:10.1136/bjsports-2021-104081.
39. Imbeault MA, Mantha OL, Haman F. Shivering modulation in humans: effects of rapid changes in environmental temperature. *J Therm Biol* 2013;38(8):582-587. doi:10.1016/j.jtherbio.2013.10.002.
40. Gagge AP, Gonzalez RR. Mechanisms of heat exchange: biophysics and physiolgy, Chapter 4. In: Fregly MJ, Blatteis C, eds. *Handbook of Physiology – Environmental Physiology*, Bethesda, MD, American Physiological Society, 1996.
41. Kingma BR, Frijns AJ, Schellen L et al. Beyond the classic thermoneutral zone: including thermal comfort. *Temperature* 2014;1(2):142-149. doi:10.4161/temp.297027-9.
42. Blondin DP, Tingelstad HC, Mantha OL et al. Maintaining thermogenesis in cold exposed humans: relying on multiple metabolic pathways. *Compr Physiol* 2014;4(4):1383-1402. doi:10.1002/cphy.c130043.
43. Eyolfson DA, Tikuisis P, Xu X et al. Measurement and prediction of peak shivering intensity in humans. *Eur J Appl Physiol* 2001;84(1–2):100-106. doi:10.1007/s004210000329.
44. Scott CG, Ducharme MB, Haman F et al. Warming by immersion or exercise affects initial cooling rate during subsequent cold water immersion. *Aviat Space Environ Med* 2004;75(11):956-963.
45. Young AJ, Sawka MN, Neufer PD et al. Thermoregulation during cold water immersion is unimpaired by low glycogen levels. *J Appl Physiol* 1989;66(4):1809-1816. doi:10.1152/jappl.1989.66.4.1809.
46. Haman F, Legault SR, Rakobowchuk M et al. Effects of carbohydrate availability on sustained shivering II. Relating muscle recruitment to fuel selection. *J Appl Physiol* 2004;96(1):41-49. doi:10.1152/japplphysiol.00428.2003.
47. Petrone P, Asensio JA, Marini CP. Management of accidental hypothermia and cold injury. *Curr Probl Surg* 2014;51(10):417-431. doi:10.1067/j.cpsurg.2014.07.004.
48. Parsons K. Measurement methods and assessment techniques, Chapter 5. *Human thermal environments: The effects of hot, moderate, and cold environments on human health, comfort and performance*, 2nd ed., London, Taylor & Francis, 2003. in.
49. Tikuisis P. Finger cooling during cold air exposure. *Bull Am Meteorol Soc* 2004;85(5):717-724.
50. Haman F, Péronnet F, Kenny GP et al. Partitioning oxidative fuels during cold exposure in humans: muscle glycogen becomes dominant as shivering intensifies. *J Physiol* 2005;566(Pt 1):247-256. doi:10.1113/jphysiol.2005.086272.
51. Arnold JT, Hemsley Z, Hodder SG et al. Reliability and validity of methods in the assessment of cold-induced shivering thermogenesis. *Eur J Appl Physiol* 2020;120(3):591-601. doi:10.1007/s00421-019-04288-2.
52. Bell DG, Tikuisis P, Jacobs I. Relative intensity of muscular contraction during shivering. *J Appl Physiol* 1992;72(6):2336-2342. doi:10.1152/jappl.1992.72.6.2336.
53. Haman F, Blondin DP. Shivering thermogenesis in humans: origin, contribution and metabolic requirement. *Temperature* 2017;4(3):217-226. doi:10.1080/23328940.2017.1328999.
54. Gordon K, Blondin DP, Friesen BJ et al. Seven days of cold acclimation substantially reduces shivering intensity and increases nonshivering thermogenesis in adult humans. *J Appl Physiol* 2019;126(6):1598-1606. doi:10.1152/japplphysiol.01133.2018.
55. Blondin DP, Labbé SM, Tingelstad HC et al. Increased brown adipose tissue oxidative capacity in cold-acclimated humans. *J Clin Endocrinol Metab* 2014;99(3):E438-E446. doi:10.1210/jc.2013-3901.
56. Okla M, Kim J, Koehler K et al. Dietary factors promoting Brown and beige fat development and thermogenesis. *Adv Nutr* 2017;8(3):473-483. doi:10.3945/an.116.014332.
57. Montgomery RE, Hartley GL, Tyler CJ et al. Effect of segmental, localized lower limb cooling on dynamic balance. *Med Sci Sports Exerc* 2015;47(1):66-73. doi:10.1249/MSS.0000000000000379.
58. Heus R, Daanen HA, Havenith G. Physiological criteria for functioning of hands in the cold: a review. *Appl Ergon* 1995;26(1):5-13. doi:10.1016/0003-6870(94)00004-i.
