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Metabolic Therapy at the Crossroad: How to Optimize Myocardial Substrate Utilization?

Stephen C. Kolwicz Jr. and Rong Tian*

There has been growing interest in targeting myocardial substrate metabolism for the therapy of cardiovascular and metabolic diseases. This is largely based on the observation that cardiac metabolism undergoes significant changes during both physiologic and pathologic stresses. In search for an effective therapeutic strategy, recent studies have focused on the functional significance of the substrate switch in the heart during stress conditions, such as cardiac hypertrophy and failure, using both pharmacologic and genetic approaches. The results of these studies indicate that both the capacity and the flexibility of the cardiac metabolic network are essential for normal function; thus, their maintenance should be the primary goal for future metabolic therapy. (Trends Cardiovasc Med 2009;19:201–207) © 2009, Elsevier Inc.

• Introduction

The heart requires a continually high level of energy supply to maintain its mechanical function throughout life. The amount of adenosine triphosphate (ATP) generated and consumed by a human heart daily is more than 15 times its own weight (Ingwall 2002) and is primarily generated through complex metabolic pathways that supply carbon substrates to the mitochondria for oxidative phosphorylation (Figure 1). Mitochondria occupy ~30% of the volume of a cardiac myocyte, ensuring the great oxidative capacity of the system. To meet the high energetic demand, the cardiac metabolic network has developed into an

extremely versatile system, capable of metabolizing all carbon substrates, that is, lipids, carbohydrates, and amino acids, for energy production.

An important feature of cardiac metabolism is that it is highly adaptable throughout the life cycle as well as under physiologic or pathologic stressors. In utero, the fetal heart relies on carbohydrate substrates for ATP generation (Fisher 1984). As the heart matures, in parallel to the increase of mitochondrial volume and higher circulating fatty acids levels, fatty acids become the dominant energy substrate (Lopaschuk et al. 1994). During conditions of fasting or diabetes, the adult heart can become even more dependant on fatty acids (Belke et al. 2000; Mazumder et al. 2004). This is in contrast to the hypoxic or failing heart, where the relative use of carbohydrate, especially glucose, is increased (Allard et al. 1997; Barger and Kelly 1999; Tian 2003). Although much attention has focused on the use of glucose and fatty acids in cardiac metabolism, the heart is also capable of utilizing ketones, lactate, and endogenous substrates, that is, glycogen and triglycerides, as fuel (Figure 1). These observations underscore the flexibility of cardiac metabolism in response

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1050-1738/09/\$-see front matter

PII S1050-1738(09)00181-9

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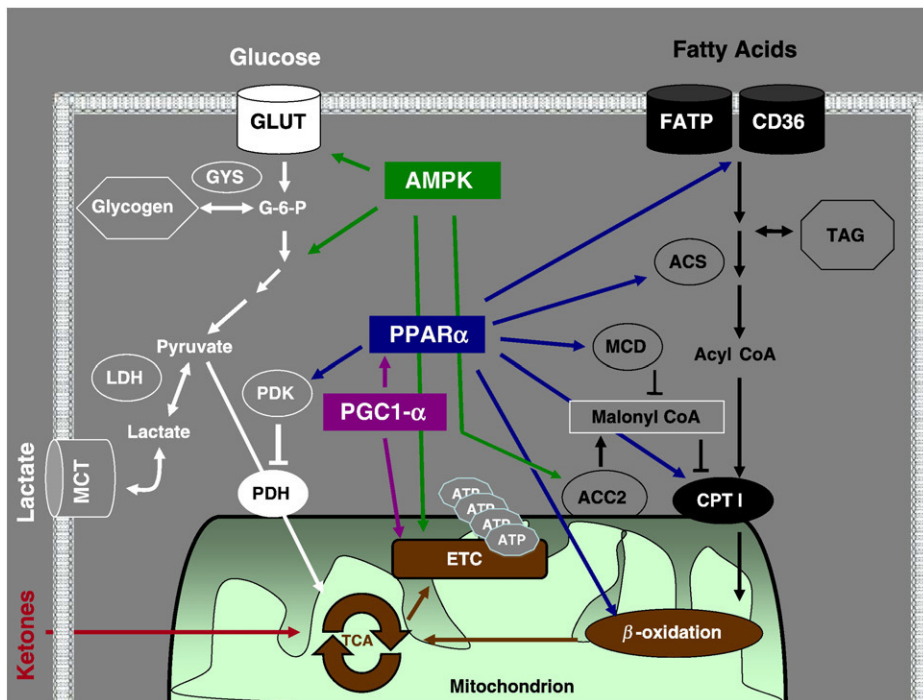


