

REVIEW

Open Access



Heat stress-induced mitochondrial damage and its impact on leukocyte function

Toshiaki Iba^{1*} , Julie Helms^{2,6} , Isao Nagaoka³ , Ricard Ferrer⁴ and Jerrold H. Levy⁵

Abstract

Heatstroke is characterized by systemic inflammation, immune dysregulation, and multiorgan failure, in which mitochondrial damage in leukocytes plays a pivotal role. This review examines the mechanisms by which heat stress induces leukocyte mitochondrial dysfunction and its downstream effects on immunity, coagulation, and organ integrity. Exposure to heat stress activates leukocytes through damage-associated molecular patterns (DAMPs), triggering the release of proinflammatory cytokines, reactive oxygen species (ROS), and neutrophil extracellular traps (NETs). These responses disrupt endothelial integrity, promote microvascular thrombosis, and contribute to the development of disseminated intravascular coagulation (DIC). Prolonged heat exposure further shifts the immune landscape toward immunosuppression, marked by monocyte deactivation and lymphocyte apoptosis. Mitochondrial dysfunction is central to this biphasic immune response. Heat stress reduces mitochondrial membrane potential, increases ROS production, and promotes the release of mitochondrial DNA and cytochrome c, amplifying inflammation and initiating cell death pathways, including apoptosis, pyroptosis, and ferroptosis. Biomarkers such as reduced mitochondrial membrane potential ($\Delta\Psi_m$), elevated mitochondrial ROS, cytochrome c, circulating mitochondrial DNA (mtDNA), and altered expression of mitophagy regulators (e.g., PINK1 and Parkin) provide insights into mitochondrial integrity and function in leukocytes. In addition to immune disruption, mitochondrial injury exacerbates coagulation abnormalities by promoting platelet activation and endothelial dysfunction, fostering a prothrombotic environment. In the microcirculation, leukocyte adhesion, NET formation, and endothelial damage create a self-amplifying cycle of ischemia and inflammation, ultimately leading to organ dysfunction, including hepatic failure, acute kidney injury, acute lung injury, and gastrointestinal barrier breakdown. Therapeutic strategies aimed at preserving mitochondrial function include antioxidants (e.g., N-acetylcysteine and MitoQ), mitochondrial biogenesis inducers (e.g., PGC-1 α activators), and mitophagy enhancers. Understanding the central role of leukocyte mitochondrial damage in heat stress provides a foundation for the development of targeted diagnostics and interventions to prevent organ failure and improve clinical outcomes.

Keywords Heat stress, Leukocyte, Mitochondria, Cell death, Organ dysfunction

*Correspondence:

Toshiaki Iba

toshiiba@juntendo.ac.jp

Full list of author information is available at the end of the article



© The Author(s) 2025. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

Introduction

Under heat stress, leukocytes undergo profound functional and phenotypic changes that contribute to systemic inflammation and organ dysfunction, forming a critical component of heat stroke pathophysiology [1]. Initially, exposure to excessive heat triggers the release of damage-associated molecular patterns (DAMPs), such as heat shock protein (HSP) 70, HSP72, and High Mobility Group Box 1 (HMGB1), from injured tissues, which in turn activate pattern recognition receptors (PRRs), such as toll-like receptors (TLRs) on leukocytes [2]. This leads to rapid activation of neutrophils and monocytes, resulting in the release of proinflammatory cytokines, including IL-1 β , IL-6, and TNF- α . These cytokines amplify the systemic inflammatory response, further promoting endothelial activation, increased vascular permeability, and further leukocyte recruitment into tissues [3].

Neutrophils play a central role by releasing reactive oxygen species (ROS), proteases, and forming neutrophil extracellular traps (NETs), which, while initially protective, can damage endothelial cells and exacerbate microvascular thrombosis [4]. Concomitantly, the heat stress induces lymphocyte apoptosis, particularly of CD4⁺ and CD8⁺ T cells, leading to a compromised adaptive immune response [5]. As the condition progresses, many patients shift from a hyperinflammatory state toward immunoparalysis, characterized by monocyte deactivation and further lymphopenia [6], increasing susceptibility to secondary infections and delaying inflammation resolution.

In addition, altered leukocyte trafficking and dysregulated expression of adhesion molecules further disrupt immune surveillance and tissue repair. The number and function of leukocytes can vary during the clinical course: an early leukocytosis with neutrophilia may be followed by leukopenia, particularly in severe or prolonged cases of heat stress, with these changes often correlating with a worse prognosis [7]. Importantly, many of these leukocyte responses mimic those seen in sepsis and sterile systemic inflammatory response syndrome (SIRS), highlighting the overlap between infectious and non-infectious inflammatory triggers [8]. Moreover, the dysregulated immune response under heat stress contributes to coagulation abnormalities, such as disseminated intravascular coagulation (DIC), further complicating clinical outcomes [9]. Emerging studies also suggest that mitochondrial dysfunction and oxidative stress within leukocytes themselves may impair their phagocytic and signaling capacities, reinforcing both the inflammatory and immunosuppressive phases of heat-related illness [10].

Taken together, leukocyte dysregulation under heat stress reflects a complex immune response in which

hyperinflammatory and immunosuppressive features may coexist. While a biphasic model of early hyperactivation followed by subsequent exhaustion has been described, recent evidence, including reduced monocyte HLA-DR expression at admission in exertional heat-stroke [6] and similar findings in sepsis, indicates that inflammation and immunosuppression can overlap from the outset. This evolving view underscores the heterogeneity of immune responses in heat stroke and highlights the need for further investigation.

This review provides an updated synthesis of the effect of heat stress on mitochondria and its consequences for leukocyte dysfunction and death.

Mechanism

Mitochondrial damage

Heat stress causes profound mitochondrial dysfunction in leukocytes, acting as a central mediator of immune dysregulation and systemic inflammation in heat-related illnesses [11, 12]. Mitochondria are crucial for energy production, maintaining redox balance, and regulating immune signaling. Elevated temperatures disrupt mitochondrial structure and function, compromising their ability to support normal leukocyte activity [13, 14].

One of the primary consequences of heat exposure is the loss of mitochondrial membrane potential ($\Delta\Psi_m$), which impairs ATP synthesis and diminishes energy-dependent leukocyte processes, such as migration, phagocytosis, and cytokine production [11, 13]. Heat stress also accelerates mitochondrial reactive oxygen species (mtROS) generation, resulting in oxidative damage that affects mitochondrial membranes, enzymes, and DNA [13, 15]. Although mtROS are involved in antimicrobial responses under physiological conditions, their excessive accumulation promotes cellular injury and pro-inflammatory signaling [16, 17].

Damaged mitochondria release immunostimulatory molecules, such as mitochondrial DNA (mtDNA) and cytochrome c, which act as DAMPs (Fig. 1). These molecules activate innate immune receptors, such as TLR9 and cGAS-STING (cyclic GMP-AMP synthase-stimulator of interferon genes), amplifying inflammation and triggering cell death pathways, including apoptosis and pyroptosis [18, 19]. For instance, mitochondrial outer membrane permeabilization leads to the release of cytochrome c and the initiation of the intrinsic apoptotic cascade, reducing lymphocyte viability and contributing to immunosuppression [20, 21].

The impact of mitochondrial damage varies across leukocyte subsets. Neutrophils exhibit impaired chemotaxis and altered NET formation under mitochondrial stress, while monocytes demonstrate reduced antigen-presenting capacity and disrupted metabolic

