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Cardiac Energy Metabolism in Health and Disease

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This book is dedicated to Professor John H. McNeill for his outstanding leadership and contribution to cardiovascular research. Professor McNeill is internationally recognized for his research in understanding the cardiovascular complications associated with diabetes. He has published over 500 research papers, 98 review articles, and has edited five books. His work has been cited nearly 11,000 times placing him in the top 1 % of all scientists in the world. He has been recognized by the Pharmacological Society of Canada, the Association of Faculties of Pharmacy, and the University of British Columbia by receiving their top research awards. He has been elected as a Fellow of the Royal Society of Canada, the International Academy of Cardiovascular Sciences, the American Academy of Nutrition, and the Canadian Academy of Health Sciences. As Dean of the Faculty of Pharmaceutical Sciences at UBC (from 1985 to 1996) he distinguished himself as an administrator and is noted for curriculum changes, the introduction of the first Doctor of Pharmacy program in Canada and his stewardship of the Faculty. He also has demonstrated outstanding undergraduate teaching skills (winner of the Killam Award for Teaching) and graduate training skills (25 Ph.D. students, 31 M.Sc. students, 24 postdoctoral fellows, and 11 Visiting Professors). Many of the individuals he

trained have gone on to successful academic or professional careers in the area of cardiovascular energy metabolism. This book dealing with the regulation of cardiovascular energy metabolism in health and disease pays a special tribute to Professor McNeill for his distinguished contributions to this research field.

Preface

The heart has a very high energy demand but very little energy reserves. As a result, the heart has to continually produce a large amount of ATP necessary to sustain contractile function. The heart utilizes free fatty acids mainly and carbohydrates to some extent as substrates for making energy and any change in this energy supply can seriously compromise cardiac function. It has emerged that alterations in cardiac energy metabolism are a major contributor to the development of a number of different forms of heart disease. It is also now known that optimizing energy metabolism in the heart is a viable and important approach to treating various forms of heart disease.

This book describes the research advances that have been made in understanding what controls cardiac energy metabolism at molecular, transcriptional, and physiological levels. It also describes how alterations in energy metabolism contribute to the development of heart dysfunction, and how optimization of energy metabolism can be used to treat heart disease. The topics covered include a discussion of the effects of myocardial ischemia, diabetes, obesity, hypertrophy, heart failure, and genetic disorders of mitochondrial oxidative metabolism on cardiac energetics. The treatment of heart disease by optimizing energy metabolism is also discussed, which includes increasing overall energy production as well as increasing the efficiency of energy production and switching energy substrate preference of the heart.

The chapters are written by leaders in the field of research dealing with cardiac energy metabolism. The first part deals with how cardiac energy metabolism is controlled in the heart. The chapter by *Taegtmeyer* describes the role of carbohydrate metabolism in cardiac energy production whereas that by *Drosatos and Goldberg* presents an eloquent review of the role of lipoproteins in providing fatty acids and other essential lipids to the heart. This is followed up by a chapter by *Wan and Rodrigues* that examines the role of coronary vascular lipoprotein lipase in fatty acid delivery to the heart, and how an increase in its activity is a prelude for the cardiovascular complications seen in diabetes. The chapter of *Glatz and Liuken* then describes how fatty acids are transported into the myocardium, and the pivotal role of CD36 in the overall regulation of myocardial fatty acid uptake and utilization. *Lopaschuk* reviews the metabolic alterations that occur in heart failure associated

with obesity and diabetes, and the molecular mechanisms responsible for these changes, while *Vega*, *Leone, and Kelly* summarize our current understanding of the perturbations in the gene regulatory pathways that occur during the development of heart failure. This is followed by a chapter from *Aasum* that focuses on our current understanding of how myocardial substrate supply and/or utilization contribute to altered cardiac efficiency. The chapter of *Sack* discusses the key role of acetylation and its regulatory control is explored in the context of the control of mitochondrial integrity and metabolic functioning.

The second part explores alterations in cardiac energy metabolism that can occur in heart disease. *Sharma and McNeill* summarize the results of studies investigating how β-adrenergic signaling controls cardiac metabolism, and the significance of these mechanisms in diabetes. *Lygate and Neubauer* describe the complexities of changes in energy metabolism in heart failure, and the central role that alterations in creatine kinase have on these processes. *Kolwicz and Tian* focus on advances in the understanding of cardiac metabolic plasticity in pathological cardiac hypertrophy and heart failure as well as therapeutic strategies based on these observations. The key role of mitochondrial dysfunction with respect to energy production due to ischemia reperfusion injury is discussed by *Dhalla and his coworkers* and the involvement of oxidative stress and intracellular Ca^{2+}-overload in these processes has been emphasized. *Byrne*, *Sung, and Dyck* review the role of AMPK in the control of cardiomyocyte growth and discuss the potential benefits and pitfalls that may accompany the approach of pharmacologically activating AMPK to control the pathogenesis of cardiac hypertrophy. *Ussher* focuses on the potential role of incomplete fatty acid β-oxidation in the heart as a mediator of cardiac insulin resistance.

Finally, the third part of this book explores the exciting concept that optimizing energy metabolism may be a clinical approach to treat heart disease. *Marzilli and associates* discuss the clinical data demonstrating that inhibiting fatty acid oxidation and increasing glucose oxidation as an approach to treat ischemic heart disease. The chapter by *Gao and coworkers* reviews the existing evidence for inhibition of fatty acid oxidation to treat heart failure. Further studies are needed to confirm the potential benefit of modulating these metabolic targets as an approach to treating heart failure in clinical settings. The chapter by *Ralphe and Scholz* then examines the role of the neonatal myocyte in the heart disease, and approaches to improve myocardial preservation that is an essential part of open-heart surgery in infants and children. In the end *Portman and Olson* have presented transcriptional modulation of fatty acid oxidation as a potential therapy for heart failure.

A major contributor to our understanding of how diseases such as diabetes can impact cardiac energy metabolism is Professor John McNeill. Fittingly, this book is dedicated to Professor McNeill in order to recognize his many contributions to this important research area. Indeed, we are grateful to all contributors for providing state of the art articles and thus making this book a reality. Our thanks are also extended to both Dr. Vijayan Elimban and Ms. Eva Little at the St. Boniface Hospital Research Centre for the time and efforts which they devoted on this project. We appreciate the help of Ms. Diana Ventimiglia as well as the staff at the

Springer Media, New York in the preparation of this book. It is our sincere hope that this book will be a valuable source of information to graduate students, postdoctoral fellows, and investigators in the field of experimental cardiology as well as biochemists, physiologists, pharmacologists, cardiologists, cardiovascular surgeons, and other health professionals.

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Part I
Control of Energy Metabolism

A Primer on Carbohydrate Metabolism in the Heart

Heinrich Taegtmeyer

Abstract As a biological pump the heart is a self-renewing engine serving the efficient conversion of chemical to mechanical energy. Among others, glucose, lactate and glycogen are providing substrates for the heart. Glucose homeostasis in the cardiomyocyte is tightly regulated by hemodynamic factors, neurohumoral factors, and oxygen availability. The efficiency of carbohydrates for oxidative mitochondrial ATP production is well recognized both *in vivo* and *ex vivo*. Glucose and glycogen also provide a small, but sometimes critical, amount of ATP through substrate level phosphorylation in the glycolytic pathway. Glucose, lactate and glycogen compete with fatty acids as substrates for oxidative metabolism and provide anaplerotic substrate for the Krebs cycle in the form of pyruvate carboxylation. The product of the first reaction in the glycolytic pathway, glucose 6-phosphate (G6P) is also the substrate for phosphoglucomutase (the first step in glycogen synthesis), for the pentose phosphate pathway, and for the hexosamine biosynthetic pathway. G6P is also part of the nutrient signaling in the mTOR pathway. Collectively, carbohydrate metabolism is a highly regulated, integral part of energy transfer and metabolic signaling in heart muscle.

Keywords Glucose • Glycogen • Lactate • Glucose 6-phosphate • Insulin resistance

1 Introduction

As a biological pump the heart is a self-renewing engine serving the efficient conversion of chemical to mechanical energy. Glucose, lactate and glycogen are among energy providing substrates for the heart. Glucose homeostasis in the

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cardiomyocyte is tightly regulated by hemodynamic factors, neurohumoral factors, and oxygen availability. This chapter highlights principles of myocardial carbohydrate metabolism, and includes newly discovered roles of intermediary metabolites that regulate cardiac cell function. The chapter also highlights metabolic signatures as they relate to emerging concepts in metabolic regulation at the transcriptional, translational, and post-translational level, and discusses the putative role of glucose-specific metabolic signals in the regulation of myocardial protein turnover. In the age of molecular biology metabolic pathways seem a distant memory. Yet the importance of fuel metabolism in the heart is newly appreciated in heart disease, cancer, and diabetes. The chapter begins therefore with a brief review of glucose metabolic pathways.

2 Carbohydrate Metabolism in Perspective

Like in any living organ, it is impossible to separate function from metabolism in the heart. The bulk of the heart's energy for contraction, ion movements, and the cellular turnover of its constituents is provided by the metabolism of oxidizable substrates. The final product of oxidative metabolism in the mitochondria is ATP. A decrease in the flux of metabolic energy (in) results in a decrease in ATP production; a decrease in contractile function (out) *vice versa*, an increase in energy demand increases ATP turnover and an increase in oxygen consumption.

The heart's requirement for calcium, nutrients, and oxygen has been recognized for well over a century [1, 2]. The field of cardiac metabolism has a rich history of integrative and molecular physiology [3]. By measuring arteriovenous differences in the heart–lung preparation [4] of the dog or in the human heart through cannulation of the coronary sinus [5], early investigators have laid the foundation for current concepts of myocardial energy substrate metabolism. The more recent reviews survey the knowledge gained through isolated heart preparations, and isolated cell preparations, as well as isolated cell organelles [3, 6–15]. As will be discussed later in the chapter, the toolbox for the discovery of metabolic regulation in genetic animal models and in cardiomyocytes has grown even more dramatically than any clinically useful instrumentation. As more mechanisms of metabolic regulation are exposed so are the opportunities for targeting specific metabolic steps by pharmacological compounds.

Cardiac metabolism maintains a dynamic state of equilibrium for efficient energy transfer at the site of ATP production in the mitochondria and at the site of ATP usage by the cross-bridged cycle. This has three consequences. First, the rate of energy turnover, and not the tissue content of ATP, is the main driving force of energy metabolism [8, 16–18]. The greater the work output, the higher the rate of ATP turnover, the higher the rate of oxygen consumption, and the higher the rate of substrate input and utilization. Secondly, energy transfer in the heart obeys the first law of thermodynamics, i.e. within a closed system, energy can only be converted from one form into another. Third, when the heart's ability to convert chemical into mechanical energy is impaired *for any reason* [19] the consequences manifest themselves as functional and metabolic decline in the rest of the body.

3 A General Roadmap of Glucose Metabolism

A *metabolic pathway* is defined as a series of enzyme-catalyzed reactions beginning with a flux-generating step (usually a reaction catalyzed by a non-equilibrium reaction or transport of the metabolite across a membrane) and ending with the removal of a product [20, 21]. Characteristic of most metabolic pathways is that, once flux has been initiated, there is a rapid and concerted response of the entire pathway. In this system of flux, metabolite levels control enzyme activities and, in turn, enzyme activities are controlled by metabolite levels. Control is the power to change metabolic flux in response to an external signal, whereas regulation is the inherent capacity of enzymes geared toward maintaining a constant internal state [22]. In such a system, large changes in the flux through metabolic pathways correspond to only very small changes in myocardial metabolite concentration [23]. Regulatory sites of metabolism, or pacemaker enzymes [24], have become targets for the manipulation of metabolism with drugs [25, 26]. A case in point is the glycolytic pathway, which begins with the transport of glucose into the cell, and which ends with the formation of pyruvate as lactate.

4 Glucose, Lactate, and Glycogen Metabolism in Focus

Although long chain fatty acids are the predominant fuel for energy provision to the postnatal heart, carbohydrates are the fuel for the fetal heart [27] and also for the stressed adult heart in the state of exercise or pressure overload [28, 29]. The reasons for such a "hierarchy" of fuels can be deduced from a number of observations:

1. In the normal, i.e. the non-diabetic, mammalian organism glucose levels in the blood are tightly regulated at around 5 mM or (90 mg/dL).
2. When we exercise and blood lactate levels rise, lactate replaces all other substrates as fuel for respiration of the heart [28].
3. When the normal heart is stressed, it oxidizes first glycogen, then glucose and lactate [30, 31].
4. Glucose is an anaplerotic substrate for the citrate cycle [32, 33] and glucose is essential for the initiation of fatty acid oxidation in heart [34].

Isotopic tracer studies have demonstrated that the normal human heart produces lactate at the same time it oxidizes lactate [30, 35]. It appears also that a large portion of exogenous glucose, when taken up by the cardiomyocytes, is shunted into first glycogen before it is oxidized [36, 37].

5 Regulation of Glucose Metabolism

There are three energy-yielding stages of glucose metabolism: the glycolytic pathway leading to pyruvate, oxaloacetate, and lactate; the Krebs cycle; and the respiratory chain. Each state is regulated by its own set of checkpoints, so that overall flux

through the pathways (which may be assessed externally on a second-by-second time-scale with the glucose tracer analogue FDG) [38] proceeds at a rate just sufficient to satisfy the heart's beat-to-beat needs for ATP. We discuss the regulatory sites of metabolism in more detail because dysregulation of one of these steps can affect the rate and efficacy of energy transfer and, hence, contractile function of the heart.

5.1 Glucose Entry and Metabolism in the Cardiomyocyte

The uptake of glucose by the cardiomyocyte follows Michaelis–Menten kinetics. The transport of glucose occurs along a steep concentration gradient and is regulated by specific transporters [39]. The stereospecificity of the transporter for sugars of the carbon configuration is not matched by the same degree of selectivity, and various tracer analogues, including 2-deoxyglucose and FDG, are transported in the same way as glucose.

Glucose transport, the rate-limiting step in myocardial glucose utilization [40], requires facilitative glucose transporters. The family of glucose transporters (GLUT) is conserved over a wide range of organisms, suggesting a common evolutionary origin [41–44]. GLUT-1 and GLUT-4 are the major glucose transporter isoforms expressed in the heart [45, 46]. GLUT-4, the insulin-sensitive transporter, is also expressed by skeletal muscle and in adipose tissue [41]. Recruitment of GLUT-4 from a microsomal cytosolic pool to the sarcolemma by insulin (or ischemia or adrenergic stimulation) [47–50] increases the maximal velocity of glucose transport. Alpha-adrenergic stimulation uses the same signaling pathway as insulin to promote glucose uptake [51], whereas the effects of ischemia, beta-receptor stimulation, and insulin on glucose uptake are additive [52].

Cardiomyocytes also express the GLUT-1 transporter isoform, which is presumably independent of insulin regulation and predominates in fetal, hypertrophied, atrophied and failing myocardium [42, 45, 53]. GLUT-1 is the first gene whose transcription is dually stimulated in response to hypoxia and inhibition of oxidative phosphorylation [54], and overexpression of GLUT-1 prevents the functional decline of hypertrophied heart [55]. Both transporters have a K_m for glucose (i.e., the concentration at which the rate of glucose transport is half maximal) that is in the range of plasma glucose concentrations under fasting conditions [56]. The normal heart also expresses a low amount of GLUT-3, which has a K_m below the normal plasma glucose concentration [57]. Over the years a number of novel GLUTs have been identified in heart muscle including GLUT-8, GLUT-11, and GLUT-12 [58]. A role of these proteins in myocardial glucose metabolism has not yet been firmly established.

Phosphorylation of glucose by hexokinase becomes rate limiting for glycolysis at high rates of glucose transport. Rates of glucose phosphorylation measured *in vitro* are more than twice as high as the maximal measured rates of glucose utilization by the heart at a physiologic workload and with glucose as the only substrate [59]. However, intracellular glucose concentrations rise with starvation, in diabetes, and with the concomitant oxidation of fatty acids, ketone bodies, or lactate, which

indicates inhibition of the phosphorylation step. This is most likely to be due to accumulation of glucose-6-phosphate, which is an allosteric inhibitor of hexokinase II, the cardiac isoform of hexokinase. Reduction of hexokinase II levels results in decreased cardiac function and altered remodeling after ischemia and reperfusion [60].

Glucose 6-phosphate is at the branch point of four distinct pathways:

1. Degradation via the Embden–Meyerhof pathway (also termed the *glycolytic pathway* when it entails metabolism of glucose to lactate only);
2. Conversion to glycogen via the glycogen synthase reaction;
3. Metabolism (oxidation) via the pentose-phosphate pathway, which yields ribose and the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) [61];
4. Entry into the hexosamine biosynthetic pathway [62].

While the latter two pathways are of quantitatively lesser importance in heart muscle than are the former two, they provide glycogen.

5.2 Glycogen

Glycogen metabolism is the first paradigm for the molecular basis of hormone action [63]. In this pathway the control of enzyme activity by an allosteric regulator (activation of phosphorylase by adenosine monophosphate [AMP]) was first described [64], enzyme regulation by covalent modification was discovered [65], and the molecular basis of hormone action by signal transduction was elucidated by the discovery of cyclic adenosine monophosphate (cAMP) [66]. Although the enzyme glycogen synthase kinase 3β (GSK3β), which phosphorylates and inhibits glycogen synthesis is a well-known regulator of cardiac growth, its role in intermediary metabolism must not be overlooked. Hypertrophic stimuli inhibit GSK3β to regulate changes in metabolism, gene expression, and cytoskeletal integrity needed to promote cell growth [67]. The role of glycogen metabolism in the heart is still not completely understood [68]. Here two points should be made. Firstly, the vast amount of glycogen in fetal cardiac muscle probably allows the heart to maintain its contractile activity in the face of severe hypoxia [69] during birth. Secondly, glycogen and glycogen phosphorylase are closely associated with the sarcoplasmic reticulum [70] and in skeletal muscle a decreased glycogen content results in a reversible reduction in force, Ca^{2+} release from the sarcoplasmic reticulum, and contractile protein function [71].

5.3 Glycolysis

Glucose is special among the energy-providing substrates for the heart in its ability to provide a small, but significant, amount of ATP through substrate-level phosphorylation in the glycolytic pathway. This occurs especially in the setting of hypoxia and

ischemia when flux through the glycolytic pathway is enhanced resulting in the formation of lactate as well as alanine [72]. Other stimulants of flux through the glycolytic pathway are increases in cardiac work, either acutely with exercise [30, 73] or chronically with pressure overload without [74] or with hypertrophy [29, 75]. Both hypertrophy and atrophy are associated with increased glucose oxidation rates in the face of decreased insulin responsiveness of the heart [76]. In ischemia the accumulation of glycolytic intermediates may worsen contractile function [77] and acute hyperglycemia may abolish ischemic preconditioning *in vivo* [78] while provision of glucose together with insulin and potassium improves contractile function in the acutely ischemic, reperfused myocardium [79–82].

The first step committing glucose to the glycolytic pathway is 6-phospho-fructo-1-kinase (PFK-1), which catalyzes the phosphorylation of fructose-6-phosphate to fructose 1,6-bisphosphate. Because of the complex allosteric regulation of PFK, this is a rate-limiting step (pacemaker enzyme) for glycolysis [83]. ATP, citrate, and protons are negative allosteric effectors, whereas AMP and fructose 1,6-bisphosphate are positive effectors [84–86]. Fructose 2,6-bisphosphate is the main activator of PFK-1 in normoxic heart [87].

Further down in the glycolytic pathway, the oxidation of the triose-phosphate glyceraldehydes 3-phosphate to 1,3-diphsophoglycerate couples in the energy-conserving step in the glycolytic pathway that leads to the nonoxidative formation of ATP with high cardiac work [88] or ischemia [89] when PFK becomes strongly activated, glycolysis is controlled further downstream, at the triose-phosphate dehydrogenase step.

5.4 Pyruvate Metabolism: An Intermediate at the Crossroads

The last glycolytic intermediate, pyruvate, is substrate for another branch point in metabolism, as the following discussion shows. Pyruvate can be reduced to lactate (which completes the glycolytic pathway), transaminated to alanine [72], carboxylated to oxaloacetate or malate [90, 91], or, most importantly, oxidized to acetyl-CoA. In well-oxygenated, working heart muscle, however, the bulk of pyruvate enters the mitochondrion through a transporter which can be inhibited by 4-hydroxy-alpha-cyanocinnamate [92]. Once inside the mitochondrial matrix, pyruvate is either decarboxylated to acetyl-CoA or carboxylated to oxaloacetate [91]. The capture of metabolically produced carbon dioxide from the pyruvate dehydrogenase reaction to form oxaloacetate is an example for the efficient use of one substrate supplying two precursors for citrate synthesis and for the efficient recycling of carbon dioxide.

Oxidative decarboxylation of pyruvate is highly regulated by activation and inactivation of the pyruvate dehydrogenase complex (PDC) [93]. The conversion of pyruvate to acetyl-CoA requires the sequential action of three different enzymes: pyruvate dehydrogenase, dihydrolipoyl transacetylase, and dihydrolipoyl dehydrogenase. The reaction also requires five different coenzymes or prosthetic groups: thiamine

pyrophosphate, lipoic cid, uncombined coenzyme A (CoA SH), FAD^+, and NAD^+. These enzymes and coenzymes are organized into a multi-enzyme cluster.

Much work was done in the 1970s on the regulation of PDC by covalent modification through a phosphorylation and dephosphorylation cycle [6]. Multi-site phosphorylation of the pyruvate dehydrogenase component of the complex provides an indirect means by which the entire complex is regulated by the relative activities of the PDC kinase and phosphatase reactions. Of the metabolite pairs ATP–ADP, acetyl-CoA–CoA SH, NADH–NAD^+, and lactate–pyruvate, the first member either activates the kinase or serves as substrate, and the second member inhibits the enzyme. Ca^{2+} and Mg^{2+} both inhibit the kinase and activate the phosphatase reaction (i.e., lead to PDC activation). The effects of fatty acids or ketone body oxidation are likely to be mediated by the increase in acetyl-CoA because the primary effect or the inhibition-inactivation of PDC by fatty acids or ketone bodies in the acetyl-CoA–COA SH ratio [94]. Conversely, an increase in cardiac work may inhibit the PDC kinase owing to a decrease in NADH, acetyl-CoA, and ATP, leading to activation of PDC [95].

5.5 Dysregulated Glucose Metabolism

Glucose and its metabolites also have multiple functions in the cardiac myocyte besides energy provision. Failure to adequately control levels of intracellular glucose metabolites has been implicated in the development of insulin resistance and in the generation of reactive oxygen species (ROS). Compared to the liver [96], relatively little is known about the effects of glucose metabolites on gene expression in the heart. Through investigations of the glucose/carbohydrate response elements (GIRE/ChoRE) in the promoter regions of various glucose-regulated genes, a number of candidate transcription factors have been identified that are believed to be involved in glucose-mediated gene expression. In the liver and in fat cells upstream stimulatory factor (USF), stimulatory protein 1 (Sp1), and sterol regulatory element binding protein 1 (SREBP1) play a role in glucose sensing [97], and it is reasonable to assume that the same transcription factors play similar roles in the heart [98].

Excessive accumulation of glucose metabolites is also associated with various cardiac pathologies. Although studied mainly in the vasculature, the four main mechanisms proposed for hyperglycemia induced diabetic complications [99] are likely to be operative in the cardiomyocyte as well. The four hypotheses are increased polyol pathway flux (via aldose reductase), increased intracellular formation of advanced glycation end-products (AGE) and AGE-induced ROS generation activation of protein kinase C mostly through activation by the lipid second messenger diacylglycerol (DAG), and increased flux through the hexosamine biosynthetic pathway [99]. As mentioned, many of these hypotheses have thus far only been tested in vascular tissue and await further confirmation in cardiomyocytes. However, it is already known that *O*-linked β-*N*-acetylglucosamine (*O*-GlcNAc) modifies many different nuclear and cytoplasmic proteins and that *O*-GlcNAc plays

an important role in signal transduction [100]. The basis is a "spillover" of intermediates of glucose metabolism into the hexosamine biosynthetic pathway and into the pentose phosphate pathway [98].

Lastly, increases in the rate of glycogen and glucose oxidation are the first responders to hemodynamic stress in the heart [30, 101, 102], except in the metabolic state of exercise [28]. When hearts are subjected to a sustained hemodynamic pressure, the left ventricle remodels both metabolically and structurally. Remodeling is, to some extent, driven by the mammalian target of rapamycin (mTOR), a regulator of myocardial protein synthesis downstream of Akt in the insulin signaling pathway. A mismatch between myocardial glucose uptake and oxidation results in the intracellular accumulation of glucose 6-phospate (G6P), which has been implicated both, in insulin stimulated and load-induced mTOR activation and the endoplasmic reticulum stress response [103, 104]. G6P has also been implicated in enhanced mTOR activation and hypertrophy in hearts with impaired fatty acid oxidation [105]. Sustained activation of these pathways results in decreased contractile efficiency which can be reversed (or prevented) by either rapamycin or metformin. In the end, targeting glucose metabolism may once again become an attractive option for supporting the failing heart. In a more physiologic context we are also speculating that insulin resistance protects the heart from fuel overload in dysregulated metabolic states [106].

6 Conclusions

Glucose metabolism is an integral part of energy substrate metabolism and supports the function of the heart as consumer and provider of energy. Glucose and its metabolites have pleiotropic roles. The bulk of the energy for contraction of the heart comes from oxidative phosphorylation of ADP which glucose and/or lactate oxidation contribute to varying degrees. A vast network of highly regulated metabolic pathways matches demand and supply with precision. Glucose metabolites are also regulators of enzyme activities and cardiac growth. The complexity of intermediary metabolism is also a rich source of speculation on impaired energy transfer as either cause or consequence of impaired cardiac function. All is in flux and nothing endures but change (Heraclitus of Ephesus 535–475 B.C.).

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Lipoproteins: A Source of Cardiac Lipids

Konstantinos Drosatos and Ira J. Goldberg

Abstract Lipids are the major substrate for cardiac ATP production and they are derived from adipose tissue or lipoprotein triglycerides. Lipoproteins are synthesized in the liver and they obtain their mature form following interaction with enzymes that are present in the circulation. Lipoprotein-derived fatty acids are released by lipoprotein lipase and are then taken up by cardiomyocytes either passively or via fatty acid receptors, such as CD36. Uptake of remnant lipoproteins via cardiomyocyte lipoprotein receptors is also possible. Besides fatty acids, other hydrophobic molecules such as cholesteryl esters, retinyl esters and vitamins are delivered by lipoproteins to the heart. While lipids are important for normal cardiac function, excessive lipid uptake, also known as lipotoxicity, may lead to cardiac abnormalities. This chapter focuses on the role of lipoproteins in providing fatty acids and other essential lipids to the heart in healthy conditions as well as in cardiac disease.

Keywords Lipoprotein triglyceride • Lipoprotein lipase • Fatty acid receptors • Fatty acid uptake • Cardiomyocytes • Cardiac lipoprotein receptors

1 Introduction

The heart can obtain energy from several sources including lipids, glucose, ketones and lactate. 70 % of cardiac ATP is thought to be produced via fatty acid (FA) oxidation [1]. Triglycerides (TGs) are the primary source of FAs in circulation and

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constitute, not surprisingly, the primary lipid source used for cardiac energy production [2]. Over 90 % of plasma FAs are esterified within either TG or phospholipids. Lipoprotein-associated FAs are derived from dietary fat, as well as from endogenous hepatic de novo FA synthesis. The heart primarily obtains FAs after their release from TG-rich lipoproteins by the enzyme lipoprotein lipase (LpL). Some cardiac FAs as well as hydrophobic lipids such as cholesteryl esters and retinyl esters are dissociated from lipoproteins or they are obtained via internalization of whole lipoprotein particles by lipoprotein receptors. Cardiac FA uptake occurs either via passive non-receptor transport, also known as "flip-flop" [3], or via cell membrane receptors, such as cluster of differentiation (CD) 36 [4, 5] and fatty acid transport protein (FATP) [6, 7]. The exuberant use of lipids by the heart makes it an ideal organ to gain insights into the routes of uptake of lipid from liver, gut, and circulation. This chapter will focus on reviewing the role of lipoproteins in providing FAs and other essential lipids to the heart.

2 General Structure and Physiologic Role of Lipoproteins

Lipoproteins are water-soluble macromolecules that transfer lipids amongst different tissues. They consist of a polar surface of phospholipids, free cholesterol and apolipoproteins (Apos) and a non-polar lipid core that contains primarily cholesteryl ester and TGs as well as fat soluble vitamins. There are two major classes of TG-rich lipoproteins: chylomicrons, which are produced from dietary fat, and very low density lipoproteins (VLDL) that have hepatic origin and are carriers of endogenously produced FAs (Fig. 1). The major cholesterol-containing lipoproteins are low density lipoproteins (LDL) and high density lipoproteins (HDL).

