JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 7, NO. 11, 2022

STATE-OF-THE-ART REVIEW

Inefficient Batteries in Heart Failure

Metabolic Bottlenecks Disrupting the Mitochondrial Ecosystem

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HIGHLIGHTS

- **Mitochondrial abnormalities have long been described in heart failure.**
- **Reducing mitochondrial function to only a few measures limits our understanding of the entire mitochondrial ecosystem.**
- **Systems biology approaches and comprehensive assessments of the multiple facets of mitochondrial function are needed to advance our understanding of the disturbed mitochondrial ecosystem in heart failure.**

SUMMARY

Mitochondrial abnormalities have long been described in the setting of cardiomyopathies and heart failure (HF), yet the mechanisms of mitochondrial dysfunction in cardiac pathophysiology remain poorly understood. Many studies have described HF as an energy-deprived state characterized by a decline in adenosine triphosphate production, largely driven by impaired oxidative phosphorylation. However, impairments in oxidative phosphorylation extend beyond a simple decline in adenosine triphosphate production and, in fact, reflect pervasive metabolic aberrations that cannot be fully appreciated from the isolated, often siloed, interrogation of individual aspects of mitochondrial function. With the application of broader and deeper examinations into mitochondrial and metabolic systems, recent data suggest that HF with preserved ejection fraction is likely metabolically disparate from HF with reduced ejection fraction. In our review, we introduce the concept of the mitochondrial ecosystem, comprising intricate systems of metabolic pathways and dynamic changes in mitochondrial networks and subcellular locations. The mitochondrial ecosystem exists in a delicate balance, and perturbations in one component often have a ripple effect, influencing both upstream and downstream cellular pathways with effects enhanced by mitochondrial genetic variation. Expanding and deepening our vantage of the mitochondrial ecosystem in HF is critical to identifying consistent metabolic perturbations to develop therapeutics aimed at preventing and improving outcomes in HF. (J Am Coll Cardiol Basic Trans Science 2022;7:1161-1179)

A highly energetic tissue, the heart is sensitive to perturbations in mitochondrial function. The average heart consumes an estimated 5,600 L of oxygen and approximately 6 kg of adenosine triphosphate (ATP) per day.[1-3] Fatty acids are the primary source of fuel for the heart in the normal resting state.[4] The heart's constant contractile function is deftly maintained via metabolic fuel flexibility—the ability to use a variety of substrates (fatty acids, carbohydrates [glucose, lactate], ketones, amino acids) to produce the

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

Manuscript received January 24, 2022; revised manuscript received March 25, 2022, accepted March 29, 2022.

ISSN 2452-302X https://doi.org/10.1016/j.jacbts.2022.03.017

ABBREVIATIONS AND ACRONYMS

ADP = adenosine diphosphate
ANT1 = adenine translocator 1
ATP = adenosine triphosphate
CVD = cardiovascular disease
DCM = dilated cardiomyopathy
DRP-1 = dynamin-related protein 1
EET = epoxyeicosatrienoic acid
$FADH_2$/FAD = flavin adenine dinucleotide
HETE = hydroxyeicosatetraenoic acid
HF = heart failure
HFpEF = heart failure with preserved ejection fraction
HFrEF = heart failure with reduced ejection fraction
HIF1α = hypoxia-inducible factor 1α
iPLA2γ = Ca^{2+}-independent mitochondrial phospholipase
LV = left ventricle
LVAD = left ventricular assist device
LVEF = left ventricular ejection fraction
mPTP = mitochondrial permeability transition pore
mtDNA = mitochondrial DNA
NADH/NAD^+ = nicotinamide adenine dinucleotide
OPA1 = optic atrophy protein 1
OXPHOS = oxidative phosphorylation
PGC1-α = peroxisome proliferator-activated receptor gamma coactivator 1 alpha
SIRT1-7 = sirtuins 1-7

reduced intermediates nicotinamide adenine dinucleotide (NADH) and flavin adenine dinucleotide ($FADH_2$) as donors for mitochondrial electron transport and ATP generation. This metabolic elasticity enables the heart to adapt to acute stressors, but prolonged bottlenecks in multiple metabolic pathways result in an inability for the heart to sustain the ATP levels needed for cardiac function, contributing to heart failure (HF).

Metabolic and energetic alterations are well described in HF.[5,6] Mitochondrial perturbations and abnormalities in HF include a down-regulation of mitochondrial transcription, decrease in oxidative phosphorylation (OXPHOS), mitochondrial oxidative stress, increased mitochondrial DNA (mtDNA) damage, alterations in mitochondrial networks, and mitochondrial morphologic abnormalities.[7-13] However, most studies to date have assessed each of the various components of mitochondrial function in isolation, without simultaneous, comprehensive phenotyping of the complete mitochondrial system. This siloed approach limits our understanding of the effects of metabolic changes on key components of the mitochondrial network itself. Within this review, we seek to outline the many components of the mitochondrial "ecosystem" and how disturbances in one aspect of the mitochondrial environment influence the entire system. Only by understanding the mitochondrial derangements within the ecosystem will we be able to effectively target metabolic pathways in HF and further our understanding of the metabolic heterogeneity in HF subtypes.

ENERGETIC INSUFFICIENCY IN HF

Phosphocreatine allows for the rapid mobilization of ATP in the heart. In cardiomyocytes, ATP is primarily generated by OXPHOS within the mitochondria, but this ATP must be transferred to the cytosol for cellular use through the mitochondrial creatinine kinase system. A phosphate group from ATP is transferred to creatine, generating phosphocreatine, which can readily cross the mitochondrial membrane. Once in the cytosol, the phosphate on phosphocreatine is transferred back to adenosine diphosphate (ADP) to regenerate ATP for use by the contractile apparatus or to power the ATPase pumps needed to maintain the proper electrochemical gradients for cardiac function.

Advances in ^{31}P-magnetic resonance spectroscopy in the 1990s enabled in vivo imaging of phosphocreatine-to-ATP ratios in various HF etiologies in humans. Most studies have shown that phosphocreatine/ATP ratios are lower in individuals with HF, regardless of etiology. Importantly, the lower phosphocreatine/ATP ratio correlates with symptoms, disease progression and severity, and outcomes.[14-17] Not only is ATP lower in the heart in HF, phosphocreatine and creatinine are also reduced, likely matching the decline in ATP.[1,14,18-20] Importantly, the phosphocreatine/ATP ratio is unaffected if both phosphocreatine and ATP both decline in a proportional manner, which has been observed in individuals with dilated cardiomyopathy (DCM), and may result in the underestimation of an energetic insufficiency.[18] The phosphocreatine/ATP ratio also confers independent prognostic information on cardiovascular mortality beyond New York Heart Association functional class or left ventricular (LV) ejection fraction (LVEF).[17,20-22] Overall, phosphocreatine/ATP ratios are lower in symptomatic HF and associated with a decline in LVEF, suggesting that a loss of ATP contributes to HF progression.

IMPAIRMENTS IN MITOCHONDRIAL RESPIRATION IN HF

DOWN-REGULATION OF OXPHOS. To meet the energy needs of the heart, OXPHOS uses the reducing equivalents NADH and $FADH_2$, primarily derived from fatty acid oxidation, to generate ATP efficiently. When oxygen is limited, such as during ischemic conditions, mitochondrial respiration is suppressed.[23] In the short term, suppression of mitochondrial respiration is considered protective because it limits damage caused by reactive oxygen species generation. However, prolonged inhibition or down-regulation of mitochondrial respiration results in a limited ability for the heart to increase ATP production to match its energy needs, especially during states of increased energy demand.

Animal models of HF consistently show a transcriptional down-regulation of OXPHOS, although the timeline for the down-regulation and subsequent up-regulation of glucose metabolism differs across models.[24-27] Peroxisomal proliferator-activated receptor gamma coactivator 1 alpha (PGC1-α), the master regulator of mitochondrial respiration, up-regulates nuclear respiratory factors 1 and 2, which subsequently up-regulate the expression of citric acid cycle and the OXPHOS enzymes.[24] PGC1-α is down-regulated in the cardiac tissue of animal HF models, along with subsequent declines in both mitochondrial

and nuclear genome-encoded OXPHOS subunit levels and activities of the individual enzymes.[28-30] Isolated and perfused hearts from PGC1-α-knockout mice were unable to increase cardiac workload in response to dobutamine, a β-adrenergic agonist, in the presence of glucose and pyruvate or physiologic levels of fatty acids, glucose, ketones, and lactate.[31] The inability to increase cardiac workload, despite multiple different sources of reducing equivalents, suggests that increasing flux through alternative metabolic pathways may not be sufficient to sustain cardiac function without also restoring mitochondrial respiration. In addition, transverse aortic constriction-induced HF was accelerated in PGC1-α-knockout mice, suggesting that impairments in mitochondrial respiration likely play a key role in HF pathogenesis.[32] PGC1-α is also post-translationally regulated through phosphorylation by 5′ adenosine monophosphate–activated protein kinase (AMPK) and acetylation by sirtuins (SIRTs),[33] but the role of posttranslational modifications in the setting of HF is not known.