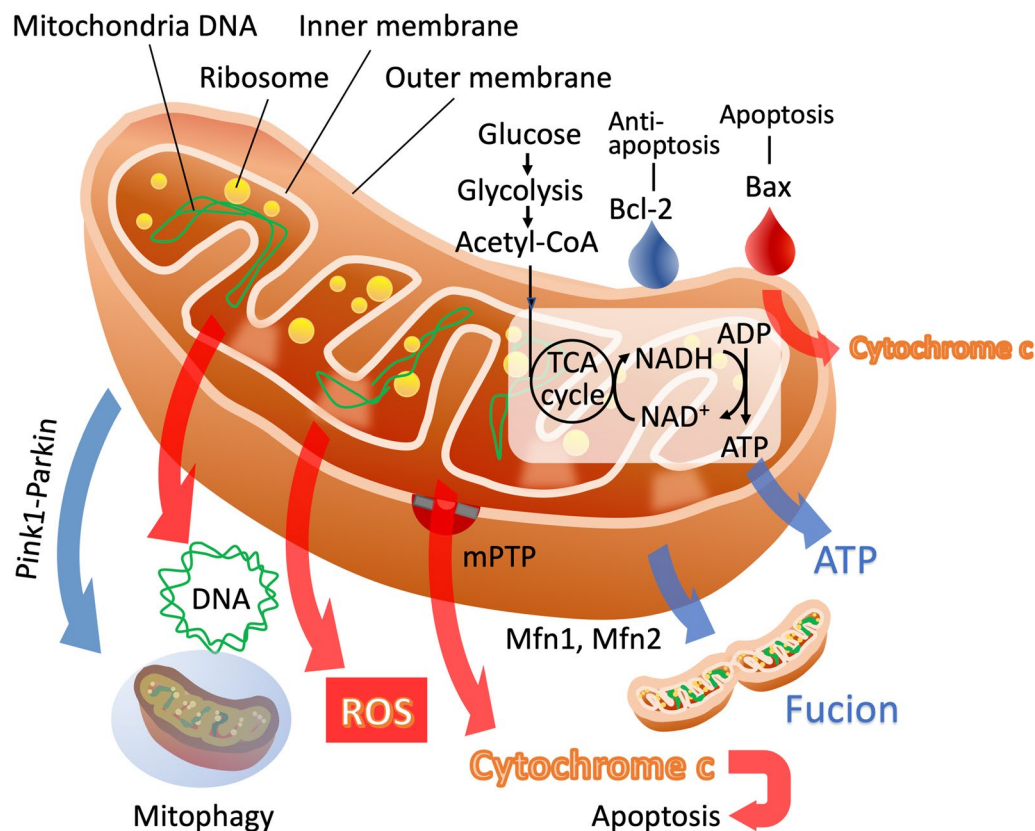


Fig. 1 Mitochondrial responses to heat stress. Heat stress induces mitochondrial damage, leading to multiple intracellular responses. The figure illustrates the mitochondrion's inner and outer membranes, mitochondrial DNA (mtDNA), ribosomes, and critical biomolecules, such as cytochrome c, adenosine triphosphate (ATP), and reactive oxygen species (ROS). Heat-induced mitochondrial dysfunction triggers the release of ROS and cytochrome c, leading to oxidative damage, mitochondrial DNA disruption, and apoptosis. The Tricarboxylic Acid (TCA) cycle is impaired, reducing ATP production. Damaged mitochondria are targeted for mitophagy, primarily through the Pink1–Parkin pathway. Meanwhile, the dynamic processes of mitochondrial fusion and fission modulate the mitochondrial network to maintain homeostasis or propagate damage under excessive stress. Bcl-2: B-cell lymphoma 2; Bax: Bcl-2-associated X protein; mPTP: mitochondrial permeability transition pore; Mfn: mitochondria fusion; Blue arrow: protective response; Red arrow: injurious response

reprogramming [22]. These changes compromise immune surveillance and resolution of inflammation, contributing to the biphasic immune response seen in heatstroke: an initial hyperinflammatory phase followed by immunosuppression [6, 23]. On the other hand, recent evidence suggests mitochondrial dysfunction in heatstroke operates within a bidirectional loop rather than a single linear cascade. Direct thermal stress can impair electron transport chain function, promote, and precipitate permeability transition, leading to loss of membrane potential and excess ROS generation [11].

Compounding this injury, heat stress impairs mitophagy, the selective degradation of dysfunctional mitochondria, allowing damaged organelles to accumulate [12, 24]. This further promotes oxidative

stress, cytokine production, and leukocyte dysfunction. Experimental studies using microscopy and functional assays confirm the presence of mitochondrial fragmentation, swelling, and depolarization in leukocytes exposed to heat [25]. The longitudinal experimental observations provide sequential evidence that decreased mitochondrial depolarization precedes mitochondrial degradation and subsequent leukocyte death, thereby supporting the temporal cascade (Figs. 2, 3 and 4, Suppl. Figures 1, 2 and 3).

Altogether, these mitochondrial alterations in leukocytes under heat stress initiate a feedback loop of energy depletion, inflammation, and immune cell death, contributing to multiorgan dysfunction and poor clinical outcomes [14, 26]. Targeting mitochondrial integrity and function may offer novel therapeutic avenues for managing heat-related syndromes [27, 28].

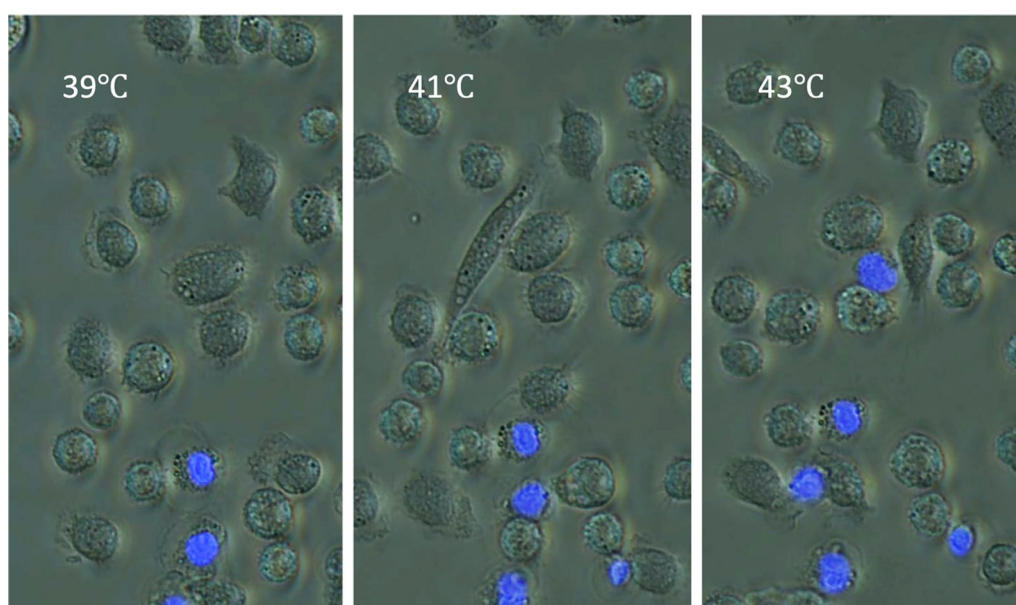


Fig. 2 Temperature-dependent increase in leukocyte cell death following heat exposure. Leukocytes were isolated from the abdominal cavity and cultured. The temperature of the culture medium was increased almost linearly from 37 °C to 43 °C in 3 h (The temperatures 39 °C, 41 °C, and 43 °C mean times 1, 2, and 3 h) at temperatures ranging from 39 °C to 43 °C. Intracellular DNA was stained with DAPI (4',6-diamidino-2-phenylindole, blue), and phase-contrast imaging was used to assess cellular morphology. While phase-contrast images revealed minimal morphological changes even at 43 °C, DAPI staining showed a progressive increase in the number of positively stained cells with rising temperature, indicating a temperature-dependent increase in cell death

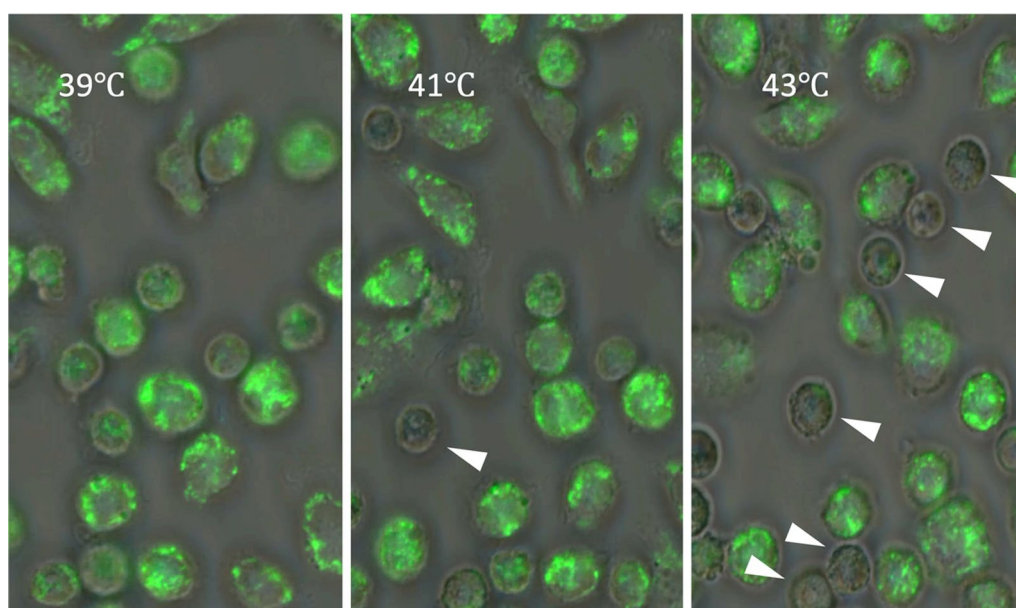


Fig. 3 Temperature-dependent loss of mitochondrial staining in leukocytes. Mitochondria in leukocytes were visualized using MitoBright LT™ (Dojindo, Kumamoto, Japan), a small-molecule fluorescent dye that selectively stains mitochondria via electrostatic interactions. This staining method allows for the assessment of mitochondrial distribution, abundance, and morphology. Leukocytes were exposed to increasing temperatures (from 39 °C to 43 °C in 3 h), and mitochondrial integrity was assessed by fluorescence microscopy. As the temperature increased, a growing number of cells showed a loss of mitochondrial staining (indicated by arrows), suggesting heat-induced mitochondrial depolarization or degradation