Lipoproteins were originally differentiated by their floatation in the ultracentrifuge as salt was added to the serum, hence their names. Lipoproteins can also be separated based on their size using gel filtration, and by charge using electrophoresis. They can also be distinguished by NMR. The largest and most buoyant lipoproteins contain the greatest amount of lipid, which is predominantly TG. The smaller more dense lipoproteins have cholesteryl ester as the major non-polar lipid. The lipids that each lipoprotein class carries reflect its major physiologic role: delivery of dietary or hepatic produced TG or cholesterol.

3 Chylomicrons

Dietary TGs are packaged in chylomicrons. In most cases FAs not esterifed to glycerol, free FAs (FFAs), but not TGs cross cell membranes. Dietary TG is converted into FFAs by intestinal lipases and then re-esterified within the enterocyte. TG and the ester forms of other lipids such as cholesterol and retinol are packaged into particles via the actions of microsomal TG transfer protein (MTTP). The major structural protein of chylomicrons is ApoB48, which is an edited version of the longer hepatic ApoB100 (Fig. 1).

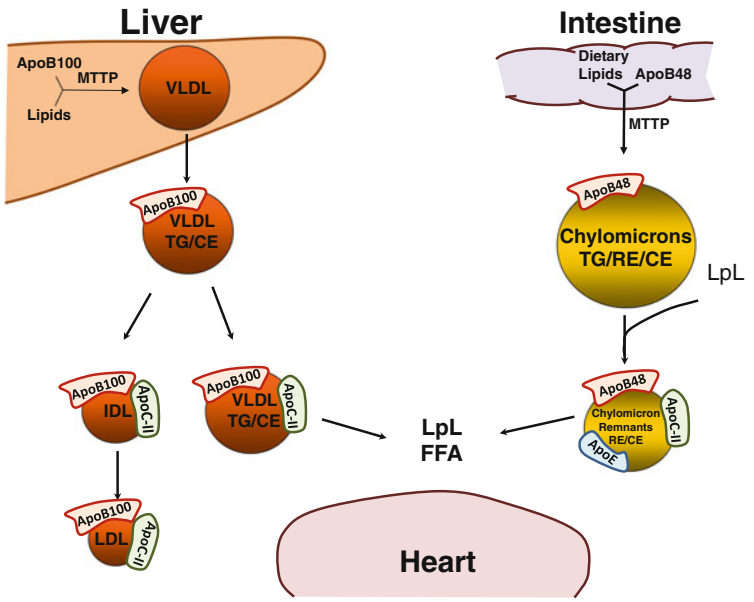

Fig. 1 Biosynthesis of VLDL and chylomicrons—VLDL production in the liver requires and transport ApoB and microsomal triglyceride transfer protein (MTTP). VLDL are carriers of triglycerides (TG) and cholesteryl ester (CE). Gradual lipoprotein lipase (LpL)-mediated hydrolysis of TG leads to conversion of VLDL to IDL and LDL. Chylomicrons are produced in the intestine and transfer TG, CE and retinyl esters. Following hydrolysis of TG by LpL chylomicron remnants are formed. Lipolysis accounts for most FA uptake by the heart

Chylomicrons are not secreted directly into the circulation, but arrive into the superior vena cava via the lymphatic system. Thus, they reach the heart prior to their exposure to the liver or peripheral tissues such as skeletal muscle and adipose tissue. Once in the bloodstream, chylomicron surface proteins, such as ApoC-II, the LpL activator, are exchanged with other lipoprotein proteins. Chylomicrons deliver cholesterol and FAs to the heart as shown by studies in vivo [8–10] and in isolated hearts of rats that were perfused with chylomicrons enriched in radiolabelled cholesterol and/or FAs [8–10].

FFAs obtained by the heart can serve as fuel or components of structural lipids and lipid droplets. While the vast majority of albumin-bound FFAs are used for oxidation, chylomicron-derived cardiac FAs are used evenly for oxidation and storage [10].

4 VLDL

Hepatic TGs are secreted as a component of VLDL, TG-rich lipoproteins that are smaller and less buoyant than chylomicrons (Fig. 1). VLDL-associated TGs are from three sources, which differ under physiologic and pathologic conditions. Some

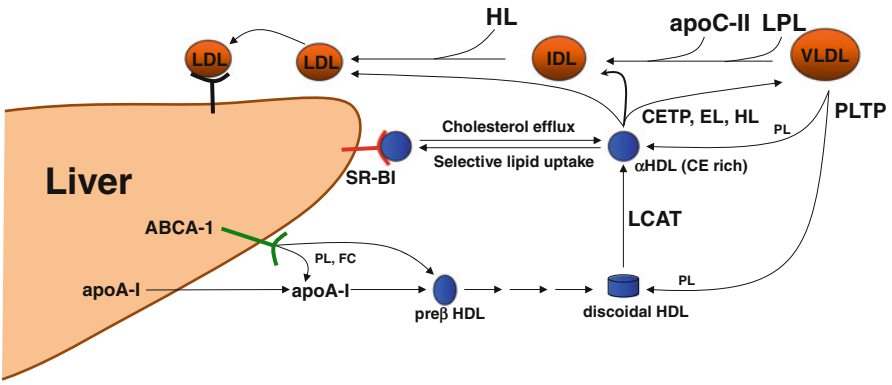

Fig. 2 Lipoprotein metabolism – HDL and VLDL are produced in the liver. HDL biosynthesis begins with gradual addition of phospholipids (PL) and free cholesterol (FC) on ApoA-I that leads to formation of discoidal HDL particles. Discoidal HDL are converted to spherical HDL via contribution of lecithin cholesterol acyl-transferase (LCAT) and addition of PL by phospholipid transfer protein (PLTP). Transfer of cholesteryl ester (CE) to VLDL is mediated by CE transfer protein (CETP). Gradual hydrolysis of TG by LpL, which is activated by ApoC-II, and hepatic lipase (HL), liberates lipids and apolipoproteins that transfer to HDL. SR-BI mediates HDL uptake and will obtain some HDL lipids without ApoA-I; this is known as selective uptake

TGs are produced via de novo synthesis that converts glucose or amino acids to FAs. Other FAs are products of lipolysis in the periphery or are derived from TG returned to the liver via uptake of partially digested TG-rich lipoproteins, termed remnants. The third source of hepatic FAs are those liberated from intracellular lipolysis of TG stored in adipocytes; adipocytes TG lipolysis is mediated by adipose TG lipase (ATGL) and hormone sensitive lipase (HSL). These enzymes are inhibited by insulin and activated by catecholamines and thyroid hormone.

The packaging of liver TGs with ApoB100 is regulated by insulin, FFAs, and liver inflammation [11–13]. After secretion, VLDL undergoes gradual TG hydrolysis first by LpL and then by hepatic lipase (HL) leading to conversion of VLDL to IDL and LDL (Fig. 2).

5 LDL

Although LDLs are the major cholesterol carrier in human blood, HDLs are the greater carriers of circulating cholesterol in wild type rodents. Mice with defects in the LDL receptor or ApoE, both of which should reduce cholesterol uptake by the liver, do not have a cardiac phenotype. In part this might be because the heart is one of the least important sites of LDL uptake [14]. In addition heart synthesizes very little cholesterol [15]. Perhaps, as shown in isolated perfused hearts [9], the robust metabolism of TG-rich lipoproteins, as they circulate through the heart, is sufficient to provide cholesterol for the myocardium.

6 HDL

HDL biosynthesis takes place in the liver and small intestine. ApoA-I is secreted by the liver and interacts with hepatic ABCA1 transporter (Fig. 2). ApoA-I is then lipidated with phospholipids and cholesterol and forms discoidal HDL particles. Discoidal HDL is converted to spherical HDL following the action of the enzyme lecithin cholesterol acyl transferase (LCAT). LCAT esterifies cholesterol with FA to form cholesteryl ester, which is forced to the inner core of HDL. Loss of either LCAT [16] or ABCA1 [17, 18] prevents formation of mature HDL and leads to lower HDL cholesterol levels.

HDL is removed from the circulation via cell surface receptors, following the action of two lipases: HL [19] and endothelial lipase (EL) [20]. Most HDL lipid is returned to the liver via scavenger receptor receptor-B-I (SR-BI) [21]. However, HDL proteins are degraded in both the liver and kidney; the latter is most important in removal of smaller relatively lipid-poor HDL [22].

7 Apolipoproteins

These lipid-binding proteins are amphipathic, thus able to interact with aqueous and non-aqueous media. Apos allow interaction of lipoproteins with cell surface receptors and metabolic enzymes.

7.1 ApoB

ApoB is the main structural protein of chylomicrons and VLDL and remains attached to these lipoproteins throughout their formation and catabolism. Two ApoB species have been described: hepatic ApoB100 and intestinal ApoB48, which corresponds to the N-terminal 48 % of ApoB100. Following gradual TG hydrolysis by LpL and conversion of VLDL to IDL and subsequently LDL, ApoB100 is recognized by the LDL receptor, which mediates LDL uptake by the liver. The heart expresses both ApoB and MTTP [23], but is generally believed to secrete only small amounts of lipoproteins. However, it has been proposed that secretion of cardiac TG-enriched ApoB-containing lipoproteins may occur as a defensive mechanism to protect from cardiac lipotoxicity in obesity [24], diabetes [25] and heart failure [25].

7.2 ApoE

ApoE is present in chylomicrons, VLDL, IDL, LDL and HDL and is recognized by lipoprotein receptors, such as LDL receptor (LDLr) [26], LDL receptor relate protein (LRP)-1 [27], VLDL receptor (VLDLr) [28], ApoE receptor 2 [29], SR-BI [30,

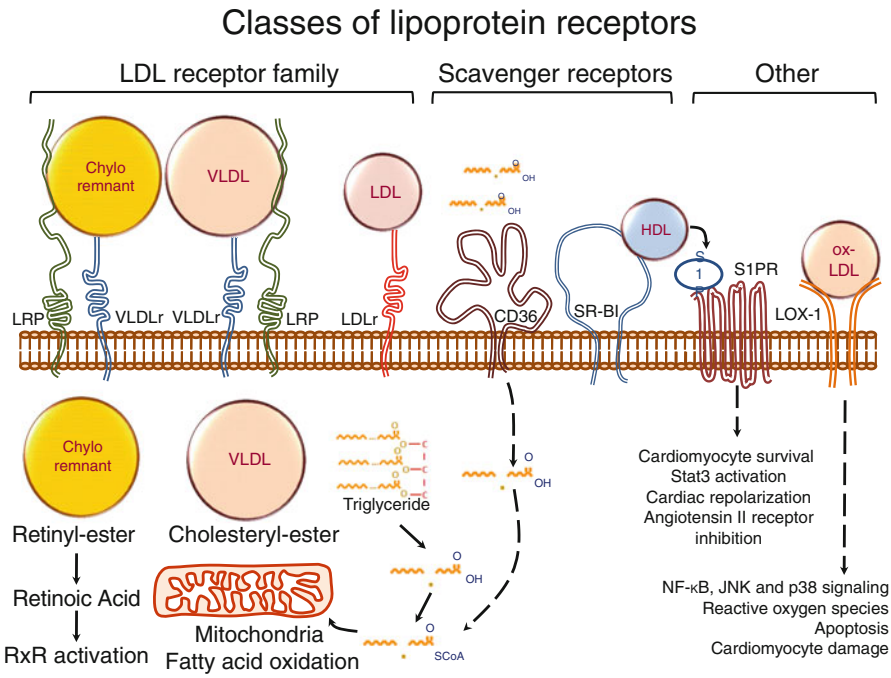

Fig. 3 Classes of cardiac lipoprotein receptors

31] and ABCA-1 [32] (Fig. 3). ApoE is primarily expressed in the liver [33], but also in peripheral tissues, including the intestine and the heart [34]. Among the three human ApoE isoforms, ApoE2 and ApoE3 show a preference for binding on HDL, while ApoE4 has higher affinity for VLDL and LDL [35]. As cardiomyocytes express VLDLr [36] and LRP1 [37, 38] and low levels of LDLr [36] and SR-BI [39], ApoE may be a component of the lipoprotein-derived cardiac lipid uptake process.

7.3 ApoCs

ApoCs are short polypeptides that are associated with chylomicrons, VLDL and HDL [40]. ApoC-I activates LCAT and increases cholesterol and TG levels, perhaps because it inhibits uptake of remnant lipoproteins [40]. LpL is also regulated by ApoCs. Specifically, ApoC-II activates LpL [41], while ApoC-III is inhibitory and promotes hypertriglyceridemia [42]. Loss of ApoC-III prevents hypertriglyceridemia in some situations, such as rodent diabetes [43]. ApoC-III is also thought to regulate lipoprotein uptake by receptors.

8 Enzymes that Modulate Lipoproteins

Following secretion in the circulation, chylomicrons and VLDLs interact with a number of enzymes that modulate their size and affect their interaction with lipoprotein receptors that mediate lipoprotein catabolism (Fig. 2), such as lipases, phospholipid transfer protein (PLTP) and cholesteryl ester transfer protein (CETP). Lipoprotein-associated TGs are hydrolyzed by LpL within the circulation, allowing cardiomyocytes to take up released FFAs for β-oxidation or activation of transcriptional factors, such as PPARα [44, 45]. Although cardiac LpL is produced primarily by cardiomyocytes [46], it is thought to be most active when associated with endothelial cells [47, 48]. At least in the mouse, heart LpL accounts for a significant portion of circulating TG catabolism. Mice that express LpL in cardiomyocytes but not in adipose tissue and skeletal muscle have normal plasma TG levels [49]. Also deletion of LpL only in cardiomyocytes leads to hypertriglyceridemia [50].

Endothelial cell-associated LpL is likely bound to both glycosyl phosphatidylinositol-anchored high-density lipoprotein-binding protein 1 (GPIHBP1) [47, 51] and heparin sulfate proteoglycans (HSPGs) [52, 53]. A variety of proteins have been identified as either activators of LpL-mediated TG hydrolysis, such as Apo-CII [54] and Apo-AV [55, 56], or inhibitors, such as Apo-CIII [57, 58], ApoA-II [59], angiopoietin-like protein (ANGPTL) 3 [60], ANGPTL4 [61] and ANGPTL8 [62]. ANGPTL4, which is the predominant isoform in the heart and adipose tissue, exerts its inhibitory function by converting catalytically active LpL dimers to inactive monomers [63].

HL and EL also mediate intravascular TG hydrolysis [64]. HL hydrolyzes chylomicron remnant-, IDL- and HDL-associated TGs [65], while endothelial lipase catalyzes hydrolysis of HDL phospholipids [66]. Neither lipase has been associated with cardiac lipid uptake under normal conditions. However, a recent study showed increased endothelial lipase and reduced LpL levels in a pressure overload-induced cardiac hypertrophy animal model [67]. Despite downregulation of LpL, endothelial lipase-expressing hearts had increased ATP levels and improved function as compared to endothelial lipase deficient hearts [67]. This observation indicates a potential role for endothelial lipase and HDL in providing FAs to the heart when LpL-mediated lipolysis is compromised during cardiac hypertrophy. More details on lipolysis and its regulation are included in another chapter.

PLTP facilitates transfer of phospholipids from VLDL to HDL, which affects the size of HDL [68]. Hepatic PLTP also modulates VLDL secretion [69]. CETP mediates exchange of cholesteryl ester within HDL and LDL for TG in VLDL and chylomicrons [70].

9 Cardiac Lipoprotein Receptors

Some lipid-loaded lipoproteins or lipoprotein remnants likely enter cardiomyocytes via lipoprotein receptors (Fig. 3), but the physiologic importance of these receptors is unclear. Many receptors that primarily bind to ApoB100 or ApoE are expressed

in the heart. These include the LDLr [36], VLDLr [36] and LRP1 [37, 38], as well as the HDL receptor SR-BI [39]. Furthermore, failing hearts express lectin-like oxidized-LDL receptor-1 (LOX-1) [71]. However, genetic deletion of each of these receptors in isolation leads to no obvious cardiac phenotype.

VLDLr is a member of the LDLr superfamily that binds ApoE-TG-rich lipoproteins such as VLDL [72] and chylomicrons [73]. VLDLr is expressed in heart [36] and particularly in cardiomyocytes [74, 75], as well as in endothelial cells [76]. Besides and contributes to lipid uptake. Besides remnant lipoprotein uptake, VLDLr are important for transportation of LpL to the luminal surface of vascular endothelial cells [77], as well as for enhancing cardiac LpL activity [78]. However, $Vldlr^{-/-}$ mice do not have lower cardiac TG levels; neither do they develop cardiac dysfunction [74].

LRP1 was initially described as the back-up receptor that allowed chylomicron remnant uptake by the liver in LDLr deficient mice [79–81]. However, there are no data indicating a role for LRP1 in cardiac lipid metabolism under normal conditions. Treatment of isolated cardiomyocytes with increasing doses of LDL and VLDL in normoxic conditions led to increased levels of VLDLr and LRP1 expression, while LDLr expression did not change [82]. When LRP1 was knocked down in isolated cardiomyocytes that were treated with LDL, cholesteryl ester uptake continued although at a significantly slower rate [82].

Although cardiomyocytes express receptors for HDL, these receptors do not seem to have a role in cardiac function under normal conditions. The receptor for HDL, SR-BI [39], is expressed in cardiomyocytes although this lipoprotein class does not seem to have a role in acquisition of lipids and cardiac energy production. HDLs provide sphingosine-1-phosphate to cardiomyocytes via the S1P receptor [83], which seems to be protective during cardiac stress [84]. Thus, cardiac SR-BI may serve as a "docking station" for HDL to allow lipolysis and release of S1P. SR-BI may contribute in cardiomyocyte cholesterol efflux to HDL, although this process has been shown to be predominantly mediated by cardiac ABCA1 and ABCG1 [85]. It is likely that much of the lipid needed for cardiac metabolism is supplied during lipolysis of TG-rich lipoproteins.

10 Role of Lipoprotein-Carried Vitamins in Cardiac Metabolism and Function

Lipoproteins also serve as carriers of vitamins that are important for cardiac energetics and function such as β-carotene [86], a precursor of vitamin A, vitamin A [87], vitamin D [88], α-tocopherol (vitamin E) [89] and vitamin K1 [90]. The importance of these vitamins for maintaining normal cardiac function has been demonstrated in several studies. Tissue accumulation of vitamin A is via uptake of retinol or retinyl ester. Dietary vitamin A is absorbed as retinyl esters within chylomicrons [91, 92]. Following LpL-mediated hydrolysis of retinyl esters [93, 94] retinol is released and enters tissues. In cardiomyocytes some retinyl esters are also taken up by lipoprotein receptors [8] without being cleaved [94]. LpL-mediated

conversion of chylomicrons to chylomicron remnants is necessary as shown by compromised cardiac uptake of retinyl esters in LpL-deficient hearts [8]. Retinol can also be obtained from its major circulating pool that is associated with retinol binding protein.

Fat soluble vitamins affect a number of basic cardiac metabolic pathways. Retinol is converted to retinoic acid that activates RxR, the transcriptional partner of PPARα, which is a major regulator of cardiac fatty acid oxidation [95]. Vitamin K1 is also important for cardiac energetics as it provides derivatives that serve electron transportation between mitochondrial electron-donating and electron-accepting enzyme complexes that facilitate ATP production in cardiomyocytes [96]. Vitamin E with its anti-oxidant properties is important for alleviating the effects of cardiac oxidative stress [97, 98] by inhibiting lipid peroxidation [99, 100], stress signaling pathways [101] and apoptosis [102, 98, 101]. Regarding vitamin D, although there is not much information about its role in cardiac fatty acid oxidation, it has been associated with increased fatty acid oxidation in other organs such as liver [103] and bone cartilage [104]. Thus, vitamin D may have a positive effect on cardiac fatty acid oxidation.

11 Cardiac Lipoprotein Metabolism in Disease

11.1 Cardiac Hypertrophy

Cardiometabolic diseases compromise several components of the lipoprotein metabolism pathways. Pressure overload-induced left ventricular hypertrophy switches the metabolic pattern of the heart to a fetal profile characterized by reduced FA oxidation and increased glucose catabolism [105]. Although this is not associated with marked reduction in circulating lipoproteins, the uptake of lipids into the heart should be reduced due to downregulation of LpL and CD36 expression that has been observed in human [106] and mouse [67, 107] hearts. Reduced PPARα activation may be a major event that accounts for the changes in LpL and CD36 [108]. Similarly, reduced LpL and VLDLr levels and increased glucose utilization were observed in hearts of spontaneously hypertensive rats-stroke prone, an animal model for hypertension-induced cardiac hypertrophy [109, 75].

Oxidized-LDL and their receptor, (Lox-1) may play a role in heart failure. Cardiomyocyte LOX-1 expression is increased by endothelin and norepinephrine [71] and it seems to aggravate heart failure. Activation of LOX-1 by oxidized LDL leads to increased release of reactive oxygen species [110], apoptosis [71], cardiomyocyte damage [111] and elevation of heart failure biomarkers, such as brain natriuretic peptide and monocyte chemoattractant protein-1 [112]. LOX-1 expression is induced by angiotensin-mediated hypertrophy [113, 114] and is inhibits by curcumin and rosuvastatin, which also inhibits cardiomyocyte growth [113, 114]. The cause and effect relationship of these effects is uncertain.

Although HDL does not seem to have a role in providing FAs for cardiac energy production, an exception may occur during pressure overload-induced cardiac hypertrophy, when downregulation of LpL is compensated by EL upregulation that mediates HDL lipolysis and provides phospholipids [67]. Induction of pressure overload-mediated cardiac hypertrophy in $EL^{-/-}$ mice resulted in a more severe systolic dysfunction accompanied by lower levels of cardiac fatty acid oxidation-related gene expression and ATP levels as compared to wild-type mice [67].

11.2 Ischemia

Hypoxic hearts show reduced cardiac TG utilization in rats [115] and increased TG accumulation in dogs [116] and mice [74]. This may involve the VLDLr [74], although changes in cardiac lipid uptake and accumulation during ischemia might be model specific. In one report, myocardial infarction in mice led to a marked increase in expression of VLDLr and accumulation of intracellular lipids; this was prevented by with VLDLr deficiency [74]. Increased VLDLr expression levels due to ischemia may be driven by hypoxia-inducible factor (HIF)-1α, which is elevated in ischemic hearts [82] and is a positive regulator of VLDLr expression [74, 117]. However, in rat models of low-flow ischemia cardiac FA uptake and TG content were reduced [118].

VLDLr-mediated lipoprotein uptake in hypoxic cardiomyocytes is facilitated by LRP1 [37]. Increased LRP-1 expression occurs in ischemic cardiomyopathy patients [82] who have increased cardiac TG and cholesterol, as well as in isolated cardiomyocytes during hypoxia [37]. siRNA-mediated knock-down of LRP1 prevented hypoxia-induced VLDL-cholesteryl ester uptake in isolated neonatal rat ventricular myocytes and a mouse cardiomyocyte cell line [37]. LRP1 protein expression levels increase in patients with ischemic cardiomyopathy [82].

LOX-1 is upregulated by ischemia-reperfusion [119, 120]. Abrogation of LOX-1 in an animal model of chronic ischemia reduced infarct size, improved cardiac hemodynamics, prevented cardiac remodeling and fibrosis and improved survival [121]. Besides chronic ischemia, LOX1 may also be detrimental for cardiac damage that occurs in ischemia-reperfusion. Specifically, LOX-1 increased in rat cardiomyocytes following ischemia-reperfusion, while administration of anti-LOX-1 antibody reduced myocardial infarction size [120] and LOX-1 genetic deletion reduced ischemia-driven collagen accumulation [121]. Therefore, LOX-1 appears to be involved in ischemic heart failure as it activates stress signaling kinases, such as JNK and ERK [112]. Accordingly, treatment of mouse cardiomyocytes with JNK or ERK inhibitors prevented ox-LDL-induced increase of BNP [112]. The beneficial effect of LOX-1 inhibition in ischemia has been attributed to reduced myocardial oxidative stress and inhibition of pathological NF-κB, JNK and p38 MAPK signaling pathways [121].

HDL interact with the sphingosine-1-phosphate (S1P) receptor to provide S1P to cardiomyocytes [83]. S1P improves cardiomyocyte survival during hypoxia [122],

protects against doxorubicin toxicity [123], promotes phosphorylation of connexin 43 [124] and activates Stat3, a transcription factor with an important role in adaptation of myocardium to stress [125, 126].

The increased expression profile of lipoprotein and FA receptors in ischemia may seem contradictory to the observed reduction in TG and FFA in hearts of patients with advanced heart failure [127]. This discrepancy suggests that increased uptake of lipoprotein-carried FAs, cholesteryl ester and vitamins may be an acute compensatory response of the myocardium to ischemia, which is attenuated as of the myocardium fails. Thus, ischemic cardiomyopathy is associated with increased lipoprotein catabolism, at least during the early stages of the disease. This may reflect increased need of the ischemic heart for provision of fuel and nutrients that may be important for healing processes in the damaged myocardium.

11.3 Cardiomyopathy in Both Type I and II Diabetes

Both type 1 and type II diabetes have been associated with abnormal lipoprotein metabolism and increased cardiac lipid uptake and accumulation [128, 129]. The increased lipid uptake might result from greater circulating levels of FFA and TG and induction of FA uptake pathways. Diabetic hearts consume primarily VLDL and chylomicron remnants [130]. Cardiac utilization of VLDL was shown to increase in isolated-perfused hearts of diabetic mice [131]; this was associated with increased cardiac LpL secretion [132] and activity [133], as well as enhanced CD36 expression levels [134, 135]. There is no evidence that the increased heart TG in the setting of diabetes is due to upregulation of lipoprotein receptors. In fact, diabetic hyperlipidemia reduces heart VLDLr protein [136] due to post-translational regulation. This change may represent a compensatory response that occurs in advanced stages of diabetes aiming to counterbalance cardiac lipid uptake and lipotoxicity that occur in diabetic hearts [137].

11.4 Sepsis

Sepsis is a systemic inflammatory disease that begins with bacterial infection and is associated with altered lipoprotein metabolism characterized by elevated plasma TG and FFA [138–141] and suppression of energy production in several organs, including the heart [138, 142, 143]. Increased plasma TG levels are due to compromised intravascular lipolysis [144, 145] and not defective hepatic lipoprotein production [146]. Sepsis also increases cardiac lipid accumulation but this is primarily due to reduced expression levels of PPAR nuclear receptors [138, 147] and suppression of FA oxidation [138, 142]. Cardiac LpL activity is reduced due to lower LpL and increased Angptl4 gene expression levels [138, 148]. Cardiac VLDLr and CD36 expression levels are also reduced in sepsis. These changes would be expected

to compromise lipid uptake and cardiac function [149]. Genetic and pharmacologic interventions that increase cardiac FA oxidation during sepsis, such as PPARγ or PGC-1β activation and JNK inhibition, prevent heart dysfunction [143, 142, 138] and improve survival [138].

Efficient lipoprotein clearance from circulation during sepsis can contribute to the removal of bacterial endotoxins, as the latter stick on lipoproteins [150], particularly chylomicrons [151]. Thus, the observed reduced expression of lipoprotein receptors during sepsis may be a defense to prevent cardiac lipid overload as FA oxidation is reduced. In addition, since lipopolysaccharide is carried on lipoproteins, the delivery of these toxins to the heart may be inhibited via downregulation of the lipoprotein receptors.

12 Conclusions

Although the heart avidly utilizes other sources of energy, such as glucose, FFA, lactate, and ketones, the bulk of circulating energy substrates are within lipoproteins. Gain and loss of function studies of LpL confirm that lipoprotein metabolism within the heart is required for its normal acquisition of FA as well as esterified lipids such as cholesteryl esters and retinyl esters. The heart expresses several lipoprotein metabolic pathways and a number of lipoprotein receptors. While a single receptor does not appear to be essential for normal heart function, lipoprotein receptors may contribute to development of cardiac abnormalities with ischemia, hypertrophy, diabetes and sepsis.

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Role of Lipoprotein Lipase in Fatty Acid Delivery to the Heart

Andrea Wan and Brian Rodrigues

Abstract Diabetes is rampant across the globe, with 5–6 % of Canadians currently diagnosed with diabetes. In people with diabetes, inadequate pharmaceutical management predisposes the patient to heart failure, which is the leading cause of diabetes related deaths. One instigator for this cardiac dysfunction is change in fuel utilization by the heart. Thus, following diabetes, when cardiac glucose utilization is impaired, the heart undergoes metabolic transformation wherein it switches to using fats as an exclusive source of energy. Although this switching is geared to help the heart initially, in the long term, this has terrible end results. These include the generation of noxious by products which kill cardiac cells, reduce cardiac function and ultimately result in an increased morbidity and mortality. A key perpetrator that may be responsible for organizing this metabolic disequilibrium is lipoprotein lipase (LPL), the enzyme responsible for providing fat to the hearts. Either exaggeration or reduction in its activity following diabetes could lead to heart dysfunction. The objective of this article is to describe the biology of LPL during diabetes. By gaining more insight into the mechanism(s) by which cardiac LPL is regulated, new therapeutic strategies can be devised that may assist in restoring metabolic equilibrium, to help prevent or delay heart disease seen during diabetes.

