Molecular biomarkers for assessing the heat-adapted phenotype: a narrative scoping review

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Abstract

Heat acclimation/acclimatisation (HA) mitigates heat-related decrements in physical capacity and heat-illness risk and is a widely advocated countermeasure for individuals operating in hot environments. The efficacy of HA is typically quantified by assessing the thermo-physiological responses to a standard heat acclimation state test (i.e. physiological biomarkers), but this can be logistically challenging, time consuming, and expensive. A valid molecular biomarker of HA would enable evaluation of the heat-adapted state through the sampling and assessment of a biological medium. This narrative review examines candidate molecular biomarkers of HA, highlighting the poor sensitivity and specificity of these candidates and identifying the current lack of a single 'standout' biomarker. It concludes by considering the potential of multivariable approaches that provide information about a range of physiological systems, identifying a number of challenges that must be overcome to develop a valid molecular biomarker of the heat-adapted state, and highlighting future research opportunities.

Keywords Heat, Acclimation, Acclimatisation, Hormone, Heat shock protein

Introduction

Humans are often required to operate and perform in hot and or humid environments. Compared to a cool environment, these conditions reduce physical work capacity [52], impair performance [69, 90] and increase the risk of heat-related illnesses [9]. Unfortunately, due to the warming effects of climate-change, exposure to this type of environment is likely to increase in the future [41]. However, repeated frequent exposure to

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environmental conditions that are sufficient to elicit pronounced thermo-physiological strain result in within-life phenotypic adaptations that improve an individual's ability to maintain thermal homeostasis when subsequently exposed to a hot environment [38]. This process is termed heat *acclimatisation* when occurring in a natural environment and heat *acclimation* when undertaken in a controlled (laboratory) environment [84]. Heat acclimatisation/acclimation (HA) is an effective intervention for mitigating decrements in physical work capacity and performance in the heat [16], and reducing the risk of heat-related illnesses [183]. As such, HA is widely advocated for individuals operating in these conditions [160, 170].

The phenotypic adaptations to heat are commonly quantified by exposure to a standard exercise-heat stress, often termed a 'heat acclimation state test' (HAST) [169, 211], where the physiological outputs (e.g. sweating rate, cutaneous blood flow) and disturbances (e.g. skin and deep body temperature) to a fixed thermal input (e.g.

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ambient thermal stress and work rate) [164] are measured, with deep body temperature regarded as paramount due to its association with heat illness [9] (see Sect. "The heat-adapted phenotype" for more detailed discussion). However, quantification of these physiological indices requires carefully controlled environmental conditions (e.g. environmental chamber), work rate standardisation and specialist equipment, can be invasive (e.g. measurement of deep body (rectal or oesophageal) temperature), may lack objectivity, and is typically based upon change scores (i.e. pre vs. post comparisons) rather than absolute values. Less-invasive deep body temperature measurements (e.g. sub-lingual, axillary, tympanic) lack validity [136], whilst the utility of other non-invasive physiological indices of heat adaption (e.g. cardiovascular indices) has only been evidenced under carefully standardised environmental conditions [49, 202]. Together these factors can negate the practical utility of implementing a HAST, particularly in contexts where large numbers of personnel may need to be evaluated on a short time scale and with significant logistical constraints (e.g. defence [160]) and limit the use of physiological indices of heat adaption (e.g. assessment of thermoeffector outputs and physiological disturbances) in circumstances where the input conditions are not strictly controlled.

A biomarker is '*a defined characteristic that is measured as an indicator of normal biological processes, pathogenic processes or responses to an exposure or intervention*' [45], p. 43). Biomarkers can be derived from histologic, radiographic, physiological, or molecular characteristics [21], with the latter having biophysical properties, which allow their measurements in biological samples such as plasma, urine or sweat [107]. Within the context of HA, a valid molecular biomarker may negate the need for a HAST, or reduce the technical requirements and reliance on the assessment of 'hallmark" physiological indices of adaption, i.e. physiological biomarkers [164, 165], and enable an evaluation of whether an individual had acquired a heat-adapted phenotype through the sampling and assessment of a biological medium. Such a molecular biomarker may have important utility in circumstances where the assessment of physiological biomarkers is not viable, where reliable molecular markers of adaptation could not only provide assurance of the efficacy of HA, but also reduce heat illnesses and lead to more personalised approaches to adaptation [160]. Although, to date, such a molecular biomarker has not been identified, technological advances in non-invasive and minimally invasive biosensing [60, 158], low-cost easy-to-use analyte measurement approaches [88], continuous and remote monitoring wearable technology [184], and multi-molecule biomarker development [48],

make identifying molecular biomarkers of heat adaptation a timely research challenge.

We commence this narrative review by briefly reviewing the thermo-physiological adaptations occurring with HA, including both the whole-body and cellular adaptations that are the 'hallmark' physiological characteristics of the heat-adapted phenotype. We subsequently review the literature that has examined changes in a number of candidate biomarkers within the context of HA, with a focus on molecules implicated in aspects of the adaptive response to heat, including fluid regulation, energy homeostasis, and sympatho-adrenal balance, as well as indices of inflammation, sudomotor adaption and acquired thermal tolerance. We conclude by highlighting a number of challenges that future research will need to overcome in order to develop a valid biomarker of HA, as well as future research opportunities that may prove to be fruitful in the development of a biomarker of HA.

The heat-adapted phenotype

The adaptations occurring with HA have been extensively described previously and the reader is guided to reviews by Périard et al. [164, 165] for detailed descriptions. Briefly, repeated daily heat exposures of at least 60 min duration at an intensity sufficient to elevate deep body temperature and skin temperature to a level eliciting pronounced sweating invokes a multi-system array of adaptions occurring over different time courses [53]. Within 4 to 5 days a pronounced increase (> 5%) in plasma volume is observed (e.g. [14, 143, 185]), mainly underpinned by the fluid conserving effects of the hormones aldosterone and arginine-vasopressin (e.g. [2, 54, 140, 146]) as well as an oncotic effect from intravascular protein influx [162, 185]. The resultant hypervolemia increases stroke volume and, possibly in conjunction with a reduction in sympathetic nervous system excitability [81, 83, 192], results in a lowering of heart rate and improved cardiovascular stability [216].

An increased sweating rate can be detected from the second day of HA, but it is generally believed that > 10 days is required to obtain the complete changes in the sweating threshold and gain (sensitivity) and sweat electrolyte concentration that constitute 'full' sudomotor adaptation [175, 210]. The threshold for cutaneous vasodilatation is also reduced [175], which enables greater internal heat transfer as a result of increased convective heat transport from the 'core' to skin, whilst the sudomotor adaptations support increased wet-heat transfer from the skin to the environment (under conditions permitting evaporation). There is also some evidence for a shift towards a more metabolically efficient phenotype, although these data are primarily derived from long-term HA studies of rodents [131, 132]. Nevertheless, although

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it has been highlighted that the research is equivocal [29], a number of human studies employing typical HA durations (e.g. 7–14 days) have shown an improved metabolic efficiency during exercise following HA (e.g. [181, 188, 224]), with some suggesting that the resultant reduction in metabolic heat production plays a substantial role in the lower thermal strain that is evident following an HA intervention [177].

HA also induces a range of cellular adaptions that confer improved cytoprotection [139]. This acquired thermal tolerance (ATT) appears to share a common pathway with the systemic adaptations to HA that is mediated by heat shock proteins (HSP) [104], a conserved group of proteins that serve as molecular chaperones and accelerate cellular repair from heat stress, ischaemia and endotoxic shock [101, 108]. Compared to our understanding of the time course of the systemic adaptations to heat, understanding of the intracellular adaptations is relatively limited. Nevertheless, a recent meta-analysis suggests that the number of days of HA is a significant moderator of the intracellular HSP (iHSP) response [142]. This is exemplified by data from Marshall et al. [122] who showed that iHSP levels in peripheral blood mononuclear cells (PBMCs) were unchanged following two consecutive daily 2-h bouts of exercise-heat stress, and Yamada et al. [218] who reported elevated baseline iHSP levels from day 6 through to day 10 during a 10-day (100 min day^{-1}) HA program.

Together, the multi-systemic adaptations occurring with HA, including expanded plasma volume, reduced heart rate, and enhanced skin blood flow and sweating, result in a lower skin and deep body temperature and an improved perceptual response, and in conjunction with ATT, constitute the 'hallmark indices' of the heat-adapted phenotype [164, 165].

Candidate biomarkers

It is well established that acute exposure to a hot environment that is sufficient to elevate thermal strain also elicits a pronounced neuroendocrine response [125]. Likewise, the role of the neuroendocrine system in the long-term adaptation to heat has long been recognised [27, 148], as has the multi-systemic nature of adaption to heat and the interconnection between the systems controlling thermoregulation and those regulating fluid balance (e.g. renin–angiotensin–aldosterone system), energy homeostasis (e.g. hypothalamic–pituitary–thyroid axis [200]) and sympatho-adrenal activity (e.g. sympathetic–adrenomedullary and hypothalamic–pituitary–adrenal axis) [83]. Accordingly, the initial focus of this section is the evaluation of research that has measured the concentration of molecules that are associated

with these physiological systems, or related processes, in response to HA. Given the notable thermoregulatory differences between species [225], we generally constrain our review to literature examining changes in the concentration of relevant molecules in the biological samples of humans undertaking HA, and the consideration of candidate biomarkers where there is a biologically plausible rationale for their measurement. We subsequently extend the scope of our review to encompass research that has examined changes in inflammatory biomarkers with HA, and the measurement of putative heat adaptation biomarkers in other biological mediums, including the measurement of intracellular heat shock proteins, as well as use of sweat biomarkers.

Fluid balance and regulation

Adaption to heat causes an array of phenotypic alterations that improve the regulation of water and electrolyte balance, including an increase in total body water and plasma volume expansion, and a reduction in electrolyte losses [22]. These adaptations are primarily controlled by the action of the renin–angiotensin–aldosterone hormone system [8]. In response to a decreased perfusion of the juxtaglomerular apparatus in the kidneys, renin is released, which enables the conversion of angiotensinogen to angiotensin-I and subsequently to angiotensin-II by the action of angiotensin converting enzyme [8]. Angiotensin-II causes constriction of resistance vessels to elevate blood pressure, reabsorption of sodium in the proximal tubules of the kidney, and the release of aldosterone by the adrenal gland and arginine-vasopressin (AVP) from the posterior pituitary gland; the latter is also released in response to hyperosmolality detected in the pre-optic anterior hypothalamus as well as in response to baroreceptor firing from hypovolemia [79, 207]. Aldosterone acts on the mineralocorticoid receptors in the distal tubule and collecting duct of nephrons in the kidney, directly impacting sodium absorption and potassium excretion, resulting in elevated plasma sodium and the osmotic retention of water in the blood, whereas AVP mainly acts on aquaporin channels in the kidney tubules increasing water reabsorption [79]. Plasma protein levels may also play a role in the expansion of plasma volume through an oncotic effect that may be mediated by the combined actions of the lymphatic system [155], increased albumin synthesis [221] and reduced total albumin losses [76], although this appears to be a supportive rather than dominant mechanism [162]. The potential utility of a range of a number of putative biomarkers associated with these processes is discussed below.

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Aldosterone

Although plasma aldosterone concentration increases may occur in response to intense exercise performed in temperate conditions [115], this effect is potentiated during exercise in the heat [135] and with hypohydration [56]. When plasma aldosterone is measured following a standard exercise-heat exposure (i.e. HAST) the aldosterone concentration is often shown to be diminished following an HA intervention (e.g. [54, 61, 96, 146, 189, 191]), likely as a result of increased sensitivity to the effects of aldosterone to changes in plasma osmolality [22]. However, this effect is no longer evident 1 week after HA [61] and the decline in plasma aldosterone during a post-HA HAST has not been observed in all studies (e.g. [10, 47, 100, 147]). Moreover, as detailed in Sect. "Introduction", the practical utility of undertaking a HAST may be limited in many contexts [160], and baseline changes in the concentration of fluid regulatory hormones are likely to be more relevant in terms of a viable candidate biomarker of HA.

A number of studies have shown a significant increase in the resting baseline concentration of plasma aldosterone following an HA intervention [54, 143, 146, 147]. This is exemplified by Nielsen et al. [146] who demonstrated that mean (SD) baseline plasma aldosterone concentration increased from 96.6 (12.8) pg mL^{-1} to 156.0 (15.3) pg mL^{-1} following 9–12 consecutive days exercise in dry heat (90 min day^{-1}), and Francesconi et al. [54] who demonstrated a significant increase in baseline plasma aldosterone concentration from ~12 to 17 ng dL^{-1} following a 10-day HA intervention (100 min day^{-1}) in a combination of hot-dry and hot-wet environments. This effect of HA on baseline aldosterone does not appear to be significantly affected by the superimposition of modest dehydration (average daily body mass loss − 2.71 kg) during the HA regime [143], but may be affected by the level of dietary sodium, with high dietary sodium consumption associated with an abolished aldosterone response and low dietary sodium associated with a potentiated aldosterone response [2]. However, it is important to note that some studies have shown unchanged (e.g. [10, 47, 61, 96, 100, 189, 191]) or even reduced [161] baseline plasma aldosterone concentration with HA. It has been speculated that these apparently divergent findings may be related to differences in sodium balance, as well as different experimental models, including the mode and method of HA and training status of the participants [22], seasonal effects on aldosterone levels have also been reported [47, 94], whereas the extent to which systemic aldosterone concentration reflects the concentration at the renal mineralocorticoid receptors is unclear. Nevertheless, a recent meta-analysis concluded that, although there was high inter-investigation variability, there was a small significant increase (+25±35%) in baseline plasma aldosterone concentrations following HA [205]. However, at present there is a paucity of data examining the time course of changes in resting aldosterone concentration with sufficient resolution over the induction and decay of HA and an increased understanding of the temporal relationship between plasma aldosterone and the plasma volume changes occurring with HA will be necessary to establish the utility of aldosterone as a HA biomarker.

Arginine vasopressin

It is generally accepted that, similar to plasma aldosterone concentration, plasma AVP concentration is acutely increased during intense exercise [79], but during exercise in the heat significant increases in plasma AVP are observed at lower work rates [64]. There is some evidence that the magnitude of increase in plasma AVP during exercise in the heat is diminished following HA [64, 146], although conversely, others studies have suggested that the increase in AVP might be larger following HA [147]. Similarly, Mudambo et al. [140] demonstrated that resting baseline plasma AVP levels were significantly elevated (+61%, $P<0.001$) in 24 soldiers following 30 days of field-based HA, but interventions of this duration are uncommon and this finding appears to be in the minority, with the majority of studies suggest that baseline plasma AVP levels are unchanged following an HA intervention (e.g. [61, 64, 146, 147, 161]). Overall, these data are consistent with a recent meta-analysis, which concluded that there was no effect of HA (− 5±15%) on resting plasma AVP concentration [205]. Nevertheless, this conclusion, as well as the apparently conflicting findings from different studies may, at least in part, be a consequence of the short half-life of AVP (<30 min), whilst the fact that AVP is unstable, even in isolated plasma [190], combined with its small size, means it cannot be measured by sandwich immunoassay, but only by less sensitive competitive immunoassays [137].

Copeptin

Given the challenges posed by plasma AVP measurement there has been a growing interest in the potential of surrogate measures of AVP concentration [190]. Copeptin is a 39-amino acid glycopeptide that forms the c-terminal part of the AVP precursor protein preprovasopressin [137]. It is released in an equimolar fashion with AVP from magno- and parvo-cellular neurons of the hypothalamus and parallels the increase in AVP in response to alterations in tonicity and blood pressure [25]. However, in contrast to AVP, copeptin has a longer half-life [15], is stable in serum or plasma for days [137] and is less technically challenging to assay [25]. Together

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these factors may overcome some of the issues associated with the measurement of AVP.

Stacey et al. [190] examined the plasma copeptin concentrations in humans in response to thermal stress, demonstrating a significant rise in copeptin [10.0 (6.3) vs. 16.7 (9.6) pmol L^{-1}, $P < 0.001$] among a group of 15 soldiers undertaking a 3.5 h simulated combat assault in a hot environment (27 °C), as well as evidence of a threshold effect, indicated by a negligible copeptin increase in individuals who did not exceed a deep body temperature of 38 °C. Moreover, the post-assault copeptin concentration was strongly related to the plasma osmolality ($r = 0.70$, $P < 0.01$), as well as the accumulated thermal stress (area under the curve for a deep body temperature > 37 °C; $r = 0.78$, $P < 0.01$). In a subsequent study, Stacey et al. [191] measured copeptin in a group of 23 soldiers over a 23-day natural HA intervention and demonstrated that neither the resting baseline, nor post-HAST, copeptin concentration changed over the HA period, although there was a rightward shift in the serum osmolality–copeptin relationship following HA. In a study by the same group, Omassoli et al. [156] again demonstrated that baseline copeptin was unchanged over the course of the 23-day HA intervention—a finding that has since been corroborated by another research group [152] employing a prolonged HA intervention ($\sim 28 \times 60$ min exercise-heat stress exposures over 5.5 weeks). However, in contrast to their previous study, Omassoli et al. [156] also reported that the post-HAST copeptin concentration was reduced over the course of the HA intervention, but their analyses do not clarify when the reduction in copeptin became significant, although the nadir value occurred on day 9 of the 23-day HA intervention.