Unbiased transcriptomic and proteomic analyses have revealed a down-regulation in mitochondrial metabolic transcripts and proteins associated with cardiomyopathies and HF in humans.[34,35] OXPHOS and citric acid cycle enzymes were among the most prominently affected pathways in endocardial samples from patients with DCM (LVEF of <40%) compared to tissue from referent individuals with normal left ventricular function.[34] The lower levels of OXPHOS and citric acid cycle proteins associated with DCM were further supported by lower transcript levels in paired endocardial tissue from the same patients, suggesting a transcriptional down-regulation of mitochondrial metabolism.[34] The alterations in the OXPHOS transcript and protein levels spanned all OXPHOS complexes.[34] However, complex I subunits were down-regulated, whereas complex II subunits were up-regulated, suggestive of a potential redirection of electron input through complex II via $FADH_2$. This is consistent with findings that ischemia induction in heart perfusion models increases complex II activity as a result of increased citric acid cycle activity, driving reverse electron flow through complex I, thereby increasing oxidant generation.[36,37] Complex III was also down-regulated at both the transcriptional and proteomic levels, which, combined with the decreased complex I, is intriguing because these 2 OXPHOS complexes are the primary sites of oxidant generation within the chain.[38] Consistent with the down-regulation of gene expression and proteomic data, several studies have shown lower complex III and IV activities in left ventricular tissue from patients with idiopathic or ischemic DCM.[39,40]

A single-cell RNA-sequencing analysis revealed that genes involved in metabolism and contractile machinery were the predominant pathways differentially expressed in cardiomyocytes from HF patients (n = 2) compared to cells from referent heart tissue.[35] Both of the HF patients in this study underwent left ventricular assist device (LVAD) implantation, but only 1 of the patients had improved cardiac function post-implantation.[35] The patient with HF that improved post-LVAD showed a more favorable metabolic transcriptional profile, more similar to that of the referent individuals, whereas the metabolic pathway disturbances persisted in the HF patient who showed no improved cardiac function.[35] Although the small sample size will require additional studies to replicate these findings, this study suggests that metabolic disturbances are a key feature in cardiomyopathies and HF and that therapeutic improvements in cardiac function may be tied to improved metabolic function but require validation.

Intriguingly, a recent publication describing the transcriptome of right ventricular cardiac tissue from patients with HF with preserved ejection fraction (HFpEF) and HF with reduced ejection fraction (HFrEF) illustrated that the transcriptomic alterations in OXPHOS were one of the primary distinguishing features between HFpEF and HFrEF.[41] In cardiac tissue of HFpEF patients, genes involved in OXPHOS were elevated, whereas the OXPHOS genes were down-regulated in tissue from HFrEF patients. After adjustment for body mass index, the higher OXPHOS expression in HFpEF was attenuated, suggesting that obesity may contribute to the elevated OXPHOS gene expression in HFpEF.[41] Whether the transcriptomic differences in OXPHOS expression with HFpEF are matched by similar alterations in protein levels and respiration have yet to be determined.

MITOCHONDRIAL RESPIRATION. As might be expected from lower OXPHOS expression, mitochondrial respiration is suppressed in cardiac tissue from HF animal models and tissues from patients with cardiomyopathies and HF.[29,42-45] In Zucker/spontaneously hypertensive F1 hybrid obese rats that develop an HFpEF-like phenotype, complex I–mediated mitochondrial respiration was impaired in permeabilized cardiac fibers.[46] The suppression of mitochondrial respiration could, in part, be caused by increased mitochondrial matrix Ca^{2+} levels, which would contribute to a lower proton motive force for ATP generation, but mitochondrial membrane potential was not measured.[46] In addition, markers of oxidative

stress were higher in cardiac tissue from pacing-induced HF canines compared to control animals, which was likely driven by increased oxidant generation at complex I, related to a suppression of mitochondrial respiration.[47]

Baseline (state 2), ATP production–related (state 3), and uncoupled respiration were lower in permeabilized cardiac fibers from patients with HF (all with LVEF of <45%) compared to fibers from the referent group.[48] The suppressed mitochondrial respiration in the cardiac fibers from HF patients was observed across all substrates, including complex I substrates (glutamate/malate, pyruvate), complex II substrate succinate, and fatty acid oxidation substrates (palmitoylcarnitine, octanoylcarnitine).[48] However, permeabilized fibers from patients with cardiovascular disease (CVD) but no or mild HF had similar levels of mitochondrial respiration as the referent group across all mitochondrial substrates, suggesting that the suppression of mitochondrial respiration may occur later in the development of HF.[48] Citrate synthase levels were lower in cardiac tissue from HF patients compared to non-HF referent individuals, suggestive of lower mitochondrial mass in tissue from patients with HF.[48] This study suggests that impairments in mitochondrial respiration likely occur later in the development of HF in humans and may be attributable to lower mitochondrial content, although the sample sizes were small, raising the need for additional investigation.[48]

ALTERATIONS IN MITOCHONDRIAL RESPIRATORY SUPERCOMPLEX ASSEMBLY IN HF MODELS. In addition to the transcriptional down-regulation of OXPHOS, alterations in the formation of respiratory supercomplexes have been described in HF models.[11,25] Microembolism-induced HF in canines resulted in decreased state 3 and maximal mitochondrial respiration, despite similar levels of OXPHOS complex expression and activities as control animals.[43] However, complex I/complex III dimer/complex IV supercomplexes were lower in cardiac tissue from microembolism-induced HF animals, suggesting that supercomplex formation or instability may underlie the impaired mitochondrial respiration.[11] A follow-up study by the same group demonstrated a deficiency in the phosphorylation of threonine sites on complex IV as an additional potential mechanism, possibly resulting in decreased incorporation of complex IV into the respirasome.[25] The role of impaired mitochondrial supercomplex formation and differences in supercomplex composition in human HF have not yet been explored.

REGIONAL VARIATION IN MITOCHONDRIAL ABNORMALITIES. Cardiac regionality with differential mitochondrial characteristics and metabolism has been shown in animal models of cardiomyopathy and HF. In a canine DCM model, left bundle branch block ablation and pacing resulted in a down-regulation of OXPHOS, citric acid cycle, and fatty acid oxidation genes. The down-regulation in the metabolic pathways with cardiomyopathy was more pronounced in the anterior LV wall than the posterior LV wall, suggesting that regional variation in metabolic disturbances within the heart may relate to the LV contraction pattern.[49] Mitochondria from the LV of canines with pacing-induced HF had lower complex I, III, and V activities compared to tissue from control animals. In contrast, left atrial tissue from canines with cardiomyopathy had higher complex IV activity and lower complex V activity, despite similar levels of citrate synthase, suggesting that the metabolism in the various chambers of the heart are differentially affected.[50] Complex III levels were found to be lower in interfibrillar mitochondria from lateral LV tissue of paced animals compared to tissue from nonpaced animals.[49] However, mitochondrial subpopulations across other sites of the heart were unaffected by pacing, suggesting that the mitochondrial subpopulations in different cardiac walls are differentially affected by stressors.[49] Whether regional variation and subpopulations are differentially affected in human HF has not been studied.

MITOCHONDRIAL GENETICS IN HF

The OXPHOS complexes are encoded by both the nuclear and mitochondrial genomes. Located in the mitochondrial matrix, mtDNA encodes 13 subunits that form the catalytic cores of the OXPHOS complexes I and III-V as well as its own translational machinery (2 ribosomal RNAs and 22 transfer RNAs). The nuclear genome encodes the remaining approximately 88 subunits of the OXPHOS complexes, with complex II being the only entirely nuclear-encoded OXPHOS complex.[51,52] The nuclear genome also encodes several mitochondrial chaperones and import machinery that aid in the trafficking of nuclear-encoded subunits into the mitochondrion, as well as other metabolic enzymes, including additional electron donators such as electron transfer flavoprotein dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase. Hence, regulation of mitochondrial biogenesis requires the coordination of the gene expression of both genomes, coordinated by PGC1-α.[51,52]

The mtDNA has a much higher mutation rate than the nuclear genome, which has largely been attributed to replication errors, limited DNA repair capabilities, and the absence of protective wrapping around histones.[51,53,54] Unlike the nuclear genome, the mtDNA is exclusively maternally inherited, does not undergo recombination, and is almost entirely coding, with the exception of the D-loop.[51] Consequently, despite being only 16.6 kilobases in length, the genetic variation of the mtDNA is several orders of magnitude higher than that of the nuclear genome, and variants occur more frequently within protein-encoding genes.[51,55-57] mtDNA sequences can be grouped into haplogroups, consisting of collections of similar mtDNA sequences that all contain haplogroup-defining variants and a shared ancestor that is reflective of prehistoric human migrations.[58]

The mtDNA is present in approximately 6 to 10 copies per mitochondrion, and depending on the number of mitochondria per cell, each cell contains hundreds to thousands of mtDNA copies.[51] The number of mtDNA copies is proportional to the energy needs of the cell. Because of the high energy demands of the heart, cardiomyocytes contain hundreds of mitochondria and, consequently, thousands of copies of mtDNA.

