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Metabolic Stress in the Myocardium: adaptations of gene expression

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Abstract

The heart is one of the highest ATP consuming organs in mammalian organisms. Its metabolic function has evolved a remarkable degree of efficiency to meet high demand and plasticity in response to varying changes in energy substrate supply. Given the high flux of energy substrates and the centrality of their appropriate use for optimal cardiac function, it is not surprising that the heart has intricate signaling mechanisms through which it responds to metabolic stress. This review focuses on the changes in gene expression in myocardial and vascular tissues during metabolic stress that affect mRNAs and subsequent protein synthesis with an eye toward understanding the manner in which these changes effect adaptive and maladaptive responses of the heart.

Keywords

diabetic cardiomyopathy; pathological hypertrophy; ischemia-reperfusion; nutrient deprivation; metabolic adaptation and maladaptation; nuclear receptor transcription factor; histone deacetylation; miRNA; protein translation

To maintain bioenergetic homeostasis, the myocardium is poised to reconfigure its metabolic program during states that alter caloric content and abundance, hemodynamic load, and oxygenation. Cytoplasmic signal transduction cascades and nuclear programming drive mechanisms that regulate protein synthesis and turnover, organelle function, and metabolic flux. The purpose of this review is to reveal the myocardial adaptations of gene regulation that are enlisted during three commonly-encountered states of metabolic stress: (1) metabolic substrate excess and diabetes; (2) pressure overload; and (3) ischemia-reperfusion. Evidence for these adaptations comes from physiological studies of humans and rodent models as well as from biochemical and genetic analyses of the various cell types resident in the heart. We will focus on dynamic gene regulation that directly influences metabolic flux and substrate selection in these contexts, and will integrate DNA-bound transcription factors with chromatin modifications, non-coding RNA regulatory mechanisms, and translational regulation.

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Disclosures

None.

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1. Cellular responses to States of Myocardial Metabolic Stress

While omnivorous and metabolically flexible, the normal heart predominantly exploits the high-energy yield of fatty acid β -oxidation to generate ATP for its mechanical, electrical, and homeostatic activities [1, 2]. However, prolonged ingestion of excess calories through overnutrition increases delivery of fatty acids and undermines systemic lipid homeostasis secondary to insulin resistance. The heart is initially able to increase fatty acid oxidation, but when this capacity is exceeded, fatty acids are diverted to neutral lipid pools and to alternate metabolic fates within the myocardium. These responses contribute to metabolic inflexibility, bioenergetic inefficiency, insulin resistance of the myocardium itself, cellular damage and contractile dysfunction [3–6]. Moreover, these metabolic abnormalities may underlie, in part, increased heart failure and worse outcomes in coronary artery disease that significantly impact morbidity and mortality in diabetes [7–9]. Metabolic adaptations of the myocardium to caloric excess and diabetes are classically accompanied by gene expression programs that support increased cellular processing of fatty acids: uptake and esterification to CoA (CD36, fatty acid transport protein 1, and long-chain acyl-CoA synthetase 1), intracellular transport (fatty acid binding protein), esterification to glycerol backbones (glycerol-3-phosphate acyltransferase and diacylglycerol acyltransferase), and β -oxidation [carnitine palmitoyltransferase 1 (CPT1) and medium chain acyl-CoA dehydrogenase]. Furthermore, augmentation of the mRNAs encoding mediators of mitochondrial uncoupling (mitochondrial thioesterase 1, uncoupling proteins 2 and 3) and inhibition of glucose oxidation (pyruvate dehydrogenase kinase 4) are also observed [5, 10, 11]. Related shifts in gene expression occur in the setting of nutrient deprivation, when the myocardium becomes highly dependent on fatty acids and ketone bodies, reducing its glucose utilization [12].

Pathological hypertrophy commonly occurs in response to pressure (e.g., hypertension, aortic coarctation) or volume (e.g., left-to-right shunt) overload, or after myocardial infarction. The compensated hypertrophic phase is accompanied by marked changes in gene expression, and is considered an adaptive response to diminish wall stress associated with increased strain [13]. Pathological hypertrophy is coordinated by cytoplasmic signal transduction cascades, including multiple arms of the mitogen-activated protein kinase pathway, the calcineurin pathway, the PKA and PKC pathways, the phosphatidylinositol 3-kinase (PI3K)/Akt/glycogen synthase kinase (GSK) pathway, and the Ca^{2+} /calmodulin-dependent protein kinase II pathway, all of which are engaged by both ‘stretch’ sensors, and by locally-produced and systemically circulating neuroendocrine mediators. In turn, these pathways transduce a subset of their signals to the nucleus, resulting in marked reprogramming of the cardiomyocyte at the level of gene expression and chromatin structure. From prognostic, physiological, and molecular mechanistic perspectives, it is important to differentiate pathological from the process of physiological hypertrophy, which occurs in trained athletes. While the cytoplasmic and nuclear signaling pathways engaged by these programs are distinct, there is overlap. Details of the metabolic shifts that differentiate these two forms of hypertrophy were recently reviewed [14, 15]. In particular, the serine/threonine kinase Akt, downstream of insulin/insulin receptor substrate/PI3K signaling, serves as an integrative node in the development of physiological and pathological forms of hypertrophy. When constitutively active (via myristoylation) Akt1 is conditionally overexpressed in cardiomyocytes in vivo, the heart initially exhibits an adaptive growth phase with preserved systolic function and enrichment of myocardial transcripts that are associated with physiological growth [16, 17]. Moreover, cardiomyocyte Akt1 is required for the development of physiological hypertrophy [18]. Conversely, chronic constitutive Akt1 activation results in the development of pathological hypertrophy that includes fibrosis, altered homeostasis of glucose uptake, and systolic dysfunction [16, 19] – but Akt1 knockout mice also develop an exacerbated form of pathological hypertrophy [18]. The provocation of similar phenotypes in both absence and excess of discrete cellular signals is

