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Selecting heart failure patients for metabolic interventions

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ABSTRACT

Introduction: Heart failure (HF) has become the cardiovascular epidemic of the century and now imposes an immense burden on health care systems. While our understanding of the pathophysiology of HF has increased dramatically, the translation of knowledge into clinical practice has been disappointing. Metabolic dysfunction in HF has been studied for eight decades, but these efforts have not resulted in effective therapies. This paucity in clinical translation probably results from the variable contribution of metabolic dysfunction to the underlying heart disease. A major unmet need in cardiac drug development is therefore the ability to identify a homogeneous subset of patients in whom HF is driven by a specific mechanism that can be targeted.

Areas covered: The available literature was evaluated to describe maladaptive metabolic perturbations that occur in failing hearts and may cause metabolic inflexibility, oxidative stress and cardiac energy depletion. Furthermore, the potential utility of various biomarkers and molecular imaging techniques to detect and quantify specific metabolic dysfunctions in HF were compared. Finally, the authors propose ways to utilize these techniques to select patients for specific metabolic interventions.

Expert commentary: Metabolic dysfunction is among the most promising therapeutic targets in HF. Meticulous patient-selection with molecular imaging techniques and specific biomarkers appears indispensable for the effective translation of decades of scientific knowledge into clinical therapeutics.

1. Heart failure: the scope of the problem

Over the past four decades, heart failure (HF) patients have derived substantial benefits from the advances in our understanding of the pathophysiology of this devastating disease. Multiple fundamental discoveries in cardiovascular biology have fueled novel treatment paradigms that have successfully been translated into clinical applications. As a result, HF has transformed from a simple descriptive entity treated with palliative measures into a well-defined syndrome that can be treated with a vast array of life saving drugs, interventions, and devices [1]. Paradoxically, these advances have done little to reduce the population disease burden, including its economic impact. For instance, the rate of hospital admissions for acute HF in the USA has remained around 1 million per year since the beginning of the century. While the prognosis of HF has improved, the 5-year mortality rate is still worse than for most types of cancer [2]. In fact, HF is currently responsible for one in nine deaths in the USA and Europe [3]. In 2012, HF affected 2% of the US adult population and 9% of those over 60 years of age, and the associated healthcare costs were estimated at 30.7 billion per year [3]. While healthcare systems are barely coping with the immense healthcare burden of HF as it is, the prevalence of HF and the associated costs are projected to increase by 50% within the next 15 years [3]. HF may thus be regarded as the most pressing cardiovascular epidemic of this century. New strategies to treat or prevent HF are therefore urgently needed.

2. Heart failure therapy: is it time to transcend neurohormonal blockade?

The evolution of HF treatment is regarded as the epitome of evidence-based medicine because current pharmacological regimens have all survived the scrutiny of randomized controlled trials in relatively unselected patient populations. With some exceptions, all HF patients are treated with the same drugs and devices irrespective of the underlying disease mechanism. The ability to treat such a heterogeneous disorder in such a homogeneous fashion may be explained by the fact that these drugs essentially target the systemic response to cardiac failure. Indeed, while HF drugs target very specific components of the sympathetic nervous system, the renin-angiotensin system or natriuretic peptide biology, they can all be regarded as interventions intended to restore the systemic neurohormonal balance. After 15 years without any progress in HF therapy, the PARADIGM-HF trial recently demonstrated that the combination of a neutral endopeptidase inhibitor with an angiotensin-receptor blocker was superior to the well-established angiotensin converting enzyme-inhibitor enalapril [4]. As both of these drugs target the neurohumoral system, the PARADIGM-HF trial clearly indicates that there are still viable treatment targets left within the neurohumoral system. However, the increasing time-window between subsequent pharmacological innovations does suggest that we are approaching the asymptote for...
neurohormonal blockade [2]. Accordingly, there is a broad precedent to refocus drug development efforts from nonselective blockade of the systemic consequences of HF, toward interventions that target the specific underlying disease mechanisms within the heart [5].

Over the years, multiple perturbations in the cardiac muscle have been identified that can cause HF or contribute to disease progression, including but not limited to altered cardiac excitation-contraction (EC) coupling, aberrant activation of signal transduction pathways, and dysfunctional myocardial metabolism [6–11]. While the role of these mechanisms in HF development has been firmly established, their contribution to the underlying disease may vary among HF patients, as well as during different stages of disease progression within an individual patient. For instance, the expression and activity of the key calcium cycling molecule sarcoplasmic reticulum calcium ATPase (SERCA2a) is not uniformly reduced in myocardial tissue samples from HF patients and the degree of metabolic dysfunction (as specified below) also varies [11,12]. An intervention that targets a specific myocardial defect will probably not be effective or may even be harmful in patients in whom that particular mechanism is not causal. This may be one of the explanations for the lack-off effect of SERCA2a gene transfer in the CUPID-II trial, as a reduction in myocardial SERCA2a expression was not an entry criterion for participation in this study [13]. Whereas routine interrogation of myocardial SERCA2a expression levels may cross ethical and practical boundaries, there are ample opportunities to evaluate metabolic dysfunction in HF that could routinely be incorporated in trial design for metabolic therapies. In the following sections, we will therefore discuss major metabolic changes that occur in the failing heart and briefly mention metabolic therapies that are in various stages of clinical development. Current therapeutic concepts to restore cardiac metabolism are summarized in Figure 1.

