

Published in final edited form as:

J Mol Cell Cardiol. 2021 August 08; 161: 106–115. doi:10.1016/j.yjmcc.2021.08.003.

With a grain of salt: Sodium elevation and metabolic remodelling in heart failure

Dunja Aksentijevic^{a,*}, Michael J. Shattock^b

^aWilliam Harvey Research Institute, Centre for Biochemical Pharmacology, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, Charterhouse Square, London, UK

^bSchool of Cardiovascular Medicine and Sciences, British Heart Foundation Centre of Research Excellence, King's College London, The Rayne Institute, St Thomas' Hospital, London, UK

Abstract

Elevated intracellular Na (Na_i) and metabolic impairment are interrelated pathophysiological features of the failing heart (HF). There have been a number of studies showing that myocardial sodium elevation subtly affects mitochondrial function. During contraction, mitochondrial calcium (Ca_{mito}) stimulates a variety of TCA cycle enzymes, thereby providing reducing equivalents to maintain ATP supply. Na_i elevation has been shown to impact Ca_{mito}; however, whether metabolic remodelling in HF is caused by increased Na_i has only been recently demonstrated. This novel insight may help to elucidate the contribution of metabolic remodelling in the pathophysiology of HF, the lack of efficacy of current HF therapies and a rationale for the development of future metabolism-targeting treatments. Here we review the relationship between Na pump inhibition, elevated Na_i, and altered metabolic profile in the context of HF and their link to metabolic (in)flexibility and mitochondrial reprogramming.

Keywords

Sodium; Cardiac metabolism; Heart failure; Sodium pump; Energetics

1 Introduction

Heart failure (HF) imposes an enormous worldwide medical and economic burden. With few effective treatments available, heart failure (HF) affects over 64 million people worldwide carrying the annual death toll of 17.5 million lives [1]. Despite the advancements in diagnostic tools and therapies, with an ever-ageing population its prevalence is on

This work is licensed under a [CC BY 4.0](https://creativecommons.org/licenses/by/4.0/) International license.

*Corresponding author at: William Harvey Research Institute, Centre for Biochemical Pharmacology, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, Charterhouse Square, London EC1M 6BQ, UK. d.aksentijevic@qmul.ac.uk (D. Aksentijevic).

Author contribution

D.A. and M.J.S drafted the manuscript. D.A. prepared the figures.

Disclosures

None.

continuous rise. The current COVID-19 pandemic has added to this chronic disease burden as the patients with underlying cardiovascular disease face significantly poorer prognosis [2–6]. Thus, there is a clear and rapidly increasing requirement for improved understanding of fundamental cellular mechanisms in HF which can, in turn, help the development of improved treatments and innovative diagnostic techniques.

Numerous molecular mechanisms have been proposed that could contribute to the development of HF and these include an energy deficit following metabolic reprogramming. In a series of precisely regulated enzymatic reactions, heart muscle highly efficiently converts chemical into mechanical energy [7]. This fact is easily obscured by the complexities of myocardial anatomy, haemodynamics and coronary flow. Despite myocardial metabolism and function being inseparably linked, substrate metabolism as a paradigm for the development of novel HF therapies has been mostly overlooked [8]. In addition to the changes in cardiac metabolism, alterations in excitation–contraction (*E-C*) coupling including Na_i elevation are characteristic features of pathological cardiac remodelling and underpin contractile dysfunction in HF.

2 Intracellular Na regulation

In the healthy mammalian heart, cytoplasmic Na, Ca, and H concentrations are lower than their electrochemical equilibrium values. In the myocardium of most large animals the intracellular Na concentration is typically around 8–10 mM [9–11] (Table 1). In the murine heart (rats and mice) Na_i is reported to be significantly higher at 10–20 mM (reviewed in [9]) (Table 1). Due to differences in experimental methodologies, absolute values of measured Na_i may vary (Table 1). This elevated intracellular Na is associated with a range of other physiological adaptations including a higher heart rate, a shorter action potential and EC-coupling that is less dependent on transsarcolemmal Ca flux and more dependent on intracellular Ca cycling [17]. In murine species, these adaptations appear to contribute to a relatively flat force–frequency relationship and the phenomenon of post-rest potentiation [12].

The low intracellular Na is maintained by the activity of the Na/K ATPase or Na/K pump (NKA). The energy invested by the cell in driving the Na/K ATPase, and maintaining this high transmembrane electrochemical gradient, creates a ‘battery’ that the cell then exploits to drive ions, substrates and amino acids into (symports or co-transporters) or out of (antiports or exchangers) the cell (Fig. 1). Each of these transmembrane transporters will tend to dissipate the Na gradient. As the Na/K ATPase is the only quantitatively significant efflux pathway, its activity, and capacity, needs to exceed the sum total of all these influx pathways combined (Fig. 1) [13].

The principal regulator of the activity of Na/K ATPase (NKA) is phospholemman (PLM) and plays a crucial role in regulation of cardiomyocyte contractility. NKA is a transmembrane protein consisting of α and β subunits found in the peripheral sarcolemma as well as T-tubules. α subunit is a highly conserved 110 kDa non-glycosylated protein. On the inner membrane it contains the ATPase domain and on the outer membrane ion-binding and ouabain-binding domains. β subunit is a ~55 kDa glycoprotein [14]. The Michaelis-Menten

constant (K_m) for ATP for the catalytic site is disputed, but is estimated to be $\ll 1$ mM [14]. In addition to ATP catalytic site, NKA also possess a low-affinity allosteric ATP-binding site. Both subunits comprise a family of isoforms whose expression varies during different stages of development, between atria and ventricles as well as in pathophysiological states including HF [14,15].

NKA activity is strongly influenced by intracellular Na as the K_m for activation typically sits close to resting Na_i . The K_m for NKA (and hence the prevailing Na_i) is itself dynamically regulated by its accessory protein phospholemman (PLM) (FXD1) which exerts a tonic inhibition on NKA [16]. This inhibition is relieved, and the K_m for Na reduced, by the phosphorylation of the cytoplasmic tail of PLM - principally by Protein Kinases A and C (Fig. 1) [17]. PLM phosphorylation is necessary for the active control of Na_i during sudden changes in heart rate or during disease and plays a vital role in Na regulation during ‘flight or fight’ and adrenergic stimulation [18]. During the normal cardiac contractile cycle, NKA hydrolyses ATP to power the transport of Na and K across cell membranes. The stoichiometry of 3 Na extrusion in exchange for 2K import accompanied by the hydrolysis of 1 ATP, is strictly maintained. Furthermore, due to the sarcolemmal localization as well as high ATP demand required for its activity, NKA has been associated with preferential use of glycolytically-derived ATP [19–21]. Previous studies have suggested that cytosolic Na can also profoundly influence the feed-forward coupling between mechanical contraction and mitochondrial Ca-dependent ATP production (Fig. 2) [12,22]. Nonetheless, until recently the extent to which the mismatch in myocardial ATP supply–demand could arise as the consequence of elevated Na_i remained unclear.

3 Energy metabolism in the healthy heart

The heart has an enormous energy demand—it burns through 6 kg of ATP daily, consuming 2% of its total energy reserves per beat and turning over its total ATP pool in <1 min [23–26]. Despite this continuous dependence on ATP, its capacity to store ATP is miniscule: a 300 g human heart stores 30 mg ATP compared with the ATP utilization demand of 30 mg/s to sustain baseline cardiac function [27]. Therefore, it is predominantly reliant on aerobic metabolism for a continuous supply of ATP to fuel its mechanical work (Fig. 1). Mitochondria which occupy third of the cardiomyocyte volume account for more than 90% of generated ATP [28]. The large myocardial ATP demand is mainly related to EC coupling energy-dependant processes. Approximately 70–75% of total ATP turnover is used for force generation powering cardiac output, and the residual 25–30% used for the maintenance of basal metabolism [29,30]. In terms of force generation and ion regulation in the beating heart, it is estimated that the actomyosin ATPase accounts for 57%, SERCA (sarcoendoplasmic reticulum Ca ATPase) 11% and NKA for 7% of total ATP expenditure [29,31]. Glucose, lactate, free fatty acids (FFA), ketone bodies and, under rare circumstances, amino acids, compete as catabolic substrates in order to meet constantly varying myocardial ATP demand [32]. This renders the healthy adult heart a “metabolic omnivore” and enables high degree of fuel flexibility [32]. Metabolic substrate selection is dynamic and in addition to changes in myocardial workload it is driven by O_2 concentration and substrate availability [7,9].

The adult heart in vivo converts chemical energy predominantly stored in FFAs (60–90%) and pyruvate (10–40% derived from carbohydrates glucose and lactate) into contractile work [28]. However, the fate of metabolic substrates contributing to myocardial ATP provision is ultimately governed by multiple physiological factors: ATP demand, O₂ supply/availability, availability and the type of carbon substrate heart is exposed to, hormonal influences, transcriptional, translational, and posttranslational control of the various components of metabolic pathways [33]. Combination of these factors ensures careful matching of myocardial ATP supply and demand beat-to-beat.