Keywords Cardiomyocytes • HSPG • Heparanase • VEGF • GPIHBP1

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1 Introduction

The incidence of diabetes has reached epidemic proportions, and globally, approximately 366 million people are affected by this disease. This number is projected to grow to 552 million by 2030 (7.8 % of the adult population) (International Diabetes Federation). Cardiovascular disease is the leading cause of diabetes-related death [1]. Although atherosclerotic vascular disease is a primary reason for this cardiovascular dysfunction, a susceptibility to heart failure in patients with diabetes might also be an outcome of a specific impairment of the heart muscle itself (labeled diabetic cardiomyopathy) [2–4]. The etiology of diabetic cardiomyopathy is complex, with changes in cardiac metabolism being considered a principal culprit. The earliest change that occurs in the diabetic heart is reduced glucose consumption, with a switch to utilization of fatty acids (FA) predominantly as an energy resource [5]. A majority of FA provided to the heart comes from the breakdown of circulating lipoprotein-triglycerides (TG) [6], a process catalyzed by lipoprotein lipase (LPL) located at the vascular lumen. In models of moderate Type 1 diabetes, when circulating albumin-bound FA has yet to increase, an augmented vascular LPL activity is observed [7]. Although this adaptation might be beneficial in the short-term, it is potentially catastrophic over a protracted duration, given the malicious effects produced by increased FA oxidation and myocyte TG accumulation [8–10]. Intriguingly, following severe diabetes when circulating albumin-bound FA also increases, luminal LPL is "turned off" to avoid lipid overload [11]. Paradoxically, chronic cardiac-specific deletion of LPL is also associated with a decreased cardiac ejection fraction (LPL function is irreplaceable) [12–14]. In this chapter, we will examine the role of coronary vascular LPL in fatty acid delivery to the heart, and how an increase in its activity is a prelude for the cardiovascular complications seen with diabetes.

2 Cardiac Metabolism

With uninterrupted contraction being a unique feature of the heart, cardiac muscle has a high demand for energy. As a consequence, this organ demonstrates substrate promiscuity, enabling it to utilize multiple sources of energy, including FA, carbohydrates, amino acids and ketones [15]. Among these, carbohydrates and FA are the major participants from which the heart derives most of its energy. Accordingly, in a basal aerobic setting, glucose and lactate account for approximately 30 % of energy, whereas 70 % of ATP generation is through FA oxidation [15]. However, the heart has a limited capacity to synthesize FA, and thus relies on an exogenous supply. FA delivery and utilization by the heart involves: (a) release from adipose tissue and transport to the heart after complexing with albumin, (b) provision through breakdown of endogenous cardiac triglyceride (TG) stores, and (c) lipolysis of circulating TG-rich lipoproteins (VLDL and chylomicrons) to FA by lipoprotein lipase (LPL) positioned at the endothelial cell (EC) surface of the coronary lumen (Fig. 1).

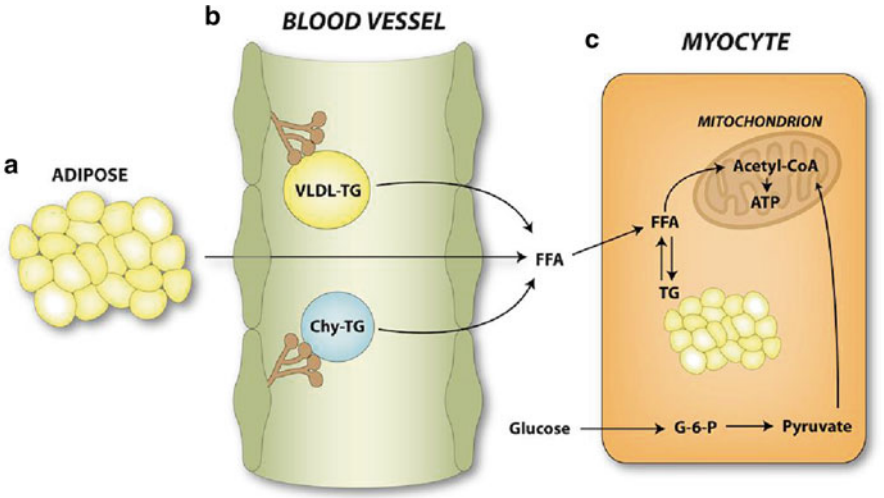

Fig. 1 Substrate utilization by the heart. Glucose entry into cardiomyocytes occurs through transporters. Once inside, it undergoes glycolysis and pyruvate is formed, which is transported into the mitochondria. At this location, pyruvate undergoes decarboxylation to acetyl-CoA, which is eventually used for ATP generation. The other major substrate that is utilized by the heart is fatty acid (FA). FA is delivered to the heart from three major sources: (**a**) adipose tissue lipolysis with release of FA into the plasma, (**b**) LPL mediated breakdown of TG-rich lipoproteins from the liver (VLDL) and gut (chylomicron), and (**c**) endogenous triglyceride (TG) breakdown within the heart. Specifically related to LPL, this enzyme is located at the apical side of endothelial cells, and hydrolyzes circulating TG to release FA that are taken up into the cardiomyocyte by FA transporters. This FA can be stored as TG or undergo β-oxidation to generate acetyl-CoA, which is further oxidized to generate ATP

3 Lipoprotein Lipase (LPL)

Regarding LPL, (a) greater than 90 % of plasma FA are contained within lipoprotein-TG, with LPL having a pivotal role in hydrolysis of this TG to FA, (b) compared to other tissues, the heart has the most robust expression of LPL, and (c) LPL-mediated lipolysis of TG-rich lipoproteins is suggested to be a principal source of FA for cardiac utilization. Lipoprotein-TG clearance by LPL proceeds at the apical surface of EC that line the coronary lumen. Despite this critical function, EC do not synthesize LPL [16]. Instead, this enzyme is synthesized in cardiomyocytes and processed to dimeric [17], catalytically active enzyme, an obligatory step for ensuing secretion. Transfer to the coronary lumen requires movement of LPL to the cardiomyocyte plasma membrane [18] by AMP-activated protein kinase [19], protein kinase D, and p38 MAPK [20]. Activation of these kinases facilitates LPL vesicle formation, in addition to promoting cytoskeletal rearrangement for secretion onto cell surface heparan sulfate proteoglycans (HSPG) [20], where the enzyme is

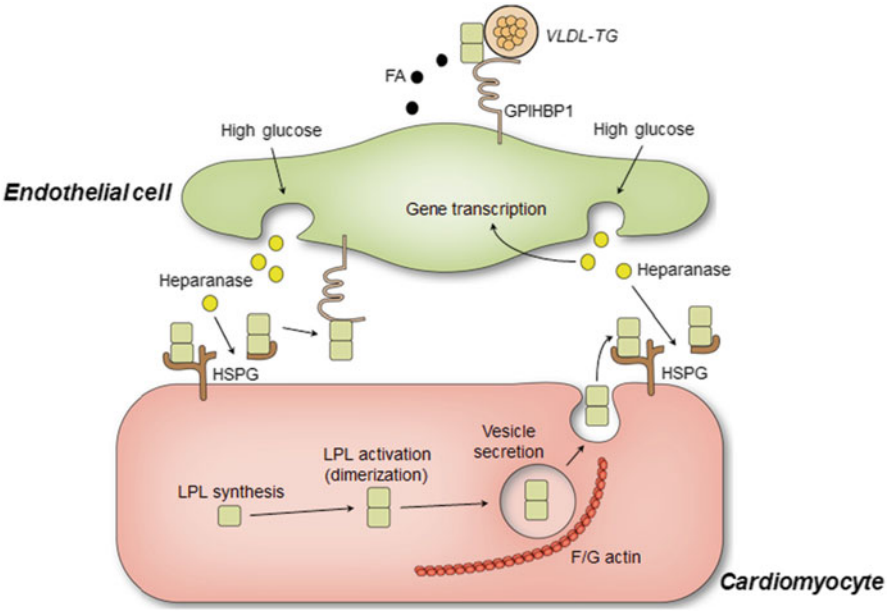

Fig. 2 Synthesis, activation and transport of LPL in the heart. Following its synthesis as an inactive monomer in cardiomyocytes, LPL undergoes several post-translational processes to be activated (dimerized). Fully processed dimeric LPL is sorted in vesicles that are targeted to the cell surface (for secretion) by moving along the actin cytoskeleton. At the plasma membrane, LPL docks with heparan sulfate proteoglycan (HSPG) binding sites. For its onward movement to the luminal side of the endothelial cell (EC), detachment of LPL from HSPG is a prerequisite. EC heparanase facilitates transfer of LPL from the cardiomyocyte to the vascular lumen. In the EC lysosomes, active heparanase is stored in a stable form. In normal conditions, heparanase released from the basolateral side of EC towards the cardiomyocyte can initiate cleavage of myocyte HSPG side chains. Following this, the oligosaccharide-bound LPL released into the interstitial space is then transported towards the apical side of EC. Out here, LPL is responsible for lipoprotein-TG hydrolysis. Following diabetes, increased circulating glucose can enhance heparanase secretion from the EC, which can cleave more HSPGs. The released LPL is transferred to the lumen. The end effect is to increase TG hydrolysis, bringing more FA to the cardiomyocyte, leading to lipotoxicity

momentarily located [21, 22] (Fig. 2). For its onward movement across the interstitial space to the apical side of vascular EC, detachment of LPL from the myocyte surface is a prerequisite, and is likely mediated by enzymatic cleavage of cardiomyocyte surface HSPG by heparanase [23, 24].

4 Heparanase

HSPG are ubiquitous macromolecules present in every tissue compartment but particularly the extracellular matrix, cell surface, intracellular granules and nucleus [25]. They consist of a core protein to which several linear heparan sulphate (HS)

side chains are covalently linked, and function, not only as structural proteins but also as anchors [26]. The latter property is implicitly used to bind a number of different proteins [chemokine's, coagulation factors, enzymes like LPL, and growth factors such as vascular endothelial growth factor (VEGF)] [27]. Attachment of these bioactive proteins is a clever arrangement, providing the cell with a rapidly accessible reservoir, precluding the need for *de novo* synthesis when the requirement for a protein is increased. Heparanase is an endoglycosidase, exceptional in its ability to degrade HS, thereby instigating release of ligands [28].

Heparanase is initially synthesized as a latent (inactive) 65 kDa proheparanase enzyme that undergoes cellular secretion followed by reuptake facilitated by HSPG [29, 30]. After undergoing proteolytic cleavage (removal of a 6 kDa linker peptide in lysosomes), a 50 kDa polypeptide is formed that is ~100-fold more active than the 65 kDa inactive proheparanase [31, 32]. Within the acidic compartment of lysosomes (that have a predominantly perinuclear localization), active heparanase is stored in a stable form until mobilized. In the presence of high glucose (HG, 25 mM), we reported a clear redistribution of lysosomal heparanase from a perinuclear location towards the plasma membrane of EC, together with an elevated heparanase secretion into the incubation medium (Fig. 2). We also determined that ATP release, purinergic receptor activation, cortical actin disassembly and stress actin formation were essential for HG-induced heparanase secretion [23].

Although a role for heparanase in physiology (e.g., embryonic morphogenesis) has been described, it was intensive research focused on cancer progression that hinted towards a unique responsibility in cardiac metabolism. In cancer, degradation of HS chains by the increased expression of heparanase is associated with extracellular matrix and basement membrane disruption [33]. The loss of this physical barrier facilitates tumor cell invasion [34]. However, it was the reported liberation of LPL anchored to HS that was the most provocative. In the heart, subsequent to its synthesis and transfer to the myocyte surface, it was uncertain as to how LPL journeys to its site of action, the vascular lumen. We established that LPL detachment from the cell surface is a requirement, and is made possible by heparanase. Given its strategic location, EC can operate as a first responder, informing the underlying myocytes about hyperglycemia following diabetes. We proved that HG is a potent stimulator of heparanase secretion from EC, and in so doing, empowered the myocyte to send LPL to the vascular lumen (in search of lipoprotein-TG) as an adaptation to contest the impending loss of glucose consumption. EC secretes both latent and active heparanase. We questioned why a cell would release inactive enzyme followed by reuptake and subsequent activation. This practice would appear counterintuitive and wasteful, unless latent heparanase has a function. Indeed, compelling data from our lab suggests that latent heparanase generates signals in myocytes to reload LPL from an intracellular pool, to replenish the surface reservoir released by active heparanase [24]. One interesting feature of myocyte HSPG is its capability to electrostatically bind proteins other than LPL. As such, we questioned whether these proteins could be released by heparanase, and further focused on those that would be able to aid in LPL-derived fatty acid delivery and oxidation. VEGF is one such protein that fits this profile.

5 Vascular Endothelial Growth Factor (VEGF)

Following diabetes, the heart rapidly increases LPL at the vascular lumen by transferring this enzyme from the underlying cardiomyocyte. We implicated heparanase in this process, an enzyme that cleaves HSPG to facilitate bioactive protein release from this attachment receptor. We reported that within 30 min of hyperglycemia, active heparanase was secreted into the interstitial space, and was able to detach LPL from the sub-endothelial myocyte cell surface, for onward movement to the vascular lumen. In the heart, cardiac myocytes are also a major source of VEGF. As the predominant forms of VEGF, VEGFA ($VEGF_{164}$) and VEGFB ($VEGF_{167}$) have a heparin binding domain, it is possible that in addition to an intracellular location, VEGF can be captured by myocyte cell surface HSPG following its secretion. Such a location would allow for a rapid release when there is a requirement for this growth factor. If true, then it is conceivable that given this comparable location of LPL and VEGF at the myocyte cell surface, heparanase will also dislodge VEGF. This would be an elegant mechanism, with VEGF assisting in LPL-derived FA uptake (by its effects on FABP and FATP), in addition to supporting cardiac flexibility that is desirable to oxidize these FA (through its promotion of angiogenesis; FA oxidation needs ample oxygenated blood).

Of the many different VEGF isoforms, VEGFA and VEGFB are notable standouts abundantly expressed in the myocardium, but more critically, are related to metabolic regulation [35, 36]. Upon secretion, they have a variable affinity for HSPG, an interaction that transpires by way of a heparin binding domain (HBD, amino acids rich in basic residues) on VEGF [37, 38]. Although this liaison protects VEGF against degradation, it also allows the cell matrix to retain a pool of readily accessible bioactive growth factors that can be freed, either by ionic displacement (employing heparin) or more physiologically, by matrix proteolysis (through the action of heparanase).

VEGFA: From a single human VEGFA gene, alternative splicing generates a number of isoforms. The predominantly expressed $VEGF_{165}$ (mouse $VEGF_{164}$) is the main effector of VEGF action, and is partially tethered (50–70 %) to the extracellular matrix by its HBD. VEGFA has a proven role in vascular permeability (through its formation of intracellular gaps and fenestrations), vasodilatation (due to the induction of eNOS and NO production), and blood vessel formation (encompassing angiogenesis, vasculogenesis, and arteriogenesis) [39, 40]. VEGFA is also capable of promoting FA binding protein 4 (FABP4) expression, a FA transporting protein abundantly expressed in microvascular EC in the heart [41].

VEGFB: Of the isoforms identified, $VEGF_{167}$ constitutes more than 80 % of the total VEGFB transcript, and also possesses a HBD. Unlike other VEGF family members, VEGFB is not required for physiological angiogenesis nor does it influence EC permeability [42, 43]. Nevertheless, in the heart, although located predominantly in myocytes, this cytokine executes a unique metabolic function in EC. By stimulating FA transport proteins (FATP3 and 4) in these cells, VEGFB promotes

FA uptake and transport across the EC layer. To recapitulate, the effects of VEGF would be expected to not only indirectly support LPL-derived FA transport across EC but to also provide O_2 needed for mitochondrial oxidative phosphorylation of FA, the main source of cardiomyocyte ATP.

Having been released by heparanase, LPL needs to be transferred from the basolateral to the apical (luminal) side of EC where the enzyme is functional. This transfer across the EC is facilitated by glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein 1 (GPIHBP1).

6 Glycosylphosphatidylinositol-Anchored High Density Lipoprotein-Binding Protein 1 (GPIHBP1)

GPIHBP1 is a glycoprotein abundantly expressed in the heart, exclusively on capillary EC [44, 45]. On the apical side of these cells, its ability to avidly bind both lipoprotein-TG and LPL allows it to serve as a platform for TG (chylomicrons, VLDL) lipolysis along the luminal surface of capillaries [46–48]. At the basolateral side of EC, GPIHBP1 operates as a transporter, collecting LPL from the interstitial spaces surrounding myocytes and shuttling it across EC to the capillary lumen [47] (Fig. 2). Given these critical functions, complete absence or mutations of GPIHBP1 cause mislocalization of LPL (enzyme remains trapped in interstitial space) and severe hypertriglyceridemia [49]. The earliest evidence of regulated GPIHBP1 expression came from experiments investigating the effects of fasting/refeeding [50]. Fasting amplifies coronary luminal LPL activity [19] and cardiac GPIHBP1 expression, effects that are reversed 6 h after refeeding [50]. As GPIHBP1 levels change quickly, it is conceivable that to increase FA delivery to the diabetic heart, this protein participates in the accelerated transfer of LPL from the cardiomyocyte to the vascular lumen (Fig. 2). We tested whether EC respond to hyperglycemia by increasing GPIHBP1, and our preliminary evidence indicates that diabetes stimulates cardiac LPL activity and GPIHBP1 gene and protein expression.

7 Aberrant Fuel Metabolism in Diabetes

In diabetes, as glucose uptake and oxidation are impaired, the heart is compelled to use FA exclusively for ATP generation. Multiple adaptive mechanisms, either whole body or intrinsic to the heart, operate to make this achievable. These include augmented adipose tissue lipolysis, where breakdown of stored TG in fat cells increases circulating FA that are transported to the heart. If delivered to the liver, these FA can raise circulating lipoprotein concentrations as hepatic FA availability is a rheostat for VLDL synthesis. In so doing, VLDL-TG is an additional and major resource to increase FA delivery to the heart for oxidation. Innate to the cardiac muscle, the uptake of albumin-bound FA is driven by plasma membrane FA transporters

(for example CD36), which are increased following diabetes. Diabetes also enhances adipose triglyceride lipase leading to mobilization of the storage pool of TG within cardiomyocytes. Finally, the utilization of VLDL-TG as a FA source by the diabetic heart is influenced, not only by elevated plasma concentrations of VLDL, but also by the vascular content of LPL, the rate-limiting enzyme in circulating TG clearance. We were the first to report higher luminal LPL activity following diabetes [51].

8 Physiological and Pathophysiological Regulation of LPL

LPL synthesis and activity are altered in a tissue specific manner by physiological conditions like cold exposure, lactation, or feeding and fasting [52, 53]. In fasting, with ensuing hypoinsulinemia, LPL activity decreases in the adipose tissue but increases in the heart. As a result, FA from circulating TG is diverted away from storage to meet the metabolic demands of cardiomyocytes. Hence, LPL fulfills a "gate-keeping" role by regulating the supply of FA to meet the metabolic requirements of different tissues.

We described a robust expansion in the coronary pool of LPL following diabetes, likely an effect to address the urgent need for FA to compensate for decreased ATP production from glucose. This increase in LPL activity was immediate and unrelated to LPL gene expression [54]. We consequently described potential mechanisms to explain this novel finding in the diabetic heart. Its prelude includes exaggerated LPL processing to dimeric, catalytically active enzyme, an obligatory step for ensuing secretion [17]. Transfer of this active enzyme to the coronary lumen however requires movement of LPL to the cardiomyocyte plasma membrane [18]. The signaling behind the passage of LPL in cardiomyocytes was the responsibility of AMP-activated protein kinase (AMPK) [19]. Downstream, transit control of LPL by AMPK embraced a) activation of protein kinase D, whose zinc-finger domain is known to interact with trans Golgi membranes, allowing for LPL vesicle formation [7], and b) p38 MAPK activation and actin cytoskeleton polymerization, thus providing the LPL cargo a transport infrastructure for secretion onto myocyte plasma membrane HSPG [20]. For its onward movement across the interstitial space to the apical side of vascular EC, LPL detachment from the myocyte cell surface is a prerequisite, and is likely mediated by enzymatic cleavage of cardiomyocyte HSPG by high-glucose induced heparanase release [23].

9 Cardiomyopathy in Diabetes

Heart disease is not only a leading cause of death, but also a substantial driver of health care costs among people with diabetes. Coronary vessel disease and atherosclerosis are the primary reasons for this increased incidence of cardiovascular dysfunction. However, Type 1 (T1D) and Type 2 (T2D) patients have also been

diagnosed with reduced or low-normal diastolic function and left ventricular hypertrophy in the absence of coronary heart disease (cardiomyopathy) [55]. Evidence of cardiomyopathy has also been reported in animal models of T1D and T2D. Cardiomyopathy is a complicated disorder, and several factors have been associated with its development. These include an accumulation of connective tissue and insoluble collagen, impaired endothelium function and sensitivity to various ligands (e.g., β-agonists), and abnormalities of various proteins that regulate ion flux, specifically intracellular calcium [56, 57]. The view that diabetic cardiomyopathy could occur as a consequence of early alterations in cardiac metabolism has also been put forward, and linked to LPL.

10 Role of LPL in Cardiomyopathy

Cardiac-specific deletion [14] or overexpression [6, 58, 59] of LPL is associated with heart dysfunction. In this context, consider that although the loss of cardiomyocyte LPL in adult mice increased glucose metabolism (typically, increased FA uptake by cells slows down glucose oxidation pathways), neither this effect, nor albumin bound FA could replace the action of LPL, and cardiac contractility decreased [14]. Cardiac specific overexpression of LPL caused severe myopathy characterized by lipid oversupply and deposition, muscle fiber degeneration, excessive dilatation, and impaired left ventricular function [6, 58, 59]. The latter experiments demonstrate that in the absence of any vascular defects, selective overexpression of LPL in the heart is sufficient to cause lipotoxicity and cardiac failure, a situation comparable to that seen with diabetic cardiomyopathy. Although this increase of LPL in diabetes serves to guarantee FA supply and consumption when glucose utilization is compromised, it unintentionally provides a surfeit of FA to the diabetic heart, sponsoring a setting where FA uptake exceeds the mitochondrial oxidative capacity. Chronically, the resulting increase in the conversion of FA to potentially toxic FA metabolites, including ceramides, diacylglycerols, and acylcarnitines, paired with increased formation of reactive oxygen species secondary to elevated FA oxidation, can provoke cardiac cell death (lipotoxicity).

11 Conclusion

Pharmaceutical management of diabetes can never completely duplicate the exquisite control of glucose observed in healthy humans and hence, patients with diabetes who have imperfect control of their glucose are exposed to repeated bouts of hyperglycemia. As a consequence, changes in cardiac metabolism (decreased glucose utilization forces the heart to increase LPL at the vascular lumen to augment FA) arise, are predicted to occur rapidly, and can influence the development of cardiovascular disease if sustained. The amplification in LPL emerges as an

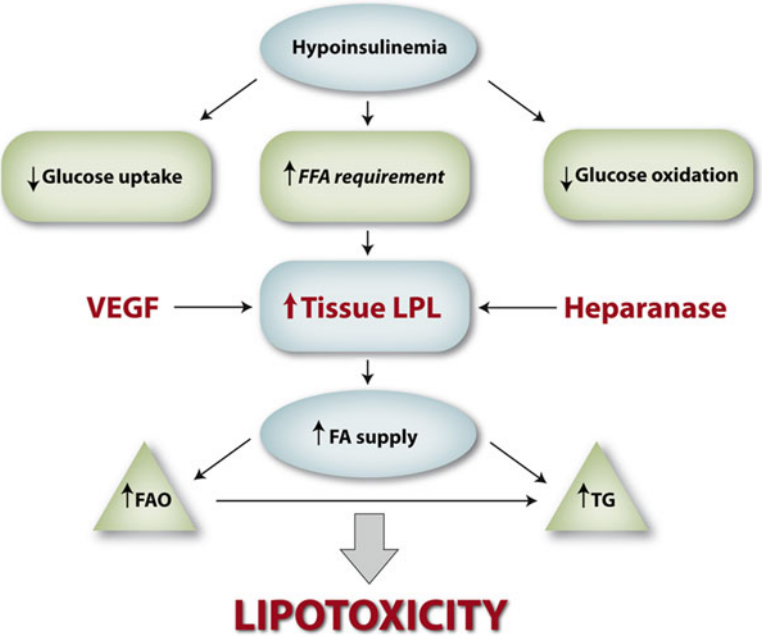

Fig. 3 Metabolic basis for diabetic heart disease. The earliest change that occurs in the diabetic heart is altered energy metabolism. Hence, in the presence of reduced glucose utilization, the heart switches to predominantly use FA for energy. The protein "ensemble" (heparanase-VEGF-LPL) cooperates in the diabetic heart to make this possible. This abnormal FA utilization by cardiac tissue may have lethal consequences under the umbrella called "lipotoxicity". Specifically, FA accumulates and can, either by themselves or via the production of second messengers such as ceramides, provoke cell death. Understanding how heparanase cooperates with its accomplices, VEGFs and LPL, would be valuable to restore metabolic equilibrium and limit lipotoxicity and diabetes-related cardiac damage

outcome of enzyme movement to the cardiomyocyte cell surface, with subsequent forward transfer to the coronary lumen. We suggest that this occurs due to high glucose induced secretion of endothelial heparanase (the first responder) that has concurrent functions. These include stimulation of myocyte HSPG bound LPL, and VEGF release. The latter effect contributes towards augmenting LPL action, and amplifying FA delivery and utilization by the diabetic heart. At the apical side of EC, LPL is responsible for VLDL-TG hydrolysis to FA (the preferred substrate for the diabetic heart), which we propose can result in lipotoxicity (Fig. 3). By gaining further insight into the mechanism(s) by which diabetes alters endothelium-bound LPL, we can attempt to piece together a part of the cascade of events leading to diabetic heart disease. Appreciating the mechanism of how the heart regulates LPL following diabetes should allow the identification of novel targets for therapeutic intervention, to restore metabolic equilibrium and limit lipotoxicity and diabetes-related cardiac damage.

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Control of Myocardial Fatty Acid Uptake

Jan F.C. Glatz and Joost J.F.P. Luiken

Abstract Carbohydrates and long-chain fatty acids are the predominant substrates for cardiac energy production. While the mechanism and regulation of myocardial carbohydrate (glucose, lactate) uptake have been unraveled in detail in the 1990s, insight into fatty acid uptake originates from more recent studies. Fatty acid movement across the sarcolemma is facilitated by membrane-associated proteins, specifically CD36, membrane-associated fatty acid-binding protein ($FABP_{pm}$) and selected fatty acid transport protein (FATP) isoforms, and is up- or downregulated through changes in sarcolemmal content of (primarily) CD36. The recruitment of CD36 from an endosomal storage pool to the sarcolemma, which is under the control of various physiological stimuli (including insulin and contraction), represents a pivotal step in the overall regulation of myocardial fatty acid uptake and utilization. Dysregulation of the intracellular cycling of CD36 underlies various cardiac metabolic diseases. As a result, the mechanism and regulation of myocardial glucose uptake by GLUT4 cycling and of fatty acid uptake by CD36 cycling are very similar. Likely, manipulation of the presence and/or activity of substrate transporters for glucose and fatty acids in the sarcolemma holds promise as therapeutic approach to alter cardiac substrate preference in disease so as to regain metabolic homeostasis and rectify cardiac functioning.

Keywords Fatty acid uptake • CD36 • Fatty acid-transport protein • Fatty acid-binding protein • Substrate preference • Metabolic modulation

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1 Introduction

The importance of long-chain fatty acids (hereafter referred to as 'fatty acids') for myocardial homeostasis is well recognized. Apart from their role as a fuel for myocardial energy production, fatty acids are incorporated into phospholipids forming the core of biological membranes and serve in selected signal transduction pathways to alter gene expression. However, because of their hydrophobic properties, fatty acids also exert detrimental effects and may cause (acute) cellular injury. As a result, the transport of fatty acids among tissues and their handling within tissue cells need to occur through specific mechanisms that allow their rapid and controlled distribution without the possible harmful effects associated with their detergent-like properties.

Fatty acids are delivered to the heart either as fatty acids bound to albumin or esterified in triacylglycerols forming part of lipoproteins (chylomicrons, very low density lipoproteins) from which they are released after hydrolysis by lipoprotein lipases located at the surface of the capillaries. During their subsequent movement across the endothelium and interstitium into the cardiac myocyte, fatty acids pass several biomembranes. Fatty acid transport across these membranes has long been considered to occur by simple (passive) diffusion, but has now been established to be facilitated by a number of membrane-associated fatty acid-binding proteins (termed 'fatty acid transporters' for convenience). Following their entry into the cardiac myocyte, fatty acids are accepted by heart-type cytoplasmic fatty acid-binding protein (H-$FABP_c$) which facilitates the intracellular aqueous transport of fatty acids, mainly to mitochondria. Studies in both humans and animal models have disclosed the involvement of these various proteins not only in the healthy heart but also in the etiology of metabolic aberrations and pathologies of the heart.

In this chapter we discuss our current understanding of the mechanism and the regulation of myocardial fatty acid uptake, focusing on both the acute and chronic regulation of fatty acid uptake and its derangement in cardiac metabolic disease. The significance of the cellular uptake process in the overall regulation of myocardial fatty acid utilization will also be discussed. Finally, examples will be described of manipulating myocardial fatty acid uptake as therapy for chronic cardiac metabolic disease.