In cardiac tissue from HF patients, the mtDNA copy number is reduced, reflective of a loss of mitochondria, which likely contributes to the observed down-regulation of mitochondrial respiration.[59-61] One study found that the number of mtDNA copies in circulating blood samples was associated with elevated risk of hospitalization and cardiovascular death in HF patients.[62] Additionally, mtDNA copy number in circulating blood cells is associated with LV diastolic volume and thickness, suggesting that a loss of mtDNA copies is associated with cardiac remodeling.[59] However, evaluation of temporal changes in mtDNA copy number in relation to HF development in humans has not yet been performed. However, cardiomyocyte-specific genetic ablation of mitochondrial transcription factor A (TFAM), the mtDNA transcription factor, results in mtDNA depletion and OXPHOS down-regulation, concomitant with LV dilation and increased cardiac mass, further suggesting that the loss of mtDNA copies, or mitochondria in general, contributes to cardiac remodeling.[63]

Large-scale mtDNA deletions are associated with CVD and higher risk of all-cause mortality.[64,65] LV cardiac tissue from patients with DCM had a higher number of mtDNA copies with deletions compared to tissue from either age-matched control individuals or patients with end-stage ischemic cardiomyopathy.[66] Interestingly, the frequency of large-scale mtDNA deletions was lower in cardiac tissue from patients with DCM who had received an LVAD, suggesting that ventricular pressure unloading may increase the turnover of mitochondria with mutant mtDNAs.[66] Transgenic expression of a human mutant DNA polymerase-γ that introduces deletions into the mtDNA in a murine model resulted in mtDNA depletion, impaired OXPHOS, and elevated oxidative stress.[67] Transgenic DNA polymerase-γ mice developed DCM with LV dysfunction and bradycardia and, ultimately, had a reduced lifespan.[67] These studies collectively suggest that declines in mtDNA copy number and increased mtDNA deletions may contribute to cardiomyopathy and HF.

Because of the presence of multiple mitochondria in a cell, different mtDNAs often coexist within an individual mitochondrion or population of mitochondria within a tissue, a condition termed heteroplasmy.[51] Heteroplasmic mtDNA variants can undergo clonal expansion, resulting in genetic mosaicism within a tissue or within different regions of an organ.[68-70] Although cardiomyocytes are nonreplicative cells, mtDNA replication and biogenesis are not restricted to particular phases of the cell cycle. Consequently, mitochondria with specific mtDNA heteroplasmic mutations may undergo selection during the life of the cardiomyocyte or during hypertrophy. A recent study using single-cell sequencing of blood samples from patients with a mitochondrial syndrome showed that the number of T cells carrying the pathogenic mtDNA variant was much lower than the variant allele frequencies of the pathogenic variant in other blood cell types, suggestive of cell-type-dependent purifying selection.[71] In brain tissue, particular regions have a greater accumulation of heteroplasmic mtDNA variants than others, which may contribute to the development of neurodegenerative disease.[68-70] Whether such regional variation in the mtDNA exists in the human heart has yet to be evaluated.

Mitochondrial diseases can result from sequence variation within the mitochondrial genes in either genome and typically result in cardiomyopathy, often with subsequent HF.[72] Variants in the nuclear-encoded OXPHOS subunits, mitochondrial chaperones, import machinery, mitochondrial metabolic pathways, and mtDNA replication and transcription genes are implicated in mitochondrial cardiomyopathies (**Table 1**). Furthermore, genetic manipulation of the mtDNA replication and transcriptional machinery in animal models induces maladaptive cardiac remodeling and dysfunction.[31,32,63,67]

CVD is underdiagnosed in patients with mitochondrial disease, who often show signs of cardiac

TABLE 1 Mitochondrial Mutations That Cause Cardiomyopathy and Heart Failure

Gene	Protein	Mutations
Mitochondrial translation and assembly		
GATB	Glutamyl-tRNA-amidotransferase subunit B	580_581del, 408T>G
GATC	Glutamyl-tRNA-amidotransferase subunit C	233T>G
GTPBP3	Mitochondrial GTP binding protein 3	32_33delinsGTG, 1291dupC, 1375G>A, 476A>T, 964G>C, 1009G>C
FOXRED1	FAD-dependent oxidoreductase	1054C>T
AARS2	Mitochondrial alanyl-tRNA synthetase	1774C>T, 464T>G, 647insG
ATAD3A	ATPase family AAA domain containing 3A	1582C>T
C12ORF62	Cytochrome c oxidase assembly factor COX14	88G>A
COA5	Cytochrome c oxidase assembly factor 5	157G>C
COA6	Cytochrome c oxidase assembly factor 6	
CLPB	Caseinolytic mitochondrial matrix peptidase chaperone subunit B	1249C>T
ELAC2	elaC ribonuclease Z 2	631C>T, 1559C>T, 460T>C, 1267C>T
MIPEP	Mitochondrial intermediate peptidase	1745T>G, 212T>A, 916C>T, 1804G>T, 1027A>G, 1534C>G
MRPL3	Mitochondrial ribosomal protein L3	950C>G, 49delC
MRPL44	Mitochondrial ribosomal protein L44	467T>G
MRPS14	Mitochondrial ribosomal protein S14	322C>T
MT-RNR1	Mitochondrially encoded 12S RNA	1555A>G
MT-TE	Mitochondrially encoded tRNA-glutamic acid	m.14674T>C
MT-TG	Mitochondrially encoded tRNA-glycine	9997T>C
MT-TH	Mitochondrially encoded tRNA-histidine	12192G>A
MT-TI	Mitochondrially encoded tRNA-isoleucine	4269A>G, 4284G>A, 4291C>T, 4295A>G, 4300G>A, 4317A>G
MT-TK	Mitochondrially encoded tRNA-lysine	8363G>A
MT-TL1	Mitochondrially encoded tRNA-leucine (UUR)	3243A>G, 3303C>T, 3260A>G
MT-TL2	Mitochondrially encoded tRNA-leucine (CUN)	12297T>C
MT-TV	Mitochondrially encoded tRNA-valine	1624C>T
MT-TW	Mitochondrially encoded tRNA-tryptophan	5545C>T
MT-TY	Mitochondrially encoded tRNA-tyrosine	5843A>G
MRPS22	Mitochondrial ribosomal protein S22	509G>A, 644T>C
MTO1	Mitochondrial tRNA translation optimization 1	1858dupA, 1282G>A, 1430G>A, 1232C>T, 1858_dupA
NDUFAF1	NADH:ubiquinone oxidoreductase complex assembly factor 1	1001A>C, 1140A>G, 631C>T, 733G>A
NDUFAF4	NADH:ubiquinone oxidoreductase complex assembly factor 4	194T>C
QRSL1	Glutaminyl-tRNA amidotransferase subunit QRSL1	398G>T, 850-3-A-G, 555C>A
RMND1	Required for meiotic nuclear division 1 homolog	713A>G, 613G>T
SCO1	Synthesis of cytochrome c oxidase 1	394G>A
SCO2	Synthesis of cytochrome c oxidase 2	1280C>T, 1541G>A, 1797C>T, 1634C>T, 1391C>T, 1521G>A, 107G>A, 1312-1321_10BPdup
TMEM70	Transmembrane protein 70	317A>G, 366A>T, 238C>T, 578delCA
TMEM126B	Transmembrane protein 126B	635G>T
TRMT5	tRNA methyltransferase 5	312_315del, 1156A>G
TSFM	Mitochondrial Ts translation elongation factor	856C>T, 944A>G, 997C>T
XPNPEP3	X-prolyl aminopeptidase 3	931delAACA
YARS2	Tyrosyl-tRNA synthetase 2	156C>G, 1303A>G

