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Interspecies Differences in Mitochondrial Structure and Function: Implications for Cardiovascular Physiology in Translational Research

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Abstract

The heart is among the most energy-hungry organs in the body, and almost all of that energy is supplied by mitochondria. For this reason mitochondrial biology sits close to the centre of cardiovascular physiology and, increasingly, of cardiovascular drug discovery. Yet most of what we know about cardiac mitochondria has been pieced together from a handful of laboratory species – mostly mice and rats – whose mitochondria are not simply scaled-down versions of our own. This review examines how mitochondrial structure and function differ between the species that populate translational cardiovascular research, and why those differences matter when results are carried from the bench to the clinic. We consider the conserved architecture of the organelle alongside the features that vary: the lipid composition of the inner membrane and its link to metabolic rate, the magnitude of proton leak and mild uncoupling, the balance between reactive oxygen species production and antioxidant capacity, the handling of calcium, and the sensitivity of the permeability transition pore. Each of these axes tends to scale, often steeply, with body mass and life history, so that a mouse cardiomyocyte beating some six hundred times a minute operates under bioenergetic constraints quite unlike those of a resting human heart. We use the repeated failure of mitochondria-targeted cardioprotection in clinical trials, despite striking success in rodents, as a cautionary illustration. We argue that progress depends less on finding a single "best" model than on interpreting each model in light of its mitochondrial idiosyncrasies, and on combining small animals, large animals and human cell-based systems thoughtfully.

➢ *Highlights*

- Cardiac mitochondria share a deeply conserved architecture across vertebrates, yet differ markedly in membrane lipids, coupling efficiency and ion handling between species.
- Mass-specific metabolic rate, heart rate and membrane polyunsaturation scale with body size, shaping species-specific mitochondrial bioenergetics and redox balance.
- Differences in calcium handling and permeability-transition sensitivity help explain why cardioprotection that works in rodents has translated poorly to patients.
- Rational model selection – and the complementary use of large animals and human iPSC-derived cardiomyocytes – is essential for credible translation.

Keywords: *Mitochondria; Cardiac Physiology; Interspecies Differences; Translational Research; Oxidative Phosphorylation; Reactive Oxygen Species; Calcium Handling; Permeability Transition Pore; Membrane Lipids; Animal Models.*

I. INTRODUCTION

Few organs illustrate the dependence of physiology on mitochondria as plainly as the heart. A human heart never pauses, and to keep contracting it hydrolyses an enormous amount of ATP – on the order of several kilograms per day – almost all of which is regenerated by oxidative phosphorylation within mitochondria that occupy roughly a third of the volume of each adult cardiomyocyte [1]. Because the stored pool of ATP would

last only seconds, supply and demand must be matched on a beat-to-beat basis, and any disturbance of mitochondrial function is quickly felt as contractile or electrical dysfunction [1,2]. It is therefore unsurprising that mitochondria have become a focus of research into heart failure, ischaemia–reperfusion injury, cardiomyopathy and the ageing heart.

Almost inevitably, that research has been done in animals. Genetically tractable rodents in particular have driven decades of mechanistic discovery, and they remain the workhorses of preclinical cardiovascular science [3]. The difficulty is that mitochondria are not invariant across the tree of life. While the core machinery of the organelle – the respiratory complexes, the ATP synthase, much of the proteome – is conserved to a remarkable degree [4], many functionally important properties are not. Membrane lipids, proton leak, the rate at which reactive oxygen species are generated and removed, and the thresholds that govern calcium overload all vary between species, frequently in tight relation to body size and longevity. A mouse and a human differ in heart rate by roughly an order of magnitude, and their cardiomyocytes are tuned accordingly [3].

This review surveys those interspecies differences in mitochondrial structure and function, with an emphasis on the cardiovascular system and on the species used in translational work. Our aim is not to rank models but to clarify how each one departs from the human heart, so that findings can be read with the caution they deserve.

II. THE MITOCHONDRIAL BLUEPRINT: CONSERVED ARCHITECTURE, DIVERGENT DETAIL

At first glance a cardiac mitochondrion looks much the same whether it comes from a zebrafish, a mouse or a human. The double membrane, the folded cristae that house the electron transport chain, the matrix with its own dedicated genome – all of this is ancient and broadly conserved. Inventories of the mitochondrial proteome bear this out: the great majority of the roughly 1,100–1,200 proteins that make up the mammalian organelle are shared across species and tissues, and the catalytic subunits of the respiratory complexes are among the most conserved proteins known [4]. The mitochondrial DNA itself, encoding a small but essential set of oxidative-phosphorylation subunits together with the ribosomal and transfer RNAs needed to translate them, follows the same compact organisation in fish, rodents and primates [5].