2 Fatty Acids in Aqueous Compartments

The hydrophobic nature of (long-chain) fatty acids dictates specific requirements to their presence and transport in aqueous compartments. In blood plasma and the interstitium fatty acids are avidly bound by albumin (68 kDa) or are present as fatty ester in lipoproteins. Intracellularly, fatty acids are bound by cytoplasmic heart-type (H-) $FABP_c$ (FABP3; 15 kDa) which acts as the intracellular counterpart of plasma albumin. The FABPs are members of the intracellular lipid-binding protein (iLBP) family and comprise at least nine distinct types each showing a specific tissue

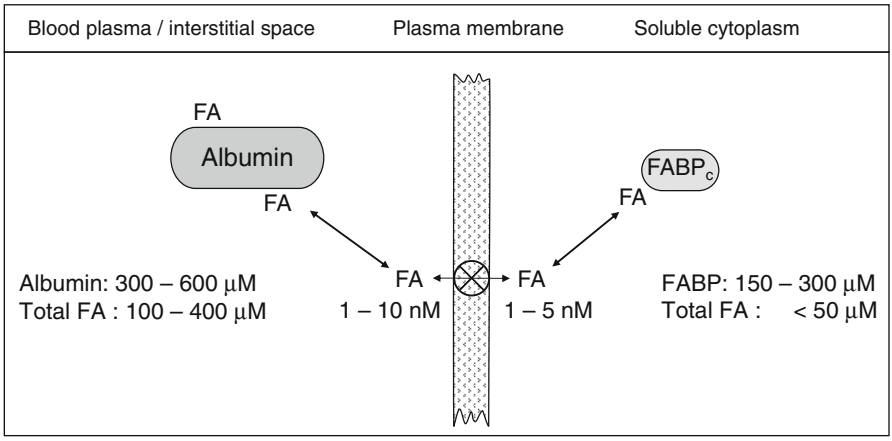

Fig. 1 Fatty acid transport across the plasma membrane. Comparison of concentrations of soluble binding proteins, i.e., albumin (68 kDa) in plasma or interstitial space and cytoplasmic FABP (15 kDa) in the cellular cytoplasm, and (non-protein bound) fatty acids on both sides of the plasma membrane of hepatocytes or cardiac myocytes. *FA*, long-chain fatty acid, *FABP* fatty acid-binding protein

distribution pattern [1–3]. Albumin and H-FABP$_c$ each bind fatty acids with such high affinities that virtually all of the fatty acids present in the aqueous compartment are protein-bound. Thus, in plasma and interstitium the total fatty acid concentration is 100–400 µM while the concentration of non-protein bound fatty acids is several orders of magnitude lower and amounts to only 1–10 nM (Fig. 1) [4, 5]. Similarly, for cardiomyocytes it has been estimated that in the soluble cytoplasm the total fatty acid concentration is up to 50 µM (depending on the metabolic state of the cell) while the non-protein bound fatty acid concentration is only 1–5 nM [6, 7]. As a result, albumin and H-FABP$_c$ each provide a buffer for fatty acids, as each fatty acid that is metabolized or undergoes transmembrane transport to another compartment is immediately replenished by the release of another fatty acid from the protein binding site. It should be emphasized that the abundance of albumin in plasma (approximately 600 µM) and interstitium (300 µM) and that of H-FABP$_c$ in the soluble cytoplasm (150–300 µM in cardiomyocytes) presents with a total buffering capacity that markedly exceeds the total fatty acid concentration in each compartment (Fig. 1). The latter assures that the non-protein bound fatty acid concentration remains low, even under mild pathological conditions (e.g. mild ischemia), so as to keep fatty acids from exerting potential detrimental effects [7].

3 Transendothelial Fatty Acid Transport

Unlike other tissues such as liver, the heart is characterised by a non-fenestrated (continuous) endothelium. The interendothelial clefts are too narrow to allow for permeation of the relatively large albumin molecule at a speed that could explain the

observed high rates of myocardial fatty acid uptake [8]. Therefore, fatty acids first need to dissociate from plasma albumin prior to their transport through the endothelium. The precise mechanism by which the subsequent transendothelial transport of fatty acids occurs has long been unclear. This contrasts to the significant increase in knowledge on the mechanism and regulation of fatty acid uptake by cardiomyocytes (to be discussed below). However, some recent studies shed new light on the role of the microvascular endothelium in the overall process of myocardial fatty acid uptake including mechanistic insight into transendothelial fatty acid transport.

The microvascular endothelium in the heart abundantly expresses two types of cytoplasmic FABP, i.e., FABP4 and FABP5 [1, 9]. Mice with a deletion of both FABP4 and FABP5 show markedly impaired rates of myocardial fatty acid uptake with a concomitant increase in glucose uptake and utilization [10]. These findings suggest a pivotal role for cytoplasmic FABP4 and FABP5 in myocardial fatty acid uptake whereby the fatty acids, on their route from the luminal to the abluminal endothelial membrane, most likely bind to FABP4/5 and traverse the endothelial cytoplasm rather than laterally diffuse within the plasmalemma of the endothelial cell. The mechanism underlying the uptake of fatty acids into the plasma membrane of endothelial cells, their subsequent transmembrane translocation, and binding to FABP4/5 has remained understudied. A recent report showed that vascular endothelial growth factor (VEGF)-B controls endothelial uptake of fatty acids via transcriptional induction of the membrane fatty acid transport proteins FATP3 and FATP4 [11]. The presence in capillary endothelial cells of these and other membrane-associated fatty acid-binding proteins, such as CD36 [12, 13], could indicate that endothelial fatty acid uptake involves a molecular mechanism that is similar to the mechanism that operates in cardiac myocytes. The nature and characteristics of such mechanism will be discussed in detail below.

4 Mechanism of Myocardial Fatty Acid Uptake

With respect to the uptake of fatty acids from the interstitium into cardiac myocytes, albumin acts merely as a large-capacity buffer for non-protein bound fatty acids while H-FABP$_c$ functions as a sink for incoming fatty acids. Studies with genetically manipulated mouse models have shown that FABP displays a permissive action (rather than a regulatory role) in cellular fatty acid uptake. Thus, in skeletal muscle of mice with an homozygous deletion of H-FABP$_c$, the fatty acid uptake rate was reduced by 42–45 % while in skeletal muscle from heterozygous mice, in which the H-FABP$_c$ protein expression was 34 % of that of wild-type mice, fatty acid uptake was not altered compared to that in wild-type animals [14]. As a result, H-FABP$_c$ plays an important, yet permissive, role in fatty acid uptake into (cardiac) muscle.

In the past two decades there has been considerable debate on the mechanism by which fatty acids traverse the plasma membrane to enter the soluble cytoplasm [15]. The dispute centered around the rate-limiting kinetic step in this process and

Table 1 Membrane-associated proteins facilitating myocardial fatty acid uptake

Protein (current designation)	Molecular mass	References
Plasma membrane fatty acid-binding protein ($FABP_{pm}$)	40–43	[18–20]
Fatty acid transport protein (FATP) (isoforms FATP1, FATP4 and FATP6)	63	[21]
Fatty acid translocase/CD36	88[a]	[12, 22, 23]

The proteins share the feature of facilitating the transmembrane translocation of (long-chain) fatty acids. Commonly, for these proteins the designation 'fatty acid transporter' is used for convenience but does not necessarily imply a classic transmembrane transport mechanisms such as that of GLUT4

[a]Glycosylated protein mass. The mass of the non-glycosylated protein is 53 kDa

whether one or more membrane proteins could facilitate and/or regulate the overall uptake process. When considering the cellular uptake of fatty acids, the physical transport can be regarded to comprise three steps:

1. Adsorption: entry of the fatty acid into the outer leaflet of the lipid bilayer, whereby the hydrocarbon chain intercalates between the chains of the phospholipid and the carboxyl group localizes at the aqueous interface;
2. 'Flip-flop': transfer of the fatty acid from the outer to the inner leaflet, whereby the polar carboxyl group moves through the bilayer interior and re-positions at the opposite interface;
3. Desorption: movement of the fatty acid into the aqueous phase and its hydration.

Detailed biophysical studies have disclosed that the fatty acid adsorption step and the subsequent 'flip-flop' of fatty acids in a phospholipid bilayer are very fast for virtually all fatty acid types, but that desorption from the membrane will be the rate-limiting step of transmembrane transport [16]. Fatty acid desorption also is strongly dependent on chain length and degree of unsaturation of the fatty acid [17]. For dietary fatty acids measured values of desorption kinetics have revealed half-life times in the msec to sec range, which would be fast enough to support intracellular metabolism [17]. Based on these findings it was concluded that the lipid bilayer of the plasma membrane does not represent a barrier for fatty acids and that, therefore, cellular fatty acid uptake can occur by (passive) diffusion without the need for membrane proteins to facilitate the process [16].

From a theoretical perspective it would be undesirable to have fatty acids pass biological membranes without control. In addition, such mechanism may not meet (changes in) cardiac metabolic demand, e.g. upon a rapid increase in the rate of contraction. Thus, since the early 1980s several investigators have searched for membrane-associated proteins able to bind fatty acids and that may function to facilitate transmembrane fatty acid transport. To date, various membrane proteins have been identified that facilitate the cellular uptake of fatty acids (Table 1 and Fig. 2):

1. Plasma membrane fatty acid-binding protein ($FABP_{pm}$) is a peripheral protein of approximately 43 kDa with a ubiquitous tissue occurrence [18–20].

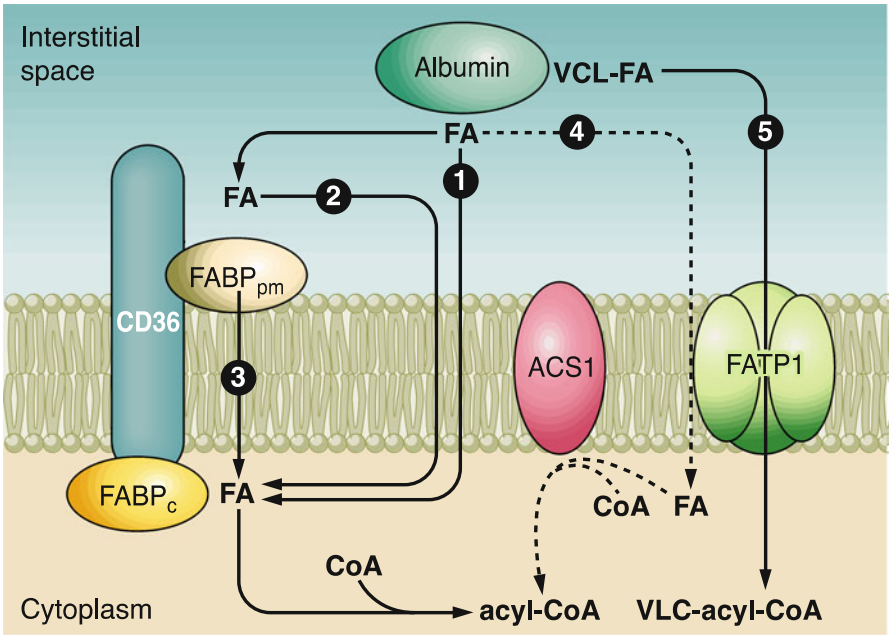

Fig. 2 Putative molecular mechanism(s) for the cellular uptake of long-chain fatty acids (FA) and of very-long-chain fatty acids (VLC-FA). (1) In view of their hydrophobic nature, fatty acids could dissociate from their albumin binding sites and cross the plasma membrane by simple diffusion (referred to as uptake by passive diffusion). (2) Alternatively, membrane-associated proteins, such as the peripheral membrane protein $FABP_{pm}$ or the transmembrane protein CD36, could act, either alone or together, as acceptor for fatty acids to increase their concentration at the cell surface and thus enhance the number of fatty acid diffusion events. (3) CD36 itself may also facilitate the transport of fatty acids across the phospholipid bilayer (uptake by facilitated diffusion). Once at the inner side of the cell membrane, fatty acids are bound by cytoplasmic FABP ($FABP_c$) before entering metabolic or signaling pathways. (4) Additionally, a minority of fatty acids are thought to be transported by FATP1 and rapidly activated by plasma membrane acyl-CoA synthetase (ACS1) to form acyl-Co esters. (5) VLC-FA are preferentially transported by FATP1 (or other FATPs) and by action of the synthetase activity of FATP1 directly converted into VLC-acyl-CoA esters (uptake by vectorial acylation). Reproduced from [22], with permission

2. A family of so-called 'fatty acid-transport proteins' (FATP; 63 kDa) consists of 6 members (FATP1–6) each displaying a characteristic tissue distribution [21]. The FATPs are trans-membrane proteins showing acyl-CoA synthetase activity and merely function in the uptake of very long-chain fatty acids (chain length >22) which then are converted directly into very long-chain acyl-CoA esters [21, 22].
3. CD36, also referred to as fatty acid translocase (FAT), is a class B scavenger receptor protein with multiple functions such as the binding of thrombospondin, oxidized low-density lipoprotein (LDL), and anionic phospholipids, and its action as a gustatory lipid sensor [12, 22, 23]. CD36 has a hairpin membrane topology with two transmembrane spanning regions, and is heavily glycosylated bringing the 472-amino acid protein (53 kDa) to 88 kDa.

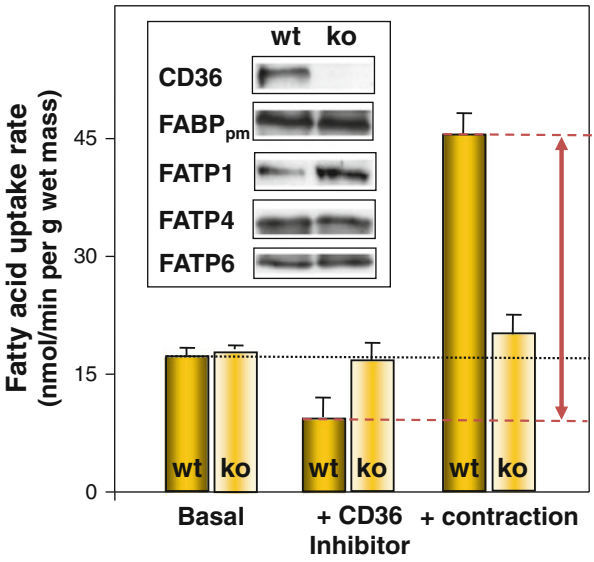

Fig. 3 Contraction-induced myocardial fatty acid uptake is virtually blunted in mice with a targeted deletion of CD36. Isolated mouse cardiac myocytes from wild-type (WT) and CD36 null mice (KO) were incubated in the absence or presence of the CD36 inhibitor sulfo-*N*-succinimidyl-oleate (SSO) (middle panel) and in the absence or presence of oligomycin (to mimic the metabolic effects of contraction), whereafter the rate of radiolabeled palmitate uptake was measured. Western blot analyses revealed a twofold upregulation of FATP1 in hearts of CD36 null mice, which may explain the maintained rate of fatty acid uptake in CD36 deficient cardiomyocytes under basal conditions. The vertical arrow illustrates that in oligomycin-treated cardiomyocytes the contribution of CD36-mediated fatty acid uptake to total fatty acid uptake is about 70 %. Data obtained from [24]

In myocardium, $FABP_{pm}$, three of the six types of FATP (i.e., FATP1, –4 and –6), and CD36 are co-expressed. Although studies in cell lines and/or in genetically altered animals have demonstrated that each of these proteins facilitates cellular long-chain fatty acid uptake, in the heart CD36 appears quantitatively most important. Thus, studies on fatty acid uptake by cardiac myocytes isolated from CD36 null mice and incubated in the presence of oligomycin (to mimic the metabolic effects of contraction) revealed sarcolemmal CD36 to be responsible for about 70 % of myocardial fatty acid uptake (Fig. 3) [24, 25].

With respect to the molecular mechanism of myocardial fatty acid uptake, the prevalent view is that the fatty acid transporter CD36 acts as an acceptor for fatty acids wherafter the fatty acids make their way through the cell membrane by simple diffusion (route 2 in Fig. 2). At the inner site of the membrane, the (transmembrane) protein may provide a docking site for H-$FABP_c$ or for enzymes that act on fatty acids such as acyl-CoA synthetase (Fig. 2). Thus, CD36 may function to sequester fatty acids in the membrane, and help organize them within specific membrane domains so as to make the fatty acids readily available for subsequent aqueous transport and/or enzymic conversion.

At the extracellular site CD36 shows protein–protein interaction with plasma membrane fatty acid-binding protein ($FABP_{pm}$), and at the intracellular site with cytoplasmic FABP that acts as a lipid chaperone by binding the incoming fatty acids and facilitating their transport to sites of utilization, as discussed above. The nature of the role served by $FABP_{pm}$ in the cellular fatty acid uptake process is still obscure; for instance, it may function to help create a local high concentration of fatty acids to drive their uptake or to localize CD36 to domains of the plasma membrane where fatty acid uptake preferentially would occur. Such latter role is also envisaged for the caveolins, small integral membrane proteins (22 kDa) that are the defining protein constituents of caveolae (specialized microdomains of the plasma membrane). Caveolins have been suggested to be potential fatty acid transporters [26], but may function in fatty acid uptake in an indirect manner, i.e., by offering plasma membrane docking sites for CD36 [22].

In case of the FATPs, the acyl-CoA synthetase activity of these transporters converts the fatty acids directly into their acyl-CoA ester, resulting in so-called metabolic trapping of fatty acids. This facilitatory action of the FATPs on cellular fatty acid uptake is also referred to as vectorial acylation.

5 Myocardial Fatty Acid Uptake and Control of Fatty Acid Utilization

With the disclosure of the role of membrane-associated proteins—particularly CD36—in myocardial fatty acid uptake, a discussion was started on the rate-limiting step of the overall process of cardiac fatty acid utilization. In the heart as well as in liver and skeletal muscle, carnitine palmitoyl-transferase-I (CPT-I) is well-established to provide a major site of acute regulation of fatty acid β-oxidation via its physiological inhibitor malonyl-CoA. The enzymatic activity of CPT-I is generally proposed to be the overall rate-limiting step in long-term cellular fatty acid utilization [27, 28]. However, recent work has questioned this role for CPT-I. First, the concentration of malonyl-CoA in the heart has been estimated to be 1–10 μmol/L [29, 30], which greatly exceeds the half maximal inhibitory concentration of M-CPT-I for malonyl-CoA (0.02 μmol/L). Hence, β-oxidation would, theoretically, be permanently blocked if CPT-I is rate-limiting for β-oxidation [31]. Second, in perfused hearts from *db*/*db* mice, fatty acid oxidation was increased 4-fold, independent of changes in CPT-I activity [32]. These observations suggest that CPT-I activity may not be rate-limiting for cardiac β-oxidation. Third, in CD36-null mice, in which CPT-I protein expression is not altered, fatty acid oxidation is impaired, particularly during a metabolic challenge [33]. This indicates that sarcolemmal fatty acid transporters, notably CD36, are major determinants of the rate of fatty acid oxidation. Finally, partial inhibition of CPT-I activity *in vivo* in rats, induced by subjecting the animals to daily injections with etomoxir, a specific inhibitor of CPT-I, for 8 days and resulting in a 44 % reduced cardiac CPT-I activity, failed to alter the

rates of cardiac fatty acid uptake and/or oxidation [34]. Together, these data indicate that CPT-I does not act as a major rate-controlling site in total cardiac fatty acid flux, and suggest that cellular fatty acid uptake (trans-sarcolemmal transport) is the primary site of fatty acid flux regulation.

6 Regulation of Myocardial Fatty Acid Uptake

The presence of CD36 not only on the cell membrane but also in intracellular compartments, notably endosomes, has triggered studies to identify whether regulation of fatty acid transport could occur by translocation of CD36 from endosomes to the cell membrane to increase fatty acid uptake. Such a mechanism would be analogous to the well-known regulation of glucose uptake which involves the translocation of the glucose transporter GLUT4 from an intracellular storage depot to the cell membrane. A series of studies showed that in heart and skeletal muscles both muscle contraction and insulin stimulate, within minutes, the translocation of CD36 from an endosomal compartment to the cell membrane to markedly (up to twofold) increase fatty acid uptake [35–37]. The contraction-induced translocation is mediated by AMP-kinase and occurs independently of the insulin-induced translocation which is mediated by PI3 kinase. In each case, both CD36 and GLUT4 are recruited within the same time frame resulting in increased uptake rates for both fatty acids and glucose [35, 38, 39]. Similar to what is known for GLUT4, the translocation of CD36 is a vesicle-mediated process (involving a.o. various proteins such as coat proteins, rab proteins, and vesicle-associated membrane proteins (VAMPs), as well as specific cytoskeletal networks) and is rapid and reversible, with CD36 being internalized on the same time scale (minutes) as its recruitment [35, 40]. Taken together, the regulation of fatty acid uptake by heart and muscle appears to display a striking similarity with the regulation of glucose uptake, in both cases involving the recycling of substrate transporters that is under the control of (at least a number of) the same triggers (Fig. 4).

Others have confirmed for skeletal muscle that contractile activity increases fatty acid transport via the translocation of CD36 [41]. Contraction also increases the translocation to the cell membrane of $FABP_{pm}$, FATP1, and FATP4 in muscle [42] and $FABP_{pm}$ in cardiac myocytes [43]. Nevertheless, as mentioned above, studies with cardiac myocytes obtained from CD36 null animals have indicated that CD36 is fundamental to enabling the contraction-induced increase in fatty acid uptake, as its ablation almost completely blunted this increase in rate of uptake [24].

Considerable effort is being made to unravel both the signalling and trafficking pathways involved in the recycling of CD36, in particular in relation to that of GLUT4. Knowledge on these pathways is relevant (1) to understand which factors influence cellular substrate uptake through substrate transporter recycling and (2) to learn to what extent CD36 and GLUT4 recycling share the same triggers and trafficking machinery and what signals would selectively influence either CD36-mediated fatty acid uptake or GLUT4-mediated glucose uptake (which would alter

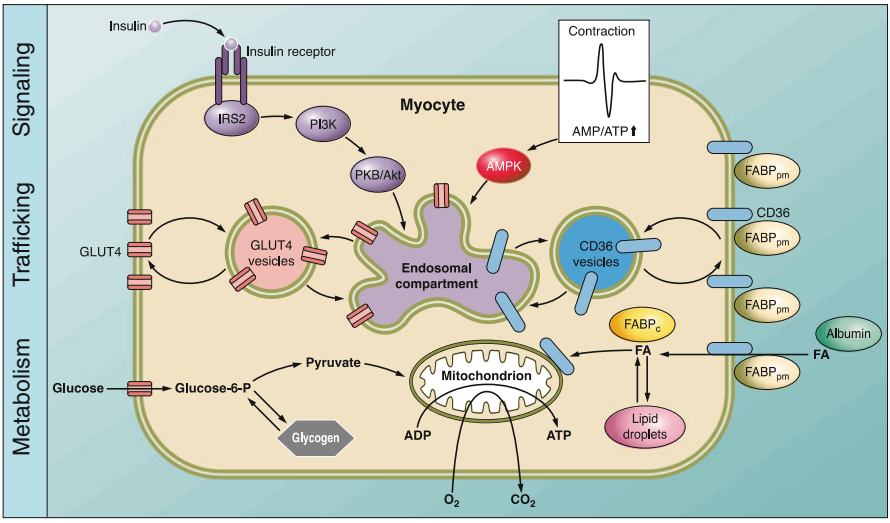

Fig. 4 Schematic presentation of the regulation of cellular uptake of glucose and (long-chain) fatty acids in heart and skeletal muscle. In response to stimulation with insulin or during increased contractile activity both the glucose transporter GLUT4 and the fatty acid transporter CD36 translocate to the plasma membrane to increase glucose and fatty acid uptake, respectively. Note that GLUT4 and CD36 may be mobilized from distinct stores within the endosomal compartment. For clarity, the involvement of GLUT1 in glucose uptake and the putative recycling of other fatty acid transporters ($FABP_{pm}$ and FATPs) are not shown. *FA* long-chain fatty acid. Reproduced from [22], with permission

substrate preference; see below). The signalling pathways involved in CD36 and GLUT4 recycling appear to be very much alike (reviewed in [22]) but the trafficking pathways used for CD36 and for GLUT4 translocation show clear differences with respect to vesicle-associated membrane proteins (VAMPs) involved [44]. Thus, of the five VAMP isoforms occurring in the heart, three VAMPs are necessary for both GLUT4 and CD36 translocation, while one isoform (VAMP7) is specifically involved in GLUT4 traffic and another isoform (VAMP4) specifically in CD36 traffic [44].

With respect to the functioning of CD36, recent data indicate that the fatty acid facilitatory activity of CD36 not only is controlled by intracellular recycling of the protein but also by post-translational modification, in particular *N*-glycosylation [45] and ubiquitination [46, 47]. In addition, more recent work suggests that CD36 translocation to the sarcolemma and effectuating this translocation into enhanced fatty acid uptake are separate events (Angin Y, Luiken JJFP, unpublished observations). These findings propose a mechanism whereby an additional quantity of 'inactive' CD36 already is recruited to the sarcolemma to have these available to enter a functional pool of CD36 in order to respond rapidly to conditions that instantaneously require increased cardiac energy demands.

7 Chronic Physiological Changes in Myocardial Fatty Acid Uptake

Whole-body lipid homeostasis requires a fine tuning of fatty acid transport and utilization by metabolically active tissues. Because of both their facilitatory and regulatory roles in cellular fatty acid uptake and utilization, membrane fatty acid transporters form an integral part of this homeostatic system. As a result, physiological as well as pathophysiological changes in lipid metabolism likely will influence the functioning of fatty acid transporters, while changes in fatty acid transporter content or functioning—for instance as induced by dietary or pharmacological interventions—may have an impact on whole-body lipid metabolism and potentially elicit a pathological state.

Long term changes in the cellular contents of fatty acid transporters involve *de novo* protein synthesis and/or breakdown which effects are mediated by specific transcription factors and coactivators. For instance, CD36 expression in the heart is under the transcriptional control of nuclear peroxisome proliferator-activated receptor (PPAR) α [48], although in an indirect manner [49]. Because fatty acids and selected fatty acid metabolites are the physiological ligands for the PPARs, they 'prepare' the cell for the uptake and utilization of larger amounts of fatty acids. Fatty acid transporters take part in this process in a special fashion as they could promote a positive-feedback loop to further increase gene transcription.

Myocardial fatty acid uptake and utilization are known to change markedly in response to development and ageing, fasting, specific hormones (e.g., insulin, leptin), and exercise training. In each of these conditions, these changes were found to associate with concomitant changes in the myocardial contents of fatty acid transporters in the sarcolemma [22]. For instance, the rapid increase in oxidation capacity and mitochondrial enzymes during postnatal development of rat heart is accompanied by a similar rapid adaptation in total CD36 protein expression [50]. Insulin not only (acutely) induces the translocation of CD36, but upon longer exposure also increases the expression of total CD36 protein but not of $FABP_{pm}$ protein [51]. Chronic changes in sarcolemmal transporter content can be accomplished also by a redistribution of these proteins in the cell without a change in total cellular contents. The latter may explain why exercise training elicits an increase in cardiac fatty acid oxidation without an altered expression of either CD36 or $FABP_{pm}$ [52].

8 Pathophysiological Changes in Myocardial Fatty Acid Uptake

Alterations in cardiac lipid metabolism as occur in pathological states have also been found to be accompanied by concomitant adaptations in the expression and/or subcellular localization of selected fatty acid transporters [22]. Two examples will be discussed, i.e., acute changes in cardiac substrate metabolism during ischemia

and reperfusion, and chronic changes in cardiac substrate metabolism during the development of high fat diet-induced cardiac insulin resistance and diabetic cardiomyopathy.

During ischemia, when cardiac metabolism rapidly adapts to the limited oxygen supply by changing its substrate preference from fatty acids towards glucose, a reorganization occurs of sarcolemmal substrate transporters with CD36 moving away and the glucose transporter GLUT4 moving toward the sarcolemma [53]. These changes support the increased utilization of glucose to generate sufficient ATP anaerobically to ensure cardiomyocyte survival, while preventing intracellular lipid overload that could cause contractile dysfunction. Interestingly, during reperfusion this relocation of CD36 and GLUT4 was maintained to enable replenishing of glycogen stores as a priority, presumably to equip the heart should a second ischemic insult follow [53].

