Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol.14 , Issue 2 (2025)
https://www.jneonatalsurg.com

Threshold-Dependent Redox Network Rewiring Links Oxidative Stress to Chronic Inflammatory and Metabolic Disease: Mitochondrial ROS Amplification as a Tipping Point

Dr. Arafat Ahmad*[1], Dr. Mohammad Arif[2], Dr. Samina Wasi[3], Dr. Nazia Hasan[4], Dr. Satyendra Kumar Sinha[5], Arati Gupta[6] & Dr. Feroz Ahmad[7]

[1]Assistant Professor, Department of biochemistry , KMC medical college and hospital , Maharaj Ganj UP
Email ID: arfatahmad2110@gmail.com

[2]Assistant Professor, Department of biochemistry, Madhubani Medical college and hospital, Madhubani Bihar
Email ID: arifmd269@gmail.com

[3]Assistant Professor , Department of Biochemistry, College of Medicine, Imam Abdul Rahman bin Faisal University, Dammam 31441,P.O Box 1982. Kingdom of Saudi Arabia.
Email ID: skiasi@iau.edu.sa

[4]Assistant Professor, Lnjpit(lok Nayak jai prakash institute of technology)
Email ID: naziakaleem1111@gmail.com

[5]Assistant professor, Department of Zoology, Jagdam college chapra, JPU
Email ID: satty2007@gmail.com,
Email:ID: satyendraksinha@yahoo.com

[6]Tutor, department of biochemistry, KMC medical college and hospital, MaharajGanj UP.
Email:ID: guptaarati786@gmail.com

[7]Assistant professor , Department of psychology ,Jagdam college chapra, J.P.University
Email ID: lamsarufa@gmail.com

Corresponding author:
Dr. Arafat Ahmad

Cite this paper as Dr. Arafat Ahmad, Dr. Mohammad Arif, Dr. Samina Wasi, Dr. Nazia Hasan, Dr. Satyendra Kumar Sinha, Arati Gupta & Dr. Feroz Ahmad (2025) Threshold-Dependent Redox Network Rewiring Links Oxidative Stress to Chronic Inflammatory and Metabolic Disease: Mitochondrial ROS Amplification as a Tipping Point., Journal of Neonatal Surgery, 14, (2) 353-367

ABSTRACT

No more as toxic products of aerobic metabolism Reactive oxygen species (ROS) are now viewed as signals that are compartmentalized and time-limited, and that signal ROS, and ROS particularly H2O2, regulates metabolic fluxes, immune activation and cell-fate pathways. In the meantime, oxidative stress is also getting identified as something more than more ROS, and this has been defined as either a perturbation of redox signalling and regulation, or an accretion of molecular damage when the production of oxidants becomes larger than its buffering capacity. According to this systemic strategy, it is this redoxome reprogramming threshold-dependent connection between oxidative stress and chronic inflammatory -metabolic disease that this review proposes as its central idea. Mitochondrial production of ROS (mtROS) is extremely sensitive to bioenergetics such as electron flux or Δp, NADH/NAD +, ubiquinone redox, access to substrates and O 2 (Figure 1 A) and mitochondrial elimination of ROS is highly reliant on the NADPH-linked glutathione and thioredoxin/peroxiredoxin networks that may be saturated during chronic stress. We extrapolate mechanistic indications of nonlinearity and tipping-point mitochondrial behaviour by (i) burst-like amounts/production of mtROS in stationary likely defined respiratory conditions (e.g. reverse electron transport [RET] at Complex I or ROS-release at Complex III), (ii) the magnitude-enhancing positive feedback on a mitochondrial scale with subsequent stippling network propagation [depolarization waves, ROS-induced ROS release], and (iii) experimentally observed and modelled switch-like/mitigated mtRO We then add pathway level coupling, in which the pattern of connecting the nodes of the inflammatory and metabolic pathways -NF-kB, NLRP3 inflammasome, HIF-1α, insulin signalling by redox sensivity phosphatases (e.g., PTP1B/PTEN), AMPK energy sensing mTOR) - is to maintain steady disease states. Finally, we take into account methodological difficulties in quantification of compartmental redox states and the implications of successful interventions the necessity of spatial

.

control of intervention, timing to proximate-to-threshold biomarker, and the requirement of restoration of redox-control mechanisms instead of nonselective disabling downstream oxidants..

Keywords: *Mitochondrial ROS; oxidative stress; redox signalling; redox network rewiring; tipping point; threshold dynamics; bistability; hysteresis; reverse electron transport (RET)*

INTRODUCTION

The field of redox biology has evolved into a systems viewpoint that acknowledges the use of reactive species, especially hydrogen peroxide (H2O2), as spatially and time-regulated signaling that conveys metabolism, immune system functionalities, cell destinies, etc. The most widespread definition in use today is that of oxidative stress as the imbalance between the oxidants and antioxidants in favour of the former leading to redox signalling and control and /or molecular damage. Of importance about this definition is that it recognises that the pathological condition is not merely an elevation in oxidants, but rather the failure to control network-wide redox signalling and control.

The special place in this network representation belongs to mitochondria. They are not just an important location of cellular oxygen uptake and of ATP production, but also of a regulated source of reactive oxygen species (ROS), which can be transduced to the nucleus and cytosol. The proximal ROS so generated is mitochondrial superoxide (O 2 -), which is usually generated due to the leakage of electrons at specific sites (especially complexes I and III, but there are other sites also likely to be involved in substrate oxidation) and is converted by the superoxide dismutases to H2O2, an oxidant that is more membrane-permeable and signalling-competent. The geography (where is ROS produced in mitochondria?), topology (on which side of the inner membrane?), and conditionality (which respiratory modes?) of production of mitochondrial ROS have been optimized over the last 15-20 years of research.

In this case, the oxidative load above a certain threshold is neutralized by the action of high-capacity antioxidant and repair mechanisms (glutathiene and thioredoxin/peroxiredoxin circuits, NADPH donation, enzymatic antioxidants, and repair pathways). Beyond this point the qualitative behaviour of the system will change: redox couples will be repositioned, redox-sensitive proteins will change states, redox inflammatory/metabolic signalling nodes will reconfigured, feedback loops will stabilise a new inflammatory/metabolic regime. The arguments behind this are (i) switch-like ROS behaviour and hysteretic responses observed and modeled during metabolic stress, (ii) ultrasensitive responses in peroxiredoxin/thioredoxin signal networks, and (iii) ROS induced ROS release (RIRR) phenomena under metabolic stress and network propagation centres around the mitochondria.

The mechanism of the tipping point of the mitochondrial radicals as a point where inflammatory and metabolic pathophysiology intersect can be offered at least in three recurring logics:

To begin with, signal amplification: small amounts of augmented production of ROS in response to conditions of specific bioenergetic states (such as reverse electron transport at complex I during conditions of high ubiquinone pool reduction and protonmotive force) can give rise to disproportionate burst of ROS. A well-known example of such amplification is the complex I reverse electron transport chain in the presence of succinate, but analogous circumstances can be observed in chronic overload of metabolism (as in altered substrate supply, maintained high membrane potential, insufficient demand of ADP equivalent, etc).

Second, network coupling and feed-forward loops: the activation of inflammatory pathways (e.g., NF-κB and NLRP3 inflammasome) via the activation of the mtROS can also further augment ROS by cytokine signalling, NADPH oxidase activation, mitochondrial dysfunction and release of mitochondrial-derived danger-associated molecules (e.g., oxidised mtDNA). One striking example is the disulphide bridge between cysteines 54 and 347 of the IKK regulatory subunit NEMO, formed as a consequence skin bullshit catcher helldesked upon sensing on your cucky cuckface sissy cuck scum spineless pussy cowtits mother repeated by proxy violator castration bedwetter perverted sex offender pigfuck censor shutthefuckup your mouth is good only for sucking public urinal cock induced mtROS in infected macrophages (38) allowing proinflammatory NF-kB complement signalling and showing how a mitochondrial redox product can link to an ancient inflammatory transcriptional programme.

Third, state change and bistability: states of chronic inflammation and metabolic disease (e.g. persistent low-grade inflammation in the form of insulin resistance' dysfunctional lipid handling) are self-perpetuating which may make transition difficult. Both experimental and modelling research indicates that cellular dynamics of ROS will have bistable control structure when responding to nutrient stress in a threshold mode.

The report is designed to support this thesis in (i) conceptual support (what are redox networks, what is oxidative stress, what are redox network thresholds, what are redox network tipping points), (ii) molecular redox network generation and amplification pathways, (iii) the evidence and ideas of redox network rewiring, (iv) redox network-level coupling of redox networks to chronic inflammation and metabolic disease (including NF- 8 -B, NLRP3, HIF-1 8 -, insulin signalling, AMP.