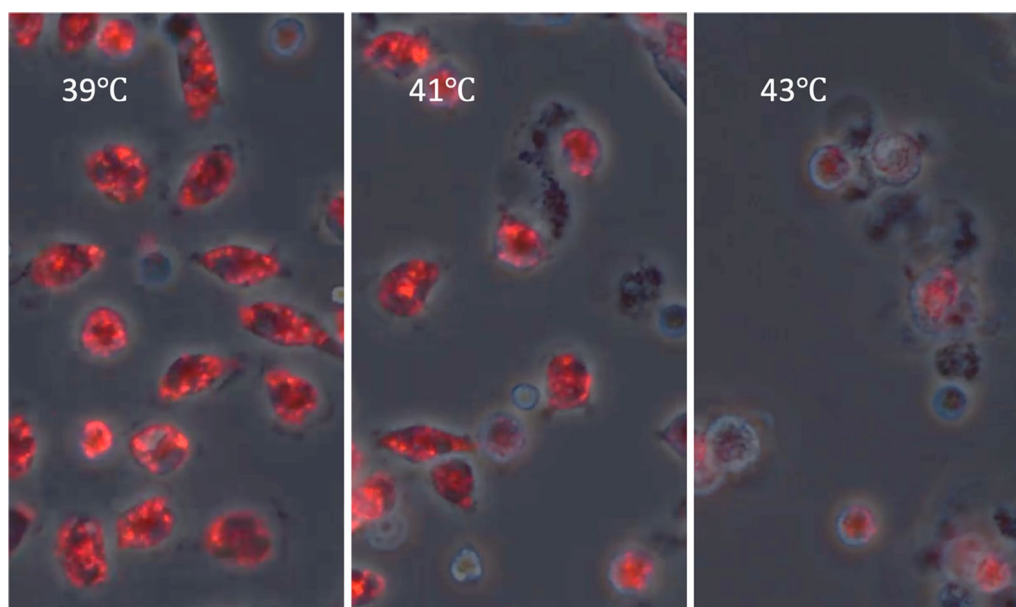


Fig. 4 Heat stress induces a temperature-dependent loss of mitochondrial membrane potential. Mitochondria are critical for cellular energy production through the generation of adenosine triphosphate (ATP). Their function is tightly linked to mitochondrial membrane potential ($\Delta\psi_m$), which reflects the organelle's capacity to produce ATP. JC-1 (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolylcarbocyanine iodide), a cationic fluorescent dye, was used to visualize $\Delta\psi_m$. Under normal conditions, JC-1 aggregates in mitochondria and emits red fluorescence, whereas depolarized mitochondria show green fluorescence due to monomeric JC-1. The figure illustrates a progressive, temperature-dependent decline in membrane potential with increasing heat stress (from 39 °C to 43 °C in 3 h), suggesting mitochondrial dysfunction under elevated thermal conditions. Loss of mitochondrial membrane potential precedes mitochondrial degradation and cell death, suggesting it may play a causative role in initiating these processes

Leukocyte cell death under heat stress

Leukocyte death is a pivotal consequence of heat stress and plays a central role in immune dysfunction, systemic inflammation, and organ failure. Various modes of cell death—apoptosis, pyroptosis, necrosis, necroptosis, ferroptosis, and NETosis—can be activated in leukocytes, depending on the intensity of stress, mitochondrial status, and metabolic context [29, 30]. Among these, mitochondria-mediated apoptosis is a major contributor, especially in lymphocytes. Heat stress disrupts mitochondrial membrane integrity, promoting the release of cytochrome c and the activation of the intrinsic apoptotic cascade through caspase-9 and caspase-3, which leads to DNA fragmentation and cellular dismantling [20, 21, 31]. This reduces lymphocyte populations, notably CD4⁺ and CD8⁺ T cells, impairing adaptive immunity and predisposing patients to secondary infections [32, 33].

Beyond apoptosis, mitochondrial dysfunction also predisposes monocytes and neutrophils to necrotic and necroptotic death, particularly when ATP production is compromised [34, 35]. These forms of cell death release intracellular DAMPs, including mtDNA, which propagates inflammation via TLR9 and cGAS–STING pathways, further amplifying systemic immune activation [18, 19].

Pyroptosis, driven by inflammasome activation (e.g., NLRP3), is especially relevant in monocytes and macrophages under thermal injury [36]. Heat-induced mitochondrial ROS promotes inflammasome assembly, activating gasdermin D and inducing pore formation, resulting in IL-1 β and IL-18 release [16, 37]. This pro-inflammatory cell death not only exacerbates leukocyte dysfunction but also reinforces endothelial damage and vascular leakage, linking directly to the coagulopathy described in Sect. “Coagulation disorders and mitochondrial damage under heat stress”.

NETosis, a neutrophil-specific response to oxidative stress, involves the release of chromatin-based NETs. Although NETs can sequester pathogens, their excessive release under heat stress damages endothelial barriers and promotes thrombosis, contributing to DIC [4, 38]. These processes highlight a convergence between cell death and coagulation, emphasizing the need for integrated therapeutic approaches.

Furthermore, ferroptosis, an iron-dependent, lipid peroxidation-driven cell death, has been observed under oxidative thermal stress, although its role in leukocytes remains under investigation [29]. The balance between survival and cell death is modulated by mitochondrial integrity, redox status, and anti-apoptotic factors, such as

Bcl-2 and HSP70, whose expression can delay or inhibit death signaling [39].

In clinical settings, elevated leukocyte apoptosis and impaired monocyte function correlate with poor outcomes in heatstroke patients [35]. As such, leukocyte cell death is not merely a marker of heat-related damage but also a driver of disease progression. Therapeutic interventions aimed at preserving mitochondrial function, inhibiting inflammasome activation, or blocking executioner caspases may hold promise in attenuating immune collapse and mitigating organ injury in severe heat-related illnesses.

Coagulation disorders and mitochondrial damage under heat stress

Coagulation abnormalities are a critical complication of heat stress and are closely linked to mitochondrial dysfunction across various cell types, including leukocytes, endothelial cells, and platelets. Mitochondria not only regulate cellular energy metabolism but also modulate redox signaling, inflammation, and programmed cell death, all of which influence hemostasis. Under heat stress, mitochondrial injury disrupts $\Delta\Psi_m$, impairs oxidative phosphorylation, and accelerates the generation of mtROS, thereby promoting procoagulant responses [11, 17, 27, 35].

In endothelial cells, mitochondrial damage increases oxidative stress, reduces ATP production, and impairs endothelial barrier function. This leads to enhanced vascular permeability and the exposure of subendothelial procoagulant factors, such as tissue factor (TF) and collagen, which initiate the extrinsic and intrinsic coagulation cascades [15, 27]. Concurrently, mitochondrial dysfunction promotes the release of circulating mtDNA, a potent DAMP that activates innate immune receptors, such as TLR9 and cGAS–STING, upregulating TF expression and priming monocytes and platelets for procoagulant activity [18, 40].

Platelets, when exposed to elevated temperatures, undergo mitochondrial depolarization, mtROS accumulation, and apoptosis-like changes. These mitochondrial alterations enhance platelet activation, aggregation, and the release of procoagulant microvesicles, collectively increasing thrombin generation and fibrin deposition [35, 41]. In parallel, leukocyte activation under heat stress contributes to NET formation, which further promotes microvascular thrombosis and the development of DIC [4, 38].