Conservation, however, is not the same as uniformity. The heart packs its mitochondria into two spatially and biochemically distinct populations – subsarcolemmal mitochondria beneath the cell membrane and interfibrillar mitochondria wedged between the myofibrils – and the proportions and properties of these subpopulations are not identical across species, or even across regions of the same heart [6]. Respiratory complexes, moreover, do not float about independently but assemble into higher-order supercomplexes, or respirasomes, whose abundance influences electron flux and the slip of electrons onto oxygen; the degree of supercomplex assembly differs between tissues and is remodelled in disease, and there is little reason to assume it is fixed across mammals [7]. Even cristae density, which sets the surface area available for oxidative phosphorylation, tends to track the metabolic intensity of the tissue, and is generally greater in the hearts of small, fast-hearted mammals than in large ones. The efficiency of the process – how much ATP is recovered per oxygen consumed – and the isoform complement of carriers such as the adenine nucleotide translocase also show tissue- and species-related variation, so that two hearts running what is nominally the same respiratory chain need not extract the same useful work from a given quantity of fuel. The conserved blueprint, in other words, is built out to different specifications depending on the animal (Figure 1) – a point easy to overlook when a result obtained in one species is generalised to another.

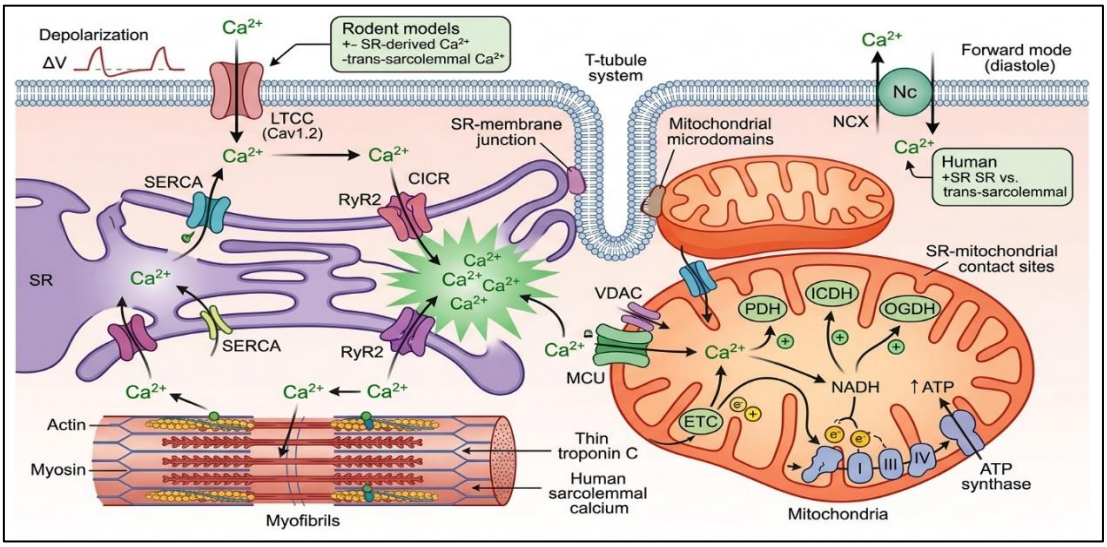

Fig 1 Conserved Architecture and Species-Divergent Features of the Cardiac Mitochondrion. Cardiac Mitochondria form Two Populations – Subsarcolemmal (SSM) Beneath the Sarcolemma and Interfibrillar (IFM) Between the Myofibrils. The Magnified Organelle shows the Double Membrane, the Cristae that House the Respiratory Chain (Cristae Density is Species-Divergent) and the Assembly of Complexes I, III and IV into a Supercomplex.

III. BODY MASS, METABOLIC RATE AND THE ALLOMETRIC SETTING OF CARDIAC BIOENERGETICS

One of the oldest quantitative laws in physiology is that whole-animal metabolic rate scales with body mass raised to a power of about three-quarters, while mass-specific metabolic rate falls as animals grow larger [8]. A shrew and an elephant are built from broadly similar cells, but the shrew's tissues respire far faster per gram. This allometry is mirrored almost perfectly in the cardiovascular system. Resting heart rate declines steeply with body size, from several hundred beats per minute in small rodents to around seventy in humans and fewer still in very large mammals, with the result that the number of heartbeats over a lifespan is, very roughly, conserved across mammals [9]. The heart of a small animal is thus a faster, more metabolically intense pump, and its mitochondria must sustain a correspondingly higher rate of ATP turnover (Table 1).

Table 1 Comparative Cardiovascular and Mitochondrial Parameters Across Species Commonly Used in Translational Research

Species	Body mass	Resting HR (bpm)	Dominant ventricular MHC	Mito. volume density (%)	Membrane polyunsaturation / peroxidation	Relative proton leak
Mouse	~25–30 g	~500–600	α-MHC	~32	High	High
Rat	~250–400 g	~300–450	α-MHC (predominant)	~28	High–intermediate	High–intermediate
Rabbit	~2–5 kg	~180–250	β-MHC	[≈25–30]*	Intermediate	Intermediate
Dog	~10–30 kg	~70–120	β-MHC	~22	Intermediate–low	Low
Pig	~50–150 kg	~70–120	β-MHC	[≈22–28]*	Low	Low
Human	~70 kg	~60–100	β-MHC (~90%)	~22–28	Low	Low