2. Redox biology conceptual underpinning and thresholds

On the contrary, it has an interlocking redox pair (NADH/NAD(sh as a near-equilbrium catabolic couple; NADPH/NApD + as a reductant supply couple) and enzyme circuitry organization functioning at different potentials and timescales (glutathione (GSH/GSSG), thioredoxin systems as control system redoxes; protein thiol proteomes in biology which provide swithcable layers). This architecture of several layers is codified in the redox code, whose premise states that it is not the molecular damage per se but a failure of its organisational logic which mediate system failure and disease.

2.1 Definitions

One of the most recent definitions of oxidative stress focuses on the impairment of redox signalling and regulation and/or molecular damage, as opposed to the presence of more ROS. The reason this definition is so helpful is that it allows physiological oxidative eustress, and yet controlled oxidant signalling in a physiological regime, but keeps oxidative distress in other regimes that become dysregulated and result in damage. Simultaneously, redox signalling can be conceptualized as controlled oxidation-reduction reactions (usually of protein cysteines) that convey information, and the redox network to the entire collection of interacting oxidant sources, sinks, redox couples, and redox-responsive targets across compartments.

It follows (i) that in vivo antioxidants are largely an enzymic system with their substrates and cofactors, not a non-substrate free radical scavenger, (ii) a particular alteration of the redox regulation circuit has qualitative effects different from those in general ROS suppression. It is why antioxidant supplementation has never been found to be one of benefit.- and indeed in some studies was associated with harm; both of these findings consistent with the notion that not all ROS are all bad when itcomes to specific forms of adaptive signalling.

2.2 Thresholds and tipping points in redoxsystems

There are three kinds of thresholds in complex networks (): (1) saturation of scavenging or buffering capacity; (2) a regulatory node changing its state (such as redox sensor KEAP1/NRF2, thiol switches on phosphatases/kinases); and (3) nonlinearity amplifications via feedback loops. The tipping point is the situation in which the attractor of the system changes so that instead of being regulated signalling and homeostasis, one obtains an environment of chronically disrupted signaling/metabolism. Such tipping points for redox biology almost surely exist, because the majority of events of redox regulation follow saturation kinetics with autocatalytic or feed forward interactions and/or compartmentally localized barriers to diffusion, which generate local accumulation.

How redox systems might work in this way is no longer merely a theoretical case. A study combining modeling with single-cell experiments observed a ROS response to glucose deprivation in form of switch-like all-or-none behavior, at concentrations lower than even physiological levels of glucose and it induced hysteresis and irreversibility which is characteristic for bistable behavior.

2.3 Mitochondria as a redox decision module

The mitochondria are optimally positioned to make use of thresholds as the production of mitochondrial ROS is highly sensitive to bioenergetic parameters (membrane potential, redox state of NADH and ubiquinone pools, oxygen availability, substrate supply), whereas mitochondrial elimination of mitochondrial ROS is reliant on NADPH-linked scavenging networks and antioxidant enzyme activities.

3. Molecular processes of the production of mitochondrial reactive oxygen species and amplification

3.1 Canonical location and topography of the production of mtROS

The Mitochondrial ROS generation is a result of the leakage of electrons to oxygen at predetermined points related to the oxidation of substrates and oxidative phosphorylation. Notably, topology is different: some places produce superoxide to the matrix side exclusively, and complex III and mitochondrial glycerol-3-phosphate dehydrogenase may produce superoxide on both sides (matrix and intermembrane space). This is important since intermembrane-space ROS can easier serve its purpose on cytosolic targets.

3.2 Bioenergetic regulation and the ROS generation modes in mitochondria

The point that the production of mtROS is not consistently similar is one of the main mechanistic arguments. It is much dependent on electrochemical gradient (protonmotive force), reduction state of NADH/NAD + and ubiquinone/ubiquinol pools, and oxygen concentration. The conventional synthesis emphasizes the observation that isolated mitochondria exhibit increased generation of superoxide particularly (i) when the mitochondria are not synthesising ATP (high protonmotive force, low coenzyme Q pool), and (ii) when the ratio of mitochondrial matrix NADH/NAD + is high. The generation of ROS can also be greatly decreased in the instance of actively producing ATP (state 3 respiration) mitochondria, and the sites which contribute most to it may vary.

The outcome of this bioenergetic dependency is a natural nonlinearity. A computational model of ROS production and redox balance of cardiac mitochondria had shown that under conditions of state 4 complex III ROS can grow exponentially with the membrane potential. The other nonlinearity of the same modelling framework is that, when the membrane potential falls (e.g. due to an increase in proton leak), ROS production can decrease and then rose again, due to depletion of scavenging capacity, which is characteristic of a redox homeostasis failure in spite of an increasingly nonmonotonically rising production of ROS.

3.3 ROS bursting and reverse electron transport

The reverse electron transport (RET) is also another significant amplification process at the complex I: electrons are transported backwards through a highly reduced ubiquinone pool into complex I to generate high flux of superoxide at a specific site in complex I that is specified in the presence of high membrane potential. Which prevents the buildup of succinate or RET inhibits the production of ROS and injury in models. Despite being an acute environment, I/R can be conceptually correlated to chronic metabolic overload and heterogeneity of mitochondria in tissues through the application of RET-like conditions (high succinate, high membrane potential, impaired ADP utilisation).

3.4 ROS-release and permeability transition ROS release and permeability transition is a propagating amplifier

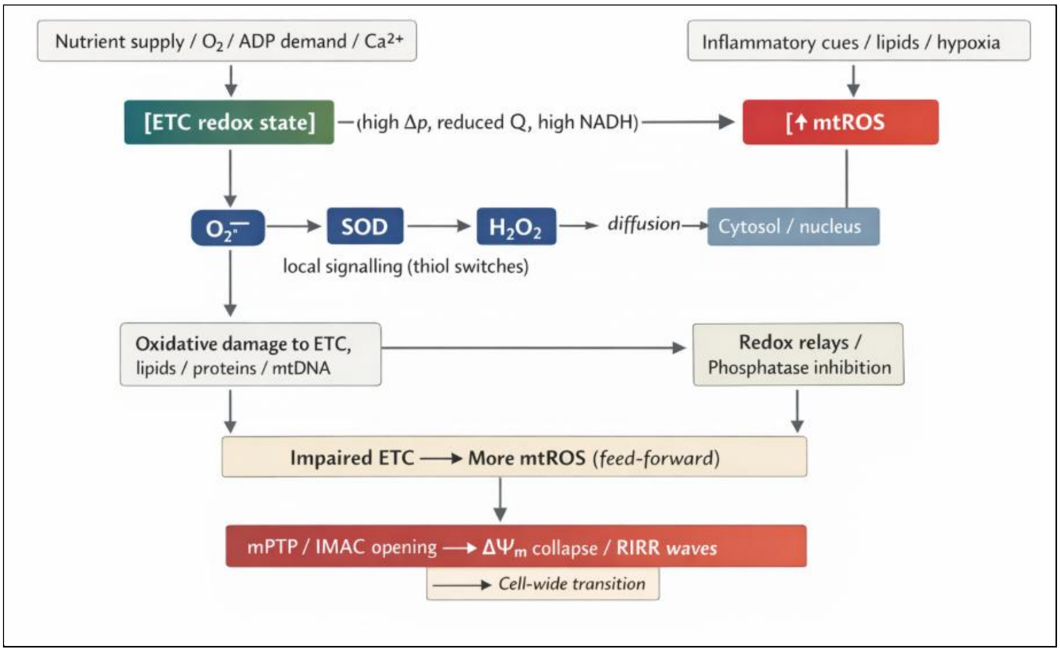
In addition to the single-mitochondrion bioenergetics, mitochondria may act as a coupled network. An experimental study which was seminal reported the ROS-induced ROS release (RIRR): photodynamically induced ROS triggered mitochondrial permeability transition (MPT) and a burst of ROS release by mitochondria, which could be coordinated between chains of adjacent mitochondria, implying cooperativity. The delay time of triggering ROS to induce permeability transition was found to be dependent on intrinsic cellular scavenging ability, especially glutathione, and had a direct relationship between mitochondrial amplification and network level antioxidant thresholds.

This was further extended to a reaction diffusion model of RIRR in mitochondrial networks to reproduce depolarisation waves and be sensitive to parameters including superoxide diffusion, production and scavenging.

3.5 Nonlinear buffers as scavenging systems, and threshold foundations

The capacity and correlation of the scavenging systems as well as the production is not the sole way of regulating the mitochondrial levels of H2O2. Two H2O 2 scavengers, experimentally-computationally correlated to be the two main mitochondrial H2O 2 scavengers, are the glutathione (GSH) and thioredoxin/peroxiredoxin pathways, and both of the pathways depend on NADPH as the electron donor. Such models emit a small amount of H2O2 until the overall capacity of the system is attained when the emission becomes rampant as would be predicted by a buffering-to-failure transition. These mechanistic observations confirm the idea of thresholds in the signalling of the mtROS arise as limits in capacity of NADPH-dependent antioxidant networks and their time-dependence.