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remodeling in the absence of overt disease.[73,74] The mtDNA m.3243A>G variant is one of the most common mtDNA disease mutations and is associated with LV dysfunction.[75,76] In patients with mitochondrial disease caused by the m.3243A>G variant, with no known clinical CVD, the m.3243A>G variant levels correlated with increased LV mass index and reduced endocardial circumferential strain.[74] Interestingly, the m.3243A>G variant can result in either LV diastolic or LV systolic dysfunction, suggesting that the same mtDNA variant may result in different cardiac phenotypes. Cells depleted of their mitochondria with ethidium bromide and repopulated with mitochondria containing the m.3243A>G variant differentially expressed genes involved in regulating the epithelial-mesenchymal transition and fetal cardiac genes, suggestive of cardiac remodeling and fetal reprogramming, as has been observed in HF.[77] Cardiomyopathy in the setting of mitochondrial diseases often presents as increased wall thickness along with, or concomitant with, ventricular dilation, although variation in cardiac phenotypes even among those sharing the same causal variant has been noted.[78-80]

TABLE 1 Continued

Gene	Protein	Mutations
Oxidative phosphorylation		
TKFC	Triokinase and FMN cyclase	1628G>T
FLAD1	Flavin adenine dinucleotide synthetase 1	526_537delinsCA
NDUFA2	NADH:ubiquinone oxidoreductase subunit A2	IVS2DS, G-A, +5
NDUFS2	NADH:ubiquinone oxidoreductase core subunit S2	683G>A, 686C>A, 1237T>C
NDUFS4	NADH:ubiquinone oxidoreductase subunit S4	44G>A, 316C>T
NDUFS8	NADH:ubiquinone oxidoreductase core subunit S8	236C>T, 305G>A, 229C>T, 476C>A
NDUFA10	NADH:ubiquinone oxidoreductase subunit A10	1A>G, 425A>G
NDUFB11	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 11	IVS1DS, G-A, +5, 262C>T, 402delG
NDUFV2	NADH:ubiquinone oxidoreductase core subunit V2	IVS2+5_+8delGTTA, 669_670insG
SDHA	Succinate dehydrogenase complex flavoprotein subunit A	1664G>A
SDHD	Succinate dehydrogenase complex subunit D	275A>G
COQ4	Coenzyme Q4	433C>G, 421C>T, 718C>T, 197_198delGCinsAA, 202G>C
MT-CYB	Mitochondrially encoded cytochrome b	15498 G>A
UQCRFS1	Ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1	IVS1AS, G-C, -1, 610C>T
MT-CO2	Mitochondrially encoded cytochrome c oxidase subunit 2	7896G>A
COX6A2	Cytochrome c oxidase subunit 6A2	117C>A
COX6B1	Cytochrome c oxidase subunit 6B1	58C>T
COX10	Cytochrome c oxidase subunit 10	791C>A, 1211A>T
COX14	Cytochrome c oxidase subunit 14	88G>A
COX15	Cytochrome c oxidase subunit 15	700C>T, C447-3G
MT-ATP6	ATP synthase F0 subunit 6	8993T>G, 8528T>C
MT-ATP8	ATP synthase F0 subunit 8	8528 T>C
ATP5F1D	ATP synthase F1 subunit delta	245C>T
MC5DN6	ATP synthase membrane subunit DAPIT	87+1G>C, +1
PPA2	Inorganic pyrophosphatase 2	c.280A>G, 318G>T, 380C>T, 500C>T, 514G>A, 683C>T
Fatty acid oxidation		
ACAD9	Acyl-CoA dehydrogenase family member 9	130T>A, 797G>A, 1594C>T, 509C>T, 1687C>G, 4-bpINS -44TAAG promoter
ACADVL	Acyl-CoA dehydrogenase very long chain	IVS11DS, G-A, +1; IVS5AS, 1-BP del, G, -1
CPT2	Carnitine palmitoyltransferase 2	1992C>T, 439C>T, 2399A>C, 680C>T
ECHS1	Enoyl-CoA hydratase, short chain 1	473C>A, 414+3G>C
HADHA	Hydroxyacyl-CoA dehydrogenase trifunctional multienzyme complex subunit alpha	IVS3, G-A, +1; IVS3DS, A-G, +3
HADHB	Hydroxyacyl-CoA dehydrogenase trifunctional multienzyme complex subunit beta	1-BP INS, 36-BP DEL; 1364T>G
LCHAD	Hydroxyacyl-CoA dehydrogenase trifunctional multienzyme complex subunit alpha	1528G>C
Lipid and glycerolipid metabolism		
AGK	Acylglycerol kinase	IVS3DS, T-C, +2; 1170T>A, 3G>C, 517C>T, 306C>T, 841C>T, 672C>A, 1137_1143del
HSD17B10	Hydroxysteroid 17-beta dehydrogenase 10 (essential for mitochondrial tRNA processing)	257A>G
LIAS	Lipoic acid synthetase	746G>A
Branched-chain amino acid metabolism		
DLD	Dihydrolipoamide dehydrogenase	1436A>T
Apoptosis		
AIFM1	Apoptosis inducing factor mitochondria associated 1	422C>T
Glucose metabolism		
GYS1	Glycogen synthase	1384C>T
Mitochondrial dynamics		
ATAD3A	ATPase family AAA domain containing 3A	1582C>T
OPA1	OPA1 mitochondrial dynamin like GTPase	1601T>G
Cholesterol metabolism		
ATAD3A	ATPase family AAA domain containing 3A	1582C>T
Mitochondrial protein import		
DNAJC19	DnaJ heat shock protein family (Hsp40) member C19/mitochondrial import inner membrane translocase subunit TIM14	IVS3AS, G-C, -1; 1-BP DEL, 300A

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TABLE 1 Continued

Gene	Protein	Mutations
Mitochondrial DNA maintenance and replication		
FBXL4	F-box and leucine-rich repeat protein 4	1555C>T, 1303C>T, 614T>C, 106A>T
MGME1	Mitochondrial genome maintenance exonuclease 1	456G>A, 698A>G
POLG	DNA polymerase gamma, catalytic subunit	1399G>A
TOP3A	DNA topoisomerase III alpha	2271dup, 2428del
Iron-sulfur assembly		
FXN	Frataxin	(GAA)n repeat expansion, IVS1; 1-BP DEL, 157C; TGG-to-GGG in exon 5a; 371_376del6ins15
ISCU	Iron-sulfur cluster assembly enzyme	149G>A
BOLA3	bolA family member 3	123dupA
Citric acid cycle		
IDH2	Isocitrate dehydrogenase (NADP(+))2	419G>A
MLYCD	Malonyl-CoA decarboxylase	4BP DEL, 638GTGA, 119T>C
Mitochondrial structure		
MICOS13	Mitochondrial contact site and cristae organizing system subunit 13	44delC, 30-1G>A
TAZ	Tafazzin	441C>G, 1-BP INS, NT868; 1-BP DEL in exon 8; 877G>A; IVS1DS, G-C, +5; IVS3DS, G-A, +110; 352T>C, 398-2A-G, C>A substitution in exon 3; 4-BP DEL, AGTG; IVS8AS, G-C, -1; 1006G>A
Antioxidants		
NNT	Nicotinamide nucleotide transhydrogenase	211C>T
SOD2	Superoxide dismutase 2	47C>T
Carriers/shuttles		
SLC25A3	Solute carrier family 25 member 3 [mitochondrial phosphate carrier]	215G>A
SLC25A4	Solute carrier family 25 member 4	239G>A, 703C>G, 368C>A; IVS1DS, G>A, +1
SLC25A20	Solute carrier family 25 member 20	558C>T, 712A>G

Genes and mutations in the table were curated from Online Mendelian Inheritance in Man by performing a search for "mitochondrial cardiomyopathy" on January 4, 2020.

A = adenine; AAA = ATPases associated with diverse cellular activities; ATP = adenosine triphosphate; C = cytosine; CoA = coenzyme A; del = deletion; dup = duplication; FAD = flavin adenine dinucleotide; FMN = flavin mononucleotide; G = guanine; GTP = guanosine triphosphate; ins = insertion; NADH = nicotinamide adenine dinucleotide; NADP = nicotinamide adenine dinucleotide phosphate; T = thymine; tRNA = transfer RNA.

Few studies have evaluated the relation of mitochondrial genetic variation with HF. A cross-sectional study in a Spanish sample found that mitochondrial haplogroup H was overrepresented among patients undergoing heart transplantation and that individuals belonging to haplogroup UK had more frequent complications post-cardiac transplantation.[81] In a volume-overload murine HF model, mitochondrial genetic variation was shown to contribute to cardiac remodeling, mitochondrial dysfunction, and oxidative stress.[82] The abnormalities were more pronounced in mice with the C57BL/6J mitochondrial genome, irrespective of nuclear genetic background, suggesting that mitochondrial genetic variation may be an important contributor to HF pathogenesis.[82] However, whether specific mitochondrial mutations are associated with HF or modify HF onset and severity in humans is not well studied.