*Body mass, resting heart rate (HR) and dominant ventricular myosin heavy-chain (MHC) isoform compiled from comparative cardiac physiology [3,9,35]. Mitochondrial volume densities (% of cardiomyocyte volume) from cardiac ultrastructural morphometry (mouse ≈32%, rat ≈28%, dog ≈22%, human ≥20%; documented mammalian range ≈22–37%, with the smallest species showing the highest values, correlating with heart rate and basal O_2 consumption). *Bracketed values for rabbit and pig are interpolated from this range/trend and are not directly measured. Membrane polyunsaturation/peroxidation index and relative proton leak follow the membrane-pacemaker framework [10–15]; qualitative descriptors (high/intermediate/low) denote relative, not absolute, values.*

These differences are not cosmetic; they reach down to the organelle. To support rapid cycling, rodent cardiomyocytes carry a higher mitochondrial volume density, express contractile and ion-handling proteins tuned for speed, and rely on a different balance of repolarising currents than human myocytes do [3]. The dominant myosin heavy-chain isoform differs as well – the faster α-isoform predominates in the mouse ventricle, the slower β-isoform in the human – with direct consequences for the energetic cost of each contraction. Because so much of basal metabolic rate is spent maintaining transmembrane gradients across the mitochondrial inner membrane and the plasmalemma, the steep scaling of metabolic rate with size implies, almost of necessity, parallel scaling in the properties of those membranes [10]. This is the bridge between whole-animal allometry and the molecular composition of mitochondria, and it is the subject of the next section. For the translational researcher the immediate lesson is blunt: a mouse heart is not a slow human heart sped up; it is a different bioenergetic device.

IV. MEMBRANE LIPID COMPOSITION AND THE "MEMBRANE PACEMAKER"

If metabolic rate scales with size, what sets it at the molecular level? An influential answer is the membrane pacemaker theory, developed largely by Hulbert and Else, which holds that the fatty-acyl composition of cellular and mitochondrial membranes is a major determinant of an animal's metabolic rate [11]. The central observation is robust and slightly counter-intuitive: the membranes of small, metabolically fast mammals are richer in highly polyunsaturated fatty acids – docosahexaenoic acid (22:6n-3) in particular – than those of large, slow ones, and the same contrast separates warm-blooded mammals from cold-blooded reptiles of similar size [11,12]. Highly polyunsaturated acyl chains make bilayers more fluid and more permeable, and they appear to accelerate the membrane-bound proteins embedded in them, from ion pumps to the components of oxidative phosphorylation [13]. Membrane lipids, on this view, act as a kind of physical pacemaker, setting the tempo at which membrane-dependent processes run.

The heart fits the pattern. Cardiac phospholipids were among the first tissues in which docosahexaenoic acid was shown to decline with increasing body mass [12], the implication being that the inner mitochondrial membrane of a small mammal's heart is intrinsically "faster" and leakier than that of a large one. This matters beyond metabolic rate, because membrane polyunsaturation is a double-edged property: the very double bonds that confer

fluidity are chemically vulnerable to oxidation, linking membrane composition to susceptibility to lipid peroxidation and, ultimately, to ageing [11]. A direct functional read-out of this membrane divergence is mitochondrial proton leak – the dissipation of the proton-motive force across the inner membrane without ATP synthesis. Basal proton conductance correlates both with metabolic rate and with membrane fatty-acyl composition, and early comparative work found that liver mitochondria from a reptile leaked protons several-fold more slowly than those of a mammal of the same size, in step with their lower respiration [14,15]. Some of this leak is mild, inducible uncoupling through the adenine nucleotide translocase and the uncoupling proteins, and it is now thought to serve a protective role: a small, regulated leak lowers the membrane potential just enough to restrain the generation of reactive oxygen species in active tissues [15] (Figure 2). The heart, working continuously at high electron flux, has good reason to exploit this mechanism, and the extent to which it does so is unlikely to be identical across species with such different membranes. For comparative and translational purposes the framework supplies a unifying explanation for a whole suite of correlated differences – metabolic rate, proton permeability, peroxidation susceptibility – that would otherwise read as a disconnected list. It also issues a warning: a drug that acts on, or is influenced by, the lipid environment of mitochondrial membranes may behave quite differently in the polyunsaturated, leaky membranes of a mouse heart than in the comparatively saturated membranes of a human one.