Inflammatory cytokines (e.g., IL-1 β and IL-18) produced by pyroptotic monocytes and other immune cells exacerbate endothelial activation and coagulation, establishing a feed-forward loop between inflammation and thrombosis [37, 42]. This process is further aggravated

by suppressed anticoagulant systems, including reduced expression or activity of antithrombin, protein C, and tissue factor pathway inhibitor (TFPI), all of which are negatively influenced by oxidative stress and mitochondrial dysfunction [5, 43].

At the same time, heat stress impairs endothelial production of anticoagulant and fibrinolytic mediators, such as thrombomodulin and prostacyclin, due to mitochondrial ATP depletion and ROS-induced signaling defects [5, 15]. These imbalances tip the hemostatic scale toward a prothrombotic state. In advanced stages, this may evolve into consumptive coagulopathy, where platelets and coagulation factors are depleted, increasing the risk of bleeding—an ominous feature in fulminant heatstroke [7].

Taken together, mitochondrial dysfunction under heat stress serves as a central link connecting oxidative stress, inflammatory signaling, and coagulation activation. The interaction between mitochondrial injury and coagulopathy creates a vicious cycle in which inflammation and microthrombosis impair tissue perfusion, exacerbate organ damage, and further perpetuate mitochondrial dysfunction [12, 26]. Targeted therapies aimed at preserving mitochondrial integrity—such as antioxidants, mitochondrial reactive oxygen species scavengers, and mitochondrial membrane stabilizers—may offer promising strategies to interrupt this cycle and reduce coagulation-related complications in severe heat-related illnesses. Biomarkers such as circulating mtDNA, platelet activation markers, and thrombin–antithrombin complexes may aid in the early detection and monitoring of heat-induced coagulopathy [40, 41].

Microcirculatory damage under heat stress

Microcirculatory dysfunction is a fundamental pathophysiological feature of heat-related illness, linking leukocyte activation, endothelial injury, and organ failure. The microcirculation, including capillaries, arterioles, and venules, plays a critical role in oxygen and nutrient delivery and waste removal. Under heat stress, this finely tuned network is disrupted by thermal injury, hemodynamic instability, and systemic inflammation, leading to tissue hypoperfusion and metabolic collapse [42, 44] (Fig. 5), (Suppl. Figure 4).

Direct thermal damage to endothelial cells impairs cytoskeletal organization, induces protein denaturation, and disrupts intercellular junctions, resulting in barrier breakdown and increased vascular permeability [45]. These changes are compounded by oxidative stress and ATP depletion caused by mitochondrial dysfunction within endothelial cells, which further compromise the integrity of tight junctions and increase capillary leakage [12, 27]. Simultaneously, activated leukocytes, especially

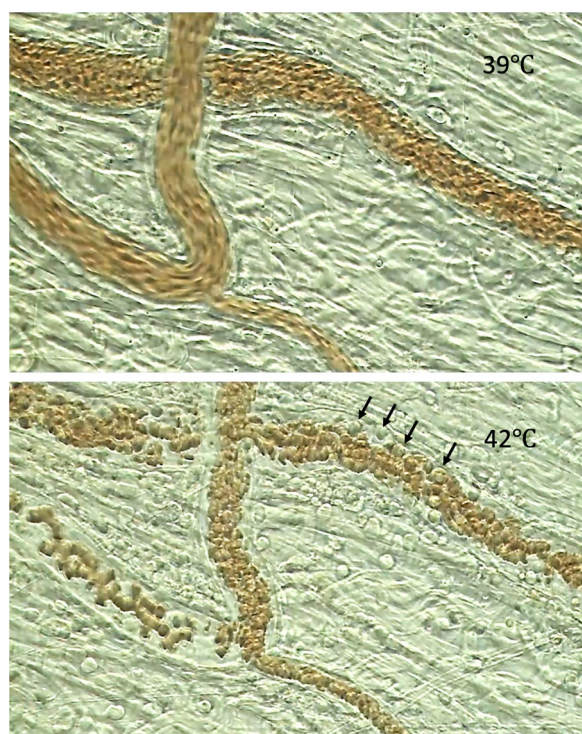


Fig. 5 Temperature-dependent alterations in mesenteric microcirculation during heat stress. Intravital microscopy was used to assess mesenteric microcirculation in a rat subjected to progressive heat exposure. At a core body temperature of 39 °C (top panel), blood flow appeared smooth with minimal leukocyte–endothelium interaction. As the temperature increased, leukocytes (indicated by black arrows) became rounded and began adhering to the vascular endothelium. At 42 °C (bottom panel), significant leukocyte adhesion led to disrupted blood flow, reflecting microcirculatory disturbance induced by heat stress

neutrophils and monocytes, adhere to the damaged endothelium via upregulated adhesion molecules, such as ICAM-1 and VCAM-1, triggering localized inflammation and amplifying vascular injury [12, 46].

A hallmark of microcirculatory damage under heat stress is the formation of NETs. While NETs help trap pathogens, their excessive release obstructs capillary flow, promotes microthrombosis, and directly injures endothelial cells, contributing to DIC and further exacerbating vascular dysfunction [4, 47, 48].

These events culminate in capillary leak syndrome, leading to tissue edema, impaired oxygen diffusion, and localized hypoxia. Organs with high metabolic demands, such as the brain, liver, kidneys, and lungs, are particularly vulnerable to these effects, which manifest clinically as encephalopathy, hepatic injury, acute kidney injury, and non-cardiogenic pulmonary edema [26, 49].

Hemodynamic instability, resulting from systemic vasodilation and hypovolemia, further impairs microvascular perfusion. Even after normalization of systemic

blood pressure, heterogeneous perfusion and “no-reflow” phenomena persist due to endothelial swelling, microthrombi formation, and leukocyte plugging—especially in renal and splanchnic territories [50, 51]. These regional ischemic zones contribute to irreversible organ damage and are not corrected by conventional fluid resuscitation or vasopressor therapy alone.

Mitochondrial damage in both leukocytes and endothelial cells plays a central role in this process by driving oxidative injury, reducing cellular resilience, and fueling proinflammatory and procoagulant signaling [12, 42]. The interplay between mitochondrial dysfunction, leukocyte activation, and endothelial disruption forms a self-perpetuating cycle of microcirculatory collapse.

In summary, microcirculatory injury under heat stress is a multifactorial process involving thermal endothelial injury, mitochondrial failure, immune cell activation, and microthrombosis. These alterations lead to inadequate tissue perfusion, organ ischemia, and ultimately multiorgan failure. Therapeutic strategies aimed at preserving endothelial and mitochondrial function, limiting leukocyte activation, and restoring capillary integrity are crucial for mitigating these downstream effects and improving outcomes in severe heat-related illnesses.

Organ dysfunction under heat stress and its relation to leukocyte damage

Multiorgan dysfunction is a defining feature of severe heat-related illness and is closely linked to leukocyte activation, mitochondrial injury, and systemic inflammation. Upon exposure to extreme temperatures, injured tissues release DAMPs that trigger leukocyte activation via pattern recognition receptors, initiating a cascade of proinflammatory responses. Activated leukocytes, particularly neutrophils and monocytes, release cytokines (e.g., IL-1 β and TNF- α), ROS, and proteolytic enzymes, contributing to endothelial injury, capillary leak, and microvascular thrombosis [1, 4, 52].

In parallel, mitochondrial dysfunction within leukocytes compromises ATP production and redox balance, promoting cell death through apoptosis, pyroptosis, and NETosis [12, 19, 20]. These forms of leukocyte death not only reduce immune cell numbers, especially lymphocytes, but also release secondary DAMPs and inflammatory mediators, amplifying systemic inflammation and propagating tissue injury. This feedback loop between mitochondrial damage and immune dysregulation accelerates organ failure across multiple systems.

The kidneys are especially vulnerable, frequently exhibiting acute tubular necrosis and ischemia due to both hypoperfusion and leukocyte-driven inflammation within the renal microvasculature [26]. In the liver, histological changes include sinusoidal congestion, leukocyte

infiltration, and hepatocellular necrosis, reflecting both systemic inflammation and oxidative damage [53]. Pulmonary complications, including non-cardiogenic pulmonary edema and acute respiratory distress syndrome (ARDS), result from increased endothelial permeability and leukocyte-induced alveolar–capillary barrier disruption [54]. In the central nervous system, elevated cytokine levels and compromised blood–brain barrier integrity contribute to neuronal dysfunction and cerebral edema, manifesting clinically as confusion, delirium, or coma [55].