Figure 1. Cardiac metabolic network for substrate utilization. Highlighted in *solid colors* are key regulatory sites for carbohydrate and lipid metabolism. The molecular targets of several transcriptional and/or signaling pathways in the regulation of substrate selection are also illustrated. ACC2 indicates acetyl CoA carboxylase 2; ACS, acyl-CoA synthetase; AMPK, adenosine monophosphate-activated protein kinase; CD36, cluster of differentiation 36 (fatty acid transporter); ETC, electron transport chain; FATP, fatty acid transport protein; G-6-P, glucose-6-phosphate; GYS, glycogen synthase; LDH, lactate dehydrogenase; MCD, malonyl CoA decarboxylase; MCT, monocarboxylate transporter; PDH, pyruvate dehydrogenase; PDK, pyruvate dehydrogenase kinase; TAG, triacylglycerol; TCA, tricarboxylic acid cycle.

to a variety of environmental changes. Studies in the last decade have revealed a number of mechanisms that remodel the metabolic pathways at the molecular level to enable such adaptations, for example, peroxisome proliferator-activated receptors (PPARs), adenosine monophosphate-activated protein kinase, and peroxisome proliferator-activated receptor- γ coactivator 1 (PGC1) (Figure 1). As our understanding of the molecular mechanisms regulating metabolic flexibility advances, it becomes increasingly attractive to consider metabolic modulations as means to maintain or improve cardiac function under pathologic conditions.

This review will focus on recent advances in the understanding of the functional significance of alterations in the myocardial substrate utilization that accompany cardiac hypertrophy/heart failure and obesity or diabetes. In addition, metabolic manipulations that have been attempted for these conditions in animal models and patients will be discussed.

• The Fetal Metabolic Profile in Cardiac Hypertrophy and Failure

It has been widely recognized that pathologic hypertrophy is associated with the reappearance of the fetal gene expression pattern (Buttrick et al. 1994). The metabolic profile of the hypertrophied heart also reverts to the fetal pattern, showing decreased fatty acid oxidation and increased reliance on carbohydrate fuel sources (Barger and Kelly 1999; Razeghi et al. 2001). The switch in the metabolic profile in animal models of heart failure is associated with down-regulation of PPAR α and up-regulation of enzymes involved in glucose utilization (Tian 2003). As pathologic hypertrophy progresses to heart failure, the shift of substrate preference to glucose is closely associated with impairment of myocardial energetics and loss of contractile reserve (Neubauer 2007). These observations have raised the question whether the metabolic switch toward glucose is maladaptive for cardiac hypertrophy and failure.

To determine a causal relationship between altered cardiac metabolism and the development of heart failure, genetically altered mouse models have been used to recapitulate or manipulate the metabolic phenotype in cardiac hypertrophy and failure (Table 1). Because of the space restraint, the discussion will be limited to a few models. We used transgenic mice expressing the insulin-independent glucose transporter (glucose transporter 1 [GLUT1]) in the heart that led to increased glucose uptake, glycolysis, and glucose oxidation, with decreases in fatty acid oxidation in the heart (Liao et al. 2002; Luptak et al. 2005). Despite the fetal-like cardiac metabolic profile, the GLUT1 mice lived a normal life span with unaltered cardiac function and, when subjected to pressure overload by ascending aortic constriction, were protected against contractile dysfunction and left ventricular dilation (Liao et al. 2002; Luptak et al. 2007). These studies demonstrate that increased reliance on glucose per se is not detrimental to the heart.