At present, the reason for these discrepant findings are unclear, particularly given the commonalities between the studies. Moreover, plasma copeptin concentration appears to be affected by a number of non-thermal stimuli and disease conditions [137] and may therefore lack sufficient sensitivity and specificity to be utilised as a biomarker of HA. Thus, at present, the available research does not support the measurement of resting plasma copeptin concentration as a viable biomarker of HA, although there may be some utility if plasma copeptin is assessed following a standard HAST. Further research is needed to reconcile discrepancies between the limited number of available studies and to examine plasma copeptin levels over more common HA durations (e.g. 8–10 days; [205]) than those that have been studied to date (e.g. 23–30 days; [152, 156, 189, 191]). In addition, future studies measuring copeptin concentration should consider the fact that, while the longer half-life relative to AVP provides greater measurement stability [15], this could result in a temporal dissociation between these molecules in situations eliciting rapid changes in AVP concentration.

Renin

An increase in plasma renin concentration has frequently been demonstrated following exercise-heat stress [10, 33, 47, 54, 146, 147], with some evidence that the magnitude of increase in plasma renin may be attenuated following HA [47, 146] and that this effect may be greater than for other fluid regulatory hormones such as aldosterone [54], but this has not been shown to occur consistently [10, 33, 64, 146]. The reasons for these equivocal findings are not immediately clear, although Francesconi et al. [54] reported a more pronounced attenuation of the increase in plasma renin levels following HA when HASTs were performed in the hypohydrated state, compared to the euhydrated state, which was attributed to the combined effects of expanded plasma volume, attenuated renal vasoconstriction and a decreased sympathetic response to exercise-heat stress when heat adapted. Therefore, as has been described for other hormones involved in fluid regulation [140], variations in hydration levels may affect the influence of HA on renin levels during a HAST, with effects generally appearing to be more pronounced when hypohydration is superimposed on the exercise-heat stress.

Based upon the data of Finberg and Berlyne [47], Davies et al. [33], Armstrong et al. [10] and Nielsen et al. [146] a recent narrative review concluded that the baseline plasma renin concentration is unaffected by HA [22]. However, the data from Finberg and Berlyne [47], which demonstrated a small but statistically significant reduction in resting plasma renin concentration following a 7-day HA intervention, may have been misinterpreted. Similarly, data from Nielsen et al. [147], which claimed to show an increase in resting renin concentration with an 8 to 13-day HA regime consisting of daily exercise to exhaustion in hot-humid conditions, were not included in this review, although within this study there appears to be discrepancy between the text conclusion and the data presented.

Therefore, the data are again somewhat equivocal for the effect of HA on resting and post-HA HAST plasma renin concentration. Whilst protocol differences may influence these findings, it is worth noting that in a number of the studies demonstrating a null effect (e.g. [33, 146]), data were obtained from a limited number (≤ 6) of participants, which will have resulted in low statistical power and an increased likelihood of Type II error.

Atrial natriuretic peptide and related hormones

Atrial natriuretic peptide (ANP) is a small peptide that is secreted by the atria of the heart as result of atrial distension due to a high systemic blood pressure. ANP counteracts some of the effects of aldosterone, decreasing reabsorption of sodium from the inner medullary collecting duct and increasing urinary sodium excretion, as well as increasing vascular protein efflux through effects on capillary permeability [214], resulting in a decreased plasma volume [31]. Pro-ANP is the precursor to ANP, whereas brain natriuretic peptide (BNP) is excreted by the ventricular cardiomyocytes and exerts similar effects to ANP, but has a longer half-life and lower receptor biding affinity [141, 197], with both binding to natriuretic peptide receptor A to induce cyclic guanylyl monophosphate as a second messenger in the target cells [214]. Despite their role in fluid regulation, to date, very few studies have examined changes in plasma ANP, pro-ANP, and BNP levels following HA in humans. Mudambo et al. [140] demonstrated a small, statistically significant, reduction in resting plasma ANP concentration following HA, whereas plasma BNP was unchanged although, as mentioned previously, this study employed an atypical duration of HA (30 days *c.f.* 8–10 days) and was undertaken in a field setting. In contrast, Kraemer et al. [100] reported no changes in baseline plasma ANP over an 8-day HA intervention, and Patterson [161] reported no statistically significant difference in resting baseline plasma ANP among 12 individuals after either 8 days or 22 days of an HA intervention, with a numerical *increase* of 32% apparent on day 22, which might be reflective of the sustained hypervolemia induced by their experimental model [162]. Again, these divergent findings likely stem from differences in the types of HA intervention in these studies, which include natural and laboratory HA, different thermal forcing functions (e.g. controlled hyperthermia, fixed work rate) and various durations, amounts of exercise, and dietary and hydration conditions. Pro-ANP does not appear to be affected by HA [152], but has received limited attention in the context of HA.

Other fluid regulatory molecules

Other molecules that are involved in the control of fluid balance and may play a role in the adaptive responses seen with exposure to heat have received relatively little attention; we are unaware of any research that has examined the effects of HA on the resting plasma concentrations of angiotensinogen, angiotensin-I and angiotensin-II in humans. Changes in the plasma concentrations of these molecules have been documented during acute exposure to heat [99], but cross-sectional observational research suggests that there does not appear to be a seasonal effect on baseline angiotensin-II, even when these is some

evidence of acclimatisation [94]. Nevertheless, given the involvement of these molecules in the fluid-regulatory pathways that are linked to aspects of the heat-adapted phenotype, future investigation of these molecules may prove fruitful in the context of a biomarker of the heat-adapted state, although their utility could be limited by extremely short circulating half-lives [3, 80] and susceptibility to changes in hydration state [168].

Markers of kidney function and kidney injury

Heat stress represents a profound challenge to kidney function and can increase the risk of acute kidney injury (AKI) [182], which is augmented with increasing thermal strain and dehydration [23]. A number of recent studies have examined the effects of HA interventions on biomarkers of kidney function and AKI to establish if there is protective adaptation of the kidney with repeated heat exposure; biomarkers evidencing such an effect may have utility in identifying the heat-adapted phenotype. Based upon increases in serum creatinine and reductions in estimated glomerular filtration rate (calculated from serum creatinine concentration), Pryor et al. [167] reported that 75% of individuals undertaking 2 h of exercise in the heat exceeded the clinical threshold for AKI before a 4-day HA intervention, with only 58% of individuals meeting this threshold after the HA intervention. However, neither the mean baseline nor the mean post-exercise serum creatinine concentration were significantly altered by the HA intervention. Similarly, Omassoli et al. [156] reported a reduction in the number of individuals attaining serum creatinine levels exceeding the threshold for clinical AKI from 14/20 to 1/20 individuals following 23 days of HA. However, in contrast to Pryor et al. [167], a reduction in mean serum creatinine was also reported following a standard HAST, although baseline serum creatinine was unchanged. In a subsequent study, Ravanelli et al. [171] reported that neither glomerular filtration rate (calculated as the product of urine creatinine concentration and urine flow rate divided by serum creatinine concentration) nor its constituent analytes were altered by a 7-day passive HA intervention.

More recent research examining the effects of HA on AKI [73] has assessed the urine concentrations of neutrophil gelatinase-associated lipocalin (NGAL), a biomarker of renal tubular damage [133] and urinary kidney injury molecule-1 (KIM-1), a transmembrane glycoprotein that indicates proximal tubule injury [72]. This research indicated that urinary NGAL was unaffected by a HAST and did not differ pre- to post-HA, whereas KIM-1 was elevated following the HAST, but to a similar extent before and after HA, with no change in baseline concentration [73]. Thus, whilst there is some limited evidence that HA

may reduce the achievement of serum creatinine levels associated with AKI during a HAST, and that longer HA interventions may lower the post-HAST serum creatinine concentration, other biomarkers of AKI (e.g. NGAL, KIM-1) appear unaffected by HA. Moreover, none of these biomarkers of kidney of AKI have shown baseline changes following HA, indicating that they may have limited utility in the assessment of the heat-adapted phenotype. Finally, it is important to note that, to date, no studies examining the effect of HA on AKI have assessed urine concentrations of insulin-like growth factor binding protein 7 (IGFBP7) and tissue inhibitor of metalloproteinase 2 (TIMP-2), which appear to outperform other biomarkers of AKI in clinical settings [95]; these biomarkers should be considered in future research examining renal adaptions to HA.

Haematological indices associated with altered fluid balance

The hypervolemia that is a characteristic of the fluid shifts occurring with the heat-adapted phenotype typically results in a pronounced haemodilution, leading to a reduction in both the blood haematocrit (volume percentage of red blood cells in blood) and blood haemoglobin concentration [17, 74]. Because this hypervolemia is partly driven by an oncotic effect, to which albumin is the biggest contributor [180, 185], the expanded intravascular volume is also often associated with an increase in the plasma protein and plasma albumin content [162, 185]. This effect may become evident after 2 days of HA [185] and has been shown to persist for as long as 22 days when a controlled hyperthermia HA regime is used [162]. Although some studies have not reported an increase in plasma protein content with HA (e.g. [17]), this may be due to differences in the method of expression, with changes in total protein content typically more pronounced than changes in protein concentration [162], presumably because the latter acts as the stimulus for the plasma volume expansion. As a consequence, these simple haematological parameters may have some utility in identification of the heat-adapted state, but their time course of acquisition may precede the development of other aspects of this phenotype such as sudomotor changes [164]. Moreover, whilst within-individual reductions in haematocrit and haemoglobin concentration are consistently observed following an HA intervention [17], there is pronounced inter-individual variability in the baseline haematocrit and haemoglobin, which may be related to a range of physiological (e.g. sex [20]; training status [145], genetics [119]), environmental (e.g. hypoxic exposure[114]) and nutritional factors (e.g. hydration [26], protein and carbohydrate consumption [154]).

Similarly, recent research has shown that a prolonged HA regimen (5.5 weeks) can result in an increased haemoglobin mass [152], although this does not appear to be the case with shorter HA regimens [172], and the effects of HA on erythropoietin levels are equivocal [152, 172]. Moreover, plasma albumin and protein levels can increase with training intensification, independent of HA [152], and may be altered by dietary protein consumption [67]. Each of these factors could adversely impact on the utility of these simple haematological indices as a biomarker of HA and suggests that, in isolation, these measures may not be appropriate, but they may provide valuable information in situations where it is possible to control for the aforementioned potential confounders.

Energy homeostasis

Although the role of energy homeostasis in thermoregulation is well accepted [200], it has mainly been studied within the context of exposure to cold, and in the control of the production of additional heat via the influence of hypothalamic-pituitary-thyroid axis on the metabolic processes that constitute facultative (non-shivering) thermogenesis [195]. Similarly, the role of the prostaglandin–cyclooxygenase system is well established in febrile temperature regulation [86] and has recently received some attention in the context of exposure to cold [43, 50]. However, there is some evidence suggesting that these processes may also play a role in the adaptive processes to chronic heat exposure and as such may be suitable for further investigation in the context identifying a biomarker of the head adapted phenotype.

Thyroid hormones

Thyroid hormones play a central role in the control of heat generation from biological processes that are inherent to homeothermic species, including humans [87, 195]. The circulating levels of the thyroid hormones triiodothyronine (T3) and thyroxine (T4) are regulated by the hypothalamic–pituitary–thyroid axis. In response to low T3 and T4 levels the hypothalamus releases thyrotropin-releasing hormone (TRH), which stimulates the release of thyroid stimulating hormone (TSH) from the anterior pituitary. This results in the secretion of thyroid hormones from the thyroid gland (primarily T4) and the further production of the more potent T3 through the action of deiodinase on T4 in peripheral organs, including the liver. T3 and T4 are both active in their 'free' state (i.e. unbound to thyroxine-binding globulin, transthyretin, or albumin), and inhibit TRH and TSH, thus creating a negative feedback loop which regulates circulating levels [187]. T3 and T4 have multifarious effects including on development [130] and cardiovascular function [44], as well as on body temperature regulation [194], the latter appears to be mainly related to effects on metabolic rate and non-shivering thermogenesis [87, 195]. Nevertheless,

more than 50 years ago Weiner and Collins [27] suggested that *'a reduction in thyroid activity in hot conditions might play a part in maintaining thermal balance by bringing about a slowing in metabolism and heat output.'* (p. 790). Subsequent animal research demonstrated that rats treated with propylthiouracil to lower circulating T3 and T4 have a lower deep body temperature at rest and during heat stress [219], and that after a 4-week heat acclimation intervention blood T3 and T4 levels were spontaneously reduced in rodents [132]. These changes in thyroid hormone levels appeared to be causally related to the development of a more metabolically efficient phenotype during HA, as determined through measurements of cardiac mechanics, with the adaptations blunted when propylthiouracil was administered to maintain a euthyroid state throughout the HA [131], although it remains to be established if the improved metabolic efficiency reported after some human HA studies (e.g. [181, 188, 224]) is related to the action of thyroid hormones.

In humans, cross-sectional analysis of a large data-set of deep-body temperature measurements demonstrated a linear relationship between TSH levels and resting deep-body temperature [153]. A recent meta-analysis of studies in healthy euthyroid adults evidenced significant seasonal variation in thyroid hormones, including lower circulating TSH and T3 levels during the summer compared to the winter [105]. Beyond these cross-sectional observational studies, a limited number of studies have measured thyroid hormone levels in humans over the course of a HA regimen. An early study conducted by Sridharan et al. [196] reported a reduction in resting plasma T3 and T4 after 4, 2-h daily heat exposures (45 °C, 30% R.H.), accompanied by a reduction in tympanic temperature and increased sweating rate, with further reductions in plasma T3 evident after the 8 day of heat exposure. More recently, in an unpublished study, McIntyre [128] demonstrated that the change in resting plasma total T4 after a 6-day HA was related to the magnitude of the accumulated endogenous thermal 'dose' (total area under the curve for rectal temperature >38.5 °C, $r = -0.32$, $P = 0.021$), with a larger endogenous thermal dose resulting in a greater reduction in plasma total T4. In a subsequent study examining two different 12-day HA regimens, the same group demonstrated a significant reduction ($P = 0.006$) in resting plasma free T3 following a 12-day hot water immersion HA regimen [129], but no significant change in plasma T4 levels. Interestingly, in unpublished data from this group [128], it appears that the plasma free T3 level was related to the reduction in resting rectal temperature ($r = 0.47$, $P = 0.044$), as well as the endogenous thermal dose accumulated during a 12-day HA regimen ($r = -0.57$, $P = 0.017$).

Whilst these data suggest that the measurement of thyroid hormones may have utility in the assessment of heat-adapted phenotype, some caution is warranted. For instance, McIntyre [128] noted that the reduction in rectal temperature preceded the change in thyroid hormone levels during HA, indicating that either the thyroid hormones were not causally linked to the reduction in deep body temperature, or that they are only mechanistically relevant for the longer-term adaptive response. Moreover, circulating plasma T3 may be reduced after a single heat exposure [42], raising the possibility that measurements made in close proximity to an acute heat exposure could be similar to those found in the resting state following chronic heat exposure, but without incurring the adaptive benefits. There is also some evidence of sex differences in the thyroid hormone responses to a thermal stimulus [105], whilst non-thermal factors including calorific restriction [37], stress [78] and disease [89] also affect thyroid hormone levels and may therefore impact on their utility as a biomarker of heat adaptation.

The prostaglandin–cyclooxygenase system
Prostaglandins are potent bioactive lipid messengers belonging to the eicosanoids, a family of oxygenated 20 carbon fatty acids [111, 159]. There are four principal bioactive prostaglandins generated in vivo: prostacyclin (PGI_2), prostaglandin D_2 (PGD_2) prostaglandin E_2 (PGE_2) and prostaglandin F2α (PGF2α) [174] with PGE_2 the most common [111]. Prostaglandins are synthesised from arachidonic acid in most mammalian tissues via the actions of prostaglandin synthases and two cyclooxygenase (COX) isoenzymes: COX-1, which preferentially oxygenates exogenous arachidonic acid and is responsible for regulating baseline prostaglandin levels and mediates a range of 'housekeeping' functions, such as gastric epithelial cytoprotection and homeostasis [174]; and COX-2 which acts on endogenously produced arachidonic acid and controls prostaglandin synthesis in response to inflammation [159].