59. North Atlantic Treaty Organization. Management of heat and cold stress, *Guidance to NATO Medical Personnel, RTO Technical Report TR-HFM-187, Science and Technology Organization*, 2013.
60. Sullivan-Kwantes W, Haman F, Kingma BRM et al. Human performance research for military operations in extreme cold environments. *J Sci Med Sport* 2021;24(10):954-962. doi:10.1016/j.jsams.2020.11.010.
61. Potter AW, Looney DP, Friedl K. *Modeling Cold Stress – Russian Soldiers in Ukraine. USARIEM Technical Note TN22-02*. U.S. Army Research Institute of Environmental Medicine, Natick, MA. 10.13140/RG.2.2.28147.81447
62. Osczevski R, Bluestein M. The new wind chill equivalent temperature chart. *Bull Am Meteorol Soc* 2005;86(10):1453-1458. doi:10.1175/BAMS-86-10-1453.
63. International Organization for Standardization. *ISO 7243: Ergonomics of the Thermal Environment – Assessment of Heat Stress Using the WBGT (Wet-Bulb Globe Temperature Index)*, Geneva Switzerland, ISO, 2017.
64. Havenith G, Fiala D. Thermal Indices and Thermophysiological Modeling for Heat Stress. *Compr Physiol* 2015;6(1):255-302. doi:10.1002/cphy.c140051. Erratum in: Compr Physiol. 2016;6(2):1134.
65. Xu X, Tikuisis P. Thermoregulatory modeling for cold stress. *Compr Physiol* 2014;4(3):1057-1081. doi:10.1002/cphy.c130047.
66. Payne S, Macintosh A, Stock J. The influence of digit size and proportions on dexterity during cold exposure. *Am J Phys Anthropol* 2018;166(4):875-883. doi:10.1002/ajpa.23486.
67. Selkirk GA, McLellan TM. Influence of aerobic fitness and body fatness on tolerance to uncompensable heat stress. *J Appl Physiol* 2001;91(5):2055-2063. doi:10.1152/jappl.2001.91.5.2055.
68. Epstein Y, Moran DS, Shapiro Y et al. Exertional heat stroke: a case series. *Med Sci Sports Exerc* 1999;31(2):224-228. doi:10.1097/00005768-199902000-00004.
69. Roberts WO, Armstrong LE, Sawka MN et al. ACSM expert consensus statement on exertional heat illness: recognition, management, and return to activity. *Curr Sports Med Rep* 2021;20(9):470-484. doi:10.1249/JSR.0000000000000878.
70. Horowitz M, Robinson SD. Heat shock proteins and the heat shock response during hyperthermia and its modulation by altered physiological conditions. *Prog Brain Res* 2007;162:433-446. doi:10.1016/S0079-6123(06)62021-9.
71. Bruchim Y, Ginsburg I, Segev G et al. Serum histones as biomarkers of the severity of heatstroke in dogs. *Cell Stress Chaperones* 2017;22(6):903-910. doi:10.1007/s12192-017-0817-6.
72. Lim CL, Mackinnon T. The role of exercise-induced immune system disturbances in the pathology of heat stroke: the dual pathway model of heat stroke. *Sports Med* 2006;36(1):39-64. doi:10.2165/00007256-200636010-00004.
73. Patra AK, Kar I. Heat stress on microbiota composition, barrier integrity, and nutrient transport in gut, production performance, and its amelioration in farm animals. *J Anim Sci Technol* 2021;63(2):211-247. doi:10.5187/jast.2021.e48.
74. Hall DM, Buettner GR, Oberley LW et al. Mechanisms of circulatory and intestinal barrier dysfunction during whole body hyperthermia. *Am J Physiol Heart Circ Physiol* 2001;280(2):H509-H521. doi:10.1152/ajpheart.2001.280.2.H509.
75. Lambert GP. Role of gastrointestinal permeability in exertional heat-stroke. *Exerc Sport Sci Rev* 2004;32(4):185-190. doi:10.1097/00003677-200410000-00011.
76. Gathiram P, Wells MT, Brock-Utne JG et al. Antilipopolysaccharide improves survival in primates subjected to heat stroke. *Circ Shock* 1987;23(3):157-164.
77. Dokladny K, Zuhl MN, Moseley PL. Intestinal epithelial barrier function and tight junction proteins with heat and exercise. *J Appl Physiol* 2016;120(6):692-701. doi:10.1152/japplphysiol.00536.2015.
78. Xiong Y, Yi H, Wu Q et al. Effects of acute heat stress on intestinal microbiota in grow-finishing pigs, and associations with feed intake and serum profile. *J Appl Microbiol* 2020;128(3):840-852. doi:10.1111/jam.14504.