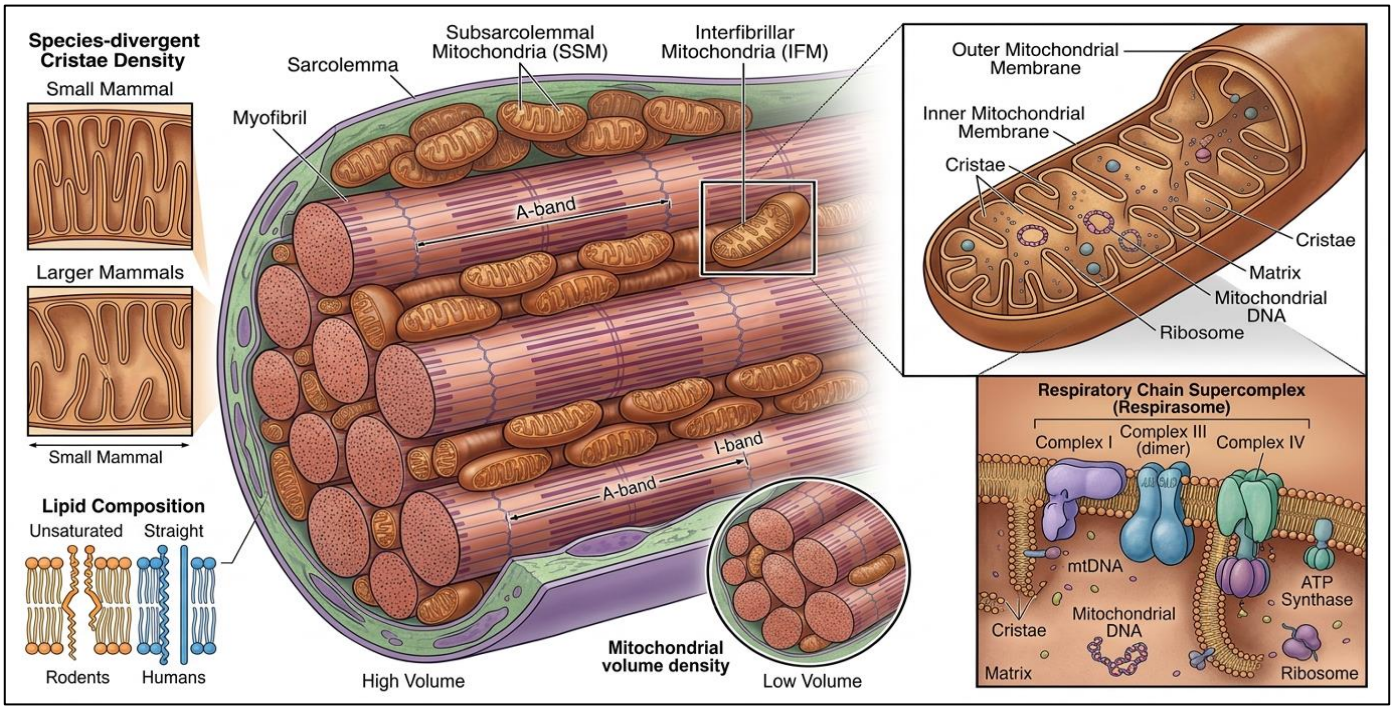

Fig 2 The Membrane Pacemaker of Metabolism. The Acyl-Chain Composition of Cellular and Mitochondrial Membranes Scales with Body Mass: the Membranes of Small, Metabolically Fast Mammals are Richer in Highly Polyunsaturated Fatty Acids (Notably Docosahexaenoic Acid, DHA), which Raises Proton Leak and Susceptibility to Lipid Peroxidation, whereas the Less-Unsaturated Membranes of Large Mammals are More Stable and Leak Less. Membrane Composition thus Tunes the Intrinsic Tempo of Membrane-Dependent Metabolism.

V. REACTIVE OXYGEN SPECIES, ANTIOXIDANT CAPACITY AND LESSONS FROM EXTREME MODELS

Mitochondria are the principal intracellular source of reactive oxygen species (ROS), which leak from the respiratory chain as a by-product of electron transport. At controlled levels these molecules act as signals; in excess they damage lipids, proteins and DNA, and they have been implicated in virtually every form of cardiac pathology, as well as in ageing itself [16]. The long-standing mitochondrial free-radical theory of ageing proposed that long-lived species should generate less ROS than short-lived ones, and a generation of comparative studies set out to test it [16]. The results have been famously equivocal. The clearest lesson to emerge is that what distinguishes species may be less the rate at which ROS are produced than the capacity to dispose of them, and the resistance of their tissues to oxidative damage.

The naked mole-rat has become the emblem of this revision. This mouse-sized rodent lives for more than three decades – an order of magnitude longer than a mouse – and remains strikingly free of the cardiovascular and neoplastic disease that afflicts old mice [17]. Yet its mitochondria do not obviously produce less hydrogen peroxide than those of a mouse, and some of its tissues sustain considerable oxidative damage from a young age; what appears to set it apart includes an enhanced capacity to consume ROS within the mitochondrion and an unusual resistance to the pro-apoptotic effects of oxidative stress [17]. The cardiovascular relevance is direct, and it cuts to the heart of model choice: two rodents of nearly identical

size can occupy completely different positions on the trade-off between ROS production and removal.

That the balance matters more than the source is reinforced by the cardiac ageing literature in mice. Targeting the antioxidant enzyme catalase to mitochondria, rather than to its usual cytosolic location, attenuates the age-related decline of the mouse heart and extends lifespan, demonstrating that it is mitochondrial ROS specifically – and the cell's ability to neutralise them – that drives the phenotype [18,19]. For translational work the implication is twofold. First, antioxidant strategies that succeed in one species may fail in another whose baseline redox handling is different. Second, the choice of an unusually short-lived or unusually long-lived model can bias conclusions about oxidative mechanisms in ways that are easy to miss (Table 2).

Table 2 Reactive Oxygen Species Production Versus Antioxidant and Scavenging Capacity in Selected Short- and Long-Lived Species.