However, as discussed previously, increased glucose utilization has been shown to be associated with impaired myocardial energetics in the hypertrophied and failing heart. Similarly, PPAR α -null hearts, which had permissive increases in glucose oxidation as a result of impaired fatty acid oxidation, also failed to maintain myocardial energetics and function during a high workload challenge (Luptak et al. 2005). In addition, the PPAR-null mice develop cardiomyopathy at an old age (Watanabe et al. 2000). Interestingly, the loss of energetic and contractile reserves in the PPAR-null heart could be rescued by overexpressing GLUT1, which markedly expanded the capacity for glucose uptake and utilization (Luptak et al. 2005). These findings suggest that the inherent capacity for glucose utilization in an adult heart, when fatty acid oxidation is severely impaired, is insufficient for sustaining normal energy supply under stress. Overexpression of GLUT1 under these conditions expanded the capacity and provided the optimal substrate in the face of impaired ability to oxidize fatty acids. There has been significant amount of evidence suggesting that the failing heart is insulin resistant (Ashrafian et al. 2007; Witteles et al. 2004). Because the capacity for glucose uptake and

Table 1. Mouse models of altered cardiac metabolism

<i>Mouse model</i>	<i>Metabolic profile</i>	<i>Cardiac phenotype</i>	<i>References</i>
LVH/HF in wild type	Switch to fetal metabolic profile with ↑ GOX and glycolysis and ↓ FAO	Decreased function, energetics, and switch to fetal metabolic phenotype	(Allard et al. 1997; Barger and Kelly 1999; Tian 2003)
GLUT1 overexpression	↑ GOX and glycolysis with ↓ FAO	Improved function, energetics, and survival	(Liao et al. 2002; Luptak et al. 2005)
PPAR α overexpression	↑ FAO at the expense of glucose	Development of cardiomyopathy	(Finck et al. 2002)
PPAR α null	↓ FAO with ↑ GOX and lactate oxidation	Impaired function and energetics with increased workload, age-associated development of cardiac fibrosis	(Campbell et al. 2002; Loichot et al. 2006; Luptak et al. 2005; Watanabe et al. 2000)
PGC1 α $-/-$	↓ FAO	Reduced function at baseline and impaired with physiologic challenge; accelerated heart failure	(Arany et al. 2005, 2006)
PGC1 α overexpression	↑ in mitochondria number with abnormal ultrastructure	Development of cardiomyopathy	(Lehman et al. 2000; Russell et al. 2004)
PDK4 overexpression	↑ in FAO and ↓ GOX	Exacerbation of heart failure in cardiomyopathy model; decreased survival	(Zhao et al. 2008)
PDK4 $-/-$	No change in GOX or glycolysis	Preserved cardiac function and decreased fibrosis	(Wende et al. 2009)
CIRKO	↑ GOX and glycolysis with ↓ FAO	Reduced heart mass with lower cardiac function	(McQueen et al. 2005; Sena et al. 2009)
MCD $-/-$	No change in metabolism at baseline; ↑ GOX during reperfusion	Normal cardiac function at baseline; improved function after ischemia	(Dyck et al. 2006)
GLUT4 null	Normal glucose uptake with ↓ fatty acid metabolic genes	Marked cardiac hypertrophy and fibrosis; reduced longevity	(Katz et al. 1995; Stenbit et al. 2000)
GLUT4H $-/-$	↑ basal glucose uptake and ↓ insulin-stimulated glucose uptake	Modest cardiac hypertrophy with preserved function, poor response to ischemia/reperfusion	(Abel et al. 1999; Tian and Abel 2001)
M/MtCK $-/-$	↓CK activity and ↓phosphocreatine levels	Impaired energetics with ↑ workload; development of hypertrophy and LV dilation	(Nahrendorf et al. 2005; Saupe et al. 1998)
CD36 $-/-$	↑ GOX and ↓ FAO	↓ lipid accumulation, improves lipotoxic cardiomyopathy; no change or decreased function after ischemia	(Irie et al. 2003; Koonen et al. 2007; Kuang et al. 2004; Yang et al. 2007)