PGE_2 is a principal pyrogenic mediator of the elevation in core temperature occurring with fever [86]. The upregulation of PGE_2 during fever is functionally coupled to the inducible COX-2 isoform [178] with the first febrile-phase initiated by PGE_2 originating in peripheral tissues and the subsequent phase caused by the binding of PGE_2 to a specific EP3 receptor in the median pre-optic area to affect hypothalamic neurons that regulate thermoregulation. Although the role of this pathway in non-febrile temperature regulation is less well established, COX-2 gene deficient mice are unable to effectively defend their deep body temperature during cold exposure [116]. Research in humans has shown a thermogenic response of the COX-2/PGE_2 pathways during

acute (120 min) cold exposure in humans and a relationship between rectal temperature and PGE_2 during acute exposure to cold and thermoneutral conditions, although no effects were evident during acute exposure to hot (40 °C; 40% R.H.) conditions [43]. Similarly, administration of acetaminophen, a COX enzyme inhibitor, reduced core temperature of humans during an acute (120 min) passive exposure to 20 °C, 40% R.H [51] as well as during running [19] and cycling [126] exercise in the heat (30 °C, 50% R.H.); this latter finding has been challenged [28], whilst studies that have used forearm dermal microdialysis of COX inhibitors indicate that both COX isoforms may be involved in sudomotor regulation in the heat [58], although in contrast to studies of oral COX inhibitors (which may influence central and peripheral thermoregulatory mechanisms), microdialysis administration will only exert peripheral effects.

Given the purported role of COX/PGE_2 pathway on temperature regulation it is surprising that, as far as we are aware, only one study has examined these molecules in humans undertaking HA. Shin et al. [198] demonstrated that both serum PGE_2 and COX-2 were significantly reduced ($-$ 18.5% and $-$ 17.3%, respectively) following a 3-week HA intervention, with both of these molecules explaining a significant proportion of the variance in mean body temperature (PGE_2 $r^2 = 0.495$; COX-2 $r^2 = 0.369$). Interestingly, the concentration of serum orexin, a neuropeptide that is produced in the hypothalamus [34], was also reduced ($-$ 17.7%) and was also related to the variance in mean body temperature ($r^2 = 0.382$). Orexin serves a number of functions, including regulating sleep and wake states, feeding behaviour, reward systems, mood and cognition [24], with evidence from animal studies indicating that orexin is also implicated in the regulation of body temperature at rest [134, 223] and during exercise [123]. However, whilst the findings of Shin et al. [198] are promising, it has been noted that this study did not adjust for the plasma volume changes occurring with HA and should therefore be interpreted cautiously [43]. Moreover, they are based on a limited sample size ($n = 9$), and an atypical HA regime consisting of 10 bouts of 30-min hot water immersion over a 3-week period, as well as deep and mean body temperature calculation from tympanic measurement which is of questionable validity (e.g. [1]). Therefore, although this HA regime was apparently effective at inducing hallmark induced of heat adaption (reduced deep body temperature and increasing sweating), it remains to be seen if these molecules demonstrate the same responses over more traditional HA regimens in air, when deep body temperature is assessed using gold standard method (e.g. rectal or oesophageal), and when plasma volume changes are accounted for.

Sympathetic–adrenomedullary and hypothalamic–pituitary–adrenal axis

Evidence from HA studies suggests that the activity of the sympathetic–adrenomedullary (SAM) axis [46, 81, 118, 146, 156] and hypothalamic–pituitary–adrenal (HPA) axis [156, 192, 206, 208] may be altered in the heat-adapted state. The SAM and HPA axis work in coordination to regulate the 'stress response' over acute and chronic time scales [62, 204]. The processing of physiological stress signals by the brainstem and hypothalamus activates the neurocircuitry generating the SAM axis response, which is regarded as the most rapidly responding system of the stress response [62]. SAM activity is mainly mediated by the secretion of the catecholamines adrenaline and noradrenaline from the adrenal medulla and the release of noradrenaline from the sympathetic nerves [62], which initiates a range of physiological responses to prepare the body to respond to the stressor [204]. In contrast to the SAM axis, the HPA axis is a hormone system, which results in activation over a slower timescale than the SAM axis, but with more protracted effects [179]. Activation of the HPA axis is elicited by the paraventricular nucleus of the hypothalamus, which releases corticotropin-releasing hormone, and the arcuate nucleus which secretes growth hormone releasing hormone. Together this stimulates the secretion of adrenocorticotropic hormone and growth hormone from the anterior pituitary gland [7, 204]; AVP can also act synergistically with corticotropin-releasing hormone to stimulate adrenocorticotropic hormone secretion [106]. Thereafter, growth hormone binds to the growth hormone receptor and activates multiple downstream intracellular signalling cascades [7], whereas adrenocorticotropic hormone binds to receptors in the adrenal cortex of the adrenal gland, resulting in the release of the glucocorticoid hormone cortisol, which is the end hormonal product of the HPA axis [62, 204] and elicits a range of metabolic and immune functions that support the bodies response to stress [204].

Catecholamines

Sympathetic nervous system activity is implicated in thermoregulation during heat stress [150] and a change in SAM axis activity, typified by a reduction in sympathetic activity [81] and possibly increased parasympathetic activity [49], is characteristic of the heat-adapted state. Catecholamines have commonly been employed as molecular biomarkers of SAM axis activity [63, 68] and it appears that, compared to exercise in cool conditions, exercise in the heat results in an acute elevation in plasma catecholamine levels [46], with some evidence that this increase may become attenuated following HA [46, 81, 146, 166]. For example, Nielsen et al. [146]

reported a significant increase in plasma adrenaline and noradrenaline concentrations during HASTs undertaken before and after 9–12 days of 90-min daily exercise-heat stress, with the concentration of both catecholamines reduced after 30 min of exercise in the post-HA HAST. Subsequent research has examined the time course of the reduction in plasma noradrenaline concentration following daily 90-min bouts of walking at 40% of maximal oxygen uptake in the heat, with the post-exercise reduction in noradrenaline becoming evident from the third day onwards and paralleling the time course of reductions in exercise heart rate [81]. Similarly, Febbraio et al. [46] reported that the plasma adrenaline concentration was reduced at the end of a 40-min HAST following a 7-day HA intervention, although noradrenaline levels during the HAST were not changed, whereas Nielsen et al. [147] reported no change in either plasma catecholamine following HA, although this study utilised an exercise to exhaustion HAST which lasted longer post-HA and may have masked any effects on catecholamine concentration. However, none of the aforementioned studies reported changes in the resting baseline concentrations of either plasma catecholamine.

Given the short circulating half-life of 1–2 min, as well as the influence of postural changes and the stress of venepuncture on plasma catecholamines [163], urine catecholamine measurements may be preferable in some situations, but they are influenced by the duration over which samples are obtained [68, 163] and do not offer the same temporal resolution as plasma measurements. Studies measuring urine catecholamine concentrations over the course of HA have reported conflicting results. Using an 8-day HA intervention consisting of 90 min day^{-1} of exercise-heat stress, Maher et al. [118] demonstrated that urinary noradrenaline excretion, assessed from samples collected from the onset of exercise to 1 h after exercise completion, was reduced during the last 3 days of the HA intervention, compared to the first 3 days. In contrast, Leppäluoto et al. [112] demonstrated no effect on resting urine adrenaline concentration (collected from 20:00 to 0800), but a slight increase in resting urine noradrenaline in participants exposed to dry heat (in a Finnish sauna; 80 °C) for 1 h, twice a day, for 7 days. The reasons for these conflicting findings are unclear, but should be interpreted cautiously given that potential analytical challenges have been identified in the assessment of urine catecholamines [68], the differences in temporal resolution for the urine samples between the studies, as well as notable protocol differences (e.g. active vs passive HA). In addition, Leppäluoto et al. [112] noted high individual variation in their data, and changes to fluid consumption practice mid-way through their study may have confounded their data.

Nephrines

Given the reported challenges with plasma and urine catecholamine measurement it has been suggested that metanephrine and normetanephrine, the o-methylated extra neuronal metabolites of adrenaline and noradrenaline, respectively, may offer appropriate alternatives for the measurement of sympatho-adrenal activity [39], overcoming many of the limitations associated with catecholamine measurement [163]. Plasma nephrine measures are more stable than those of the catecholamines [36] and demonstrate good correlation with both the plasma concentration and urinary excretion of their parent compounds [176]. However, to date, only a small number of research studies, from the same research group, have examined the concentration of plasma nephrines in response to HA. In a published abstract, Stacey et al. [189] reported that the post-HAST concentrations of plasma normetanephrine and metanephrine were both significantly reduced ($P < 0.0001$) following a 23-day natural HA (plasma normetanephrine: 948 (328) vs 461 (132) pmol L^{-1}; plasma metanephrine: 302 (103) vs 230 (91) pmol L^{-1}). Moreover, both the Δ normetanephrine and Δ metanephrine were correlated with the reduction in physiological strain index (calculated from deep body temperature and heart rate) over the HA period, which was interpreted as evidencing reduced sympathetic activation and led the authors to conclude that plasma nephrines could have utility as a biomarker of heat tolerance. In a subsequent study, Stacey et al. [192] demonstrated that the plasma free normetanephrine concentration was increased during a HAST, with the increase attenuated on day 6 and 23 of the intervention (but not on day 9) which, in conjunction with heart rate variability analyses, was interpreted as indicating a transition towards diminished sympathetic activity. In contrast, plasma free metanephrine did not increase over each HAST, although a significant main effect of HAST day was reported, but the location of this effect was not identified. Most recently, Omassoli et al. [156] reported significant main effects for both baseline and post-HAST free plasma metanephrine concentration over a 23-day HA, again, the locations of any effects were not reported, but the numerical nadir for the resting baseline free plasma metanephrine concentration occurred on day 2, whereas the post-HAST value declined progressively, reaching a nadir on day 23. However, it is important to note that there is high similarly between the methods of Stacey et al. [189], Stacey et al. [192] and Omassoli et al. [156], and these studies may be reporting data from the same participant pool, with possible overlap between the studies.

Cortisol

In addition to the measurement of plasma nephrines, Stacey et al. [189], Stacey et al. [192] and Omassoli et al. [156] each reported reductions in the post-HAST plasma cortisol levels over the course of their HA regimen, with Stacey et al. [192] indicating that this effect was significant from day 6 onwards and Stacey et al. [189] reporting a relationship between the Δ cortisol ($r = 0.62$) and the reduction in physiological strain index over the HA regime. These findings are consistent with a number of others studies that have also reported a reduction in post-HAST plasma cortisol levels following both passive (e.g. [112, 166]), and active (e.g. [10, 33, 201, 206, 208]), HA protocols. For example, Pilch et al. [166] reported that the rise in cortisol over a sauna was attenuated in the final exposure in a group of females undertaking 7×30-min saunas over a 14-day period. Similarly, Armstrong et al. [10] reported a significant increase in plasma cortisol levels during a HAST undertaken on day 1 of an 8-day active HA regimen, which was not evident on the final day. However, it is important to note that, with the exception of Tamm et al. [201], these studies reported no change in the resting baseline plasma cortisol levels, and whilst the majority of studies report reductions in post-HAST plasma cortisol following HA, some studies have shown no effect (e.g. [212, 213]). Although the reasons for these discrepant findings are not immediately clear, cortisol displays strong circadian effects [215] which may influence pre–post HAST measures. Additionally, hydration appears to potentiate the cortisol response to exercise-heat stress [30] and may also mediate the adaptive responses with HA; Francesoni et al. [55] reported that an increase in plasma cortisol during a HAST was only evident when participants were hypohydrated, but not when euhydrated, and that when the HAST was repeated in the hypohydrated state following a period of HA the increase in plasma cortisol was attenuated.

Other HPA-axis hormones

Compared to studies examining the cortisol response, a limited number of studies have measured other HPA-axis hormones in response to HA, with most focusing on adrenocorticotropic hormone (e.g. [112, 166, 201, 206]) or growth hormone (e.g. [55, 146, 147, 157]); we were unable to identify studies reporting changes in corticotropin-releasing hormone during HA in humans. Leppäluoto et al. [112] reported that plasma adrenocorticotropic hormone levels, measured post-sauna were unchanged over a twice-daily, 7-day sauna HA intervention, with similar data presented by Pilch et al. [166] when the same number of saunas were undertaken over 14 days. In contrast, Timpmann et al. [206] reported that plasma adrenocorticotropic hormone levels were attenuated during a HAST performed after an 8-day active HA, with similar data also reported by Tamm et al. [201] following a 10-day active HA. In each instance, baseline plasma adrenocorticotropic hormone levels were unchanged by HA. The discrepancies between these studies in the post-HAST changes may be related to differences in both the HA regimes (passive vs active) and HAST, with the exercise-heat stress approach employed by Timpmann et al. [206] and Tamm et al. [201] likely eliciting a significantly greater thermo-physiological strain than the passive sauna approach of Leppäluoto et al. [112] and Pilch et al. [166]. Circulating growth hormone levels are known to increase with hyperthermia [57] and thus a decrease in growth hormone concentration might be hypothesised following a period of HA concomitant with the reduction in deep body temperature. However, data on the effect of HA on growth hormone levels are similarly equivocal, with studies showing no effect following a HAST [146, 147], an attenuated response [157] or inconsistent findings [55], although these differences are not easily attributable to protocol differences given that all employed active HA and HAST approaches, they might be influenced by the pulsatile nature of growth hormone release [75]. Nevertheless, baseline plasma growth hormone responses were consistent, being unaffected by HA in each of these studies.

Taken together, the majority of studies suggest that HA does not have any substantial effect on resting baseline SAM or HPA-axis hormones, but may reduce the post-HAST levels, particularly with HAST approaches that elicit a pronounced thermo-physiological strain (e.g. active approaches). Given the established effect of an elevation in deep body temperature on SAM and HPA-axis hormone release [125], the post-HAST reductions in SAM and HPA-axis hormones following HA are likely reflective of attenuated thermo-physiological strain in the heat-adapted state. Interestingly, this effect does not appear to be evident in the resting state, despite the fact that resting deep body temperature is typically reduced with HA [164, 165], which may indicate a potential floor effect for these hormones. Therefore, SAM and HPA-axis hormones appear to have limited utility as a biomarker of the heat-adapted phenotype when only a resting baseline measurement is possible, but may have some utility in contexts where individuals can be exposed to a standardised thermal stressor, i.e. a HAST.

Inflammatory markers

The most commonly measured inflammatory markers in the context of HA are the interleukins (IL), IL-6 and IL-10 as well as tumour necrosis factor (TNF) α, and C-reactive protein; other inflammatory markers have received

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scant attention. Interleukins are a type of cytokine that are expressed by leukocytes, as well as other cells, and regulate immune and inflammatory actions through multifarious actions on immune cells; including activation, differentiation, proliferation, maturation, migration, and adhesion. IL-6 is regarded as having both anti- and pro-inflammatory effects with IL-10 being considered an anti-inflammatory cytokine [91]. TNF α is an inflammatory cytokine produced by macrophages/monocytes during acute inflammation [85] and C-reactive protein is an acute phase reactive protein that is induced by IL-6 and has both pro- and anti-inflammatory properties [144].

Interleukin-6 and interleukin-10

The plasma concentration of both IL-6 and IL-10 has been shown to increase in response to an acute exercise-heat stress (e.g. [30, 70, 71, 104, 110, 173], although some studies have failed to report an increase [92, 149]. These differences between-studies could be the result of a number of factors including the magnitude of thermal stress that the participants were exposed to during the HAST [173], which is likely to be influenced by the HAST protocol as well as the health and fitness of the participants. IL-6 appears to be the most commonly investigated cytokine in the context of HA and whilst a number of studies investigating the effect of HA interventions on IL-6 have shown that neither the baseline nor the post-HAST IL-6 concentrations are different following a period of HA ([30, 71, 93, 213], others have indicated that plasma IL-6 concentration may be altered with HA. For instance, Lee and Thake [110] reported that the increase in IL-6 during exercise-heat stress was reduced on the 10th day of HA. Omassoli et al. [156] also reported significant main effects for both the baseline and post-HAST IL-6 concentration measured on days 0, 2, 6, 9 and 23 of HA, but the exact days when the differences occurred was not reported, which limits the conclusions that can be drawn. Similarly, although Kuennen et al. [104] reported that the immediate and 2 h post-HAST IL-6 data were unchanged following HA, there was evidence for a more-rapid return to baseline 2 h post-HAST. We were unable to identify any studies that have reported a reduction in baseline plasma IL-6 concentrations following a period of HA.

Compared to IL-6, a smaller number of studies have investigated the effect of HA on IL-10. Yamada et al. [218] showed no increase in IL-10 in HASTs undertaken before or after HA, whilst conversely, Kuennen et al. [104] demonstrated that the increase in IL-10 that was observed in an initial HAST was not evident after 7 days of HA, with similar data reported by Lee and Thake [110] on the 10th day of HA. Closer inspection of Kuennen et al [104] data suggests that following HA the baseline IL-10

concentration was approximately halved (from $\sim 10 \pm 1$ to $\sim 5 \pm 1$ pg·mL^{-1}), but their statistical analysis, which does not appear to include a between group component, did not evaluate this. As far as we are aware, there are no studies that have reported a statistically significant reduction in baseline plasma IL-10 concentration. The apparent discrepancies between HA studies that have measured plasma cytokine concentrations can be explained by the research of Rhind et al. [173], which demonstrated that cytokine concentration increases during exercise are only evident when an individual becomes hyperthermic, and may be abolished when deep body temperature is clamped at resting levels during the same exercise task. Although the threshold deep body temperature for promoting the release of the various cytokines is unknown, these data provide a plausible mechanism accounting for the lack of increase in cytokine levels during the initial HAST in some studies (i.e. the thermal threshold for cytokine release was not surpassed) as well as the reduction seen in the post-HAST cytokine levels following a period of HA, where an elevation in cytokine levels was seen in the initial HA (i.e. the lower thermal strain during the HAST when heat adapted means that the individual no longer surpasses the thermal threshold for cytokine release).