Species	**Approx. max lifespan**	**Relative mito. ROS / H_2O_2 production**	**Antioxidant / scavenging capacity & damage resistance**	**Notes**
Mouse	~3–4 yr	High	Moderate	Standard short-lived model
Rat	~3–4 yr	High–moderate	Moderate	—
Human	~80–120 yr	Low–moderate	High	Long-lived large mammal
Naked mole-rat	> 30 yr	Not consistently lower than mouse	High ROS-consuming capacity; resistant to oxidative-stress–induced apoptosis	Exceptional longevity; paradoxical tolerance of oxidative damage
Pigeon (long-lived bird)	~30–35 yr	Low (low radical leak per O_2)	High	~Rat body size but ~10× lifespan
Little brown bat	> 30 yr	Low	High	Long-lived for body size

Lifespans are approximate maxima. Relative reactive-oxygen-species (ROS) production and antioxidant/scavenging capacity follow the comparative mitochondrial free-radical literature [16]; the naked mole-rat phenotype – exceptional longevity with paradoxical tolerance of oxidative damage – from [17]. Targeting catalase to mitochondria attenuates murine cardiac ageing and extends lifespan, underscoring the primacy of ROS disposal over production [18,19]. Descriptors are relative, not absolute.

VI. MITOCHONDRIAL CALCIUM HANDLING AND EXCITATION–CONTRACTION COUPLING

Calcium ties the mitochondrion to the beating of the heart. Each contraction is triggered by a cytosolic calcium transient, and a portion of that calcium is taken up by mitochondria through the mitochondrial calcium uniporter, where it stimulates the dehydrogenases of the Krebs cycle and so accelerates ATP production to match demand – the elegant arrangement known as mechano-energetic coupling [2]. The molecular identity of the uniporter was established only in 2011 [20,21], and work since then has shown that cardiac-specific manipulation of the channel tunes the heart's ability to ramp up output under acute stress [22]. Mitochondrial calcium uptake is thus not a passive overflow but an integral part of how the heart meets a changing workload.

The trouble for translation is that the cytosolic side of this system – excitation–contraction coupling itself – is among the most species-variable aspects of cardiac physiology [23]. The fraction of activator calcium drawn from the sarcoplasmic reticulum versus across the sarcolemma differs substantially between species: small rodents lean heavily on sarcoplasmic-reticulum stores, the sodium–calcium exchanger contributing comparatively little, whereas larger mammals, humans included, depend more on trans-sarcolemmal flux [23,3]. The architecture that delivers calcium also differs – the density and regularity of the transverse-tubular network, which couples surface depolarisation to release deep inside the cell, varies between species and is sparse or disordered in many small-animal and stem-cell-derived myocytes. Because mitochondrial calcium uptake competes with, and is shaped by, these cytosolic fluxes, the mitochondrial calcium signal that accompanies each beat cannot be assumed to be quantitatively equivalent across species. The kinetics differ too: a mouse mitochondrion must track a calcium transient repeating six hundred times a minute, a human one roughly a tenth as often.

These differences matter most acutely when calcium handling fails. Mitochondrial calcium overload is a central trigger of the cell death that follows ischaemia and reperfusion, and the threshold at which overload becomes lethal is governed by a structure whose species variability has had real consequences for drug development – the permeability transition pore, to which we now turn [2] (Figure 3).