In addition, heat stress impairs gastrointestinal barrier function, allowing bacterial translocation and further activation of innate immune responses, which can exacerbate systemic inflammation and worsen organ injury [56]. The cumulative impact of leukocyte activation, endothelial dysfunction, and mitochondrial impairment results in widespread tissue hypoxia, metabolic failure, and ultimately, multiorgan failure.

A distinctive feature of the immune response to heat stress is its biphasic nature. The initial hyperinflammatory phase is characterized by massive cytokine release and leukocyte activation, while the later immunosuppressive phase involves lymphocyte apoptosis and monocyte deactivation (e.g., reduced HLA–DR expression), resulting in impaired pathogen clearance and increased susceptibility to secondary infections [6, 23]. Mitochondrial injury plays a crucial role in both phases, promoting early immune activation through the release of DAMPs and driving later immunoparalysis via energy failure and the apoptotic loss of immune cells.

Biomarkers for mitochondrial damage

Identifying biomarkers of mitochondrial dysfunction in leukocytes provides valuable insight into the pathophysiology of heat-related illnesses and offers tools for diagnosis and therapeutic monitoring [57]. Mitochondrial damage affects energy production, redox homeostasis, and immune signaling, and several measurable indicators have been developed to assess these alterations.

One major class of biomarkers involves $\Delta\Psi_m$, a surrogate for mitochondrial function and ATP-generating capacity. Decreased $\Delta\Psi_m$ can be detected using fluorescent dyes, such as JC-1, TMRE (tetramethylrhodamine ethyl ester), or Rhodamine 123 via flow cytometry, indicating compromised mitochondrial integrity [58, 59]. A decline in $\Delta\Psi_m$ is correlated with impaired leukocyte function and increased susceptibility to programmed cell death [13, 21].

MtROS levels, another key indicator of oxidative stress, are commonly measured using probes, such as MitoSOX Red™ (Mitochondrial Superoxide Indicator, for live-cell imaging, ThermoFisher Scientific Inc., Waltham, MA)

[53]. Elevated mtROS levels reflect excessive oxidative activity and are associated with leukocyte dysfunction, inflammasome activation, and inflammatory cell death [16, 17]. However, the clinical application of mtROS as a biomarker is limited by its extremely short half-life, and thus surrogate indicators of oxidative stress may provide more reliable diagnostic value.

Similarly, the release of cytochrome c into the cytosol or plasma serves as a biomarker for mitochondrial outer membrane permeabilization and the initiation of intrinsic apoptosis [60].

Circulating or intracellular mtDNA is a potent DAMP and can be quantified via qPCR techniques. Elevated mtDNA levels are indicative of mitochondrial disruption and systemic inflammation [40, 61]. These changes may also point to impaired mitophagy and the accumulation of damaged mitochondria [12]. Complementary markers include mitophagy-related proteins, such as PINK1 (PTEN-induced kinase 1), Parkin, and LC3B, which can be measured via immunoblotting or flow cytometry. The altered expression of these proteins reflects the status of mitochondrial quality control and correlates with disease severity [24, 62].

In addition to structural and redox biomarkers, metabolic indicators such as reduced ATP content or altered NAD⁺/NADH ratios provide insight into mitochondrial metabolic failure. These can be evaluated using enzymatic or luminescent assays, offering insight into the energetic capacity of leukocytes during heat stress [63, 64].

Collectively, these biomarkers offer a comprehensive assessment of mitochondrial health, encompassing functional, structural, and metabolic aspects. Their integration into clinical and research settings may aid in early diagnosis, risk stratification, and evaluation of therapeutic responses targeting mitochondrial preservation in heat-related illnesses [6, 33].

Although $\Delta\Psi_m$, mtROS, and circulating mtDNA have been proposed as promising indicators of mitochondrial dysfunction, their translation into routine clinical use faces several obstacles. Measurement of $\Delta\Psi_m$ typically requires fluorescent probes in freshly isolated cells, limiting its applicability at the bedside, where real time, minimally invasive assays are preferable. Similarly, direct quantification of mtROS is hampered by their extremely short half-life and the need for specialized probes or imaging platforms, restricting current use to research laboratories. Circulating cell-free mtDNA can be measured using quantitative PCR or next-generation sequencing approaches, but results are influenced by pre-analytical variables (sample type, processing time, storage) and analytical variability across laboratories [5]. Standardization of protocols and establishment of

clinically meaningful cutoffs remain unresolved. Cost is another consideration, as high-throughput molecular assays may not be feasible in all healthcare systems.

Management of mitochondrial damage under heat stress

Effective management of mitochondrial damage is crucial for improving outcomes in heat-related illnesses, where mitochondrial dysfunction leads to immune dysregulation, coagulopathy, and organ failure. Heat stress disrupts $\Delta\Psi_m$, enhances the production of mtROS, and induces the release of pro-apoptotic and proinflammatory molecules, such as cytochrome c and mtDNA, initiating cascades of inflammation, cell death, and tissue injury [11, 12, 65].

Therapeutic strategies aim to preserve or restore mitochondrial integrity, limit oxidative damage, and promote mitochondrial quality control mechanisms. One core approach involves antioxidant therapy, which targets excessive mtROS and prevents oxidative injury to mitochondrial membranes, proteins, and DNA. Agents such as N-acetylcysteine (NAC), coenzyme Q10, vitamin C, and mitochondria-targeted antioxidants like mitoquinone (MitoQ) have shown efficacy in experimental models, reducing oxidative stress and preserving mitochondrial function under thermal injury [66–68] (Table 1).

Enhancing mitochondrial biogenesis is another key therapeutic avenue. Pharmacologic activators of PGC-1 α (peroxisome proliferator-activated receptor gamma coactivator 1- α), such as resveratrol, AICAR (5-Aminimidazole-4-carboxamide ribotide), and melatonin, stimulate mitochondrial replication and metabolic recovery, improving cellular energy balance during and after heat stress [69–71]. Similarly, supporting mitophagy, the selective clearance of damaged mitochondria, is crucial for maintaining mitochondrial homeostasis and preventing inflammatory signaling. Compounds that activate the PINK1/Parkin pathway or AMPK (AMP-activated protein kinase) signaling may help eliminate dysfunctional mitochondria and reduce leukocyte-mediated tissue injury [24].

Mitochondria-targeted peptides, such as SS-31 (elamipretide), represent an emerging class of therapies. SS-31 localizes to the inner mitochondrial membrane, where it stabilizes cardiolipin, reduces membrane permeability, and preserves ATP production under stress conditions [72]. In addition, inhibitors of the mitochondrial permeability transition pore (mPTP), such as cyclosporin A, may prevent mitochondrial swelling, cytochrome c release, and necrotic cell death in heat-stressed tissues [73].

Beyond direct mitochondrial interventions, supportive care remains essential. Rapid cooling is the most effective

method to prevent further mitochondrial damage by halting the thermal insult. Maintenance of adequate tissue perfusion through fluid resuscitation, vasopressors, and, if needed, renal replacement therapy is critical to minimize secondary ischemic injury. Metabolic support, including glucose, glutamine, or pyruvate, may serve as alternative energy substrates to sustain mitochondrial function during recovery [64].

Finally, biomarkers of mitochondrial dysfunction, such as circulating mtDNA, reduced $\Delta\Psi_m$ in leukocytes, or elevated mtROS, may aid in early diagnosis, risk stratification, and therapeutic monitoring in clinical settings [6, 61]. Incorporating these markers into clinical workflows could enable precision-targeted interventions and improve prognosis in patients with heat-related syndromes.

In summary, managing mitochondrial damage under heat stress requires a multifaceted approach combining antioxidant therapy, mitochondrial biogenesis and mitophagy support, temperature control, and organ support strategies. Targeted mitochondrial therapeutics represent a promising frontier in improving cellular resilience and limiting immune-mediated tissue injury in heat-related illnesses.