In mouse models, mtDNA genetic variants can also act synergistically with nuclear DNA mutations. Mice with an *ND6* missense mutation (P25L), in addition to the c.523delC mutation in the adenine translocator 1 (*ANT1*) gene, have impaired complex I activity, increased oxidative stress, greater mitochondrial fission, increased sensitivity of the permeability transition pore opening, and greater mtDNA mutational burden, all of which contribute to DCM and reduced lifespan.[83] However, mice with the c.523delC mutation in *ANT1* that have the same mtDNA but lack the *ND6* missense mutation are phenotypically unaffected.[83] Furthermore, mice lacking *NNT* were unaffected by the *COI* V421A mutation, whereas the *ND6* P25L mutation acts synergistically with *NNT* genetic ablation, resulting in earlier onset and more severe hypertrophic cardiomyopathy.[84]

In humans, mitochondrial haplogroup U modifies the severity and age of onset of cardiomyopathy in a single family with a frameshift in the cardiac-specific isoform of ANT, *ANT1*.[85] Individuals with the *ANT1* frameshift variant who belong to the mitochondrial haplogroup U had earlier onset of symptoms and required cardiac transplantation at younger ages. In contrast, individuals with the *ANT1* variant belonging to mitochondrial haplogroup H had a milder form of cardiomyopathy that did not require cardiac

transplantation.[85] Collectively, these studies suggest that mtDNA variants can modify the effects of nuclear DNA variants on cardiac phenotypes, but whether mitochondrial-nuclear genetic interactions contribute to HF subtypes in humans is not known.

MITOCHONDRIAL DYNAMICS, MITOPHAGY, AND BIOGENESIS

The lifecycle of a mitochondrion is regulated through a continuous flux of fission, fusion, mitophagy, and biogenesis. Mitochondrial networks continuously break down through fission to isolate dysfunctional sections of the network for targeted removal by mitophagy, and the remaining network reforms through fusion. Mitochondrial degradation through mitophagy is usually coordinated with mitochondrial biogenesis to replace the damaged sections of the mitochondrial network. Mitochondrial structure and networks are intimately linked with mitochondrial function, with smaller, rounder mitochondria generated through fission, producing greater amounts of oxidants and less ATP, whereas a well-integrated mitochondrial network through fusion is thought to have high membrane potentials coupled with ATP production.[86]

In the setting of HF, an imbalance toward mitochondrial fission and the accumulation of abnormally shaped mitochondria is thought to contribute to mitochondrial dysfunction and impaired cardiac function. In animal models of HF, mitochondrial fission proteins, including DRP-1 and FIS-1, are often up-regulated, while mitochondrial fusion proteins such as optic atrophy protein 1 (OPA1) are down-regulated.[87] Treatment of adult murine cardiomyocytes with mitochondrial division inhibitor 1, a DRP-1 inhibitor, reduced cell death and mitochondrial permeability transition pore opening after simulated ischemia-reperfusion. However, another study found that DRP-1 inhibition had mixed effects on cell death, despite mitochondrial and metabolic preservation.[88]

LV cardiac tissue collected from the anterior wall from patients with DCM or ischemic cardiomyopathy had lower levels of complex II and IV activities indicative of impaired OXPHOS compared to tissue from nonfailing hearts.[66] LV cardiac tissue from patients with DCM had a higher number of fragmented mitochondria, which was associated with myofibril displacement.[66] The alteration in mitochondrial morphology observed in the cardiac tissue from patients with DCM was associated with an up-regulation of PGC1-α, mitochondrial fusion proteins OPA1 and mitofusin 2, and both mitochondrial and nuclear-encoded OXPHOS peptides.[66] However, nuclear DNA- and mtDNA-encoded OXPHOS subunits, mitochondrial fusion, and biogenesis genes were not different or were down-regulated in cardiac tissue from ischemic cardiomyopathy compared to tissue from nonfailing hearts.[66] Hence, mitochondrial abnormalities differ by cardiomyopathy subtype.

Mitochondrial fragmentation and loss of cristae have been observed in subepicardial LV tissue from HF patients.[89] Mitochondrial area, measured using electron microscopy, was lower in cardiac tissue from both HFpEF and HFrEF patients compared to tissue from nonfailing hearts.[89] In HFrEF cardiac tissue, DRP-1, a mitochondrial fission protein, was up-regulated, whereas PGC1-α was down-regulated, suggestive of increased mitochondrial fission and reduced mitochondrial biogenesis. These alterations in gene expression were not observed in HFpEF patients, indicating that the lower mitochondrial volume associated with HFpEF may be mediated through alternative pathways.[89] Studies of cardiac tissue from patients with HFpEF are extremely limited, likely because of challenges in obtaining cardiac tissue from HFpEF patients, as opposed to patients with HFrEF, who undergo more procedures that lead to cardiac tissue obtainment.

Recent work using focused ion beam scanning electron microscopy has shown that mitochondria form more intricate structures within myocytes than previously appreciated.[90,91] In fact, within cardiomyocytes, multiple individual mitochondria make up subnetworks that form the mitochondrial reticula, which run longitudinally around the cell with very few perpendicular connections between the subnetworks.[91] Targeted depolarization of a small section of one subnetwork, using a photoactivated uncoupler, revealed a limited spread of the depolarization beyond the immediately affected region.[91] The limited spread of the depolarization across the network was caused by retraction of the inter-mitochondrial junctions between the dysfunctional and functional mitochondrial sections, thereby preserving the function of the surrounding subnetworks.[91] Following electrical isolation, the depolarized section of the mitochondrial reticulum retracted and was consolidated into a larger, round mitochondrion.[91] The fate of such mitochondria is not yet known, although it is speculated that these mitochondria are likely removed by mitophagy.[91]

How the mitochondrial reticulum functions in the setting of cardiovascular pathophysiology has yet to be explored.

MITOCHONDRIAL PERMEABILITY TRANSITION PORE AND HF

The mitochondrial permeability transition is the increase in the permeability of the mitochondrial inner membrane mediated by the opening of the mitochondrial permeability transition pore (mPTP). The mPTP is a nonspecific pore that allows the passage of compounds up to 1,500 Da in size. Opening of the mPTP dissipates the mitochondrial membrane potential, uncoupling respiration from ATP synthesis. The mPTP can open in short bursts, postulated to decrease Ca^{2+} in the mitochondrial matrix, or it may remain open, leading to mitochondrial swelling and necrotic cell death initiation.[92,93] The loss of cardiomyocytes caused by mPTP opening carries significant ramifications because cardiomyocytes are not replaced upon loss, resulting in permanent damage and fibrosis.

Formation and activation of the mPTP occurs in response to elevations in mitochondrial matrix Ca^{2+} and oxidants.[92,94] NADH, Mg^{2+}, protons, and ADP lower the sensitivity of the mPTP for Ca^{2+}, linking the metabolic state of the cell with cell death.[95-98] The mechanisms for mPTP activation have yet to be fully elucidated, and even the molecular composition of the mPTP remains elusive, with a number of models currently proposed.[99] Adenine nucleotide transporter, phosphate carrier, F_1-F_O-ATP synthase dimers, and the F_1-F_O-ATP synthase c-ring have been proposed as the inner membrane components of the mPTP and the voltage-dependent anion channel as the outer membrane component.[99] Cyclophilin D has been identified as a key activator of the mPTP, with inhibitors of cyclophilin D reducing cell death in response to mPTP stimuli, including ischemia-reperfusion.[99-102] Activators of the mPTP are characteristic of the cardiomyocyte milieu in HF, rousing interest in investigating the role of the mPTP in HF.[95-98]

The intricacies of cyclophilin D's interaction with the mPTP in HF pathogenesis remain unclear. Cyclophilin D-deficient mice have accelerated CVD in response to chronic pressure overload, characterized by increased hypertrophy and mortality in response to forced exercise.[103] Conversely, another study showed that cyclophilin D-deficient mice subjected to prolonged cardiac ischemia had smaller infarcts and reduced mortality compared to control animals.[104] Thus, our understanding of cyclophilin D in the setting of HF may be currently model dependent and requires additional investigation.