In order to avoid ATP waste and overall energetic inefficiency, the balance between carbohydrate and FFA utilization is carefully regulated by the Randle cycle (glucose-fatty acid cycle) [34]. Despite the complexity of converging metabolic pathways, myocardial ATP generation can be broken down into four principal stages. Metabolic substrate delivery (Stage 1), substrate selection, uptake and oxidation (Stage 2) to generate acetyl-CoA for TCA cycle entry (Stage 3). The 4th stage is by far the most important mechanism for aerobic ATP biosynthesis. It consists of two coupled processes: electron transport and oxidative phosphorylation (OXPHOS). Under aerobic conditions, mitochondrial OXPHOS accounts for ~90% ATP synthesis whilst the O₂ supply-independent substrate level phosphorylation accounts for the residual ~10% [33]. In addition to O₂ availability, mitochondrial OXPHOS is contingent on the availability of reducing equivalents (H⁺ and electrons) which are transferred from various energy-providing substrates to the mitochondria by the reduced forms of nicotinamide adenine dinucleotide (NAD—H⁺) and flavin adenine dinucleotide (FADH—H⁺), generated by dehydrogenase reactions that occur in the stepwise degradation of energy-providing substrates. Kreb's cycle (TCA cycle) plays the role of the central metabolic hub and reducing equivalent provider by converging multiple metabolic pathways as the acetyl Co-A entering the Krebs cycle originates from the plethora of metabolic substrates (FFA, carbohydrates, amino acids and ketone bodies). Electron transport involves oxidation of NADH and FADH₂ accompanied by the transport of electrons through a chain of oxidation/reduction reaction involving cytochromes (ETC) until donated to O₂. The transport of electron drives H pumps in complexes I, III and IV. H are extruded from the mitochondrial matrix leading to matrix side of the membrane becoming negatively charged. This difference in electrochemical potential provides the energy for ATP synthesis when H return to the matrix through the F₀ proton channel, thereby driving F₁ ATP synthase (complex V) (Fig. 1). Upon synthesis, adenine nucleotide translocase (ANT) mediates ADP-ATP exchange across the inner mitochondrial membrane. This process initiates further cytosolic propagation of ATP/ADP disequilibria mediated by enzymes creatine kinase and adenylate kinase. Phosphotransfer therefore ensures ATP delivery from mitochondrial sites of synthesis to cytosolic ATP sinks. (Fig. 1) [35].

4 Na pump and regulation of Na_i in heart failure

Many studies of the failing myocardium report significantly elevated intracellular Na concentrations (reviewed in [9]) and, in part, this may be mediated by changes in NKA expression, activity and PLM phosphorylation [36]. The pathological consequences of elevation of intracellular Na include cellular Ca overload, a negative force–frequency relationship, impaired relaxation and arrhythmias [37]. While an increase in Na influx

may be an important component of elevated Na_i [11], each individual influx pathway (such as Na/H exchanger, or slowly inactivating Na channel current) is quantitatively small in comparison to the NKA capacity. Hence, the reduction of NKA pump function, and/or expression, and PLM dephosphorylation, may be quantitatively more significant [16,18,36–38].

Originally identified in the early 20th century, myocardial energy starvation hypothesis has been widely accepted paradigm in HF. It postulates that chronic metabolic perturbations precede, initiate and maintain contractile dysfunction in HF [26]. Advances in technologies including use of nuclear magnetic resonance spectroscopy (NMRs) for pioneering human cardiac studies have improved mechanistic insights into “engine out of fuel” hallmark of HF and helped to classify alterations causing energetic deficit into those related to key steps of cardiac metabolism: substrate utilization, intermediary metabolism and energy reserve [39]. Decades of cardiac metabolism research have further developed and refined our understanding of the detrimental metabolic remodelling that characterizes HF. This helped to formulate our contemporary definition of metabolic perturbation in HF as a sum of chronic metabolic inefficiencies and lack of metabolic flexibility including alterations of intermediate substrate metabolism and oxidative stress, rather than an ATP deficit per se (reviewed in [40]).

Upon acute stress, in order to meet its ATP demand, heart readily shifts its “glucose-fatty acid cycle” to dominant carbohydrate catabolism [41]. When the heart is subject to persistent stress, such as chronic haemodynamic overload, it also reactivates foetal gene expression programme [42]. Thus, the switch from adult to foetal metabolic phenotype leads to extensive metabolic remodelling [32,43,44]. Even if initially adaptive, this switch ultimately leads to a loss of insulin sensitivity and consequent loss of metabolic flexibility [45]. The onset of the substrate switch as well as the stage at which it could be therapeutically targeted is subject to debate. Some studies suggest that cardiac energetics is only impaired during advanced stages of HF (ATP <30–40%) with ATP levels maintained during the initial stages of metabolic remodelling [46–50]. Nevertheless, series of seminal in vivo ^{31}P NMRs studies [26,51–54] have helped to identify the reduction of the myocardial energy reserve defined as phosphocreatine-to-ATP ratio (PCr/ATP of <1.6) led to 44% increase in death from cardiovascular causes vs 5% of DCM patients with a PCr/ATP of >1.6. Collectively, these pioneering ^{31}P NMRS studies helped to establish cardiac energetics as a powerful and reliable prognostic indicator in HF [55]. Furthermore, there have also been many preclinical and clinical studies describing the role of mitochondrial dysfunction: mitochondrial misalignment, reduced density, aggregation, disorganized cristae and membrane disruption in hypertrophy and HF [56–58]. Dysfunctional mitochondrial ETC in relation to ATP synthesis was shown to intensify ROS damage of proteins, lipids and DNA leading to cardiomyocyte loss [57].

The relationship between NKA function and metabolism is bidirectional. That is, as a significant consumer of ATP and the main determinant of Na_i , NKA both responds to, and influences, metabolism. As an energy-dependent pump it is reasonable to hypothesise that declining ATP concentrations in the failing or ischaemic heart might compromise ion transport. In ischaemia, both PCr and ATP fall precipitously and, in the failing heart, the

substrate switch from fatty acids to glucose leads to a decline in cytosolic PCr/ATP reserve limiting the ATP supply. However, even during severe metabolic stress, Na_i rises at a time when the total ATP concentration greatly exceeds the K_m for the pump ($\sim 0.1\text{--}0.8$ mmol/l) and the G of ATP exceeds that required for NKA activity (~ 44 kJ/mol) [59]. In hypoxia and ischaemia, the accumulation of cytosolic inhibitors of NKA activity, and redox changes may inhibit ion transport long before ATP supply becomes limiting [59]. There is, therefore, little evidence to suggest that a failure of metabolism and energy supply limits NKA activity. However, there is accumulating evidence for the contrary; that is, NKA inhibition and the associated increase in Na_i can directly influence mitochondrial metabolism [43,60]. In addition, both NKA and SERCA have been shown to preferentially depend on glycolytic metabolism [61] and, in the failing heart, the switch to a glycolytic phenotype could reflect changes in their activity an attempt to maintain ion homeostasis [19,62,63].

5 Metabolic remodelling and myocardial Na_i elevation: a causality dilemma

Despite the extensive evidence for the concomitance of remodelled metabolism and elevated Na_i in HF, studies investigating their interaction are scarce and mostly limited to isolated organelles. Increase in cytosolic Na (12.5 mM to 25 mM) was shown to reduce state 3 respiration in isolated rat mitochondria potentially impacting ATP synthesis [64]. Other isolated mitochondria studies have shown that extra-mitochondrial Na addition ($1\text{--}10$ mM) led to a dose-dependent decrease in OXPHOS which was reversed by addition of Ca or by diltiazem inhibition of Na/Ca_{mito} (NCLX) exchanger [65].

^{31}P -NMR spectroscopy assessment of superfused mitochondria embedded in agarose beads showed that $3\text{--}30$ mM increase in Na significantly reduced ATP synthesis, particularly in type 2 diabetic mitochondria [66]. Collectively, these studies suggested that supra-physiological Na elevation leads to abnormalities in OXPHOS but neither elucidated the beat-to-beat kinetics of Ca_m transport nor explained its link to mitochondrial ATP synthesis.

Numerous studies examining the relationship between mitochondrial transport of Na and Ca and ATP synthesis demonstrated that increase in Ca_m stimulates ATP production [64,67–69]. The Ca_m uniporter (MCU) accounts for the majority of Ca_m uptake, while NCLX the principal mechanism for Ca_m extrusion (Fig. 2) [70]. In series of rabbit cardiac mitochondria experiments Cox and Matlib [67] have studied the impact of Na_i on Ca_m and measured Ca_m by using fura-2. Incubating mitochondria with increasing concentrations of NaCl showed that decrease in Ca_m reduces state 3 respiration and NADH production. Inhibition of NCLX with three inhibitors of different potency (lowest to highest): d-cis-diltiazem < clonazepam < CGP-37157 and the ruthenium red MCU inhibitor significantly increased Ca_m, state 3 respiration and NADH production in a dose-dependent manner. This outcome suggested that Na_i overload impairs the matching between ATP supply and demand. However, isolated mitochondria experiments should be interpreted with caution, as the measurements are made in the absence of the key ATP utilizers including myosin ATPase, NKA and SERCA, intracellular compartments and metabolic pathways including glycolysis.

In isolated guinea pig cardiomyocytes Maack et al. [60] have shown that Na_i elevation significantly reduces both diastolic and systolic Ca_m . Consistently, the percentage of NAD(H) in the control group was maintained at ~62%, but in the high Na_i group was significantly reduced. Despite changes in Ca_m and [NADH], Na_i elevation did not change the mitochondrial membrane potential (Ψ_m). Another premise of their work was that failure to maintain pyridine nucleotide reduction (NADPH) and in turn antioxidant flux due to high Na_i contributes to reactive oxygen species (ROS) overflow which is readily corrected by lowering Na_i in failing heart cells [71]. Collectively these results supported the argument that Na_i plays an important role in regulation of cardiac energy homeostasis. However, which metabolic pathways are most affected by Na_i overload and whether any of these changes were truly reflective of the regulatory mechanism in the beating heart remained to be shown.

A recent study by Hernansanz-Agustin et al. [72] has offered an additional mechanistic insight into the effect of Na on mitochondrial function and OXPHOS. Under the conditions of metabolic stress such as acute hypoxia which is typical of ischaemia/reperfusion injury, Na was shown to act as a second messenger. Their study identified that hypoxia-mediated activation of $\text{Na}/\text{Ca}_{\text{mito}}$ increases Na_{mito} and leads to release of Ca_m from calcium phosphate precipitates as well as Na-dependant reduction of the fluidity in the inner mitochondrial membrane. Consequently, alterations in the membrane fluidity caused a reduction in ubiquinone mobility between ETC II and III leads to ROS production. Inhibition of $\text{Na}/\text{Ca}_{\text{mito}}$ was shown to block all these events.

