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# Genomics and proteomics of heat acclimation

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## 1. ABSTRACT

Plasticity of the thermoregulatory system is a key factor for the induction of heat acclimation. Temperature-adaptive shifts in gene expression play an essential role in the processes involved. This review attempts to bridge the gap between the classical physiological heat acclimation profile and the molecular/cellular mechanisms underlying the evolution of the acclimated phenotype. Essential acclimatory modifications linked with thermal tolerance are (i) neuronal plasticity (ii) cytoprotection. Leftward and rightward threshold shifts in these respective functional categories expand the dynamic thermoregulatory range of the acclimated phenotype. Neural plasticity depends on changes in hypothalamic warm/cold sensitive neuron ratio and excitability. Over the course of acclimation, there is marked upregulation of transcripts encoding voltage dependent $K^+$ and $Ca^{2+}$ channels, neurotransmitters and/or their receptors. Temperature threshold for thermal injury is associated with progressive enhancement of inducible cytoprotective networks including the essential acclimatory components HSP70, HSF1 and HIF-1. Via cross-tolerance, achieved through shared on-call cytoprotective networks, acclimation also renders protection against novel stressors. Collectively, heat acclimation is a within life evolutionarily beneficial phenomenon with a memory, imprinted via epigenetic mechanisms.

## 2. INTRODUCTION

Among the various physical environmental stressors, "temperature is ecologically most important, for it is a factor that is all pervasive, and in most environments, lacks spatial or temporal constancy" (1). Concomitantly, when exposed to altered temperatures for prolonged periods, most animals can adapt physiologically and biochemically. This process is termed thermal acclimatization, when natural, e.g. if the effects are due to seasons or geographical zones or acclimation when the phenotypic adaptations occur under controlled experimental conditions (2) and, if successful, enhances heat tolerance by extending the limits of tolerable temperature and the duration of survival at that temperature (3). Acclimation is only achieved following prolonged exposures, and has long lasting effects.

The first references to heat acclimation are from the 18th century, coinciding with the emerging interests in tropical climate and diseases [e.g. Lind J (1773) (4), Jackson R, (1779) (5), Jousset A, (1884) (6), Virchow R (1885)(7)], but experimentation using humans and animals only began in the 20th century [Sumner (1913), Sundstroem (1925) (8, 9)]. Comprehensive reviews appeared shortly after; e.g. (i) Hisato Yoshimura, 1960 (10) in his review on human acclimation to heat and cold concludes that "

increased excitabilities of thermoregulatory center and increased activities of endocrine glands seem to be the main processes underlying acclimatization…"; (ii) Ushakove, (11) discussing the effects of thermal acclimatization on the thermal stability of cells, concluded that "….the possibility exists that in some poikilothermal animals there occur at the cellular level thermal adaptations which are a consequence of a local effect of high temperature on the organs…". [1]

Acclimation is a reversible, "within life time" mechanism of the individual organism which may have a genetic basis. Taken together, the time-span required for acclimation and our current concepts of the role of transcriptome remodeling in functional performance, we suggest that acclimation, which gives rise to a new phenotype, involves altered/reprogrammed gene expression and translational processes. This review focuses on the accumulating genomic data with respect to heat acclimation, and attempts to bridge the gap between the classical physiological acclimation profile and the underlying molecular and cellular mechanisms responsible for acclimatory homeostasis using an integrated approach. For detailed acclimatory physiology the reader will be referred to many excellent reviews (12-16).

## 3. HEAT ACCLIMATION: PHYSIOLOGICAL CRITERIA AND ACCLIMATION DYNAMICS

In terms of the physiology of regulatory effectors, acclimatory criteria are (i) reduced metabolic and heart rates as well as basal body temperature, (ii) lower temperature-thresholds for activating heat dissipation effectors and (iii) increased cardiovascular reserves and capacity of the evaporative cooling system. The increased cellular hardening achieved by heat acclimation, namely greater cellular responsiveness to heat stress (e.g. faster recruitment of heat shock proteins and/or other cytoprotective molecules) delays thermal injuries (3, 17). Apparent heat acclimation is already observed after the short-term acclimation phase (2-5 days, STHA). However, during STHA, temperature homeostasis is achieved primarily via augmented excitability of the autonomic nervous system that overrides the desensitization of cell membrane receptors and signaling pathways, leading to impairments in peripheral cellular performance (3, 18-23). In the rat salivary gland, the major evaporative cooling organ of this species, impaired responsiveness is exemplified by increased levels of high-affinity muscarinic receptors and reduced glandular response to muscarinic agonists such as pilocarpine (notably, salivation cooling is cholinergically controlled). When acclimatory homeostasis has been achieved (long term heat acclimation-LTHA, ~1mo), the number of receptors as well as tissue response to pilocarpine returns to control levels (21). Enhanced metabolic and functional performance of the peripheral effector organs are the characteristics of the new acclimated phenotype (3, 17, 24). Acclimation, thus, is a transition from an *early transient, "inefficient" state – the hallmark of STHA – to a state where cellular machinery and integrative processes are highly efficient;* when acclimatory homeostasis has been reached (3, 17, 18) (25).

The two essential aspects of acclimatory modifications related to thermal endurance are central regulation and cytoprotection. The leftward and rightward shifts in thresholds for activation of thermoregulatory effectors and onset of thermal injuries, respectively, expand the dynamic regulatory range of the acclimated individual. An orchestrated continuum of peripheral-central feedback interactions, employing modulations in both the left and the right arms of the loop are responsible for this outcome (12, 26) as discussed in the following sections.