3. Metabolic dysfunction in Heart failure

The heart pumps 10 tons of blood around the body each day for which it requires up to 30 times its own weight in adenosine triphosphate (ATP). The cardiac ATP stores are, however, only sufficient to sustain three heart beats. Cardiovascular function and myocardial ATP production are therefore heavily intertwined and small perturbations in the provision of carbon-based fuels and the efficiency of mitochondrial respiration can have major consequences for myocardial performance. The first indication that energy reserves are reduced in HF dates back more than eight decades, when Herrmann and Decherd discovered that creatine levels in failing hearts are reduced [14]. Based on these observations, it was hypothesized that energy deprivation leads to cardiac dysfunction, equivalent to an engine running out of fuel [11]. With few exceptions, the majority of the evidence gathered since then has supported this hypothesis. In the following paragraphs, we will provide an overview of the major metabolic changes that occur in the failing heart and briefly mention metabolic therapies that are in various stages of clinical development. Current therapeutic concepts to restore cardiac metabolism are summarized in Figure 1.

3.1. Cardiac substrate preference

The heart has considerable metabolic flexibility as it can transform chemical energy stored in various carbon-based substrates, including fatty acids, glucose, lactate, ketones, and amino acids, into ATP through oxidative phosphorylation [15]. In healthy

Figure 1. Therapeutic concepts to restore metabolic dysfunction in heart failure. Simplified schematic depicting cellular energy metabolism from substrate utilization to oxidative phosphorylation and energy transfer to the sites of energy consumption. Current therapeutic concepts to improve energetic efficiency in heart failure either target proportional substrate use by decreasing fatty acid metabolism (left panel), increasing glucose oxidation (right upper panel), or augmenting energy transfer (right lower panel). ADP: adenosine diphosphate; ATP: adenosine triphosphate; CPT: carnitine palmitoyltransferase; Cr: free creatine; FFA: free fatty acids; GLUT4: glucose transporter 4; PCr: phosphocreatine; PDH: pyruvate dehydrogenase; MCD: malonyl-CoA decarboxylase; NAD⁺, NADH: oxidized and reduced forms of nicotinamide adenine dinucleotide; TG: triglycerides; β-ox: beta oxidation.
resting myocardium, 60–90% of the total ATP production is derived from β-oxidation of fatty acids. The second major fuel is pyruvate, which can be generated by glycolysis or lactate oxidation. During exercise as well as the initial stages of most cardiac pathologies, the heart shifts from fatty acids to glucose as the primary source of energy. This shift in substrate preference is generally considered to be protective as it improves the stoichiometric ratio of oxygen consumption to ATP production [16]. β-oxidation of fatty acids also promotes uncoupling of the proton motive force generated by the mitochondrial respiratory chain and ATP synthesis, thereby reducing oxygen utilization efficiency even further [10,17]. While the amount of energy generated per gram of substrate is higher for fatty acids compared to carbohydrates, the oxygen utilization efficiency is much more favorable for pyruvate—than for β-oxidation [17].

An additional aspect favoring carbohydrate metabolism in failing hearts is that two ATP molecules are generated anaerobically within the cytosol during glycolysis, i.e. the transformation of glucose into pyruvate. Glycolysis is the only source of ATP production under hypoxic conditions, but it is very inefficient as the net ATP yield is about 8% of that obtained from oxidative phosphorylation of pyruvate. In advanced HF, glycolytic enzymes and glycolytic activity are markedly induced while the mitochondrial capacity to oxidize pyruvate and other major fuels becomes impaired. This phenomenon results in uncoupling of glycolysis and glucose oxidation and promotes anaerobic glycolysis as the primary cardiac route of ATP production [18].

Because anaerobic glycolysis is an inefficient pathway for energy production, augmentation of glycolysis further aggravates the myocardial energy deficit. Accordingly, cardiac ATP depletion typically develops when glucose oxidation diminishes, and increased levels of circulating pyruvate and lactate can be detected in plasma samples of HF patients at this point [19–21].