6 Chronic and acute Na_i overload reprogram cardiac metabolism

In order to ensure that ATP supply matches consumption during increased cardiac contractility, mitochondria readily respond to increases in the cytosolic Ca_m [73]. Resultant rise in mitochondrial matrix calcium (Ca_{mito}) activates key Ca-sensitive TCA cycle regulatory enzymes ($\text{CaDH}_{\text{mito}}$) including pyruvate dehydrogenase (PDH), α -ketoglutarate dehydrogenase and the NAD-linked isocitrate dehydrogenase (Fig. 2) as well as ETC complex V [74] [68].

$\text{CaDH}_{\text{mito}}$ activation leads to enhanced production of NADH, a critical intermediate for the electron transport chain (ETC) as well as redox signalling regulator (Fig. 2) [22].

This critical nexus of ATP supply-demand has been proposed to be affected by elevated Na_i , which activates NCLX, decreasing Ca_m and leading to compromised NADH/NAD redox cycling and energetic inefficiency [22,75,76] (Fig. 2).

In our recently published work [43], we examined whether elevated myocardial Na_i regardless of its duration (30 min, 5-week or 25-week Na_i overload) or aetiology (pharmacological, transgenic or hypertrophy-mediated NKA inhibition, Fig. 4) results in myocardial metabolic derangement. Our study combined mouse hearts perfusions under physiological conditions including temperature and metabolic substrate availability with non-invasive in situ NMR spectroscopy: multiple quantum filtered ^{23}Na to assess sodium, ^{31}P to assess cardiac energetics (Fig. 3), steady-state ^{13}C assessment of metabolic substrate flux

and utilization. We combined our perfusion NMR experiments with ex vivo high-resolution ^1H NMRs and LC GC MS/MS metabolomic profiling as well as with in silico modelling to examine the impact of elevated Na_i on mitochondrial ATP provision.

We observed that elevated Na causes common metabolic alterations that *precede* the onset of impaired energy reserve (PCr/ATP) and deterioration in function. This was observed in models of chronic and acute Na elevation: PLM^{3SA} mouse with NKA inhibition, left ventricular hypertrophy (TAC model) and pharmacological NKA inhibition with ouabain (Fig. 4). All these models shared a common ‘fingerprint’ of metabolic switch towards enhanced carbohydrate oxidation (Fig. 4). This switch appeared to be adaptive as high energy phosphate (PCr/ATP) and redox metabolite pools (NADH) were not compromised (Fig. 4).

However, given that we have observed depletion of the metabolic substrates including lactate as well as depletion of amino acids, it is possible that the metabolic adaptation observed in the long run may not be sufficient to maintain PCr/ATP ratio comparable to the healthy hearts. Mathematical simulations support this hypothesis based on flux analysis. However, additional tracer experiments are needed to assess flux distributions under these conditions. In silico work showed that the impact of elevated Na_i on mitochondrial ATP provision is mechanistically more complex than previous studies using isolated cells and organelles suggested. CardioNet mathematical modelling predicted maintenance of ATP supply by enhanced aerobic glucose metabolism and anaplerosis. The pivotal finding of our study was no evidence of impaired energetics - at least when Na overload is acute, or early in the hypertrophic process (Fig. 4). However, although PCr/ATP may not be impacted during early cardiac remodelling in our study, the kinetics of CK flux may still be disproportionately altered, as previously observed in HF patients [77]. Of note, acute CGP37157 inhibition (30 min) of NCLX attenuated all the changes in substrate utilization profile (palmitate vs glucose oxidation) as well as restored the levels of depleted metabolic intermediates (Fig. 4). Our results suggest that in a healthy heart this change in fuel supply can be switched on and off by simply changing cytosolic Na_i concentration. If this process is truly reversible, developing drugs that lower the elevated Na_i in HF will not only treat some of the EC coupling problems (i.e. diastolic dysfunction) but may also improve the ATP supply.

In our perfused murine preparations glucose was the preferred substrate under physiological conditions. Whilst surprising, it is consistently observed that murine hearts perfused in isolation have a baseline preference for carbohydrates, potentially due to differences in oxygen needed to fully oxidize carbohydrates vs fatty acids (e.g., palmitate) [78–82]. Furthermore, the first-rate limiting step of glycolysis, hexokinase, operates under maximum velocity even at very low glucose concentrations ($K_m = 0.02$ mM), which ensures that the heart never runs out of fuel. Thus, flux increases for glycolysis in perfused hearts are possible under physiological conditions. What is clear from our studies is that compared to the prevailing baseline in isolated rat and mouse hearts, sodium elevation enhances glucose use and reduces fatty acid contribution over and above baseline.

7 Therapeutic potential

Since William Withering's recognition of the efficacy of cardiac glycosides, Na_i has inadvertently been a HF therapeutic target for at least 200 years. Glycosides are not only present in plants such as the foxglove (*digitalis purpurea*), but are also endogenously found in animals under physiological conditions (e.g. ouabain, digoxin and bufalin) and are elevated in chronic kidney disease patients [83] and HF [84]. The positive inotropic effects of cardiac glycosides are well understood and involve NKA inhibition [85], the elevation of Na_i and the resetting of Na/Ca exchange leading to cellular Ca loading. While historically inotropic agents were considered to offer symptomatic relief to HF patients, it is relatively recently that this was recognised as 'flogging a dead horse' [86]. Recent clinical experience has shown that decreasing, rather than increasing, cardiac workload is a far more effective therapeutic strategy with pharmacological interventions such as beta blockers, diuretics (to reduce preload and afterload) and heart rate slowing agents (such as ivabradine) shown to have much improved outcomes [87]. Lowering intracellular sodium rather than elevating it, would therefore seem to be a desirable therapeutic aim in HF. As described, pathological Na elevation in the failing heart impairs metabolism, causes substrate switching, increases oxidative stress, increases cardiac work, impairs relaxation contributing to diastolic dysfunction, and is proarrhythmic - a plethora of reasons why it should be therapeutically targeted. Reducing Na influx with drugs like ranolazine or cariporide have been trialled with mixed results but since these target quantitatively small transmembrane Na fluxes (relative to the large efflux capacity of the NKA) targeting the NKA itself may be a more effective strategy [88,89]. The problem is that as yet we have no effective activators of the NKA.

While therapeutically reducing elevated Na may have multiple benefits, it may also be possible to target the consequences of Na elevation more directly and specifically to target deranged metabolism. In spite of the substantial data to support the pharmacological targeting of the substrate switch, successful translation to the clinic has been limited, with the exception, perhaps, of the cluster of drugs which target post-ischaemic myocardial energetic efficiency [90]. These include drugs targeting fatty acid oxidation inhibition via CPT1 such as etomoxir, ethyl-2-tetradecyl glycidate and oxfenicine [91,92]. However, etomoxir has the narrow therapeutic window, as in skeletal muscle CPT I inhibition leads to excess triglyceride accumulation and lipotoxicity [93]. Partial fatty-acid oxidation (PFox) inhibitors such as piperazine derivatives ranolazine and trimetazidine have been prescribed as anti-anginal drug in France [94]. They inhibit fatty-acid oxidation but also act indirectly by increasing the activity of pyruvate dehydrogenase to enhance glucose use. Trimetazidine is prescribed to DCM patients but only modestly improves cardiac function [95,96]. Pyruvate dehydrogenase kinase inhibition by sodium dichloroacetate improved contractile performance in HF patients, but a vehicle control group was omitted from the study design [97,98]. Therefore, given the currently limited success of targeting substrate metabolism, it is important to continue to investigate the therapeutic potential of alternative aspects of cardiac metabolism, including intermediary ATP supply pathways including intracellular NAD cycling [99,100]. Drugs targeting intermediary metabolism such as insulin-sensitizing (thiazolidinediones), lipid-lowering drugs (statins) and anaplerosis-

targeting agents (propionyl L-carnitine) could also further help to elucidate the role these metabolic alterations play in transition from compensated hypertrophy to HF [101].

Given that mitochondrial Na/Ca exchange inhibitor CGP37157 would treat a consequence of sodium elevation rather than a primary cause, our recently published work suggests that reducing Na_i overload by pharmacological activation of NKA may be a more superior therapeutic strategy for attenuation of metabolic remodelling in HF with potentially fewer off-target effects [17,102]. Our study outcome also further underlines the unsuitability of cardiac glycosides such as digoxin for HF treatment as the elevation of Na_i would be expected to exacerbate pre-existent metabolic remodelling. Inhibitors of renal sodium-glucose co-transporter (SGLT2i) have been used at the successful type 2 diabetes treatment with cardioprotective, glycaemic control-independent effects observed in HF patients. It was widely postulated that SGLT2i produce their positive effects in HF by reducing Na_i by direct inhibition of cardiac NHE1 activity [103,104]. In our recently published study, we have used an integrated experimental approach at the cell (isolated rat ventricular myocytes cSNARF1 fluorescence imaging) and perfused organ level (perfused ^{31}P and ^{23}Na NMRS of the mouse, rat and guinea pig hearts) to assess the impact of SGLT2i empagliflozin on intracellular Na and NHE1. Our study has shown that SGLT2 inhibitors have no effect on Na_i concentration nor NHE1. This has been confirmed for the whole range of therapeutic empagliflozin doses and other SGLT2i's. Therefore, any beneficial effects of SGLT2inhibitor family in failing hearts cannot be attributed to their action on myocardial NHE1 or intracellular Na [105].