Arachidonic acid and the eicosanoids derived from arachidonic acid metabolism by cyclooxygenases, lipoxygenases, and cytochrome P450s have differential effects on the mPTP. Hydroxyeicosatetraenoic acids (HETEs) produced by Ca^{2+}-independent mitochondrial phospholipase (iPLA2γ) in response to Ca^{2+} increase the sensitivity of mPTP opening, which leads to further iPLA2γ activation in a positive feedback loop that ultimately leads to prolonged mPTP opening, cell death, and progression of HF in a murine model.[105] Similarly, Ca^{2+} challenge of isolated mitochondria from cardiac tissues of patients with HFrEF and from nonfailing hearts activated different phospholipases, rendering mitochondria from failing hearts more susceptible to swelling and mPTP opening.[106] Isolated mitochondria from cardiac tissue of individuals with HFrEF produced higher levels of HETEs upon Ca^{2+} stimulation and lower epoxyeicosatetraenoic acid (EET) production because of the preferential activation of the phospholipase iPLA2γ.[106] The elevation in HETEs increased mitochondrial swelling, indicative of greater sensitivity of mPTP opening in isolated mitochondria from cardiac tissue of HFrEF patients. In contrast, in isolated mitochondria from nonfailing human hearts, Ca^{2+}-dependent mitochondrial phospholipase (cPLA2ζ) was activated by Ca^{2+}, resulting in EET over HETE production from arachidonic acid, which was associated with no alterations in mitochondrial swelling or mPTP opening.[106] Pharmacologic inhibition of iPLA2γ or lipoxygenases attenuated mPTP opening in isolated mitochondria of failing human myocardium, whereas cytochrome P450 inhibition of epoxygenases stimulated opening of the mPTP, underscoring the cardioprotective nature of EETs.[105,107]

To date, most studies have focused on the role of the mPTP in the setting of ischemia-reperfusion injury, with few studies on the contribution of the mPTP in HF. The role of the mPTP in HF continues to be poorly understood, in part, because of limitations in our understanding of the molecular composition of the pore.

THE ROLE OF OXIDANTS IN HF

Mitochondria are an important source of oxidants in the failing heart. The balance of oxidants is important for regulating cellular activities. Lower levels of oxidants serve as important cellular signaling molecules activating transcription factors such as hypoxia-inducible factor 1α (HIF1α) and nuclear

factor erythroid 2-related factor 2 (NRF2) to upregulate antioxidants and certain metabolic pathways.[108] Such pathways are also activated by transient bursts of oxidant generation during brief periods of ischemia during preconditioning, which is cardioprotective.[109-111] Although cells have an antioxidant detoxification system in place to maintain the redox environment, if the antioxidant system is overwhelmed, the excess oxidants induce oxidative stress, resulting in damage to lipids, proteins, and DNA. Sustained or high enough oxidant production may even trigger cell death pathways, as observed in ischemia-reperfusion models.[37,112]

Most studies of the role of mitochondrial oxidative stress in HF are in the setting of ischemic cardiovascular disease. During ischemia, OXPHOS is inhibited because of the loss of oxygen, resulting in a depolarization of the mitochondrial membrane and a switch from fatty acid oxidation to glucose utilization via glycolysis, driving increases in metabolic byproducts such as lactate and succinate.[37,112] Upon reperfusion, oxygen stimulates OXPHOS, but the reinstatement of oxygen leads to a rapid increase in oxidants that may trigger the mPTP with subsequent cell death and fibrosis.[37]

UPSTREAM METABOLIC PATHWAYS CONVERGE ON OXPHOS

Blockade or impairments in OXPHOS drive upstream alterations in metabolic pathways to sustain ATP levels and limit oxidant production. Blockade in the metabolic pathways is often the result of a decline in the availability of cofactors or product inhibition as a result of the accumulation of metabolic intermediates. The alternations in the levels of metabolic cofactors and intermediates drives posttranslational modifications and the activation or inhibition of pathways that are regulated by the metabolites, driving transcriptional changes. Because metabolic pathways are closely intertwined via the shared reducing equivalents (NADH, $FADH_2$, NADPH) and common substrates such as acetyl coenzyme A (acetyl-CoA), the impact of one metabolic pathway has a ripple effect, altering the rest of the integrated metabolic system, with important implications in HF.

SUBSTRATE SWITCHING IN HF. Initiated more than 106 years ago, seminal work characterizing the metabolism of the heart has laid the foundation and framework of the core tenets in cardiac metabolism.[113,114] The hierarchy of mitochondrial substrate preference of fatty acids over glucose, termed the "Randle effect," was first described in 1963.[115] The cardiac fuel preference for fatty acids was later validated in classic studies of cardiac metabolism.[116,117] Since the 1990s, researchers have evaluated the role of myocardial metabolism in cardiomyopathy and HF with increasing granularity.

The heart's constant contractile function is deftly maintained through metabolic fuel flexibility, the ability to utilize a variety of substrates to produce NADH and $FADH_2$, the electron donors for mitochondrial electron transport and ATP generation, and NADPH for maintaining a redox homeostasis. The reducing equivalents are required for multiple metabolic processes; consequently, the reducing equivalents link the metabolic pathways together, ensuring a coordinated and sustained generation of ATP. In pathologic states, such as ischemia and HF, the demand for ATP outweighs the supply, which is coupled with a loss in the elasticity of substrate utilization and an imbalance in oxidized to reduced NAD^+/NADH, FAD/$FADH_2$, and NADP/NADPH. How the imbalance in reducing equivalents contributes to the loss of metabolic flexibility in HF is not well understood.

Comprehensive reviews of cardiac metabolism in various animal models of HF are beyond the scope of this review and have been discussed elsewhere[118-120]; however, important overarching themes have emerged. Under homeostatic conditions, the heart relies on fatty acids for 60% to 90% of its fuel substrate, followed by glucose and other substrates.[4] Hormones (insulin, catecholamines) and substrate availability are the key factors that direct fuel utilization. In conditions that deplete the preferred fatty acid substrates (fasting, strenuous exercise), the heart adeptly switches to alternative substrates, including ketones and lactate, to sustain ATP generation. In contrast, the metabolic elasticity to shift substrates is lost in cardiomyopathies.[6] In HF animal models, cardiac fatty acid oxidation is reduced with a concomitant increased dependence on glucose.[121-123]

METABOLOMIC PROFILING IN HUMAN HF. Metabolites not only serve as clinical biomarkers of CVD, but the metabolomic patterns also provide mechanistic insight into upstream pathway aberrations.[124] An accumulation of intermediary metabolites within a pathway is often indicative of a metabolic bottleneck. Alterations in the flux of metabolites are revealed by lower levels in the primary pathway and an accumulation of intermediates in the alternative metabolic pathway, thereby pointing to possible mechanisms of metabolic abnormalities.

Circulating acylcarnitines are elevated in patients with HF, reflective of an impairment in fatty acid oxidation. Acylcarnitine and β-hydroxybutyrylcarnitine are elevated with LV dysfunction in humans following ischemia-reperfusion, supporting

the concept of a down-regulation of oxidative fuel metabolism with redirection toward anaerobic metabolism.[125] Using a targeted metabolomics approach, elevations in acylcarnitines were similar between individuals with HFrEF, regardless of diabetic status, across all species, with the exception of C2-carnitine, which was higher in patients with HFrEF and diabetes.[126] In a subgroup and validation cohort, very-long-chain acylcarnitines were found to be higher in plasma from patients with HFrEF, and a number of the long- and very-long-chain acylcarnitines delineated individuals with HF from non-HF referent individuals. The elevation in very-long-chain acylcarnitines with HFrEF suggests a possible impairment in peroxisomal fatty acid metabolism, a process that requires oxygen, which has yet to be further explored.

Circulating metabolomic patterns have been used to discriminate between different subtypes of HF. Using the CATHGEN (CATHeterization GENetics) biorepository, well-selected, clinically stable individuals were classified as HFpEF, HFrEF, and non-HF referent individuals, and 60 plasma metabolites were quantified in a targeted approach. Long-chain acylcarnitines were found to increase linearly with declining LVEF.[127] Although the source of the circulating long-chain acylcarnitines remains to be determined, the acylcarnitines may be key mediators in inflammation, metabolic inflexibility, cell stress, and arrhythmic disturbances.[127]

Zordoky et al[128] corroborated the findings of increased long-chain acylcarnitines in HFpEF compared to non-HF referent individuals and those with HFrEF. Octanoyl-carnitine, arginine, and sphingomyelin (C20:2), along with N-terminal pro-B-type natriuretic peptide, reliably distinguished HFpEF from non-HF referent individuals and may represent novel biomarkers for HFpEF.[128] Long-chain acylcarnitines, short-chain dicarboxylacylcarnitines, and amino acids (asparagine/aspartic acid) were increased in the sildenafil treatment arm compared to the placebo arm of a negative trial of sildenafil treatment for exercise outcomes in HFpEF. This additional analysis provided the insight that mitochondrial dysfunction, heightened with sildenafil treatment, may have contributed to the lack of effect observed in the trial.[129] Furthermore, this study illustrates the utility of metabolomics for monitoring the effectiveness of interventions in HFpEF.

The metabolic profiles of earlier stages of HF have been characterized by sampling the circulation rather than cardiac tissue. In stage C HFrEF, a 4-metabolite panel consisting of histidine (lower), phenylalanine (higher), spermidine (higher), and phosphatidylcholine C34:4 (lower) improved predicted diagnosis of HF above conventional methods and showed increased strength for prognostication, including mortality over B-type natriuretic peptide.[130] Importantly, individuals with stage C HF in this study largely had reduced LVEF and were hospitalized individuals with acute or decompensated HF.