not uncommon, as this review will highlight, creating challenges in the development of targeted therapies.

Classically-described alterations of gene expression during cardiac hypertrophy include induction of the encoded fetal sarcomeric isoforms, the natriuretic peptides, mediators of inflammation and fibrosis, glycolysis, and apoptosis [20]. While the time course of measured metabolic shifts in the pathologically hypertrophied heart remain debated, fatty acid β -oxidation becomes reduced, and this diminution is accompanied by decreased mRNA abundance of encoded enzymes in the pathway, including CPT1. While reduction of glucose oxidation rates appears to be a later component in the evolution from compensated to decompensated hypertrophy, glycolysis rates, and its encoded mediators, are increased [15]. Interestingly, anaplerotic entry of pyruvate into the tricarboxylic acid (TCA) cycle, relative to entry rates of pyruvate through acetyl-CoA into the cycle via citrate synthase, is augmented in hypertrophied hearts, which can be linked to increased expression of the cytoplasmic isoform of the malic enzyme [21]. In the failing heart, rates of oxidative metabolism and indices of normal mitochondrial function are both reduced, as the heart becomes more reliant on the lower yield glycolytic pathway [5, 15, 22, 23].

Much of the myocardial metabolic response to acute ischemia occurs through signaling cascades independent of changes in gene expression: increased peripheral lipolysis and mobilization of free fatty acids, activation of the AMP-activated protein kinase (AMPK), cardiomyocyte plasma and mitochondrial inner membrane remodeling due to phospholipase activation, impairment of fatty acid β -oxidation and oxidative phosphorylation, and shifts of intracellular ionic and redox potentials [5]. Acute reperfusion is associated with re-activation of β -oxidation. In the post-infarction remodeling phase, myocardial β -oxidation is reduced, which can be linked to reduced expression of mediators of fatty acid transport and oxidation [24]. Numerous transcription factors influence remodeling post ischemia or infarction. Taken together, modifications of gene regulation fundamentally reprogram cell function and adaptation to multiple forms of metabolic stress.

2. Adaptation to metabolic stress by transcription factors

2a. Nuclear Receptor Transcription Factors

A plethora of transcription factors coordinate the metabolic responses to stress in the myocardium (Table 1). The peroxisome proliferator-activated receptors (PPARs), nuclear receptor transcription factors that bind to site-specific DNA sequences as heterodimers with retinoid X receptors (RXR), serve as integrated coordinators of multiple forms of metabolic stress. For PPARs, *cis*-elements are organized as repeated 'half-site' hexad sequences separated by a single nucleotide, with one half-site bound per monomer of the PPAR-RXR complex [25]. These 'direct repeat 1' elements are commonly encountered in the promoters of genes encoding mediators of fatty acid transport and β -oxidation [26]. Constitutively, PPARs repress transcription through interaction with a complex of co-repressor histone deacetylases (HDACs), maintaining chromatin in a condensed conformation. When fatty acid and eicosanoid ligands bind PPARs and induce conformational changes in the PPAR-RXR heterodimer, co-repressors are released, and co-activator histone acetyltransferases are recruited, opening chromatin and favoring transcriptional activation in a site-specific manner [25, 27]. Three mammalian PPAR family members, PPAR α , PPAR δ , and PPAR γ , are encoded by distinct genes, and exhibit unique tissue distributions and ligand specificities – PPAR α , is a target for the hypolipidemic fibrate drug class, and PPAR γ is a target for insulin-sensitizing thiazolidinediones [28]. All three PPARs are expressed in cardiomyocytes to varying extents, and demonstrate overlapping and distinct properties in the context of metabolic stress, which will be described below.