The reductions in glucose oxidation in advanced HF are at least partially caused by mitochondrial damage or dysfunction (discussed below). In addition, a functional block at the level of the pyruvate dehydrogenase (PDH) complex has also emerged as a central driver of this process [17]. PDH is considered the rate-limiting step in glucose oxidation as it transforms pyruvate into acetyl-CoA, which subsequently enters the Krebs cycle. The negative regulator of PDH, PDH kinase, is strongly activated in failing hearts essentially blocking the cardiac capacity to oxidize carbohydrates [22]. It must be stressed, however, that these metabolic adaptations are not exclusive to cardiomyocytes or HF models as they occur in virtually all cell types subjected to hypoxic or metabolic stress [23].

Furthermore, while the data from animal experiments are more or less consistent, there is a large variation in substrate utilization in patients with cardiac hypertrophy or HF [19,20]. Finally, comprehensive metabolomics profiling recently indicated that the failing heart relies more on ketone bodies than on pyruvate, suggesting that advances in biochemical techniques may further refine contemporary paradigms [24]. In summary, the initial stages of pathological cardiac stress are accompanied by a marked augmentation of glycolysis and glucose oxidation, while advanced HF is associated with a reduced capacity to metabolize all major fuels and increased dependence on anaerobic glycolysis.

### 3.2. Oxidative stress in Heart failure

Oxidative stress is another key element of the pathophysiology of HF. Since the metabolic changes in HF are inextricably linked to the production of reactive oxygen species (ROS) by mitochondria, oxidative stress can be a cause as well as a consequence of HF. Even under physiological conditions, mitochondria produce superoxide (O$_2^-$) as a by-product of respiration [25]. Preferably, this free radical is broken down to water by manganese-dependent superoxide dismutase (MnSOD) and nicotinamide adenine dinucleotide phosphate (NADPH)-dependent enzymatic reduction [25]. In HF, defective EC coupling and mitochondrial ion homeostasis impede activation of the Krebs cycle [26]. This not only disturbs regeneration of NADH to supply the electron transport chain (ETC) with electrons, but also that of NADPH required for adequate antioxidant capacity [26]. The consequent oxidative stress leads to a vicious circle in which oxidative damage to mitochondrial DNA causes increased electron leakage from the ETC and oxidation of proteins contributes to further derangement of EC coupling [26,27]. Adaptive changes associated with HF, including activation of the sympathetic nervous system and the renin-angiotensin-aldosterone system, aggravate this process by increasing cardiac energy supply-demand mismatch as well as direct stimulation of ROS production [28,29]. Mitochondrial ROS (mtROS) triggers increased mitochondrial permeability by activation of the mitochondrial permeability transition pore, among other pores and channels, allowing ROS to escape to the cytosol [30].

On emission, mtROS not only induce oxidative damage to non-mitochondrial structures, but also stimulate ROS production by other sources, including NADPH oxidase (Nox), uncoupled NO synthase (NOS), and xanthine oxidase (XO) [30]. Reciprocally, ROS produced by these sources promote the production of mtROS [30]. ROS production in HF is summarized in Figure 2.

The relative contribution of different cell types remains unclear and may well vary based on underlying disease mechanisms. In fact, comorbidity-related proinflammatory signaling and consequent coronary endothelial inflammation and ROS production have been implicated in the pathophysiology of HF with a preserved ejection fraction (HF-pEF), whereas in HF with a reduced ejection fraction (HF-rEF) ROS are assumed to primarily originate from cardiomyocytes [31]. Reflecting the heterogeneous nature of HF pathology, these variations in ROS production emphasize the need for a personalized approach to improve the treatment of patients suffering from this syndrome.

### 3.3. Mitochondrial dysfunction and myocardial energy depletion

Mitochondria from failing hearts exhibit major structural and functional defects that diminish their capacity to generate ATP and increase superoxide release from the respiratory chain. The activity of respiratory chain complexes and ATP synthase is significantly decreased in HF and the sensitivity to endogenous regulators of oxidative phosphorylation becomes
diminished [32]. The contribution of mitochondria to other cellular processes such as cardiac calcium handling, cellular signaling, and the regulation of cell death also becomes severely perturbed [33]. Mitochondrial defects are generally considered to result from ROS-mediated damage to mitochondrial DNA and proteins. However, uncontrolled activation of mitochondrial signaling pathways, transcriptional mitochondrial reprogramming, and reduced elimination of damaged mitochondria by defective mitophagy are now increasingly recognized as additional causal factors [10, 34, 35]. Nevertheless, current pharmacological strategies to alleviate mitochondrial dysfunction are primarily designed to attenuate mitochondrial oxidative stress with mitochondria-targeted antioxidants, such as Szeto-Schiller peptide (SS-31) and MitoQ [36, 37]. Further advances in our understanding of mitochondrial regulatory mechanisms in HF may provide novel treatment paradigms that will allow us to specifically target the underlying disease mechanisms rather than its consequences. For instance, the endogenous regulation of ATP synthase is still poorly understood and several key regulatory mechanisms have only recently been discovered [38–40].