Fig. 1 - Mechanistic diagram: sources, sinks, and positive feedback of ROS in the mitochondria



Major characteristics that have been supported by experimental and modelling studies are higher levels of RET-induced high levels of mitROS during high membrane potential and low ubiquinone concentrations, capacity-limited scavenging, which alters system behaviour upon saturation, RIRR/MPT-linked propagation through mitochondrial networks.

4. Redox network rewiring and threshold logic evidence

4.1 What is Redox network rewiring?

This expression is used to stress the fact thatchronic oxidative stress does not just passively addup damage, but also activates different steering routes for electrons, signals and membrane traffic about which we will have to know quite a lot in order tounderstand cell life. The rewiring could include: [i] the expression and localisation of oxidant sources (sites and mitochondria; NADPH oxidases)or sinks (Thpx s, Gpxs, Trx systems); [ii] source reassessment itself or how they are paired to their NADP+ reducer; iii-iv) transfection or transfer via different ETC-chain types, such as pendulous-type since that elicits proton exits from site 1Qo to other ones under varying redox conditions in relation with v-vi] module of ROS cellular sender ([NF- k ′ B; KEAP1/NRF2 axis; p53; ATM), following specific command from cell.

Redox rewiring is clinically important when it is state dependent: the network is different at redox levels below and above a redox threshold. This can be caused by saturable scavenging, redox switches, and feedback loops which produce ultrasensitivity and bistability.

4.2 Peroxiredoxin ‘floodgate’ signaling pathway and local microdomain of H2O2

One of the most enduring redox signalling puzzles is that H2O2 can serve as a particular signal when it is present in large quantities when it is efficiently eliminated by overabundant antioxidants. The local control is one of the suggested resolutions: local transient inactivation of high-capacity peroxidases permits a microdomain of H2O2 to form around membranes where signalling components are located, and maintains global H2O2 at low levels. It was demonstrated experimentally that peroxiredoxin I, which is present in membranes, can be transiently phosphorylated (Tyr194) and inactivated in response to growth factor or immune receptor stimulation to allow local accumulation of H2O2 to be used in signalling. It is a real-life instance of redox network rewiring: it is by post-translational control of a major sink that rewiring takes place, changing spatial routing of oxidant signals.

More importantly these mechanisms add a thresholding element: when peroxiredoxin capacity is inhibited or saturated at a point then the local signal of H2O2 can switch between regulated signalling and unregulated oxidation which may invoke stress programmes.

4.3 KEAP1/NRF2 in the role of sensor-effector threshold module

The KEAP1/NRF2 pathway is one of the major lines of anti-oxidant/electrophile defence. Reactive cysteines on KEAP1 serve as sensors and NRF2 is a transcription factor that activates cytoprotective gene programmes. That this system is in some sense a threshold logic is indicated by its being designed to remain relatively quiescent under basal conditions but will produce strong gene induction activity when the redox insults are so severe that they modify enough cysteine residues on KEAP1 to stabilise NRF2. This could lead to a new redox signaling set point through repeated or persistent activation, which is characterized by modification in the antioxidant and metabolic phenotype.

It is often referred to as protective when expressed in the context of disease states, however, extended activation may also become unbeneficial for pathological states58 (e.g. survival under pro-oxidant conditions or facilitating serial metabolic needs). The caveat of this review therefore is that NRF2, as a mechanism feeding into redox rewiring, can operate across a threshold(the transient activation below which may facilitate adaptation -above it or chronic induction- triggering a transcriptional remodelling to reprogram metabolism and inflammatory outcomes).

4.4 Proteome scale data: redox switches and mitochondrial-cytosolic communication

Transcriptional responses are not the only redox rewired ones. Quantitative redox proteomics has been able to reveal the state-dependent cysteine oxidation and specific redox switches have been discovered. An experimental proteomics paper has characterized redox switches to control global translation in reaction to mitochondrially produced ROS, showing that the result of the mitochondrial ROS can be connected to nontraditional cellular reactions.

Such findings are in support of a systems viewpoint: the oxidation of biomolecules by the mtROS is not accidental, but can be targeted to specific redox-sensitive nodes, which take regulatory decisions.

4.4 Redox network threshold response and bistability

Evidence There is a set of facts which indicate the possibility of threshold-dependent redox behaviour:

- At back cellular level, a nutrientredox model of experimental ROS measurements of single cell lines demonstrated switch ROS dynamics, which is irreversible and hysterical during glucose depletion. This provides a direct support of bistable redox control in human cells which is directly compatible with the concept of a tipping-point.
- At the antioxidant circuit level, peroxiredoxin/ thioredoxin/ sulfureredoxin systems have been studied using modelling, showing that there are inherent causes of ultrasensitivity as well as diminished H2O2 elimination on high peroxiredoxin hyperoxidation. Specifically, the bistability of a regime of parameters in such a circuit can provide a mechanistic explanation of the switch-like redox signalling.
- Experimental and computational oscillation and reaction-diffusion propagation of ROS at the scale of the

mitochondrial network has demonstrated that the mitochondrion can experience sudden and orchestrated phenomena (e.g. depolarisation waves) that are controlled by ROS production and removal.

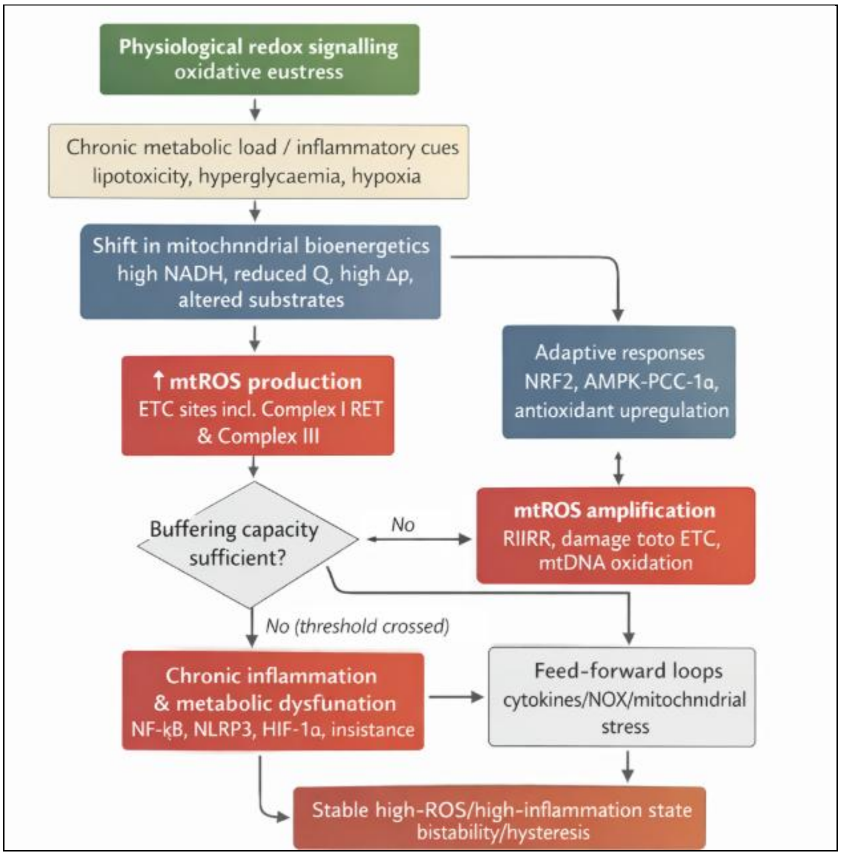


Fig 2. Mermaid flowchart: a "tipping point" model for mtROS-driven disease linkage

This evidence-based model of bioenergetic regulation of mitROS formation, antioxidant buffering set points and feed-forward failure mechanisms, ROS-dependent propagation by mitochondrial network effects, and hysteresis-based switch-like redox behaviour underlies the model.

5. nodes of mtROS-linked signalling between chronic inflammation and metabolic disease.

5.1 Overview

Consistent among most of these chronic inflammatory and metabolic diseases is prolonged disruption in energy balance, nutrient sensing,and immune activity — similar to what often develops under low-grade inflammation (or metaflammation) and tissue-restricted insulin resistance. mtROS might couple these compartments capable of sensing metabolic state and signaling pathways to regulate inflammat.), inflammasome activation, hypoxic responses and insulin/energy sensing.