Genetic gain- and loss-of-function tools have provided insight into the roles of these nuclear receptors in cardiovascular pathophysiology. When specifically overexpressed in cardiomyocytes of transgenic mice using an α -myosin heavy chain (α -MHC) promoter, PPAR α causes increased expression of the genes encoding mediators of fatty acid uptake and oxidation, decreased expression of the insulin-responsive glucose transporter type 4 (GLUT4), decreased glucose oxidation, and predisposition to the development of cardiomyopathy – all of which mimic features of diabetic cardiomyopathy [29, 30]. Given the importance of the GLUT1 transporter in cardiomyocyte glucose transport [31], which is less insulin-responsive than GLUT4 [32, 33], the role of *Glut4* mRNA diminution is likely to be a partial contributor to this decreased glucose oxidation phenotype. Cardiomyocyte-specific loss of HDAC3, a negative regulator of PPAR α -induced transcription, recapitulates much of the α -MHC-PPAR α phenotype [34]. On the other hand, therapeutic PPAR α ligands (fibrates) have not been associated with cardiotoxicity, a finding attributed to their relative selectivity on liver, which also abundantly expresses PPAR α [35]. Additional insight into the relationship between myocardial lipid flux and PPAR α may be gleaned from examination of germline knockout mice for adipose triglyceride lipase (ATGL) [36, 37]. Instead of suffering from excess of lipid delivery, this model of neutral lipid storage disease reveals the consequences of decreased turnover of myocellular triacylglycerols, provides indirect evidence that PPAR α ligands derive from these stores, and suggests that maintenance of homeostatic PPAR α activity is critical for appropriate myocardial lipid handling. The hearts of ATGL knockout mice exhibit decreased expression of PPAR target genes, including the co-activators PPAR γ co-activator (PGC)-1 α and PGC-1 β , which are key regulators of mitochondrial bioenergetic function [38–40]. ATGL knockout mice exhibit severe myocardial lipid accumulation, and lethal cardiomyopathy that are reversed by administration of synthetic PPAR α ligand. These results suggest that intracellular genesis of PPAR α ligands requires ATGL for production and/or delivery to the receptor, and underscore the importance of PPAR α in myocardial lipid metabolism. To confirm the cardiomyocyte autonomous nature of ATGL knockout mouse phenotypes, highly penetrating analysis of the novel cardiomyocyte-specific knockout will be very useful in the future, because PPAR α deficiency does not phenocopy the effects of ATGL deficiency [41, 42]. Moreover, development of tissue-specific PPAR α knockout mouse strains will provide additional mechanistic insight into the role of systemic versus cell-specific PPAR α signaling in myocardial lipid metabolism, mitochondrial function, and cardiac function.

Mice that overexpress PPAR γ specifically in cardiomyocytes exhibit a similar phenotype to that observed in α -MHC-PPAR α mice, i.e. increased expression of genes that encode mediators of fatty acid oxidation, increased triglyceride storage, disrupted mitochondrial function, dilated cardiomyopathy, and lethal ventricular arrhythmias [43, 44]. Insulin responsiveness is maintained in the hearts of α -MHC-PPAR γ mice, in keeping with the insulin sensitizing effects of PPAR γ . Intriguingly, increased myocardial fatty acid oxidation, and elevated triacylglycerol, diacylglycerol, and ceramide pools persist in myocardium of α -MHC-PPAR γ mice that are bred onto a PPAR α knockout background. However, several important features are improved in this genetic context: myocardial acylcarnitine content is decreased, protein kinase C (PKC) activation and reactive oxygen species (ROS) are decreased, and the dilated cardiomyopathy improves [45]. These findings support the notion that the three myocardial PPARs function through overlapping, but intricately refined mechanisms. Lipotoxicity caused by myocardial PPAR γ overexpression in mouse models may provide insight into the cardiotoxicity that is observed in humans managed on thiazolidinedione therapy for diabetes. However, this connection should be drawn cautiously, as some thiazolidinediones appear to influence PPAR γ -independent targets, and other compound classes that target PPAR γ function are not associated with cardiotoxicity [46–49]. It is also possible that in select metabolic states, PPAR γ activation in cardiomyocytes may be adaptive.

PPAR γ influences the course of pressure overload-mediated hypertrophy through cardiomyocyte autonomous and non-autonomous mechanisms. Through a mechanism dependent on transcriptional induction of its gene by the basic helix-loop-helix transcription factor hypoxia-inducible factor-1 (HIF)-1, PPAR γ activates genes that encode mediators of fatty acid uptake and glycerolipid biosynthesis [50]. While thiazolidinediones cause cardiac hypertrophy – one reason their clinical use has been markedly curtailed – cardiomyocyte-specific PPAR γ knockout mice also spontaneously develop cardiac hypertrophy [51]. Moreover, cardiomyocyte-specific PPAR γ knockout mice retain a subset of myocardial responses to the thiazolidinedione rosiglitazone, which possibly signals via myocardial fibroblasts. One role of cardiomyocyte PPAR γ is to hold the maladaptive transcription factor nuclear factor κ -light-chain-enhancer of activated B cells (NF- κ B) in check via ligand-dependent transrepression [25]. NF- κ B is a dimeric transcription factor consisting of two subunits from the following five components: c-Rel, RelA (p65), RelB, p50, and p52. Under unstimulated conditions, NF- κ B is retained in the cytoplasm by I κ B, but after stimulation, I κ B is phosphorylated and thereby targeted for proteosomal degradation, releasing NF- κ B to the nucleus [52]. Studies of loss-of-function mouse models that targeted NF- κ B subunits, or used NF- κ B inhibitors did not definitively identify the role of NF- κ B in the pathogenesis of pathological hypertrophy. Recent studies, however, indicate a physical interface between NF- κ B and nuclear factor of activated T-cells (NFAT), a dynamic regulator of the pathological hypertrophy program, which is retained in the nucleus when dephosphorylated by calcineurin. The NF- κ B-NFAT complex is required for NF- κ B-mediated progression of pressure overload-induced cardiac hypertrophy [53].