The transport of ATP from mitochondria to the sites of ATP consumption within the cell is facilitated through the creatine kinase (CK) shuttle. To allow effective diffusion of energy throughout the cell, one phosphate group is transferred from ATP to creatine by mitochondrial CK to generate phosphocreatine. Phosphocreatine rapidly diffuses throughout the cell and ATP is subsequently regenerated from phosphocreatine by CK at the sites of ATP consumption. In addition to the catalysis of ATP transfer, the CK shuttle also acts as a cellular energy buffer. Whereas cardiac ATP levels only drop in the more advanced stages of HF, an energy deficit expressed as the phosphocreatine/creatine ratio, already becomes apparent before overt cardiac dysfunction develops [41]. Accordingly, the energy status of the heart can be expressed as the phosphocreatine/creatine ratio. As described above, the energy depletion hypothesis in HF was based on creatine depletion in failing hearts, perhaps making this the best-studied metabolic defect in HF. Despite eight decades of research and several clinical trials, there are no therapies that can specifically restore cardiac energy levels or ATP transfer [42]. For instance, creatine supplementation failed to alleviate HF development in multiple clinical scenarios [43, 44]. The importance of the cardiac CK shuttle was recently scrutinized in a mouse model of systemic creatine knockdown. While the hearts of these mice were fully depleted of creatine, cardiac function and exercise performance were not altered [45]. Despite the lack of successful interventions in ATP transfer mechanisms, and the debate surrounding its physiological relevance, the phosphocreatine/creatine ratio may still prove...
valuable as a biomarker to select patients for metabolic interventions.

4. Biomarkers for metabolic dysfunction in Heart failure

4.1. Biomarkers for cardiac substrate preference and energy depletion

As described above, severe perturbations in cardiac glucose oxidation in advanced HF are associated with increased circulating lactate and pyruvate levels and these biomarkers have been used to monitor metabolic interventions in the past [46]. Nevertheless, such biomarkers to monitor myocardial substrate preference have limited sensitivity and specificity as they reflect a combination of nutritional intake, gastrointestinal uptake, and systemic substrate utilization in all organs. Likewise, it will be very hard to untangle cardiac and extra-cardiac contributions to circulating levels of markers for ATP depletion, such as circulating ADP or AMP levels. Advances in metabolomics profiling have more recently been adopted to discover biomarkers with higher specificity for cardiac energy homeostasis. For instance, long-chain acylcarnitines have emerged as very promising biomarkers for disrupted β-oxidation, but require further validation [47,48]. A more direct approach to evaluate cardiac substrate utilization is the comparison of metabolite levels in aortic and coronary sinus blood samples, which allows more detailed analysis of cardiac substrate use, including metabolic substrate flux and ATP production efficiency [49]. The obvious drawback of this technique is the invasive nature and this type of analysis is typically reserved to monitor the effect of invasive procedures such as cardiac resynchronization therapy.

4.2. Biomarkers for oxidative stress

As extensively described elsewhere, several biomarkers are commonly used to assess oxidative stress in HF. These include oxidized low-density lipoprotein, malondialdehyde, and myeloperoxidase in plasma, and isoprostane in plasma and urine [50]. The gut microbe-generated metabolite trimethylamine-N-oxide (TMAO), which is adversely associated with HF prognosis, has also been proposed to be related to oxidative stress [51]. Some of these traditional biomarkers, particularly aldehydes, have been suggested to further increase ROS formation, although studies on their role in pathophysiology of cardiovascular diseases (CVDs) are conflicting [50]. Aldehydes are formed during many processes including lipid peroxidation [52]. During HF, increased ROS production decreases the activity of aldehyde dehydrogenase 2 [53]. This causes an accumulation of aldehydes, including malondialdehyde and 4-hydroxy-2-nonenal (HNE), which localize to mitochondria and further increase mitochondrial ROS formation through reactions with nucleophilic protein residues [52,54,55]. Aldehyde levels can be detected in the plasma and several studies have indicated that plasma aldehyde levels can serve as biomarkers for oxidative stress. For instance, lipid peroxidation has been investigated as a biomarker for atherogenesis [56]. Nevertheless, it should be noted that, regardless of their pathological significance, aldehydes are products of the reaction of ROS with lipids, and thus arise secondarily to the process that initiated oxidative stress. Thus, while aldehydes may serve as biomarkers, it is unlikely that they will serve as a nodal point for metabolic interventions [50]. Consequently, these biomarkers can be used as a marker for oxidative stress, but may not for the selection of patients for specific metabolic interventions.