## 4. ACCLIMATORY NEUROPLASTICITY –SHIFTS IN TEMPERATURE THRESHOLDS

### 4.1. Evidence from physiological/electrophysiological studies

Alteration of temperature thresholds for activation of the thermoregulatory effectors is one important manifestation of heat acclimation Figure 1 and references (12, 22, 27, 28) . It is plausible that altered thresholds may stem from localized changes in the sensitivity of specific organs, e.g. due to post synaptic alterations in receptor sensitivity. However, the coordinated concurrent shifts in all thermoregulatory effectors seen when heat acclimatory homeostasis is achieved imply central modulation.

Despite the large body of evidence on heat acclimation-mediated shifts in temperature-thresholds, most studies are at the integrative level [for reviews see e.g. (12, 20, 22, 27, 28)] and there are a limited number of direct studies on central controller plasticity. Briefly, in rats, Pierau's group (29) showed that heat acclimation leads to a considerable decrease in the number of warm-sensitive neurons with a diminished number having a very low temperature coefficient (0.6-0.8 imp/s/$^{\circ}$C) compared to matched populations of neurons in hypothalami of cold-acclimated rats. An additional clear effect of warm acclimation was the ability to convert a larger number of insensitive neurons to warm sensitive neurons by mediators, e.g. bombesin (29). In guinea-pigs, Hinckel *et al.*, (30) recorded changes in the tonic activity of neurons in the nucleus raphe magnus and subcoeruleus regions after cold and warm adaptations in response to reciprocal ambient stimulation. In an intact body experimental model, Attias *et al.*, (31) used the auditory evoked response (ABR-which reflects compound action potentials of the brainstem auditory pathway) to investigate brainstem activity, and demonstrated that following STHA, the time intervals between successive waves are prolonged, whereas 60 days of acclimation resulted in shorter time intervals. This was accompanied by significant increases in the compound action potential of the auditory nerve indicating more rapid activity and enhanced firing synchronization between the axons contributing to the compound amplitude. The suprachiasmic nucleus (SCN) acts as an endogenous oscillator and plays a critical role in circadian rhythm generation and other physiological functions. Based on studies by Boulant's group (32) on the SCN and temperature sensitive integrator neurons in the preoptic area of the hypothalamus, the approach of Shido and his group, to examine thermoregulatory neuroplasticity via

