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Peripheral Blood Mononuclear Cell Bioenergetics as Biosensors of Heat Stress Resilience in Dairy Cows: A Review

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Abstract: *Global warming and frequent heatwaves subject high-yielding dairy cows to severe heat stress (HS), exceeding thermoregulatory capacity at Temperature-Humidity Index (THI) values ≥ 65–68. Beyond reducing dry matter intake (DMI), hyperthermia directly induces systemic mitochondrial dysfunction, oxidative stress, and immunosuppression. Because assessing solid-organ mitochondrial function (liver, mammary gland, endometrium) requires invasive biopsies unsuitable for herd-level screening, this review evaluates the bioenergetic "liquid biopsy" paradigm using peripheral blood mononuclear cells (PBMCs) to quantify metabolic and immune resilience during post-HS recovery. Synthesizing peer-reviewed Open Access literature, this review formulates a harmonized working protocol replacing mechanical blood mitochondria isolation with intact PBMC isolation over Ficoll-Paque PLUS gradients, platelet depletion via low-speed washing (120–200 × g), quantification in phosphate-buffered saline (PBS) or RPMI-1640 before MiR05 suspension, and in situ respirometry on cells selectively permeabilized with digitonin (3–6 µg/10^6 cells) or saponin (~50 µg/mL) across Oroboros O2k (Oxygraph-2k) and Strathkelvin Mitocell (MT200/MT200A) platforms. Published transcriptomic, proteomic, and oxygraphic studies collectively demonstrate that HS downregulates mitochondrial DNA (mtDNA) protein-coding genes and Electron Transfer System (ETS) Complex I (NDUFS8) and IV (COX4I1) subunits, alongside hypothalamic–pituitary–adrenal (HPA) axis activation (2–3-fold cortisol increase) and gut endotoxin translocation (~3-fold serum lipopolysaccharide [LPS] increase). Across reviewed studies, these alterations manifest in PBMCs as suppressed fatty acid oxidation, inactivated T-cell receptor (TCR) signaling, pro-inflammatory Th17 (IL-17A) polarization, decreased coupled oxidative phosphorylation (NP and NSP), elevated proton leak (L), depleted spare respiratory capacity (SRC = E − R), and reduced Bioenergetic Health Index (BHI). Conversely, comparative literature on resilient bovine phenotypes (High Immune Responders, HSP70 C/- genotypes, and high feed-efficient cows) documents superior maximal respiration in PBMCs and CD4$^+$ T cells (56.7 vs. 37.9 pmol/min), robust acute HSP70 induction (42 °C) with baseline normalization within 18 h post-recovery, and favorable mito-nuclear interactions. Ultimately, synthesized evidence suggests that in situ PBMC respirometry using Substrate–Uncoupler–Inhibitor Titration (SUIT) protocols provides a minimally invasive, reproducible biomarker for selecting and managing climate-resilient dairy cattle.*

- Introduction

Global climate change and intensifying heatwaves severely threaten the welfare, sustainability, and economic viability of the modern dairy industry. Intensive genetic selection for high milk yield has elevated basal metabolic rates and endogenous thermogenesis in dairy cows, compromising homeothermy under high ambient temperature and humidity. Although herd-level heat stress (HS) thresholds are traditionally defined at a Temperature-Humidity Index (THI) of 68–72—above which milk yield drops by 23% within four days and up to 53% during severe thermal loads—high-producing cows exhibit systemic redox imbalance, negative energy balance, elevated respiratory rates, and hyperthermia at a THI as low as 65. In the United States dairy sector alone, HS causes $897 million to $1.2 billion in direct annual losses, compounded by $810 million from dry-period carry-over effects that impair mammary development and multigenerational productivity. While summer declines in lactation and reproduction were historically attributed to reduced dry matter intake (DMI), pair-fed thermoneutral models demonstrate that nutrient restriction explains only 35% to 50% of yield losses; the remaining 50% to 65%—alongside a two- to threefold increase in clinical mastitis, retained placenta, and postpartum metritis—stem directly from hyperthermia-induced disruptions in cellular bioenergetics, epithelial barrier integrity, and host immunity.

At the epicenter of this cellular pathology lies the mitochondrion, which generates approximately 90% of cellular adenosine triphosphate (ATP) via oxidative phosphorylation (OXPHOS) while regulating intracellular Ca^{2+} homeostasis, redox signaling, and intrinsic apoptosis. Under thermal stress, mitochondrial networks undergo excessive fission, cristae swelling, and electron transport uncoupling across complexes I–IV, overproducing reactive oxygen species (ROS) such as superoxide anions ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2). Concurrently, systemic immune activation in a lactating Holstein cow consumes more than 1 kg of glucose within 12 hours, forcing an immunometabolic switch from OXPHOS to aerobic glycolysis and creating intense substrate competition between host defense and lactogenesis.