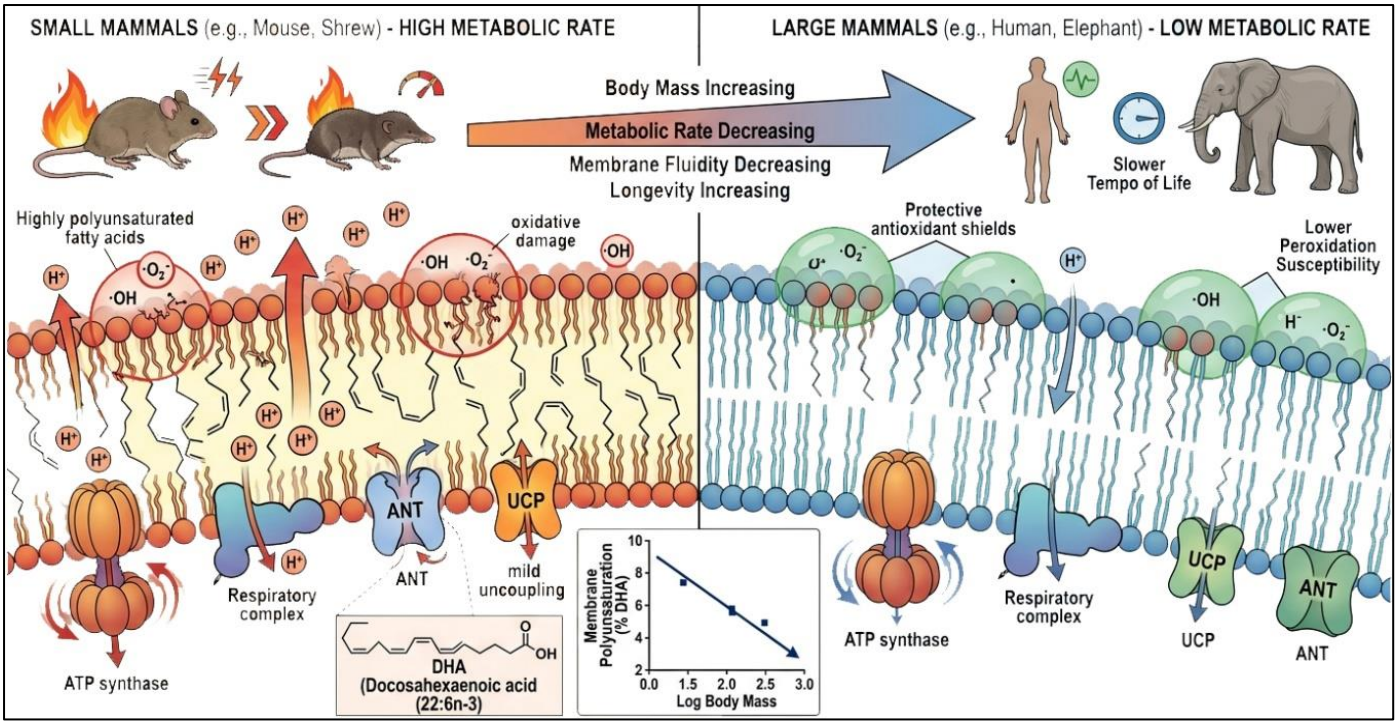

Fig 3 Mitochondrial Calcium Handling and Mechano-Energetic Coupling in the Cardiomyocyte. Depolarisation Admits Ca^{2+} Through L-Type Channels (LTCC), Triggering Ca^{2+}-Induced Ca^{2+} Release from the Sarcoplasmic Reticulum (SR) Via the Ryanodine Receptor (RyR); the Cytosolic Transient Drives Contraction and is Reset by SERCA and the Na^{+}/Ca^{2+} Exchanger (NCX). At SR–Mitochondrial Microdomains, Ca^{2+} Enters the Matrix Through VDAC and the Uniporter (MCU), Stimulating the Dehydrogenases (PDH, ICDH, OGDH) that Raise NADH Supply to the Respiratory Chain. The Balance of SR Versus Trans-Sarcolemmal Flux and T-Tubule Density are Species-Divergent.

VII. THE PERMEABILITY TRANSITION PORE AND THE CARDIOPROTECTION TRANSLATION GAP

When a cardiomyocyte is overloaded with calcium and assaulted by oxidative stress during reperfusion, a large, non-selective channel can open in the inner mitochondrial membrane – the mitochondrial permeability transition pore. Its opening collapses the proton-motive force, uncouples oxidative phosphorylation and swells the organelle, and it is widely regarded as a decisive event in the transition from reversible to lethal reperfusion injury [24]. The pore is regulated by cyclophilin D, which is inhibited by cyclosporine A, and herein lies one of the most instructive stories in translational cardiology [24,25].

In isolated mitochondria, in cardiomyocytes and in the hearts of small animals, inhibiting the permeability transition is robustly cardioprotective; cyclosporine A and related compounds reduce infarct size across numerous rodent studies, and an early proof-of-concept trial in patients even suggested benefit [25]. On that basis a large, well-designed clinical trial, CIRCUS, tested cyclosporine A in patients with acute myocardial infarction undergoing primary angioplasty. It was negative: the drug did not reduce death, heart failure or adverse remodelling [26]. The disappointment was sharp enough that some commentators asked whether it amounted to “a kiss of death” for the whole strategy [27].

The reasons are still debated, but the episode exposes how treacherous interspecies extrapolation can be. The preclinical evidence was itself less consistent than its reputation suggested: systematic review found that cyclosporine reduced infarct size variably and inconsistently across experimental models, and it failed outright in some pig and rat studies even before the clinical trials [28,29,30]. Pharmacokinetics, the presence of comorbidities and co-medications in real patients, and differences in the timing and depth of ischaemia all contribute. But beneath all of this lies the simple fact that the rodent heart in which the concept was forged differs from the human heart in mitochondrial calcium handling, redox balance and the very sensitivity of the pore – precisely the variables the drug was meant to act upon [31] (Figure 4). Cardioprotection has become, in effect, a case study in why mechanistic success in one species is no promissory note for another.