5.2 The NF-κB as a redox-integrating hub

4.6 NF-KB plays a central role in mediating expression of inflammatory genes, and the oxidant–NF-KB relationship is bidirectional)91).ROS, for instance can activate as well as inhibit NF-KB activity (the stimuli-induced ROS effects are also concentration- and time-dependent; they vary with specific cell types and subcellular localizations.) It has been observed in reviews that: NF- - itself is a regulator of antioxidant as well as pro-oxidant target genes (including subunits of NADPH oxidase) and the regulation of such genes serves to create feedback loops which can serve to stabilise certain redox states. And this very bidirectionality is why thresholds are constantly needed: low ROS may either precondition or even set off NF-KB activation pathways, but high oxidative stress can shut down the elements or block proteasomal steps and we would have had different results.

An example of mechanistically instructive interactions is a direct interaction between mtROS and canonical NF- -activation; in macrophages responding to bacterial infection, the IKK complex regulatory subunit NEMO was directly connected to Cys54 and Cys347 by intermolecular disulphide linkage, a connection necessary to activate IKK and subsequent ERK1/2 and NF- signalling to secretion of proinflammatory cytokines. This offers a tangible chemical pathway of how the action of the mtROS as a regulatory signal as opposed to diffuse damage.

The identical cysteines (Cys54/Cys347) had previously been implicated in oxidant-enhanced NEMO dimerization, and suggested a more generalized model that oxidants can regulate IKK/NF- kB by thiol chemistry. These arguments give an incentive to a threshold model: at low concentrations, it can be tolerated that the mtROS is permissive but there are routes of NF-κB which can be occupied or be prominent at higher concentrations.

5.3 NLRP3 inflammasome activation through mitochondrial dysfunction and mitochondrial-associated signals of mitochondrial respiratory chain dysfunction

The NLRP3 inflammasome combines danger signals and provides a role in the IL-18/IL-1b maturation and pyroptosis. Mitochondria have become also important players in the NLRP3 activation in various ways, such as the production of ROS, mitochondrial localisation of NLRP3/ASC upon activation, mitophagy regulation, mitochondrial lipid signalling (cardiolipin), and mitochondrial lipid release/oxidation.

A major study suggested that inhibiting mitophagy/autophagy leads to accumulation of dysfunctional, ROS-producing mitochondria that subsequently activates the NLRP3 inflammasome; mitochondrial pathways inhibition prevents the formation of ROS and activation of the NLRP3 suggests that NLRP3 is a sensor of mitochondrial dysfunction.

Mitochondrial cardiolipin is shown to bind NLRP3 and is required to stimulate the inflammasome in a model environment, linking mitochondrial inner membrane lipid biology to inflammatory signalling. This is beneficial to the case that mitochondrial dysfunction does not merely supply ROS, but also supplied ligands and platforms to assemble inflammasomes.

Subsequently, mechanistic work has clarified the mechanism through which oxidised mtDNA may get into the cytosol during non-apoptotic circumstances. Repaired/processed oxidised mitochondrial DNA fragments can be released via channels of mPTP-dependent and VDAC-dependent release, which activates NLRP3 inflammasome and cGAS-STING interferon signalling. This provides a direct bridging pathway of mitochondrial oxidative stress to innate immune activation, which is consistent with a tipping-point model in which the self-amplifying inflammatory state is occasioned by miROS-mediated mitochondrial oxidation and mitochondrial DNA release.

5.4 HIF-1alpha, hypoxic signalling, and immunometabolic programing

HIF-1a is a global controller of hypoxic responses, and can also determine immune and metabolic phenotypes (e.g. glycolytic reprogramming of inflammatory macrophages). One of the conventional models put forward that hypoxia-induced stabilisation of HIF-1alpha is dependent on mitochondrial ROS generated at complex III. Initial analyses indicated that mitochondria-derived ROS were necessary and adequate to stimulate HIF-1α stabilisation and that mitochondrial ROS generation (e.g. by depleting mitochondrial DNA) was necessary to suppress hypoxic HIF responses.

This was later expanded into immunometabolism: in lipopolysaccharide-stimulated macrophages, the stabilisation of HIF-1alpha by succinate accumulation and the stimulation of IL-1 beta production links TCA cycle remodelling, mitochondrial functioning and cytokine inflammatory programmes. This applies directly to chronic inflammatory conditions and inflammation related to obesity in which disturbed metabolite pools can be maintained to perpetuate inflammatory signalling.

The relation between the HIF and the ROS, however, is controversial. A recent investigation based on peroxide reporter strategies contended that the stabilisation of HIF1 in hypoxia is not triggered by oxidants, and that peroxide concentrations did not necessarily rise in hypoxia, indicating that ROS might be permissive, and not initiating.

5.5 Insulin signalling and redox-sensitive phosphatase regulation

There are many points at which redox chemistry regulates insulin signalling. In a classic mechanistic study, the insulin-stimulated H_2O_2 was reported to reverse-acting of PTP1B in vivo stimulating the early cascade of insulin action. This evidence favours the idea that insulin action has some oxidant signalling, but not antagonistic signalling.

In line with this, it was reported that deletion of glutathione peroxidase 1 (Gpx1) was able to protect mice against insulin resistance due to high-fat diet with greater insulin signalling being associated with more intense oxidation of PTEN; deletion of the antioxidant reversed the augmented insulin sensitivity. These findings are causal evidence of ROS as a positive stimulator of insulin signalling in vivo.

In comparison, persistent or excessive mtROS may be a cause of insulin resistance. It has been shown by evidence of work correlating the increased potential of mitochondrial H_2O_2 emission and an oxidised cellular redox environment with diet-induced insulin resistance in rodents and humans, and of mitochondrial catalase overexpression maintaining insulin sensitivity in the face of high-fat diet.

This paradox is explained by a threshold model: low, brief ROS (such as that provoked by insulin) can be beneficial to signalling, whereas high or persistent ROS causes signalling to switch to stress-kinase and inflammatory regimes (e.g. JNK activation, serine phosphorylation of IRS proteins), and can also change mitochondrial metabolism and inflammatory signalling.

5.6 AMPK and mTOR as integrators of energy/redox-states

AMPK is a cellular energy sensor and functions generally to replenish energy. It is also redox sensitive. One of the reports stated that mitochondrial ROS have the ability to physiologically activate AMPK, which AMPK activation elicited an antioxidant response mediated by PGC-1 that limited the production of mitochondrial ROS; and that AMPK activity loss enhanced mitochondrial ROS production and premature senescence. Notably, control of AMPK-PGC-1α of mitROS was linked to AMPK-deficient control of HIF-1alpha stabilisation and metabolic reprogramming (Warburg-like effects) by the same work. An example here of a feedback architecture is: mtROS triggers AMPK which reduces the amount of mtROS, stabilising redox homeostasis, until the system is overwhelmed.

mTOR combines signals of nutrient and growth factors; it is controlled by stress signals such as ROS via upstream nodes such as ATM and AMPK. It has been demonstrated that in response to high levels of ROS, ATM can stimulate TSC2 through the LKB1/AMPK pathway in the cytoplasm to inhibit mTORC1. This offers a mechanistic pathway through which oxidative stress can modify anabolic signalling and autophagy in response to this, impacting on mitochondrial quality control and energetic state.

The mTOR and AMPK controls in the cases of chronic metabolic diseases might also be maladaptive: the chronic nutrient excess might maintain mTOR signalling at high levels and inhibit autophagy/mitophagy, enhancing mitochondrial dysfunction and mitochondrial ROS, further supporting inflammation and insulin resistance.

6. Contexts of cells and tissues

A threshold-dependent redox rewiring model has to take into consideration cell type since both the production of mitROS and redox control architecture vary across tissues and since the functional implications of redox signals are diverse (e.g. antimicrobial defence in phagocytes versus insulin-stimulated glucose uptake in muscle).

6.1 Immunocytes (macrophages, dendritic cells, neutrophils, T cells)

ROS are not just the by-products of innate immunity: they are operational effectors and signalling intermediates. The engagement of Toll-like receptor may cause the recruitment of mitochondria to phagosomes of macrophages and enhance the production of mROS, which promotes bactericidal activity. It depicts controlled production of mtROS as immune activity.

The signal to NF-KB can be regulated by the mtROS at the signalling level, where NEMO disulphide connection is activated during infection, and it appears to be involved in a direct connection between the redox outputs of the mitochondrion and transcription of inflammatory responses.

Inflammasome pathways are also activated in immune activation. NLRP3 can be activated by mitochondrial damage and ROS-producing mitochondria, cardiolipin association and oxidised mtDNA escape via mPTP/VDAC channels may provide ligands and triggers to inflammasome and interferon pathways. These mitochondrial signals provide a reasonable explanation of how temporary immune activation may turn into chronic when repair/mitophagy is not successful or when metabolic stress results in chronic mitochondrial dysfunction.