Quantifying mitochondrial resilience in real time remains limited by the surgical and ethical constraints of invasive hepatic, mammary, or skeletal muscle biopsies, which preclude repeated herd-level screening. Consequently, circulating peripheral blood mononuclear cells (PBMCs) and platelets have emerged as a minimally invasive bioenergetic "liquid biopsy"; because PBMCs continuously recirculate through the vasculature, their simultaneous exposure to elevated core temperatures, hypothalamic–pituitary–adrenal (HPA) glucocorticoids (cortisol), translocated gut endotoxins (LPS), and plasma metabolome shifts allows them to mirror both solid-organ bioenergetics and systemic immunometabolic competence. Accordingly, the objectives of this review are threefold: (1) to standardize Ficoll-Paque density-gradient PBMC isolation and in situ oxygraphic analysis using Oroboros O2k and Strathkelvin Mitocell platforms, resolving misconceptions regarding mechanical mitochondrial isolation and pre-analytical media artifacts; (2) to synthesize the molecular and pathophysiological mechanisms linking HS to altered mitochondrial respiration across blood cells and supporting organs in dairy cattle; and (3) to define respirometric and genetic biomarkers of post-HS resilience applicable to precision breeding and nutritional mitigation.mitigation.

Material and method

To construct this methodological and pathophysiological review, a systematic search of international indexed databases (PubMed/MEDLINE, PubMed Central, Web of Science, Scopus, and DOAJ) was conducted using Boolean operators and targeted search strings combining heat stress, dairy cows, mitochondrial respiration, peripheral blood mononuclear cells, and high-resolution respirometry platforms. Priority was assigned to peer-reviewed Open Access primary studies and consensus methodological protocols published between 2014 and 2026.

Methodological synthesis evaluated three biological preparation types defined by the MitoEAGLE consortium:

Intact Living Cells: Routine respiration is measured prior to plasma membrane permeabilization, governed by physiological intracellular ATP turnover and endogenous substrate supply, where exogenous impermeant substrates or ADP cannot cross the intact membrane.

Selectively Permeabilized Cells In Situ: Intact cells are converted into permeabilized cells inside the oxygraph chamber by titrating a mild detergent (digitonin at 3–6 µg/10^6 cells or saponin at ~50 µg/mL) in an intracellular-like respiration medium (MiR05 or MRB). This selectively perforates the plasma membrane while preserving all mitochondrial subpopulations in situ, enabling external control of specific pathway and coupling states via Substrate–Uncoupler–Inhibitor Titration (SUIT) protocols and direct measurement of maximal oxidative phosphorylation capacity.

Mechanically Isolated Mitochondria: Obtained through cell disruption and differential centrifugation, requiring large sample volumes and risking selective loss of damaged mitochondrial subpopulations, network fragmentation, and outer membrane rupture.

Instrumentally, three major respirometry platforms were compared:

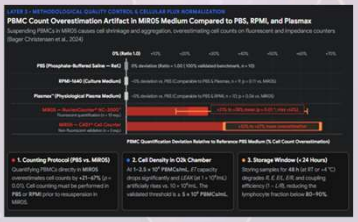
Oroboros O2k: Utilizes two closed 2.0 mL or 0.5 mL glass chambers under continuous magnetic stirring at 750 rpm and high-precision Peltier temperature control (37 °C or 38.5 °C for bovine normothermia), representing the gold standard for mechanistic dissection of respiratory complexes in intact and permeabilized cells isolated via Ficoll-Paque gradients.

Strathkelvin Mitocell (MT200 / MT200A): A Clark-electrode micro-respirometer equipped with a glass water jacket and polycarbonate plunger, enabling in situ respirometry in sample volumes of 50 to 100 µL (requiring as few as 5 × 10^4 to 5 × 10^5 cells) or up to 1.0 mL.

Seahorse XF Analyzer: A microplate-based extracellular flux platform that measures real-time Oxygen Consumption Rate and Extracellular Acidification Rate in intact cells immobilized with Poly-D-Lysine or Poly-L-Lysine using standardized stress test injection protocols.