4.3. Serum free thiols

Recently, the serum free thiol level regained interest as an indicator of oxidative stress in the context of chronic HF. Reactive species can oxidize their targets by reacting with thiols, i.e. functional groups composed of a sulfur and a hydrogen atom. Because free (or reduced) thiols are readily oxidized by reactive species, their level may be seen as a direct reflection of the balance between oxidants and antioxidant capacity, and thus the overall level of oxidative stress [57,58]. What makes free thiols different from other biomarkers is that apart from reflecting the overall level of oxidative stress, thiols are critical active components of the antioxidant defense, which may be receptive to therapeutic modulation. So far, most studies looking into antioxidant machinery have focused on low-molecular-weight thiols, in particular glutathione and cysteine. For example, a recent study has shown an association between higher cystine (i.e. oxidized cysteine) and lower glutathione levels and an increased mortality risk of coronary artery disease patients [59]. However, whereas these low-molecular-weight thiols are key actors in intracellular antioxidant defense, in the extracellular compartment they are strongly outnumbered by protein thiols [60]. In fact, the single sulphydryl group of albumin, the most abundant serum protein, accounts for the majority of thiols in serum [60]. Hence, compared to the concentration of low-molecular-weight thiols, the total free thiol level may be a more relevant circulatory biomarker of oxidative stress.

Depletion of circulatory free thiols has been shown in patients with CVD, including acute myocardial infarction [57,61,62]. Also, thiol oxidation has been linked to established CVD risk factors, such as aging, smoking, and obesity [63]. Moreover, our group has recently reported a positive association between protein-adjusted serum free thiols and a favorable disease outcome in a small cohort of 101 stable chronic HF patients [64]. This finding suggests that serum free thiols provide a robust reflection of redox status and warrants confirmation in a larger cohort.

4.4. Gaseous signaling molecules NO and H₂S

Reversible oxidative modifications of protein thiols by several small molecules may protect proteins from irreversible oxidative damage and, in some cases, alter protein function [58]. Among these small molecules are gasotransmitters nitric oxide (NO) and hydrogen sulfide (H₂S).

NO, also known as endothelium-derived relaxing factor, has long been acknowledged for its versatile role in cardiac physiology [65]. As the alias suggests, it is an important regulator of vascular tone, among numerous other bodily processes.
In HF, NO bioavailability is assumed to be reduced [66,67]. At the same time, increased levels of NO metabolites, nitrite and nitrate (NOx) have been described in HF, possibly reflecting impaired renal excretion [68,69]. More recently, H$_2$S has been recognized as a cardiovascular signaling molecule, similar to NO. Like NO, H$_2$S is involved in the regulation of various (patho)physiological processes and features vasodilatory, antioxidant, and anti-inflammatory properties [70].

Unfortunately, reliable methods for direct detection of NO and H$_2$S in biological samples are lacking. Alternatively, quantification of their metabolites – i.e. NOx for NO and thiosulfate and sulfate for H$_2$S – may be used to evaluate changes in the metabolism of these gaseous signaling molecules. Naturally, other factors that may be of influence, such as intake and renal function, should be considered in this process. To date, very few studies have looked into concentrations of NO and H$_2$S metabolites in relation to outcome of CVD. One paper describes a higher plasma concentration of NOx 24 hours after onset of ST-segment elevation myocardial infarction to be associated with an increased risk of one-year all-cause mortality or rehospitalization [71]. Another study has found both urinary thiosulfate and sulfate concentrations of renal transplant recipients to be positively associated with a favorable cardiovascular risk profile and patient survival [72].

5. Molecular imaging of cardiac metabolism

5.1. Positron emission tomography

Positron emission tomography (PET) is a three-dimensional imaging technique that can monitor biological processes in vivo through the use of molecules that have been labeled with positron emitting radionuclides. PET already has a central role in clinical cardiac diagnostics, for the evaluation of myocardial perfusion and viability in ischemic heart disease, the differential diagnostics of cardiomyopathies, and the diagnosis and risk stratification of endocarditis. PET also holds great promise for cardiac drug development as virtually every molecule can be modified to be detectable by PET [73]. The most abundantly used clinical PET tracer, $^{18}$F-FDG, was specifically designed to monitor glycolytic activity and tracers for $\beta$-oxidation have also been developed for clinical application. These tracers accurately monitor changes in glycolysis and $\beta$-oxidation in experimental animals, as well as in patients with HF [74–77]. In particular, $^{18}$F-FDG PET is a sensitive marker for cardiac glucose uptake, and appears to have reasonable accuracy for the prediction of a treatment response to various interventions [78–80]. More studies are, however, required to further define the utility of PET as a biomarker for cardiac substrate utilization in HF.