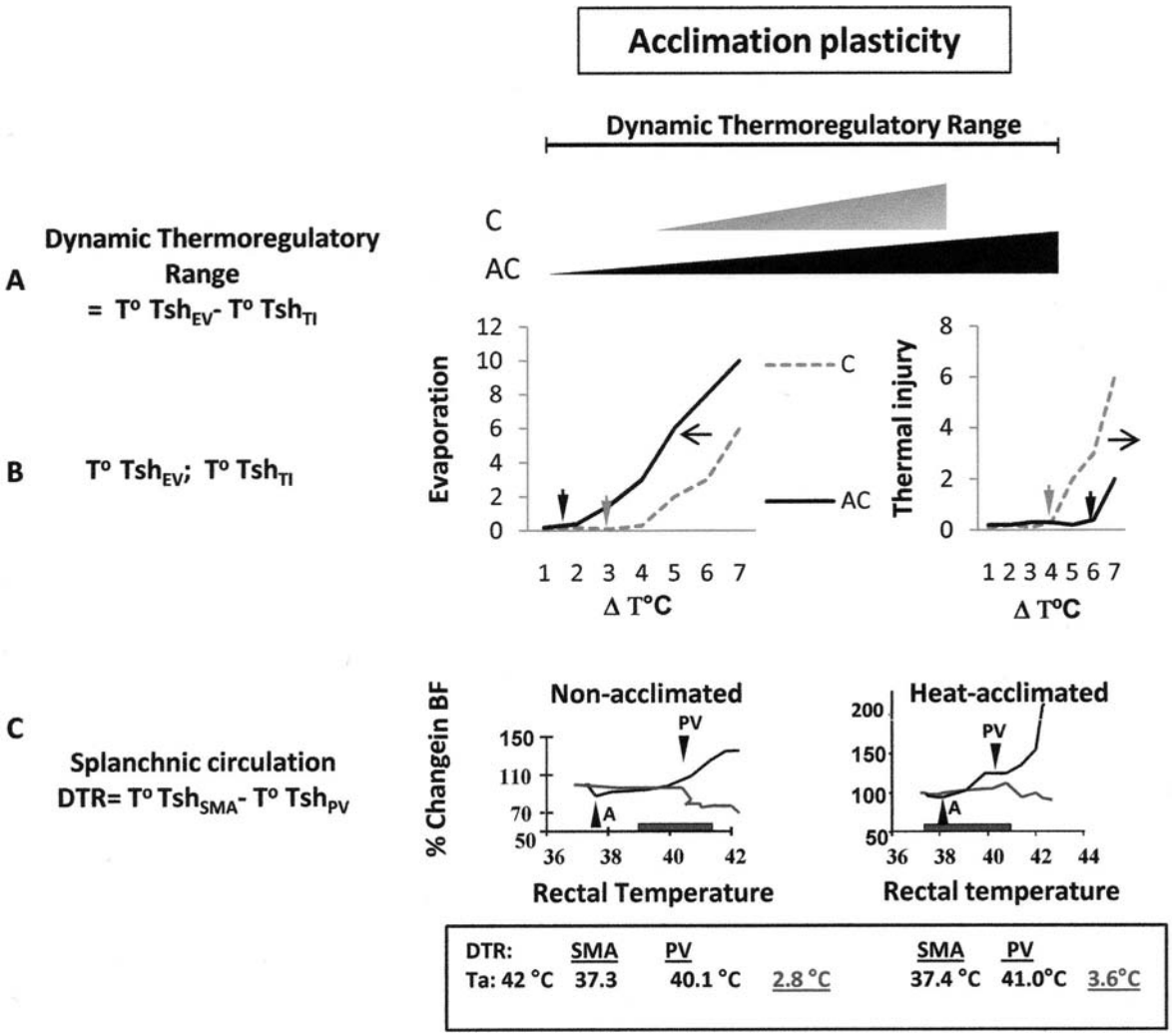


**Figure 1.** Acclimation plasticity, mirrored by changes in thermoregulatory thresholds. A. Heat acclimation expands the dynamic thermoregulatory range [Temperature threshold for the onset of evaporative cooling ($T^oTsh_{EV}$) - Temperature threshold for failure of thermoregulation ($Tsh_{TI}$)]. B. Water volume secreted for evaporative cooling (left), thermal injury (right) per degree rise in Tb (body temperature). Black lines-heat acclimated (AC), dashed lines-non acclimated (C). Vertical arrows T-Tsh. C. Dynamic thermoregulatory range (DTR) before and after heat acclimation in the splanchnic vascular bed [superior mesenteric artery (SMA, red) and portal vein (PV, black) blood flow (BF)]. The onset of thermoregulatory-induced vasomotor reflex is depicted by SMA vasoconstriction. Failure of this reflex is denoted by abrupt vasoconstriction of SMA and vasodilatation of the PV. DTR is markedly longer in the acclimated animal. Vertical arrows denote Tsh. Reproduced from Horowitz 2002 (17), Horowitz *et al.*,1983 (22) and Haddad and Horowitz (90).

studying the effects of the SCN on core temperature shifts in acclimated rats, is interesting. Maruyama *et al.*, (2007) (33) used rats with lesions in the SCN and demonstrated that a functioning SCN is essential for heat stress circadian memory but has no effect on acclimation temperature shifts. A similar phasic-acclimation protocol used by the same authors to study whether the clock gene affects thermal responses of the peripheral vasculature did not yield positive results (34).

The pharmacological approach also can be used to determine whether heat acclimation induced neuroplasticity is due to threshold alteration. Thus, Christman and Gisolfi, 1980; Christman *et al.*, 1985 (35, 36) demonstrated that prior acclimation enhances hypothalamic sensitivity to norepinephrine, a consensus neurotransmitter in temperature regulation, while Horowitz *et al.*, (37), and Schwimmer *et al* (38) shed light on the neuromodulatory role of the angiotensinergic system via its disruption by intracerebroventricular administration of losartan (an AT1-receptor blocker). Horowitz *et al.*, (37), and Schwimmer *et al.*, (38) demonstrated the interconnectivity of two antagonistic Ang II signaling pathways, that of AT1 receptors (which decrease the evaporative-cooling temperature threshold and core temperature) and AT2 receptors (which elevate body

temperature). In the heat acclimated phenotype the influence of AngII is routed to enhance the AT2 signaling pathway. Heat acclimation increases total AT2 receptor density and the AT2 membrane/AT1 cytosolic receptor ratio (via cellular trafficking) (38). In this way, the AT2 receptors oppose the drop in thermoregulatory thresholds induced by the AT1 receptors (37). It is likely that changes in outer membrane Angiotensin II receptor densities fine-tunes a "rudimentary" regulatory pathway, abolishing extreme deviations in integrative physiological responses [For further details the reader is referred to ref (18)].

**4.2. Genomic responses**

Two recent advances – (i) the implication that long-term accommodation to a changing environment involves functional neuronal remodeling associated with transcriptional reprogramming (39-43) and (ii) the understanding that there is neurogenesis in the hypothalamus (39), have introduced new concepts to the studies of heat acclimation. These developments gave rise to the hypothesis that the adjustability of the T-Tsh in response to chronic (environmental) sensory inputs, implies that long-term molecular and cellular processes are at least part of the underlying mechanisms of neuronal plasticity.

**4.2.1. Does heat acclimation enhance hypothalamic progenitor cell proliferation?**

Matsuzaki *et al.*,2009 (44) demonstrated that long term heat acclimation generated a significant number of new mature neurons in the hypothalamus. In congruence with Piraue's findings these neurons might play a role in thermoregulation. In the same vein, Schwimmer and Horowitz, (unpublished and in, http://www.ncbi.nlm.nih.gov/geo/ GEO Series accession number GSE2890), used a cDNA array and demonstrated up-regulation of gene transcripts associated with cell cycle and division in the hypothalami of long term heat acclimated rats.

**4.2.2. Understanding the hypothalamic transcriptome throughout heat acclimation**

During acclimation the variations in T-Tsh for major heat-dissipation mechanisms occur in a biphasic manner, with an initial marked depression followed by steady state stabilization characterizing acclimation (22). This temperature profile correlates with the biphasic remodeling of hypothalamic gene expression in several distinct functional groups [GO (gene ontology) for biological processes] thus allowing genomic-physiological linkage interpretations (18, 45). At the onset of heat acclimation (STHA) (i) the marked transient up-regulation in transcription is confined predominantly to genes encoding voltage-gated ion channels, ion pumps, or transporters, as well as hormone or transmitter receptors and cellular messengers, and implicates enhanced membrane depolarization, leading to the release of transmitters and neuronal excitability at this acclimation phase; and (ii) the transient down-regulation of genes participating in intracellular protein trafficking, metabolism, or phosphorylation processes implies a perturbation in cellular maintenance(46, 47).