LVH indicates left ventricular hypertrophy; HF, heart failure; GOX, glucose oxidation; FAO, fatty acid oxidation; $-/-$, knockout; PDK4, pyruvate dehydrogenase kinase 4; CIRKO, cardiomyocyte-selective insulin receptor knockout; MCD, malonyl CoA decarboxylase; GLUT4, glucose transporter 4; GLUT4H, cardiac-specific glucose transporter 4; M/MtCK, muscle and mitochondrial isoforms of creatine kinase; CD36, cluster of differentiation 36 (fatty acid transporter).

utilization in an adult heart is highly insulin dependent, impaired insulin signaling in combination with decreased fatty acid oxidation can result in severe limitations of substrate oxidation in heart failure. The benefit of overexpressing GLUT1, an insulin-independent glu-

cose transporter, may be partially attributable to the relief of limitations in substrate supply associated with insulin resistance. Thus, for failing hearts, an adaptive metabolic profile must be able to fully support the energetic demand among other metabolic considerations.

• Pharmacologic Approaches to Optimize Cardiac Metabolism in Heart Failure

Along the line of promoting glucose utilization of the heart, an established protein target is the muscle form of the

carnitine palmitoyl transferase-I (CPT-1, Figure 1), the enzyme responsible for the uptake of long-chain fatty acids into the mitochondria. Several CPT-1 inhibitors—oxfenicine, etomoxir, and perhexiline—have been shown to partially reduce fatty acid oxidation and promote glucose oxidation of the heart. In rodent and large animal models of heart failure, these compounds delayed the onset of decompensated failure while preventing the transcriptional down-regulation of key enzymes in cardiac energy metabolism (Lionetti et al. 2005) and improving the rate of sarcoplasmic reticulum calcium uptake (Rupp and Vetter 2000). Furthermore, a recent study showed that short-term treatment with perhexiline, in addition to standard medication, improved cardiac function and peak exercise oxygen consumption in chronic heart failure patients. Other partial fatty acid oxidation inhibitors, for example, trimetazidine, also showed benefit in a small study of elderly heart failure patients with coronary heart disease (Vitale et al. 2004). One potential mechanism for the benefit of replacing fatty acid oxidation with glucose is the higher oxygen efficiency during ATP synthesis. Theoretically, glucose oxidation requires 11% to 13% less oxygen than fatty acid oxidation for ATP synthesis (Opie 2004). However, acute depletion of free fatty acid supply to the heart resulted in ~25% oxygen sparing in normal mouse and human hearts (How et al. 2005; Tuunanen et al. 2006), consistent with the notion that high levels of fatty acids may stimulate mitochondrial uncoupling proteins resulting in a decline in oxygen efficiency beyond what is expected from the ATP to oxygen ratio (Boudina et al. 2007).

A different class of molecules, such as glucagon-like peptide (GLP), promotes myocardial glucose utilization via stimulation of insulin secretion and its insulin-mimetic effects. The GLP stimulates insulin signaling, enhances myocardial glucose uptake, reduces circulating fatty acid levels, and hence promotes glucose utilization via a distinct mechanism from the partial inhibition of fatty acid oxidation (D'Alessio et al. 1994). Both animal experiments and clinical studies using GLP for short-term treatment have demonstrated improvement of cardiac function in heart failure (Nikolaidis et al. 2004; Poornima

et al. 2008; Sokos et al. 2006). Taken together, pharmacologic treatments that enhance cardiac glucose utilization appear to protect against the progression of heart failure, although the molecular mechanisms remain to be fully defined. An outstanding challenge is to determine whether such metabolic modulations alter the long-term clinical outcome, that is, survival rate in heart failure patients.