Monitoring and quantification of focal oxidative stress in tissues has several potential clinical applications and several PET probes have been developed for the detection of hypoxia or ROS production, mostly for oncological or for cardiovascular applications [81–83]. Unfortunately, the cardiac uptake of these tracers has been disappointing as they provide insufficient signal intensity to detect the mild hypoxia or ROS production in HF. There are, however, several promising novel tracers in development and these may prove more reliable for application in HF [84,85]. Of particular interest for ROS monitoring in HF is radiolabeled vitamin C ([$^{11}$C]Ascorbic acid), which exhibits ROS-dependent cellular accumulation that could result in significant augmentation of signal intensity. Accordingly, PET holds great promise for the selection and monitoring of patients for metabolic interventions.

5.2. Magnetic resonance spectroscopy

Cardiovascular magnetic resonance imaging (MRI) has emerged as the primary method for myocardial tissue characterization in clinical cardiology, but few people realize that MRI is in fact a molecular imaging technique. Traditional MRI employs the combination of a high magnetic field to align hydrogen nuclei, the most abundant MR active nuclei in the body, and radio waves that causes these nuclei to resonate. When hydrogen nuclei relax from their resonance, they emit radio waves that can be detected. Differences in relaxation time in various tissues result in differences in resonance intensity that can be transformed to create images. These images can be used to evaluate myocardial tissue characteristics, such as the presence of fibrosis or edema, and also allows for detailed quantification of cardiac volumes and function in HF. Nuclear magnetic resonance spectroscopy (MRS) utilizes the same hardware and radio waves, but rather than creating an image from the signal that it receives, it detects distortions in magnetic resonance frequency within the tissue. This so-called chemical shift results from the interactions among neighboring nuclei and electrons in the tissue. As specific molecules generate a specific shift in resonance frequency, detailed analysis of the frequencies can be used to quantify the concentrations of various abundant metabolites in tissues. (Figure 3) MRS can be used to measure the concentrations of molecules rich in protons ($^1$H) but also less abundant nuclei such as phosphorus ($^{31}$P), carbon ($^{13}$C), or sodium ($^{23}$Na). While several metabolites can be detected with MRS, for human applications, $^1$H MRS has primarily been used to detect cardiac creatine and triglyceride content, $^{31}$P MRS to detect cardiac ATP, phosphocreatine, and phosphocreatine/creatinine ratios and $^{23}$Na to monitor cardiac sodium content (Figure 3) [86]. Despite its vast potential, cardiac MRS is a challenging technique as measurements need to be adjusted for cardiac and respiratory motions. The time required to measure MRS spectra varies between 30 seconds and 20 minutes for $^1$H MRS and 20 and 30 minutes for $^{31}$P MRS, depending on the gating technique and the pulse sequences employed. Even though it can be performed on most commercially available scanners, currently, MRS is primarily used as a research tool in specialized centers. The most commonly studied MRS-derived parameters are myocardial triglyceride content and phosphocreatine/creatinine ratios [86]. The latter appears to have remarkable specificity for changes in cardiac energy stores. For instance, a study in type 2 diabetic patients was capable of detecting a transient, 12% reduction in cardiac phosphocreatine/creatinine ratio during exercise [87]. MRS has been used to monitor cardiac energy levels for more than 25 years and can detect changes in high-energy phosphate levels during treatment [88]. As such, it could be regarded as the gold standard for the evaluation of energy stores.

The application of MRS as a tool to study cardiac ROS is limited, although myocardial oxygen levels can be measured.
with MRI using blood oxygen level-dependent sequences [89].

In theory, $^{13}$C MRS holds a vast potential for metabolic imaging because cellular energy is derived from carbon-based fuels. Unfortunately, the clinical application of native $^{13}$C MRS is limited by the low abundance and low sensitivity of $^{13}$C nuclei to MR pulses. To circumvent this limitation, a hyperpolarization technique has been developed that can increase the signal of specific $^{13}$C-containing molecules by more than 10,000-fold, using a combination of free radicals and intense freezing under high magnetic field strengths. These hyperpolarized metabolites are subsequently injected into the bloodstream and their cardiac uptake and transformation into downstream metabolites can be monitored. Hyperpolarization has a short half-life and cannot be used to enhance native molecules within the heart. It can be used to monitor metabolic processes in vivo, including the PDH flux, β-oxidation, and the Krebs cycle [90]. In experimental animals, hyperpolarized $^{13}$C MRS can effectively monitor changes in substrate utilization during the progression of HF and the effects of interventions in cardiac substrate utilization [20,91,92]. Regulatory approval of hyperpolarized $^{13}$C MRS for application in humans is pending and, considering its potential, eagerly anticipated.