The third PPAR family member, PPAR δ , exerts distinct regulation of myocardial metabolism – driving a glucose utilization program, rather than that of fatty acid utilization and storage [54]. Moreover, unlike mice overexpressing PPAR α and PPAR γ within cardiomyocytes, PPAR δ -overexpressing mice do not develop cardiomyopathy. However, cardiomyocyte-specific PPAR δ knockout mice demonstrate a dilated cardiomyopathy with decreased expression of mediators of fatty acid oxidation, and increased lipid storage [55]. Because dilated cardiomyopathies are generally accompanied by these metabolic alterations, it is not clear if these aspects of the phenotype are primarily due to PPAR δ deficiency [5].

As dynamic regulators of myocardial lipid metabolism, PPARs also modify the response to ischemia. Through mechanisms at least partially explained by an improved ability to engage glucose metabolism, PPAR α knockout mice show improved post-ischemic recovery [56]. Nonetheless, systemic PPAR α , PPAR δ , or PPAR γ activation via administration of selective synthetic ligands all improve myocardial responses to ischemia-reperfusion, through mechanisms linked to increased Akt activation, limitation of ROS, curtailed inflammatory cascades, and enhanced nitric oxide signaling [57–59]. The ability to link these findings to cardiomyocyte-specific PPAR family members, however, is currently limited.

PGC-1 α and estrogen-related receptor α (ERR α) are key mediators of mitochondrial biogenesis and oxidative phosphorylation, and play an integrative role in the response to pressure overload. ERR α is a nuclear receptor that (like the PPARs) recruits the co-activators PGC-1 α and PGC-1 β , but unlike the PPARs, has no known ligand – and is therefore an orphan receptor. The mammalian genome also encodes ERR β and ERR γ , with ERR γ serving an important role in the metabolic adaptation of the neonatal heart to birth [60]. Unlike PPARs, which recruit PGC-1 co-activators when ligand-bound to direct repeat 1 sequences, ERRs recruit PGC-1s and activate transcription from *cis*-response elements that consist of only one hexad half-site, which are distributed through many genes that encode mediators of the mitochondrial inner membrane electron transport chain and oxidative phosphorylation [61]. In response to pressure overload, as the myocardium progresses from a compensated (concentric hypertrophy with preserved systolic function) to

a decompensated state (chamber dilatation and systolic dysfunction), expression of $ERR\alpha$ and $PGC-1\alpha$ is reduced [15]. Analysis of $ERR\alpha$ knockout mice indicates that this orphan nuclear receptor is required for mitochondrial homeostasis in the setting of pressure overload: after transverse aortic constriction (TAC), $ERR\alpha$ knockout mice develop decompensated hypertrophy and deranged energy metabolism [62]. Through distinct target gene programs, independent loss of both $PGC-1\alpha$ (which targets oxidative phosphorylation) and $PGC-1\beta$ (which influences glucose metabolism) also yield accelerated heart failure in pressure-overloaded hearts [63, 64]. While gene targets of $ERR\alpha$ - $PGC-1\alpha$ signaling broadly include mediators of mitochondrial inner membrane electron transport and oxidative phosphorylation, one target, the nuclear-encoded DNA/RNA nuclease endonuclease G (ENDOG) has recently emerged as an intriguing component of mitochondrial homeostasis in the prevention of maladaptive cardiac hypertrophy [65]. Identified in a quantitative genetic mapping experiment in a rat model of blood-pressure independent left ventricular hypertrophy, ENDOG knockout mice exhibit myocardial mitochondrial dysfunction and increased ROS, but only mild cardiac hypertrophy – much milder than that exhibited by mice that lack mitochondrial transcription factor A (TFAM), a nuclear-encoded transcriptional activator of mitochondrial gene transcription and genome replication [66, 67]. The full scope of ENDOG mechanistic targets remains to be determined, as does the integration of this intriguing nuclease into the landscape of modifiers of cardiac hypertrophy.