Few studies have evaluated the metabolic profile of human cardiac tissue. Cardiac tissue from patients with late-stage HF (stage D) obtained at the time of LVAD placement or cardiac transplantation was used to evaluate the underlying metabolic abnormalities associated with HF.[131] Consistent with prior studies, mitochondrial respiration was lower in cardiac tissue from patients with advanced HF, suggestive of impaired OXPHOS. The lower OXPHOS capacity was coupled with lower medium-chain fatty acid oxidation, which supports preclinical research of myocardial substrate shifts away from fats toward glucose in HF.[132] Elevated intracellular lactate levels were observed in pre-LVAD myocardial biospecimens compared to referent tissue, indicative of an increased reliance on glycolysis.[133] In paired tissue from the same individuals collected at both the time of LVAD placement and later, at heart transplantation, metabolomics analysis revealed an increase in glycolysis without concomitant improvements in mitochondrial respiration with LVAD unloading of the heart, although significant variation in responses was noted.[131] Whether mitochondrial function improved in "responders" to LVAD therapy was unclear because of the small sample size and will require further study.

To date, most of the metabolomic studies performed in humans were targeted analyses, primarily restricted to metabolites of fatty acid oxidation, in plasma. Untargeted metabolomics approaches may reveal novel metabolic mechanisms in HF. Metabolomic analysis of cardiac tissue from non-HF referent individuals and patients with HF could lend additional insights into the pathobiology of HF, especially across HF subtypes.

REDUCTIVE STRESS: THE NADH/NAD$^+$ RATIO. The ratio of NADH/NAD$^+$ serves as a key regulator of many of the metabolic enzymes. Metabolites reflective of the NADH/NAD$^+$ ratio have been proposed as biomarkers of mitochondrial dysfunction.[134] NAD$^+$ is required for glycolysis and pyruvate oxidation to acetyl-CoA before entry into the citric acid cycle. NAD$^+$ is also essential at various points in the citric acid cycle and may be obtained by anaplerosis to sustain citric acid cycle flux. The metabolic pathways converge on OXPHOS by supplying the electron

donors in the form of NADH and $FADH_2$ for electron transport, driving proton pumping across the inner mitochondrial membrane with subsequent ATP generation at ATP synthase. Although $FADH_2$ and NADPH are critical coenzymes for the reduction-oxidation reactions in living cells, the NADH/NAD^+ ratio, signifying reductive stress, is particularly important in HF and will be the center point of this focused discussion. Comprehensive discussions regarding FAD(H) and NADP(H) in cellular function and energy metabolism are reviewed elsewhere.[135-137]

The mammalian mitochondrial membrane is impervious to NADH,[135,138] and consequently, 2 main NADH cellular pools exist—the cytosolic and the mitochondrial pools—with approximately 75% of the cellular pool residing in the mitochondria in cardiomyocytes.[135] Two NADH shuttles, the malate-aspartate shuttle and the glycerol 3-phosphate shuttle, transport NADH into the mitochondrial matrix.[139] NAD(H) is predominantly bound to intracellular proteins, and the free concentrations are lower than the total concentrations.[139] Importantly, the NADH/NAD^+ mitochondrial pool is maintained in a more reduced state to optimize the thermodynamic favorability of OXPHOS in mitochondria and glycolysis in the cytosol.

The activation of sirtuins by NAD^+ has been a key rationale of targeting NAD^+ to combat metabolic disturbances and mitochondrial dysfunction in HF. Sirtuins are NAD^+-dependent deacetylases that reverse acetyl modifications of lysine residues on histones and other proteins.[139] Mitochondrial protein acetylation caused by an NADH/NAD^+ imbalance is associated with mitochondrial dysfunction in animal models and cardiac tissue from patients with ischemic or DCM.[140] Sirtuins alter cellular metabolism to promote OXPHOS and fatty acid oxidation. For example, SIRT1 deacetylates and activates FOXO1 and PGC1-α, whereas SIRT3 deacetylates complexes I and II and long-chain acyl coenzyme A dehydrogenase.[136] A novel murine model of HFpEF induced using a combination of high-fat diet and constitutive inhibition of nitric oxide synthase is characterized by reductive stress, and treatment with an NAD^+ donor or with direct NAD^+ biosynthesis stimulation improved cardiac function and exercise tolerance.[141,142] The effects of NAD^+ repletion on improved cardiac function were partly mediated by SIRT3 reactivation with subsequent deacetylation of a key enzyme involved in fatty acid oxidation.[141]

Growing evidence suggests that an NADH/NAD^+ imbalance plays a role in HF, but the translation to humans has been challenging because of limited access to human cardiac tissue. Because sampling compartmental NADH/NAD^+ ratios in tissues (ie, myocardium) is extremely challenging in humans, measurement of circulating metabolites in plasma reflective of cytosolic and mitochondrial NADH/NAD^+ levels may circumvent the need for tissue sampling.[143] However, such findings will require validation at the tissue level because circulating metabolites are likely reflective of whole-body metabolism and may not reflect cardiac metabolism. Substrates and products of NAD(H)-linked dehydrogenases can be utilized to estimate NADH/NAD^+ ratios, provided the selected dehydrogenase catalyzes a near-equilibrium reaction that is confined to 1 subcellular compartment.[139] The lactate/pyruvate ratio captured extracellularly (eg, plasma) harnesses the lactate dehydrogenase reaction for the cytosolic redox state. Similarly, α-ketobutyrate/α-hydroxybutyrate was found to be reflective of NADH-reductive stress in a study of inherited mitochondrial disease.[143]

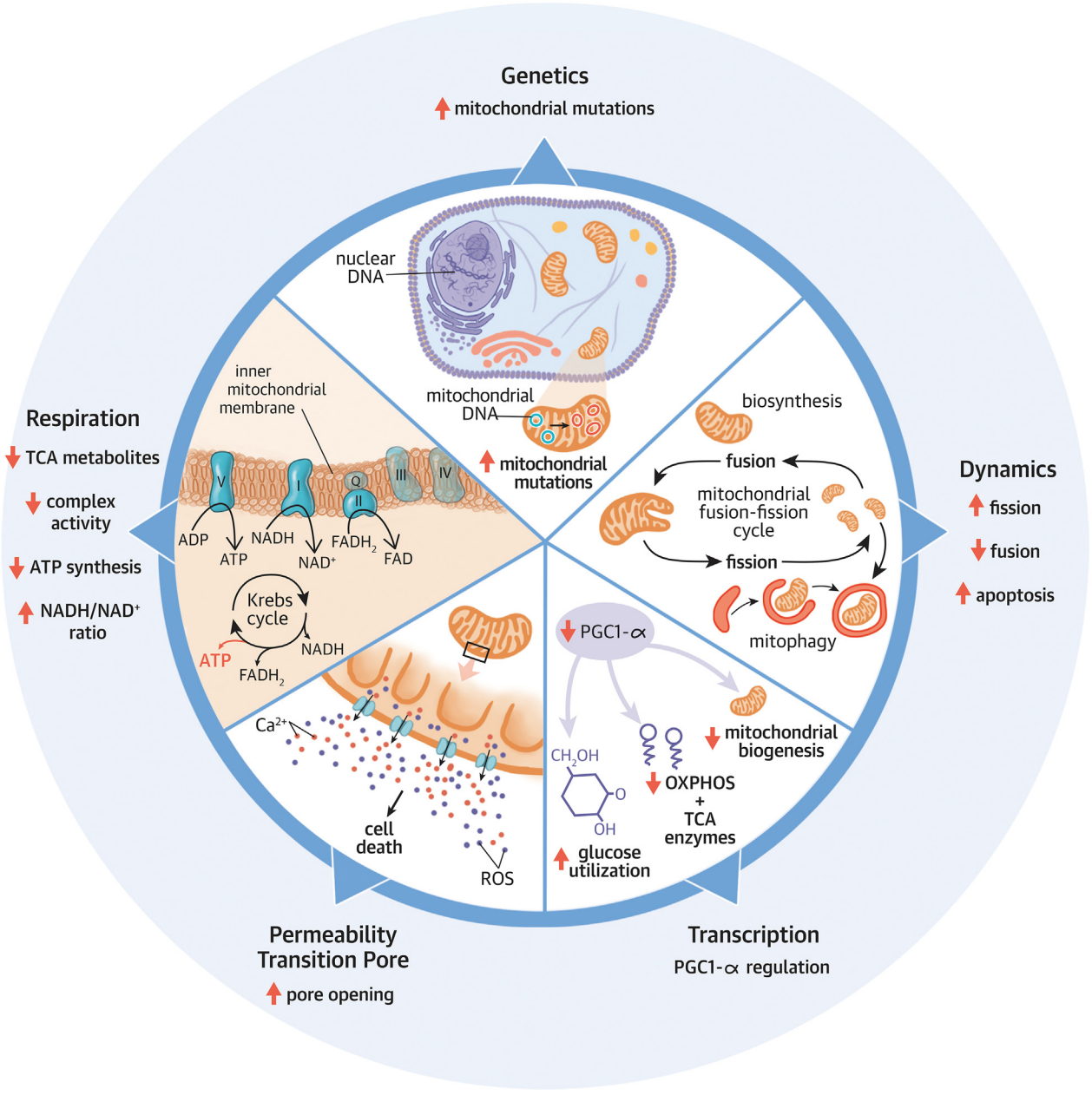
Few studies have quantified circulating metabolites indicative of reductive stress in the setting of HF. Because of the impairment in OXPHOS, one would expect NADH/NAD^+ ratios to be elevated in the setting of HF. High levels of NADH/NAD^+ would result in product inhibition of a number of metabolic enzymes across pathways, which may drive increases in the intermediates of the citric acid cycle and fatty acid oxidation, as is commonly observed in HF. Utilization of nontargeted metabolomics across well-matched and phenotyped patients with HF will be needed to determine the role reductive stress may play in HF.