In summary, PET is a well-established technique that could be regarded as the gold standard to monitor cardiac substrate utilization and is a promising technique for myocardial ROS detection. Systemic redox status can be measured with circulating and urinary biomarkers, although cardiac specificity remains limited. MRS is the gold standard for the detection of cardiac energy levels and holds great promise for the detection of cardiac substrate utilization through hyperpolarization techniques, but clinical validation is still pending. Accordingly, we propose that PET should be employed for patient selection and monitoring when interventions in cardiac substrate utilization are considered, whereas biomarkers, such as total serum free thiols, should be used to select patients for antioxidant treatments. Finally, MRS could be used to monitor interventions in cardiac ATP production, but may also serve to monitor the efficacy of other metabolic interventions. A scheme depicting the potential value of biomarkers, PET, and MRS to select HF patients for metabolic interventions during various stages of disease progression is summarized in Figure 4.

### 6. Therapeutic interventions in cardiac metabolism

#### 6.1. Therapeutic interventions in cardiac substrate preference

As the shift from fatty acid to glucose oxidation is generally considered a physiological adaptation to stress, and several therapeutic strategies have been developed that aim to promote glucose oxidation in failing hearts [16]. These drugs either promote cellular glucose uptake, augment glucose oxidation by restoring PDH complex functionality or inhibit fatty acid β-oxidation (as summarized in Figure 1 and extensively reviewed in ref. 17) [17]. While there have been some encouraging improvements in symptoms and exercise performance...
with these drugs such as dichloracetate and etomoxir, phase III clinical trials have been neutral or were stopped early due to drug toxicity or increased HF events [17, 93–96]. While this might be regarded as proof that reductions in glucose oxidation reflect an adaptive mechanism or that the therapeutic interventions were poorly designed, it should be borne in mind that defective cardiac substrate utilization was not an inclusion criterion for these studies. In selected patients, the outcomes of these studies may have been positive.

6.2. Therapeutic interventions to alleviate myocardial oxidative stress

As outlined above, excess ROS production is a key factor in the pathophysiology of HF. At the same time, redox signaling is an integral part of physiology and lower levels of ROS are involved in all kinds of beneficial cellular processes, including immunity, cell growth, and apoptosis [97]. Besides, recurrent episodes of oxidative stress have been found to upregulate endogenous antioxidant mechanisms, which in turn may promote health and longevity [97]. Thus, disturbance of the physiological functions of ROS is likely to have contributed to the contradicting findings of studies evaluating uncontrolled and untargeted antioxidant therapies, including N-acetylcysteine (NAC) and vitamins, in cardiac disease [98, 99]. Also, when a specific ROS producing component is disarmed, this may adversely affect other parts of the redox network. For example, this may in part explain the disappointing results of treatment with an XO inhibitor in HF, as this substance prevents XO from producing O$_2^-$, but also decreases NO production [100]. Perhaps, combination therapy of an XO inhibitor and an NO donor can resolve this problem. Moreover, physiological regulatory processes may prevent administered antioxidants from reaching adequate bioavailability, unless there is a deficiency to overcome [97]. Finally, oxidation of an antioxidant substance before or during ingestion may not only abate its efficacy, but in fact induce oxidative stress [97].

Despite these challenges, in patients with aberrant endogenous antioxidant capacity, antioxidant therapies may well be beneficial. For example, restoring redox status by therapeutic modulation of free thiols may hold promise to improve disease outcome in HF. Indeed, in multiple clinical trials, the cysteine derivative NAC has been shown to both directly reduce disulfide bonds and act as a glutathione precursor [101]. Other thiol compounds, with similar modes of action, have also been described [102]. Although therapeutic results have been inconsistent, these compounds may prove to be effective in selected patients with low free thiol concentrations. Alternatively, pharmacological stimulation of molecules that induce reversible protein modifications, including NO and H$_2$S, may offer opportunities to influence the amount of free thiols, as well as favorably steer protein function [58, 65, 70]. One H$_2$S releasing compound, AP39, may be of particular interest as it is specifically targeted to mitochondria, one of the most important sources of ROS in HF. In experimental models, AP39 has been shown to attenuate H$_2$O$_2$-induced cytotoxicity and to positively affect vascular tone and intracellular calcium homeostasis [103]. However, further research is needed to prove its clinical applicability. Other mitochondria-targeted antioxidants, including MitoQ and SS-31, have also shown promise in experimental HF. Moreover, SS-31 is currently investigated in a clinical trial focused on myocardial reperfusion injury and a trial studying this compound in the context of HF will soon begin [36, 104].

7. Conclusions

Metabolic dysfunction is among the most promising therapeutic targets in HF. Meticulous patient selection with molecular imaging techniques and specific biomarkers appears indispensable for the effective translation of decades of scientific knowledge into clinical therapeutics.