Limitations

The difficulty in researching heatstroke is that it requires extensive use of animal experiments. Although rodent and cell culture studies have provided crucial mechanistic insights into mitochondrial dysfunction, reactive oxygen species generation, and cellular injury during heat stress, their direct translation to human pathophysiology remains challenging. Animal models often employ extreme hyperthermic conditions and genetically homogeneous populations that do not fully capture the heterogeneity of human responses. Moreover, the immunological and metabolic responses in rodents may differ substantially from those in humans, limiting the extrapolation of therapeutic targets. Cell culture systems, while valuable for dissecting molecular pathways, lack the complex interactions among immune, vascular, and endocrine systems that shape human disease outcomes. Existing clinical data remain sparse, but reports of mitochondrial damage markers, such as circulating cell-free mitochondrial DNA, in critically ill or heatstroke patients suggest that mitochondrial dysfunction is also relevant in human pathology [5, 74]. Nevertheless, robust human studies, ideally integrating clinical biomarkers with mechanistic endpoints, are urgently needed. Bridging this gap will require translational research that validates preclinical findings in carefully phenotyped patient populations, using advanced molecular and imaging tools.

Table 1 Mitochondria-targeted therapies

Agent (class)	Mechanism (target)	Key experimental/clinical evidence	Clinical status (2025)	Main limitations
MitoQ/Mitoquinone	Mitochondria-targeted antioxidant; scavenges ROS	Preclinical organ protection; small human feasibility studies show bio-marker modulation	Investigational; small clinical trials ongoing, outcome benefit unproven	Clinical efficacy not established; PK in critically ill uncertain; tissue delivery may be limited
SS-31/Elamipretide	Cardiolipin-binding peptide; stabilizes ETC, reduces ROS, preserves ATP	Robust preclinical cardioprotection; phase 2–3 trials in myopathies and HF show mixed signals	Advanced clinical development in rare mitochondrial disorders, cardiomyopathies	High cost; neutral results in some trials; dosing in shock unvalidated
Resveratrol/PGC-1α activators	SIRT1 activator; enhances PGC-1α → mitochondrial biogenesis	Multiple preclinical studies; small RCTs with heterogeneous biomarker effects	Supplement available; clinical trials heterogeneous; not an approved therapy	Poor bioavailability; slow mechanism may limit use in acute illness
AICAR (AMPK activator)	Activates AMPK; stimulates mitophagy; biogenesis, metabolic reprogramming	Preclinical protection against mitochondrial dysfunction and stress; limited translational work	Experimental; occasional early human pharmacology studies	Limited human safety/PK data; optimal dosing in critical illness unknown
Melatonin	Direct antioxidant; regulates mitophagy, fusion/fission, mitochondrial enzymes	Extensive preclinical; human trials in oxidative stress show biomarker/physiologic effects	Widely available, safe, inexpensive; multiple small clinical trials	Variable dosing; limited data on mortality or organ protection in shock
N-acetylcysteine (NAC)	Replenishes glutathione; scavenges ROS, modulates redox	Long history of clinical use; RCTs in sepsis/SIRS show mixed outcomes	Approved for acetaminophen toxicity; widely available	No consensus on dose for mitochondrial rescue; inconsistent outcome benefit
Cyclosporine A	Inhibits cyclophilin D → prevents mPTP opening	Strong preclinical ischemia/reperfusion protection; clinical trials in MI largely neutral	Approved immunosuppressant; tested experimentally in acute injury	Immunosuppressive, nephrotoxic; neutral outcomes in cardioprotection trials

ROS: reactive oxygen species, PK: pharmacokinetics, HF: heart failure, SIRT1: silent information regulator 1, PGC-1α: peroxisome proliferator-activated receptor gamma coactivator 1-α, RCTs: randomized controlled trials, AICAR: 5-Aminimidazole-4-carboxamide ribotide activator, AMPK: adenosine monophosphate-activated protein kinase, NAC: N-acetylcysteine, SIRS: systemic inflammatory response syndrome, mPTP: mitochondrial permeability transition pore, MI: myocardial infarction

Conclusion

Mitochondrial damage in leukocytes plays a central and multifaceted role in the pathogenesis of heat-related illness, linking immune dysfunction, systemic inflammation, microcirculatory collapse, and coagulation abnormalities. Heat stress disrupts mitochondrial homeostasis, leading to energy failure, excessive ROS production, and the release of proinflammatory and prothrombotic mediators. These changes trigger a biphasic immune response—initial hyperactivation followed by immunosuppression—which contributes to multiorgan dysfunction and poor clinical outcomes. Identifying mitochondrial biomarkers such as mtROS, $\Delta\Psi_m$, and mtDNA can offer valuable tools for early diagnosis and monitoring. Therapeutic strategies aimed at preserving mitochondrial integrity, enhancing mitophagy, and limiting oxidative stress hold promise in mitigating disease severity. Interventions such as antioxidant therapy, mitochondrial-targeted agents, and immune modulation may improve cellular resilience and patient recovery. A deeper understanding of mitochondrial–immune interactions under heat stress could pave the way for precision medicine approaches in managing severe heat-related syndromes.

Abbreviations

DAMPs	Damage-associated molecular patterns
ROS	Reactive oxygen species
NETs	Neutrophil extracellular traps
DIC	Disseminated intravascular coagulation
$\Delta\Psi_m$	Mitochondrial membrane potential
HSP	Heat shock protein
HMGB1	High Mobility Group Box 1
PRRs	Pattern recognition receptors
TLRs	Toll-like receptors
ROS	Reactive oxygen species
NETs	Neutrophil extracellular traps
SIRS	Systemic inflammatory response syndrome
mtROS	Mitochondrial reactive oxygen species
mtDNA	Mitochondrial DNA
cGAS–STING	Cyclic GMP–AMP synthase–stimulator of interferon genes
TF	Tissue factor
TFPI	Tissue factor pathway inhibitor
ARDS	Acute respiratory distress syndrome
TMRE	Tetramethylrhodamine ethyl ester
PINK1	PTEN-induced kinase 1
NAC	N-acetylcysteine
PGC-1 α	Peroxisome proliferator-activated receptor gamma coactivator 1- α
AICAR	5-Aminoimidazole-4-carboxamide ribotide
AMPK	AMP-activated protein kinase
mPTP	Mitochondrial permeability transition pore

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s40560-025-00832-9>.

Supplementary Material 1: Changes in leukocyte cell death. Time-lapse observation of leukocytes at 37°C for 4 hours. Intracellular DNA was stained with DAPI (4',6'-diamidino-2-phenylindole, blue), and phase-contrast imaging was used to assess cellular morphology. Both phase-contrast view and DAPI staining revealed minimal morphological changes for this period.

Supplementary Material 2: Changes in mitochondrial staining in leukocytes. Mitochondria in leukocytes were visualized using MitoBright LT™ (Dojindo, Kumamoto, Japan) and observed for 4 hours at 37 °C. This staining method allows for the assessment of mitochondrial distribution and function. As time went on, some cells showed a loss of mitochondrial staining (indicated by arrows).

Supplementary Material 3: Changes in mitochondrial membrane potential. Morphological changes of the leukocytes were observed under the microscope at 37°C for 4 hours. No significant change was recognized (upper row). JC-1 (5,5',6,6'-tetrachloro-1,1',3,3'-tetraethylbenzimidazolyl-carbocyanine iodide), a cationic fluorescent dye, was used to visualize membrane potential ($\Delta\Psi_m$). Under normal conditions, mitochondrial membrane potential was maintained for 4 hours (lower column).

Supplementary Material 4: Alterations in mesenteric microcirculation. Intravital microscopic observation revealed minimal changes in mesenteric flow in a rat. Blood flow appeared smooth with minimal leukocyte–endothelium interaction throughout the study period.

Acknowledgements

None.

Author contributions

T. Iba and J. Helms wrote the draft. I. Nagaoka, R. Ferrer, and J. H. Levy reviewed and revised the manuscript, and all of the authors reviewed and approved the final version.

Funding

None.

Data availability

Not applicable.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

¹Faculty of Medical Science, Juntendo University, 6-8-1 Hinode, Urayasu, Chiba 279-0013, Japan. ²Medicine Faculty, Medical Intensive Care Unit NHC,

Strasbourg University, University Hospital Federation (FHU) TARGET, Strasbourg University Hospital, Strasbourg, France. ³Faculty of Medical Science, Juntendo University, 6-8-1 Hinode, Urayasu, Chiba, Japan. ⁴Intensive Care Department, Hospital Universitari Vall d'Hebron Universitat Autònoma de Barcelona, Barcelona, Spain. ⁵Department of Anesthesiology, Critical Care, and Surgery, Duke University School of Medicine, Durham, NC, USA. ⁶INSERM (French National Institute of Health and Medical Research, UMR 1260, Regenerative Nanomedicine (RNM), FMTS, Strasbourg, France.