In contrast to the STHA phase, a decrease in the expression of specific LTHA-activated genes related to various metabolic activities, including mitochondrial energy metabolism and cellular maintenance processes, together with the resumption of pre-acclimation transcript levels of genes encoding proteins involved in ion movement and membrane or cellular signaling, is noteworthy. An additional significant finding is the constitutive down-regulation of genes associated with energy metabolism and food intake and the marked up-regulation of a large group of genes linked with the immune response (45).

Collectively, the marked initial drop in T-Tsh (22) and increased effector organ excitability (20), signifying perturbed peripheral-effector cellular performance (19-22) match the hypothalamic transcriptome map established by Schwimmer *et al.*, (45). The metabolic features of the acclimated state also fit with the transcriptome map, suggesting that neuromodulation affecting thermoregulatory thresholds, involves the reprogramming of gene expression, and occurs during acclimation.

Notably, hypohydration interferes with heat acclimation. There are changes in the hypohydrated hypothalamic transcrictome maps during the course of heat acclimation (45). The predominant transcriptional activation could be categorized into genes associated with transmembrane ion transport, including those associated with $K^+$ currents, sodium and calcium conductance, and neuronal signaling as well as vasopressin and angiotensin receptors, cytochrome *c* oxidase, and several transporters characterizing supraoptic nucleus activation. Downregulation of a large number of gene transcripts associated with maintaining homeostatic cellular processes. This profile resembles that characterizes brain traumatic situations associated with cellular energy exhaustion and, in turn, with depolarization, transmitter release. However, currently our data are insufficient to link central-peripheral thresholds and support failure at the level of the peripheral effectors (46, 47).

A conceptual model describing hypothalamic peripheral organ cross-talk, which ultimately determine thermoregulatory thresholds, is presented in Figure 2.

**4.2.3. Does Heat acclimation affect the epigenetic code?**

Recent data from our laboratory established an "imprinting" of the effects of acclimation (48). Measurements of $T_{core}$ profile in heat acclimated, de-acclimated and re-acclimated rats during heat stress demonstrated that re-acclimation is markedly faster than the initial acclimation session, even after a 2 month period of de-acclimation, suggesting heat acclimation memory. Acclimation memory studied in the heart substantiated the involvement of chromatin remodeling and transcription in this process (48) and Tetievsky and Horowitz (unpublished). We hypothesize that similar processes occur centrally. This hypothesis is supported by Yossifoff *et al.*, (49) who studied the frontal hypothalamus of chicks and showed transient changes in the expression of brain-derived