Tumour necrosis factor α and C-reactive protein

Although TNF α levels have been shown to increase when exercise in undertaken in the heat compared to cooler conditions [193], a limited number of studies have reported plasma TNF α levels in response to HA. Both Kuennen et al. [104] and Lee and Thake [110] observed no increase in plasma TNF α concentrations in HASTs undertaken before or after HA, in each instance the HASTs employed were sufficient to elevate the concentration of other cytokines (e.g. IL-6, IL-10) suggesting that TNF α is less sensitive to heat stress than other cytokines. Similarly, Costello et al. [30] reported that C-reactive protein was unchanged in HASTs undertaken before or after 10 days of HA. Kaldur et al. [92] reported an in increase in the log C-reactive protein concentration during an endurance exercise task following HA, but the open-ended nature of their endurance task resulted in an increased test duration following the HA and limits the ability to compare these findings to other studies. In each of the aforementioned studies there was no evidence for baseline changes in the TNF α or C-reactive protein levels. This observation, as well as the high reported daily variability of a number of these inflammatory biomarkers [70], and the fact that inflammation is a generalised immune response which may be elicited numerous non-thermal stimuli, will likely negate the use of inflammatory

markers in the context of a biomarker of the heat-adapted phenotype.

Heat shock proteins

HSPs are a conserved group of proteins that vary in molecular mass from ∼ 15 to 110 kDa [101]. The expression of these proteins can be induced by a variety of stressors, including exercise, hypoxia, energy depletion, acidosis, reactive oxygen and high body temperatures [101, 103], with the 72-kDa protein (HSP72 or HSP70) appearing to be the most inducible and thermosensitive HSP [101], as well as the most studied HSP in the context of HA. Within cells these HSPs (iHSP) prevent apoptosis and inflammation [222] and act as a molecular chaperone, accelerating cellular repair from heat stress, ischaemia and endotoxic shock and providing cytoprotection against subsequent, more extreme, and potentially lethal exposure to these stressors [6, 101, 108]. HSPs can also be released into the systemic circulation from a variety of cells (eHSP), where they appear to serve a range of immunological functions [103].

Extracellular heat shock proteins

A number of studies have examined the effect of HA on extracellular HSP (eHSP) levels in plasma and serum, with the possibility of using eHSP as a biomarker of the HA phenotype proposed by Kresfelder et al. [102], who demonstrated that individuals who were able to effectively adapt to a 6-day HA regimen had a concomitant reduction in resting eHSP70. However, subsequent studies demonstrated mixed results. For instance, Yamada et al. [218] and Neal et al. [143] both reported that the resting baseline eHSP70 concentration was unchanged following 10–11 days of HA, with Yamada et al. [218] also demonstrating that eHSP levels did not increase in HASTs performed before and after the HA. In contrast, Marshall et al. [121] demonstrated a small post-exercise increase in eHSP on each of 2 consecutive days of exercise-heat stress, as well as a significant reduction in baseline eHSP70 levels on the day after the second heat exposure, but this likely proceeded the development of many of the whole-body indices of adaptation and so the physiological relevance of this change as a biomarker of HA is questionable. However, some studies have demonstrated that HA attenuates the eHSP response to a HAST. For example, Magalhães et al. [117] reported that although baseline eHSP70 was unaffected by an 11-day HA regime, a significant increase in eHSP70 observed in the HAST undertaken before HA was abolished in the post-HA HAST; a similar attenuation of the eHSP response to exercise-heat stress was reported by Lee and Thake [110] following a 10-day HA regime.

The reasons for the differences in eHSP response between these studies is not clear, although the data of Magalhães et al. [117] demonstrated that the increases in eHSP70 following a HAST had returned to baseline within 1 h, indicating that sample timing may influence findings. In addition, the eHSP concentration during exercise appears to be closely related to the peak deep body temperature achieved [117], which may need to exceed 38.5 °C to elicit an increase in eHSP70 levels [65]. Thus, taken together, it appears that there is some evidence for a post-HA reduction in eHSP levels when measured after a HAST, which may be a function of a lower deep body temperature when heat adapted, but this has not been universally demonstrated. However, baseline eHSP levels do not appear to be consistently reduced in the heat-adapted state. This conclusion is in keeping with the trivial effect-size of HA on resting eHSP70 (Hedges' $g=0.18$) reported in a meta-analysis [205]. Moreover, eHSP may also be elevated in inflammatory clinical conditions, unrelated to heat adaption (e.g. [109]), whilst the immunological functions of eHSP have led to the suggestion that it might have utility as a biomarker of overtraining [103] and data from Guy et al. [70] have shown a high within-participant variability in eHSP (37%) when measured within a 7-day period. Taken collectively, these factors may limit the sensitivity of eHSP measurements for identifying individuals who have acquired the heat acclimated phenotype.

Intracellular heat shock proteins

Although sample collection is slightly more technically challenging than for the measurement of eHSP, intracellular HSP70 (iHSP) content has been quantified within PBMCs in a large number of HA studies (e.g. [5, 110, 117, 122, 127, 218]), although more invasive measurements of muscle HSP content are sometimes reported [208]. Research examining iHSP levels during HA in humans has shown that shorter durations of HA, e.g. 1 to 2 days [122, 218] do not typically result in a measurable increase in iHSP70 levels, whereas significant increases in iHSP70 content may develop from 3 days onwards [110], and are commonly reported over 6 to 10 days of active HA [5, 110, 117, 122, 127, 218]) where a progressive accumulation of iHSP70 may be evident [110]. Indeed, a recent meta-analysis concluded that HA has a large significant effect overall on iHSP70 protein expression (Hedges' $g=0.97$), with the number of days of HA a significant moderator of the iHSP response [142]. However, it is interesting to note that Watkins et al. [208] did not detect any significant increase in muscle HSP content after completion of a 7-day active HA intervention, but the reasons for the differences between this study and those utilising the measurement of iHSP70 levels in

PBMCs is not clear. Likewise, Morton et al. [138] demonstrated that passively elevating core and muscle temperature to levels encountered during aerobic exercise did not increase skeletal muscle iHSP, implying non-thermal factors elicit the elevations in muscle HSP content seen with exercise in the heat.

In addition, whilst the majority of research has focused on iHSP70/HSP72, a small number of studies have examined the effect of HA on iHSP90. First among these were McClung et al. [127] who demonstrated that the increase in iHSP90 (21.1%) was similar to the increase in iHSP72 (+17.7%) over a 10-day HA intervention. Similarly, Gibson et al. [66] demonstrated that iHSP90α mRNA changes over the course of HA paralleled the changes in iHSP70 mRNA, with both increasing to a similar extent during exercise-heat stress on day 1 and 10 of an HA intervention. McClung et al. [127] also reported a significant correlation ($r^2 = 0.89$) between the percentage change in AUC for a deep body temperature of > 38.0 °C and ex vivo HSP90 inducibility between days 1 and 10 of the HA intervention, but not HSP72, suggesting that iHSP90 could have some benefit over iHSP70 in terms of identifying individuals who have acquired a given level of adaptation to heat, possibly because HSP90 plays a functionally important role in the regulation of cutaneous blood flow during exercise-heat stress [59]. However, it is also important to note that other stressors such as exercise [113] and hypoxia can result in increased iHSP, albeit possibly at a lower level than that induced by exposure to heat stress [110] and there is also some evidence for seasonal variation in iHSP levels [217].

Sweat biomarkers

Whilst biomarker development has traditionally focused on blood and urine analysis, there is a growing realisation of the potential of sweat analysis in a biomarker context [199], driven in part by technological developments in wearable sensor technology [151]. Although sweat is primarily composed of water, it also contains a mixture of chemicals in varying concentrations, including electrolytes (Na^+, Cl^-, K^+, Ca_2^+, Mg_2^+, Fe_2^+), micronutrients (vitamins), metabolites (e.g. glucose, lactate, ammonia, urea, bicarbonate, amino acids, ethanol), cytokines, and cortisol [13]. The recent application of high-throughput metabolomic and proteomic approaches (e.g. [82, 186]), has enabled further quantification of an extensive range of molecules in sweat, which may have future utility in a biomarker context (see Sect. "Challenges and opportunities" for further discussion of 'omics' approaches). However, whilst the vast majority of these molecules have yet to be examined within the context of HA, reductions in the electrolyte content of eccrine sweat, and specifically sweat sodium (Na^+) and chloride (Cl^-), with HA are well established [205] and may result from the increased eccrine gland responsiveness to aldosterone [96] which will increase sweat Na^+ reabsorption by increasing the activity of Na^+–K^+-ATPase on the basolateral membrane in the eccrine sweat duct [13].

Recently, the changes in sweat electrolyte concentration following HA have been assessed by meta-analysis with both Na^+ and Cl^- demonstrating a large effect-size for the reduction in the concentration of both sweat Na^+ (Hedges' g = − 0.94) and sweat Cl^- (Hedges' g = − 2.02) [205]. This meta-analysis also concluded that the concentration of sweat K^+ is unchanged with HA, a finding that is consistent with recent primary research examining the daily time course of changes in sweat electrolyte concentration over a 10-day heat acclimation programme [97], and demonstrating that the reductions in sweat Na^+ and Cl^- concentration were significant from the third day of HA onwards. Klous et al. [97] also measured sweat lactate concentration over the course of the HA intervention, which broadly paralleled the time course of reductions in sweat Na^+ and Cl^-, becoming significant from day 6 onwards and appearing to be caused by a greater dilution due to an increased local sweat rate, rather than a reduced excretion rate [97]. The observation of a reduced sweat lactate concentration with HA has been replicated by others (e.g. [209]). Given that the changes in the concentration of the various sweat electrolytes may occur through different mechanisms, each may provide subtly different information about the adaptive process to heat. For instance, sweat Na^+ concentration may yield information about the reabsorption rates of Na^+ by sweat ducts, which may be linked to fluid-regulatory balance, whilst sweat lactate concentration may provide useful information about alterations in sweating rate, which is linked to maximum evaporative heat loss potential, although the latter is also influenced by metabolic stress [4].

The reduction in sweat Na^+ concentration appears to persist with longer HA regimes > 20 days [189, 191], decay when the HA is stopped [97, 143] and be less affected by diet than plasma aldosterone concentration [2]; it can also be re-established with a period of re-acclimation [97]. Together this suggests that the concentration of certain sweat electrolytes may usefully track changes in heat acclimation state, but further research is required to verify this. However, caution is warranted due to reported substantial inter and intra-individual variability in sweat Na^+ concentration [11]. Sweat composition is influenced by multifarious factors not related to HA, including training status, genetics/disease, exercise intensity, diet, hydration status, sweat collection method and collection site [12], each of these factors may adversely impact on the utility of sweat as a biomarker of the heat-adapted state. From a practical perspective, because of

the low rate of resting eccrine sweat secretion, some form of exercise-heat stress (e.g. HAST) is typically required in order to stimulate sufficient sweat production to enable collection; as we detailed in Sect. "Introduction", this may be neither desirable nor practical. However, a significant reduction in resting sweat Na^+ concentration has been reported following both 6- and 23-day HA programmes using pilocarpine iontophoresis [35, 189, 191]. Similarly, new technological advances incorporating the use of microfluidics and electrochemical sensing into wearable sweat patch development may support improved resting sweat collection in the future [151, 199].

Challenges and opportunities

Based upon the information presented in Sect. "Candidate biomarkers" it is clear that, following a period of HA, the concentration of a number of readily measurable biomolecules may be reduced during exercise-heat stress (i.e. a HAST), reflecting the reduction in thermophysiological strain across numerous body systems (see Table 1). These biomolecules might, therefore, have potential utility as biomarkers of the heat-adapted phenotype. However, beyond negating the need for invasive deep-body temperature measurements, the practical advantage of measuring these biomolecules in this way is limited, because the considerable logistical challenges and personnel burden of administering a suitably standardised HAST remain. Moreover, the extent to which such a biomarker would provide additional information over a range of simple and minimally invasive physiological measures that are already commonly employed in a HAST, such as the assessment of whole-body sweating rate (through body mass change) or sweat Na^+ concentration, cardiovascular strain, plasma volume changes (through fingertip capillary blood sampling) or skin temperature measurement, is questionable.

Among the various biomolecules that we have reviewed, there is some evidence that the plasma, serum, sweat or intracellular concentration of a small subset of these may be altered in the resting state following HA (see Table 1). For the reasons outlined above, a change in the resting concentration of a given biomolecule is likely to be of greater practical relevance in the context of developing a molecular biomarker of the heat-adapted state than changes observed during a HAST. However, as we have highlighted, for many of these putative molecular biomarkers the data are limited at present to a small number of studies, or are not conclusive and further research is needed to confirm the robustness of reported baseline changes; in the majority of cases relevant data are lacking from female volunteers. Moreover, given the pronounced hypervolemia that occurs with HA, it is interesting that some of the studies reviewed have not reported appropriate adjustment of biomarker concentrations to

account alterations in plasma volume [124]. Without these adjustments, for a fixed circulating amount of a molecule the resting plasma/serum concentration will typically be reduced following a period of HA, but as a consequence of the hypervolemia rather than a change in the amount of the circulating molecule. Differences in the application of this adjustment among the studies that we have reviewed may have contributed to some of the inter-study variability.

Moreover, HA has been described as a continuum of many parallel processes [120]. For example, fluid regulatory changes and the associated haematological effects may be observed very quickly (e.g. 2–3 days; [14]), whereas cytoprotective (>3 days; [142]) and sudomotor adaptions (e.g. 7–14 days; [175]) typically develop over longer timescales. Thus, whilst the measurement of a given biomolecule may provide useful information about changes in a particular biological system, it may not necessarily provide information about the integrated adaptive process to heat, i.e. the overall state of 'heat readiness'. For instance, a change in a biomarker indicative of expanded plasma volume (e.g. haemoglobin concentration) might precede a change in a biomarker of cytoprotection (e.g. iHSP70) or sudomotion (e.g. sweat Na^+ concentration). Temporal dissociation between changes in the concentration of some of these putative biomarkers and the induction of the 'hallmark' indices of the heat-adapted state may confound the development of a biomarker of the heat-adapted state. Allied to this, we are not aware of any research that has examined the relationship between the rate of decay in hallmark indices of HA that occurs once the heat stressor is removed and the kinetics of many of these biomolecules, but it is known that, similar to the induction time-course, the decay rate of the various hallmark indices of heat adaption is also variable [32]. Therefore, research is needed to better understand the temporal relationship between changes in biomolecule concentration and the "hallmark" indices of the heat-adapted phenotype during, and following, an HA programme.

From the information presented in Sect. "Candidate biomarkers", it is also apparent that the concentration of many of these putative biomarkers has the potential to be altered by a variety of non-thermal stressors. This is perhaps unsurprising given the multi-systemic nature of the adaptions to heat, but will likely result in commonalities in the biochemical changes induced by heat exposure and those occurring with exposure to other stressors such as: exercise; infection; dietary change (calorific intake or composition); disease; psychological stress; other environmental stressors such as hypoxia. Such an overlap is likely to adversely impact on the sensitivity and specificity of a given biomolecule in providing useful information about the heat-adapted phenotype.