6.2 Adipose tissue (adipocytes and infiltrating immune cells)

Obesity is defined by a chronic inflammation of low grade in adipose tissue, which entails peristubular adipocyte stress, adipokines distortion, and immune cell infiltration. These investigations did not lower the biology to the cause of disease by ROS but directed to the danger signals in the metabolism (e.g. ceramides) and immune activation. The mitochondrial rewiring of the redox rewiring lens is relevant as a source of mitochondrial component release (oxidised mtDNA) and as an authorising or enhancing signal to activate inflammasomes and generate cytokines. The fact that there are feed-forward loops (adipose inflammation to systemic cytokines to tissue insulin resistance to more metabolic stress) enhances the probability of threshold behaviour in which adipose tissue switches to an inflammatory attractor.

6.3 Liver (hepatocytes, Kupffer cells and NAFLD/NASH progression)

The liver is a central metabolic gland prone to oxidative/inflammatory stress due to the high concentration of mitochondrial content, centrality of lipid/glucose metabolism, and endotoxin signal and circulating metabolites. The NAFLD reviews are concentrated on complicated cause: it is still argued whether the cause or the effect is the mitochondrial dysfunction and oxidative stress. Such uncertainty can facilitate a tipping-point perspective: redox changes in the mitochondrion may not cause NAFLD but can be decisive in the development of inflammatory NASH and fibrosis through the amplification of inflammatory signalling, mitochondrial injury and cellular responses to stress.

Inflammasome pathways are triggered by the liver resident macrophages (kupffer cells). Mitochondrial DNA release has been proposed to trigger NLRP3 in Kupffer cells fatty acids, and it has been suggested that lipid overload is a mechanistic contribution of mitochondrial stress and innate immune activation. These processes are in line with redox network rewiring where lipid-induced mitochondrial dysfunction attains a threshold that causes the release of mitochondrial DNA and the activation of inflammasomes, which promotes the development of chronic inflammation in the liver.

6.4 Skeletal muscle (insulin resistance, mitochondrial H2O2 emission and redox buffering)

The skeletal muscle is the primary location of glucose disposal and is one of the primary locations of insulin resistance. A groundbreaking study associated high-fat diet with higher mitochondrial H2O2 emission capacity and a more oxidised cellular redox environment in muscle, and demonstrated that mitochondrial H2O2 emission targeting prevented insulin insensitivity despite a high-fat diet. This paper is especially relevant to threshold models in the sense that it presents mitochondrial H2O2 emission as a gauge of energy balance and as a controller of the cellular redox environment, as opposed to a marker of damage.

Human trials also support adaptation between beneficial and detrimental chronic ROS in muscle, with evidence that benefits of antioxidant supplementation can inhibit exercise-induced gain of insulin sensitivity as well as prevent activation of endogenous antioxidant defence regulators. This is in favour of a position where certain redox stress is necessary to achieve positive remodelling and that inhibiting it can cause network behaviour to change towards negative directions.

6.5 Endothelium and vascular tissue

Oxidative stress and inflammation are significant factors in endothelial dysfunction, which is a significant cause of cardiovascular disease. Mitochondrial ROS is suggested to be a major mediator of age-related vascular dysfunction; translation to human models demonstrates that chronic oral supplementation of the mitochondria-targeted antioxidant MitoQ enhanced brachial artery flow-mediated dilation and was linked to reversal of age-associated inhibition of endothelial function by mitochondrial ROS. Although this is not conclusive evidence that the mitochondrial oxidative pathway is causal in all endothelial dysfunction, these outcomes suggest that, by targeting mitochondrial oxidative pathways, clinically relevant vascular functional outcomes can be altered.

Endothelial and vascular smooth muscle cells are also sensitive to inflammatory (TNF-α, IL-1b) and metabolic (hyperglycaemia) signals that may raise the level of mitochondrial ROS and trigger the downstream signalling pathways involved in vascular complications. A review on mitochondrial ROS in diabetes explains the action of high glucose on the rise in mitochondrial ROS in endothelial cells and the inactivation of pathways involved in diabetic complications by intervention that normalises mitochondrial ROS. It is consistent with the tipping-point model: the prolonged metabolic stress can keep the endothelial cells in a redox state above a redox threshold, in which the pathways of inflammation and damage become predominant.

7. Data of threshold-dependent responses

threshold dependent redox responses are based on a number of experimental motifs:

1. Buffering to failure: experiments and models show that antioxidants systems can operate to their maximum capacity by maintaining low emissions of H2O2. The mitochondrial GSH and thioredoxin/peroxiredoxin systems serve as important buffering systems that have NADPH dependence when saturated or blocked either and in this case the emission of H2O2 increases tremendously.
2. All-or-none redox switching of antioxidant circuits: peroxiredoxin systems can be ultrasensitive and even bistable in parameter space. This provides a mechanistic account of abrupt variability in H2O2 signalling and peroxiredoxin oxidation conditions.
3. Sudden transformations in mitochondrial networks: Sudden transient changes in mitochondrial membrane potential and ROS release can be induced by ROS-induced permeability transition as well as ROS-induced ROS release. The network coupling can orchestrate interactions between mitochondria, transforming local stress to global changes of state.

Hysteresis means that the input (e.g. glucose) may not be immediately restored to the previous redox state, the system can be stuck in a new state. Micro Switch-like ROS responses reported in a nutrient-redox modelling and experimental study were irreversible and hysterical under glucose deprivation. Although it is not a direct demonstration of chronic inflammation in vivo, it is a demonstration of principle that cellular redox networks can promote sustained states and state transitions which would be directly applicable to chronic inflammatory and metabolic diseases which are clinically persistent and difficult to remit.

A number of modelling methods are applicable:

- Mechanistic ODE modeling of mitochondrial ROS production. The ROS and redox balance computational model of cardiac mitochondria was used to investigate how the production of electron transport chain ROS vary in state 3 versus state 4 respiration, and how the production in combination with scavenging of the scavenging can produce non-monotonic ROS behavior when scavenging is depleted. This modelling is consistent with the opinion that threshold-like properties of ROS outputs can be obtained without having to appeal to the complicated processes of biology beyond the established bioenergetics and scavenging kinetics.
- Mitochondrial propagation reaction-diffusion network models. Reactiondiffusion model of RIRR in

mitochondrial networks replicated the depolarisation waves and gave new understanding of the emergent macroscopic properties of mitochondrial networks under metabolic stress that offered a mechanistic explanation of cell-wide effects as network effects and not single-organelle effects.

Whole-cell nutrient-redox models containing multistability. A nutrientredox model highlighted mutually inhibitory interactions between reduced glutathione and ROS, which are coupled to upstream nutrient pathways and downstream feedback loops (calcium, NADPH oxidases), and which exhibit switch-like behaviour and hysteresis. These models are good contenders to reconcile molecular redox specifics to cell fate choice and chronic state alterations.

Table 1. Comparing representative studies illustrating threshold logic, amplification, and rewiring

Study	Model System	Context	Evidence for Threshold / Amplification
Zorov et al. (2000)	Cardiac myocytes	Photo-triggered ROS	ROS-induced MPT triggers ROS burst (RIRR)
Zhou et al. (2010)	Computational + experimental	Metabolic stress	ΔΨm depolarisation waves sensitive to ROS diffusion
Huang et al. (2021)	Human cells + in silico	Glucose deprivation	Switch-like ROS response; hysteresis
Anderson et al. (2009)	Rodents + humans	High-fat diet	mtH_2O_2 links fat intake to insulin resistance
Chouchani et al. (2014)	Cardiomyocytes + in vivo heart	Ischaemia–reperfusion	Succinate-driven RET causes ROS burst
Xian et al. (2022)	Macrophages	Mitochondrial stress	Ox-mtDNA release activates NLRP3, cGAS–STING
Herb et al. (2019)	Macrophages infection model	Infection-induced mtROS	mtROS induces NEMO disulphide for NF-κB activation

The quality of threshold models is limited by the quality of measurements to detect nonlinear behaviour; mtROS is difficult to measure due to (i) the short lifetime of superoxide and non-membrane permeability; (ii) the long lifetime of H2O2 and rapid degradation and compartmentalisation; (iii) artefacts, off-target oxidation or pH effects of most fluorescent probes; and (iv) the perturbation of the redox state by measurements.

Probes and caveats of fluorescent. Mitochondrial superoxide probes based on ethidium have been applied to detect mitochondrial superoxide, and a landmark paper outlines selective fluorescent imaging methods and comments on technical issues. Although it is widely used, MitoSOX assays should be carefully controlled because of possible non-specific oxidation and localisation. These considerations are reflected in protocol literature which means it is popular but not infallible.