THE MITOCHONDRIAL ECOSYSTEM

Mitochondria not only supply the ATP necessary for contraction and relaxation, but they also play a role in Ca^{2+} buffering and storage, apoptosis, and heme and steroid hormone biosynthesis. Furthermore, mitochondria play central roles in signaling pathways through metabolic intermediates and byproducts, such as NADH/NAD^+ and oxidants, allowing for rapid responses to environmental stimuli that integrate the stress response across the entire metabolic system. For example, increased Ca^{2+} uptake by the mitochondria serves to match metabolism with the energetic needs of the heart by stimulating citric acid cycle dehydrogenases, which regenerate oxidized NAD^+ and FAD to increase electron flux into the respiratory chain. The citric acid cycle is also an important source of NADPH, which is required for regenerating glutathione and peroxiredoxin, key mitochondrial antioxidants. Increased mitochondrial

JACC: BASIC TO TRANSLATIONAL SCIENCE VOL. 7, NO. 11, 2022
NOVEMBER 2022:1161–1179

CENTRAL ILLUSTRATION **The Mitochondrial Ecosystem in Heart Failure**



Schwartz B, et al. J Am Coll Cardiol Basic Trans Science. 2022;7(11):1161–1179.

The mitochondrial ecosystem consists of an intricate system of metabolic pathways and dynamic changes in mitochondrial networks that exist in a delicate balance. Perturbations in any section of the mitochondrial ecosystem has a ripple effect, altering upstream and downstream metabolic and cellular signaling pathways that are modified by mitochondrial genetic variation and mutations. ADP = adenosine monophosphate; ATP = adenosine triphosphate; $FADH_2$/FAD = flavin adenine dinucleotide; NADH/NAD^+, nicotinamide adenine dinucleotide.

Ca^{2+} uptake in HF leads to impaired mitochondrial respiration with a decrease in mitochondrial membrane potential, resulting in lower levels of reducing equivalents (NADH, $FADH_2$), anaplerotic scavenging, increased sensitivity to mPTP opening, and redox imbalances.

Although short bursts of ischemia and reperfusion are cardioprotective because of the activation of the

nuclear respiratory factors and HIF1α, the longer durations of ischemia with subsequent reperfusion in ischemic heart disease drives an increase in mitochondrial oxidant production that is damaging to the cellular components, including mitochondrial enzymes, and may even trigger cell death.[37,109-112] Without removal of dysfunctional mitochondria from the network/reticulum through mitophagy, impaired respiration results in reverse electron flow through the chain, increasing oxidant production through complex I.[37,112,144] Without a functional respiratory chain, accumulating NADH and acetyl CoA inhibit fatty acid oxidation and key enzymes in the citric acid cycle. As a result of inhibition of the citric acid cycle, pyruvate accumulates and is shunted toward lactate production. Furthermore, elevated NADH to NAD^+ inhibits pyruvate dehydrogenase, thereby inhibiting glucose oxidation and driving glycolysis.

Alterations in metabolic pathways and the redirection of metabolites may also modify the substrates available for posttranslational modifications of proteins, particularly OXPHOS complexes and histones, resulting in changes in signaling pathways and nuclear gene expression that promote cardiac remodeling. An imbalance in the NADH/NAD^+ alters the transcriptional activity of the sirtuins, which require NAD^+ for activation. The sensitivity of mitochondria to environmental stressors may be enhanced and augmented by mitochondrial genetic variation, although our understanding of the underlying mechanisms remain limited. Finally, mitochondrial morphology, networks, and reticula within the cardiomyocytes are associated with changes in metabolic activities that have only recently been appreciated. Thus, one small alteration has a ripple effect of disturbances across the entire mitochondrial ecosystem (**Central Illustration**).

CONCLUSIONS AND FUTURE DIRECTIONS

Mitochondrial abnormalities have long been described in the setting of cardiomyopathies and HF, yet the advancement in our understanding of the mechanisms and spatial and temporal changes in mitochondrial function remain poorly understood. Most of the literature to date has focused on a switch from fatty acid oxidation to glucose utilization, related to the down-regulation of mitochondrial respiration to maintain ATP generation. Ischemic injury in the heart results in a down-regulation in mitochondrial respiration, aimed to alleviate oxidative stress to prevent further cell damage and death. The down-regulation in mitochondrial respiration drives up-stream alterations in metabolic pathways, resulting in a greater dependence on glycolysis and amino acid scavenging via anaplerosis. Anaerobic respiration has the advantage of limiting reactive oxygen species generation, but it is also an ineffective means for generating ATP. The high energetic demands of the heart, especially following ischemic injury and in the setting of volume overload, place a greater cardiac demand for ATP that cannot be sufficiently met by anaerobic respiration.

Many unanswered questions remain regarding the role mitochondrial dysfunction plays in HF largely because of the limitations in animal models, collapsing all HF subtypes together despite distinctly different phenotypes, and few studies using cardiac tissue from humans. Our understanding of the subtypes of HFpEF has been limited by animal models that do not adequately recapitulate the spectrum of phenotypes observed in patients. In the limited number of studies evaluating cardiac tissue from patients, different mitochondrial abnormalities are present in the setting of different cardiomyopathies and HF phenotypes. However, a majority of studies continue to group HFpEF and HFrEF together, not distinguishing between the 2 very different pathogenic processes that likely have differing underlying mechanisms. The HF phenotypes are likely modified by the comorbidities, especially in HFpEF, although defining the heterogeneous subtypes remains uncertain. Furthermore, cardiac tissue sampling in HF patients has largely been limited to HFrEF because of greater procedural access, thus limiting insights into the cardiac phenotype in HFpEF.

The metabolic processes within mitochondria are interconnected, allowing for adaptations to stressors and substrate switching to sustain ATP levels. The metabolic pathways are coordinately regulated by ADP, metabolites, oxidants, and Ca^{2+}, thereby altering the entire ecosystem. Mitochondrial genetic variation likely modifies the mitochondrial signaling and response to stressors, which may contribute to differences in cardiac resilience and remodeling. However, to date, studies evaluating the role of individual pathways or mechanisms have failed to take this into account.

With the recent surge in studies relating mitochondrial dysfunction to HF, multiple strategies for the modulation of mitochondrial function have been proposed. Targeting mitochondrial function as a therapy for HF is no small task insofar as it requires consideration of the mitochondrion's complex and dynamic biology. Furthermore, it requires devising

reliable and meaningful clinical study endpoints to assess the response in HF. Nevertheless, therapies targeting the mitochondrion and cardiac metabolism represent an exciting divergence from current neurohormonal therapies and have the potential to provide both prognostic and symptomatic benefit in HF.

Reducing mitochondrial function to 1 or 2 measures provides a limited snapshot into the greater complexity and pathophysiology of HF. Our understanding of the mechanisms for mitochondrial dysfunction in HF phenotypes is nuanced and requires multidisciplinary, translational approaches. Rather than a reductionist view, it will be critical that we take a systems biology approach to advancing our understanding of the different HF phenotypes and mechanisms underlying the disturbed mitochondrial ecosystem in cardiac pathobiology.

FUNDING SUPPORT AND AUTHOR DISCLOSURES

This research was supported by the National Institutes of Health Multidisciplinary Training for Promoting Research in Medical Residency (1R38HL143584 to Dr Schwartz); National Heart, Lung, and Blood Institute (K01 HL143142 to Dr Fetterman); and an American Heart Association grant (17FTF33670369 to Dr Gopal). All authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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KEY WORDS **cardiomyopathy, heart failure, metabolism, mitochondria, mitochondrial ecosystem**