Table 1 Summary of candidate biomarkers for assessing the heat acclimated phenotype at rest and following a Heat Acclimation State Test (HAST)

Biomarker	Medium	Post-HA baseline change			Post-HA HAST change	
Fluid regulatory		Results			Results	
Aldosterone	Blood	↑**	↓*	↔**	↓**	↔**
Arginine-vasopressin	Blood	↑*		↔**	↓**	↔**
Copeptin	Blood			↔**	↓*	↔*
Renin	Blood	↑*	↓*	↔**	↓**	↔**
Atrial natriuretic peptide	Blood		↓*	↔**		
Pro-Atrial natriuretic peptide	Blood			↔*		
Brain natriuretic peptide	Blood			↔*		
Renal stress and injury						
Creatinine	Blood			↔**	↓**	↔**
Neutrophil gelatinase-associated lipocalin	Urine					↔*
Kidney injury molecule-1	Urine					↔*
Haematological						
Haematocrit	Blood		↓**			
Haemoglobin concentration	Blood		↓**			
Haemoglobin mass		↑*	↔*			
Plasma protein	Blood	↑**		↔*		
Erythropoietin	Blood	↑*		↔*		
Energy homeostasis						
Triiodothyronine (T3)	Blood		↓**	↔*		
Thyroxine (T4)	Blood		↓**	↔*		
Orexin	Blood		↓*			
Prostaglandin E2	Blood		↓*			
Cyclooxygenase-2	Blood		↓*			
Sympathetic–adrenomedullary axis						
Adrenaline	Blood			↔**	↓**	↔*
Adrenaline	Urine			↔*		
Noradrenaline	Blood			↔**	↓**	↔**
Noradrenaline	Urine	↑*			↓*	
Metanephrine	Blood			↔*	↓*	↔*
Normetanephrine	Blood		↓*	↔*	↓**	
Hypothalamic–pituitary–adrenal axis						
Cortisol	Blood		↓*	↔**	↓**	↔*
Adrenocorticotropic hormone	Blood			↔**	↓**	↔**
Growth hormone	Blood			↔**	↓*	↔**
Interleukin-6	Blood			↔**	↓**	↔**
Interleukin-10	Blood			↔**	↓*	
Tumour necrosis factor α	Blood			↔**		↔**
C-reactive protein	Blood			↔*		↔*
Heat shock						
Intracellular heat shock protein 70	PBMC	↑**				
Intracellular heat shock protein 70	Muscle			↔*		
Extracellular heat shock protein 70	Blood		↓**	↔**	↓**	↔**
Intracellular heat shock protein 90	PBMC	↑*				
Sudomotor						
Sodium	Sweat		↓**		↓**	
Chloride	Sweat				↓**	
Potassium	Sweat					↔**
Lactate	Sweat				↓**	

Table 1 (continued)

↑ = increase in concentration or level of biomolecule; ↓ = decrease in concentration or level of biomolecule; ↔ = no change in concentration or level biomolecule

HA heat acclimation/acclimatisation, *PBMC* peripheral blood mononuclear cell

*Study supporting identified change when heat adapted

**Multiple studies supporting identified change when heat adapted

However, it has been recognised that exposure to different stressors may invoke common adaptive pathways (e.g. heat and hypoxia), and that the adaptions occurring through exposure to one stressor may confer both acclimatory (e.g. whole body) and cytoprotective (e.g. cellular) effects when exposed to another stressor [40]. As such, it is unclear to what extent, if any, the potential overlap between changes in biomolecules occurring with exposure to non-thermal stressors would present a meaningful problem, given that such changes may still be indicative of an improved ability to tolerate heat stress. Similarly, whilst seasonal changes may occur in a number of the biomolecules that we have identified, these may simply be reflective of natural acclimatisation to an increased ambient temperature, and would therefore be expected to improve tolerance to thermal stress.

Taken together, the evidence presented in this review suggests that, at present, no single biomolecule (i.e. a univariable approach) will provide sufficient information or demonstrate adequate sensitivity and specificity to act as a biomarker of the heat-adapted phenotype. However, the use of multivariable modelling approaches has been successful employed in other fields to develop more powerful biomarkers with superior diagnostic ability (e.g. [48]). Such an approach may be particularly well suited to the multi-system adaptions that are characteristic of HA. Ensuring that such a model includes variables indicative of a range of body systems associated with HA, as well as potentially including information from other factors known to influence thermoregulation such as anthropometric (e.g. body mass, body composition, body surface area) and demographic factors (e.g. age and gender) [77]), might overcome some of the challenges identified.

Moving beyond multivariable approaches incorporating small numbers of variables (e.g. < 10 variables), the future application of 'omics' approaches including metabolomics and transcriptomics may prove fruitful. The metabolome is directly linked to changes in cell metabolism that are caused by environmental stimuli, as well as diseases, and utilises the measurement of hundreds to thousands of metabolites within a biological medium. Whilst this approach has already proved fruitful in biomarker development [98], it is not without challenges including strict technical requirements related to sample storage and analysis, high inter-individual variability, the potential confounding effects of a range of demographic factors, and the need for large samples sizes (e.g.

n = 200–400) to protect against false discovery rate [203]. The 'n' for most HA studies is an order of magnitude lower than that recommended for metabolomics studies owing to the time and resource intensive nature of this type of work [205].

The transcriptome is the complete set of all RNAs transcribed by certain tissues or cells and has emerged as a powerful approach for investigating the molecular response to environmental stressors [220]. Indeed, the transcriptomic response of human PBMCs to heat has recently been reported, and is characterised by significant gene suppression and differentially expressed genes encoding proteins involved in proteostasis, energy metabolism, cell growth and proliferation, and cell death and survival [18]. However, the significance of these observations in terms of developing a biomarker of heat adaptation is, as yet, unclear. Finally, technological advances in the development of sensors for non-invasive and minimally invasive continuous analyte monitoring are now enabling the development of wearable devices that are overcoming historical challenges for biological sample analysis [60], and in combination with real time biomonitoring techniques and more complex analysis approaches (e.g. the use of resting heart rate variability; [49]), may pave the way for addressing the aforementioned temporal problems.

Conclusions

Quantifying the level of phenotypic adaption to heat from the measurement of a biomolecule(s) may be desirable in a number of contexts (e.g. defence, occupational, sporting), particularly with emerging technological developments. Such a biomarker may have utility to characterise the 'heat readiness' of an individual, thereby reducing heat-illness risk and increasing operational and training capacity. This narrative review of literature has shown that the concentration of a number of biomolecules implicated in fluid regulation, energy homeostasis, sympatho-adrenal balance, inflammation, sudomotor adaption and acquired thermal tolerance may be altered when an individual is exposed to a standard heat stress following a period of HA. However, only a small subset of these biomolecules appears to be altered at baseline. Moreover, for many of these putative biomarkers the data are equivocal and/or limited at present to a small number of studies and (male) participants. Given the multi-systemic nature of adaption to heat, we speculate that no

single biomolecule is likely to have sufficient sensitivity and specificity as a biomarker of the heat-adapted phenotype when used in isolation. Thus, we propose that future research should verify the robustness of the change in the baseline concentration of the identified biomolecules following a period of HA and their temporal association with different 'hallmark indices' of the heat-adapted phenotype (i.e. physiological biomarkers, including appropriate deep body temperate measurements). The use of multivariable approaches that include biomarkers providing information about a range of physiological systems should also be explored.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
JSY has undertaken contract research for Takeda and Boston Scientific, collaborated with Pfizer on an EU FP7 grant, INComb, undertaken consultancy for Tena and Essity, and received funding for a research conference from Astallas. The other authors declare that they have no competing interests.

References

1. Aadal L, Fog L, Pedersen AR (2016) Tympanic ear thermometer assessment of body temperature among patients with cognitive disturbances: an acceptable and ethically desirable alternative? Scand J Caring Sci 30(4):766–773. https://doi.org/10.1111/scs.12303
2. Allsopp AJ, Sutherland R, Wood P, Wootton SA (1998) The effect of sodium balance on sweat sodium secretion and plasma aldosterone concentration. Eur J Appl Physiol Occup Physiol 78(6):516–521. https://doi.org/10.1007/s004210050454
3. Al-Merani SA, Brooks DP, Chapman BJ, Munday KA (1978) The half-lives of angiotensin II, angiotensin II-amide, angiotensin III, Sar1-Ala8-angiotensin II and renin in the circulatory system of the rat. J Physiol 278:471–490. https://doi.org/10.1113/jphysiol.1978.sp012318
4. Ament W, Huizenga JR, Mook GA, Gips CH, Verkerke GJ (1997) Lactate and ammonia concentration in blood and sweat during incremental cycle ergometer exercise. Int J Sports Med 18(1):35–39. https://doi.org/10.1055/s-2007-972592
5. Amorim F, Yamada P, Robergs R, Schneider S, Moseley P (2011) Effects of whole-body heat acclimation on cell injury and cytokine responses in peripheral blood mononuclear cells. Eur J Appl Physiol 111(8):1609–1618. https://doi.org/10.1007/s00421-010-1780-4
6. Amorim FT, Fonseca IT, Machado-Moreira CA, Magalhães FC (2015) Insights into the role of heat shock protein 72 to whole-body heat acclimation in humans. Temperature 2(4):499–505. https://doi.org/10.1080/23328940.2015.1110655
7. Anderson T, Berry NT, Wideman L (2019) Exercise and the hypothalamic–pituitary–adrenal axis: a special focus on acute cortisol and growth hormone responses. Curr Opin Endocr Metabol Res 9:74–77
8. Antunes-Rodrigues J, de Castro M, Elias LL, Valença MM, McCann SM (2004) Neuroendocrine control of body fluid metabolism. Physiol Rev 84(1):169–208. https://doi.org/10.1152/physrev.00017.2003
9. Armstrong LE, Casa DJ, Millard-Stafford M, Moran DS, Pyne SW, Roberts WO (2007) American College of Sports Medicine position stand: exertional heat illness during training and competition. Med Sci Sports Exerc 39(3):556–572. https://doi.org/10.1249/MSS.0b013e31802fa199
10. Armstrong LE, Francesconi RP, Kraemer WJ, Leva N, De Luca JP, Hubbard RW (1989) Plasma cortisol, renin, and aldosterone during an intense heat acclimation program. Int J Sports Med 10(1):38–42. https://doi.org/10.1055/s-2007-1024871
11. Baker LB (2017) Sweating rate and sweat sodium concentration in athletes: a review of methodology and intra/interindividual variability. Sports Med 47(Suppl 1):111–128. https://doi.org/10.1007/s40279-017-0691-5
12. Baker LB, De Chavez PJD, Ungaro CT, Sopeña BC, Nuccio RP, Reimel AJ, Barnes KA (2019) Exercise intensity effects on total sweat electrolyte losses and regional vs. whole-body sweat [Na+], [Cl-], and [K+]. Eur J Appl Physiol 119(2):361–375. https://doi.org/10.1007/s00421-018-4048-z
13. Baker LB, Wolfe AS (2020) Physiological mechanisms determining eccrine sweat composition. Eur J Appl Physiol 120(4):719–752. https://doi.org/10.1007/s00421-020-04323-7
14. Bass DE, Kleeman CR, Quinn M, Henschel A, Hegnauer AH (1955) Mechanisms of acclimatization to heat in man. Medicine 34(3):323–380. https://doi.org/10.1097/00005792-195509000-00002
15. Beglinger S, Drewe J, Christ-Crain M (2017) The circadian rhythm of copeptin, the C-terminal portion of arginine vasopressin. J Biomark 2017:4737082. https://doi.org/10.1155/2017/4737082
16. Benjamin CL, Sekiguchi Y, Fry LA, Casa DJ (2019) Performance changes following heat acclimation and the factors that influence these changes: meta-analysis and meta-regression. Front Physiol 27(10):1448. https://doi.org/10.3389/fphys.2019.01448
17. Bonner RM, Harrison MH, Hall CJ, Edwards RJ (1976) Effect of heat acclimatization on intravascular responses to acute heat stress in man. J Appl Physiol 41(5 Pt. 1):708–713. https://doi.org/10.1152/jappl.1976.41.5.708
18. Bouchama A, Aziz MA, Mahri SA, Gabere MN, Dlamy MA, Mohammad S, Abbad MA, Hussein M (2017) A model of exposure to extreme environmental heat uncovers the human transcriptome to heat stress. Sci Rep 7(1):9429. https://doi.org/10.1038/s41598-017-09819-5
19. Burtscher M, Gatterer H, Philippe M, Krüsmann P, Kernbeiss S, Frontull V, Kofler P (2013) Effects of a single low-dose acetaminophen on body temperature and running performance in the heat: a pilot project. Int J Physiol Pathophysiol Pharmacol 5(3):190–193
20. Cable RG, Steele WR, Melmed RS, Johnson B, Mast AE, Carey PM, Kiss JE, Kleinman SH, Wright DJ, NHLBI Retrovirus Epidemiology Donor Study-II (REDS-II) (2012) The difference between fingerstick and venous hemoglobin and hematocrit varies by sex and iron stores. Transfusion 52(5):1031–1040. https://doi.org/10.1111/j.1537-2995.2011.03389.x
21. Califf RM (2018) Biomarker definitions and their applications. Exp Biol Med 243(3):213–221. https://doi.org/10.1177/1535370217750088

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22. Chapman CL, Johnson BD, Parker MD, Hostler D, Pryor RR, Schlader Z (2020) Kidney physiology and pathophysiology during heat stress and the modification by exercise, dehydration, heat acclimation and aging. Temperature 8(2):108–159. https://doi.org/10.1080/23328940.2020.1826841

23. Chapman CL, Johnson BD, Vargas NT, Hostler D, Parker MD, Schlader ZJ (2020) Both hyperthermia and dehydration during physical work in the heat contribute to the risk of acute kidney injury. J Appl Physiol 128(4):715–728. https://doi.org/10.1152/japplphysiol.00787.2019

24. Chieffi S, Carotenuto M, Monda V, Valenzano A, Villano I, Precenzano F, Tafuri D, Salerno M, Filippi N, Nuccio F, Ruberto M, De Luca V, Cipolloni L, Cibelli G, Mollica MP, Iacono D, Nigro E, Monda M, Messina G, Messina A (2017) Orexin system: the key for a healthy life. Front Physiol 31(8):357. https://doi.org/10.3389/fphys.2017.00357

25. Christ-Crain M, Morgenthaler NG, Fenske W (2016) Copeptin as a biomarker and a diagnostic tool in the evaluation of patients with polyuria-polydipsia and hyponatremia. Best Pract Res Clin Endocrinol Metab 30(2):235–247. https://doi.org/10.1016/j.beem.2016.02.003

26. Coffman KE, Mitchell KM, Salgado RM, Miller GD, Kenefick RW, Cheuvront SN (2020) Potential for dehydration to impact the athlete biological passport. Drug Test Anal 12(8):1206–1211. https://doi.org/10.1002/dta.2811

27. Collins KJ, Weiner JS (1968) Endocrinological aspects of exposure to high environmental temperatures. Physiol Rev 48(4):785–839. https://doi.org/10.1152/physrev.1968.48.4.785

28. Coombs GB, Cramer MN, Ravanelli NM, Morris NB, Jay O (2015) Acute acetaminophen ingestion does not alter core temperature or sweating during exercise in hot-humid conditions. Scand J Med Sci Sports 25(Suppl 1):96–103. https://doi.org/10.1111/sms.12336

29. Corbett J, Neal RA, Lunt HC, Tipton MJ (2014) Adaptation to heat and exercise performance under cooler conditions: a new hot topic. Sports Med 44(10):1323–1331. https://doi.org/10.1007/s40279-014-0212-8

30. Costello JT, Rendell RA, Furber M, Massey HC, Tipton MJ, Young JS, Corbett J (2018) Effects of acute or chronic heat exposure, exercise and dehydration on plasma cortisol, IL-6 and CRP levels in trained males. Cytokine 110:277–283. https://doi.org/10.1016/j.cyto.2018.01.018

31. Curry FR (2005) Atrial natriuretic peptide: an essential physiological regulator of transvascular fluid, protein transport, and plasma volume. J Clin Invest 115(6):1458–1461. https://doi.org/10.1172/JCI25417

32. Daanen HAM, Racinais S, Périard JD (2018) Heat acclimation decay and re-induction: a systematic review and meta-analysis. Sports Med 48(2):409–430. https://doi.org/10.1007/s40279-017-0808-x

33. Davies JA, Harrison MH, Cochrane LA, Edwards RJ, Gibson TM (1981) Effect of saline loading during heat acclimatization on adrenocortical hormone levels. J Appl Physiol Respir Environ Exerc Physiol 50(3):605–612. https://doi.org/10.1152/jappl.1981.50.3.605

34. de Lecea L, Kilduff TS, Peyron C, Gao X, Foye PE, Danielson PE, Fukuhara C, Battenberg EL, Gautvik VT, Bartlett FS 2nd, Frankel WN, van den Pol AN, Bloom FE, Gautvik KM, Sutcliffe JG (1998) The hypocretins: hypothalamus-specific peptides with neuroexcitatory activity. Proc Natl Acad Sci USA 95(1):322–327. https://doi.org/10.1073/pnas.95.1.322

35. Delves SK, Kelly JS, Gledhill M, Warke R, Fallowfield JL (2015) The effect of acclimation to the heat on the resting sweat response. Extrem Physiol Med 4(1):1–2

36. Deutschbein T, Unger N, Jaeger A, Broecker-Preuss M, Mann K, Petersenn S (2010) Influence of various confounding variables and storage conditions on metanephrine and normetanephrine levels in plasma. Clin Endocrinol 73(2):153–160. https://doi.org/10.1111/j.1365-2265.2009.03761.x

37. de Vries EM, van Beeren HC, Ackermans MT, Kalsbeek A, Fliers E, Boelen A (2015) Differential effects of fasting vs food restriction on liver thyroid hormone metabolism in male rats. J Endocrinol 224(1):25–35. https://doi.org/10.1530/JOE-14-0533

38. Dreosti AO (1935) Symposium-Problems arising out of temperature and humidity in deep mining on the Witwatersrand: The results of some investigations into the medical aspect of deep mining on the Witwatersrand. J South Afr Inst Min Metall 36(5):102–129

39. Eisenhofer G, Friberg P, Pacak K, Goldstein DS, Murphy DL, Tsigos C, Quyyumi AA, Brunner HG, Lenders JW (1995) Plasma metadrenalines: do they provide useful information about sympatho-adrenal function and catecholamine metabolism? Clin Sci 88(5):533–542. https://doi.org/10.1042/cs0880533

40. Ely BR, Lovering AT, Horowitz M, Minson CT (2014) Heat acclimation and cross tolerance to hypoxia: Bridging the gap between cellular and systemic responses. Temperature 1(2):107–114. https://doi.org/10.4161/temp.29800