The invention of genetically encoded probes allowed better spatiotemporal resolution and targeting of sub cells. HyPer probe was presented as a highly specific fluorescent probe which is a genetically-encoded probe of intracellular H2O2, amenable to cytosolic and mitochondrial targeting. Later evolution led to HyPer7, which is an ultra-fast, ultrasensitive and pH-insensitive ratiometric H2O2 reporter, which overcomes some of the major limitations (particularly pH sensitivity) of the previous probes. These methods allow H2O2 dynamics to be mapped with enhanced fidelity and form the basis of studying threshold behaviour in living cells.

The roGFP-based sensors offer complementary information on the glutathione redox potential (EGSH) dynamics or H2O2 dynamics by fusion with redox relay elements. Grx1-roGFP2 probe provides the opportunity to equilibrate the glutathione redox couple in real time. These probes are essential in determining the redox thresholds based on redox potential and not exclusively on ROS concentration.

One of the significant advances is the creation of mitochondria-targeted ratiometric mass spectrometry probes like MitoB that accumulates in mitochondria and reacts with H2O2 to form MitoP; the proportion of MitoP/MitoB can be measured using LCMS/MS to infer mitochondrial matrix H2O2 in vivo. This method was to measure mitochondrial H2O2 in living Drosophila and has been utilized in vivo to ageing models. Protocols of this kind can be useful in threshold models since they have the potential to provide quantitative, compartment-specific read-outs less subject to certain fluorescence artefacts but there are limitations (delivery, calibration, kinetics).

Redox status can also be established by using low molecular weight thiol/disulphide couples and their redox potentials, such as: GSH/GSSG and cysteine/cystine. Methodological synthesis. Methodological synthesis discusses assay, biological significance of the GSH/GSSG redox potential as an indicator ofÂ redox stateÂ and its use in assessing metabolically relevant physiologic function and compartmentation. Their systemic character could make them functional as some kind of “systemic” markers, albeit not strictly representative of the mtROS microdomains.

OxICAT is applied to site-specifically measure cysteine oxidation – in vivo – for assisting the identification of redox switches and global redox remodelling. Such networks are important for implementing redox network rewiring beyond the level of ROS: they show which functional protein nodes change in oxidation status under certain conditions.

8. Therapeutic approaches, controversies and future

8.1 Therapeutic logic

According to the threshold-dependent rewiring view, successful treatment should be based on the issue of where, when, and how to intervene. Antioxidant supplementation around the globe can suppress positive redox signalling and adaptive mechanisms. An experiment involving controlled clinical trial demonstrated that vitamin C and vitamin E intake inhibited exercise-induced enhancement in insulin sensitivity and inhibited the activation of ROS-sensitive transcriptional regulators and endogenous antioxidant enzymes, which are both in line with the concept of mitohormesis, as well as the idea that physiological oxidative stress may be beneficial.

In addition, systematic reviews have found that antioxidant supplement interventions (beta-carotene, vitamins A/C/E, selenium) are not consistently found to decrease mortality and may in fact lead to increased mortality, but these conclusions are controversial and very sensitive to trial selection, bias, and heterogeneity. Nevertheless, these results generally support the idea that antioxidant strategies, which are not specifically identified, may not work or even be pernicious, which is in line with the complexity of redox networks.

8.2 Mitochondria-targeted redox modulators and antioxidants

A more specific approach is to provide antioxidant effectiveness to mitochondria. MitoQ is a ubiquinone analog analogue, which is mitochondria-targeted, and accumulated at the inner membrane under the influence of a lipophilic cation (triphenylphosphonium). The clinical and translational evidence indicates the possible advantages in particular settings: oral MitoQ 6 weeks enhanced flow-mediated dilation in a randomised crossover study, which is in line with decreased suppression of endothelial activity by the mitochondrial reactive species. Mitoquinone was found to decrease liver enzyme markers in hepatitis C patients in a phase II study, indicating possibly a decrease in hepatic necroinflammation but no change in viral load.

MitoTEMPO and similar compounds are other mitochondria-targeted antioxidants. MitoTEMPO, in an animal model of metabolic dysfunction, has been reported to inhibit insulin resistance and cardiac dysfunction in certain situations, so that in such models, it is possible that mitochondrial oxidative stress may be a causative factor.

Notably, mitochondria-targeted approaches continue to have their problems: (i) mitochondrial accumulation is no guarantee of proper microdomain targeting; (ii) antioxidants can become pro-oxidant under some redox cycling conditions; (iii) the redox state of interest may vary between tissues and disease stages; and (iv) the redox state of interest may have physiological signalling functions, hence dose, timing, and patient selection may be important. These reflections are based on the fundamental biology that there is a regulated range of oxidative eustress (mtROS) and pathological ranges of oxidative distress.

8.3 Metabolic modulators which indirectly tune off rewiring and mtROS

The second pharmacological groups are metabolic modulators that modify the mitochondrial bioenergetics and thus, mitROS production. These mechanisms are but not limited to: decreasing the excessive mitochondrial membrane potential, matching ADP, promoting mitophagy or changing substrate utilization. The reasoning is that the mtROS production is very bastion bioenergetic-dependent, shifting the flux shift can translocate such system below a ROS threshold without necessarily eliminating ROS.xxx31xxx32 This is consistent with the finding that AMPK activation normalizes redox homeostasis and promotes antioxidant defence beyond mitochondrial reactive species (mtROS) 17,18, as well as the central role of succinate and metabolic reprogramming in inflammatory macrophages (the succinate–HIF-1a–IL-1B axis), supporting a potential to modulate inflammation through metabolic interventions.

8.4 Targeting inflammasomes and mitochondrial danger signalling

Since it is possible that mitochondrial-derived signals (oxidised mtDNA, cardiolipin) are partially responsible in the activation of the inflammasomes, the inhibition of inflammasome-activation or mitochondrial-DNA release is a possible approach. NLRP3 activation has also been suggested as a cause of insulin resistance and inflammation in obesity settings;

thus, there is interest in inflammasome-based strategies (direct inhibitors, IL-1 pathway blockers). Although not all of them are mitochondria-specific, the knowledge of mtROS as an upstream signal can inform mechanism-based strategies of combination.

Table 2. Comparing therapeutic strategies targeting mtROS or redox rewiring

Strategy Class	Representative Interventions	Mechanistic Target	Evidence Highlights
Conventional antioxidants	Vitamins C/E, selenium, beta-carotene	Global ROS scavenging	Blocked exercise-induced insulin sensitivity gains
Mitochondria-targeted antioxidants	MitoQ, MitoTEMPO	Inner mitochondrial membrane ROS	Improved endothelial function; reduced liver enzymes; prevented insulin resistance (model-specific)
Redox-sensor modulation	NRF2 activation	KEAP1–NRF2 cytoprotective pathway	Reprogrammes antioxidant/detox genes
Energy-sensing modulation	AMPK activation; mTOR suppression	Energy–redox integration	mtROS activates AMPK; AMPK enhances antioxidant defence; represses mTORC1

A number of current issues define the basis for future studies:

- Coronary metabolic syndrome Goes It Is Not Confounding, Causality Versus Correlation in Chronic Metabolic Diseases and Does a Flame Have to Be Hot? Even in relatively well-understood circumstances like insulin resistance, the extent to which mitochondrial dysfunction and free radical damage are causes versus consequences is still a matter of both speculation and debate.
- We need better tools and careful inference to assign mitochondrial site, occupied compartment and contribution of different sites/ compartments to signalling in a particular disease.
- Hypoxia signalling controversy. Although there is a long history of the association between mitochondrial ROS and HIF-1α stability, two recent studies suggested that peroxide elevations are not upstream signals in hypoxia. Not “ROS or not” but threshold/permissive model may be a better representation -basal redox state would allow HIF regulation and can be a permissive then oxygen-sensing enzymes become the main trigger YYSTACK:Argumentation. Addressing this problem will require better compartmentalized monitoring of ROS in more physiological relativedesigns with heterogenious O 2 gradients.
- Heterogeneity and single-cell variability. The threshold behaviour might appear different between cells in a tissue than to varying mitochondrial load, substrate exposure and hypoxia-sensitivity, antioxidant supply or inflammatory tone among the cells. Minority and majority rule of the median times were on the order of six and two orders, respectively, too long with respect to an in vivo detection. Single cell redox imaging or 3D-RED-256 could thus be required for the robust detection of tipping points in vivo.

9. FUTURE DIRECTIONS

A programme of research that corresponds to the thesis of this review would emphasise:

1. Redox mapping attractorstates in relevant cells and tissues (immune cells, adipo- cytes, hepatocytes, myocytes and endothelium) by means of holo-redox potential probes together with mtROS reporters andquantitative proteomics for identicationof stable regimes is anobjective that now can be addressed; ✓#x002d7 Mapping✓the redox landscape of biological representa- tive cell types constitutes identicationng of stable edges§ Redox dynamics in acute liver failure:MTB geometry determining cellular fate During ALF the stripped geometrical occurrences willrun amok at cellular level.
2. Mechanistic description of amplification loop (i.e., RET driving forces under condition of chronic metabolic overload, mitochondrial network propagation, mtDNA oxidation/export pathways) and their dependence on antioxidant buffering capacity.