Given the complexity of heart metabolism, it remains to be seen whether early prevention of myocardial Na_i elevation could either preclude the origin or change the course of metabolic remodelling in HF. This hypothesis warrants precision medicine approach and extensive further work including development of pharmacological agents that target these interconnected pathophysiological events.

Acknowledgments

Figures created with BioRender.com.

Funding

DA acknowledges British Heart Foundation Accelerator Award [AA/18/5/34222], Diabetes UK Grant (19/0005973), Barts Charity Grant (MRC 0215), Wellcome Trust [221604/Z/20/Z] for funding her work. MJS: British Heart Foundation Programme Grant [RG/12/4/29426].

Abbreviations

EC	excitation-contraction
Na_i	intracellular sodium
HF	heart failure
Ca	calcium (usually indicating Ca^{2+} ions in free solution)
Na	sodium (usually indicating Na^+ ions in free solution)

Ca_{mito}	intramitochondrial calcium
NMR	nuclear magnetic resonance
NKA	sodium/potassium ATPase
PLM	phospholemman
NCX	sarcolemmal sodium-calcium exchanger
NHE	sodium-proton exchanger
SERCA	sarcoendoplasmic reticulum Ca ATPase
FFA	free fatty acids
TCA	tricarboxylic acid cycle
ETC	electron transport chain
OXPHOS	oxidative phosphorylation
MCU	mitochondrial calcium uniporter
NCLX	mitochondrial sodium-calcium exchanger
Na_{ex}	extramitochondrial sodium
SBFI	sodium-binding benzofuran isophthalate
Tm(DOTP)	Thulium (III) 1,4,7,10-Tetraa-zacyclododecane-1,4,7,10-tetra(methylenephosphonate)
DCM	dilated cardiomyopathy.

References

- [1]. WHO. Cardiovascular Diseases. World Health Organization; 2017.
- [2]. Shi S, Qin M, Shen B, Cai Y, Liu T, Yang F, et al. Association of Cardiac Injury with Mortality in hospitalized patients with COVID-19 in Wuhan, China. *JAMA Cardiol.* 2020; 5 :802–810. [PubMed: 32211816]
- [3]. Guo T, Fan Y, Chen M, Wu X, Zhang L, He T, et al. Cardiovascular implications of fatal outcomes of patients with coronavirus disease 2019 (COVID-19). *JAMA Cardiol.* 2020; 5 :811–818. [PubMed: 32219356]
- [4]. Inciardi RM, Lupi L, Zacccone G, Italia L, Raffo M, Tomasoni D, et al. Cardiac involvement in a patient with coronavirus disease 2019 (COVID-19). *JAMA Cardiol.* 2020; 5 :819–824. [PubMed: 32219357]
- [5]. Li H, Liu L, Zhang D, Xu J, Dai H, Tang N, et al. SARS-CoV-2 and viral sepsis: observations and hypotheses. *Lancet.* 2020; 395 :1517–1520. [PubMed: 32311318]
- [6]. Ruan Q, Yang K, Wang W, Jiang L, Song J. Clinical predictors of mortality due to COVID-19 based on an analysis of data of 150 patients from Wuhan, China. *Intensive Care Med.* 2020; 46 :846–848. [PubMed: 32125452]
- [7]. Taegtmeyer H. Energy metabolism of the heart: from basic concepts to clinical applications. *Curr Probl Cardiol.* 1994; 19 :59–113. [PubMed: 8174388]

- [8]. Taegtmeyer H. Cardiac metabolism as a target for the treatment of heart failure. *Circulation*. 2004; 110 :894–896. [PubMed: 15326079]
- [9]. Aksentijevic D, O'Brien BA, Eykyn TR, Shattock MJ. Is there a causal link between intracellular Na elevation and metabolic remodelling in cardiac hypertrophy? *Biochem Soc Trans*. 2018; 46 :817–827. [PubMed: 29970448]
- [10]. Harrison SM, McCall E, Boyett MR. The relationship between contraction and intracellular sodium in rat and guinea-pig ventricular myocytes. *J Physiol*. 1992; 449 :517–550. [PubMed: 1522523]
- [11]. Despa S, Islam MA, Weber CR, Pogwizd SM, Bers DM. Intracellular Na(+) concentration is elevated in heart failure but Na/K pump function is unchanged. *Circulation*. 2002; 105 :2543–2548. [PubMed: 12034663]
- [12]. Shattock MJ, Bers DM. Rat vs. rabbit ventricle: Ca flux and intracellular Na assessed by ion-selective microelectrodes. *Am J Phys*. 1989; 256 :C813–C822.
- [13]. Fuller W, Tulloch LB, Shattock MJ, Calaghan SC, Howie J, Wypijewski KJ. Regulation of the cardiac sodium pump. *Cellular and molecular life sciences : CMLS*. 2013; 70 :1357–1380. [PubMed: 22955490]
- [14]. Ingwall JS, Balschi JA. Energetics of the Na(+) pump in the heart. *J Cardiovasc Electrophysiol*. 2006; 17 (Suppl 1) :S127–S33. [PubMed: 16686667]
- [15]. Muller-Ehmsen J, Wang J, Schwinger RH, McDonough AA. Region specific regulation of sodium pump isoform and Na,Ca-exchanger expression in the failing human heart-right atrium vs left ventricle. *Cell Mol Biol (Noisy-le-grand)*. 2001; 47 :373–381. [PubMed: 11357898]
- [16]. Boguslavskiy A, Tokar S, Prysyzhna O, Rudyk O, Sanchez-Tatay D, Lemmey HA, et al. Phospholemman phosphorylation regulates vascular tone, blood pressure and hypertension in mice and man. *Circulation*. 2021; 143 (11) :1123–1138. DOI: 10.1161/CIRCULATIONAHA.119.040557 [PubMed: 33334125]
- [17]. Pavlovic D, Fuller W, Shattock MJ. Novel regulation of cardiac Na pump via phospholemman. *J Mol Cell Cardiol*. 2013; 61 :83–93. [PubMed: 23672825]
- [18]. Verdonck F, Volders PG, Vos MA, Sipido KR. Intracellular Na⁺ and altered Na⁺ transport mechanisms in cardiac hypertrophy and failure. *J Mol Cell Cardiol*. 2003; 35 :5–25. [PubMed: 12623296]
- [19]. Cross HR, Radda GK, Clarke K. The role of Na⁺/K⁺ ATPase activity during low flow ischemia in preventing myocardial injury: a ³¹P, ²³Na and ⁸⁷Rb NMR spectroscopic study. *Magn Reson Med*. 1995; 34 :673–685. [PubMed: 8544687]
- [20]. Murphy E, Eisner DA. Regulation of intracellular and mitochondrial sodium in health and disease. *Circ Res*. 2009; 104 :292–303. [PubMed: 19213964]
- [21]. Okamoto K, Wang W, Rounds J, Chambers EA, Jacobs DO. ATP from glycolysis is required for normal sodium homeostasis in resting fast-twitch rodent skeletal muscle. *Am J Physiol Endocrinol Metab*. 2001; 281 :E479–E488. [PubMed: 11500303]
- [22]. Liu T, Brown DA, O'Rourke B. Role of mitochondrial dysfunction in cardiac glycoside toxicity. *J Mol Cell Cardiol*. 2010; 49 :728–736. [PubMed: 20620145]
- [23]. Balaban RS. Cardiac energy metabolism homeostasis: role of cytosolic calcium. *J Mol Cell Cardiol*. 2002; 34 :1259–1271. [PubMed: 12392982]
- [24]. Mootha VK, Arai AE, Balaban RS. Maximum oxidative phosphorylation capacity of the mammalian heart. *Am J Phys*. 1997; 272 :H769–H775.
- [25]. Marelli-Berg FM, Aksentijevic D. Immunometabolic cross-talk in the inflamed heart. *Cell Stress*. 2019; 3 :240–266. [PubMed: 31440740]
- [26]. Neubauer S. The failing heart-an engine out of fuel. *N Engl J Med*. 2007; 356 :1140–1151. [PubMed: 17360992]
- [27]. Neely JR, Morgan HE. Relationship between carbohydrate and lipid metabolism and the energy balance of heart muscle. *Annu Rev Physiol*. 1974; 36 :413–459. [PubMed: 19400669]
- [28]. Stanley WC, Recchia FA, Lopaschuk GD. Myocardial substrate metabolism in the normal and failing heart. *Physiol Rev*. 2005; 85 :1093–1129. [PubMed: 15987803]
- [29]. Gibbs CL. Cardiac energetics: sense and nonsense. *Clin Exp Pharmacol Physiol*. 2003; 30 :598–603. [PubMed: 12890186]