The importance of $ERR\alpha$ in the evolution of pathological hypertrophy is further underscored by recent results indicating that $ERR\alpha$ - $PGC-1\alpha$ complexes, which transcriptionally activate genes encoding mediators of oxidative metabolism, can be displaced from $ERR\alpha$ -bound half-sites by $PPAR\alpha$ monomers during pressure overload, leading to inhibition of $ERR\alpha$ target genes, and thus the initiation of a maladaptive metabolic response [68]. Pressure overload increases the expression of not only $PPAR\alpha$, but also the NAD^+ -dependent histone deacetylase silent mating type information regulation 2-homolog 1 (SIRT1), which upon recruitment to $PPAR\alpha$ bound to $ERR\alpha$ elements, suppresses transcription of $ERR\alpha$ target genes. Because this molecular interplay also occurs normally during fasting, these molecular events may reflect the execution of a genetic program under a hemodynamic circumstance that yields a maladaptive response. However, the results of this study remain to be reconciled with prior studies that revealed decompensated hypertrophy in $PPAR\alpha$ knockout mice, which could be selectively linked in the knockout to pressure overload-induced gene programs of inflammation, oxidative stress, and extracellular matrix remodeling [69, 70]. While these studies were performed using mice that lack $PPAR\alpha$ in all tissues, they also underscore the role of balance in nuclear receptor signaling. Transcriptional programming molecules such as $PPAR\alpha$ engender complex network-wide effects when tested in gain- and loss-of-function experiments, which reflect both loss of ligand-induced $PPAR\alpha$ -dependent transactivation and $PPAR\alpha$ -dependent recruitment of transcriptional co-repressors on many target gene promoters. Single nucleotide polymorphisms (SNPs) in the human *PPAR\alpha* locus also support an intersection between this nuclear receptor and both hypertrophic growth and diabetic cardiomyopathy [71, 72].

2b. Other Important Transcription Factors and Chromatin Remodeling

Myocyte enhancer family 2 (MEF2) transcription factors transcriptionally activate a number of markers of pathological hypertrophy and mitochondrial function [73, 74]. In addition, the zinc-finger transcription factors GATA4 and GATA6 overlap in function and are fundamental programmers of the myocardial response to pressure overload, although the roles of these mediators of pathological hypertrophy in the metabolic response to pressure overload have not been fully elucidated [75–80]. One key regulator of the fetal gene

expression program during pathological hypertrophy was recently determined to be an ATP-dependent chromatin-remodeling protein, Brahma-related protein 1 (BRG1), which interacts with HDACs and poly (ADP-ribose) polymerase, repressing the gene encoding α -MHC and activating the gene encoding fetal β -MHC, which exhibits intrinsically reduced ATPase activity [81]. BRG1 expression is absent in normal adult myocardium, but present during pathological hypertrophy and in embryonic cardiomyocytes. Another important transcriptional regulator of this switch may be Ccaat-enhancer-binding protein (C/EBP)- β , a transcription factor that can complex with BRG1[82] and was recently shown to be down-regulated in the myocardium with exercise training [83]. Furthermore, mice heterozygous for C/EBP β deficiency constitutively manifest a physiological hypertrophy phenotype that includes both cardiomyocyte hypertrophy and increased markers of cell cycle activity, and these haploinsufficient mice are also resistant to pressure overload-mediated pathological hypertrophy. Future experiments will delineate a prospective role of C/EBP β in the metabolic underpinnings of pathological hypertrophy.

In addition to the role of the chromatin-remodeling protein BRG1, the epigenetic regulation of gene expression in the pathological hypertrophy phenotype has been extensively evaluated in mouse models with genetic manipulation of the HDACs. Through inhibition of MEF2, GATA4, and NFAT, Class IIa HDACs (i.e., HDACs 4, 5, 7, and 9) suppress cardiac hypertrophy – knockouts exhibit enhanced pathological hypertrophy [84, 85]. Class I HDACs, which interact with BRG1 and include HDACs 1–3, and 8, tend to promote hypertrophy (knockout of HDAC2 attenuates the pathological hypertrophy program), at least in part through inhibition of Krüppel like factor 4 and inositol polyphosphate-5-phosphatase signaling, with the latter serving as a key regulator of the cell growth mediator GSK-3 β [86, 87]. HDAC Class segregation is not absolute, however – as described above, cardiomyocyte-specific HDAC3 knockout mice exhibit cardiac hypertrophy and myocardial steatosis, similar to PPAR α -overexpressing mice [34]. Furthermore, transgenic overexpression of HDAC3 results in increased cardiomyocyte proliferation without hypertrophy [88]. The distinct phenotypes observed among genetically modified models of HDAC Class I family members suggest that therapeutic strategies based upon rational pan-HDAC Class drug design could be problematic [15].

Recent findings have uncovered the ability of the forkhead box FOXO transcription factor family to link insulin signaling to multiple forms of metabolic stress in the myocardium. Downstream of insulin receptor substrate-Akt signaling, FOXO transcription factors regulate cell size, viability, and metabolism. Cardiomyocyte-specific loss of FOXO1 protects the myocardium from the lipotoxic effects of caloric excess from high-fat diet, shifts its preference from fatty acids to glucose, and prevents the development of diabetic cardiomyopathy [89]. Further studies will be needed to assess the relationship between FOXO1 and PPAR signaling in this model and determine whether relatively unopposed and sustained glucose utilization can become associated with high-fat diet-dependent adverse myocardial remodeling, as has been reported in α -MHC-GLUT1 transgenic mice maintained on a high-fat diet [90].