3. Intervention timing and patient selection using markers of proximity to a redox tipping point rather than nonspecific “oxidativestress” surrogates.

Systems integrating metabolism, inflammation and redox with adequate non-linearities and empirically determined predictions necessary for applying translationalinference to thresholds and bistability in human disease.

REFERENCES

1. Alexander, A., Cai, S.-L., Kim, J., Nanez, A., Sahin, M., MacLean, K. H., Inoki, K., Guan, K.-L., Shen, J., Person, M. D., Kusewitt, D., Mills, G. B., Kastan, M. B., & Walker, C. L. (2010). ATM signals to TSC2 in the cytoplasm to regulate mTORC1 in response to ROS. *Proceedings of the National Academy of Sciences of the United States of America, 107*(9), 4153–4158. doi:10.1073/pnas.0913860107
2. Albrecht, S. C., Barata, A. G., Großhans, J., Teleman, A. A., & Dick, T. P. (2011). In vivo mapping of hydrogen peroxide and oxidized glutathione reveals chemical and regional specificity of redox homeostasis. *Cell Metabolism, 14*(6), 819–829. doi:10.1016/j.cmet.2011.10.010
3. Anderson, E. J., Lustig, M. E., Boyle, K. E., Woodlief, T. L., Kane, D. A., Lin, C.-T., Price, J. W., Kang, L., Rabinovitch, P. S., Szeto, H. H., Houmard, J. A., Cortright, R. N., Wasserman, D. H., & Neufer, P. D. (2009). Mitochondrial H_2O_2 emission and cellular redox state link excess fat intake to insulin resistance in both rodents and humans. *The Journal of Clinical Investigation, 119*(3), 573–581. doi:10.1172/JCI37048
4. Belousov, V. V., Fradkov, A. F., Lukyanov, K. A., Staroverov, D. B., Shakhbazov, K. S., Terskikh, A. V., & Lukyanov, S. (2006). Genetically encoded fluorescent indicator for intracellular hydrogen peroxide. *Nature Methods, 3*(4), 281–286. doi:10.1038/nmeth866
5. Bjelakovic, G., Nikolova, D., Gluud, L. L., Simonetti, R. G., & Gluud, C. (2012). Antioxidant supplements for prevention of mortality in healthy participants and patients with various diseases. *Cochrane Database of Systematic Reviews*, (3), CD007176. doi:10.1002/14651858.CD007176.pub2
6. Brand, M. D. (2016). Mitochondrial generation of superoxide and hydrogen peroxide as the source of mitochondrial redox signaling. *Free Radical Biology and Medicine, 100*, 14–31. doi:10.1016/j.freeradbiomed.2016.04.001
7. Chandel, N. S., McClintock, D. S., Feliciano, C. E., Wood, T. M., Melendez, J. A., Rodriguez, A. M., & Schumacker, P. T. (2000). Reactive oxygen species generated at mitochondrial complex III stabilize hypoxia-inducible factor-1α during hypoxia: A mechanism of O_2 sensing. *The Journal of Biological Chemistry, 275*(33), 25130–25138. doi:10.1074/jbc.M001914200
8. Chouchani, E. T., Pell, V. R., Gaude, E., Aksentijević, D., Sundier, S. Y., Robb, E. L., Logan, A., Nadtochiy, S. M., Ord, E. N. J., Smith, A. C., Eyassu, F., Shirley, R., Hu, C.-H., Dare, A. J., James, A. M., Rogatti, S., Hartley, R. C., Eaton, S., Costa, A. S. H., … Murphy, M. P. (2014). Ischaemic accumulation of succinate controls reperfusion injury through mitochondrial ROS. *Nature, 515*(7527), 431–435. doi:10.1038/nature13909
9. Cochemé, H. M., Logan, A., Prime, T. A., Abakumova, I., Quin, C., McQuaker, S. J., Patel, J. V., Fearnley, I. M., James, A. M., Porteous, C. M., Smith, R. A. J., Hartley, R. C., & Murphy, M. P. (2011). Measurement of H_2O_2 within living *Drosophila* during aging using a ratiometric mass spectrometry probe targeted to the mitochondrial matrix. *Cell Metabolism, 13*(3), 340–350. doi:10.1016/j.cmet.2011.02.003
10. Cortassa, S., Aon, M. A., Winslow, R. L., & O’Rourke, B. (2004). A mitochondrial oscillator dependent on reactive oxygen species. *Biophysical Journal, 87*(3), 2060–2073. doi:10.1529/biophysj.104.041749
11. Gane, E. J., Weilert, F., Orr, D. W., Keogh, G. F., Gibson, M., Lockhart, M. M., Frampton, C. M., Taylor, K. M. W., Smith, R. A. J., & Murphy, M. P. (2010). The mitochondria-targeted anti-oxidant mitoquinone decreases liver damage in a phase II study of hepatitis C patients. *Liver International, 30*(7), 1019–1026. doi:10.1111/j.1478-3231.2010.02250.x
12. Go, Y.-M., & Jones, D. P. (2013). Thiol/disulfide redox states in signaling and sensing. *Critical Reviews in Biochemistry and Molecular Biology, 48*(2), 173–181. doi:10.3109/10409238.2013.764840
13. Herb, M., Gluschko, A., Wiegmann, K., Farid, A., Wolf, A., Utermöhlen, O., Krut, O., Krönke, M., & Schramm, M. (2019). Mitochondrial reactive oxygen species enable proinflammatory signaling through disulfide linkage of NEMO. *Science Signaling, 12*(568), ea ar5926. doi:10.1126/scisignal.aar5926
14. Herscovitch, M., Comb, W., Ennis, T., Coleman, K., Yong, S., Armstead, B., Kalaitzidis, D., Chandani, S., & Gilmore, T. D. (2008). Intermolecular disulfide bond formation in the NEMO dimer requires Cys54 and Cys347. *Biochemical and Biophysical Research Communications, 367*(1), 103–108. doi:10.1016/j.bbrc.2007.12.109