- [30]. Gibbs CL, Loiselle DS. Cardiac basal metabolism. *Jpn J Physiol.* 2001; 51 :399–426. [PubMed: 11564278]
- [31]. Schramm M, Klieber HG, Daut J. The energy expenditure of actomyosin-ATPase, Ca(2+)-ATPase and Na+,K(+)-ATPase in guinea-pig cardiac ventricular muscle. *J Physiol.* 1994; 481 (Pt 3) :647–662. [PubMed: 7707233]
- [32]. Murashige D, Jang C, Neinast M, Edwards JJ, Cowan A, Hyman MC, et al. Comprehensive quantification of fuel use by the failing and nonfailing human heart. *Science.* 2020; 370 :364–368. [PubMed: 33060364]
- [33]. Taegtmeier H, Young ME, Lopaschuk GD, Abel ED, Brunengraber H, Darley-Usmar V, et al. Assessing cardiac metabolism: a scientific statement from the American Heart Association. *Circ Res.* 2016; 118 :1659–1701. [PubMed: 27012580]
- [34]. Hue L, Taegtmeier H. The Randle cycle revisited: a new head for an old hat. *Am J Physiol Endocrinol Metab.* 2009; 297 :E578–E591. [PubMed: 19531645]
- [35]. Aksentijevic D, Zervou S, Eykyn TR, McAndrew DJ, Wallis J, Schneider JE, et al. Age-dependent decline in cardiac function in Guanidinoacetate-N-Methyltransferase knockout mice. *Front Physiol.* 2019; 10 1535 [PubMed: 32038270]
- [36]. Boguslavskiy A, Pavlovic D, Aughton K, Clark JE, Howie J, Fuller W, et al. Cardiac hypertrophy in mice expressing unphosphorylatable phospholemman. *Cardiovasc Res.* 2014; 104 :72–82. [PubMed: 25103111]
- [37]. Pieske B, Maier LS, Piacentino V 3rd, Weisser J, Hasenfuss G, Houser S. Rate dependence of [Na+]i and contractility in nonfailing and failing human myocardium. *Circulation.* 2002; 106 :447–453. [PubMed: 12135944]
- [38]. Pogwizd SM, Sipido KR, Verdonck F, Bers DM. Intracellular Na in animal models of hypertrophy and heart failure: contractile function and arrhythmogenesis. *Cardiovasc Res.* 2003; 57 :887–896. [PubMed: 12650867]
- [39]. Hudsmith LE, Neubauer S. Magnetic resonance spectroscopy in myocardial disease. *JACC Cardiovasc Imaging.* 2009; 2 :87–96. [PubMed: 19356540]
- [40]. Bertero E, Maack C. Metabolic remodelling in heart failure. *Nat Rev Cardiol.* 2018; 15 :457–470. [PubMed: 29915254]
- [41]. Goodwin GW, Taylor CS, Taegtmeier H. Regulation of energy metabolism of the heart during acute increase in heart work. *J Biol Chem.* 1998; 273 :29530–29539. [PubMed: 9792661]
- [42]. Depre C, Shipley GL, Chen W, Han Q, Doenst T, Moore ML, et al. Unloaded heart in vivo replicates fetal gene expression of cardiac hypertrophy. *Nat Med.* 1998; 4 :1269–1275. [PubMed: 9809550]
- [43]. Aksentijevic D, Karlstaedt A, Basalay MV, O'Brien BA, Sanchez-Tatay D, Eminaga S, et al. Intracellular sodium elevation reprograms cardiac metabolism. *Nat Commun.* 2020; 11 4337 [PubMed: 32859897]
- [44]. Aksentijevic D, McAndrew DJ, Karlstadt A, Zervou S, Sebag-Montefiore L, Cross R, et al. Cardiac dysfunction and peri-weaning mortality in malonylcoenzyme a decarboxylase (MCD) knockout mice as a consequence of restricting substrate plasticity. *J Mol Cell Cardiol.* 2014; 75 :76–87. [PubMed: 25066696]
- [45]. Taegtmeier H, Golfman L, Sharma S, Razeghi P, van Arsdall M. Linking gene expression to function: metabolic flexibility in the normal and diseased heart. *Ann N Y Acad Sci.* 2004; 1015 :202–213. [PubMed: 15201161]
- [46]. Akki A, Seymour AM. Western diet impairs metabolic remodelling and contractile efficiency in cardiac hypertrophy. *Cardiovasc Res.* 2009; 81 :610–617. [PubMed: 19028723]
- [47]. Chandler MP, Kerner J, Huang H, Vazquez E, Reszko A, Martini WZ, et al. Moderate severity heart failure does not involve a downregulation of myocardial fatty acid oxidation. *Am J Physiol Heart Circ Physiol.* 2004; 287 :H1538–H1543. [PubMed: 15191896]
- [48]. Luptak I, Sverdlov AL, Panagia M, Qin F, Pimentel DR, Croteau D, et al. Decreased ATP production and myocardial contractile reserve in metabolic heart disease. *J Mol Cell Cardiol.* 2018; 116 :106–114. [PubMed: 29409987]
- [49]. Starling RC, Hammer DF, Altschuld RA. Human myocardial ATP content and in vivo contractile function. *Mol Cell Biochem.* 1998; 180 :171–177. [PubMed: 9546644]

- [50]. Cluntun AA, Badolia R, Lettlova S, Parnell KM, Shankar TS, Diakos NA, et al. The pyruvate-lactate axis modulates cardiac hypertrophy and heart failure. *Cell Metab.* 2021; 33 (3) :629–648. DOI: 10.1016/j.cmet.2020.12.003 [PubMed: 33333007]
- [51]. Bottomley PA. Noninvasive study of high-energy phosphate metabolism in human heart by depth-resolved ³¹P NMR spectroscopy. *Science.* 1985; 229 :769–772. [PubMed: 4023711]
- [52]. Weiss RG, Bottomley PA, Hardy CJ, Gerstenblith G. Regional myocardial metabolism of high-energy phosphates during isometric exercise in patients with coronary artery disease. *N Engl J Med.* 1990; 323 :1593–1600. [PubMed: 2233948]
- [53]. Bottomley PA, Weiss RG. Non-invasive magnetic-resonance detection of creatine depletion in non-viable infarcted myocardium. *Lancet.* 1998; 351 :714–718. [PubMed: 9504516]
- [54]. Bottomley PA, Hart HR, Edelstein WA, Schenck JF, Smith LS, Leue WM, et al. NMR imaging/spectroscopy system to study both anatomy and metabolism. *Lancet.* 1983; 2 :273–274.
- [55]. Neubauer S, Horn M, Cramer M, Harre K, Newell JB, Peters W, et al. Myocardial phosphocreatine-to-ATP ratio is a predictor of mortality in patients with dilated cardiomyopathy. *Circulation.* 1997; 96 :2190–2196. [PubMed: 9337189]
- [56]. Garnier A, Fortin D, Delomenie C, Momken I, Veksler V, Ventura-Clapier R. Depressed mitochondrial transcription factors and oxidative capacity in rat failing cardiac and skeletal muscles. *J Physiol.* 2003; 551 :491–501. [PubMed: 12824444]
- [57]. Sharov VG, Todor AV, Silverman N, Goldstein S, Sabbah HN. Abnormal mitochondrial respiration in failed human myocardium. *J Mol Cell Cardiol.* 2000; 32 :2361–2367. [PubMed: 11113011]
- [58]. Lemieux H, Semsroth S, Antretter H, Hofer D, Gnaiger E. Mitochondrial respiratory control and early defects of oxidative phosphorylation in the failing human heart. *Int J Biochem Cell Biol.* 2011; 43 :1729–1738. [PubMed: 21871578]
- [59]. Fuller W, Parmar V, Eaton P, Bell JR, Shattock MJ. Cardiac ischemia causes inhibition of the Na/K ATPase by a labile cytosolic compound whose production is linked to oxidant stress. *Cardiovasc Res.* 2003; 57 :1044–1051. [PubMed: 12650882]
- [60]. Maack C, Cortassa S, Aon MA, Ganesan AN, Liu T, O'Rourke B. Elevated cytosolic Na⁺ decreases mitochondrial Ca²⁺ uptake during excitation-contraction coupling and impairs energetic adaptation in cardiac myocytes. *Circ Res.* 2006; 99 :172–182. [PubMed: 16778127]
- [61]. Xu KY, Zweier JL, Becker LC. Functional coupling between glycolysis and sarcoplasmic reticulum Ca²⁺ transport. *Circ Res.* 1995; 77 :88–97. [PubMed: 7788886]
- [62]. Dizon J, Burkhoff D, Tauskela J, Whang J, Cannon P, Katz J. Metabolic inhibition in the perfused rat heart: evidence for glycolytic requirement for normal sodium homeostasis. *Am J Phys.* 1998; 274 :H1082–H1089.
- [63]. Weiss J, Hiltbrand B. Functional compartmentation of glycolytic versus oxidative metabolism in isolated rabbit heart. *J Clin Invest.* 1985; 75 :436–447. [PubMed: 3973013]
- [64]. Iwai T, Tanonaka K, Inoue R, Kasahara S, Motegi K, Nagaya S, et al. Sodium accumulation during ischemia induces mitochondrial damage in perfused rat hearts. *Cardiovasc Res.* 2002; 55 :141–149. [PubMed: 12062717]
- [65]. Babsky A, Doliba N, Doliba N, Savchenko A, Wehrli S, Osbakken M. Na⁺ effects on mitochondrial respiration and oxidative phosphorylation in diabetic hearts. *Exp Biol Med (Maywood).* 2001; 226 :543–551. [PubMed: 11395924]
- [66]. Doliba NM, Wehrli SL, Babsky AM, Doliba NM, Osbakken MD. Encapsulation and perfusion of mitochondria in agarose beads for functional studies with ³¹P-NMR spectroscopy. *Magn Reson Med.* 1998; 39 :679–684. [PubMed: 9581596]
- [67]. Cox DA, Matlib MA. Modulation of intramitochondrial free Ca²⁺ concentration by antagonists of Na⁽⁺⁾-Ca²⁺ exchange. *Trends Pharmacol Sci.* 1993; 14 :408–413. [PubMed: 8296399]
- [68]. Denton RM, McCormack JG. Ca²⁺ as a second messenger within mitochondria of the heart and other tissues. *Annu Rev Physiol.* 1990; 52 :451–466. [PubMed: 2184763]
- [69]. Eisner DA, Caldwell JL, Kistamas K, Trafford AW. Calcium and excitation-contraction coupling in the heart. *Circ Res.* 2017; 121 :181–195. [PubMed: 28684623]