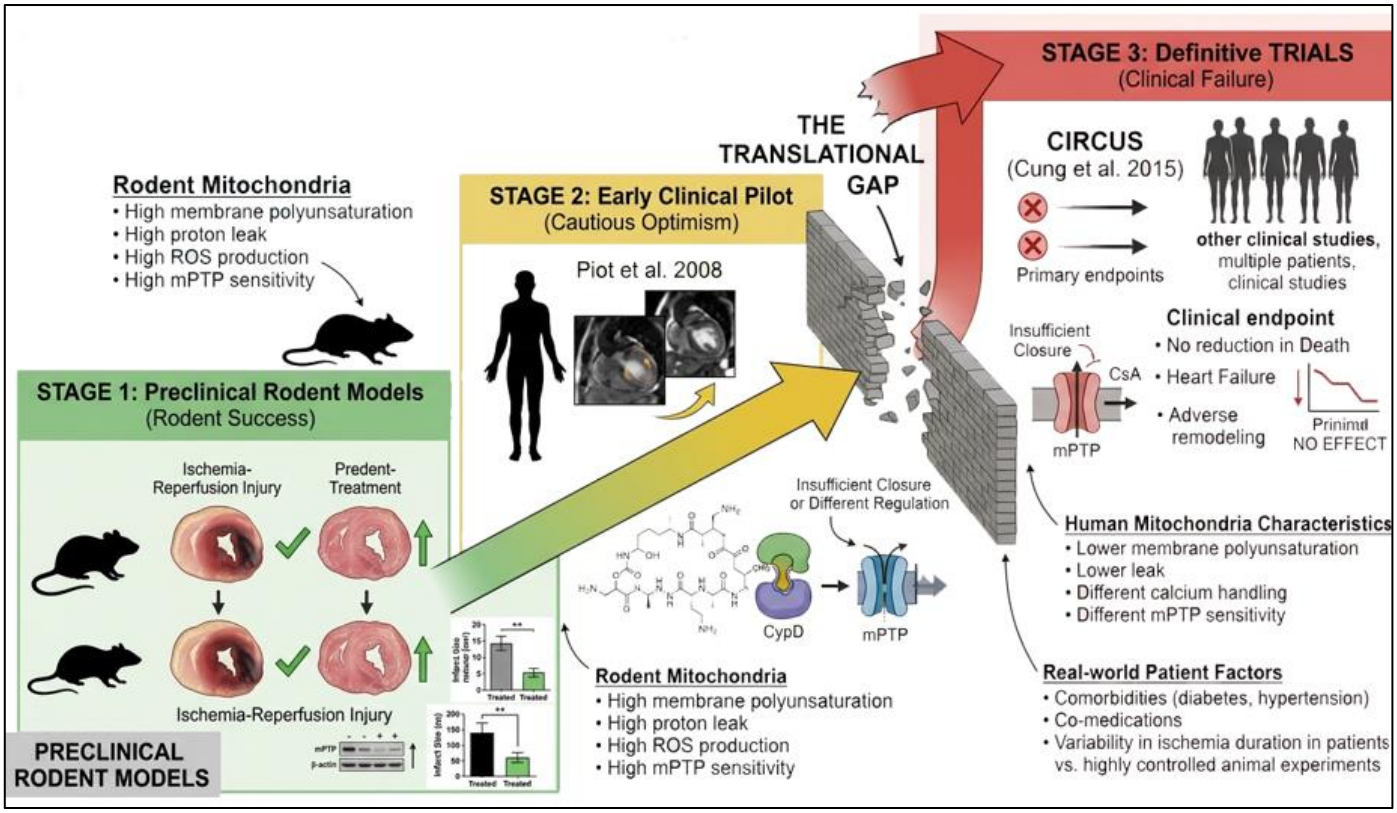

Fig 4 Mitochondria-Targeted Cardioprotection: from Rodent Concordance to Clinical Discordance. Inhibition of the Permeability Transition Pore (e.g. by Cyclosporine A) was Protective in Isolated Mitochondria and Rodent Ischaemia–Reperfusion Models, and an Early Clinical Pilot (Piot et al., 2008) Suggested Smaller Infarcts. The Definitive CIRCUS Trial (2015) and Other Clinical Studies were Neutral, Illustrating the Translational Gap Between Rodent Models and Patients.

VIII. SUBSTRATE METABOLISM, MITOCHONDRIAL DYNAMICS AND QUALITY CONTROL

The heart is metabolically omnivorous. In the adult, the bulk of acetyl-CoA feeding the Krebs cycle comes from the β-oxidation of fatty acids, with glucose, lactate and ketones making up the remainder, and the organ shifts nimbly between these fuels according to availability, workload and hormonal state [32]. This metabolic flexibility, however, is calibrated differently across species and life stages. The fetal and neonatal heart relies largely on glucose and switches towards fatty-acid oxidation after birth, and the set-point of that switch, together with the everyday balance of substrates, is influenced by diet and physiology in ways that diverge between small laboratory animals and humans [32]. Mice are typically maintained on high-carbohydrate chow and run a faster basal metabolism, so the substrate milieu of a mouse cardiomyocyte is simply not that of a human one – a discrepancy that becomes important whenever metabolism is itself the therapeutic target.

Mitochondrial mass and shape are not static either. The organelles are continually built, fused, divided and degraded, and this turnover is governed by a conserved machinery – the biogenesis programme orchestrated by the transcriptional coactivator PGC-1α, and the fission and fusion proteins that remodel the network [33]. In the heart PGC-1α coordinates mitochondrial proliferation with the demands of postnatal growth and exercise, while balanced fission and fusion maintain a healthy population [33,34]. Damaged units are culled by mitophagy, much of it routed through the PINK1–Parkin pathway, while the mitofusins and OPA1 on one side and Drp1 on the other keep the network in equilibrium [33]. Although the components of this system are shared across mammals, their expression levels, kinetics and stress responses are not guaranteed to be, and the dense, highly ordered packing of mitochondria between the myofibrils physically constrains how much remodelling a cardiomyocyte can actually undertake. Here too, then, a conserved toolkit is deployed to species-specific effect, and interventions aimed at mitochondrial biogenesis or dynamics should be expected to translate imperfectly.