Upon feeding a diet high in (saturated) fatty acids (i.e., Western type diet), myocardial substrate preference will change towards an increased utilization of fatty acids for energy production, ultimately leading to cardiac insulin resistance and contractile dysfunction, together referred to as diabetic cardiomyopathy. Cardiac insulin resistance and type 2 diabetes are known to be associated with increased intramyocardial triacylglycerol deposition [54]. Excessive accumulation of intramyocellular fatty acids and their metabolites is commonly referred to as lipotoxicity, and is a main contributor to the pathophysiology of insulin resistance and dysfunctioning of the heart [54, 55]. In animal models of insulin resistance [56–58] and in humans with insulin resistance and type-2 diabetes [59] it was found that the intramuscular triacylglycerol accumulation is associated with an increased presence of CD36 at the sarcolemma. The latter is not due to an increase in total tissue CD36 content, but rather to its permanent relocalization to the sarcolemma. Importantly, studies in rat heart [58] have elucidated that this permanent relocalization is an *early* event in the development of insulin resistance and its progression towards diabetic cardiomyopathy, and precedes a change in glucose uptake (Fig. 5). These combined observations suggest that early in the development of insulin resistance alterations occur in the signalling and/or trafficking proteins specifically dedicated to CD36 recycling, which would result in a selective and permanent relocation of CD36 to the sarcolemma, without a concurrent change in the subcellular distribution of GLUT4. The increased sarcolemmal CD36 abundance, together with an increased plasma fatty acid concentration, would elicit an increased rate of fatty acid uptake to an excessive level, in turn leading to an increased rate of fatty acid esterification into triacylglycerols and increased concentrations of fatty acid metabolites such as diacylglycerols and ceramides, as has been shown recently [60]. The latter compounds then will interfere with insulin-induced GLUT4 translocation to the sarcolemma so that GLUT4 is retained intracellularly and the rate of glucose uptake is lowered (i.e., insulin resistance) (Fig. 5). Thus, permanent CD36 relocation to the sarcolemma can be regarded as a key factor in the development of insulin resistance and cardiac contractile dysfunction.

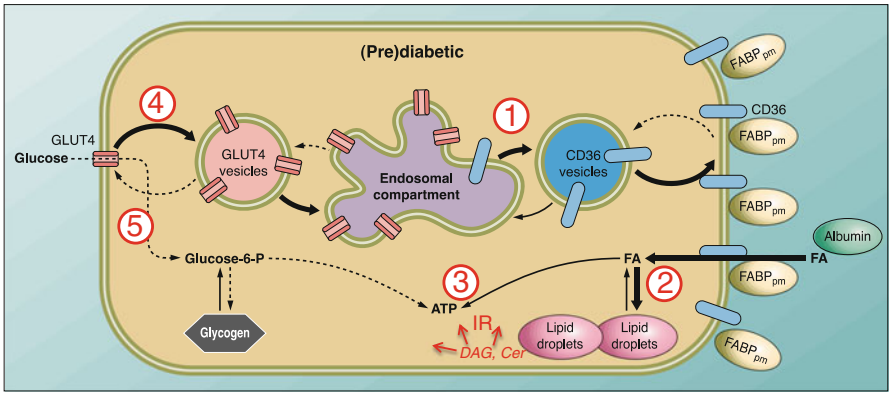

Fig. 5 Juxtaposition of the substrate transporters CD36 and GLUT4 in insulin-resistant muscle. Schematic presentation of a model for the development of an impaired GLUT4 translocation in the (pre)diabetic state. While in healthy conditions, both CD36 and GLUT4 are about equally distributed between endosomes and the sarcolemma, in the (pre)diabetic state, there is a shift in CD36 localization from the endosomes to the sarcolemma (*step 1*) resulting in enhanced fatty acid uptake and storage of fatty acids into triacylglycerols (TAGs; *step 2*). Fatty acids then become the major substrate for energy production (*step 3*). Subsequently, fatty acid metabolites such as diacylglyceroles and ceramides inhibit insulin signaling and translocation of GLUT4 from endosomes to the sarcolemma is impaired (*step 4*), resulting in lowered glucose uptake and decreased incorporation into glycogen (*step 5*). At that stage, the muscle has become insulin resistant. Adapted from [22], with permission *FA* long-chain fatty acid, *DAG* diacylglyceroles, *cer* ceramides, *IR* insulin resistance

9 Manipulating Fatty Acid Availability as Therapeutic Approach

In view of the central and often causative role of changes in cardiac fatty acid metabolism in the development of chronic cardiovascular diseases, such as the above-mentioned role of lipotoxicity in eliciting diabetic cardiomyopathy, modulating cardiac fatty acid uptake and utilization is regarded a useful therapeutic approach. Because cardiac substrate preference is regulated not only at the level of mitochondrial oxidation (Randle cycle) but predominantly at the level of sarcolemmal uptake of substrates, changes in cardiac substrate utilization can be achieved by the selective modulation of the sarcolemmal localization of the main transporters involved, i.e., CD36 and GLUT4 [61]. A number of recent studies have shown proof of concept of such approach.

Studies by Angin and co-workers [25] on isolated adult cardiomyocytes subjected to insulin-resistance evoking conditions (medium with high palmitate concentration) have shown that immunochemical inhibition of CD36 prevents lipid accumulation, insulin resistance and contractile dysfunction (Fig. 6). These studies suggest that pharmacological CD36 inhibition may be considered as a treatment

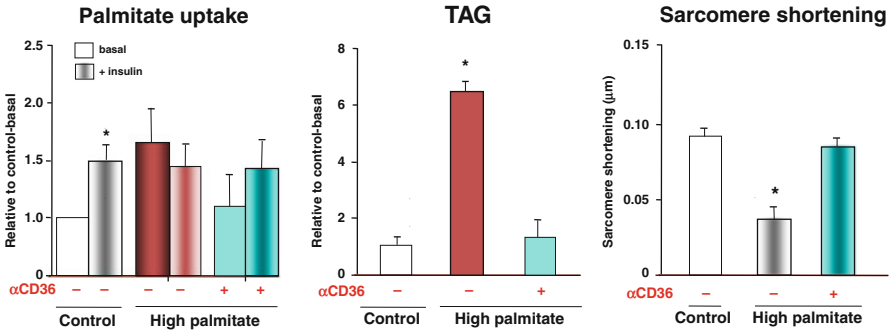

Fig. 6 Immunochemical inhibition of sarcolemmal CD36 prevents insulin resistance, lipid accumulation and contractile dysfunction in isolated cardiomyocytes. Insulin resistance and contractile function were induced in adult rat cardiomyocytes by 48 h incubation in a medium containing 200 µM albumin-bound palmitate. This resulted in elevated sarcolemmal CD36 content (not displayed), increased basal palmitate uptake (*left panel*), increased lipid accumulation (*middle panel*) and decreased sarcomere shortening (*right panel*). The addition of anti-CD36 antibodies to the medium (αCD36) prevented elevated basal palmitate uptake, triacylglycerol accumulation and contractile dysfunction. Data obtained from [25]

strategy to counteract impaired functioning of the lipid-loaded heart [62]. In line with these findings, Geloen et al. [63] described the identification of low molecular mass chemicals that can block the lipid binding and uptake functions of CD36 to subsequently show that these CD36 inhibitors reduce postprandial hypertriglyceridemia and protect against diabetic dyslipidemia and atherosclerosis in rodents. Similarly, Bessi et al. [64] reported that pretreatment of mice with EP80317, a selective synthetic peptide ligand of CD36, protected the heart against damage and dysfunction elicited by myocardial ischemia and reperfusion.

10 Conclusions

Myocardial fatty acid uptake is subject to a complex mechanism of control, involving various metabolites and a number of soluble and membrane-associated proteins that interact with fatty acids not only to facilitate their movement from the vascular space to the mitochondria in the cardiomyocytes but presumably also to protect the heart from cytotoxic effects of high concentrations of fatty acids. Hence, proper control of the bioavailability of fatty acids is of major importance. In the healthy heart, uptake and utilization of fatty acids are finely tuned. On a short-term basis, this is controlled primarily by intracellular cycling of the membrane fatty acid-binding protein CD36 between intracellular stores (endosomes) and the sarcolemma, assisted by the presence of other cytoplasmic ($FABP_c$) and membrane proteins ($FABP_{pm}$, FATPs). It should be noted that this mechanism shows a striking similarity with the regulation of myocardial glucose uptake, which has firmly been

established to be the rate-limiting step in glucose utilization and occurs through intracellular cycling of the glucose transporter GLUT4 [65]. On a long-term basis, changes in gene expression of CD36 and other fatty acid transporters will govern adaptations in the overall capacity for myocardial fatty acid uptake [66]. The driving force for fatty acid uptake is the gradient of (non-protein bound) fatty acids between the vascular compartment and the cytoplasm of the cardiomyocyte [66]. Therefore, changes in plasma fatty acid concentration (delivery) and in the rate of cellular utilization (metabolic trapping by activation into acyl-coenzyme A ester) each will impact on the rate of myocardial fatty acid uptake.

Investigations on the mechanism and regulation of myocardial fatty acid uptake have focused mostly on cardiac myocytes, rendering the microvascular endothelium with much less attention. However, a number of very recent studies suggest that the mechanistic insights disclosed for cardiomyocytes may also apply to transendothelial fatty acid transport. Thus, uptake and translocation of fatty acids through the endothelium is facilitated also by both cytoplasmic (FABP4 and FABP5) and membrane-associated proteins (CD36, FATPs). It remains to be studied whether the uptake is regulated by subcellular cycling of the membrane-associated proteins and whether and, if so, in what manner, the events occurring in capillary endothelial cells are tuned to those in cardiac myocytes.

The complexity of control of myocardial fatty acid uptake is also illustrated from the many levels of regulation of the proteins involved, which not only comprises transcriptional regulation but also translocation from intracellular storage sites to the cell membrane, post-translational modification (phosphorylation, palmitoylation, glycosylation) and protein–protein interactions (reviewed in [22, 67–69]). Upon the disclosure that the membrane fatty acid transporters are implicated in (cardiac) metabolic disease, particularly insulin resistance and its progression to diabetic cardiomyopathy, they can be regarded as promising therapeutic targets to re-direct lipid fluxes in the body in an organ-specific fashion. The potential of such an approach is illustrated by the observation that myocardial lipotoxicity and myocardial dysfunction induced experimentally by PPARα overexpression could be rescued by ablation of CD36 [70]. However, pharmacological inhibition of CD36 may not only prevent the detrimental effects of excess fatty acid uptake but may also limit the beneficial effects of omega-3 long-chain polyunsaturated fatty acid species (in particular DHA and EPA) on cardiac function [71, 72]. Hence, future studies should be aimed at fine-tuning the plasmalemmal functioning of CD36 so as to maintain a proper balance in these various processes.

Various aspects of the mechanisms by which these fatty acid transporters function in the control of myocardial fatty acid uptake still need to be uncovered in order to design therapies to selectively modulate their actions. These remaining issues include: (1) further disclosing the signalling and trafficking pathways involved in recycling of fatty acid transporters, especially in relation to that of the glucose transporter GLUT4; (2) establishing whether protein–protein interaction and posttranslational modifications among fatty acid transporters have functional significance, and whether these aspects are changed in the pathological state; (3) unraveling the

role of membrane microdomains (caveolae, rafts) in the functioning of fatty acid transporters; (4) examining whether fatty acid transporters select for specific fatty acid types and/or target fatty acids towards specific metabolic pathways (oxidation, esterification) or signalling routes (activation of fatty acid responsive genes).

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Cardiac Energy Metabolism in Heart Failure Associated with Obesity and Diabetes

Gary D. Lopaschuk

Abstract Obesity and diabetes are important risk factors for the development of heart failure. The metabolic abnormalities that accompany obesity and diabetes result in dramatic alterations in cardiac energy metabolism, which can contribute to the progression of heart failure. Elevated rates of fatty acid oxidation and depressed rates of glucose oxidation characterize the cardiac metabolic profile in the setting of obesity-induced insulin resistance and diabetes. This metabolic profile results in a marked cardiac insulin resistance, which is accompanied by decrements in both cardiac function and efficiency, and by the accumulation of potentially toxic fatty acid metabolites in the heart. Acetylation of various mitochondrial and glycolytic enzymes are altered in obesity and diabetes, which may also contribute to the pathogenesis of heart failure in obesity and diabetes. As a result, therapeutic interventions that prevent or reverse the energy metabolic switch in the heart of obese and diabetic individuals, and/or the accumulation of fatty acid metabolites may lessen the severity of heart failure. These interventions include inhibiting myocardial fatty acid oxidation, stimulating glucose oxidation, restoring myocardial insulin sensitivity, preventing myocardial fatty acid metabolite accumulation, and inhibiting the acetylation of key enzymes involved in fatty acid oxidation. This paper reviews the metabolic alterations that occur in heart failure associated with obesity and diabetes, and the molecular mechanisms responsible for these changes.

Keywords Fatty acid oxidation • Glucose oxidation • Lysine acetylation • Cardiac insulin-resistance • Lipotoxicity

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1 Introduction

Obesity and diabetes are major health concerns in our population, with the incidence rapidly rising in both Canada and the world. Among the many complications associated with obesity and diabetes is an increased risk of developing heart failure, a complex clinical syndrome [1, 2], characterized by the progressive inability of the heart to fill with, and eject, adequate amounts of blood to meet the needs of the body. This increased risk of developing heart failure in obese and diabetic individuals persists even after adjusting for independent factors including coronary artery disease and hypertension [3–5]. As a result, a considerable research effort has focused on the mechanisms responsible for the increased prevalence of heart failure in obesity and diabetes. Potential contributing factors identified thus far include: (1) increased oxidative stress [6], (2) development of cardiac autonomic neuropathies [7], (3) accelerated apoptosis [8, 9], (4) accelerated inflammatory responses [9–11], (5) accelerated fibrosis [12], (6) altered cardiac Ca^{2+} and Na^{+} handling [13], (7) production of advanced glycation end products (AGE) and receptors for AGEs activation [14], (8) increased polyol pathway activity [15], (9) activation of NADPH oxidase [16, 17], (10) increased O-linked β-N-acetylglucosamine [18], and (11) alterations in cardiac energetics (discussed below).

Heart failure in obesity and diabetes is characterized by the early development of left ventricular (LV) diastolic dysfunction [19], increased LV mass, increased LV wall thickness, and the eventual development of LV systolic dysfunction [20]. This is accompanied by changes in control of fatty acid metabolism at both the level of the heart and skeletal muscle [22–28]. As heart failure progresses, myocardial ATP and PCr content decreases [23–28], with a decrease in the PCr/ATP ratio correlating with NYHA functional class 2 [29, 30]. Defects in the rates of oxygen consumption and mitochondrial electron transport chain activity (which impact oxidative phosphorylation, and hence ATP generation) also accompany heart failure (see for review [31]). Heart failure also impairs insulin signaling [32, 33]. These deficits in insulin sensitivity and energy generation contribute to the pathogenesis and progression of heart failure in obesity and diabetes. Evidence is emerging that the exacerbation of heart failure in obesity and diabetes is due, in part, to the alterations in the use of fatty acids as a source of ATP production.

2 Cardiac Fatty Acid Use in Obesity, Diabetes, and Heart Failure

While fatty acids are a major energy source of the heart, fatty acid uptake and subsequent mitochondrial fatty acid oxidation must be coordinately regulated in order to ensure adequate, but not excessive supply, for cardiac energetic requirements (Fig. 1). The presence of diabetes and/or obesity induced insulin-resistance can markedly alter this regulation, leading to adverse consequences on cardiac

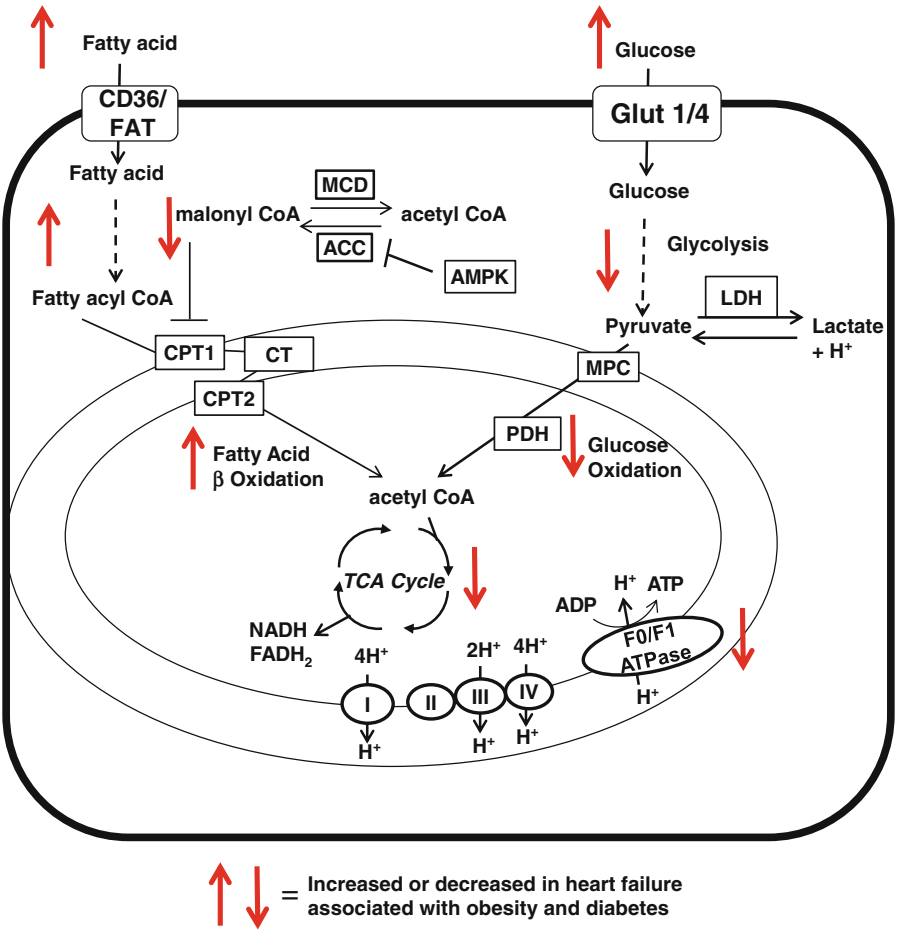

Fig. 1 Alterations in cardiac energy metabolism that occur during heart failure associated with obesity and diabetes

function. In particular, numerous clinical and experimental studies have shown that the heart switches to a greater reliance on fatty acid oxidation as a source of energy in obesity and diabetes. We and others have shown that fatty acid oxidation rates in the heart are increased in diabetic rats, as well as in rats and mice subjected to diet-induced obesity and in insulin-resistant *db*/*db* and *ob*/*ob* mice, with the increase in fatty acid oxidation occurring primarily at the expense of glucose oxidation [33–48]. Human studies using positron emission tomography and ^{11}C-palmitate imaging found that obese women and type 2 diabetics also have increased cardiac fatty acid oxidation [49, 50].

Heart failure itself results in significant alterations in cardiac energy metabolism, with the metabolic phenotype dependent on the stage/severity of the syndrome.

Fatty acid oxidation rates are normal in acute heart failure secondary to aortic banding in rats [51], in the failing canine heart [52], and in patients with asymptomatic hypertrophic cardiomyopathy [53]. However, in NYHA functional class III patients fatty acid use is increased [54], and is accompanied by elevated plasma lactate levels, indicative of fatty acid impairment of carbohydrate oxidation [54]. In clinically stable NYHA functional class II and III patients, cardiac fatty acid uptake [55, 56] and subsequent oxidation [55] is greater than that observed in healthy controls, while glucose uptake [56] and oxidation are lower [55]. Mitochondrial TCA cycle and oxidative phosphorylation are also depressed in heart failure [57]. In mice subjected to an abdominal aortic constriction (AAC), we show that the development of hypertrophy and diastolic heart failure is accompanied by early decreases in overall mitochondrial oxidative capacity [58]. An early change that occurs in heart failure is a decrease in overall mitochondrial oxidative metabolism, with a prominent decrease in carbohydrate oxidation [59–62]. Of importance is that a decrease in carbohydrate oxidation is primarily responsible for the decrease in mitochondrial oxidation, and fatty acid oxidation is only marginally decreased. In addition, a marked decrease in insulin sensitivity occurs in the heart during the development of diastolic heart failure [59–62]. We also recently showed that development of systolic heart failure secondary to pressure overload is accelerated in obese mice [62]. Of interest, is that decreasing obesity by switching mice to a low fat diet or by caloric restriction markedly increases insulin-sensitivity, decreases fatty acid oxidation, and improves glucose oxidation [62, 63]. We therefore propose that worsening of heart failure occurs secondary to obesity-induced changes in fatty acid oxidation, and an exacerbation of the insulin resistance that occurs in heart failure.

3 The Controversy of Fatty Acid Metabolism and Insulin-Resistance

High circulating levels of fatty acids, as well as increased uptake and esterification of fatty acids contributes to muscle insulin-resistance and cardiac lipotoxicity (see [64] for review). Decreasing muscle fatty acid uptake and/or esterification can decrease the accumulation of these toxic lipid intermediates [21, 22, 65–69]. However, a controversial strategy for decreasing lipid accumulation is based on enhancing fatty acid oxidation, which has been proposed to help remove cytoplasmic lipid metabolites, thereby improving insulin sensitivity [21, 22, 69, 70]. This concept is based on the observation that the size and number of mitochondria, as well as the activity of proteins in the respiratory chain are reduced in obese insulin-resistant humans, rodents [71–76], or subjects with type II diabetes [77–79]. However, contrary evidence suggests that several markers of mitochondrial function and fatty acid oxidative capacity (oxidative enzyme activity and protein expression) are elevated in muscle of high fat-fed mice and rats, obese Zucker rats (*fa/fa*), and *db/db* mice [80, 81], and direct measurements of fatty acid oxidation in the heart have shown that in insulin resistance fatty acid oxidation rates are accelerated

[39, 42, 82]. Furthermore, we and others have shown that inhibiting fatty acid oxidation in heart and skeletal muscle can increase insulin sensitivity [40, 41, 83, 84]. Products of incomplete fatty acid oxidation can also contribute to muscle insulin-resistance [41]. As a result, debate still exists as to whether stimulating or inhibiting muscle fatty acid oxidation is an approach to lessen insulin resistance, prevent lipid accumulation and improve contractile dysfunction.

4 High Fatty Acid Oxidation Decreases Cardiac Efficiency and Contractile Function in Obesity, Diabetes and Heart Failure

Cardiac efficiency (the amount of work performed by the heart per oxygen consumed) [85], is influenced by alterations in fatty acid oxidation [86]. This has potentially important consequences in heart failure, as well as in the setting of obesity and diabetes where rates of fatty acid use are markedly altered. As the majority of ATP utilized to drive cardiac contraction is generated by mitochondrial oxidative phosphorylation, cardiac efficiency itself can be influenced by both the efficiency of ATP generation and hydrolysis (i.e. the efficiency of converting chemical energy into mechanical energy). Interestingly, there are relatively few studies that have examined cardiac mechanical efficiency in heart failure, and there appears to be discrepant results between these studies. Studies have shown a preservation of cardiac efficiency secondary to decreases in MVO_2 [87, 88]; while, in contrast, others have demonstrated decreased cardiac efficiency secondary to oxygen wasting effects in the failing heart [89, 90]. The effects of obesity and/or diabetes on cardiac efficiency are less ambiguous. In murine models of obesity, insulin resistance, and diabetes (including leptin-deficient *ob*/*ob* and leptin receptor-deficient *db*/*db* mice) cardiac fatty acid use is increased, while cardiac efficiency is decreased [39, 42, 91–94]. The decrease occurs in response to increased MVO_2 [38, 39, 91], decreased LV work [39, 92] or a combination of both [39, 92].

5 Mechanism by Which Fatty Acid Oxidation is Altered in Obesity, Diabetes, and Heart Failure

5.1 Alterations in Fatty Acid Supply

Both human and animal studies have shown that a prevalent metabolic change in obesity/insulin resistance involves an elevation in circulating fatty acids and triacylglycerol (TAG) levels [38, 95–100], resulting in an increase in cardiac fatty acid uptake and oxidation. Increased fatty acid supply to the cardiomyocyte may also be

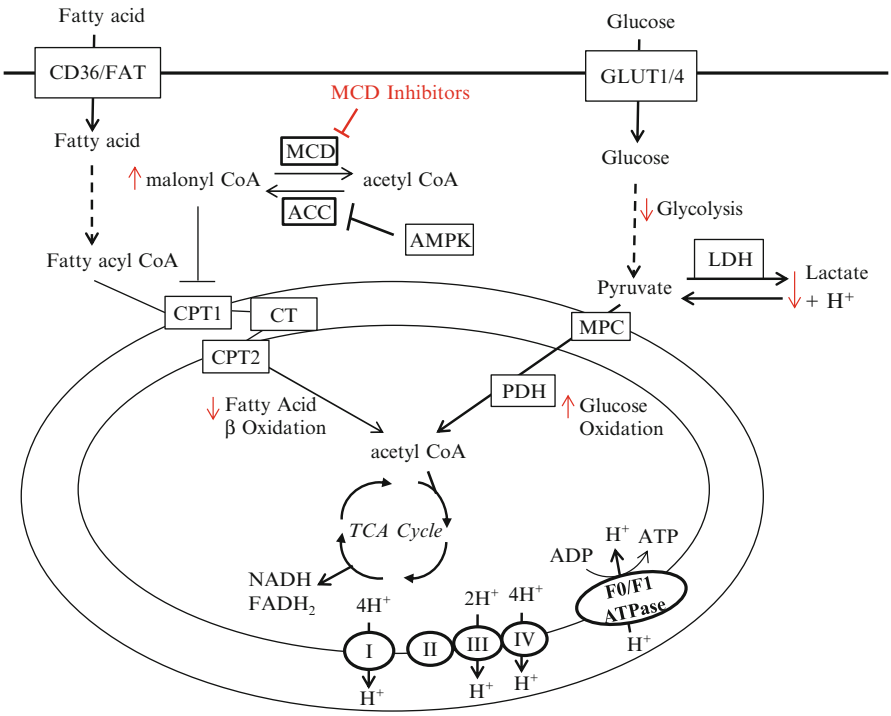

Fig. 2 Increased fatty acid uptake and oxidation in failing hearts associated with obesity and diabetes is accompanied by a marked decrease in energy production from glucose oxidation. Decreases in tricarboxylic acid (TCA) cycle activity and electron transport chain activity can lead to an increase in incomplete fatty acid oxidation. The increase in fatty acid oxidation in failing hearts associated with heart failure can be explained by: (1) an increased fatty acid supply to the heart, (2) a decrease in cardiac malonyl CoA levels resulting in a decreased inhibition of carnitine palmitoyltransferase 1 (CPT1), and/or (3) an increased acetylation of fatty acid oxidative enzymes. *FAT* fatty acid transporter, *MCD* malonyl CoA decarboxylase, *ACC* acetyl CoA carboxylase, *AMPK* AMP-activated protein kinase, *CPT* carnitine palmitoyltransferase, *CT* carnitine acylcarnitine translocase, *PDH* pyruvate dehydrogenase, *MPC* mitochondrial pyruvate carrier, *LDH* lactate dehydrogenase, *Glut* glucose transporter

due to an increase in lipoprotein lipase (LPL) activity in insulin-resistant [101], and diabetic animals [102, 103], although a consistent increase in LPL has not been found [102–106]. The uptake of fatty acids into cardiomyocytes is facilitated by the action of a number of fatty acid transporters (e.g. FAT/CD36, FABPpm, and FATPs) (Fig. 1). Translocation of FAT/CD36 to the sarcolemmal membrane is increased in the presence of insulin resistance and diabetes resulting in increased fatty acid uptake [96, 97, 107]. Increased expression and sarcolemmal localization of fatty acid transporters may also partially account for increased fatty acid supply and oxidation [108–110] (Fig. 2).

5.2 Alterations in the Intracellular Fate of Fatty Acids

This can also contribute to the high fatty acid oxidation rates in obesity and diabetes. Once transported into the cytosol, fatty acids are esterified to CoA by fatty acyl CoA synthetase (Fig. 1), forming long chain acyl CoA. Most of the long chain acyl CoAs is destined for mitochondrial fatty acid oxidation, but a small portion can be converted into intracellular lipid intermediates, such as TAG, phospholipids, diacylglycerol (DAG), and ceramide. To undergo ß-oxidation, the acyl groups from long chain acyl-CoA are transported into the mitochondria via a carnitine-dependent transport system (Fig. 1) involving carnitine palmitoyl transferase-1 (CPT-1). CPT-1 activity is controlled by malonyl CoA, a potent allosteric inhibitor of CPT-1) [111]. Malonyl CoA content, in turn, is controlled by its rate of turnover. Acetyl CoA carboxylase (ACC) catalyzes the synthesis of malonyl-CoA, whereas malonyl CoA decarboxylase (MCD) catalyzes its degradation. In diabetes malonyl CoA control of CPT-1 is decreased, due in part to an increased expression of MCD [112], resulting in increased mitochondrial uptake and oxidation of fatty acids.

5.3 Alterations in the Fatty Acid ß-Oxidative Pathway

This can also contribute to increased fatty acid oxidation in obesity and diabetes. In *db*/*db* mice, increased cardiac fatty acid oxidation [35, 43, 113, 114] is associated with a concomitant increase in the activity of enzymes of mitochondrial oxidation. This increase in fatty acid oxidative enzymes may be related to an increase in the transcriptional regulator PPARα, which increases fatty acid oxidative enzyme expression and produces a dramatic increase in fatty acid oxidation that has the potential to decrease cardiac efficiency [115]. Pharmacologically shifting the balance of oxidative metabolism from fatty acid oxidation towards glucose oxidation by targeting either (1) the cellular uptake of energy substrates, (2) transcriptional regulators of energy substrate metabolism, (3) mitochondrial fatty acid uptake, (4) mitochondrial fatty acid oxidation, and (5) glucose oxidation can improve the efficiency of ATP generation and hydrolysis (see for review [31]).