- [70]. Huser J, Blatter LA, Sheu SS. Mitochondrial calcium in heart cells: beat-to-beat oscillations or slow integration of cytosolic transients? *J Bioenerg Biomembr.* 2000; 32 :27–33. [PubMed: 11768759]
- [71]. Kohlhaas M, Maack C. Adverse bioenergetic consequences of Na⁺-Ca²⁺ exchanger-mediated Ca²⁺ influx in cardiac myocytes. *Circulation.* 2010; 122 :2273–2280. [PubMed: 21098439]
- [72]. Hernansanz-Agustin P, Choya-Foces C, Carregal-Romero S, Ramos E, Oliva T, Villa-Pina T, et al. Na⁺ controls hypoxic signalling by the mitochondrial respiratory chain. *Nature.* 2020; 586 :287–291. [PubMed: 32728214]
- [73]. Bers DM. Digitalis and Na/Ca exchange: old dog learns new mitochondrial tricks. *J Mol Cell Cardiol.* 2010; 49 :713–714. [PubMed: 20708015]
- [74]. Territo PR, Mootha VK, French SA, Balaban RS. Ca²⁺ activation of heart mitochondrial oxidative phosphorylation: role of the F₀/F₁-ATPase. *Am J Physiol Cell Physiol.* 2000; 278 :C423–C435. [PubMed: 10666039]
- [75]. Kohlhaas M, Liu T, Knopp A, Zeller T, Ong MF, Bohm M, et al. Elevated cytosolic Na⁺ increases mitochondrial formation of reactive oxygen species in failing cardiac myocytes. *Circulation.* 2010; 121 :1606–1613. [PubMed: 20351235]
- [76]. Liu T, O'Rourke B. Enhancing mitochondrial Ca²⁺ uptake in myocytes from failing hearts restores energy supply and demand matching. *Circ Res.* 2008; 103 :279–288. [PubMed: 18599868]
- [77]. Bottomley PA, Panjraht GS, Lai S, Hirsch GA, Wu K, Najjar SS, et al. Metabolic rates of ATP transfer through creatine kinase (CK Flux) predict clinical heart failure events and death. *Sci Transl Med.* 2013; 5 215re3
- [78]. Hafstad A, Solevag GH, Severson D, Larsen TS, Aasum E. Perfused hearts from type 2 diabetic (db/db) mice show metabolic responsiveness to insulin. *Am J Physiol Heart Circ Physiol.* 2006; 290 :H1763–H1769. [PubMed: 16327015]
- [79]. Zhang Y, Taufalele PV, Cochran JD, Robillard-Frayne I, Marx JM, Soto J, et al. Mitochondrial pyruvate carriers are required for myocardial stress adaptation. *Nat Metab.* 2020; 2 :1248–1264. [PubMed: 33106689]
- [80]. Chatham JC. Lactate - the forgotten fuel! *J Physiol.* 2002; 542 :333. [PubMed: 12122132]
- [81]. Karlstadt A, Zhang X, Vitrac H, Harmancey R, Vasquez H, Wang JH, et al. Oncometabolite d-2-hydroxyglutarate impairs α-ketoglutarate dehydrogenase and contractile function in rodent heart. *PNAS.* 2016; 113 :10436–10441. [PubMed: 27582470]
- [82]. Chatham JC, Gao ZP, Forder JR. Impact of 1 wk of diabetes on the regulation of myocardial carbohydrate and fatty acid oxidation. *Am J Phys.* 1999; 277 :E342–E351.
- [83]. Kolmakova EV, Haller ST, Kennedy DJ, Isachkina AN, Budny GV, Frolova EV, et al. Endogenous cardiostericoids in chronic renal failure. *Nephrol Dial Transplant.* 2011; 26 :2912–2919. [PubMed: 21292813]
- [84]. Kennedy DJ, Shrestha K, Sheehy B, Li XS, Guggilam A, Wu Y, et al. Elevated plasma Marinobufagenin, an endogenous Cardiostericoid, is associated with right ventricular dysfunction and Nitrate stress in heart failure. *Circ Heart Fail.* 2015; 8 :1068–1076. [PubMed: 26276886]
- [85]. Schatzmann HJ. Cardiac glycosides as inhibitors of active potassium and sodium transport by erythrocyte membrane. *Helv Physiol Pharmacol Acta.* 1953; 11 :346–354. [PubMed: 13142506]
- [86]. Adams KF Jr, Ghali JK, Herbert Patterson J, Stough WG, Butler J, Bauman JL, et al. A perspective on re-evaluating digoxin's role in the current management of patients with chronic systolic heart failure: targeting serum concentration to reduce hospitalization and improve safety profile. *Eur J Heart Fail.* 2014; 16 :483–493. [PubMed: 24574198]
- [87]. Wintrich J, Kindermann I, Ukena C, Selejan S, Werner C, Maack C, et al. Therapeutic approaches in heart failure with preserved ejection fraction: past, present, and future. *Clin Res Cardiol.* 2020; 109 :1079–1098. [PubMed: 32236720]
- [88]. Belardinelli L, Shryock JC, Fraser H. Inhibition of the late sodium current as a potential cardioprotective principle: effects of the late sodium current inhibitor ranolazine. *Heart.* 2006; 92 (Suppl 4) :iv6–iv14. [PubMed: 16775092]

- [89]. Theroux P, Chaitman BR, Danchin N, Erhardt L, Meinertz T, Schroeder JS, et al. Inhibition of the sodium-hydrogen exchanger with cariporide to prevent myocardial infarction in high-risk ischemic situations. Main results of the GUARDIAN trial. Guard during ischemia against necrosis (GUARDIAN) investigators. *Circulation*. 2000; 102 :3032–3038. [PubMed: 11120691]
- [90]. Taegtmeier H, Salazar R. Myocardial metabolism: a new target for the treatment of heart disease? *Curr Hypertens Rep*. 2004; 6 :414–415. [PubMed: 15527683]
- [91]. Rupp H, Jacob R. Metabolically-modulated growth and phenotype of the rat heart. *Eur Heart J*. 1992; 13 (Suppl D) :56–61. [PubMed: 1396861]
- [92]. Tutwiler GF, Brentzel HJ, Kiorpes TC. Inhibition of mitochondrial carnitine palmitoyl transferase a in vivo with methyl 2-tetradecylglycidate (methyl palmoxirate) and its relationship to ketonemia and glycemia. *Proc Soc Exp Biol Med*. 1985; 178 :288–296. [PubMed: 3969383]
- [93]. Dobbins RL, Szczepaniak LS, Bentley B, Esser V, Myhill J, McGarry JD. Prolonged inhibition of muscle carnitine palmitoyltransferase-1 promotes intramyocellular lipid accumulation and insulin resistance in rats. *Diabetes*. 2001; 50 :123–130. [PubMed: 11147777]
- [94]. Detry JM, Sellier P, Pennaforte S, Cokkinos D, Dargie H, Mathes P. Trimetazidine: a new concept in the treatment of angina. Comparison with propranolol in patients with stable angina. Trimetazidine European multicenter study group. *Br J Clin Pharmacol*. 1994; 37 :279–288. [PubMed: 8198938]
- [95]. Fragasso G, Palloshi A, Puccetti P, Silipigni C, Rossodivita A, Pala M, et al. A randomized clinical trial of trimetazidine, a partial free fatty acid oxidation inhibitor, in patients with heart failure. *J Am Coll Cardiol*. 2006; 48 :992–998. [PubMed: 16949492]
- [96]. Tuunanen H, Engblom E, Naum A, Nagren K, Scheinin M, Hesse B, et al. Trimetazidine, a metabolic modulator, has cardiac and extracardiac benefits in idiopathic dilated cardiomyopathy. *Circulation*. 2008; 118 :1250–1258. [PubMed: 18765391]
- [97]. Bersin RM, Stacpoole PW. Dichloroacetate as metabolic therapy for myocardial ischemia and failure. *Am Heart J*. 1997; 134 :841–855. [PubMed: 9398096]
- [98]. Bersin RM, Wolfe C, Kwasman M, Lau D, Klinski C, Tanaka K, et al. Improved hemodynamic function and mechanical efficiency in congestive heart failure with sodium dichloroacetate. *J Am Coll Cardiol*. 1994; 23 :1617–1624. [PubMed: 8195522]
- [99]. Walker MA, Tian R. NAD(H) in mitochondrial energy transduction: implications for health and disease. *Curr Opin Physiol*. 2018; 3 :101–109. [PubMed: 32258851]
- [100]. Lee CF, Chavez JD, Garcia-Menendez L, Choi Y, Roe ND, Chiao YA, et al. Normalization of NAD⁺ redox balance as a therapy for heart failure. *Circulation*. 2016; 134 :883–894. [PubMed: 27489254]
- [101]. Ardehali H, Sabbah HN, Burke MA, Sarma S, Liu PP, Cleland JG, et al. Targeting myocardial substrate metabolism in heart failure: potential for new therapies. *Eur J Heart Fail*. 2012; 14 (2) :120–129. DOI: 10.1093/eurjhf/hfr173 [PubMed: 22253453]
- [102]. Shattock MJ. Phospholemman: its role in normal cardiac physiology and potential as a druggable target in disease. *Curr Opin Pharmacol*. 2009; 9 :160–166. [PubMed: 19195931]
- [103]. Uthman L, Baartscheer A, Schumacher CA, Fiolet JWT, Kuschma MC, Hollmann MW, et al. Direct cardiac actions of sodium glucose Cotransporter 2 inhibitors target pathogenic mechanisms underlying heart failure in diabetic patients. *Front Physiol*. 2018; 9 1575 [PubMed: 30519189]
- [104]. Uthman L, Baartscheer A, Bleijlevens B, Schumacher CA, Fiolet JWT, Koeman A, et al. Class effects of SGLT2 inhibitors in mouse cardiomyocytes and hearts: inhibition of Na⁽⁺⁾/H⁽⁺⁾ exchanger, lowering of cytosolic Na⁽⁺⁾ and vasodilation. *Diabetologia*. 2018; 61 :722–726. [PubMed: 29197997]
- [105]. Chung YJ, Park KC, Tokar S, Eykyn TR, Fuller W, Pavlovic D, et al. Off-target effects of SGLT2 blockers: empagliflozin does not inhibit Na⁺/H⁺ exchanger-1 or lower [Na⁺]_i in the heart. *Cardiovasc Res*. 2020; cvaa323 doi: 10.1093/cvr/cvaa323 [PubMed: 33135077]
- [106]. Eykyn TR, Aksejtjevic D, Aughton KL, Southworth R, Fuller W, Shattock MJ. Multiple quantum filtered (23)Na NMR in the Langendorff perfused mouse heart: ratio of triple/double quantum filtered signals correlates with [Na]_i. *J Mol Cell Cardiol*. 2015; 86 :95–101. [PubMed: 26196304]