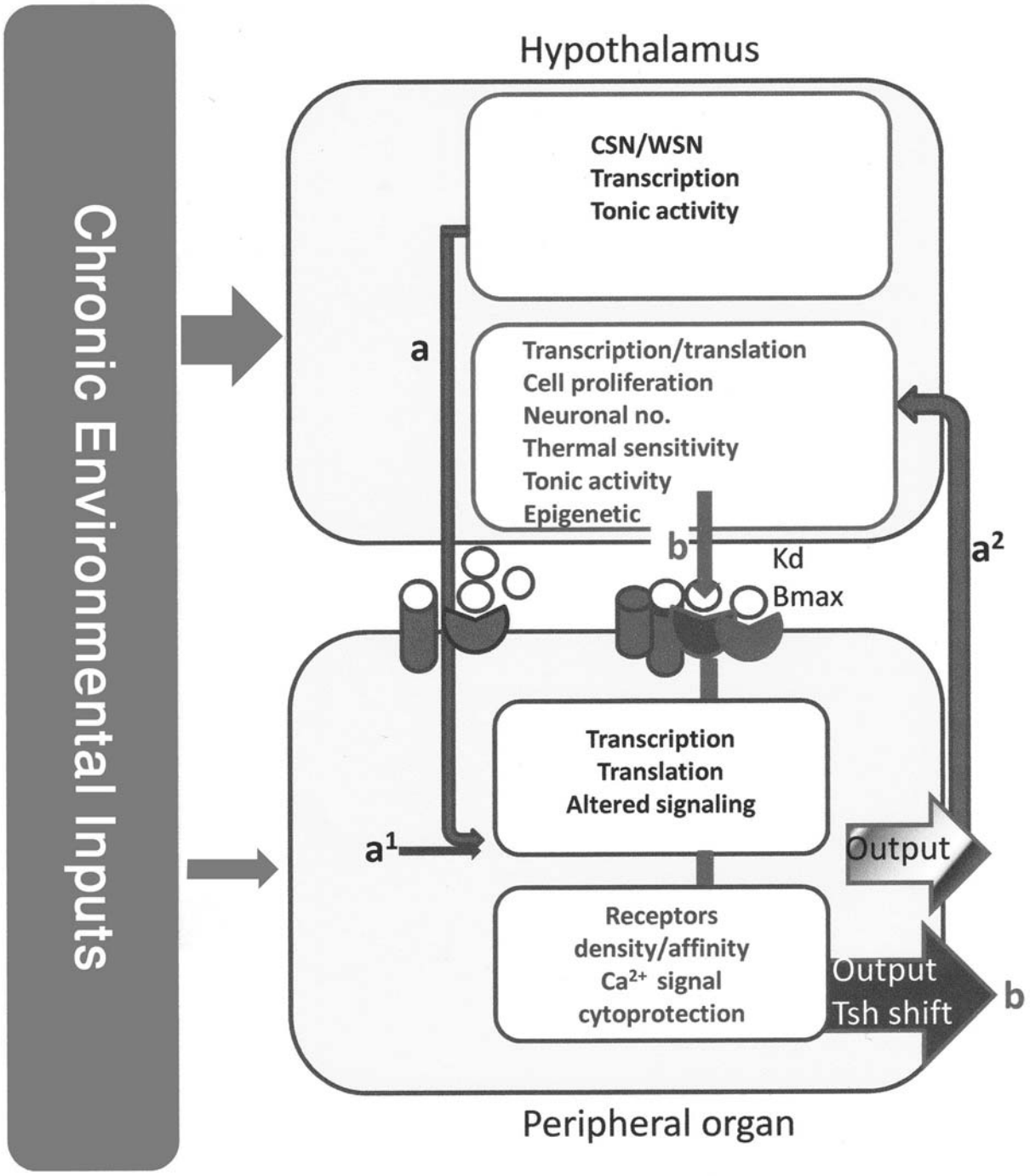


**Figure 2.** Central-Peripheral interactions during heat acclimation: a conceptual model. Chronic exposure to high ambient temperatures mobilizes the thermoregulatory system and impacts on core body temperature and warm receptors. At the onset of heat acclimation (a-loop, gray lines), increased autonomic excitability compensates for cellular impairments in peripheral effectors. Acclimatory homeostasis is displayed by the b-loop (red lines). Thermoregulatory thresholds are determined by central peripheral cross-talk. For detailed explanation see text Sec. 4). CSN/WSN – cold and warm sensitive neurons

neurotrophic factor (BDNF) during thermal conditioning and re-exposure of the conditioned chicks to heat stress. These changes were due to alterations in the epigenetic code that determines the repertoire of transcribed proteins and coincide with changes in the CpG methylation pattern in the avian Bdnf promoter region, leading to binding of the cAMP response element to the Bdnf promoter. The authors (49) suggest that complex and dynamic changes in DNA methylation are involved in the regulation of CREB expression, phosphorylation and thermotolerance

acquisition (CREB- is associated with memory storage both in the brain (50) and the heart (51). The time interval between conditioning and re-exposure (10 days) as well as the avian age (chicks) differed from the time span (1 mo)/age (adults) in the rat experiments. However, our findings of BDNF up-regulation in long term heat acclimated mice (52) and its modulation after traumatic brain injury may imply that its functional performance is similar in these species.

In sum, there is limited data regarding the underlying molecular and physiological mechanisms of acclimatory neuroplasticity and the regulation of acclimated thermoregulatory thresholds. However, the accumulated data allow us to distinguish between "fundamental" and epiphenomenon influences in acclimated threshold control as, for example, in the interaction between clock gene and acclimation. Additionally, a causal resemblance between the kinetics of ionic current genes and thermoregulatory thresholds during the course of heat acclimation is also evident, however, the central master molecular switch/es is/are still enigmatic.

## 5. ESSENTIAL AND SUFFICIENT GENES IN HEAT ACCLIMATION AND DELAYED THERMAL INJURY

In 1997 in his review on "Heat shock proteins and heat adaptation of the whole organism" (53), Moseley speculated that "Adaptation to heat may occur through acclimatization or thermotolerance (namely- heat shock response induced by heat shock proteins -Au) …" and that "…differences in their accumulation in organisms adapted to the heat suggest a role for HSPs in acclimatization as well". At the time, studies focusing on this aspect were confined to HSPs from the 90 kDa family, in poikilotherms (54). In the goby fish - *(Gilichthys mirabilis)* the threshold for HSP 90 kDa induction in the brain increased by almost 14°C following the transition from cold acclimation to warm acclimation. This was accompanied by an increased HSP90 level. Invertebrates and several other poikilotherms, evolutionary adapted to hot environment, had increased levels of HSP70 when inhabiting hot environment compared to their counterparts living in comfort habitats (55). In homeotherms, in 1997, the first report on heat acclimation and HSP72 was published, when Horowitz *et al.*, (56) studied the HSP72 profile in hearts of heat acclimating rats. The data showed that in mammal species – rats (57) and mice (58), similarly to poikilotherms, heat acclimation elevates HSP levels, suggesting that the production of a larger stock of HSP on acclimation recapitulates the evolutionary adaptive solution. In humans following an acclimation protocol (which includes heat and exercise) there was sustained elevation of HSP72 from days 6 to 10 suggesting cellular adaptation to heat acclimation that contributes to improved heat tolerance and reduced risk of heat illness (59). The novel findings derived from the rodents studies (57, 58) were that heat acclimation accelerates the rate and alters the magnitude of HSP expression upon stress. Both features coincided with improved heat endurance of acclimated rats to heat stress. Given that HSP70 is a consensus heat inducible cytoprotective molecule, its role in enhanced acclimatory thermal tolerance is unequivocal.

Is HSP70 essential and sufficient for heat acclimation? Using the genetic *Ceanorhabditis elegans* heat acclimation model Treinin *et al.*, (60) demonstrated that *C. elegans* mutant with HIF-1 (hypoxia inducible factor) loss of function cannot acclimate to heat despite elevated HSP72 levels. Likewise *C. elegans* mutants in which the heat shock transcription factor was blocked showed poor acclimation (Horowitz and Treinin, 2009, unpublished) suggesting that HSP70 might be essential but insufficient alone, for heat acclimation. HIF-1 is essential, but also, insufficient for heat acclimation.

Recently, Baird *et al.*, (61) used a hypoxic hepatic cell line and showed that induction of the heat shock pathway during hypoxia requires regulation of heat shock factor (HSF1) by hypoxia-inducible factor-1. Due to the fact that activation of HSF1 is the hallmark of the heat shock response [(62) and sec 5.1 this review)], cross talk between HIF-1 and HSF1 is intriguing and supports an interaction between HIF-1 and HSP70 in the acclimation model. This question is currently under investigation.