41. European Environment Agency (2021) https://www.eea.europa.eu/data-and-maps/indicators/heat-and-health-2/assessment. Accessed 12 Apr 2022

42. Epstein Y, Udassin R, Sack J (1979) Serum 3,5,3′-triiodothyronine and 3,3′,5′-triiodothyronine concentrations during acute heat load. J Clin Endocrinol Metab 49(5):677–678. https://doi.org/10.1210/jcem-49-5-677

43. Esh CJ, Chrismas BCR, Mauger AR, Cherif A, Molphy J, Taylor L (2021) The influence of environmental and core temperature on cyclooxygenase and PGE2 in healthy humans. Sci Rep 11(1):6531. https://doi.org/10.1038/s41598-021-84563-5

44. Fazio S, Palmieri EA, Lombardi G, Biondi B (2004) Effects of thyroid hormone on the cardiovascular system. Recent Prog Horm Res 59:31–50. https://doi.org/10.1210/rp.59.1.31

45. FDA-NIH Biomarker Working Group (2020) BEST (Biomarkers, EndpointS, and other Tools) Resource. Silver Spring (MD): Food and Drug Administration (US); Bethesda (MD): National Institutes of Health (US), www.ncbi.nlm.nih.gov/books/NBK326791/. Accessed 11 Apr 2022

46. Febbraio MA, Snow RJ, Hargreaves M, Stathis CG, Martin IK, Carey MF (1994) Muscle metabolism during exercise and heat stress in trained men: effect of acclimation. J Appl Physiol 76(2):589–597. https://doi.org/10.1152/jappl.1994.76.2.589

47. Finberg JP, Berlyne GM (1977) Modification of renin and aldosterone response to heat by acclimatization in man. J Appl Physiol Respir Environ Exerc Physiol 42(4):554–558. https://doi.org/10.1152/jappl.1977.42.4.554

48. Firouzmand S, Ajori L, Young JS (2020) New participant stratification and combination of urinary biomarkers and confounders could improve diagnostic accuracy for overactive bladder. Sci Rep 10(1):3085. https://doi.org/10.1038/s41598-020-59973-6

49. Flouris AD, Poirier MP, Bravi A, Wright-Beatty HE, Herry C, Seely AJ, Kenny GP (2014) Changes in heart rate variability during the induction and decay of heat acclimation. Eur J Appl Physiol 114(10):2119–2128. https://doi.org/10.1007/s00421-014-2935-5

50. Foster J, Mauger AR, Chrismas BC, Thomasson K, Taylor L (2015) Is prostaglandin E2 (PGE2) involved in the thermogenic response to environmental cooling in healthy humans? Med Hypotheses 85(5):607–611. https://doi.org/10.1016/j.mehy.2015.07.022

51. Foster J, Mauger A, Thomasson K, White S, Taylor L (2016) Effect of acetaminophen ingestion on thermoregulation of normothermic, non-febrile humans. Front Pharmacol 14(7):54. https://doi.org/10.3389/fphar.2016.00054. (**Erratum in: Front Pharmacol. 2016;7:93. PMID: 27014068; PMCID: PMC4789353**)

52. Foster J, Smallcombe JW, Hodder S, Jay O, Flouris AD, Nybo L, Havenith G (2021) An advanced empirical model for quantifying the impact of heat and climate change on human physical work capacity. Int J Biometeorol 65(7):1215–1229. https://doi.org/10.1007/s00484-021-02105-0

53. Fox RH, Goldsmith R, Kidd DJ, Lewis HE (1963) Acclimatization to heat in man by controlled elevation of body temperature. J Physiol 166(3):530–547. https://doi.org/10.1113/jphysiol.1963.sp007121

54. Francesconi RP, Sawka MN, Pandolf KB (1983) Hypohydration and heat acclimation: plasma renin and aldosterone during exercise. J Appl Physiol Respir Environ Exerc Physiol 55(6):1790–1794. https://doi.org/10.1152/jappl.1983.55.6.1790

55. Francesconi RP, Sawka MN, Pandolf KB (1984) Hypohydration and acclimation: effects on hormone responses to exercise/heat stress. Aviat Space Environ Med 55(5):365–369

56. Francesconi RP, Sawka MN, Pandolf KB, Hubbard RW, Young AJ, Muza S (1985) Plasma hormonal responses at graded hypohydration levels during exercise-heat stress. J Appl Physiol 59(6):1855–1860. https://doi.org/10.1152/jappl.1985.59.6.1855

57. Ftaiti F, Jemni M, Kacem A, Zaouali MA, Tabka Z, Zbidi A, Grélot L (2008) Effect of hyperthermia and physical activity on circulating growth hormone. Appl Physiol Nutr Metab 33(5):880–887. https://doi.org/10.1139/H08-073

58. Fujii N, Pastore OL, McGarr GW, Meade RD, McNeely BD, Nishiyasu T, Kenny GP (2018) Cyclooxygenase-1 and -2 modulate sweating but not cutaneous vasodilation during exercise in the heat in young men. Physiol Rep 6(17):e13844. https://doi.org/10.14814/phy2.13844

59. Fujii N, Zhang SY, McNeely BD, Nishiyasu T, Kenny GP (2017) Heat shock protein 90 contributes to cutaneous vasodilation through activating nitric oxide synthase in young male adults exercising in the heat. J Appl Physiol 123(4):844–850. https://doi.org/10.1152/japplphysiol.00446.2017

60. Gao W, Brooks GA, Klonoff DC (2018) Wearable physiological systems and technologies for metabolic monitoring. J Appl Physiol 124(3):548–556. https://doi.org/10.1152/japplphysiol.00407.2017

61. Garrett AT, Goosens NG, Rehrer NJ, Patterson MJ, Cotter JD (2009) Induction and decay of short-term heat acclimation. Eur J Appl Physiol 107(6):659–670. https://doi.org/10.1007/s00421-009-1182-7

62. Godoy LD, Rossignoli MT, Delfino-Pereira P, Garcia-Cairasco N, de Lima Umeoka EH (2018) A comprehensive overview on stress neurobiology: basic concepts and clinical implications. Front Behav Neurosci 3(12):127. https://doi.org/10.3389/fnbeh.2018.00127

63. Goldstein DS, McCarty R, Polinsky RJ, Kopin IJ (1983) Relationship between plasma norepinephrine and sympathetic neural activity. Hypertension 5(4):552–559. https://doi.org/10.1161/01.hyp.5.4.552

64. Greenleaf JE, Brock PJ, Keil LC, Morse JT (1983) Drinking and water balance during exercise and heat acclimation. J Appl Physiol Respir Environ Exerc Physiol 54(2):414–419. https://doi.org/10.1152/jappl.1983.54.2.414

65. Gibson OR, Dennis A, Parfitt T, Taylor L, Watt PW, Maxwell NS (2014) Extracellular Hsp72 concentration relates to a minimum endogenous criteria during acute exercise-heat exposure. Cell Stress Chaperones 19(3):389–400. https://doi.org/10.1007/s12192-013-0468-1

66. Gibson OR, Turner G, Tuttle JA, Taylor L, Watt PW, Maxwell NS (2015) Heat acclimation attenuates physiological strain and the HSP72, but not HSP90α, mRNA response to acute normobaric hypoxia. J Appl Physiol 119(8):889–899. https://doi.org/10.1152/japplphysiol.00332.2015

67. Goto M, Okazaki K, Kamijo Y, Ikegawa S, Masuki S, Miyagawa K, Nose H (2010) Protein and carbohydrate supplementation during 5-day aerobic training enhanced plasma volume expansion and thermoregulatory adaptation in young men. J Appl Physiol 109(4):1247–1255. https://doi.org/10.1152/japplphysiol.00577.2010

68. Grouzmann E, Lamine F (2013) Determination of catecholamines in plasma and urine. Best Pract Res Clin Endocrinol Metab 27(5):713–723. https://doi.org/10.1016/j.beem.2013.06.004

69. Guy JH, Deakin GB, Edwards AM, Miller CM, Pyne DB (2015) Adaptation to hot environmental conditions: an exploration of the performance basis, procedures and future directions to optimise opportunities for elite athletes. Sports Med 45(3):303–311. https://doi.org/10.1007/s40279-014-0277-4

70. Guy JH, Edwards AM, Miller CM, Deakin GB, Pyne DB (2017) Short-term reliability of inflammatory mediators and response to exercise in the heat. J Sports Sci 35(16):1622–1628. https://doi.org/10.1080/02640414.2016.1227464

71. Guy JH, Pyne DB, Deakin GB, Miller CM, Edwards AM (2016) Acclimation training improves endurance cycling performance in the heat without inducing endotoxemia. Front Physiol 29(7):318. https://doi.org/10.3389/fphys.2016.00318

72. Han WK, Bailly V, Abichandani R, Thadhani R, Bonventre JV (2002) Kidney injury molecule-1 (KIM-1): a novel biomarker for human renal proximal tubule injury. Kidney Int 62(1):237–244. https://doi.org/10.1046/j.1523-1755.2002.00433.x

73. Haroutounian A, Amorim FT, Astorino TA, Khodiguian N, Curtiss KM, Matthews ARD, Estrada MJ, Fennel Z, McKenna Z, Nava R, Sheard AC (2021) Change in exercise performance and markers of acute kidney injury following heat acclimation with permissive dehydration. Nutrients 13(3):841. https://doi.org/10.3390/nu13030841

74. Harrison MH, Edwards RJ, Graveney MJ, Cochrane LA, Davies JA (1981) Blood volume and plasma protein responses to heat acclimatization in humans. J Appl Physiol Respir Environ Exerc Physiol 50(3):597–604. https://doi.org/10.1152/jappl.1981.50.3.597

75. Hartman ML, Iranmanesh A, Thorner MO, Veldhuis JD (1993) Evaluation of pulsatile patterns of growth hormone release in humans: a brief review. Am J Hum Biol 5(6):603–614. https://doi.org/10.1002/ajhb.1310050603

76. Haskell A, Nadel ER, Stachenfeld NS, Nagashima K, Mack GW (1997) Transcapillary escape rate of albumin in humans during exercise-induced hypervolemia. J Appl Physiol 83(2):407–413. https://doi.org/10.1152/jappl.1997.83.2.407

77. Havenith G, van Middendorp H (1990) The relative influence of physical fitness, acclimatization state, anthropometric measures and gender on individual reactions to heat stress. Eur J Appl Physiol Occup Physiol 61(5–6):419–427. https://doi.org/10.1007/BF00236062

78. Helmreich DL, Tylee D (2011) Thyroid hormone regulation by stress and behavioral differences in adult male rats. Horm Behav 60(3):284–291. https://doi.org/10.1016/j.yhbeh.2011.06.003

79. Hew-Butler T (2010) Arginine vasopressin, fluid balance and exercise: is exercise-associated hyponatraemia a disorder of arginine vasopressin secretion? Sports Med 40(6):459–479. https://doi.org/10.2165/11532070-000000000-00000

80. Hilgenfeldt U (1988) Half-life of rat angiotensinogen: influence of nephrectomy and lipopolysaccharide stimulation. Mol Cell Endocrinol 56(1–2):91–98. https://doi.org/10.1016/0303-7207(88)90012-3

81. Hodge D, Jones D, Martinez R, Buono MJ (2013) Time course of the attenuation of sympathetic nervous activity during active heat acclimation. Auton Neurosci 177(2):101–103. https://doi.org/10.1016/j.autneu.2013.02.017

82. Hooton K, Li L (2017) Nonocclusive sweat collection combined with chemical isotope labeling lc-ms for human sweat metabolomics and mapping the sweat metabolomes at different skin locations. Anal Chem 89(15):7847–7851. https://doi.org/10.1021/acs.analchem.7b01988

83. Horowitz M (2016) Epigenetics and cytoprotection with heat acclimation. J Appl Physiol 120(6):702–710. https://doi.org/10.1152/japplphysiol.00552.2015

84. IUPS Thermal Commission, The Commission for Thermal Physiology of the International Union of Physiological Sciences (2001) Glossary of terms for thermal physiology (Third edition). Japn J Physiol 51(2):245–280

85. Idriss HT, Naismith JH (2000) TNF alpha and the TNF receptor superfamily: structure-function relationship(s). Microsc Res Tech 50(3):184–195. https://doi.org/10.1002/1097-0029(20000801)50:3%3c184::AID-JEMT2%3e3.0.CO;2-H

86. Ivanov AI, Romanovsky AA (2004) Prostaglandin E2 as a mediator of fever: synthesis and catabolism. Front Biosci 1(9):1977–1993. https://doi.org/10.2741/1383

87. Iwen KA, Oelkrug R, Brabant G (2018) Effects of thyroid hormones on thermogenesis and energy partitioning. J Mol Endocrinol 60(3):R157–R170. https://doi.org/10.1530/JME-17-0319

88. Jiang N, Ahmed R, Damayantharan M, Ünal B, Butt H, Yetisen AK (2019) Lateral and vertical flow assays for point-of-care diagnostics. Adv Healthc Mater 8(14):e1900244. https://doi.org/10.1002/adhm.201900244

89. Jonklaas J, Nsouli-Maktabi H, Soldin SJ (2008) Endogenous thyrotropin and triiodothyronine concentrations in individuals with thyroid cancer. Thyroid 18(9):943–952. https://doi.org/10.1089/thy.2008.0061

90. Junge N, Jørgensen R, Flouris AD, Nybo L (2016) Prolonged self-paced exercise in the heat— environmental factors affecting performance. Temperature 3(4):539–548. https://doi.org/10.1080/23328940.2016.1216257

91. Justiz Vaillant AA, Qurie A (2022) Interleukin. StatPearls Publishing, Treasure Island

92. Kaldur T, Kals J, Ööpik V, Zilmer M, Zilmer K, Eha J, Unt E (2014) Effects of heat acclimation on changes in oxidative stress and inflammation caused by endurance capacity test in the heat. Oxid Med Cell Longev 2014:107137. https://doi.org/10.1155/2014/107137

93. Kanikowska D, Sato M, Sugenoya J, Iwase S, Shimizu Y, Nishimura N, Inukai Y (2012) No effects of acclimation to heat on immune and hormonal responses to passive heating in healthy volunteers. Int J Biometeorol 56(1):107–112. https://doi.org/10.1007/s00484-010-0401-6

94. Kanikowska D, Sugenoya J, Sato M, Shimizu Y, Inukai Y, Nishimura N, Iwase S (2010) Influence of season on plasma antidiuretic hormone, angiotensin II, aldosterone and plasma renin activity in young

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volunteers. Int J Biometeorol 54(3):243–248. https://doi.org/10.1007/s00484-009-0275-7

95. Kashani K, Al-Khafaji A, Ardiles T, Artigas A, Bagshaw SM, Bell M, Bihorac A, Birkhahn R, Cely CM, Chawla LS, Davison DL, Feldkamp T, Forni LG, Gong MN, Gunnerson KJ, Haase M, Hackett J, Honore PM, Hoste EA, Joannes-Boyau O, Joannidis M, Kim P, Koyner JL, Laskowitz DT, Lissauer ME, Marx G, McCullough PA, Mullaney S, Ostermann M, Rimmelé T, Shapiro NI, Shaw AD, Shi J, Sprague AM, Vincent JL, Vinsonneau C, Wagner L, Walker MG, Wilkerson RG, Zacharowski K, Kellum JA (2013) Discovery and validation of cell cycle arrest biomarkers in human acute kidney injury. Crit Care 17(1):R25. https://doi.org/10.1186/cc12503

96. Kirby CR, Convertino VA (1986) Plasma aldosterone and sweat sodium concentrations after exercise and heat acclimation. J Appl Physiol 61(3):967–970. https://doi.org/10.1152/jappl.1986.61.3.967

97. Klous L, De Ruiter C, Alkemade P, Daanen H, Gerrett N (2020) Sweat rate and sweat composition during heat acclimation. J Therm Biol 93:102697. https://doi.org/10.1016/j.jtherbio.2020.102697

98. Koeth RA, Wang Z, Levison BS, Buffa JA, Org E, Sheehy BT, Britt EB, Fu X, Wu Y, Li L, Smith JD, DiDonato JA, Chen J, Li H, Wu GD, Lewis JD, Warrier M, Brown JM, Krauss RM, Tang WH, Bushman FD, Lusis AJ, Hazen SL (2013) Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. Nat Med 19(5):576–585. https://doi.org/10.1038/nm.3145

99. Kosunen KJ, Pakarinen AJ, Kuoppasalmi K, Adlercreutz H (1976) Plasma renin activity, angiotensin II, and aldosterone during intense heat stress. J Appl Physiol 41(3):323–327. https://doi.org/10.1152/jappl.1976.41.3.323

100. Kraemer WJ, Armstrong LE, Hubbard RW, Marchitelli LJ, Leva N, Rock PB, Dziados JE (1988) Responses of plasma human atrial natriuretic factor to high intensity submaximal exercise in the heat. Eur J Appl Physiol Occup Physiol 57(4):399–403. https://doi.org/10.1007/BF00417983

101. Kregel KC (2002) Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. J Appl Physiol 92(5):2177–2186. https://doi.org/10.1152/japplphysiol.01267.2001