8. Expert commentary

Although HF patients are treated with up to 5 different classes of drugs, their mode of action is roughly similar as they can all be regarded as interventions that restore the systemic neurohormonal balance. Despite continuous refinements in this strategy, multiple lines of evidence...
indicate that we are approaching the asymptote for added benefit of neurohormonal interventions. Rather than focusing on the secondary effects of HF, we believe that we should refocus drug development efforts toward interventions that target specific pathologic changes within the heart. Despite tremendous advances in our understanding of the molecular perturbations that occur within the failing heart, the translation of this knowledge into clinical practice has been disappointing. The most likely explanation is that the contribution of a given defect to the underlying heart disease is highly variable, highlighting the need to select a homogeneous subset of patients in whom HF is driven by a specific mechanism that can be targeted. This is particularly true for myocardial metabolism, which has been studied for the better part of a century, but has not proven to be amendable by specific therapeutic interventions. Perhaps the most daunting challenge for metabolic drug development is the paucity of specific tools to study cardiac metabolism in an individual patient. Metabolomic analysis of plasma lacks cardiac specificity and isolation of cardiac muscle may identify specific metabolic defects, but cannot be applied to intervention trials, as it requires ventricular biopsies. Cardiac PET is a highly versatile technique that allows virtually every molecule to be modified for detection by PET. Nevertheless, the technique is limited by variations in signal to noise, the inability to distinguish tracers from metabolites, and by the fact that it exposes subjects to ionizing radiation. Another key limitation in metabolic research in HF is the fact that there is no evidence to support that the level of energy depletion is causing cardiac dysfunction. It is possible that the metabolic changes in the myocardium reflect beneficial cardiac adaptation to stress or even an epiphenomenon.

9. Five-year view

Eight decades of metabolic research in HF have not provided us with reliable metabolic interventions for this devastating disease. Over the next 5 years, we anticipate that several metabolic interventions will be tested in clinical trials. One of the most promising and timely interventions is the mitochondria-targeted antioxidant SS-31 (also known as Bendavia), which has been established as safe and well tolerated in patients after an acute myocardial infarction [104]. While a conference abstract indicated that Bendavia is effective in HF patients as well, appropriately sized trials have not started yet. We expect that the success of these interventions depend on the individual degree of mitochondrial dysfunction and oxidative stress. Accordingly, we believe that the biomarkers described above should be employed for patient selection and monitoring. Furthermore, we expect that hyperpolarized MRS will replace PET as the gold standard for the assessment of cardiac substrate utilization, as it can link metabolism to function, differentiate between tracers and metabolites, and avoids ionizing radiation. Whether biomarker-based patient selection will illuminate pathways forward for metabolic interventions in HF remains to be established.

Key issues

- A major unmet need in drug development for heart failure is the ability to identify a homogeneous subset of patients in whom cardiac dysfunction is driven by a specific mechanism.
- The contribution of metabolic dysfunction and aberrant production of reactive oxygen species (ROS) to the underlying disease mechanism is highly variable, underscoring the need for meticulous patient selection.
- Molecular imaging techniques such as magnetic resonance spectroscopy and positron emission tomography are reliable tools to detect a variety of metabolic perturbations in HF and monitor the effect of interventions.
- Mitochondrial ROS production and the resulting oxidative stress are both a cause and a consequence of heart failure that are potentially amendable by therapeutic interventions.
- Biomarkers for oxidative stress, such as plasma free thiols, may serve to select patients for antioxidant therapies.

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Declaration of interest

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Papers of special note have been highlighted as either of interest (♣) or of considerable interest (♣) to readers.

   - Most recent, most comprehensive, and most innovative HF guidelines to date.
   - Inspiring viewpoint editorial that highlights the great achievements in HF care and also provides guidance for the daunting challenge to cope with the impeding HF epidemic.
The paper describes the discovery of metabolic dysfunction in HF that inspired eight decades of research.

Exceptional review of the systemic metabolic impairment in HF, with an online supplement that provides a detailed yet palatable summary of cardiac metabolism.

An elaborate study on the prospective changes in cardiac metabolism during HF progression using state-of-the-art hyperpolarized magnetic resonance spectroscopy.

The paper discusses the discovery of metabolic dysfunction in HF that inspired eight decades of research.

Typical review of the systemic metabolic impairment in HF, with an online supplement that provides a detailed yet palatable summary of cardiac metabolism.

An elaborate study on the prospective changes in cardiac metabolism during HF progression using state-of-the-art hyperpolarized magnetic resonance spectroscopy.


- Evaluates oxidative stress biomarkers commonly used in cardiovascular research and practice.


- First report on the association of total serum free thios and disease outcome.


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