### 5.1. HSPs affect the thermal injury threshold: an acclimatory profile

Heat shock proteins (HSPs) are chaperones, evolutionary conserved, assisting in the correction and non-covalent assembly of other polypeptide containing structures *in vivo*, but are not components of these assembled structures while performing their biological functions (63). They recognize and selectively bind non-native proteins under stressful conditions, inhibit incorrect interactions and the formation of non-functional structures (62, 64, 65). The classical cellular salvage stress-response (traditionally known as the Heat Shock Response (HSR), has 2 windows of protection, a short term one of up to 60 minutes, activating defined salvage pathways involving the adenosine receptor, various kinases and mitochondrial $_{K}$ATP dependent channels (66). The second, delayed response is longer lasting *and involves the preferential synthesis of HSPs (65) and other proteins*. The cytoprotective action of the HSPs depends on their cellular levels. Additionally, HSP70 interferes with programmed cell death (apoptosis), has a role in normal immune response (63), enhances antigen presentation to T lymphocytes and collectively, important in targeting cytotoxic cells (71). Hence, prior HSP elevation preconditions cells to harsher stressful events. HSPs are induced by variety of stressors, including heat shock and therefore, are linked with the threshold for thermal injury. Among the HSP species, HSP70 is particularly associated with heat shock and its levels are also considered as markers of the severity of the stress (68).

Long term/continuous exposure to heat (heat acclimation/heat acclimatization), constitutively elevates cellular HSP (HSP70 and HSP90) reserves. This feature is shared by all species studied, poikilo- and homeothermic, vertebrates and invertebrates, suggesting that it is evolutionarily conserved [(54, 55, 57, 61, 69) and sec 5.0

this review]. Heat acclimation alters transcription/translation thresholds and the response rate to heat stress (and several other novel stressors) (58, 70). Collectively, heat acclimation causes a two component HSP response. While increased HSP reserves suggests that the cell is now endowed with 'on-call' cytoprotective molecules *without the need for de novo HSP synthesis* the abrupt component, namely faster transcription, also improves the renewal rate of stress protein reserves.

A cDNA array of stress genes was used to examine the mRNA from heat acclimated heat stressed rats (70), and substantiated (in addition to changes in HSPs) enhancements of (i) anti-apoptotic, and (ii) anti-oxidative networks [(50), http://www.ncbi.nlm.nih.gov/geo/ GEO Series accession number GSE2890)]. In congruence, based on TUNEL and cleaved caspase 3, Assayag *et al* (71) demonstrated in heat stressed rats that heat acclimation produces an apoptosis resistant cardiac phenotype. Likewise, Horowitz *et al.*, (72) used fluorimentric techniques and measured lower levels of reactive oxygen species in heat stressed acclimated cardiomyocytes.

The build up of cellular HSP reserves is intriguing. As heat acclimation in mammals does not induce severe hyperthermia, other mediators are required to increase HSP transcription. The failure to build up cellular reserves of HSP 72 kDa during acclimation in the presence of β-adrenergic blockade led us to postulate that the excitable sympathetic system, primarily during STHA, is a mediator of the transcriptional process (73).

HSF (Heat shock factor) is a transcription factor essential to heat shock gene transcription. Among the 4 HSF species, HSF1 is considered the master regulator of the heat shock (stress) response. When the cell is stressed, the HSF1 monomer is released from the (HSPs-HSF1) complex, trimerizes in the cytoplasm, is then phosphorylated and migrates to the nucleus (62, 79). In the nucleus it binds to the heat shock response element (HSE) found in the promoters of all heat shock genes initiating gene statement, thereby increasing HSP synthesis. In the goby fish *Gillichthys mirabilis* (54) the temperature of HSF1 activation was positively correlated with acclimation temperature. The authors attributed the acclimation plasticity to HSF1 activation adaptability. In evolutionary adapted desert lizards, higher levels of the active form of HSF1 were detected. This correlates with constitutively higher HSP levels vs non desert lizard species (75). Maloyan and Horowitz (unpublished and supplementary file in Tetievsky *et al.*, (48)) detected constitutive HSF1-HSE binding in heat acclimated rats together with faster hsp transcription during heat stress. Taken together, we suggest that constitutive HSF1-HSE binding predisposes to a faster heat shock response in acclimated rats and that plasticity in HSF1 activation is linked to the temperature threshold for thermal injury.

Notably, the mRNA/HSP72 ratio in heat acclimated rodents is markedly higher compared to that of non-acclimated controls (57) suggesting hardening of the translational system.

Taken together, the delayed thermal injury threshold stems from orchestrated interactions of several cytoprotective networks, of which the HSP regulatory system predominates. The findings that HSPs interact with integrative physiological responses such as respiratory control (76) and blood pressure regulation during heat stroke (77), synaptic transmission and epithelial permeability (78), implies that there are additional aspects to their protective role and perhaps the scope of the "classical" heat shock response is broader than that currently considered. This concept is strengthen by recent evidence, derived from heat stressed cultured cells that the tight junction protein Occludin (involved in epithelial permeability) is controlled by HSF-1 (79). Similarly, Baird *et al.*, (see above, ref (61)) provided evidence that HIF-1α requires HSF-1 for its activation.