102. Kresfelder TL, Claassen N, Cronjé MJ (2006) Hsp70 Induction and hsp70 Gene polymorphisms as Indicators of acclimatization under hyperthermic conditions. J Therm Biol 31(5):406–415

103. Krüger K, Reichel T, Zeilinger C (2019) Role of heat shock proteins 70/90 in exercise physiology and exercise immunology and their diagnostic potential in sports. J Appl Physiol 126(4):916–927. https://doi.org/10.1152/japplphysiol.01052.2018

104. Kuennen M, Gillum T, Dokladny K, Bedrick E, Schneider S, Moseley P (2011) Thermotolerance and heat acclimation may share a common mechanism in humans. Am J Physiol Regul Integr Comp Physiol 301(2):R524–R533. https://doi.org/10.1152/ajpregu.00039.2011

105. Kuzmenko NV, Tsyrlin VA, Pliss MG, Galagudza MM (2021) Seasonal variations in levels of human thyroid-stimulating hormone and thyroid hormones: a meta-analysis. Chronobiol Int 38(3):301–317. https://doi.org/10.1080/07420528.2020.1865394

106. Lamberts SW, Verleun T, Oosterom R, de Jong F, Hackeng WH (1984) Corticotropin-releasing factor (ovine) and vasopressin exert a synergistic effect on adrenocorticotropin release in man. J Clin Endocrinol Metab 58(2):298–303. https://doi.org/10.1210/jcem-58-2-298

107. Laterza OF, Hendrickson RC, Wagner JA (2007) Molecular biomarkers. Drug Inf J 41(5):573–585

108. Lau SS, Griffin TM, Mestril R (2000) Protection against endotoxemia by HSP70 in rodent cardiomyocytes. Am J Physiol Heart Circ Physiol 278(5):H1439–H1445. https://doi.org/10.1152/ajpheart.2000.278.5.H1439

109. Lechner P, Buck D, Sick L, Hemmer B, Multhoff G (2018) Serum heat shock protein 70 levels as a biomarker for inflammatory processes in multiple sclerosis. Mult Scler J Exp Transl Clin 4(2):2055217318767192. https://doi.org/10.1177/2055217318767192

110. Lee BJ, Thake CD (2017) Heat and hypoxic acclimation increase monocyte heat shock protein 72 but do not attenuate inflammation following hypoxic exercise. Front Physiol 16(8):811. https://doi.org/10.3389/fphys.2017.00811

111. Legler DF, Bruckner M, Uetz-von Allmen E, Krause P (2010) Prostaglandin E2 at new glance: novel insights in functional diversity offer therapeutic chances. Int J Biochem Cell Biol 42(2):198–201. https://doi.org/10.1016/j.biocel.2009.09.015

112. Leppäluoto J, Huttunen P, Hirvonen J, Väänänen A, Tuominen M, Vuori J (1986) Endocrine effects of repeated sauna bathing. Acta Physiol Scand 128(3):467–470. https://doi.org/10.1111/j.1748-1716.1986.tb08000.x

113. Liu Y, Mayr S, Opitz-Gress A, Zeller C, Lormes W, Baur S, Lehmann M, Steinacker JM (1999) Human skeletal muscle HSP70 response to training in highly trained rowers. J Appl Physiol 86(1):101–104. https://doi.org/10.1152/jappl.1999.86.1.101

114. Lobigs LM, Sharpe K, Garvican-Lewis LA, Gore CJ, Peeling P, Dawson B, Schumacher YO (2018) The athlete's hematological response to hypoxia: a meta-analysis on the influence of altitude exposure on key biomarkers of erythropoiesis. Am J Hematol 93(1):74–83. https://doi.org/10.1002/ajh.24941

115. Luger A, Deuster PA, Debolt JE, Loriaux DL, Chrousos GP (1988) Acute exercise stimulates the renin-angiotensin-aldosterone axis: adaptive changes in runners. Horm Res 30(1):5–9. https://doi.org/10.1159/000181017

116. Madsen L, Pedersen LM, Lillefosse HH, Fjaere E, Bronstad I, Hao Q, Petersen RK, Hallenborg P, Ma T, De Matteis R, Araujo P, Mercader J, Bonet ML, Hansen JB, Cannon B, Nedergaard J, Wang J, Cinti S, Voshol P, Døskeland SO, Kristiansen K (2010) UCP1 induction during recruitment of brown adipocytes in white adipose tissue is dependent on cyclooxygenase activity. PLoS ONE 5(6):e11391. https://doi.org/10.1371/journal.pone.0011391

117. Magalhães Fde C, Amorim FT, Passos RL, Fonseca MA, Oliveira KP, Lima MR, Guimarães JB, Ferreira-Júnior JB, Martini AR, Lima NR, Soares DD, Oliveira EM, Rodrigues LO (2010) Heat and exercise acclimation increases intracellular levels of Hsp72 and inhibits exercise-induced increase in intracellular and plasma Hsp72 in humans. Cell Stress Chaperones 15(6):885–895. https://doi.org/10.1007/s12192-010-0197-7

118. Maher JT, Bass DE, Heistad DD, Angelakos ET, Hartley LH (1972) Effect of posture on heat acclimatization in man. J Appl Physiol 33(1):8–13. https://doi.org/10.1152/jappl.1972.33.1.8

119. Malczewska-Lenczowska J, Orysiak J, Majorczyk E, Sitkowski D, Starczewski M, Żmijewski P (2022) HIF-1α and NFIA-AS2 polymorphisms as potential determinants of total hemoglobin mass in endurance athletes. J Strength Cond Res 36(6):1596–1604. https://doi.org/10.1519/JSC.0000000000003686

120. Maloyan A, Horowitz M (2002) beta-Adrenergic signaling and thyroid hormones affect HSP72 expression during heat acclimation. J Appl Physiol 93(1):107–115. https://doi.org/10.1152/japplphysiol.01122.2001

121. Marshall HC, Ferguson RA, Nimmo MA (2006) Human resting extracellular heat shock protein 72 concentration decreases during the initial adaptation to exercise in a hot, humid environment. Cell Stress Chaperones 11(2):129–134. https://doi.org/10.1379/csc-158r.1

122. Marshall HC, Campbell SA, Roberts CW, Nimmo MA (2007) Human physiological and heat shock protein 72 adaptations during the initial phase of humid-heat acclimation. J Therm Biol 32(6):341–348

123. Martin T, Dauvilliers Y, Koumar OC, Bouet V, Freret T, Besnard S, Dauphin F, Bessot N (2019) Dual orexin receptor antagonist induces changes in core body temperature in rats after exercise. Sci Rep 9(1):18432. https://doi.org/10.1038/s41598-019-54826-3. (**Erratum in: Sci Rep. 2020 Apr 2;10(1):6030. PMID: 31804545; PMCID: PMC6895233**)

124. Matomäki P, Kainulainen H, Kyröläinen H (2018) Corrected whole blood biomarkers - the equation of Dill and Costill revisited. Physiol Rep 6(12):e13749. https://doi.org/10.14814/phy2.13749

125. Matsumoto T, Nishiyama T, Nishimura N, Kato M, Inukai Y, Sugenoya J, Kosaka M (2001) Endocrine responses to heat and cold stress. Thermother Neoplas Inflamm Pain. https://doi.org/10.1007/978-4-431-67035-3_26

126. Mauger AR, Taylor L, Harding C, Wright B, Foster J, Castle PC (2014) Acute acetaminophen (paracetamol) ingestion improves time to exhaustion during exercise in the heat. Exp Physiol 99(1):164–171. https://doi.org/10.1113/expphysiol.2013.075275

127. McClung JP, Hasday JD, He JR, Montain SJ, Cheuvront SN, Sawka MN, Singh IS (2008) Exercise-heat acclimation in humans alters baseline levels and ex vivo heat inducibility of HSP72 and HSP90 in peripheral blood mononuclear cells. Am J Physiol Regul Integr Comp Physiol 294(1):R185–R191. https://doi.org/10.1152/ajpregu.00532.2007

128. McIntyre RD (2021) Novel insights into heat acclimation by post-exercise hot water immersion https://research.bangor.ac.uk/portal/en/theses/novel-insights-into-heat-acclimation-by-postexercise-hot-water-immersion(edef517b-66a6-402d-b95a-a73c34d4d499).html. Accessed 7 June 2022

129. McIntyre RD, Zurawlew MJ, Mee JA, Walsh NP, Oliver SJ (2022) A comparison of medium-term heat acclimation by post-exercise hot water immersion or exercise in the heat: adaptations, overreaching, and thyroid hormones. Am J Physiol Regul Integr Comp Physiol 323(5):R601–R615. https://doi.org/10.1152/ajpregu.00315.2021

130. McNabb FA, King DB (1993) Thyroid hormone effects on growth, development, and metabolism. In: Schreibman M, Scanes CG, Pang PKT (eds) The endocrinology of growth development and metabolism in vertebrates, vol 10. Academic Press, Cambridge, pp 873–885

131. Mirit E, Palmon A, Hasin Y, Horowitz M (1999) Heat acclimation induces changes in cardiac mechanical performance: the role of thyroid hormone. Am J Physiol 276(2):R550–R558. https://doi.org/10.1152/ajpregu.1999.276.2.R550

132. Mirit E, Gross C, Hasin Y, Palmon A, Horowitz M (2000) Changes in cardiac mechanics with heat acclimation: adrenergic signaling and SR-Ca regulatory proteins. Am J Physiol Regul Integr Comp Physiol 279(1):R77-85. https://doi.org/10.1152/ajpregu.2000.279.1.R77

133. Mishra J, Ma Q, Prada A, Mitsnefes M, Zahedi K, Yang J, Barasch J, Devarajan P (2003) Identification of neutrophil gelatinase-associated lipocalin as a novel early urinary biomarker for ischemic renal injury. J Am Soc Nephrol 14(10):2534–2543. https://doi.org/10.1097/01.asn.0000088027.54400.c6

134. Monda M, Viggiano A, Viggiano A, Fuccio F, De Luca V (2004) Injection of orexin A into the diagonal band of Broca induces sympathetic and hyperthermic reactions. Brain Res 1018(2):265–271. https://doi.org/10.1016/j.brainres.2004.05.084

135. Montain SJ, Laird JE, Latzka WA, Sawka MN (1997) Aldosterone and vasopressin responses in the heat: hydration level and exercise intensity effects. Med Sci Sports Exerc 29(5):661–668. https://doi.org/10.1097/00005768-199705000-00012

136. Moran DS, Mendal L (2002) Core temperature measurement: methods and current insights. Sports Med 32(14):879–885. https://doi.org/10.2165/00007256-200232140-00001

137. Morgenthaler NG, Struck J, Jochberger S, Dünser MW (2008) Copeptin: clinical use of a new biomarker. Trends Endocrinol Metab 19(2):43–49. https://doi.org/10.1016/j.tem.2007.11.001

138. Morton JP, Maclaren DP, Cable NT, Campbell IT, Evans L, Bongers T, Griffiths RD, Kayani AC, McArdle A, Drust B (2007) Elevated core and muscle temperature to levels comparable to exercise do not increase heat shock protein content of skeletal muscle of physically active men. Acta Physiol 190(4):319–327. https://doi.org/10.1111/j.1748-1716.2007.01711.x

139. Moseley PL (1997) Heat shock proteins and heat adaptation of the whole organism. J Appl Physiol 83(5):1413–1417. https://doi.org/10.1152/jappl.1997.83.5.1413

140. Mudambo KS, Coutie W, Rennie MJ (1997) Plasma arginine vasopressin, atrial natriuretic peptide and brain natriuretic peptide responses to long-term field training in the heat: effects of fluid ingestion and acclimatization. Eur J Appl Physiol Occup Physiol 75(3):219–225. https://doi.org/10.1007/s004210050151

141. Mukoyama M, Nakao K, Hosoda K, Suga S, Saito Y, Ogawa Y, Shirakami G, Jougasaki M, Obata K, Yasue H et al (1991) Brain natriuretic peptide as a novel cardiac hormone in humans: evidence for an exquisite dual natriuretic peptide system, atrial natriuretic peptide and brain natriuretic peptide. J Clin Invest 87(4):1402–1412. https://doi.org/10.1172/JCI115146

142. Nava R, Zuhl MN (2020) Heat acclimation-induced intracellular HSP70 in humans: a meta-analysis. Cell Stress Chaperones 25(1):35–45. https://doi.org/10.1007/s12192-019-01059-y

143. Neal RA, Massey HC, Tipton MJ, Young JS, Corbett J (2016) Effect of permissive dehydration on induction and decay of heat acclimation, and temperate exercise performance. Front Physiol 23(7):564. https://doi.org/10.3389/fphys.2016.00564

144. Nehring SM, Goyal A, Patel BC (2022) C Reactive protein. StatPearls Publishing, Treasure Island

145. Neuhaus D, Gaehtgens P (1994) Haemorrheology and long term exercise. Sports Med 18(1):10–21. https://doi.org/10.2165/00007256-199418010-00003

146. Nielsen B, Hales JR, Strange S, Christensen NJ, Warberg J, Saltin B (1993) Human circulatory and thermoregulatory adaptations with heat acclimation and exercise in a hot, dry environment. J Physiol 460:467–485. https://doi.org/10.1113/jphysiol.1993.sp019482

147. Nielsen B, Strange S, Christensen NJ, Warberg J, Saltin B (1997) Acute and adaptive responses in humans to exercise in a warm, humid environment. Pflugers Arch 434(1):49–56. https://doi.org/10.1007/s004240050361

148. Nielsen B (1998) Heat acclimation–mechanisms of adaptation to exercise in the heat. Int J Sports Med 19(Suppl 2):S154–S156. https://doi.org/10.1055/s-2007-971984

149. Niess AM, Fehrenbach E, Lehmann R, Opavsky L, Jesse M, Northoff H, Dickhuth HH (2003) Impact of elevated ambient temperatures on the acute immune response to intensive endurance exercise. Eur J Appl Physiol 89(3–4):344–351. https://doi.org/10.1007/s00421-003-0809-3

150. Niimi Y, Matsukawa T, Sugiyama Y, Shamsuzzaman AS, Ito H, Sobue G, Mano T (1997) Effect of heat stress on muscle sympathetic nerve activity in humans. J Auton Nerv Syst 63(1–2):61–67. https://doi.org/10.1016/s0165-1838(96)00134-8

151. Nyein HYY, Bariya M, Tran B, Ahn CH, Brown BJ, Ji W, Davis N, Javey A (2021) A wearable patch for continuous analysis of thermoregulatory sweat at rest. Nat Commun 12(1):1823. https://doi.org/10.1038/s41467-021-22109-z

152. Oberholzer L, Siebenmann C, Mikkelsen CJ, Junge N, Piil JF, Morris NB, Goetze JP, Meinild Lundby AK, Nybo L, Lundby C (2019) Hematological adaptations to prolonged heat acclimation in endurance-trained males. Front Physiol 1(10):1379. https://doi.org/10.3389/fphys.2019.01379

153. Obermeyer Z, Samra JK, Mullainathan S (2017) Individual differences in normal body temperature: longitudinal big data analysis of patient records. BMJ 13(359):j5468. https://doi.org/10.1136/bmj.j5468

154. Okazaki K, Ichinose T, Mitono H, Chen M, Masuki S, Endoh H, Hayase H, Doi T, Nose H (2009) Impact of protein and carbohydrate supplementation on plasma volume expansion and thermoregulatory adaptation by aerobic training in older men. J Appl Physiol 107(3):725–733. https://doi.org/10.1152/japplphysiol.91265.2008

155. Olszewski W, Engeset A, Jaeger PM, Sokolowski J, Theodorsen L (1977) Flow and composition of leg lymph in normal men during venous stasis, muscular activity and local hyperthermia. Acta Physiol Scand 99(2):149–155. https://doi.org/10.1111/j.1748-1716.1977.tb10365.x

156. Omassoli J, Hill NE, Woods DR, Delves SK, Fallowfield JL, Brett SJ, Wilson D, Corbett RW, Allsopp AJ, Stacey MJ (2019) Variation in renal responses to exercise in the heat with progressive acclimatisation. J Sci Med Sport 22(9):1004–1009. https://doi.org/10.1016/j.jsams.2019.04.010

157. Oöpik V, Timpmann S, Kreegipuu K, Unt E, Tamm M (2014) Heat acclimation decreases the growth hormone response to acute constant-load exercise in the heat. Growth Horm IGF Res 24(1):2–9. https://doi.org/10.1016/j.ghir.2013.10.001

158. Pandey PC, Pandey G, Narayan RJ (2020) Minimally invasive platforms in biosensing. Front Bioeng Biotechnol 31(8):894. https://doi.org/10.3389/fbioe.2020.00894

159. Park JY, Pillinger MH, Abramson SB (2006) Prostaglandin E2 synthesis and secretion: the role of PGE2 synthases. Clin Immunol 119(3):229–240. https://doi.org/10.1016/j.clim.2006.01.016

160. Parsons IT, Stacey MJ, Woods DR (2019) Heat adaptation in military personnel: mitigating risk, maximizing performance. Front Physiol 17(10):1485. https://doi.org/10.3389/fphys.2019.01485