79. He Y, Maltecca C, Tiezzi F. Potential use of gut microbiota composition as a biomarker of heat stress in Monogastric species: a review. *Animals* 2021;11(6):1833. doi:10.3390/ani11061833.
80. Le Sciellour M, Zemb O, Hochu I et al. Effect of chronic and acute heat challenges on fecal microbiota composition, production, and thermoregulation traits in growing pigs. *J Anim Sci* 2019;97(9):3845-3858. doi:10.1093/jas/skz222.
81. Li X, Kan EM, Lu J et al. Combat-training increases intestinal permeability, immune activation and gastrointestinal symptoms in soldiers. *Aliment Pharmacol Ther* 2013;37(8):799-809. doi:10.1111/apt.12269.
82. Karl JP, Margolis LM, Madslien EH et al. Changes in intestinal microbiota composition and metabolism coincide with increased intestinal permeability in young adults under prolonged physiological stress. *AmJ Physiol Gastrointest Liver Physiol* 2017;312(6):G559-G571. doi:10.1152/ajpgi.00066.2017.
83. Huus KE, Ley RE. Blowing hot and cold: body temperature and the microbiome. *mSystems* 2021;6(5):e0070721. doi:10.1128/mSystems.00707-21.
84. Zhang Y, Sun L, Zhu R et al. Porcine gut microbiota in mediating host metabolic adaptation to cold stress. *NPJ Biofilms Microbiomes* 2022;8(1):18. doi:10.1038/s41522-022-00283-2.
85. Sepulveda J, Moeller AH. The effects of temperature on animal gut microbiomes. *Front Microbiol* 2020;11:384. doi:10.3389/fmicb.2020.00384.
86. Heled Y, Fleischmann C, Epstein Y. Cytokines and their role in hyperthermia and heat stroke. *J Basic Clin Physiol Pharmacol* 2013;24(2):85-96. doi:10.1515/jbcpp-2012-0040.
87. Lu KC, Wang JY, Lin SH et al. Role of circulating cytokines and chemokines in exertional heatstroke. *Crit Care Med* 2004;32(2):399-403. doi:10.1097/01.CCM.0000108884.74110.D9.
88. Lin M, Hu H, Zhou S et al. Soft wearable devices for deep-tissue sensing. *Nat Rev Mater* 2022:1-20. doi:10.1038/s41578-022-00427-y.
89. Lin R, Kim HJ, Achavananthadith S et al. Wireless battery-free body sensor networks using near-field-enabled clothing. *Nat Commun* 2020;11(1):444. doi:10.1038/s41467-020-14311-2.
90. Lin R, Kim HJ, Achavananthadith S et al. Digitally-embroidered liquid metal electronic textiles for wearable wireless systems. *Nat Commun* 2022;13:2190. doi:10.1038/s41467-022-29859-4.
91. Potter AW, Blanchard LA, Friedl KE et al. Mathematical prediction of core body temperature from environment, activity, and clothing: the heat strain decision aid (HSDA). *J Therm Biol*; 64:78–85. https://doi.org/10.1016/j.jtherbio.2017.01.003
92. International Organization for Standardization. *ISO 7933: Ergonomics of the Thermal Environment - Analytical Determination and Interpretation of Heat Stress Using Calculation of the Predicted Heat Strain*, Geneva, Switzerland, ISO, 2004.
93. Bröde P, Fiala D, Błażejczyk K et al. Deriving the operational procedure for the universal thermal climate index (UTCI). *Int J Biometeorol* 2012;56(3):481-494. doi:10.1007/s00484-011-0454-1.
94. Kingma BRM, Steenhoff H, Toftum J et al. ClimApp-integrating personal factors with weather forecasts for individualized warning and guidance on thermal stress. *Int J Environ Res Public Health* 2021;18(21):11317. doi:10.3390/ijerph182111317.
95. International Organization for Standardization. *ISO 11079: Ergonomics of the Thermal Environment: Determination and Interpretation of Cold Stress when Using Required Clothing Insulation (IREQ) and Local Cooling Effects*, Geneva, Switzerland, ISO, 2007https://www.iso.org/obp/ui/#iso:std:iso:11079:ed-1:v1:en.
96. Tikuisis P. Predicting survival time for cold exposure. *Int J Biometeorol* 1995;39(2):94-102. doi:10.1007/BF01212587.
97. Tikuisis P. Prediction of survival time at sea based on observed body cooling rates. *Aviat Space Environ Med* 1997;68(5):441-448.
98. Xu X, Rioux TP, Gonzalez J et al. A digital tool for prevention and management of cold weather injuries-cold weather ensemble decision aid (CoWEDA). *Int J Biometeorol* 2021;65(8):1415-1426. doi:10.1007/s00484-021-02113-0.