Thermoregulatory capacity during hyperthermia can be demonstrated using the Tc profile during exposure to heat stress. Interestingly, in heat acclimated rats subjected to heat stress at 41°C the heating rate and HSP production are accelerated. Heat stress at 43°C, in contrast, profoundly attenuates heating in the acclimated group, decreasing heat strain and in turn HSP synthesis. Considering these responses, we can conclude that increased environmental stress enhances the physiological capacity of the acclimated animals to reduce the rise in body temperature and, in turn, heat strain and HSP production (80). Whether this phenomenon is found in other species is as yet unknown.

**5.2. Does HIF-1α play a role? A lesson from the heart and the brain**

Metabolic adaptations occurring in the heat acclimated heart include (i) a doubling of cardiac glycogen reserves (81), (ii) greater glucose uptake (81) (iii) upregulation of the glucose transporters GLUT 1 and 4 (Levi and Horowitz, unpublished), (iv) overexpression of 6-phosphofructo-2-kinase-2 (PFK-2) transcript, (v) enhancement of glyceraldehyde-3-phosphate dehydrogenase (GA3PD) activation (81). These changes drew our attention to the potential involvement of the hypoxia inducible factor HIF-1α, the master regulator of oxygen homeostasis, in heat acclimation. During heat acclimation the animal does not experience hypoxic episodes. However, the HIF-1-mediated hypoxic response appeared early in metazoan evolution to regulate metabolic responses and is highly conserved (82, 83). We therefore hypothesized that HIF-1 could be exploited by a variety of physiological adaptive mechanisms requiring metabolic changes, such as heat acclimation, where the result is enhanced metabolic efficiency (84, 85)

In contrast to HSP, which is regulated via transcriptional activation, HIF-1α levels are controlled by proteosomal ubiquitination (for mechanistic details see e.g. (86, 87)). Nevertheless, heat acclimation similarly affects HSPs and HIF-1α cellular profile, namely there is an almost twofold increase in HIF-1α levels in both heart and brain tissues (85, 88). Transcriptional activation of HIF-1 in heat acclimated animals was confirmed by confocal microscopy, nuclear binding, and transcriptional activation

of consensus HIF-1 targeted genes (85). Transcriptional activation of the target genes in response to novel stressors is hastened. This response was similar to that observed for transcription and translation of HSPs. Additionally, heat acclimated rats demonstrated weak constitutive DNA binding activity of HIF-1 to the HRE (hypoxia response element). Transcriptional activation was measured for *EPO,* (85, 88) and several metabolic enzymes linked with mitochondrial performance and ATP production including PDK1, COX4-2, LON (respiratory functions) and BNIP (apoptosis) (87) (84). In an attempt to shed light on the role of HIF-1 targeted pathways in heat acclimation, transcriptional activation was challenged by oxygen deprivation, the seemingly ultimate stressor of HIF-1α (85, 88). Heat acclimation (alone) markedly increased basal levels of the HIF-1α encoded proteins while heat stress as well as ischemia hastened transcriptional activation. These data suggest that heat acclimation predisposes to enhanced metabolic efficiency. This feature is less important under basal metabolic conditions but crucial upon challenges such as heat stress, where excessive heat production (biochemical inefficiency) is deleterious. During heat stress mediated hypoxia (e.g. heat stroke induced cerebral hypoxia) increased efficiency would be beneficial in regulating cell volume e.g. by improved control of a variety of ion pumps. Given that HIF-1α is also associated with apoptotic/antiapoptotic pathways we suggest that collectively HIF-1 targets are involved with cytoprotection and delayed thermal injury.

## 6. CENTRAL-PERIPHERAL LINKAGE IN HEAT ACCLIMATION

### 6.1. Effector organs and membrane signaling

Changes in cellular processes and membranes are mirrored by alterations in organ responsiveness (3, 24, 26) and impact on central excitability (24, 26). This has been demonstrated for all the heat dissipating effector organs, and the cell membrane is the initial target of acclimatory induced changes. Unfortunately, there are few studies on this heat acclimatory phenomenon. In the submaxillary salivary gland, the major evaporative cooling organ of the rat, acclimation induced changes are manifested by the occupancy of the high affinity vs low affinity cholinergic receptors of the gland (21, 24) In the submaxillary salivary gland, the major evaporative cooling organ of the rat, heat acclimation alters the cholinergic receptor population of the cell membrane, from low affinity to high. Additionally, changes distal to the cell membrane also occur and imply G protein modulations. Elevations in $Ca^{2+}$ signal amplitude per quantum of stimulus were also measured (24). The specificity of these responses was validated via comparison to the effects of acute heat stress (89). In the vasculature, heat acclimation enhances adrenergic arterial contraction in a multifaceted mechanism. Changes occur at the adrenergic receptor level (heightened sensitivity and a different subtype profile, $\alpha_{1D} > \alpha_{1A} >> \alpha_{1B}$), as well as in $Ca^{2+}$ responsiveness and G-protein/Phosphatase dependent contraction, collectively improving contractility and increasing the efficiency of vascular function in the acclimated animal (Nir E. and Horowitz M.). A marked NO (nitric oxide) effect was measured in the splanchnic circulation, leading to maintenance of adequate splanchnic blood flow for heat convection, despite the thermoregulatory induced 'splanchic vasoconstriction' reflex (90). It is likely that environmental signals strain the membranes, resulting in a cascade of cellular compensatory responses, which affect organ responsiveness and in turn, central stimulation during thermoregulatory activities.