5.4 Acetylation Control of Cardiac Fatty Acid Oxidation in Insulin Resistance and Diabetes

Protein acetylation is an important dynamic/reversible post-translational modification involved in many cellular processes, including nuclear transcription, cell survival, apoptosis, and differentiation [116, 117]. Nuclear lysine acetylation has been extensively studied, and is linked to active gene transcription [118–120]. This post-translational modification is mediated by histone acetyltransferases (HATs) and is

reversed by histone deacetylases (HDACs). Both class 1 and class 2 HDACs play important roles in cardiac hypertrophy [116, 121–124]. Nuclear acetylation has an important role in regulating cardiac energy metabolism. For instance, PGC-1α and HIF-1α, important transcriptional regulators of genes involved in mitochondrial oxidative metabolism and glycolysis, are both under acetylation control [125–130].

Non-nuclear lysine acetylation has also emerged as an important post-translational regulator of many metabolic pathways [131–134]. This includes enzymes that are involved in mitochondrial metabolism and glycolysis and transcriptional regulation of glycolysis and mitochondrial oxidative metabolism [133–138]. Despite the recent identification of numerous acetylation sites on metabolic enzymes, the role of acetylation in regulating cardiac energy metabolism is still poorly understood. While it is generally thought that acetylation decreases enzyme activity, this is not always the case. A number of glycolytic enzymes appear to be activated by acetylation [133, 136, 138]. Acetylation of fatty acid oxidation enzymes is generally considered to inhibit fatty acid oxidation. For instance, Hirschey et al. [133, 139] proposed that acetylation inhibits liver fatty acid oxidation, via inhibition of the fatty acid oxidation enzyme, long chain acyl CoA dehydrogenase (LCAD). However, we recently showed that acetylation is actually associated with increased fatty acid oxidation in the heart. Increased acetylation of LCAD and hydroxyacyl CoA dehydrogenase (HACD) is associated with increased HACD activity under conditions where cardiac fatty acid oxidation is high. In support of this, Zhao et al. showed that acetylation of the hepatic fatty acid oxidation enzyme enoyl-CoA hydratase/3-HACD is associated with its activation [132]. Of importance, we have demonstrated that cardiac fatty acid oxidation rates are increased in SIRT3 ko mice, which occurs concomitant with a decreased glucose oxidation. The overall acetylation of myocardial proteins is enhanced in SIRT3 ko mice. Elevated acetylation of myocardial HCAD and LCAD are also evident, which is accompanied by an increased activity of LCAD. In agreement, a decrease in the acetylation of mitochondrial proteins has been reported in cells overexpressing SIRT3 [140, 141]. Further support for the concept that increased acetylation increases, rather than decreases, fatty acid oxidation was found in hindlimb muscle of fasted mice, where increased acetylation and fatty acid oxidation rates were observed [142]. Diaphragm muscle of SIRT3 ko mice also have increased fatty acid oxidation rates [142].

Multiple enzymes in the TCA cycle and ETC are also targets for acetylation. The effects of acetylation on these enzymes is poorly understood, but it is generally considered that acetylation decreases TCA cycle and ETC activity, thereby compromising mitochondrial ATP production [133, 139–141].

Another target of acetylation are enzymes in the insulin signaling and glycolytic pathway [142, 143], suggesting that alterations in acetylation may contribute to the insulin resistance seen in obesity, diabetes, and heart failure. A number of glycolytic enzymes are acetylated, and inhibition of glycolysis is associated with a reduction in SIRT1 expression [138]. Hepatic insulin resistance is also associated with reduced expression levels of SIRT1 [144–149]. Reduced SIRT1 expression also leads to reduced Akt activation and decreased insulin-induced IRS-2 tyrosine phosphorylation in several cell lines *in vitro* [150]. Conversely, SIRT1-mediated deacetylation of

Akt, and its upstream activator, phosphoinositide dependent kinase 1 (PDK1) promotes Akt activation [151]. Of importance is that low levels of SIRT1 expression negatively correlate with obesity and BMI in humans [152]. In obese mice, SIRT1 activation can also improve insulin sensitivity [153].

6 The Consequences of Altered Fatty Acid Oxidation in Obesity, Diabetes, and Heart Failure

6.1 Altered Insulin Signaling and Lipid Intermediate Accumulation

Increased myocardial uptake of fatty acids leads to accumulation of lipid metabolites, which can have a profound impact on insulin signaling and cardiac function. Diacylglycerol (DAG), and ceramides can activate kinases involved in the down-regulation of insulin action [154–158]. Activation of JNK-AP-1, IKK-NF-κB and PKC cascades by lipid intermediates has a negative feedback on insulin action, acting via serine phosphorylation of IRS-1 [159]. A negative relationship between the accumulation of intracellular lipids in skeletal muscle and insulin sensitivity has been reported in obesity in both humans [160] and rodents [161–163].

Long chain acyl CoA's (LC acyl CoA) are also potential mediators of insulin resistance [164], and elevated levels of LC acyl CoA have been associated with decreased glucose uptake in obese individuals [165]. Obesity promotes accumulation of LC acyl CoA in muscle, which is accompanied by insulin resistance. Studies have shown an inverse relationship between muscle LC acyl CoA content and insulin resistance [164, 166–170], although this relationship does not always hold [171].

Ceramide may also be a mediator of insulin resistance. Accumulation of ceramide occurs either by the hydrolysis of sphingomyelin [172] or by *de novo* synthesis from saturated fatty acids [173]. Ceramide decreases insulin-stimulated glucose uptake in skeletal muscle [174, 175], and inhibition of ceramide [176]. Interestingly, although cardiac ceramide content does not increase in obesity [4], the salutary effects of decreasing ceramide content in promoting insulin sensitivity in skeletal muscle may nonetheless be transferable to cardiac muscle. Ceramides inhibit insulin action via the inhibition of Akt phosphorylation [174, 175], while inhibition of ceramide synthesis restores phosphorylation of Akt in insulin-resistant myotubes [174].

DAG content is increased in muscle from insulin resistant rodents and humans [158, 177–179]. In human studies, accumulation of DAG in skeletal muscle of obese, and diabetic individuals is positively correlated with the increased activity of PKC-θ [180, 181], which can impair insulin signaling via serine phosphorylation of IRS-1 [158, 181].

Accumulation of lipids has been implicated as an important mediator of cardiac dysfunction [182]. The increased concentration of TAG in association with insulin

resistance is seen in hearts from obese humans and rodents, and genetically obese and type 2 diabetic rodents [176, 183–187]. Cardiac dysfunction in Zucker obese rats is positively correlated with the accumulation of myocardial TAG and ceramide [186]. Accumulation of ceramide in the rat heart following obesity has also been observed [188]. Cardiac overexpression of PPARγ in mice subjected to obesity also augments myocardial ceramide content [189], which is implicated in the development of insulin resistance and heart failure [190–192].

6.2 Incomplete Fatty Acid Oxidation in Obesity

Increases in fatty acid oxidation that exceed the ability of the mitochondria to metabolize its downstream products can lead to the failure of muscle to completely oxidize fatty acids, leading to the accumulation of acid soluble metabolites (markers of incomplete oxidation) [45, 80]. In contrast, increasing TCA cycle and ETC activity prevents incomplete fatty acid oxidation [80]. Indeed, increased fatty acid oxidation in the skeletal muscle and heart during high fat feeding contributes to the mismatch between β-oxidation and TCA cycle activity, leading to incomplete fatty acid oxidation [60, 193]. This is supported by the observation that intermediates of incomplete fatty acid oxidation accumulate in muscle from insulin-resistant animals, and that decreasing this accumulation can improve insulin sensitivity [45, 194].

In order for insulin resistance to develop, excess fatty acids have to enter the mitochondria [5], a concept supported by the observation that channeling excessive fats to storage in the form of TAG limits insulin resistance [183, 195]. Acylcarnitines, which are markers of incomplete oxidation, may contribute to the acylation and acetylation of mitochondrial proteins to alter their function [196]. We speculate that enhancing fatty acid oxidation does not increase insulin sensitivity. Rather, correcting the ‘mismatch’ between oxidation and TCA cycle activity by lowering β-oxidation may alleviate insulin resistance.

7 Conclusions

Heart failure associated with obesity and diabetes results in dramatic changes in cardiac energy metabolism. While overall mitochondrial energy production is decreased in the heart, fatty acid oxidation rates are markedly increased. This is associated with a decrease in glucose oxidation and a decrease in insulin stimulation of glucose metabolism. These metabolic changes are associated with a decrease in cardiac efficiency that can compromise cardiac function. As a result, inhibition of fatty acid oxidation in heart failure associated with obesity and diabetes has the potential to improve cardiac function and efficiency.

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Transcriptional Control of Mitochondrial Biogenesis and Maturation

Rick B. Vega, Teresa C. Leone, and Daniel P. Kelly

Abstract The mitochondrion is the main site for ATP production in the adult heart and comprises up to 40 % of the cardiac myocyte volume. It is now recognized that a complex network of nuclear transcription factors is essential for the coordinated regulation of mitochondrial biogenesis, maturation and function. These transcription factors guide developmental changes in mitochondrial number, structure, and dynamics as well as respond to various physiologic and pathophysiologic cues to meet the energetic needs of the adult heart. The peroxisome proliferator-activated receptor gamma coactivator-1 (PGC-1) orchestrates the actions of many of these transcription factors to maintain a high level of mitochondrial ATP production. There is increasing evidence that during the development of cardiac hypertrophy and in the failing heart, the activity of this network, including PGC-1, is altered. This review summarizes our current understanding of the perturbations in the gene regulatory pathways that occur during the development of heart failure. An appreciation of the role this regulatory circuitry serves in the regulation of cardiac energy metabolism may guide the development of novel therapeutic targets aimed at the metabolic disturbances that presage heart failure.

Keywords Mitochondria • PGC-1 • Transcription factors • Fatty acid oxidation • Heart failure

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1 Introduction: Nuclear Transcription Factors Controlling Cardiac Mitochondrial Biogenesis and Function

1.1 Transcriptional Control of the Mitochondrial Genome

The mitochondrial genome encodes 13 essential protein subunits of the electron transport chain (ETC) along with several rRNAs and tRNAs necessary for the translation of the mitochondrial-encoded transcripts. The human cardiac myocyte is estimated to contain approximately 7,000 mtDNA copies per diploid genome [1], reflective of the high mitochondrial density in the cell. Several nuclear-encoded factors act within the mitochondria to activate transcription of mitochondrial-encoded products. Factors such as the mitochondrial transcription factor A (Tfam), the mitochondrial transcription factor B2 (TFB2M) and others are critical for mtDNA transcription, organization, and maintenance (Reviewed in [2]).

1.2 Nuclear Respiratory Factors (NRFs)

The nuclear respiratory factors 1 and 2 (NRF-1 and NRF-2) are nuclear transcription factors that control many fundamental aspects of mitochondrial number and function. The importance of NRF-1 as a nuclear factor involved in mitochondrial biogenesis was first demonstrated by its regulation of cytochrome c gene expression [3]. Subsequently, it was shown that NRF-1 regulates expression of several proteins that act directly on regulators of the mitochondrial genome including Tfam and TFB2M [4, 5]; providing the first evidence for a direct connection between the transcriptional control of the nuclear and mitochondrial genomes. Gene deletion of NRF-1 results in embryonic lethality at E6.5 with diminished mtDNA levels and respiratory chain activity [6]. NRF-2, the human homolog of the murine GABP, is a polypeptide transcription factor containing an ETS-domain DNA subunit. NRF-1, together with NRF-2, regulates all ten nuclear-encoded cytochrome oxidase subunits [7, 8]. More recently, chromatin immunoprecipitation followed by deep sequencing (ChIP-seq) has confirmed NRF-1 binding sites in promoters of genes encoding components present in all electron transport chain complexes (Table 1) [9].

1.3 Nuclear Receptor Transcription Factors

A subset of the nuclear receptor superfamily exerts transcriptional control on cardiac mitochondrial fuel metabolism and respiratory function. Some of these nuclear receptors are ligand-activated and, as such, can respond directly to cellular signals and metabolite levels (substrate availability). One relevant family, the peroxisome proliferator-activated receptors (PPARs), was originally identified as regulators of

Table 1 Transcription factors important for cardiac mitochondrial energy production

Transcription Factor	Class	Gene targets	Phenotype
NRF-1	Nrf1 DNA-binding	• ETC components, all Cytochrome C subunits • mtDNA (*Tfam*, *Tfb2m*) • Transcription (*Mef2a*)	Embryonic lethality of KO at E6.5 with reduced mtDNA and ETC activity
NRF-2/GABP	ETS-domain	Similar to NRF-1	Embryonic lethality prior to implantation
PPARα	Nuclear receptor	• FA uptake (*Cd36*, *Slc27a1*, *Acsl1*) • FAO (*Cpt1b*, *Acadm*, *Hadha*, *Hadhb*)	• KO has reduced FAO rates • Cardiac transgene leads to increased FA uptake, storage and oxidation; recapitulates diabetic cardiomyopathy phenotype
PPARδ	Nuclear receptor	• FAO (*Cpt1b*, *Acadm*, *Hadha*, *Hadhb*) • Glucose (*Slc2a4*, *Ldhb*)	• KO has reduced FAO and glucose oxidation • Cardiac transgene has increased FAO and glucose oxidation, protected against lipotoxicity
ERRα	Nuclear receptor	• FAO (*Cpt1b*, *Acadm*, *Hadha*, *Hadhb*) • Glucose (*Slc2a1*, *Slc2a4*, *Pdk4*) • ETC (*Atp5b*, *Cycs*, *Cox6c*, *Ndufa4*) • Transcription (*Ppargc1a*, *Ppara*)	Cardiomyopathy in KO following pressure overload with phosphocreatine depletion and reduced ATP synthesis rate
ERRγ	Nuclear receptor	Similar to ERRα	Postnatal lethality in KO with inability to shift to oxidative metabolism in heart
YY1	GLI-Kruppel zinc finger	• Transcription (*Ppargc1a*) • ETC (*Cycs*)	KO displays defects in mitochondrial structure and energy production
c-Myc	Basic helix-loop-helix/leucine zipper	• Glycolysis (*Eno1*, *Ldha*) • Mito DNA (*Tfam*, *Polg*, *Polg2*)	Cardiac transgene results in mitochondrial biogenic and ETC defects leading to cardiomyopathy

peroxisomal fatty acid oxidation (FAO) enzymes [10]. The PPARs bind to cognate DNA elements as an obligate heterodimer with the retinoid X receptor (RXR). A variety of FA derivatives have been shown to serve as ligands for the PPARs, yet the endogenous ligands have not been fully delineated. All three isoforms, PPARα,

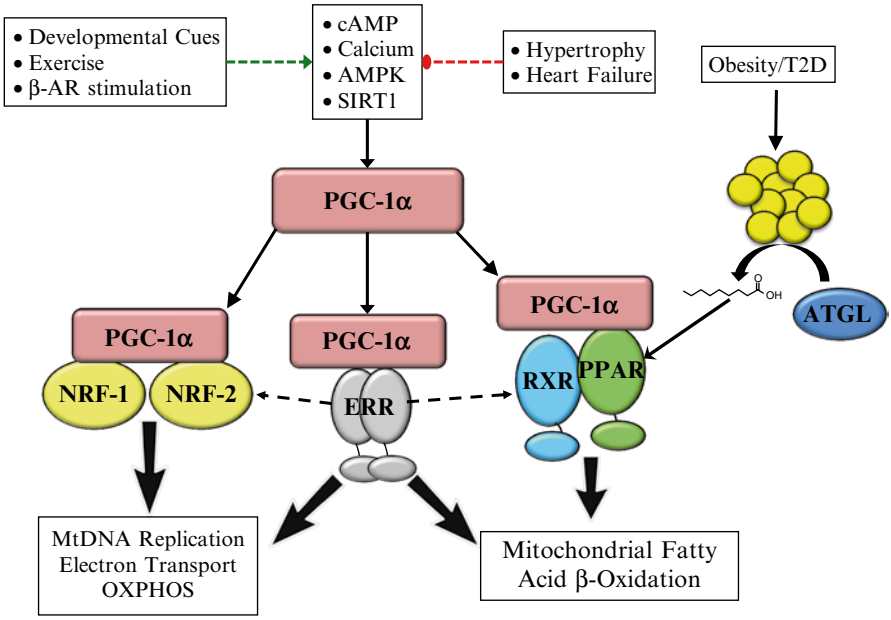

Fig. 1 The PGC-1 gene regulatory circuit controls cardiac mitochondrial function and fuel utilization capacity. PGC-1α integrates signals from diverse physiologic stimuli including developmental cues, exercise and β-adrenergic signaling. Coactivation of multiple downstream transcription factors drives the expression of proteins involved in virtually all aspects of mitochondrial energy production, regulating capacity for ATP production. Activity of PGC-1α and this circuit is inhibited during cardiac hypertrophy and heart failure. PGC-1α, PPARγ coactivator-1 alpha; PPARα, peroxisome proliferator-activated receptor alpha; RXR, retinoid X receptor; ERR, estrogen-related receptor; NRF, nuclear respiratory factor; OXPHOS, oxidative phosphorylation; ATGL, adipose triglyceride lipase; AMPK, AMP-activated protein kinase

δ(β), and γ are expressed in the cardiac myocyte with overlapping but distinct functions, with PPARα and PPARδ exhibiting the highest level of expression. PPARα target genes comprise many proteins and enzymes involved in FA uptake and mitochondrial FAO (Table 1). Insight into the function of PPARα in heart has been gained by loss- and gain-of-function mouse models. Mice lacking PPARα have reduced cardiac FAO rates [11–13] while overexpression of PPARα in the heart leads to increased uptake, storage and oxidation of fatty acids [14]. As discussed further below, the metabolic phenotype of mice overexpressing PPARα in heart recapitulates many aspects of the insulin resistant diabetic heart. Interestingly, recent evidence suggests that PPARα-activating ligands are released from intracellular triglyceride stores (Fig. 1). Specifically, adipose triglyceride lipase (ATGL) was shown to be necessary for the generation of PPARα ligands in the cardiac myocyte; ATGL knockout mice display profoundly reduced expression of PPARα target genes involved in FAO [15]. These new results also suggest that lipolysis of intracellular triglyceride stores is an important source of long-chain fatty acids for mitochondrial FAO [16]. These collective results suggest a metabolic control

mechanism whereby PPARα senses substrate availability to regulate capacity for mitochondrial FAO in heart.

PPARδ also drives high rates of mitochondrial FAO in the cardiac myocyte as its deletion reduces FAO capacity and leads to cardiac dysfunction [17]. However, unlike PPARα, cardiac overexpression of PPARδ does not lead to lipid accumulation and associated lipotoxicity. In fact, PPARδ appears to protect against myocyte lipid accumulation on a high-fat diet and cardiac dysfunction caused by pressure overload [18, 19]. In addition, PPARδ activates glucose uptake and oxidation at least in part due to its regulation of the insulin-responsive glucose transporter, Glut4 [17, 18]. Thus, PPARδ appears to drive a more balanced energy substrate utilization pattern. Cardiac-specific deletion of PPARδ also leads to a reduction of mitochondrial FAO and glucose oxidation rates resulting from a decrease in expression of enzymes in both pathways [17]. Therefore, PPARα and PPARδ share many similar targets, however these 2 nuclear receptors drive distinct metabolic pathways in the cardiac myocyte.

The estrogen-related receptors (ERRs) form a second group of nuclear receptors critical for maintaining mitochondrial function in the cardiac myocyte. All three ERR isoforms (ERRα, ERRβ, and ERRγ) are expressed in the heart and are known as "orphan" nuclear receptors due to the lack of a known ligand. ERR target genes overlap with that of the PPARs but also regulate genes involved in virtually all aspects of mitochondrial energy production including the TCA cycle, respiratory chain and oxidative phosphorylation (Table 1) [20]. Loss-of-function studies in mice have revealed roles for the ERRs in the heart. ERRα knockout mice display cardiac dysfunction when subjected to pressure-overload related to reduced capacity for maintaining phosphocreatine stores and mitochondrial ATP synthesis rates in response to energetic stress [21]. Likewise, gene deletion of ERRγ results in cardiomyopathy and death immediately following birth [22] related to the loss of the normal postnatal shift to oxidative metabolism and fatty acid utilization. As such, ERRs are required for the establishment and maintenance of cardiac energy production.

1.4 Other Nuclear Transcription Factors

Additional transcription factors have also been shown to regulate mitochondrial pathways and function in heart. A computational approach identified Yin Yang 1 (YY1) binding sites in the promoter regions of many mitochondrial genes regulated by the nutrient sensor mammalian target of rapamycin, mTOR (Table 1) [23]. YY1 deletion in skeletal muscle results in profound defects in mitochondrial structure and energy production [24]. The oncoprotein c-Myc has also been shown to activate expression of genes involved in mitochondrial biogenesis [25, 26]. Although expressed at very low levels in the normal heart, c-Myc is induced by a variety of pathologic stimuli including pressure overload [27]. The major effects of c-Myc expression in the heart are the activation of glucose utilization and downregulation of FAO (Table 1) [28]. Forced expression of c-Myc in the heart also leads to abnormal mitochondrial

biogenesis with respiratory change defects leading to cardiac dysfunction [29]. These data are consistent with a role of c-Myc in cardiac myocyte cell-cycle re-entry and activation of the fetal gene program during pathologic hypertrophy.

2 The PPARγ Coactivator 1 (PGC-1) Transcriptional Coregulators: Transducers of Physiological Cues to the Control of Cardiac Mitochondrial Biogenesis and Function

A huge breakthrough in our understanding of how mitochondrial biogenesis and function is regulated in accordance with energy demands came with the discovery of the PGC-1 coregulators. PGC-1α was first discovered in brown adipocytes as an activator of PPARγ [30]. The closely related PGC-1β [31, 32] and more distant relative, PGC-1 related coactivator (PRC) [33] comprise the other members of the family. The PGC-1 coactivators are characterized by an LXXLL motif that mediates interaction with nuclear receptors, an RNA recognition motif (RRM), and a host cell factor-1 (HCF) binding domain. PGC-1α and PGC-1β are expressed in tissues with a high oxidative capacity including the heart. Remarkably, PGC-1α is a highly inducible factor that responds to stimuli such as cold exposure and exercise [34–36]. PGC-1α expression in the heart increases during cardiac development and coincident with the large mitochondrial biogenic response that occurs just before birth [37]. Overexpression of PGC-1α in the cardiac myocyte leads to a robust mitochondrial biogenic response and increased expression of nuclear-encoded mitochondrial genes involved in multiple energy production pathways [37]. This function is orchestrated by PGC-1's interaction with and activation of PPARα [38], PPARβ/δ [39], ERRα and ERRγ [40–42]. In this manner, PGC-1α integrates multiple physiologic and developmental cues to regulate most aspects of mitochondrial function and energy production (illustrated in Fig. 1).

The importance of PGC-1 coactivators in the control of mitochondrial number and function is reinforced by loss-of-function studies in mice. Mice with loss of either PGC-1α [43, 44] or PGC-1β [45, 46] are viable and fertile with no obvious defect in cardiac energy metabolism or mitochondrial density. In the heart, pressure-overload induced hypertrophy results in accelerated heart failure in PGC-1α knockout mice [47]. A similar phenotype is also observed in PGC-1β knockouts subjected to pressure-overload hypertrophy [48]. In contrast to the single knockouts, deletion of both PGC-1α and PGC-1β results in perinatal lethality within 24 h of birth due to an arrest in mitochondrial biogenesis leading to heart failure [49]. This work was key to defining the important role played by PGC-1α/β in the perinatal cardiac mitochondrial biogenic response. In addition to its role in mitochondrial biogenesis during cardiac development, recent studies indicate that PGC-1α and PGC-1β are necessary for mitochondrial maturation including regulation of the mitochondrial fusion genes mitofusion 1 (Mfn1) and mitofusin 2 (Mfn2) among others during postnatal cardiac development [50, 51]. The regulation of Mfn1 and Mfn2 are at least in part, driven

by PGC-1 coactivation of ERRα [50, 52]. Interestingly, inhibition of mitochondrial fusion blocks cardiac myocyte differentiation [53], providing a potential link between PGC-1, mitochondrial dynamics, and cardiac development.

3 Control of Mitochondrial Function by Cellular and Metabolic Signaling

3.1 PGC-1 Responds to Cellular Signals

The PGC-1 coactivators are dynamically regulated at both transcriptional and post-transcriptional levels to integrate a variety of physiologic and pathophysiologic cues to match energy production with cardiac energy demands. Consistent with this role, multiple cellular signaling pathways converge on the PGC-1α gene including calcineurin, calmodulin-dependent kinase (CaMK), cAMP signaling, and AMP-activated protein kinase (AMPK) [54–57]. The activation of PGC-1α expression by cAMP downstream of adrenergic stimulation is mediated through direct regulation by the cAMP response element binding protein (CREB) [56].

PGC-1 activity is also modulated by post-translational modifications including phosphorylation and acetylation. AMPK has been shown to directly phosphorylate and activate PGC-1 [58]. AMPK is an energy sensing kinase that is activated by energy depletion, i.e. higher AMP/ATP ratios. Accordingly, phosphorylation of PGC-1 by AMPK provides a mechanism to boost mitochondrial energy production upon increased physiologic demands such as exercise. In addition to AMPK, the nicotinamide adenine nucleotide (NAD^+)-dependent deacetylase, Sirtuin 1 (SIRT1), is another metabolic sensor that regulates PGC-1 activity. AMPK and SIRT1 act cooperatively to activate PGC-1α activity providing a compelling link between mitochondrial biogenesis and metabolic signaling pathways sensing changes in cellular energy status [59, 60]. The effect of PGC-1 acetylation is supported by the observation that PGC-1 acetylation status is increased in muscle in mice fed a high-fat diet [61].

4 Dysregulation of Transcriptional Networks Controlling Mitochondrial Function Relevant to Heart Disease

4.1 Deactivation of Transcriptional Circuits in the Hypertrophied and Failing Heart

Similar to the expression of many structural and contractile proteins, there is a switch to the “fetal gene program” evident in metabolic enzyme gene expression during the development of cardiac hypertrophy and failure, including a decrease in

the expression of genes encoding mitochondrial FAO enzymes [62]. There is significant evidence that this fetal metabolic switch in the hypertrophied and failing heart involves alterations in the expression and activity of the transcription factors controlling mitochondrial function and biogenesis. PPARα expression is decreased in human and animal models of pressure overload-induced cardiac hypertrophy and in heart failure [62–65]. Evidence also exists that during cardiac hypertrophy PPARα, in addition to decreased expression, is regulated at the post-transcriptional level by phosphorylation via p38 to impair activity [66]. This is in agreement with the observed metabolic shift from FAO to glucose metabolism that occurs during the transition to cardiac hypertrophy and heart failure [67–69]. ERRα expression has also been shown to be decreased in animal models and human heart failure samples [70–72]. Coordinated repression of both PPARα and ERRα could represent major determinants in the regulation of mitochondrial energy production in the failing heart. As ERRα can directly regulate PPARα [73], it is tempting to speculate that repression of ERRα establishes this vicious cycle.

Given its role as a "master regulator" of mitochondrial biogenesis and function, a logical question is whether dysregulation of PGC-1 is involved in the pathogenesis of the "energy-starved" phenotype of the failing heart. Indeed, changes in fuel selection and mitochondrial function in the hypertrophied and failing heart are likely consequences of dysregulation of the PGC-1 circuit. Consistent with this notion, the expression of PGC-1α has been found to be downregulated in both animal models and human heart failure samples [62, 70, 74]. Similar findings have been reported for PGC-1β [48]. It should be noted however, that not all studies have shown a downregulation in PGC-1 in human heart failure samples [71, 72]. These differences likely reflect different etiologies, timepoints, or other variables that could influence PGC-1 expression secondarily. In addition, the role of altered PGC-1 signaling as an early (causal?) versus late event in the metabolic remodeling of heart failure remains to be determined.

4.2 Dysregulation of Transcriptional Circuits in the Diabetic Heart

The metabolic derangements that occur in the obese, insulin resistant, and diabetic patient appear to be quite distinct from that of hypertensive or ischemic heart disease. Insulin-resistant and diabetic hearts exhibit reduced capacity for glucose utilization, higher rates of mitochondrial FAO. Increased cardiac fatty acid uptake and β-oxidation have been observed in experimental models as well as obese and diabetic patients [75–78]. Interestingly, upregulation of cardiac mitochondrial FAO enzyme expression has been observed in a number of diabetic animal models consistent with an activation of FAO rates [79–81]. There is also increasing evidence that impaired glucose tolerance, even before the onset of diabetes, is associated with cardiac steatosis [82]. In the obese and diabetic patient population, myocardial lipid

accumulation has been shown by some to be associated with diastolic and, in some studies, systolic ventricular dysfunction [78, 83, 84]. Collectively, this metabolic inflexibility in the setting of neutral lipid accumulation and resulting cardiac dysfunction has been termed cardiac "lipotoxicity" [85].