Received: 23 July 2025 Accepted: 14 October 2025

Published online: 04 November 2025

References

- Iba T, Sawada T, Kondo Y, Kondo K, Levy JH. Morphological changes in blood cells in a rat model of heatstroke: a pilot study. *J Clin Med*. 2022;11(16):4821.
- Comish PB, Carlson D, Kang R, Tang D. Damage-associated molecular patterns and the systemic immune consequences of severe thermal injury. *J Immunol*. 2020;205(5):1189–97.
- Dehbi M, Uzzaman T, Baturcam E, Eldali A, Ventura W, Bouchama A. Toll-like receptor 4 and high-mobility group box 1 are critical mediators of tissue injury and survival in a mouse model for heatstroke. *PLoS ONE*. 2012;7(9):e44100.
- Zhang H, Wang Y, Qu M, Li W, Wu D, Cata JP, et al. Neutrophil, neutrophil extracellular traps and endothelial cell dysfunction in sepsis. *Clin Transl Med*. 2023;13(1):e1170.
- Dieing A, Ahlers O, Kerner T, Wust P, Felix R, Löffel J, et al. Whole body hyperthermia induces apoptosis in subpopulations of blood lymphocytes. *Immunobiology*. 2003;207(4):265–73.
- Wang F, Gong F, Shi X, Yang J, Qian J, Wan L, et al. Monocyte HLA-DR level on admission predicting in-hospital mortality rate in exertional heatstroke: a 12-year retrospective study. *Immunity Inflamm Dis*. 2024;12(4):e1240.
- Ji J, Su P, Lin W, Ouyang L, Wang C, Jia J, et al. Immune cells characteristics and their prognostic effects in exertional heatstroke patients: a retrospective cohort study. *Front Med*. 2022;9:867774.
- Sun M, Li Q, Zou Z, Liu J, Gu Z, Li L. The mechanisms behind heatstroke-induced intestinal damage. *Cell Death Discov*. 2024;10(1):455.
- Zhang Y, Deng X, Zhang J, Zhang L, Akram Z, Zhang B, et al. A potential driver of disseminated intravascular coagulation in heat stroke mice: neutrophil extracellular traps. *Int J Environ Res Public Health*. 2022;19(19):12448.
- Ferrer R, Iba T. Molecular mechanisms of heatstroke: pathophysiology and cell death pathways. *Juntendo Med J*. 2025 in press.
- Iba T, Kondo Y, Maier CL, Helms J, Ferrer R, Levy JH. Impact of hyper- and hypothermia on cellular and whole-body physiology. *J Intensive Care*. 2025;13(1):4.
- Iba T, Helms J, Maier CL, Ferrer R, Levy JH. Mitochondrial dysfunction is a major cause of thromboinflammation and inflammatory cell death in critical illnesses. *Inflamm Res*. 2025;74(1):17.
- Kikusato M, Toyomizu M. Crucial role of membrane potential in heat stress-induced overproduction of reactive oxygen species in avian skeletal muscle mitochondria. *PLoS ONE*. 2013;8(5):e64412.
- White MG, Saleh O, Nonner D, Barrett EF, Moraes CT, Barrett JN. Mitochondrial dysfunction induced by heat stress in cultured rat CNS neurons. *J Neurophysiol*. 2012;108(8):2203–14.
- Iba T, Helms J, Levi M, Levy JH. Inflammation, coagulation, and cellular injury in heat-induced shock. *Inflamm Res*. 2023;72(3):463–73.
- Sorbara MT, Girardin SE. Mitochondrial ROS fuel the inflammasome. *Cell Res*. 2011;21(4):558–60.
- Naik E, Dixit VM. Mitochondrial reactive oxygen species drive proinflammatory cytokine production. *J Exp Med*. 2011;208(3):417–20.
- Newman LE, Shadel GS. Mitochondrial DNA release in innate immune signaling. *Annu Rev Biochem*. 2023;92:299–332.
- West AP, Shadel GS. Mitochondrial DNA in innate immune responses and inflammatory pathology. *Nat Rev Immunol*. 2017;17(6):363–75.
- Bender T, Martinou JC. Where killers meet—permeabilization of the outer mitochondrial membrane during apoptosis. *Cold Spring Harb Perspect Biol*. 2013;5(1):a011106.
- Gu ZT, Wang H, Li L, Liu YS, Deng XB, Huo SF, et al. Heat stress induces apoptosis through transcription-independent p53-mediated mitochondrial pathways in human umbilical vein endothelial cell. *Sci Rep*. 2014;4:4469.
- Pérez-Hernández CA, Moreno-Altamirano MMB, López-Villegas EO, Butkeviciute E, Ali M, Kronsteiner B, et al. Mitochondrial ultrastructure and activity are differentially regulated by glycolysis-, Krebs cycle-, and microbiota-derived metabolites in monocytes. *Biology*. 2022;11(8):1132.
- Wu J, Cheng Z, Yang S, Wu Q, Yang P, Liao X, et al. Specific immune landscape of heatstroke distinguished from sepsis and aseptic inflammation. *Int J Med Sci*. 2025;22(6):1450–64.
- Wu W, Xu H, Wang Z, Mao Y, Yuan L, Luo W, et al. PINK1-Parkin-mediated mitophagy protects mitochondrial integrity and prevents metabolic stress-induced endothelial injury. *PLoS ONE*. 2015;10(7):e0132499.
- Arif A, Quds R, Mahmood R. Biotin enhances generation of ROS, damages DNA, impairs the redox system and causes mitochondrial dysfunction in human lymphocytes. *Sci Rep*. 2021;11(1):8300.
- Iba T, Connors JM, Levi M, Levy JH. Heatstroke-induced coagulopathy: biomarkers, mechanistic insights, and patient management. *EclinMed*. 2022;44:101276.
- Li L, Tan H, Yang H, Li F, He X, Gu Z, et al. Reactive oxygen species mediate heat stress-induced apoptosis via ERK dephosphorylation and Bcl-2 ubiquitination in human umbilical vein endothelial cells. *Oncotarget*. 2017;8(8):12902–16.
- Sacks B, Onal H, Martorana R, Sehgal A, Harvey A, Wastella C, et al. Mitochondrial targeted antioxidants, mitoquinone and SKQ1, not vitamin C, mitigate doxorubicin-induced damage in H9c2 myoblast: pretreatment vs. co-treatment. *BMC Pharmacol Toxicol*. 2021;22(1):49.
- McBride MA, Owen AM, Stothers CL, Hernandez A, Luan L, Burelbach KR, et al. The metabolic basis of immune dysfunction following sepsis and trauma. *Front Immunol*. 2020;11:1043.
- Perelman A, Wachtel C, Cohen M, Haupt S, Shapiro H, Tzur A. JC-1: alternative excitation wavelengths facilitate mitochondrial membrane potential cytometry. *Cell Death Dis*. 2012;3(11):e430.
- Perry SW, Norman JP, Barbieri J, Brown EB, Gelbard HA. Mitochondrial membrane potential probes and the proton gradient: a practical usage guide. *Biotechniques*. 2011;50(2):98–115.
- Smiley ST, Reers M, Mottola-Hartshorn C, Lin M, Chen A, Smith TW, et al. Intracellular heterogeneity in mitochondrial membrane potentials revealed by a J-aggregate-forming lipophilic cation JC-1. *Proc Natl Acad Sci U S A*. 1991;88(9):3671–5.
- Kauffman ME, Kauffman MK, Traore K, Zhu H, Trush MA, Jia Z, et al. MitoSOX-based flow cytometry for detecting mitochondrial ROS. *React Oxyg Sp*. 2016;2(5):361–70.
- Kalkavan H, Green DR. MOMP, cell suicide as a BCL-2 family business. *Cell Death Differ*. 2018;25(1):46–55.
- Nakahira K, Kyung SY, Rogers AJ, Gazourian L, Youn S, Massaro AF, et al. Circulating mitochondrial DNA in patients in the ICU as a marker of mortality: derivation and validation. *PLoS Med*. 2013;10(12):e1001577.
- Xin G, Wei Z, Ji C, Zheng H, Gu J, Ma L, et al. Metformin uniquely prevents thrombosis by inhibiting platelet activation and mtDNA release. *Sci Rep*. 2016;6:36222.
- Mauro-Lizcano M, Esteban-Martínez L, Seco E, Serrano-Puebla A, García-Ledo L, Figueiredo-Pereira C, et al. New method to assess mitophagy flux by flow cytometry. *Autophagy*. 2015;11(5):833–43.
- Walker MA, Tian R. NAD(H) in mitochondrial energy transduction: implications for health and disease. *Curr Opin Physiol*. 2018;3:101–9.
- Dohl J, Passos MEP, Foldi J, Chen Y, Pithon-Curi T, Curi R, et al. Glutamine depletion disrupts mitochondrial integrity and impairs C2C12 myoblast proliferation, differentiation, and the heat-shock response. *Nutr Res*. 2020;84:42–52.
- Proctor EA, Dineen SM, Van Nostrand SC, Kuhn MK, Barrett CD, Brubaker DK, et al. Coagulopathy signature precedes and predicts severity of end-organ heat stroke pathology in a mouse model. *J Thromb Haemost*. 2020;18(8):1900–10.
- Distéfano AM, Martin MV, Córdoba JP, Bellido AM, D'Ippólito S, Colman SL, et al. Heat stress induces ferroptosis-like cell death in plants. *J Cell Biol*. 2017;216(2):463–76.
- Ferrer R, Iba T. Mitochondrial damage in sepsis. *Juntendo Iji Zasshi*. 2024;70(4):269–72.