15. Huang, J.-H., Co, H. K. C., Lee, Y.-C., Wu, C.-C., & Chen, S.-H. (2021). Multistability maintains redox homeostasis in human cells. *Molecular Systems Biology, 17*(10), e10480. doi:10.15252/msb.202110480
16. Iyer, S. S., He, Q., Janczy, J. R., Elliott, E. I., Zhong, Z., Olivier, A. K., Sadler, J. J., Knepper-Adrian, V., Han, R., Qiao, L., Eisenbarth, S. C., Nauseef, W. M., Cassel, S. L., & Sutterwala, F. S. (2013). Mitochondrial cardiolipin is required for Nlrp3 inflammasome activation. *Immunity, 39*(2), 311–323. doi:10.1016/j.immuni.2013.08.001
17. Jeong, E. M., Chung, J., Liu, H., Go, Y.-M., Gladstein, S., Farzaneh-Far, A., Lewandowski, E. D., & Dudley, S. C. (2016). Role of mitochondrial oxidative stress in glucose tolerance, insulin resistance, and cardiac diastolic dysfunction. *Journal of the American Heart Association, 5*(5), e003046.
18. Jones, D. P. (2002). Redox potential of GSH/GSSG couple: Assay and biological significance. *Methods in Enzymology, 348*, 93–112. doi:10.1016/S0076-6879(02)48630-2
19. Jones, D. P. (2015). The redox code. *Antioxidants & Redox Signaling, 23*(9), 734–746. doi:10.1089/ars.2015.6247
20. Kumar, A., Vaish, M., Karuppagounder, S. S., Gazaryan, I., Cave, J. W., Starkov, A. A., Anderson, E. T., Zhang, S., Pinto, J. T., Rountree, A. M., Wang, W., Sweet, I. R., & Ratan, R. R. (2021). HIF1α stabilization in hypoxia is not oxidant-initiated. *eLife, 10*, e72873. doi:10.7554/eLife.72873
21. Lampropoulou, V., Sergushichev, A., Bambouskova, M., Nair, S., Vincent, E. E., Loginicheva, E., Cervantes-Barragan, L., Ma, X., Huang, S.-C., Griss, T., Weinheimer, C. J., Khader, S., Randolph, G. J., Pearce, E. J., Jones, R. G., Diwan, A., Diamond, M. S., Artyomov, M. N., & O'Neill, L. A. J. (2016). Itaconate links inhibition of succinate dehydrogenase with macrophage metabolic remodeling and regulation of inflammation. *Cell Metabolism, 24*(1), 158–166. doi:10.1016/j.cmet.2016.06.004
22. Loh, K., Deng, H., Fukushima, A., Cai, X., Boivin, B., Galic, S., Bruce, C., Shields, B. J., Skiba, B., Ooms, L. M., Stepto, N., Wu, B., Mitchell, C. A., Tonks, N. K., Watt, M. J., Febbraio, M. A., Crack, P. J., Andrikopoulos, S., & Tiganis, T. (2009). Reactive oxygen species enhance insulin sensitivity. *Cell Metabolism, 10*(4), 260–272. doi:10.1016/j.cmet.2009.08.009
23. Mahadev, K., Zilbering, A., Zhu, L., & Goldstein, B. J. (2001). Insulin-stimulated hydrogen peroxide reversibly inhibits protein-tyrosine phosphatase 1B in vivo and enhances the early insulin action cascade. *The Journal of Biological Chemistry, 276*(24), 21938–21942. doi:10.1074/jbc.C100109200
24. Morgan, M. J., & Liu, Z.-G. (2011). Crosstalk of reactive oxygen species and NF-κB signaling. *Cell Research, 21*(1), 103–115. doi:10.1038/cr.2010.178
25. Murphy, M. P. (2009). How mitochondria produce reactive oxygen species. *Biochemical Journal, 417*(1), 1–13. doi:10.1042/BJ20081386
26. Nishikawa, T., Kukidome, D., Sonoda, K., Fujisawa, K., Matsuhisa, T., Motoshima, H., Matsumura, T., Araki, E. (2007). Impact of mitochondrial ROS production in the pathogenesis of insulin resistance. *Antioxidants & Redox Signaling, 9*(12), 2111–2120. doi:10.1089/ars.2007.1790
27. Pak, V. V., Ezeriņa, D., Lyublinskaya, O. G., Pedre, B., Tyurin-Kuzmin, P. A., Mishina, N. M., Thauvin, M., Young, D., Wahni, K., Martínez Gache, S. A., Demidovich, A. D., Ermakova, Y. G., Maslova, Y. D., Shokhina, A. G., Ilyichova, O. V., Miliukov, V. A., Aksyonov, D. A., Lidsky, P. V., … Belousov, V. V. (2020). Ultrasensitive genetically encoded indicator for hydrogen peroxide identifies roles for the oxidant in cell migration and mitochondrial function. *Cell Metabolism, 31*(3), 642–653.e6. doi:10.1016/j.cmet.2020.02.003
28. Quinlan, C. L., Treberg, J. R., Perevoshchikova, I. V., Orr, A. L., & Brand, M. D. (2013). Sites of reactive oxygen species generation by mitochondria oxidizing different substrates. *Free Radical Biology and Medicine, 53*(9), 1807–1817. doi:10.1016/j.freeradbiomed.2012.08.015
29. Rabinovitch, R. C., Samborska, B., Faubert, B., Ma, E. H., Gravel, S.-P., Andrzejewski, S., Raissi, T. C., Pause, A., St-Pierre, J., & Jones, R. G. (2017). AMPK maintains cellular metabolic homeostasis through regulation of mitochondrial reactive oxygen species. *Cell Reports, 21*(1), 1–9. doi:10.1016/j.celrep.2017.09.026
30. Reczek, C. R., & Chandel, N. S. (2015). ROS-dependent signal transduction. *Current Opinion in Cell Biology, 33*, 8–13. doi:10.1016/j.ceb.2014.09.010
31. Ristow, M., Zarse, K., Oberbach, A., Klöting, N., Birringer, M., Kiehntopf, M., Stumvoll, M., Kahn, C. R., & Blüher, M. (2009). Antioxidants prevent health-promoting effects of physical exercise in humans. *Proceedings of the National Academy of Sciences of the United States of America, 106*(21), 8665–8670. doi:10.1073/pnas.0903485106
32. Rossman, M. J., et al. (2018). Chronic supplementation with a mitochondrial antioxidant (MitoQ) improves vascular

function in healthy older adults. *Hypertension, 71*(6), 1056–1063. doi:10.1161/HYPERTENSIONAHA.117.10787

33. Shadel, G. S., & Horvath, T. L. (2015). Mitochondrial ROS signaling in organismal homeostasis. *Cell, 163*(3), 560–569. doi:10.1016/j.cell.2015.10.001
34. Sies, H. (2015). Oxidative stress: A concept in redox biology and medicine. *Redox Biology, 4*, 180–183. doi:10.1016/j.redox.2015.01.002
35. Sies, H. (2017). Hydrogen peroxide as a central redox signaling molecule in physiological oxidative stress: Oxidative eustress. *Redox Biology, 11*, 613–619. doi:10.1016/j.redox.2016.12.035
36. Sies, H. (2020). Oxidative stress: Concept and some practical aspects. *Antioxidants, 9*(9), 852. doi:10.3390/antiox9090852
37. Sies, H. (2021). Oxidative eustress: On constant alert for redox homeostasis. *Redox Biology, 41*, 101867. doi:10.1016/j.redox.2021.101867
38. Sies, H., Berndt, C., & Jones, D. P. (2017). Oxidative stress. *Annual Review of Biochemistry, 86*, 715–748. doi:10.1146/annurev-biochem-061516-045037
39. Stienstra, R., Joosten, L. A. B., Koenen, T., van Tits, B., van Diepen, J. A., van den Berg, S. A. A., Rensen, P. C. N., Voshol, P. J., Fantuzzi, G., Hijmans, A., Kersten, S., Müller, M., van den Bout, A., van der Meer, J. W. M., Kanneganti, T.-D., Tack, C. J., & Netea, M. G. (2011). The inflammasome is a central player in the induction of obesity and insulin resistance. *Proceedings of the National Academy of Sciences of the United States of America, 108*(37), 15324–15329. doi:10.1073/pnas.1100255108
40. Tannahill, G. M., Curtis, A. M., Adamik, J., Palsson-McDermott, E. M., McGettrick, A. F., Goel, G., Frezza, C., Bernard, N. J., Kelly, B., Foley, N. H., Zheng, L., Gardet, A., Tong, Z., Jany, S. S., Corr, S. C., Haneklaus, M., Caffrey, B. E., Pierce, K., Walmsley, S., … O'Neill, L. A. J. (2013). Succinate is an inflammatory signal that induces IL-1β through HIF-1α. *Nature, 496*(7444), 238–242. doi:10.1038/nature11986
41. Topf, U., Suppanz, I., Samluk, Ł., Wrobel, L., Böser, A., Sakowska, P., Knapp, B., Pietrzyk, M. K., Chacińska, A., & Warscheid, B. (2018). Quantitative proteomics identifies redox switches for global translation modulation by mitochondrially produced reactive oxygen species. *Nature Communications, 9*(1), 324. doi:10.1038/s41467-017-02694-8
42. Vandanmagsar, B., Youm, Y.-H., Ravussin, A., Galgani, J. E., Stadler, K., Mynatt, R. L., Ravussin, E., Stephens, J. M., & Dixit, V. D. (2011). The NLRP3 inflammasome instigates obesity-induced autoinflammation and insulin resistance. *Nature Medicine, 17*(2), 179–188. doi:10.1038/nm.2279
43. Woo, H. A., Yim, S. H., Shin, D. H., Kang, D., Yu, D.-Y., & Rhee, S. G. (2010). Inactivation of peroxiredoxin I by phosphorylation allows localized H_2O_2 accumulation for cell signaling. *Cell, 140*(4), 517–528. doi:10.1016/j.cell.2010.01.009
44. Xian, H., Watari, K., Sanchez-Lopez, E., Offenberger, J., Onyuru, J., Sampath, H., … & Ting, J. P.-Y. (2022). Oxidized DNA fragments exit mitochondria via mPTP- and VDAC-dependent channels to activate NLRP3 inflammasome and interferon signaling. *Immunity, 55*(8), 1370–1385.e8. doi:10.1016/j.immuni.2022.06.007
45. Yamamoto, M., Kensler, T. W., & Motohashi, H. (2018). The KEAP1–NRF2 system: A thiol-based sensor–effector apparatus for maintaining redox homeostasis. *Physiological Reviews, 98*(3), 1169–1203. doi:10.1152/physrev.00023.2017
46. Zhou, L., Aon, M. A., Almas, T., Cortassa, S., Winslow, R. L., & O'Rourke, B. (2010). A reaction-diffusion model of ROS-induced ROS release in a mitochondrial network. *PLoS Computational Biology, 6*(1), e1000657. doi:10.1371/journal.pcbi.1000657
47. Zorov, D. B., Filburn, C. R., Klotz, L.-O., Zweier, J. L., & Sollott, S. J. (2000). Reactive oxygen species (ROS-induced) ROS release: A new phenomenon accompanying induction of the mitochondrial permeability transition in cardiac myocytes. *The Journal of Experimental Medicine, 192*(7), 1001–1014. doi:10.1084/jem.192.7.1001