The high rates of mitochondrial FAO observed in both experimental models and human patients indicate activation of transcriptional networks controlling these pathways. Indeed, mice that overexpress PPARα exclusively in the heart (MHC-PPARα) exhibit increased FAO associated with a decrease in glucose utilization resulting in an accumulation of triglyceride and a diabetic-like phenotype [14, 86, 87]. These animals exhibit left ventricular hypertrophy and cardiac dysfunction that can be inhibited by deletion of CD36 or the LDL receptor, thus depriving PPARα of activating ligands [88, 89]. Furthermore, the activity of the PGC-1/PPARα circuit is increased in the insulin resistant mouse heart [90]. However, during transition to diabetes in mouse models the activity of PGC-1α appears to fall leading to a proposed vicious cycle of mitochondrial dysfunction and myocyte lipotoxicity [91]. The relevance of this latter observation to humans remains to be determined.

5 Conclusions

The healthy heart has an amazing capacity to generate ATP and shift fuel utilization preferences in response to pathophysiologic stimuli to meet its energy needs. Much of this control occurs at the level of gene regulation through a complex circuit involving the PGC-1 coactivators and nuclear receptors. This circuit becomes constrained during the development of heart failure. The unmet needs in the heart failure arena suggest a prime opportunity to develop therapeutics that modulate mitochondrial function and fuel metabolism by targeting this circuitry. In the long-term, such therapies could, perhaps, be tailored to the etiology of heart failure and the accompanying metabolic derangements.

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Relationship Between Substrate Metabolism and Cardiac Efficiency

Ellen Aasum

Abstract The heart requires large amounts of energy to sustain its continuous pumping activity, and is highly dependent on an optimal energy substrate metabolism with efficient ATP generation and utilization. As ATP is primarily derived from mitochondrial oxidative phosphorylation, myocardial oxygen consumption (MVO_2) can be used to measure the rate of energy expenditure of the heart. Although cardiac efficiency is an ambiguous term, it commonly embraces the relationship between MVO_2 and cardiac work. There is close coupling between myocardial oxygen consumption and the factors that determine cardiac work such as wall stress (influenced by pre- and after-load), heart rate and contractile state. Substrate metabolism may also affect myocardial oxygen consumption, and has through this been considered to contribute to the pathogenesis of cardiac dysfunction in several forms of heart disease. Part of the beneficial effect of metabolic therapies may also relate to improvement of cardiac efficiency. The focus in this chapter will therefore be on our current understanding of how myocardial substrate supply and/or utilization contribute to altered cardiac efficiency.

Keywords Myocardial oxygen consumption • Mechanical efficiency • Mechano-energetics • Contractile efficiency • Mitochondrial uncoupling • Fatty acids • Oxidation • PVA • Excitation-contraction coupling • Basal metabolism

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1 Introduction

The requirement to continuously pump blood makes the heart the most active tissue in the body. Because of its high energy demand and relatively low content of high energy compounds (ATP and creatine phosphate) the heart must generate ATP at a high rate, illustrated by the fact that the human heart generates and consumes more than 15 times its own weight of ATP daily. Accordingly cardiac myocytes are the cells with high volume density of mitochondria (approximately 30 %), and the majority (>95 %) of its ATP is derived from oxidative phosphorylation. Myocardial oxygen consumption (MVO_2) is thus an appropriate measure of myocardial energy consumption. This concept was appreciated by Yeo in 1885 who advocated a relationship between oxygen consumption and mechanical activity in the dog heart [1]. Later, Starling (1917) associated the mechanical performance of the isolated mammalian heart to its metabolism [2]. Since then, a range of studies have demonstrated that the rate of myocardial respiration is linearly related to the level of its work, demonstrating the heart's ability to adjust energy production to its consumption.

The large energy requirement makes the heart highly dependent on an optimal substrate metabolism and oxygen supply, with efficient ATP generation and utilization. The term efficiency is colloquially used in the sense of ability or capability, and is generally defined as the ratio of the energy delivered by a system/machine to the energy utilized by it. Using this concept, mechanical efficiency of the heart was defined by Bing *et al*. in 1949, as the ratio between stroke work and MVO_2 [3]. Mechanical efficiency has since then been the most used measure of cardiac efficiency, and has been evaluated invasively during cardiac catheterization and non-invasively mainly using positron emission tomography. Mechanical efficiency is also commonly measured in isolated heart and cardiac tissue preparations. Impaired mechanical efficiency has been reported in various cardiovascular diseases such as acute ischemic heart disease, dilated cardiomyopathy, the hyperthyroid heart and diabetic cardiomyopathy. The relationship between oxygen consumption and cardiac work is a cornerstone in cardiac patient handling, and changes in mechanical efficiency have also been used for evaluating of the outcome of therapies.

2 Energy Conversion in the Cardiac Pump

As for any mechanical pump, only parts of the energy invested in the heart is converted to external work. Figure 1A illustrates the energy flow in the heart, where chemical energy is converted into external work. It emphasizes that at each level, part of the energy is dissipated as heat. Hence, each step will co-determine the overall mechanical efficiency of the heart. This figure also stresses that the heart uses energy for both mechanical and non-mechanical processes. The latter includes processes associated with basal metabolism which includes the oxygen used to maintain

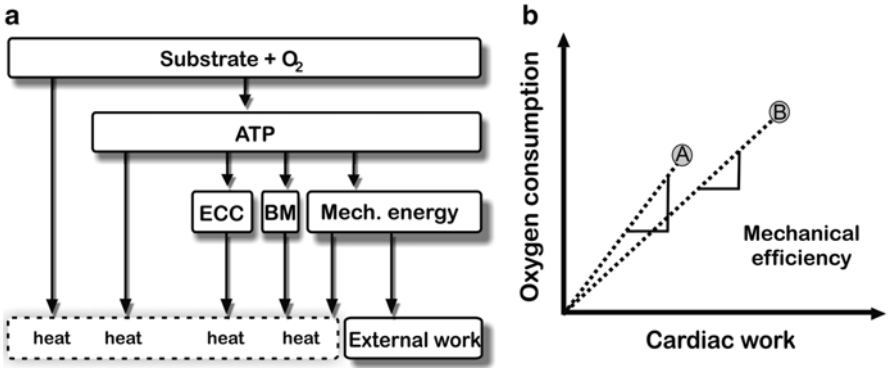

Fig. 1 *Panel A* shows an energy flow diagram of the heart as a mechanochemical transducer that converts chemical energy in myocardial substrates via oxidative phosphorylation into heat and external work. In the heart, ATP is used for non-mechanical activity (excitation-contraction coupling, ECC and basal metabolism, BM) and for generating mechanical energy. The mechanical energy (Mech. energy) includes generation of myocardial tension in the ventricular wall and pressure in the left ventricle for ejection of blood against an afterload pressure. This diagram illustrates that at each level, part of the energy is dissipated into heat and thus each step codetermines the overall mechanical efficiency. *Panel B* shows the increasing oxygen consumption in a heart when cardiac work is increased from *A* to *B*. As mechanical efficiency is defined as the ratio between these two parameters it is clear that this measure of cardiac efficiency is higher when cardiac work increases. A measure of efficiency based on single point assessment (i.e. only at one workload) it is therefore associated with limitations as it does not consider all determinants of efficiency

the mitochondrial membrane potential, for generating the ATP required for protein synthesis and for maintenance of transmembrane ion gradients, as well as, the oxygen used for non-mitochondrial purposes [4]. Basal metabolism is high in cardiac muscle as compared to other muscles in the body, and in perfused hearts, is generally measured following electrical arrest [4]. When the heart beats and the muscle contracts, its oxygen consumption immediately increases. This increase includes the energetic cost for activating the muscle; raised intracellular Ca^{2+} levels couple the excitation and contraction (i.e. E-C coupling). The energy cost associated with E-C coupling is primarily due to activity of the Ca^{2+} handling proteins and will thus increase proportionally with increases in myocardial calcium transients such as following β-adrenergic stimulation or an elevated extracellular Ca^{2+} concentration [5]. Together with basal metabolism, E-C coupling represents the non-mechanical processes within the heart as they do not generate any tension.

In addition to the energy for activation, the beating heart also uses energy for generating tension/force by the contractile machinery (i.e. crossbridge cycling). The mechanical energy generated during cross-bridge cycling is influenced by myocardial wall stress (altered by pre- and after-load) and wall thickness. Part of this energy will be forwarded as external mechanical work (stroke work (SW)/forward work), while the rest remains as energy within the myocardium. Under normal conditions, SW generally accounts for 20–25 % of total oxygen consumption (i.e. the mechanical

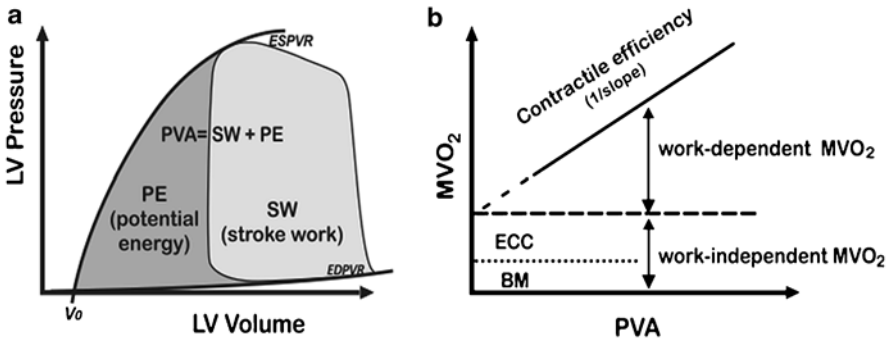

Fig. 2 *Panel A* shows ventricular mechanics and energetics in the pressure-volume (PV) framework. The area of the steady-state intra-ventricular PV loop is equivalent with the stroke work (SW), and the *triangular area* delimited by the end-systolic and end diastolic PV relationships (ESPVR and EDPVR, respectively) and the descending limb of the PV loop, defines the potential energy (PE) within the myocardium. The sum of SW and PE defines the PV area (PVA), and is equivalent to the total mechanical work performed by the heart during one cardiac cycle. *Panel B* shows the linear relationship between PVA and MVO_2, obtained by altering the loading conditions while maintaining the same contractile state. The *y*-intercept of the relationship defines the work-independent MVO_2, (which include oxygen cost for processes associated with basal metabolism (BM) and E-C coupling (ECC)). The inverse of the slope indicates the efficiency of oxygen to PVA defined as the contractile efficiency [5]

efficiency of the heart) [5]. As mechanical efficiency is the ratio between a measure of cardiac work (such as SW) and MVO_2 it is important to note that this measure of efficiency is highly dependent upon the loading conditions (Fig. 1B).

A number of indexes aimed to describe total mechanical energy generated by the left ventricle have been established. In addition to metabolic models (direct heat measurements) and mathematical models, there are a range of hemodynamic models. In 1979, Suga introduced the "pressure-volume area" (PVA) as an index for total mechanical energy generated during one cardiac cycle [5] (Fig. 2). The linear relationship between PVA and MVO_2 (per beat) describes the oxygen cost of mechanical energy (i.e. the work-dependent MVO_2). The inverse of this slope was, by Suga, defined as contractile efficiency [5], and represents the efficiency of chemo-mechanical energy transduction—the combined efficiency of mitochondrial oxidative phosphorylation and of cross-bridge cycling. The y-intercept of the relationship reflects the work-independent MVO_2 (or unloaded MVO_2) where PVA is negligible. This measure therefore corresponds to the oxygen cost for non-mechanical processes (basal metabolism and E-C coupling). The use of this framework under various experimental physiological or pathophysiological conditions has proved useful since it allows a reductionist approach to study the mechanoenergetic properties of the left ventricle, independent of load and heart rate, and it has by that expanded our understanding of the underlying mechanisms leading to altered cardiac efficiency.

3 Cardiac Efficiency and Altered Substrate Supply and Utilization

Cardiac metabolism and contraction are fundamentally integrated, thus without adequate fuel supply and/or utilization the heart is unable to meet its circulatory demands. To meet the high energetic demand, the cardiac metabolic network is a versatile system capable of metabolizing all carbon substrates for energy production. In the fasted state the heart obtains approximately 60–70 % of its energy from circulating triacylglycerol and non-esterified fatty acids [6]. When glucose and insulin increase (the fed state) myocardial fatty acid utilization falls and that of glucose increases, while lactate or ketone bodies become more prominent substrates during high intensity exercise or prolonged fasting [7]. This illustrates an important feature of the normal heart; a metabolic flexibility and ability to meet continuous energy demands through rapid changes in fuel utilization according to the substrate and hormonal environment. In various pathological cardiovascular states (acute ischemic heart disease, dilated cardiomyopathy, and diabetic cardiomyopathy) this metabolic regulation is perturbed and loss of metabolic flexibility and/or metabolic maladaptation is believed to play an important role in the development of cardiac dysfunction. These conditions are also associated with mechanical inefficiency, and there is a growing consensus around a causal link between altered metabolic substrate metabolism and mechanical inefficiency.

In support of this, decreased mechanical efficiency following an elevation of the fatty acid supply to the normal heart was first reported more than 40 years ago in the isolated rat heart [8] and in an *in situ* dog model [9]. Mjøs reported that infusion of heparin and a triacylglycerol emulsion resulted in ~30 % increase in MVO_2 without affecting left ventricle mechanical work in the dog heart [9]. Later, mechanoenergetic studies, using the PVA–MVO_2 framework confirmed these findings, and also demonstrated that elevated fatty acid levels increased the energy expenditure for non-mechanical processes without altering the contractile efficiency [10–12]. Although there are several mechanisms that may link increased levels of fatty acids to decreased cardiac efficiency, the accompanying increase in myocardial fatty acid oxidation rate is the factor most commonly referred to. This is based on the lower P/O ratio (the number of ATP molecules produced per atom of oxygen in the electron transport chain) when utilizing fat compared to glucose. Fatty acids are therefore regarded as a less "oxygen-efficient" energy substrate, as exclusive myocardial fatty acid oxidation as compared to carbohydrate, will result in 11–12 % higher MVO_2. Although increased fatty acid supply to the heart will change the substrate oxidation in favour of fat, this shift generally occurs within a mixture of carbohydrate and fat, and cannot solely explain the 30 % (or more) increase in the MVO_2. This is supported by data showing that high fatty acid concentration and not oxidation rate, is associated with increased oxygen consumption in the isolated heart [13], as well as by observations that marked switches in myocardial substrate oxidation are not necessarily associated with altered MVO_2 or cardiac efficiency [14, 15]. It is still not clear which mechanisms contribute to the oxygen wasting effects of an acute elevation of fatty acids under these conditions.

After a transient period of ischemia, when oxygen delivery has been restored, oxidative metabolism is more or less restored despite reversible contractile dysfunction. Accordingly, the stunned myocardium shows decreased mechanical efficiency [16], and load-independent evaluation in this state has demonstrated a decrease in contractile efficiency, suggesting inefficient chemo-mechanical energy transduction [17, 18]. Under these conditions fatty acids are the main source of residual oxidative metabolism [19, 20], and circulating levels of fatty acids are elevated, due to increased lipolysis in adipose tissue in response to a rapid increase in catecholamine discharge [21]. Elevated fatty acid levels are known to exaggerate ischemic injury [7, 22–24], and fatty acid-induced oxygen wastage is generally considered to be the cause. Accordingly elevated fatty acids have been reported to decrease mechanical efficiency both during mild ischemia [20] and following reperfusion [23]. Several mechanisms has been suggested contribute to this. In hypoxic cardiomyocytes challenged with fatty acids, an increase in ATP consumption due to increased release and re-esterification of fatty acids from and to the intracellular triglyceride pool, has been reported [25]. It should be noted however, that this futile cycle has also been linked to protection from lipid accumulation in the heart [26]. Thus, more research is required to better understand the role of this process and to what extent it increases MVO_2.

Since fatty acids inhibit glucose oxidation more than glycolysis, the resulting increase in lactate and H^+ production will increase the energy expenditure required to maintain ion homeostasis (i.e. futile cycling of ions) [23]. Although this does not seem to affect mechanical efficiency under aerobic conditions [20], improved mechanical efficiency following ischemia has been reported following improved metabolic coupling between glycolysis and glucose oxidation [23]. Several studies have confirmed that metabolic interventions can improve efficiency following reperfusion, although it is not clear whether these effects are related to a metabolic switch towards a more "oxygen efficient" substrate (glucose), less energy used for futile cycling, or the lipid-lowering effect often associated with these interventions [17, 27, 28].

Increased fatty acid oxidation and decreased mechanical efficiency also coexist in the diabetic heart, as demonstrated both in human [29] and animal [30–32] studies. This inefficiency has been shown to be due to increased oxygen cost for non-mechanical processes [31, 33, 34], and impaired efficiency seems to precede the development of mechanical dysfunction [29, 31, 35]. Although the obesity/diabetes-related increase in myocardial reliance on fatty acids as energy substrate induces a minor increase in MVO_2, a marked reduction of MVO_2 has been observed following interventions leading to a switch towards glucose oxidation [13, 31, 36–38]. On the other hand, decreased mechanical efficiency was not ameliorated in hearts from high-fat fed mice with cardiac-specific overexpressing GLUT1, despite showing increased myocardial glucose oxidation [39]. Thus, the significance of fat as a less "oxygen-efficient" energy substrate for this inefficiency is not fully understood. It should be noted, however, that acute elevation of fatty acid levels (despite an increased myocardial fatty acid oxidation rate), does not markedly increase MVO_2 in hearts from type 2 diabetic *db*/*db* mice [13, 33], which suggests that adaptive

mechanisms occur such that these hearts are better capable of handling an acute high-fat load.

Despite a decreased mechanical efficiency following hypertrophy [40, 41] and dilated cardiomyopathy [42, 43], there is a lack of consistency in reports related to the metabolic remodeling that occurs in human failing hearts. Although experimental studies have reported a progressive downregulation of fatty acid oxidation, it is not established whether this represents an adaptive response or is maladaptive in that it actively contributes to the development of contractile failure. A compensatory increase in anaplerosis to maintain tricarboxylic acid cycle flux can also lead to less efficient mode of carbon use for fueling energy [44]. Increased contractile efficiency has been reported in models of heart failure [45, 46], which in rodents has been shown to reflect a shift in the myosin heavy chain α isoform to the slower but more economical β isoform [46]. A high adrenergic state and insulin resistance accompanying heart failure, can lead to elevated circulating fatty acid and/or ketone levels [47, 48], which in infarcted rat hearts decreased mechanical efficiency [49]. Although experimental and pre-clinical studies have indicated that metabolic modulators might prove therapeutically advantageous, there are few and mixed results related to the effect on mechanical efficiency [43, 50–52]. This is most likely due to the different etiologies, progressive states, and diversity of the metabolic status in this group [47]. In addition, due to the decreased mechanical function load-independent evaluation of efficiency would contribute in a better understanding of the beneficial effect of these interventions.

4 Cardiac Efficiency and Mitochondria Function, ROS and Calcium Handling

Increased mitochondrial and extra-mitochondrial reactive oxygen species (ROS) generation, and impaired redox balance are regarded to be important players in the pathogenesis of cardiac dysfunction in ischemic injury, diabetic cardiomyopathy, hypertrophy and in the failing myocardium. Oxidative stress and elevated fatty acids can decrease cardiac efficiency by inducing an uncoupling between mitochondrial ATP synthesis and oxidation, due to a proton leak across the mitochondrial inner membrane believed to be mediated by uncoupling proteins (UCP), the adenine nucleotide translocase (ANT) and the matrix enzyme mitochondrial thioesterase I (MTE-I) [53, 54]. Increased uncoupling may be related to increased transcription (regulated by fatty acids-induced PPAR activation) [49, 55] and/or activation (by fatty acids, ROS and lipid peroxidation products) of these proteins [56], and their individual role under different pathophysiological conditions is clearly complex [57]. In addition, as this uncoupling, by lowering the protonmotive force, can also have a pivotal role in the regulation of mitochondrial function and cell survival, further research is required to better elucidate the precise physiological role and/or the pathophysiological consequence of mitochondrial uncoupling in the heart, and to what extent these processes contribute to altered cardiac efficiency.

The increased MVO_2 which occurs following an elevation in fatty acid supply has been associated with an increase in the oxygen cost for processes affiliated with E-C coupling [11, 16, 34]. Although an adverse effect of fatty acids on Ca^{2+} handling has been reported in isolated cardiomyocytes [58], the whole heart seems to functionally tolerate high fat supply under aerobic conditions [9, 12, 20, 54], and the underlying mechanisms related to the fatty acid induced changes in the energy cost of Ca^{2+} handling are not known. Increased O_2 cost for processes associated with Ca^{2+} handling have also been reported under pathophysiological conditions such as stunned myocardium [59, 60], and in models of hypertrophy [61], heart failure [62] and type 2 diabetes [11, 31, 34, 63]. This high energy expenditure may be related to an increased Ca^{2+} leak from the sarcoplasmic reticulum (SR) [64, 65]. In addition, as SR Ca^{2+}-ATPase (SERCA) is not only the major energy consumer, but also represents the most energetically efficient Ca^{2+} transport mechanism, less efficient compensatory Ca^{2+} transport due to reduced SERCA expression/activity, can also result in decreased cardiac efficiency [66, 67]. Accordingly SERCA2a gene transfer therapy has been shown to reduce oxygen cost for Ca^{2+} handling both in type 2 diabetic and failing aortic-banded rat hearts [66, 68], although the energetic consequence of such interventions has been questioned [69].

Impaired SR Ca^{2+} handling has also been linked to reduce mitochondrial Ca^{2+} uptake. This may hamper activation of the Krebs cycle, as well as alter the redox balance and the mitochondrial antioxidant capacity in cardiomyocytes [70]. Impaired Ca^{2+} handling may thus both directly and indirectly (via mitochondrial uncoupling) increase myocardial oxygen consumption. It should be noted, however, that acute elevation of the fatty acid supply did not increase the oxygen cost of E-C coupling in diabetic hearts [13], and there is also evidence to suggest that fatty acids are vital for the function of myocytes from these hearts [58, 71]. This highlights the point that the oxygen wasting effect of acute and long-term elevation of fatty acids levels are not necessarily via the same mechanisms.

5 Cardiac Efficiency and Energy Depletion

An imbalance between energy demand and availability, whether related to restricted substrate uptake, breakdown or entry into the Krebs cycle, impaired mitochondrial oxidative capacity and/or efficiency, or to increased energy utilization for non-contractile proposes, can ultimately lead to an energetically compromised heart with reduced working capacity. In support of this notion, decreased myocardial energetics, measured as a reduced PCr:ATP ratio, has been shown to correlate with the severity of heart failure in patients with idiopathic dilated cardiomyopathy [72, 73], and is also reported in hearts from obese and/or type 2 diabetic subjects [74, 75]. Although recent experimental studies have begun to reveal the relationship between cardiac function, high energy phosphates and efficiency [14, 15, 39], future studies are needed to elucidate the causal-relationship between inefficiency and decreased energetics in different pathophysiological conditions, as well as specifically address

the interplay between substrate metabolism and Ca^{2+} homeostasis, redox regulation and oxidative stress, in order to provide a more integrative picture of metabolic and contractile dysfunction.

6 Conclusion

Impaired efficiency is a sensitive marker of myocardial pathology. Cardiac inefficiency may not only represent a final common pathway, but may also play a causal role in the development of energetically compromised hearts with reduced working capacity and increased susceptibility to ischemia/hypoxia. While the current treatments of heart failure (ACE inhibitors, cardiac β-blockers and resynchronization therapy) aim to decrease energy demand, future strategies could focus on re-establishing the energetic balance by improving efficiency of cardiac energy production and/or ameliorating processes which adversely lead to mechanoenergetic uncoupling. Given the diversity of changes in myocardial substrate supply and utilization under both physiological and pathophysiological conditions, a better understanding of how energy substrate metabolism affects cardiac efficiency is therefore warranted.

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Acetylation in the Control of Mitochondrial Metabolism and Integrity

Michael N. Sack

Abstract Acetylation, as a post-translational modification is increasingly recognized as a nutrient-level dependent modulatory event in the control of cellular function. Non-enzymatic and enzymatic mechanisms appear to function in facilitating this modification of protein lysine residues. The functional role of protein acetylation was originally linked to lifespan regulation in simple organisms under the control of sirtuin deacetylase enzymes. In higher organisms this regulatory system is evolved to modulate in diverse cellular functions and is operational in multiple subcellular compartments. In this chapter the role of acetylation and its regulatory control is explored in the context of the control of mitochondrial integrity and metabolic functioning. Moreover, the concept that protein acetylation may function as a nutrient sensor to 'fine-tune' mitochondrial function as an underpinning of cardiac pathology will be explored.

Keywords Mitochondrial metabolism • Protein acetylation • Sirt1 • Sirt3 • Acetyl-CoA

1 Introduction

Type 2 diabetes mellitus (T2DM) and obesity are the fasting growing risk factors that adversely affect cardiovascular disease incidence and outcomes [1, 2]. The role of these nutrient-overload associated diseases with respect to global mitochondrial function, lipotoxicity and their effects on myocardial function have been extensively explored [3–5]. However, in recent years it has been recognized that

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metabolic intermediates of substrate metabolism per se can play a role in the direct and post-translational modification of metabolic and housekeeping proteins [6, 7]. The understanding of this additional level of complexity, I would argue, is paramount to dissecting out the pathophysiology of T2DM and obesity associated heart disease due to prevalence of nutrient-overload associated insulin resistant in these states. The consequence of this insulin resistance being: a diminution in the capacity to employ glucose as a fuel substrate; elevated circulating glucose and associated metabolic changes including elevations in circulating free fatty acids and triglycerides. The metabolism of these substrates and changes in their metabolic intermediates in turn alter the metabolic milieu within which the heart functions. How these metabolic intermediates, the regulatory control of their interaction with proteins and the effects they have on individual proteins and pathways is the focus of this chapter.

2 Acetylation as a Post-Translational Modification

Acetyl-CoA is present as a metabolic intermediate in mitochondria, cytosolic and nuclear compartments [8]. In mitochondria, acetyl-CoA is generated during the oxidation of pyruvate and fatty acids, and its major function is to convey carbon atoms to the tricarboxylic acid (TCA) cycle for energy production. Additionally, the TCA cycle intermediate citrate is exported from mitochondria into the cytosol and nucleus respectively. Cytosolic and nuclear citrate is converted back to acetyl-CoA by ATP citrate lyase (ACL). In the cytosol, acetyl-CoA functions as a substrate for lipogenesis and for acetyltransferase enzymes. Nuclear acetyl-CoA functions as a substrate for histone acetyltransferase reactions and thereby modulates histone acetylation and gene expression [9]. The acetylation of proteins in the mitochondria are modulated by the nutrient status [10, 11] and the function of mitochondrial proteins are modified by this post translational modification (PTM) [12].

Fasting provokes adipose tissue lipolysis resulting in elevated levels of circulating non-esterified fatty acids [13]. When levels of circulating fatty acids are high, the production of acetyl-CoA from fat breakdown may exceed cellular energy requirements and the excess acetyl-CoA can be employed in lipid, cholesterol and ketone body synthesis, and as a substrate for protein acetylation. Nutrient overload, by increasing fatty acid and/or glucose levels, can similarly increase acetyl-CoA levels resulting in similar, albeit possibly more chronic, effects. Interestingly numerous chain-length fatty acids can modify proteins via acylation and this modification can also modify protein function [14]. The regulatory control of acylation on metabolism is less well established, and will not be discussed further in this review. The role of additional TCA cycle intermediates in the post-translational modification of protein lysine residues is also evident where short-chain carbon fragments including succinyl groups and malonyl groups covalently bind to protein lysine residues [15, 16]. Similarly to the control of acylation, the regulation of succinylation and malonylation are beginning to be explored, but are beyond the scope of this chapter [15–17].

Enzymatic control of protein lysine residue acetylation and deacetylation are being actively explored and are discussed in the following sections. In addition, the recognition of non-enzymatic acetylation of proteins in the presence of acetyl-CoA is evident [18] and denatured mitochondrial proteins undergo acetylation in the presence of acetyl-CoA [19]. Furthermore, elevated levels of acetyl-CoA, coupled to the mitochondrial pH, have been shown to promote non-enzymatic protein acetylation [20]. This concept of non-enzymatic protein acetylation may be operational in diabetes where metabolic inflexibility, which is defined as the inability to switch from fatty acid to glucose oxidation during the transition from the fasted to fed state, results in part from the allosteric inhibition of pyruvate dehydrogenase by increased mitochondrial acetyl-CoA levels [21, 22]. The role of non-enzymatic protein acetylation has not been extensively investigated, although its' potentially important regulatory role has been recently been reviewed [23]. Interestingly, analysis of the mitochondrial acetylome, under various nutrient conditions and in the presence or absence of a mitochondrial deacetylation, shows evidence suggestive of both non-enzymatic and enzymatic control of mitochondrial protein acetylation [24].