43. Kennedy D, Jäger R, Mosser DD, Samali A. Regulation of apoptosis by heat shock proteins. *IUBMB Life*. 2014;66(5):327–38.
44. Huang Y, Xu W, Zhou R. Nlrp3 inflammasome activation and cell death. *Cell Mol Immunol*. 2021;18(9):2114–27. <https://doi.org/10.1038/s41423-021-00740-6>.
45. Iba T, Helms J, Levi M, Levy JH. Thromboinflammation in acute injury: infections, heatstroke, and trauma. *J Thromb Haemost*. 2024;22(1):7–22.
46. Jiang B, Liang P, Deng G, Tu Z, Liu M, Xiao X. Increased stability of Bcl-2 in HSP70-mediated protection against apoptosis induced by oxidative stress. *Cell Stress Chaperones*. 2011;16(2):143–52.
47. Wang Z, Cai F, Chen X, Luo M, Hu L, Lu Y. The role of mitochondria-derived reactive oxygen species in hyperthermia-induced platelet apoptosis. *PLoS ONE*. 2013;8(9):e75044.
48. Iba T, Helms J, Levi M, Levy JH. The role of platelets in heat-related illness and heat-induced coagulopathy. *Thromb Res*. 2023;231:152–8.
49. Iba T, Maier CL, Levi M, Levy JH. Thromboinflammation and microcirculation damage in heatstroke. *Minerva Med*. 2024;115(2):191–202.
50. Hagiwara S, Iwasaka H, Goto K, Ochi Y, Mizunaga S, Saikawa T, et al. Recombinant thrombomodulin prevents heatstroke by inhibition of high-mobility group box 1 protein in sera of rats. *Shock*. 2010;34(4):402–6.
51. Li P, Ma X, Huang G. Understanding thrombosis: the critical role of oxidative stress. *Hematology*. 2024;29(1):2301633.
52. al-Mashhadani SA, Gader AG, al Harthi SS, Kangav D, Shaheen FA, Bogus F. The coagulopathy of heat stroke: alterations in coagulation and fibrinolysis in heat stroke patients during the pilgrimage (Hajj) to Makkah. *Blood Coagul Fibrinolysis*. 1994;5(5):731–6.
53. Roberts GT, Ghebeh H, Chishti MA, Al-Mohanna F, El-Sayed R, Al-Mohanna F, et al. Microvascular injury, thrombosis, inflammation, and apoptosis in the pathogenesis of heatstroke: a study in baboon model. *Arterioscler Thromb Vasc Biol*. 2008;28(6):1130–6.
54. DuBose DA, Hinkle JR, Morehouse DH, Ogle PL. Model for environmental heat damage of the blood vessel barrier. *Wilderness Environ Med*. 1998;9(3):130–6.
55. Peng Z, Shu B, Zhang Y, Wang M. Endothelial response to pathophysiological stress. *Arterioscler Thromb Vasc Biol*. 2019;39(11):e233–43.
56. Zhang C. The role of inflammatory cytokines in endothelial dysfunction. *Basic Res Cardiol*. 2008;103(5):398–406.
57. Meegan JE, Yang X, Coleman DC, Jannaway M, Yuan SY. Neutrophil-mediated vascular barrier injury: role of neutrophil extracellular traps. *Microcirculation*. 2017. <https://doi.org/10.1111/micc.12352>.
58. Wang TT, Jabbour RJ, Girling JC, McDonald PJ. Extraluminal bowel obstruction by endometrioid adenocarcinoma 34 years post-hysterectomy: risks of unopposed oestrogen therapy. *J R Soc Med*. 2011;104(10):421–3.
59. Jin H, Chen Y, Ding C, Lin Y, Chen Y, Jiang D, et al. Microcirculatory disorders and protective role of Xuebijing in severe heat stroke. *Sci Rep*. 2018;8(1):4553.
60. Alhamdi Y, Toh CH. Recent advances in pathophysiology of disseminated intravascular coagulation: the role of circulating histones and neutrophil extracellular traps. *F1000Res*. 2017;6:2143.
61. Pan M, Liu H, Sun T, Ma C, Yang L, Dong H. Identification of liver proteins as biomarker for postmortem diagnosis of heat stroke through proteomics. *Sci Rep*. 2025;15(1):17075.
62. Su Y, Lucas R, Fulton DJR, Verin AD. Mechanisms of pulmonary endothelial barrier dysfunction in acute lung injury and acute respiratory distress syndrome. *Chine Med J Pulm Cri Care Med*. 2024;2(2):80–7.
63. Leon LR, Helwig BG. Heat stroke: role of the systemic inflammatory response. *J Appl Physiol*. 2010;109(6):1980–8.
64. Koch F, Thom U, Albrecht E, Weikard R, Nolte W, Kuhla B, et al. Heat stress directly impairs gut integrity and recruits distinct immune cell populations into the bovine intestine. *Proc Natl Acad Sci U S A*. 2019;116(21):10333–8.
65. Gu ZT, Li L, Wu F, Zhao P, Yang H, Liu YS, et al. Heat stress induced apoptosis is triggered by transcription-independent p53, Ca(2+) dyshomeostasis and the subsequent Bax mitochondrial translocation. *Sci Rep*. 2015;5:11497.
66. Ali M, Tabassum H, Alam MM, Parvez S. N-acetyl-L-cysteine ameliorates mitochondrial dysfunction in ischemia/reperfusion injury via attenuating Drp-1 mediated mitochondrial autophagy. *Life Sci*. 2022;293:120338.
67. Kikusato M, Nakamura K, Mikami Y, Mujahid A, Toyomizu M. The suppressive effect of dietary coenzyme Q₁₀ on mitochondrial reactive oxygen species production and oxidative stress in chickens exposed to heat stress. *Anim Sci J*. 2016;87(10):1244–51.
68. Mayorga EJ, Horst EA, Goetz BM, Rodriguez-Jimenez S, Abeyta MA, Al-Qaisi M, et al. Therapeutic effects of mitoquinol during an acute heat stress challenge in growing barrows. *J Anim Sci*. 2024;102:skae161.
69. Xu L, Ma X, Bagattin A, Mueller E. The transcriptional coactivator PGC1 α protects against hyperthermic stress via cooperation with the heat shock factor HSF1. *Cell Death Dis*. 2016;7(2):e2102.
70. Wenz T. Regulation of mitochondrial biogenesis and PGC-1 α under cellular stress. *Mitochondrion*. 2013;13(2):134–42.
71. Faria VS, Manchado-Gobatto FB, Scariot PPM, Zagatto AM, Beck WR. Melatonin potentiates exercise-induced increases in skeletal muscle PGC-1 α and optimizes glycogen replenishment. *Front Physiol*. 2022;13:803126.
72. Fock EM, Parnova RG. Protective effect of mitochondria-targeted antioxidants against inflammatory response to lipopolysaccharide challenge: a review. *Pharmaceutics*. 2021;13(2):144.
73. Fakharnia F, Khodaghali F, Dargahi L, Ahmadiani A. Prevention of cyclophilin D-mediated mPTP opening using Cyclosporine-A alleviates the elevation of necroptosis, autophagy and apoptosis-related markers following global cerebral ischemia-reperfusion. *J Mol Neurosci*. 2017;61(1):52–60.
74. Sonna LA, Gaffin SL, Pratt RE, Cullivan ML, Angel KC, Lilly CM. Effect of acute heat shock on gene expression by human peripheral blood mononuclear cells. *J Appl Physiol* (1985). 2002;92(5):2208–20.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.