- [107]. Gray RP, McIntyre H, Sheridan DS, Fry CH. Intracellular sodium and contractile function in hypertrophied human and guinea-pig myocardium. *Pflugers Arch.* 2001; 442 :117–123. [PubMed: 11374058]
- [108]. Grupp I, Im WB, Lee CO, Lee SW, Pecker MS, Schwartz A. Relation of sodium pump inhibition to positive inotropy at low concentrations of ouabain in rat heart muscle. *J Physiol.* 1985; 360 :149–160. [PubMed: 2580969]
- [109]. Wasserstorm JA. Intracellular sodium-ion activity in rat ventricular myocardium. *Federation Proceedings.* 1983; 42 1113
- [110]. Jelicks LA, Siri FM. Effects of hypertrophy and heart failure on $[Na^+]_i$ in pressure-overloaded guinea pig heart. *Am J Hypertens.* 1995; 8 :934–943. [PubMed: 8541010]
- [111]. Schmied R, Wang GX, Korth M. Intracellular Na^+ activity and positive inotropic effect of sulmazole in guinea pig ventricular myocardium. Comparison with a cardioactive steroid. *Circ Res.* 1991; 68 :597–604. [PubMed: 1991358]
- [112]. Wilde AA, Kleber AG. The combined effects of hypoxia, high K^+ , and acidosis on the intracellular sodium activity and resting potential in guinea pig papillary muscle. *Circ Res.* 1986; 58 :249–256. [PubMed: 3948342]
- [113]. Wang GX, Schmied R, Ebner F, Korth M. Intracellular sodium activity and its regulation in guinea-pig atrial myocardium. *J Physiol.* 1993; 465 :73–84. [PubMed: 8229860]
- [114]. Cohen CJ, Fozzard HA, Sheu SS. Increase in intracellular sodium ion activity during stimulation in mammalian cardiac muscle. *Circ Res.* 1982; 50 :651–662. [PubMed: 7074728]
- [115]. Liu T, Takimoto E, Dimaano VL, DeMazumder D, Kettlewell S, Smith G, et al. Inhibiting mitochondrial Na^+/Ca^{2+} exchange prevents sudden death in a Guinea pig model of heart failure. *Circ Res.* 2014; 115 :44–54. [PubMed: 24780171]
- [116]. Liu Y, Cabo C, Salomonsz R, Delmar M, Davidenko J, Jalife J. Effects of diacetyl monoxime on the electrical properties of sheep and guinea pig ventricular muscle. *Cardiovasc Res.* 1993; 27 :1991–1997. [PubMed: 8287408]
- [117]. Baudet S, Noireaud J, Leoty C. Intracellular Na activity measurements in the control and hypertrophied heart of the ferret: an ion-sensitive micro-electrode study. *Pflugers Arch.* 1991; 418 :313–318. [PubMed: 1831556]
- [118]. Levi AJ, Lee CO, Brooksby P. Properties of the fluorescent sodium indicator “SBFI” in rat and rabbit cardiac myocytes. *J Cardiovasc Electrophysiol.* 1994; 5 :241–257. [PubMed: 7864922]
- [119]. Donoso P, Mill JG, O’Neill SC, Eisner DA. Fluorescence measurements of cytoplasmic and mitochondrial sodium concentration in rat ventricular myocytes. *J Physiol.* 1992; 448 :493–509. [PubMed: 1593474]
- [120]. Schepkin VD, Choy IO, Budinger TF, Obayashi DY, Taylor SE, DeCampi WM, et al. Sodium TQF NMR and intracellular sodium in isolated crystalloid perfused rat heart. *Magn Reson Med.* 1998; 39 :557–563. [PubMed: 9543417]
- [121]. Lee CO, Dagostino M. Effect of strophanthidin on intracellular Na ion activity and twitch tension of constantly driven canine cardiac Purkinje fibers. *Biophys J.* 1982; 40 :185–198. [PubMed: 7183333]
- [122]. Eisner DA, Lederer WJ, Vaughan-Jones RD. The quantitative relationship between twitch tension and intracellular sodium activity in sheep cardiac Purkinje fibres. *J Physiol.* 1984; 355 :251–266. [PubMed: 6092625]
- [123]. Lee CO, Kang DH, Sokol JH, Lee KS. Relation between intracellular Na ion activity and tension of sheep cardiac Purkinje fibers exposed to dihydro-ouabain. *Biophys J.* 1980; 29 :315–330. [PubMed: 7260255]

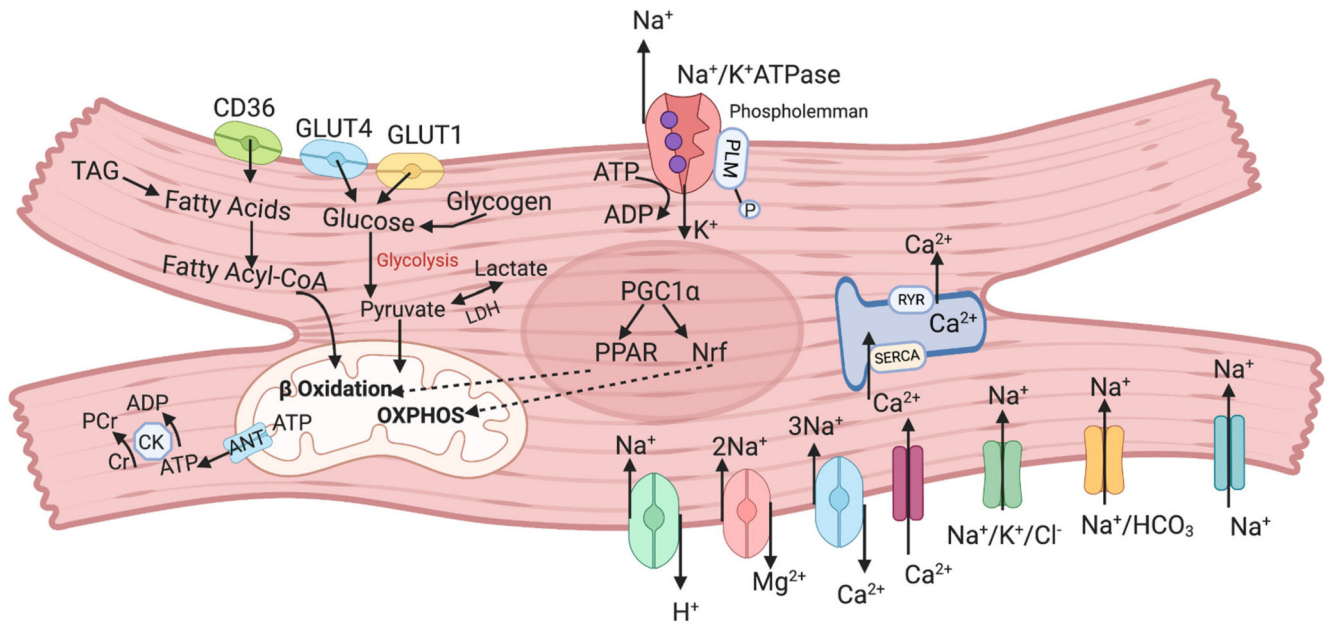


Fig. 1.

Myocardial ATP synthesis and Na_i influx/efflux pathways GLUT-glucose transporter (GLUT1 and GLUT4), CD36-fatty acid transporter, TAG-triacylglycerol, LDH-lactate dehydrogenase, CK-creatine kinase, PCr-phosphocreatine, Cr-creatine, ANF-adenine nucleotide translocase, RYR-ryanodine receptors, SERCA-Ca-ATPase in sarcoplasmic reticulum.