IX. MODEL SELECTION FOR TRANSLATIONAL CARDIOVASCULAR RESEARCH

If no single species reproduces the human heart, the practical question becomes how to choose and combine models intelligently. Each has a characteristic profile. Mice and rats offer unrivalled genetic tractability and low cost, but their very high heart rates, distinctive repolarising currents and polyunsaturated, leak-prone mitochondria make them poor mimics of human electrophysiology and, arguably, of human mitochondrial bioenergetics [3,35]. Larger mammals – rabbit, dog, pig and sheep – sit much closer to humans in heart rate, calcium handling and the balance of ionic currents, and they are correspondingly more predictive for questions of arrhythmia, infarct size

and haemodynamics; their cost, husbandry and ethical burden, however, limit their use [3,31]. Non-mammalian models add breadth: the zebrafish, with its genetic accessibility and a heart that regenerates, is valuable for development and for screening, despite a two-chambered heart and obvious physiological distance from mammals [36], while the fruit fly, whose simple dorsal vessel nonetheless relies on a conserved cardiogenic programme, has proved useful for dissecting genes and pathways [37].

The most significant recent addition is the human induced pluripotent stem cell-derived cardiomyocyte, which at last allows human cardiac cells, and patient-specific genotypes, to be studied directly [38]. Its great limitation is maturity: these cells resemble fetal rather than adult myocytes, with immature calcium handling, sarcomeric organisation and – importantly for the present argument – a glycolytic rather than fully oxidative metabolism, so that their mitochondrial phenotype is itself a moving target [38,39]. Efforts to mature them, through metabolic conditioning, electrical pacing and three-dimensional engineered tissues, are narrowing the gap, though they have not closed it [39].

A further, often neglected, source of discordance is that laboratory models are typically young, male, healthy and genetically uniform, whereas the patients who actually suffer cardiovascular disease are older, of both sexes, and burdened with hypertension, diabetes and polypharmacy – all of which alter mitochondrial function and the response to cardioprotection [31]. Humanised and comorbid models, and the routine inclusion of both sexes, are increasingly recognised as necessary if preclinical findings are to survive the journey to the clinic. The constructive conclusion is that translation is best served not by a search for the perfect organism but by triangulation: rodents for mechanism and genetics, large animals for physiology and pharmacology, and human cell-based systems for genotype and final confirmation, with each result interpreted in light of the mitochondrial peculiarities of its source [40] (Table 3). Reporting those peculiarities, rather than quietly assuming equivalence, would itself improve the reproducibility and the clinical yield of cardiovascular research.

Table 3 Strengths and Limitations of Common Translational Cardiovascular Models, with Emphasis on Mitochondrial Fidelity to the Human Heart.

Model	Key strengths	Key limitations	Mito. fidelity to human heart
Mouse	Genetic tractability; low cost; rapid breeding	Very high heart rate (~500–600 bpm); α-MHC; distinct repolarising currents	Low – polyunsaturated, leak-prone mitochondria; high mito. density
Rat	Tractable; larger than mouse for surgery and sampling	High heart rate; α-MHC predominant	Low–moderate
Rabbit	Human-like Ca^{2+} handling and repolarisation; arrhythmia models	Higher cost; limited transgenic tools	Moderate–high
Dog	Human-like electrophysiology and haemodynamics	Cost; ethical burden; outbred	Moderate–high
Pig	Close to human heart size and coronary anatomy; infarct models	Cost; husbandry; few genetic tools	High
Sheep	Large-animal haemodynamics; valve and device models	Cost; ruminant metabolism	Moderate–high
Zebrafish	Genetic accessibility; cardiac regeneration; screening	Two-chambered heart; ectotherm; physiological distance	Low (developmental/genetic use)
Drosophila	Powerful genetics; conserved cardiogenic programme	Tubular dorsal vessel; no chambers	Low (gene/pathway dissection)
Human iPSC-CM	Human genotype; patient-specific; direct human cells	Immature, fetal-like; glycolytic; disorganised sarcomeres/T-tubules	Human genotype but immature mitochondrial phenotype

Compiled from comparative model assessments [3,31,35–40]. Mitochondrial fidelity is a qualitative summary judgement relative to the adult human heart; iPSC-CM denotes induced pluripotent stem cell-derived cardiomyocyte.

X. CONCLUSION

Mitochondria are at once the most conserved and the most quietly variable feature of the vertebrate heart. The organelle's blueprint – its membranes, its respiratory chain, its genome – has changed little over hundreds of millions of years, and this is precisely what makes animal models useful at all. Yet layered on top of that conserved core is a set of differences that scale with body size and life history: the lipid composition of the inner membrane, the magnitude of proton leak, the production and disposal of reactive oxygen species, the handling of calcium, and the sensitivity of the permeability transition pore. These are not peripheral details. They are, very often, exactly the properties that a cardiovascular therapy seeks to modify, which is why interventions forged in the fast, leaky,

polyunsaturated mitochondria of a mouse have so often stumbled in the slower human heart. The remedy is not to abandon animal models but to read them more carefully – to match the model to the question, to combine small animals, large animals and human cells, and to treat mitochondrial divergence as information rather than noise.

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