• The Fatty Acid Paradox

Despite the overwhelming evidence suggesting the benefit of increasing glucose utilization in the failing heart, a recent clinical study showed that fatty acid oxidation remains essential for cardiac function. Acipimox, a nicotinic acid derivative with profound antilipolytic effects, was used to acutely lower serum fatty acid levels and hence the rate of fatty acid uptake in the heart. In patients with dilated cardiomyopathy, acipimox promoted glucose oxidation but caused significant falls of cardiac work and efficiency (Tuunanen et al. 2006). In a mouse model deficient in lipoprotein lipase in the heart, cardiac dysfunction was observed despite the up-regulation of myocardial glucose utilization (Augustus et al. 2006), suggesting a critical role of lipase-derived fatty acids in cardiac metabolism. In addition, it has been shown that endogenous triglyceride metabolism in the failing heart is impaired (O'Donnell et al. 2008), indicating that the turnover of the triglyceride pool may also represent a novel target for metabolic intervention.

Given the predominance of fatty acid oxidation in cardiac energy supply, normalization of fatty acid oxidation in the failing heart seems to be a logical strategy. However, increasing fatty acid oxidation via pharmacologic intervention in heart failure has yielded conflicting results. Chronic activation of PPAR α with fenofibrate in rats post-myocardial infarction or in dogs with pacing-induced heart failure maintained the fatty acid oxidation gene profile but had modest benefits on the development of heart failure (Labinskyy et al. 2007; Morgan et al. 2006). Conversely, Young et al. (2001) demonstrated that although PPAR α agonist treatment after aortic banding pre-

vented down-regulation of fatty acid oxidation genes, it failed to correct cardiac dysfunction. Furthermore, PPAR α agonism has been shown to worsen postischemic injury (Hafstad et al. 2009; Sambandam et al. 2006). Recently, an interesting series of studies suggested that high-fat diet (HFD) protected against the development of heart failure in a variety of animal models (Chess et al. 2009; Okere et al. 2006; Rennison et al. 2009). The mechanisms underlying the benefits of HFD are unknown, but the observation again challenges the concept that fatty acids are detrimental to the failing heart.

• Substrate Preference Switch vs Maintaining Metabolic Capacity and Flexibility

Concerns of excessive fatty acid oxidation were raised in hearts with ischemia-reperfusion injury and in cardiac dysfunction observed in animals or patients with obesity and diabetes (Aasum et al. 2003; Buchanan et al. 2005; Kudo et al. 1995). Under both conditions, the inefficiency of cardiac work and impaired contractile function associated with high fatty acid oxidation can be corrected by promoting glucose oxidation (Bersin and Stacpoole 1997; Hafstad et al. 2007). The notion that increased glucose metabolism is protective against ischemia-reperfusion injury is further supported by the observations that overexpressing GLUT1 protects the ischemic heart, whereas deletion of insulin-sensitive glucose transporter (glucose transporter 4) is detrimental (Luptak et al. 2007; Tian and Abel 2001).

The results of targeting substrate preference in diabetic hearts are quite mixed. Treatment with PPAR α or PPAR γ activators in animal models of diabetes reduced fatty acid oxidation and increased glycolysis and glucose oxidation, but yielded inconsistent outcomes with regard to functional improvement (Aasum et al. 2002, Carley et al. 2004, How et al. 2007). An important consideration here is that the commonly used type 2 diabetes rodent models (also used in these studies) have defects in leptin signaling, which cause cardiomyopathy independent of substrate metabolism (Barouch et al. 2003). Nevertheless, in type 1 diabetes model, treatments with

angiotensin-converting enzyme inhibitors or β -blockers increased cardiac glucose utilization, decreased fatty acid oxidation, and improved cardiac function, although the causal relationship could not be defined in these studies (Arikawa et al. 2007, Sharma et al. 2008).