### 6.2. Molecular - physiological linkage in the performance of peripheral organs

In the last few years, genomic technologies have improved our ability to examine the global response to environmental stressors. Hence, data regarding gene expression profiles can be derived either from studies focused on specific questions or from global genomic responses, where genes are often grouped into functional categories based on gene ontology annotation and their enrichment compared to non responding or non stressed controls. Unfortunately, the data on acclimation induced remodeling of molecular processes and cellular constitutive proteins are sporadic and confined to the special interests of particular research groups. In mammalian species, data on acclimating animals are mostly confined to contractile tissues; heart, skeletal muscles and occasionally the vasculature. The cardiac muscle of rats, acclimated under sedentary conditions, is the most extensively studied. However, several principal processes (e.g. $Ca^{2+}$ turnover/signaling, mitochondrial performance) may be shared by multiple organs (as already mentioned above; sec *5.1*). In the heart, heat acclimation improves mechanical and metabolic performance, resulting in a greater pressure generation with lower oxygen consumption. A transition to metabolically efficient V3 (slow) myosin predominance partially contributes to this feature. The hallmark of the acclimated heart is the adaptation of the contractile machinery. This is brought into play by mechanisms that increase $Ca^{2+}$ signal amplitude ($Ca^{2+}$ transient) to compensate for $Ca^{2+}$ desensitization of the myofilaments, thus increasing contractile force. These changes are accompanied by reprogramming of the expression of excitation- contraction (EC) coupling associated genes and $Ca^{2+}$ regulatory proteins. Temperature hardening allows greater pressure generation during hyperthermia whereas non-acclimated hearts fail to restitute pressure above 39$^{o}$ C (91). Reprogrammed HIF-1 targets imply improved mitochondrial aerobic capacity (84). Kodesh and Horowitz (GEO, http:// www.ncbi.nlm.nih.gov/geo- / GEO Series accession number GSE7802) initiated a comparative study on the effects of heat acclimation with and without exercise training on the heart and the soleus skeletal muscle of rats. Although analyses are still underway, it seems that acclimation under sedentary conditions vs combined heat acclimation and exercise training enriches biological functional categories differentially although both treatments enhanced contractility (92).

Collectively, the data provide evidence that heat acclimation confers long-term intrinsic adaptations; caused by the reprogramming of gene expression that matches the peripheral hemodynamic load (due to e.g. acclimation mediated plasma volume expansion and heat stress induced peripheral vasoconstriction for heat dissipation).

## 7. HEAT ACCLIMATION MEDIATED CROSS-TOLERANCE

An inseparable outcome of heat acclimation is that adjusting to one environmental stressor can increase resistance to subsequent acute exposures to novel/different stressors. Such cross-reinforcement raises the possibility of inducing adaptation to a stressor without prior exposure to it (exaptation). An important beneficial effect of heat acclimation is the induction of "cross-tolerance" against oxygen supply/oxygen demand mismatching, ischemia/reperfution insults (18, 25, 81, 93, 94), hyperoxia (95) and its consequences, as well as traumatic brain injury (96), noise (97), and ionizing radiation (Robinson, Marmary and Horowitz, in preparation). Due to the fact that heat acclimation mediated cross-tolerance is the outcome of constitutive changes that develop throughout the acclimation process, it relies on enhanced cytoprotective pathways in the acclimated phenotype as well as attenuation of detrimental processes in a two tier manner. Increased cytoportective molecule reserves confer protection without the need for *de novo* synthesis, while several other features are dynamic and are altered abruptly (up or down regulated) upon injury. The protection is achieved by enhancement of universal stress response pathways, largely independent of the type of stressor applied (e.g. HSP72, antioxidative, antiapoptotic, HIF-1 targets networks (18), however, gene chip analyses revealed subsets of differentially expressed genes, unique to each preconditioning stimulus (18, 50), suggesting that different organ/tissues may have specific cross-tolerance programs as exemplified for example for BDNF, which is upregulated in the brain, but is not visible in the heart (50, 45, respectively).

Heat acclimation induced cross-tolerance is committed to memory and reappears (even following 2 months of de-acclimation) shortly (2 days) after a return to the acclimating conditions (51). cDNA analyses of the cardiac tissue of heat acclimated, de-acclimated and re-acclimated rats pinpointed significant up- and down- regulation of genes linked to chromatin remodeling. Recent findings show histone (H4) acetylation during 30 days acclimation, de-acclimation and re-acclimation for 2 days, HSF1 binding to HSE-HSP70 promoter (98) and similar acclimatory mRNA kinetics post heat stress in these groups (51). These findings suggest that maintenance of the remodeled (open) chromatin state during the de-acclimation phase plays a pivotal role in the transcriptome profile and preconditions to rapid cytoprotection upon re-acclimation, namely it contributes to acclimation memory.

## 8. PERSPECTIVES

Plasticity of the thermoregulatory system is a key factor for coping with high/fluctuating ambient temperatures. Given the current climate changes, including global warming and more frequent heat waves, understanding the flexibility and the upper limits of our thermoregulatory system are very important. Another significant feature of heat acclimation is the cross-tolerance phenomenon, which expands the horizons of the practical applications of acclimation. Despite awareness of the importance of the molecular mechanisms underlying heat acclimation, experimentation and data gathering are still in their infancy and the sorting of specific essential acclimatory pathways are limited. Technological developments now allow transcriptome profiling of single cells, focusing the integrative genomic-physiological linkage. Less is known about the proteome and post translational modifications which play an essential role in protein functioning. As research techniques further improve, we will be able to learn more about this evolutionary conserved beneficial phenomenon.

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**Footnote 1.** The scientific distinction between the terms "acclimation" and "acclimatization" was introduced into the literature relatively recently, hence, in this review, when a citation is given the author's definition is also provided.

**Key Words:** Heat acclimation, Acclimatory neuroplasticity, Thermoregulatory threshold, Transcriptome profile, HSP70, HSF1, HIF-1α, |Cross tolerance, Review

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