161. Patterson MJ (1999) The effect of heat acclimation on body-fluid regulation. University of Wollongong. https://ro.uow.edu.au/theses/1098/. Accessed 6 June 2022

162. Patterson MJ, Stocks JM, Taylor NA (2004) Sustained and generalized extracellular fluid expansion following heat acclimation. J Physiol 559(Pt 1):327–334. https://doi.org/10.1113/jphysiol.2004.063289

163. Peaston RT, Weinkove C (2004) Measurement of catecholamines and their metabolites. Ann Clin Biochem 41(Pt 1):17–38. https://doi.org/10.1258/000456304322664663

164. Périard JD, Racinais S, Sawka MN (2015) Adaptations and mechanisms of human heat acclimation: applications for competitive athletes and

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sports. Scand J Med Sci Sports 25(Suppl 1):20–38. https://doi.org/10.1111/sms.12408

165. Périard JD, Travers GJS, Racinais S, Sawka MN (2016) Cardiovascular adaptations supporting human exercise-heat acclimation. Auton Neurosci 196:52–62. https://doi.org/10.1016/j.autneu.2016.02.002

166. Pilch W, Szygula Z, Zychowska M, Gawinek M, Szyguła Z (2003) The influence of sauna training on the hormonal system of young women. J Hum Kinet 9:19–30

167. Pryor RR, Pryor JL, Vandermark LW, Adams EL, Brodeur RM, Schlader ZJ, Armstrong LE, Lee EC, Maresh CM, Casa DJ (2020) Acute kidney injury biomarker responses to short-term heat acclimation. Int J Environ Res Public Health 17(4):1325. https://doi.org/10.3390/ijerph17041325

168. Rabbitts JA, Strom NA, Sawyer JR, Curry TB, Dietz NM, Roberts SK, Kingsley-Berg SM, Charkoudian N (2009) Influence of endogenous angiotensin II on control of sympathetic nerve activity in human dehydration. J Physiol 587(Pt 22):5441–5449. https://doi.org/10.1113/jphysiol.2009.176693

169. Racinais S, Mohr M, Buchheit M, Voss SC, Gaoua N, Grantham J, Nybo L (2012) Individual responses to short-term heat acclimatisation as predictors of football performance in a hot, dry environment. Br J Sports Med 46(11):810–815. https://doi.org/10.1136/bjsports-2012-091227

170. Racinais S, Alonso JM, Coutts AJ, Flouris AD, Girard O, González-Alonso J, Hausswirth C, Jay O, Lee JK, Mitchell N, Nassis GP, Nybo L, Pluim BM, Roelands B, Sawka MN, Wingo J, Périard JD (2015) Consensus recommendations on training and competing in the heat. Br J Sports Med 49(18):1164–1173. https://doi.org/10.1136/bjsports-2015-094915

171. Ravanelli N, Barry H, Schlader ZJ, Gagnon D (2021) Impact of passive heat acclimation on markers of kidney function during heat stress. Exp Physiol 106(1):269–281. https://doi.org/10.1113/EP088637

172. Rendell RA, Prout J, Costello JT, Massey HC, Tipton MJ, Young JS, Corbett J (2017) Effects of 10 days of separate heat and hypoxic exposure on heat acclimation and temperate exercise performance. Am J Physiol Regul Integr Comp Physiol 313(3):R191–R201. https://doi.org/10.1152/ajpregu.00103.2017

173. Rhind SG, Gannon GA, Shephard RJ, Buguet A, Shek PN, Radomski MW (2004) Cytokine induction during exertional hyperthermia is abolished by core temperature clamping: neuroendocrine regulatory mechanisms. Int J Hyperthermia 20(5):503–516. https://doi.org/10.1080/02656730410001670651

174. Ricciotti E, FitzGerald GA (2011) Prostaglandins and inflammation. Arterioscler Thromb Vasc Biol 31(5):986–1000. https://doi.org/10.1161/ATVBAHA.110.207449

175. Roberts MF, Wenger CB, Stolwijk JA, Nadel ER (1977) Skin blood flow and sweating changes following exercise training and heat acclimation. J Appl Physiol Respir Environ Exerc Physiol 43(1):133–137. https://doi.org/10.1152/jappl.1977.43.1.133

176. Roden M, Raffesberg W, Raber W, Bernroider E, Niederle B, Waldhäusl W, Gasic S (2001) Quantification of unconjugated metanephrines in human plasma without interference by acetaminophen. Clin Chem 47(6):1061–1067

177. Rivas E, Rao M, Castleberry T, Ben-Ezra V (2017) The change in metabolic heat production is a primary mediator of heat acclimation in adults. J Therm Biol 70(Pt B):69–79. https://doi.org/10.1016/j.jtherbio.2017.10.001

178. Romanovsky AA, Almeida MC, Aronoff DM, Ivanov AI, Konsman JP, Steiner AA, Turek VF (2005) Fever and hypothermia in systemic inflammation: recent discoveries and revisions. Front Biosci 1(10):2193–2216. https://doi.org/10.2741/1690

179. Rotenberg S, McGrath JJ (2016) Inter-relation between autonomic and HPA axis activity in children and adolescents. Biol Psychol 117:16–25. https://doi.org/10.1016/j.biopsycho.2016.01.015

180. Rothschild MA, Oratz M, Schreiber SS (1988) Serum albumin. Hepatology 8(2):385–401. https://doi.org/10.1002/hep.1840080234

181. Sawka MN, Pandolf KB, Avellini BA, Shapiro Y (1983) Does heat acclimation lower the rate of metabolism elicited by muscular exercise? Aviat Space Environ Med 54(1):27–31

182. Schlader ZJ, Hostler D, Parker MD, Pryor RR, Lohr JW, Johnson BD, Chapman CL (2019) The potential for renal injury elicited by physical work in the heat. Nutrients 11(9):2087. https://doi.org/10.3390/nu11092087

183. Schneider SM (2016) Heat acclimation: gold mines and genes. Temperature 3(4):527–538. https://doi.org/10.1080/23328940.2016.1240749

184. Sempionatto JR, Lasalde-Ramírez JA, Mahato K, Wang J, Gao W (2022) Wearable chemical sensors for biomarker discovery in the omics era. Nat Rev Chem 6(12):899–915. https://doi.org/10.1038/s41570-022-00439-w

185. Senay LC, Mitchell D, Wyndham CH (1976) Acclimatization in a hot, humid environment: body fluid adjustments. J Appl Physiol 40(5):786–796. https://doi.org/10.1152/jappl.1976.40.5.786

186. Serag A, Shakkour Z, Halboup AM, Kobeissy F, Farag MA (2021) Sweat metabolome and proteome: Recent trends in analytical advances and potential biological functions. J Proteomics 30(246):104310. https://doi.org/10.1016/j.jprot.2021.104310

187. Shahid MA, Ashraf MA, Sharma S (2022) Physiology, thyroid hormone. StatPearls Publishing, Treasure Island

188. Shvartz E, Shapiro Y, Magazanik A, Meroz A, Birnfeld H, Mechtinger A, Shibolet S (1977) Heat acclimation, physical fitness, and responses to exercise in temperate and hot environments. J Appl Physiol Respir Environ Exerc Physiol 43(4):678–683. https://doi.org/10.1152/jappl.1977.43.4.678

189. Stacey M, Britland S, Delves S, Burnett A, Fallowfield J, Brett S, Woods D (2016) Dynamic changes in nephrine levels with acclimatisation reflect acquisition of heat tolerance. Endocr Abstr. https://doi.org/10.1530/endoabs.44.P2

190. Stacey MJ, Delves SK, Britland SE, Allsopp AJ, Brett SJ, Fallowfield JL, Woods DR (2018) Copeptin reflects physiological strain during thermal stress. Eur J Appl Physiol 118(1):75–84. https://doi.org/10.1007/s00421-017-3740-8

191. Stacey MJ, Woods DR, Brett SJ, Britland SE, Fallowfield JL, Allsopp AJ, Delves SK (2018) Heat acclimatization blunts copeptin responses to hypertonicity from dehydrating exercise in humans. Physiol Rep 6(18):e13851. https://doi.org/10.14814/phy2.13851

192. Stacey MMJ, Delves SK, Woods DR, Britland SE, Macconnachie L, Allsopp AJ, Brett SJ, Fallowfield JL, Boos CJ (2018) Heart rate variability and plasma nephrines in the evaluation of heat acclimatisation status. Eur J Appl Physiol 118(1):165–174. https://doi.org/10.1007/s00421-017-3758-y

193. Starkie RL, Hargreaves M, Rolland J, Febbraio MA (2005) Heat stress, cytokines, and the immune response to exercise. Brain Behav Immun 19(5):404–412. https://doi.org/10.1016/j.bbi.2005.03.005

194. Silva JE (2003) The thermogenic effect of thyroid hormone and its clinical implications. Ann Intern Med 139(3):205–213

195. Silva JE (2006) Thermogenic mechanisms and their hormonal regulation. Physiol Rev 86(2):435–464. https://doi.org/10.1152/physrev.00009.2005

196. Sridharan K, Sawhney RC, Mathew L, Pichan G, Malhotra AS (1986) Thyroid gland function during cross adaptation to heat and cold in man. Int J Biometeorol 30(3):223–230. https://doi.org/10.1007/BF02189465

197. Suga S, Nakao K, Hosoda K, Mukoyama M, Ogawa Y, Shirakami G, Arai H, Saito Y, Kambayashi Y, Inouye K et al (1992) Receptor selectivity of natriuretic peptide family, atrial natriuretic peptide, brain natriuretic peptide, and C-type natriuretic peptide. Endocrinology 130(1):229–239. https://doi.org/10.1210/endo.130.1.1309330

198. Shin YO, Lee JB, Min YK, Yang HM (2013) Heat acclimation affects circulating levels of prostaglandin E2, COX-2 and orexin in humans. Neurosci Lett 10(542):17–20. https://doi.org/10.1016/j.neulet.2013.03.017

199. Sonner Z, Wilder E, Heikenfeld J, Kasting G, Beyette F, Swaile D, Sherman F, Joyce J, Hagen J, Kelley-Loughnane N, Naik R (2015) The microfluidics of the eccrine sweat gland, including biomarker partitioning, transport, and biosensing implications. Biomicrofluidics 9(3):031301. https://doi.org/10.1063/1.4921039

200. Tan CL, Knight ZA (2018) Regulation of body temperature by the nervous system. Neuron 98(1):31–48. https://doi.org/10.1016/j.neuron.2018.02.022.

201. Tamm M, Jakobson A, Havik M, Timpmann S, Burk A, Ööpik V, Allik J, Kreegipuu K (2015) Effects of heat acclimation on time perception. Int J Psychophysiol 95(3):261–269. https://doi.org/10.1016/j.ijpsycho.2014.11.004

202. Taylor HL, Henschel AF, Keys A (1943) Cardiovascular adjustments of man in rest and work during exposure to dry heat. Am J Physiol Legacy Content 139(4):583–591

203. Tolstikov V, Moser AJ, Sarangarajan R, Narain NR, Kiebish MA (2020) Current status of metabolomic biomarker discovery: impact of study

design and demographic characteristics. Metabolites 10(6):224. https://doi.org/10.3390/metabo10060224.PMID:32485899;PMCID:PMC7345110

204. Tsigos C, Chrousos GP (2002) Hypothalamic-pituitary-adrenal axis, neuroendocrine factors and stress. J Psychosom Res 53(4):865–871. https://doi.org/10.1016/s0022-3999(02)00429-4

205. Tyler CJ, Reeve T, Hodges GJ, Cheung SS (2016) The Effects of Heat Adaptation on Physiology, Perception and Exercise Performance in the Heat: A Meta-Analysis. Sports Med 46(11):1699–1724. https://doi.org/10.1007/s40279-016-0538-5. (**Erratum in: Sports Med. 2016 Nov;46(11):1771. PMID: 27106556**)

206. Timpmann S, Hackney AC, Tamm M, Kreegipuu K, Unt E, Ööpik V (2018) Influence of Rhodiola rosea on the heat acclimation process in young healthy men. Appl Physiol Nutr Metab 43(1):63–70. https://doi.org/10.1139/apnm-2017-0372

207. Verney EB (1947) The antidiuretic hormone and the factors which determine its release. Proc R Soc Lond B Biol Sci 135(878):25–106

208. Watkins AM, Cheek DJ, Harvey AE, Blair KE, Mitchell JB (2008) Heat acclimation and HSP-72 expression in exercising humans. Int J Sports Med 29(4):269–276. https://doi.org/10.1055/s-2007-965331

209. Weller RS, Buono MJ (2022) The effect of heat acclimation on the sweat lactate concentration vs. sweat rate relationship. J Therm Biol 109:103325. https://doi.org/10.1016/j.jtherbio.2022.103325

210. Weller AS, Linnane DM, Jonkman AG, Daanen HA (2007) Quantification of the decay and re-induction of heat acclimation in dry-heat following 12 and 26 days without exposure to heat stress. Eur J Appl Physiol 102(1):57–66. https://doi.org/10.1007/s00421-007-0563-z

211. Willmott AG, Hayes M, Dekerle J, Maxwell NS (2015) The reliability of a heat acclimation state test prescribed from metabolic heat production intensities. J Therm Biol 53:38–45. https://doi.org/10.1016/j.jtherbio.2015.08.008

212. Willmott AGB, Hayes M, James CA, Dekerle J, Gibson OR, Maxwell NS (2018) Once- and twice-daily heat acclimation confer similar heat adaptations, inflammatory responses and exercise tolerance improvements. Physiol Rep 6(24):e13936. https://doi.org/10.14814/phy2.13936

213. Willmott AGB, Hayes M, James CA, Gibson OR, Maxwell NS (2019) Heat acclimation attenuates the increased sensations of fatigue reported during acute exercise-heat stress. Temperature 7(2):178–190. https://doi.org/10.1080/23328940.2019.1664370

214. Wong PC, Guo J, Zhang A (2017) The renal and cardiovascular effects of natriuretic peptides. Adv Physiol Educ 41(2):179–185. https://doi.org/10.1152/advan.00177.2016

215. Wright KP Jr, Drake AL, Frey DJ, Fleshner M, Desouza CA, Gronfier C, Czeisler CA (2015) Influence of sleep deprivation and circadian misalignment on cortisol, inflammatory markers, and cytokine balance. Brain Behav Immun 47:24–34. https://doi.org/10.1016/j.bbi.2015.01.004

216. Wyndham CH, Rogers GG, Senay LC, Mitchell D (1976) Acclimization in a hot, humid environment: cardiovascular adjustments. J Appl Physiol 40(5):779–785. https://doi.org/10.1152/jappl.1976.40.5.779

217. Xiao C, Wu T, Ren A, Pan Q, Chen S, Wu F, Li X, Wang R, Hightower LE, Tanguay RM (2003) Basal and inducible levels of Hsp70 in patients with acute heat illness induced during training. Cell Stress Chaperones 8(1):86–92. https://doi.org/10.1379/1466-1268(2003)8%3c86:bailoh%3e2.0.co;2

218. Yamada PM, Amorim FT, Moseley P, Robergs R, Schneider SM (2007) Effect of heat acclimation on heat shock protein 72 and interleukin-10 in humans. J Appl Physiol 103(4):1196–1204. https://doi.org/10.1152/japplphysiol.00242.2007

219. Yang Y, Gordon CJ (1997) Regulated hypothermia in the hypothyroid rat induced by administration of propylthiouracil. Am J Physiol 272(5 Pt 2):R1390–R1395. https://doi.org/10.1152/ajpregu.1997.272.5.R1390

220. Yang X, Kui L, Tang M, Li D, Wei K, Chen W, Miao J, Dong Y (2020) High-throughput transcriptome profiling in drug and biomarker discovery. Front Genet 5(11):19. https://doi.org/10.3389/fgene.2020.00019

221. Yang RC, Mack GW, Wolfe RR, Nadel ER (1998) Albumin synthesis after intense intermittent exercise in human subjects. J Appl Physiol 84(2):584–592. https://doi.org/10.1152/jappl.1998.84.2.584

222. Yenari MA, Liu J, Zheng Z, Vexler ZS, Lee JE, Giffard RG (2005) Antiapoptotic and anti-inflammatory mechanisms of heat-shock protein protection. Ann N Y Acad Sci 1053:74–83. https://doi.org/10.1196/annals.1344.007

223. Yoshimichi G, Yoshimatsu H, Masaki T, Sakata T (2001) Orexin-A regulates body temperature in coordination with arousal status. Exp Biol Med 226(5):468–476. https://doi.org/10.1177/153537020122600513

224. Young AJ, Sawka MN, Levine L, Cadarette BS, Pandolf KB (1985) Skeletal muscle metabolism during exercise is influenced by heat acclimation. J Appl Physiol 59(6):1929–1935. https://doi.org/10.1152/jappl.1985.59.6.1929

225. Zhang L, Zhang X, Du L, Zhang C, Li H (2021) Cholinergic- rather than adrenergic-induced sweating play a role in developing and developed rat eccrine sweat glands. Exp Anim 70(2):218–224. https://doi.org/10.1538/expanim.20-0144

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