FOXO transcription factors are also important modifiers of the response to ischemia. Contrary to the contributory role that FOXO1 appears to play in the pathogenesis of diabetic cardiomyopathy, its role in ischemia-reperfusion may be protective. Mice with cardiomyocyte-specific combined deficiency of both FOXO1 and FOXO3 that are subjected to myocardial infarction via surgical coronary artery ligation exhibit reduced systolic function, increased scar formation, induction of stress-responsive signaling, and increased apoptotic cell death relative to controls [91]. Moreover, in response to an ischemia-reperfusion protocol, these combined knockout mice exhibit blunted expression of the antioxidant enzymes catalase and manganese superoxide dismutase. Future experiments will

delineate the role of FOXO1 from FOXO3, and further clarify protective versus maladaptive roles of the FOXO transcription factors in the myocardium. A key role of FOXO transcription factors in cardiomyocyte adaptation to metabolic stress may be transduced through their ability to regulate autophagy, a hallmark of many adaptive responses of the cardiomyocyte, including the responses to starvation, ischemia-reperfusion, and pressure overload [92, 93]. Deacetylation of FOXO1 by SIRT1 (a class III histone deacetylase) appears to be an important component of the cardiomyocyte autophagic response to nutrient deprivation, and perhaps other forms of metabolic stress [94]. Although additional studies will be needed to directly interconnect energy-regulating and autophagic transcriptional programs in cardiomyocytes, it is attractive to hypothesize that DNA-bound transcription factors and recruited modifiers of chromatin structure exhibit shared roles in the coordination of energy economy and autophagic flux in the adaptation to various metabolic stressors.

As described above, HIF-1 is a critical component of the myocardial response to pressure overload through the induction of PPAR γ . In addition, HIF-1 is a critical component of the response to variations of cellular oxygen tension. HIF-1 binds of *cis*-elements in promoter regions of hundreds of genes, recruiting co-activators and augmenting transcription of genes that encode protective mediators, other transcription factors, microRNAs (see below), and Jumonji domain histone demethylases. HIF-1 can also inhibit transcription of select loci [95]. The α subunit of HIF-1 is oxygen sensitive, such that under normal oxygen tensions, it is targeted for proteasomal degradation via interaction with von Hippel Lindau (VHL) protein. This HIF-1 α -VHL interaction during normoxia is dependent on hydroxylation of a proline residue on the HIF-1 α subunit by prolyl-4-hydroxylase enzymes that use oxygen and the TCA cycle intermediate α -ketoglutarate as substrates. In addition to hypoxia, mitochondrial ROS generation stabilizes HIF-1 α abundance. Through transcriptional activation of its target genes, HIF-1 reduces ROS production through mechanisms that favor mitochondrial efficiency, drive mitochondrial autophagy, and support the expression of mediators of glycolysis. Moreover, through induction of the enzymatic machinery required for adenosine production, HIF-1 is an important determinant of the ischemic pre-conditioning response. This protection by HIF-1 is supported by studies of mice with loss of prolyl-4-hydroxylase function, which exhibit relatively reduced infarct size and remodeling after experimental myocardial infarction [96].

3. Post-transcriptional regulation

microRNAs (miRNAs) are increasingly appreciated as important mediators of regulation of gene expression in the diabetic heart and vasculature. Based on observations of increased miR-223 expression in the insulin-resistant type 2 diabetic human myocardium, the effects of gain- and loss-of-function of miR-223 has been studied in neonatal rat cardiomyocytes [97]. In these cultured cells, overexpression of miR-223 is associated with increased GLUT4 protein expression and increased glucose transport, whereas loss-of-function of miR-223 leads to decreased GLUT4 expression and decreased glucose transport. The directionality of these changes and the lack of predicted miR-223 target sites in the GLUT4 3'-untranslated region (UTR) suggest that this regulation serves as an adaptive response to increase glucose utilization in the insulin-resistant heart and that this regulation is indirect. Moreover, as described above, reductions of GLUT4 expression are likely only to partially account for reductions of cardiomyocyte glucose uptake. MiR-503 is another microRNA upregulated in ischemic limb muscles of streptozotocin-treated mice and muscle tissue from amputated ischemic legs from diabetic patients [98]. In the mouse model, repression of miR-503 in the ischemic limb ameliorated the diabetes-associated impairment of angiogenesis and blood flow, through cell cycle regulatory mechanisms that are described below. In a third example, miR-125b is induced in vascular smooth muscle cells isolated from *db/db* mice (relative to

db/+ controls), targets the 3'-UTR of the chromatin histone H3 lysine-9 methyltransferase Suv39h1, and is associated with upregulation of a number of inflammatory genes [99]. Broader approaches to identify dysregulated miRNAs that contribute to the pathophysiology of cardiovascular disease in diabetes have utilized microarray technology to compare the global patterns of expression of miRNAs in diabetic and control tissues. For example, eleven miRNAs are upregulated in myocardial microvascular endothelial cells from type 2 diabetic (Goto-Kakizaki) rats compared to controls. Among these, miR-320 has been shown to impair endothelial cell proliferation and migration in vitro, although direct downstream targets remain to be elucidated [100].