In a recent study (Yan et al. 2009), the cardiac-specific GLUT1 transgenic mouse, which had demonstrated increased myocardial glucose uptake and oxidation, was fed an HFD. The wild-type animals on HFD demonstrated the expected increase in cardiac fatty acid oxidation, whereas the GLUT1 transgenic hearts maintained high glucose oxidation despite comparable levels of obesity and insulin resistance in both genotypes. The resistance to increased fatty acid oxidation in the GLUT1 transgenic heart was attributed to a number of glucose-dependent changes in the gene expression that restrict fatty acid oxidation and promote glucose oxidation. Surprisingly, the protection against the high fatty acid oxidation was associated with elevated oxidative stress and cardiac dysfunction in GLUT1 transgenic mice with diet-induced obesity. This was unexpected, given the mounting evidence suggesting that a switch of substrate utilization toward glucose would be beneficial. An important point raised by the study is that the molecular remodeling caused by excessive reliance on one substrate (glucose in the case of GLUT1 mice and fatty acids in the case of obesity and diabetes) compromises the flexibility of the metabolic network and prevents the heart from utilizing the most efficient substrate.

In conclusion, the genetic and pharmacologic studies show that optimal cardiac function depends on the ability of the heart to utilize all carbon substrates. Thus, the ultimate goal of modulating cardiac metabolism for therapeutic purposes is not to shift the substrate utilization toward one end of the spectrum or the other but rather to sustain the flexibility of the network. Metabolic therapy in the future should include treatments that improve insulin sensitivity and sustain mitochondrial function, hence satisfying the enormous energy requirement of the heart.

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PII S1050-1738(09)00183-2

TCM

The Application of Phenotypic High-Throughput Screening Techniques to Cardiovascular Research

Yoram Etzion and Anthony J. Muslin*

In traditional pure protein high-throughput drug screens, also called in vitro screens, individual compounds from a small molecule collection are tested to determine whether they inhibit the enzymatic activity or binding properties of a purified target protein. In contrast, phenotypic high-throughput drug screens, also called chemical genetic or in vivo screens, investigate the ability of individual compounds from a collection to inhibit a biological process or disease model in live cells or intact organisms. In this review, the role of phenotypic screening techniques to identify novel therapeutic agents for the treatment of cardiovascular disease will be discussed. (Trends Cardiovasc Med 2009;19:207–212) © 2009, Elsevier Inc.

• Introduction

A traditional screening method for the identification of pharmacologic agents for the treatment of human disease involves the use of a biochemical assay with a purified target protein (Burbaum and Sigal 1997; Crews and Splittgerber

1999). In this pure protein assay, the ability of a compound from a collection to alter the enzymatic activity or binding properties of the target protein is evaluated. This traditional screening approach has been successfully applied for many target proteins, and active compounds identified by this methodology are known to alter the activity of the target in question. The ability of compounds identified in pure protein high-throughput screens to modify disease progression in human patients is not known a priori and may not be related to the biochemical activity of the compound in vitro.

To identify useful compounds for the treatment of human disease in situations where the specific enzymes responsible are unknown, methods that investigate complex phenotypes in living cells or

organisms may be an attractive alternative to pure protein screens. In phenotypic screens, also called *chemical genetic* or *in vivo screens*, a biological process in live cells or intact organisms—rather than an enzymatic or binding reaction with purified protein—is assayed (Burbaum and Sigal 1997; Crews and Splittgerber 1999; Yeh and Crews 2003). The biological assay must be quantitative and reproducible, features that are characteristic of biochemical assays with pure protein, but that are not easy to achieve with biological assays. In phenotypic high-throughput screens, cells or organisms (e.g., zebrafish embryos) are placed in microtiter plates in the presence of culture or growth medium. Individual compounds from chemical libraries are pipetted into unique wells by a robotic liquid-handling device, and the biological assay is performed. Many biological assays involve the use of fluorescent or luminescent reagents that require automated microtiter plate readers with advanced data processing. In other cases, phenotypic abnormalities of embryos or cells are evaluated by light microscopy. Active compounds are identified on the basis of their ability to modify the results of the biological assay.

• Development of Phenotypic High-Throughput Screens

A variety of technical advances in biomedical research contributed to the development of chemical biology screens, including the development of chemical libraries, robotic liquid-handling devices, luminescent and fluorescent reagents and epitope tags, sensitive microtiter plate readers, and advanced data processing (Wunder et al., 2008). In one early phenotypic high-throughput screen, intact A549 cells were used to identify small molecules that could affect cell-cycle

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