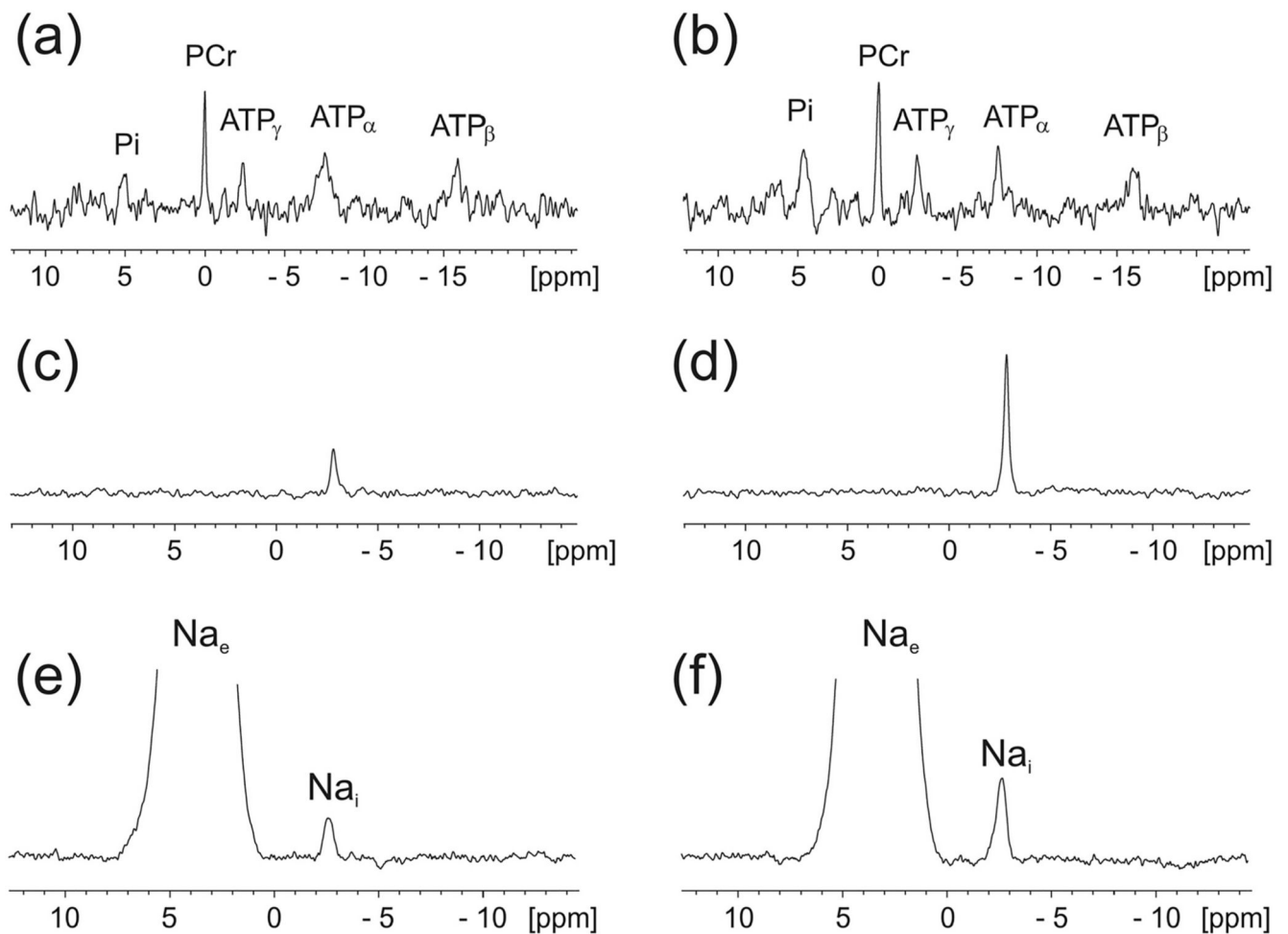


Fig. 3. Representative ^{31}P NMR, Triple Quantum Filtered ^{23}Na and 1D ^{23}Na NMR spectra from healthy and hypertrophied perfused mouse hearts (reproduced with permission from [9]).

Perfused control heart spectra are shown in panels (a,c,e) and hypertrophied heart spectra in panels (b,d,f). NMR spectra were acquired using a Bruker Avance III 400 MHz wide-bore spectrometer [106] (a-b) ^{31}P spectra, (c-d) triple quantum filtered (TQF) ^{23}Na NMR spectra. (e-f) conventional single quantum ^{23}Na NMR spectra acquired during infusion of 5 mM Tm(DOTP) shift reagent. (This figure was originally published in [9] and used with permission <https://doi.org/10.1042/BST20170508>).

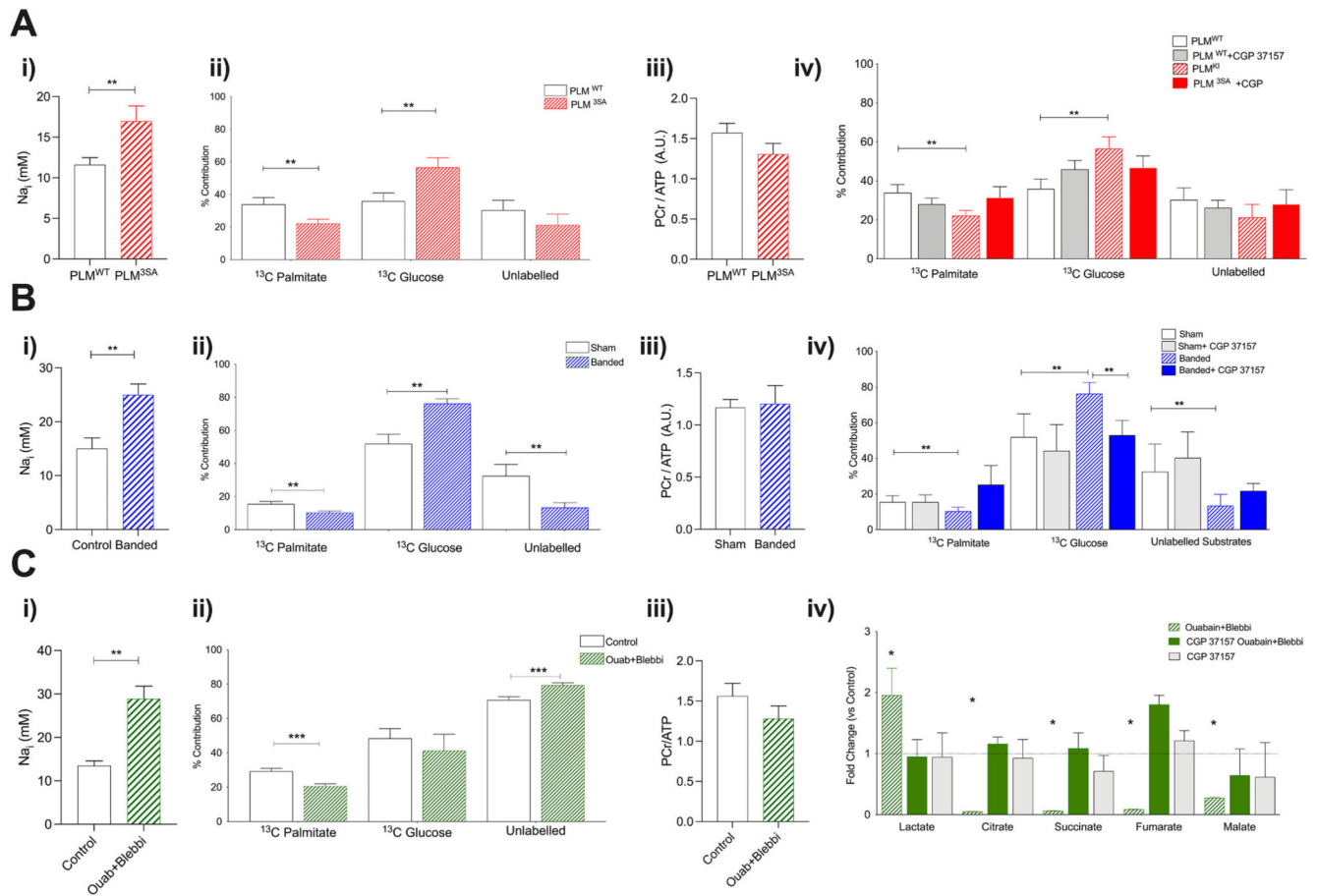


Fig. 4. Elevation of intracellular sodium reprograms cardiac metabolism [43].

Chronic transgenic (A), chronic hypertrophy (B) and acute (C) myocardial sodium elevation precedes metabolic substrate switch (% contribution to oxidative metabolism A-C ii) without compromising cardiac energetics (³¹P NMR spectroscopy A-C iii). Myocardial substrate utilization was assessed by steady state ¹³C NMR spectroscopy (A-C ii). Acute (30 min) treatment with mitochondrial Na/Ca inhibitor CGP37157 ameliorates the substrate switch (A-C iv) as well as the metabolite depletion. (Figure based on data published in [43] and used with permission).

Table 1
Myocardial Na concentrations in healthy and failing hearts Adapted from [9].

Healthy Heart				Failing Heart			
Species	[Na] _i (mM)	Assessment Technique		Species	[Na] _i (mM)	Assessment Technique	
Mouse	11.6	²³ Na NMR; perfused heart.	[106]	Mouse LVH	23	SBFI-loaded myocytes at rest.	[36]
	14	SBFI-loaded myocytes at rest.	[36]				
Rat	12.7	Na-selective microelectrodes; muscle strips at 0.5 Hz.	[12]	Guinea pig LVH	12.1	Na-selective microelectrodes; muscle strips at rest.	[107]
	8.5–30	Na-selective microelectrodes; myocytes at rest.	[108,109]		12.8	²³ Na NMR; perfused heart.	[110]
Guinea pig	4.7–8.0	Na-selective microelectrodes; muscle strips at rest.	[107,111] [112–114],	Guinea pig Heart Failure	16.8	SBFI-loaded myocytes at rest.	[115]
	6.4	²³ Na NMR; perfused heart.	[110]				
	5.1–5.2	SBFI-loaded myocytes at rest.	[10] [116]				
Ferret	7.8	Na-selective microelectrodes; muscle strips at rest.	[117]	Ferret RVH	8.0	Na-selective microelectrodes; muscle strips at rest.	[117]
Rabbit	7.2	Na-selective microelectrodes; muscle strips at 0.5 Hz.	[11,12]	Human LVH	14.2	Na-selective microelectrodes; muscle strips at rest.	[107]
	3.8–4.5	SBFI-loaded myocytes at rest.	[11,118]	Human Heart Failure	12.1	SBFI-loaded muscle strips paced at 0.25 Hz.	[37]
	5.1–21	SBFI-loaded myocytes at rest.	[11,118] [10,119],	Human MVD	11.8	Na-selective microelectrodes; muscle strips at rest.	[107]
	17.5	²³ Na NMR; perfused arrested hearts.	[120]				
Dog	8.9–10.4	Na-selective microelectrodes; Purkinje fibers at rest and 1 Hz.	[121]				
Sheep	5–6.4	Na-selective microelectrodes; Purkinje fibers at 1 Hz and at rest.	[122,123]				
	5.8–7.9	Na-selective microelectrodes; muscle strips at rest.	[114]				
Human	8.0	SBFI-loaded muscle strips paced at 0.25 Hz.	[37]				