The exposure of cultured cardiovascular cells to excess nutrients has implicated additional miRNAs in the pathogenesis of diabetic myocardial and vascular complications. Treatment of cultured neonatal mouse cardiomyocytes with palmitate to model the increased delivery of fatty acid substrates to the diabetic heart causes cardiomyocyte apoptosis [101]. Concomitantly, palmitate up-regulates miR-195, which increases oxidative stress and directly targets *Sirt1* and *bcl-2* through target sites in the 3'-UTR regions of these mRNAs, the ultimate effects of which enhance lipoapoptosis. On the other hand, hyperglycemia impairs endothelial cell functions including migration in response to stem cell factor through a mechanism involving induction of miR-221 and its inhibition of c-kit, the receptor for stem cell factor [102]. Other experiments have suggested that miR-221 and miR-222 are downregulated in the setting of high glucose and advanced glycation end products, leading to derepression of cyclin dependent kinase inhibitors 1B and 1C and ultimately slowing cell cycle progression [103]. Hyperglycemia also induces miR-503 in cultured endothelial cells, which contributes to impaired proliferation and migration of these cells in response to hyperglycemia and limiting growth factors, possibly by targeting *cdc25A* and *cyclinE1* [98]. While other miRNAs are known to be induced by nutritional excess in other cell types, including miR-133a or miR-375, it remains to be established whether they are induced or have a functional role in the myocardium in response to metabolic stress [104, 105].

The profound changes in metabolic substrate use that accompany pathological hypertrophy present a circumstance in which microRNA-mediated changes in gene expression have the potential to contribute to rapid changes in phenotype. Numerous microRNAs have been shown to contribute to the pathogenesis of pathological hypertrophy. While none of these has been directly linked to the changes in the myocardial metabolic program that accompany hypertrophy, it is intriguing that several are known to regulate metabolic pathways. miRNA-133a is downregulated in a murine model of acute TAC [106]. Although the relationship of miRNA-133a to cardiomyocyte metabolism remains to be explored, it has been shown to be induced by high glucose and to have inhibitory effects on insulin biosynthesis in human islets [105]. MiR-98/Let-7 inhibits angiotensin II-mediated cardiomyocyte cellular hypertrophy in vitro and cardiac hypertrophy in vivo [107]. In other studies, it has also been shown to play a regulatory role in glucose metabolism through repression of molecules in the insulin signaling pathway in skeletal muscle [108]. The specific effects of Let7 on myocardial metabolism and its contributions to the metabolic changes of the hypertrophic response are yet to be determined.

Expression of many microRNAs is modulated in the myocardium in the setting of myocardial ischemia-reperfusion, in the vasculature in the setting of unstable plaque and neointimal lesion formation, and in the circulation of patients with coronary artery disease [109–111]. For example, through modulation of expression of HIF-1 α , hypoxia leads to alterations of miRNAs such as miR-210 and miR-200b that are likely to serve an adaptive function in MI and ischemia-reperfusion through effects on endothelial cell function. HIF-1 α -dependent upregulation of miR-210 stimulates formation of capillary like structures on Matrigel and cell migration through a mechanism involving miR-210-mediated

downregulation of Ephrin-A3 [112]. Conversely, HIF-1 α stabilization inhibits expression of the angiogenic suppressor miR-200b, which acts through targeting the mRNA encoding pro-angiogenic ETS-1 [113]. While it is well appreciated that shifts in metabolic substrate availability and utilization occur in myocardial infarction, ischemia and ischemia-reperfusion, there is as yet little data directly linking changes in expression of these miRNAs to the shifts in utilization of fatty acids and glucose, either as a consequence of altered metabolism or as a regulator of secondary metabolic shifts.

Alternative splicing is another mechanism by which gene expression in the myocardium may be modified in response to metabolic stress, as it is clear from analysis of mRNA-seq data and exon arrays that the majority of multi-exon genes in the human heart undergo alternative splicing [114]. Metabolically-relevant genes in the heart are known to be alternatively spliced, including PGC-1 α and CPT1 [115, 116]. Similarly, five alternatively spliced forms of the murine receptor for advanced glycation end-products (RAGE) are detected in the murine heart [117]. However, the role that altered metabolic substrates in diabetes or other states of nutritional excess play in regulating alternative splicing and the potential effects of these alternatively spliced metabolic gene products on metabolic rates remains to be understood.