Across platforms, internal normalization is achieved via Flux Control Ratios—specifically ATP-linked respiration, Spare Respiratory Capacity, Coupling Efficiency, Routine Flux Control Ratio, and Uncoupling-Control Ratio—alongside intracellular mitochondrial density normalization via Citrate Synthase activity at 30 °C and calculation of the composite Bioenergetic Health Index.

- Results, discussions and Conclusions



Reliable PBMC respirometry in dairy cattle appears to depend on careful pre-analytical handling and selective in situ permeabilization. Density-gradient centrifugation over Ficoll-Paque PLUS or Histopaque-1077 yields intact PBMCs rather than free mitochondria, so permeabilization with digitonin (roughly 3-6 µg/10^6 cells on the Oroboros O2k) or saponin (about 50 µg/mL on the Strathkelvin Mitocell MT200) may be preferable to mechanical mitochondrial isolation

Intact-cell oxygraphy mainly captures routine respiration and uncoupled electron transfer capacity, because mitochondrial substrates and ADP cannot cross the plasma membrane. Permeabilization seems to overcome this limitation, retaining mitochondrial subpopulations in situ and allowing maximal OXPHOS capacity and complex-specific defects to be assessed. Detergent dosing appears to be a sensitive step: too little digitonin may underestimate phosphorylating respiration, while too much may compromise mitochondrial membranes and underestimate uncoupled capacity. Validating the dose within a full SUIT protocol could therefore be advisable, and on the MT200, saponin above roughly 100 µg/mL may damage the outer mitochondrial membrane, detectable as a rise in oxygen consumption after exogenous cytochrome c. Other pre-analytical factors may also influence normalization.

Low-speed washing (about 120-200 × g) can remove most contaminating platelets, and counting cells in PBS/DPBS or RPMI-1640 rather than MiR05 may avoid shrinkage and aggregation artifacts that seem to inflate counts by approximately 21-67%. Chamber densities of at least 5 × 10^6 cells/mL appear preferable, fresh samples seem best analyzed within about 8-24 hours, and controlled-rate cryopreservation (about -1 °C/min, then liquid nitrogen) appears to preserve complex I-IV activities, ATP content, and citrate synthase activity for at least six months. Under these conditions, PBMC readouts may mirror organ-level pathology and systemic immunometabolic reprogramming during heat stress. Hepatic and mammary data suggest downregulation of mtDNA-encoded and OXPHOS genes, reduced Complex I (NDUFS8) and Complex IV (COX4I1) expression, mitochondrial fragmentation, and Ca^{2+}/mPTP/cytochrome c-mediated apoptosis, which could present as higher LEAK respiration and a lower respiratory control ratio. Systemically, elevated cortisol (about 2-3-fold), gut-derived endotoxemia (about 3-fold LPS), and oxidative stress may suppress PBMC fatty acid oxidation, dampen T-cell receptor signaling, and bias T cells toward a pro-inflammatory Th17 profile, with reduced coupling efficiency and Bioenergetic Health Index potentially persisting for at least two weeks after an endotoxin challenge.

Taken together, these observations suggest that resilient and metabolically efficient cattle might be distinguished by a set of PBMC and CD4$^+$ T-cell biomarkers. More efficient animals appear to show higher maximal respiration (about 56.7 vs. 37.9 pmol/min) and greater spare respiratory capacity (SRC = E - R), and preserved coupling efficiency (1 - L/R) and Bioenergetic Health Index during post-stress recovery could further indicate a resilient phenotype. Lactation stage may also modulate this reserve, with late lactation and the dry period potentially associated with greater mitochondrial mass than early lactation. Heat shock protein dynamics may add complementary information: PBMCs from high immune responder cows seem to mount a rapid HSP70 (HSPA1A) increase at 42 °C, return toward baseline within roughly 18 hours, and re-induce the response on a second challenge, whereas induction may be delayed or exhausted in less resilient animals. HSP70 polymorphisms, such as the C/- genotype, have been associated with higher expression and better PBMC proliferation after heat shock, which could make them useful genetic markers of cellular recovery.

At a broader level, mito-nuclear compatibility may shape the capacity to sustain OXPHOS coupling and limit ROS under thermal stress, since most respiratory chain complexes are encoded jointly by mtDNA and nDNA. Combining this PBMC respirometric "liquid biopsy" with genomic screening (HSP70/HSPA1A, PPARGC1A, mtDNA haplotypes) could therefore offer a minimally invasive diagnostic and prognostic platform for selecting climate-resilient dairy cattle and evaluating nutritional interventions such as L-carnitine, antioxidants, and gut-barrier modulators, although validation in larger and more diverse cohorts would be needed before routine application.