4. Translational regulation

The metabolic stress of nutrient excess also impacts mRNA translation, through modulation of the effects of mammalian target of rapamycin (mTOR) complexes on general levels of mRNA translation, as well as translation of specific mRNAs involved in cell growth and proliferation [118]. In a variety of non-cardiac tissues and cell types, glucose, amino acids, and insulin signaling have been demonstrated as important regulators of the mTORC1 complex. In the heart, long-chain fatty acids increase translation initiation and protein synthesis, whereas inhibition of fatty acid β -oxidation decreases protein synthesis through inhibition of eukaryotic initiation factors (eIFs): guanine nucleotide exchange activity of eIF2B is decreased, the α -subunit of eIF2 is phosphorylated, and eIF4E-binding protein-1 and ribosomal protein S6 kinase become dephosphorylated [119]. In diabetic rats, decreased protein synthesis rates in the heart are associated with diminished phosphorylation of mTOR and its downstream targets ribosomal protein S6 kinase and eIF4E-binding protein-1 [120]. Furthermore, the mTOR pathway has downstream effects on cardiac energy metabolism through changes in gene expression. For example, cardiac ablation of raptor, an essential component of the mTOR complexes, decreases expression of PPAR α and PGC-1 α and downstream target genes in β -oxidation, and is associated with decreased fatty acid oxidation and increased glucose oxidation [121]. It is less clear, however, whether these effects are direct at the level of translation of these transcriptional regulators or indirect, through alterations in mitochondrial function or transcription.

5. Summary

Models of disease and altered nutrient supply have enabled discovery of gene expression changes in response to metabolic stress and have provided a means to understand underlying molecular mechanisms. DNA-bound transcription factors and their recruited co-regulator proteins, miRNAs, and translational modifications serve as fundamental mediators of the myocardial adaptations and maladaptations to metabolic stress. It is important to note that many of the findings presented in this review represent results from animal models of caloric overload, pressure overload, and ischemia-reperfusion. Importantly, correlative findings have emerged from studies of human SNPs within transcription factor-encoding loci (as described above) and gene array studies in failing hearts have revealed at least partial concordance of mRNA and miRNA signatures in animal models and human studies

[122–124]. Nonetheless, findings revealed by animal models have not yet been fully corroborated in humans in part because of limited accessibility of tissue for study. Another limitation of heretofore obtained results, as highlighted in this review, is that it is not uncommon to observe similar phenotypes in models designed to elicit gain or loss-of-function of the same mediator. These limitations may be overcome by parallel systems biology approaches that profile tissue function in vivo by incorporating probing network analyses. As these platforms evolve, we propose that statistical integrations derived from mutual interrogations of the myocardial mRNA and non-coding RNA transcriptomes [125], the histone acetylome and methylome, proteome, lipidome, and metabolome in animal models of disease will provide important ‘launch points’ to develop deeper understanding of dynamic metabolic states in human myocardium and in turn, usher the development of novel diagnostic and therapeutic strategies.

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HIGHLIGHTS

1. Multiple forms of metabolic stress reprogram myocardial gene expression.
2. Gene expression programs in myocardium shift through multiple molecular mechanisms.
3. Assignment of these programs as adaptive or maladaptive remains a challenge.
4. Systems biology approaches will help integrate molecular mechanisms with phenotypes.

Table 1

Key mediators of cardiovascular cell gene expression programs during metabolic stress.

Mediator(s)	Level of action	Key effects during metabolic stress	Key disease implications
PPAR α , PPAR γ	transcription factors	regulate fatty acid oxidation	nutritional excess, diabetes, hypertrophy, ischemia
ERR α	transcription factor	regulates mitochondrial biogenesis, electron transport, oxidative phosphorylation	hypertrophy
PGC-1 α	transcriptional co-activator	co-activator of PPARs, ERRs	nutritional excess, diabetes, hypertrophy
FOXO1	transcription factor	regulates fatty acid and glucose metabolism, oxidative stress, and autophagy	diabetes, ischemia
HIF-1	transcription factor	regulates response to hypoxia	ischemia, hypertrophy
NF- κ B/NFAT	transcription factors	regulate the maladaptive response to pressure overload	hypertrophy
Class IIa HDACs	histone deacetylases	repress transcription	hypertrophy
Class I HDACs	histone deacetylases	repress transcription, target cell growth	hypertrophy, diabetes
SIRT1	histone deacetylase	regulates oxidative metabolism, autophagy	hypertrophy
BRG1	ATP-dependent chromatin-remodeling protein	represses α -MHC gene, activates fetal β -MHC, which has lower ATPase activity	hypertrophy
ENDOG	DNA/RNA nuclease	mitochondrial homeostasis	hypertrophy
TFAM	transcription factor	mitochondrial homeostasis and genome replication	hypertrophy
miR-223	microRNA	increases GLUT4 expression and glucose transport in cardiomyocytes	diabetes
miR-503	microRNA	impairs endothelial cell proliferation and angiogenesis	nutritional excess, diabetes, ischemia
miR-125b	microRNA	upregulates inflammatory genes in vascular smooth muscle	diabetes
miR-320	microRNA	impairs endothelial cell proliferation	diabetes
miR-195	microRNA	promotes cardiomyocyte apoptosis	nutritional excess, diabetes
miR-221, 222	microRNAs	Regulate endothelial cell proliferation	nutritional excess, diabetes
miR-210, 200b	microRNAs	regulate angiogenesis	hypoxia