3 Enzymatic Control of Protein Acetylation

There are three major acetyltransferase families, and member proteins from each group have been implicated in the control of cellular homeostasis (reviewed [25]). Similarly, deacetylase proteins are also grouped into families. Class I, II and IV are zinc-dependent and are either localized in the nucleus or can shuttle between the nucleus and the cytoplasm and are referred to as histone deacetylases [26]. These enzymes are predominantly regulated independent of nutrient-status. In contrast, the Class III deacetylases are predominantly NAD^+-dependent, and function as sensors of the energetic status of the cell in response to the subcellular compartment levels of NAD^+ and nicotinamide and/or to the ratio of NAD^+:NADH [27–31]. The Class III enzymes are called sirtuins and mammals have seven family members designated Sirt1 through Sirt7. The mammalian sirtuins are further phylogenetically divided into five subclasses based on the homology of their 250 amino acid core domain [32]. Sirt1, 2 and 3 constitute subclass I and predominantly function in the nuclear (Sirt1), cytoplasmic (Sirt1 and 2) and mitochondrial (Sirt3) compartments. These three enzymes show closest homology to the yeast longevity protein Sir2, exhibit the most robust deacetylase activity. As the focus of this chapter explores the role of acetylation in the control of mitochondrial metabolism and function, Sirt1 and Sirt3 will be explored in this context.

The counter-regulatory acetyltransferase enzyme system is less well characterized, although in the nucleus both Gcn5 and p300 have been shown to counter the actions of Sirt1 [33]. It is interesting to note that the levels of Gcn5 are reduced when Sirt1 is genetically depleted as a putative adaptive process to 'compensate' for the reduction in the deacetylase suggesting that mechanisms exist within the cell to regulate protein acetylation status by the coordinate regulation of enzyme expression [34].

The process of protein acetylation in the mitochondria is even less well understood, although GCN5L1 has been identified as a critical molecular component of this program and its functional role is beginning to be explored [19, 35]. Recently, the mitochondrial protein acetyltransferase, acetyl-CoA acetyltransferase 1 (ACAT1), which functions in ketogenesis to combine two acetyl-CoA molecules [36] has been found to regulate the pyruvate dehydrogenase complex as a canonical acetyltransferase [37]. This finding may open the door to expanding our understanding of acetyltransferase functioning within mitochondria.

4 Nutrient Sensing in the Control of Deacetylase Enzymes

As sirtuin activity is dependent on NAD^+, it has now been established that sirtuin activation is directly linked to the energetic and redox status of the cell as measured by the ratio of NAD^+:NADH, by the absolute levels of NAD^+, NADH, and by the NAD^+ catabolite nicotinamide [27, 38, 39]. Interestingly, nicotinamide itself inhibits sirtuin activity and nicotinamide-depletion during NAD biosynthesis inversely activates sirtuins [29].

The NAD biosynthetic pathways are distinct in prokaryotes and invertebrates compared to vertebrates (reviewed [40]). We only briefly review vertebrate biochemistry here. *De novo* biosynthesis using tryptophan and nicotinic acid as precursors is the minor pathway for NAD generation. However, this pathway is induced by exercise and following the administration of peroxisome proliferator activated receptor alpha (PPARα) agonists [41, 42]. The predominant pathway to generate NAD involves the salvage of NAD using nicotinamide as the precursor. In mammals there are two intermediary steps in NAD generation, initiated by the conversion of nicotinamide to nicotinamide mononucleotide (NMN) via the nicotinamide phosphoribosyltransferase (NAMPT) enzyme. Nicotinamide/nicotinic acid mononucleotide adenylyltransferase (NMNAT) then converts NMN to NAD. These biochemical pathways are most well characterized in the nucleus, and are pivotal for the activity of SIRT1 [43]. Moreover, NAMPT has been identified as the rate-controlling step in NAD biosynthesis in that overexpression of Nampt but not Nmnat increased cellular NAD levels [43].

The investigation into the biology of NAD in the mitochondria has begun to be explored, and the identification of a mitochondrial-enriched NMNAT isoform supports the concept of subcellular compartment specific functioning of NAD biosynthesis [44]. Moreover, mitochondrial NAD^+ levels can now be measured by mass spectroscopy and it has been shown that the metabolic stress of fasting has been shown to increase mitochondrial NAMPT and to concomitantly induce mitochondrial NAD^+ levels [45]. Interestingly, now that distinct isoforms of NMNAT have been identified, the use of RNA silencing is being exploited to assay the effects of modulating distinct subcellular NAD^+ pools. The depletion of the nuclear isoform (NMNAT1) has recently been shown to play a pivotal role in the regulation of nuclear encoded mitochondrial oxidative phosphorylation enzymes in part via Sirt1 [46].

5 Sirt1 and Mitochondrial Function

Sirt1 is the most extensively explored sirtuin, and it deacetylates multiple targets in the nucleus and cytoplasm (see reviews [33, 47]). The essential role for Sirt1-mediated deacetylation of nuclear regulatory proteins and metabolic pathway enzymes is underscored in that the genetic depletion of Sirt1 results in embryonic lethality. Interestingly, Sirt1 is activated by both starvation and caloric restriction which align with its nutrient-sensing role. Sirt1's deacetylation targets include LKB1, NF-κB, PGC1-α and Foxo3a therefore linking Sirt1 activity to AMPK, nutrient sensing enzymes, inflammation, mitochondrial biogenesis and oxidant defense systems, respectively [47–49]. Sirt1 also directly regulates numerous aspects of cell metabolism through deacetylation of PPARγ (β-oxidation) and FOXO1 (gluconeogenesis).

The most extensive role uncovered regarding Sirt1 and mitochondrial biology pertains to the regulation of mitochondrial biogenesis. Peroxisome proliferator-activated receptor-coactivator-1α (PGC-1α) is a master regulator in the induction of mitochondrial proliferation [50] and the modulation of PGC-1α levels profoundly alter cardiac mitochondrial content [51]. PGC-1α is a deacetylation target of, and regulated by, Sirt1 [52, 53]. Sirt1 appears to be a major *in vitro* and *in vivo* regulator of PGC-1α deacetylation. For instance, *in vitro* knockdown of Sirt1 in hepatic cells leads to increased PGC-1α acetylation with a corresponding reduction in a set of genes that are the rate limiting enzymes responsible for hepatic gluconeogenesis [53]. Similarly, both overexpression and knockdown studies support a role for Sirt1 in regulating PGC-1α activity through reversible deacetylation, that in turn has dramatic effects on *in vivo* hepatic glucose and lipid metabolism [54, 55]. A similar relationship appears to exist in skeletal muscle. In particular, in skeletal muscle fasting was shown to lead to a Sirt1-dependent deacetylation of PGC-1α and this deacetylation appeared to be required for PGC-1α dependent gene expression including gene products required for effective mitochondrial biogenesis [56]. The role of Sirt1 in the control of mitochondrial biogenesis has been explored *in-vivo* in skeletal muscle Sirt knockout mice [34]. Surprisingly, the absence of Sirt1 in this context did not impede mitochondrial biogenesis in response to endurance exercise. However, it was found that the counter-regulatory acetyltransferase Gcn5 was coordinately downregulated in the skeletal muscle in parallel with the retention of PGC1-α deacetylation. These data highlight the coordinate control of acetylation by deacetylase and acetyltransferase enzymes. Together these studies link Sirt1, Gcn5 and PGC-1α activities in metabolically active tissues such as the liver and skeletal muscle (Fig. 1).

As germline deletion of Sirt1 is embryonic lethal, the comprehensive investigation of this deacetylase in pathology has not been extensively explored. However, tissue-specific deletion of SIRT1, including in the liver and brain, has been shown to negatively alter fat metabolism and increase susceptibility to diet-induced obesity [57, 58]. Limited copy number over-expression of SIRT1 has been shown to protect against redox stress in the brain, heart and kidneys, to ameliorate hepatic hepatosteatosis

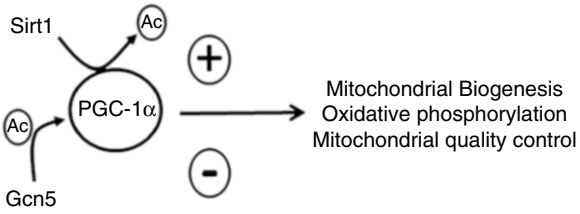

Fig. 1 Counter regulatory control of nuclear of PGC-1α acetylation by Sirt1 and Gcn5. Data support that the nuclear lysine acetylase and deacetylase enzymes may function in concert to mediate the nutrient and or redox signaling mediated control of PGC-1α. Deacetylation activates and acetylation inhibit PGC-1α, as the transcriptional coactivator in the regulation of genes encoding proteins controlling mitochondrial biogenesis, oxidative phosphorylation and mitochondrial quality control programs

that results from diet induced obesity, and to ameliorate colon cancer [59–62]. SIRT1 is also implicated, as necessary, to enable the innate adaptive reprogramming to resist ischemia-reperfusion injury, as a component of the ischemic preconditioning program [63].

6 Sirt3 and Mitochondrial Function

Sirt3 is the major mitochondrial deacetylase [64], and has been found to functions predominantly in mitochondria [65], although a few studies suggest that Sirt3 may possess extramitochondrial deacetylase activity [66–68]. Whether these extramitochondrial effects are directly attributable to Sirt3 or result from indirect effects from retrograde mitochondrial signaling has not been established, although potential mechanisms have been postulated that are amenable to investigation [69]. The depletion of Sirt3 has a subtle phenotype [64] which is unmasked in response to prolonged fasting [70], following chronic perturbations in caloric intake [11, 71, 72] and in response to redox stress [73]. Numerous proteomic approaches have been employed to identify substrates of Sirt3 deacetylation and the vast majority of proteins with alternations in acetylation are found in the mitochondria [24, 74, 75]. The functional characterization of these proteins have shown that Sirt3 mediated deacetylation regulate numerous aspects of mitochondrial function including the enzymes that regulate β-oxidation, branch-chain amino acid metabolism, ketone biology, the electron transport chain, ATP production, the urea cycle [24, 70, 75, 76] and for the control mitochondrial ROS breakdown [77, 78].

In light of the high energy demand of the heart and based on the Sirt3 targets characterized to date, the disruption of Sirt3 would be expected have cardiac consequences. Despite this, young Sirt3 knockout mice do not have any obvious phenotype [79] and young knockout mice have been reported to exhibit normal treadmill performance [80]. However, in-line with the concept that Sirt3 may play a role in

fine tuning mitochondrial biology, in response to aging Sirt3 knockout mice develop cardiac dilatation [79], and the introduction of pressure overload results in maladaptive cardiac hypertrophy [79, 81]. The mechanisms underpinning these pathologies align with the prior functions attributable to Sirt3 and include increased propensity to calcium-induced mitochondrial permeability and increased generation of reactive oxygen species [79, 81]. The ameliorative role of Sirt3 in modulating these stressors is shown in cardiomyocytes where the overexpression of Sirt3 promotes anti-apoptotic programs [67] and in cardiac-restricted Sirt3 transgenic mice, where excess Sirt3 blunts reactive oxygen species levels and enhances the activity of ROS scavengers including SOD2 and catalase [81]. An interesting additional mechanism whereby Sirt3 deficiency could potentially contribute to the pathophysiology of cardiac hypertrophy is the role of this sirtuin in fat metabolism [70]. As the loss of metabolic plasticity with the downregulation of fatty acid oxidation (FAO) is synonymous with cardiac pressure-overload mediated decompensation [82, 83], it is possible that the downregulation of FAO in Sirt3 knockout mice may play a role in the pressure-overload and aging maladaptive phenotype in the heart. This concept has not been directly explored though and warrants direct investigation.

As the regulatory control of mitochondrial protein acetylation is nutrient-level and redox-potential dependent, it is conceivable that primary perturbations within mitochondria that may modulate metabolic intermediates or redox-potential could then initiate changes in the mitochondrial acetylome. This concept has been explored in the heart in response to genetic perturbations associated with cardiovascular pathology. These include the disruption of frataxin, cyclophilin D and components of the electron transfer chain [84–86]. All of these disruptions result in the either basal or excessive pressure-overload induced cardiac dysfunction and are associated with the reduction in the NAD^+/NADH ratio and increased mitochondrial protein acetylation [84–86]. In primary cardiomyocytes, the frataxin and Complex I disruption of the acetylome are corrected with improvement in mitochondrial functioning in response to Sirt3 induction [84, 86]. Together these data support that the reciprocal control of acetylation by intrinsic mitochondria functioning can affect global mitochondrial functioning via the regulation of the mitochondrial acetylome.

7 Future Directions in Understanding the Mitochondrial Acetylome

Advances in proteomics has greatly increased our understanding of both the static and dynamic alteration of the mitochondrial acetylome in response to feeding, fasting, chronic caloric overload and in response to redox stresses [24, 87]. Additionally, these studies are beginning to identify site specific changes in lysine residue acetylation that can play important roles in: modulating protein function; in controlling synergistic PTM's; in allosteric protein interactions; in protein stability and in subcellular localization of proteins [12, 70, 74, 88]. Moreover, the stoichiometry of

proteins and the domains surrounding substrate protein lysine residues may play important regulatory roles in the interaction of acetylase and deacetylase enzymes in the acetyl-modification of these target proteins [24]. Moreover, the identification and regulation of the acetylome modifying enzymes themselves are being explored in more detail and understanding the control of the modifiers will further expand our understanding of the role of acetylation in controlling mitochondrial function [35, 77, 87].

An area of some functional discrepancy has also arisen with respect to the acetylation of specific targets within a pathway compared to the global functioning of the canonical pathway in response to acetylation. This is most vividly illustrated where fatty acid oxidation is increased in the presence of excess fat and mitochondrial protein acetylation [6, 89] in contrast to studies showing direct deacetylation of lysine residues on FAO enzymes resulting in the activation of enzyme activity [70, 90]. The mechanisms underpinning these effects and whether this may be a result of tissue distinct regulatory cues needs to be explored.

Finally, although the role of acetylation in modifying individuals proteins is the main focus of the chapter, data is emerging to show that the overall function of mitochondrial quality control and integrity, which are also modulated by nutrient levels and redox stress, including mitochondrial turnover (mitochondrial dynamics, mitophagy and biogenesis) [33, 91–93] and redox- and proteotoxic-stress amelioration effects [48, 94] are regulated by the mitochondrial acetylome. Our understanding of this biology also needs to be fleshed out and these data are beginning to explore the more integrated whole cell/organism effects of the regulation of the acetylome [7]. The schematic shown in Fig. 2 highlights some of the areas of research required to enable a more comprehensive understanding of the role of mitochondrial acetylation in the control of mitochondrial function and integrity.

8 Conclusions

The redox-stress and nutrient-sensing acetyl-modification of protein lysine residues is now recognized as abundant and complex a post-translational modification as phosphorylation [95]. Moreover, the acetyl-modification is enriched in mitochondria and is beginning to be characterized in both the regulation of mitochondrial metabolism and organelle quality control programming. The advancement in proteomic technologies and the use of genetically modified mouse models being explored to define functional acetyl-modifications and to show that this post-translational modification can be regulated in response to both extramitochondrial redox and nutrient-stressors and similarly in response to primary perturbations within mitochondria. Deeper understanding of the pathways and modulators in how acetylation controls mitochondrial integrity, regulation and function are essential in this era of caloric excess and the associated cardiovascular and metabolic diseases, in the effort to device more effective therapeutic strategies.

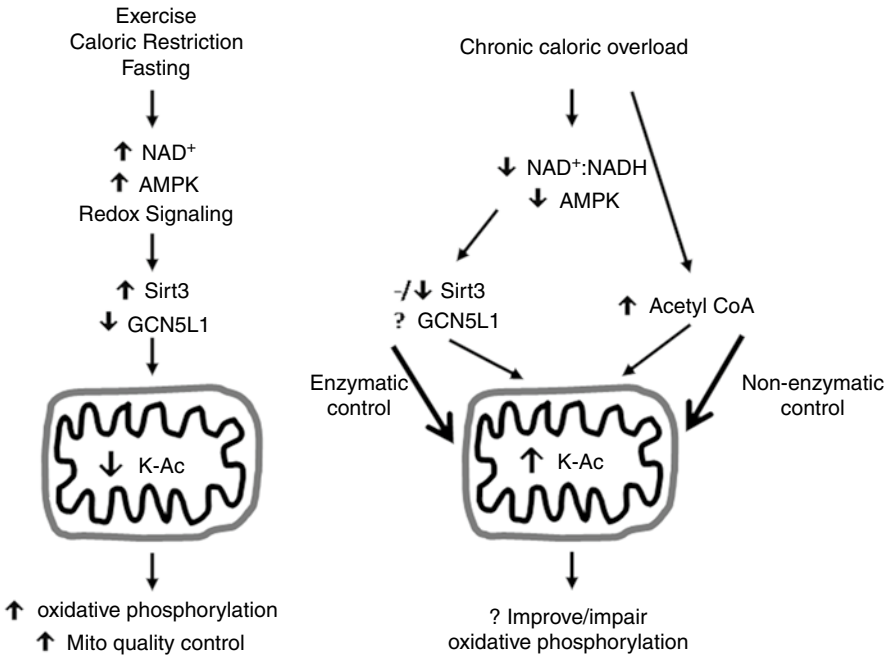

Fig. 2 The complexity of anterograde signaling in the control of mitochondrial protein acetylation. The acetylation of mitochondrial proteins can be differentially regulated by acute or chronic perturbations in nutrient load and may be controlled by enzymatic and/or non-enzymatic control. Concepts that have not been completely characterized include: (a) the relative contribution and or relevance of non-enzymatic versus enzymatic acetylation; (b) the regulatory components of functional mitochondrial acetyltransferase enzyme complexes and (c) whether the modulation of mitochondrial acetylation can have tissue specific and or nutrient-load dependent effects independent of the affects of acetylation of distinct proteins/enzymes

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Part II
Alteration in Energy Metabolism

Adrenergic Control of Cardiac Fatty Acid Oxidation in Diabetes

Vijay Sharma and John H. McNeill

Abstract Diabetes produces a direct and continuous myocardial insult even in the absence of ischemic, hypertensive or valvular disease. β-blocking agents have been shown in large-scale randomized controlled trials to reduce heart failure mortality. In this chapter, we summarise the results of our studies investigating how β-adrenergic signalling controls cardiac metabolism, and the significance of these mechanisms in diabetes. Metoprolol inhibits fatty acid oxidation but does not prevent lipotoxicity; its beneficial effects are more likely to be due to anti-apoptotic effects of chronic treatment. The range of effects produced by β-adrenergic blockade are broad and illustrate how interconnected the signalling pathways of function and metabolism are in the heart. Our initial hypothesis that inhibition of fatty acid oxidation would be a key mechanism of action was disproved. However, unexpected results have led us to some new and hitherto unexpected regulatory mechanisms of cardiac metabolism. The first is USF-2-mediated repression of PGC-1α, most likely occurring as a consequence of improved function. The second is the identification of covalent modifications which directly regulate carnitine palmitoyltransferase-1 (CPT-1) at the level of the mitochondria. We also found that β-adrenergic signalling interacts with caveolins, which could be a key mechanism of action of β-adrenergic blockade. Our experience of studying this labyrinthine signalling web illustrates that it is not necessary for initial hypotheses to be correct, and all ends foreseen, in order for valid lines of inquiry to be opened and new information revealed.

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Keywords Diabetes • Heart failure • Beta-blockers • Cardiac metabolism • Fatty acid oxidation • Adrenergic signalling • Caveolin • Apoptosis • Lipotoxicity • Mitochondria

1 Introduction

Diabetes produces a direct and continuous myocardial insult even in the absence of ischemic, hypertensive or valvular disease. The resulting pathology is diabetic cardiomyopathy, which can act synergistically with hypertension or ischemia to damage heart muscle, but can also cause heart failure independently. The disease process decreases both the compliance of the heart wall (due to increased cross-linking of collagen, cardiac hypertrophy and fibrosis [1, 2]) and contractility. Diabetic cardiomyopathy has a long clinical course which evolves in three stages [3]:

1. In the initial phase, the combination of hyperglycemia and a shift in cardiac metabolism to almost 100 % fatty acid oxidation produces oxidative stress and cellular damage due to the cytoplasmic accumulation of toxic intermediates of glucose and fatty acid metabolism (glucotoxicity and lipotoxicity) [2]. Calcium handling is impaired and the fetal gene program is activated. Ultrastructural changes in tissue architecture occur in parallel, and the extent to which these are causes or consequences of metabolic and contractile remodelling is still not entirely clear. These changes are sufficient to induce a mild diastolic dysfunction, detectable only with the use of Doppler echocardiography.
2. Left ventricular hypertrophy develops, and apoptosis and necrosis of cardiomyocytes, with ensuing myocardial fibrosis, also occurs. This results in more severe diastolic dysfunction and mild systolic dysfunction, sufficient to activate the renin-angiotensin system. The situation is further exacerbated by the development of a mild autonomic neuropathy.
3. In the final stage, microvascular disease appears, and the autonomic neuropathy becomes severe. Hypertension and ischemic heart disease frequently occur as well [3]. The result is a combined systolic and diastolic dysfunction, severe enough to cause systemic sympathetic nervous system activation.

This pathogenesis appears to be driven by the combination of hyerglycemia and shifts in substrate utilisation by the heart. It is an example of maladaptive metabolic plasticity begetting maladaptive metabolic and contractile remodelling. The main pathways involved in cardiac metabolism are summarised in Fig. 1.

All forms of heart failure are associated with activation of the sympathetic nervous system. A failing resting heart is subjected to a sympathetic drive equivalent to the maximum drive a normal heart is subjected to during severe exercise; there is increased spillover of catecholamines (as much as 50-fold), leading to an extremely large increase in cardiac and systemic catecholamine levels [4–7]. The aim of this response is to maintain systemic perfusion, but it is maladaptive and correlates inversely with survival [8]. It is for this reason that the β-blocking agents bisoprolol,

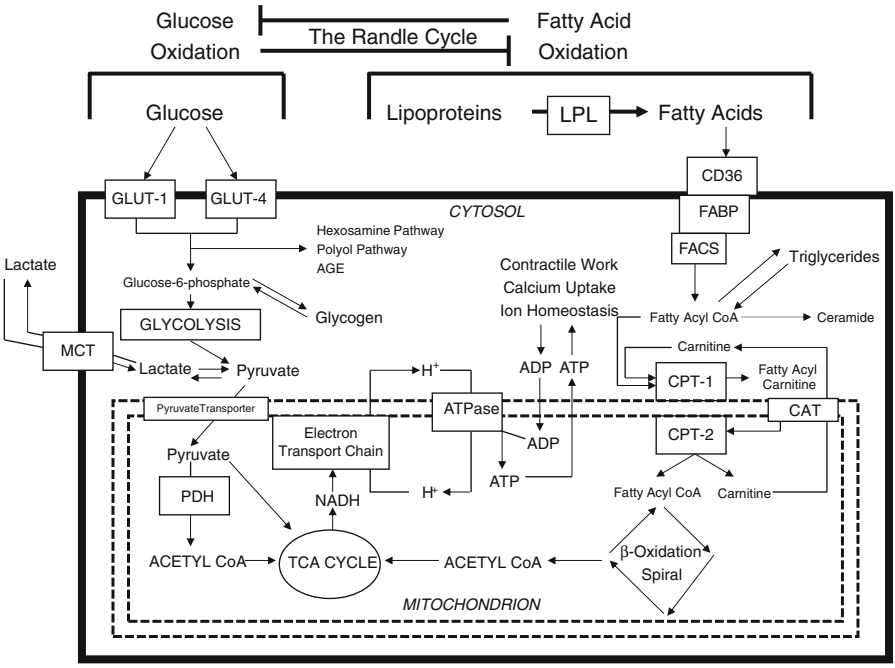

Fig. 1 Summary of fatty acid and glucose metabolism. Glucose is taken up by Glut-1 and Glut-4 transporters and is converted by glycolysis to pyruvate which enters the mitochondria to be oxidized, producing acetyl CoA. Fatty acids are liberated from lipoproteins by LPL and taken up by CD36 and FABP. LCAS converts the fatty acid to a CoA ester which is then taken up by the carnitine shuttle system to the mitochondria. The fatty acyl CoA undergoes β-oxidation, removing two carbons per turn of the cycle and generating acetyl CoA. Acetyl CoA, generated by either pathway, enters the TCA cycle to generate reducing equivalents (NADH). These pass electrons to the electron transport chain which creates an electrochemical proton gradient to drive ATP synthesis. ATP synthesis is coupled to the systems which create the ATP demand (*LPL* lipoprotein lipase, *CD36* fatty acid translocase, *FABP* fatty acid binding protein, *FACS* fatty acyl CoA synthase, *CPT* carnitine palmitoyltransferase, *CAT* carnitine acyl transferase, *CoA* coenyme A, *TCA* cycle tricarboxylic acid cycle, *AGE* advanced glycosylation endproduct, *PDH* pyruvate dehydrogenase, *MCT* monocarboxylate transporter, *PDH* pyruvate dehydrogenase, *NADH* reduced nicotinamide adenide dinucleotide, *ATP* adenosine triphosphate, *ADP* adenosine monophosphate. (Modified from Figure 1 of [117])

carvedilol and metoprolol reduce mortality, an effect which has been consistently seen in large-scale randomized controlled trials [9]. Acute administration of β-blocking drugs produces negative chronotropic and inotropic responses, and they were contraindicated in heart failure for many years for this reason. However, β-blockers were pioneered as heart failure treatments in the 1970s [10] and they are now among the agents of choice for the treatment of heart failure [9].

There are several putative mechanisms for the chronic effect of β-blockers, which are inter-related by a complex signalling web. These include antiarrhythmic effects, amelioration of cardiomyocyte hypertrophy, necrosis and apoptosis, reversal of the

fetal gene program (thereby improving calcium handling and force of contraction), increases in cardiac receptor density, anti-inflammatory effects and partial restoration of cardiac glucose oxidation [11, 12]. Metoprolol [13, 14], carvedilol [15] and bucindolol [16] induce a switch from fatty acid to glucose oxidation in non-diabetic patients with heart failure. A study in dogs with microembolism-induced heart failure revealed a potential mechanism for this effect: CPT-1 was inhibited by chronic treatment with metoprolol [17].

2 β-Adrenoceptor Signalling

The existence of two broad subtypes of adrenoceptors, α-adrenoceptors and β-adrenoceptors, was first demonstrated by Ahlquist in 1948 [18]. In the 1960s, two subtypes of β-adrenoceptors, β1 and β2, were characterized [19], and the third, β3, was characterized in 1989 [20]. In the heart, all three subtypes are expressed in the heart, but the major subtypes are β1 and β2, with the ratio of β1: β2 being approximately 60–70 %: 40–30 %, and very low β3 expression [21]. The absolute expression levels are in the femtomolar range (50–70 fmol/mg protein for the β1 adrenoceptor) so the receptor reserve is low [11]. The receptors show varying affinities for their ligands [22]:

1. β1 (adrenaline = 4 μM, noradrenaline = 4 μM, isoproterenol = 0.2 μM)
2. β2 (adrenaline = 0.7 μM, noradrenaline = 26 μM, isoproterenol = 0.5 μM)
3. β3 (adrenaline = 130 μM, noradrenaline = 4 μM, isoproterenol = 2 μM).

β-adrenoceptors are G-protein coupled receptors which, in the classical β-adrenoceptor pathway, act via Gs to produce an acute positive inotopic response mediated by increased cAMP levels and stimulation of protein kinase A (PKA). PKA subsequently phosphorylates and activates L-type calcium channels and ryanodine receptors, increasing calcium uptake and release, and phospholamban, relieving inhibition of SERCA and increasing sarcoplasmic reticulum calcium uptake [23–25]. In parallel, phosphorylation of troponin I and myosin binding protein B by PKA increases the calcium sensitivity of myofilaments [26, 27]. Phosphorylation of protein phosphatase inhibitor-1 by PKA prevents dephosphorylation of PKA target proteins and sustains its effects [28].

β-adrenoceptor signaling is temporally and spatially organized. β-adrenoceptors, and most particularly the β2-adrenoceptor, desensitize by uncoupling from their G-proteins. This dissociation is initiated by receptor phosphorylation and is mediated by β-arrestins acting together with G protein-coupled receptor kinases or PKA itself [29–31]. β-adrenoceptors can also change their coupling to downstream signaling pathways, usually in response to prolonged activation. At the β1 adrenoceptor, this causes a switch from PKA to calcium/calmodulin dependent protein kinase-II (CAMK II)—dependent signaling which is maladaptive, leading to CAMK-II mediated apoptosis and pathological hypertrophy [32]. In contrast, at the β2-adrenoceptor, it causes a switch in G-protein coupling from Gs to Gi, which is cardioprotective

due to activation both of phosphodiesterase-4, which ameliorates cAMP/PKA signaling, and of the pro-survival PI3 kinase/Akt pathway [33, 34]. A role for the extracellular-signal-regulated kinase (ERK) 1/2 in mediating β2-adrenoceptor-Gi cardioprotection has also been proposed [35]. Gi-mediated signaling therefore produces functional antagonism of Gs-mediated signaling. β1 adrenoceptor signaling is widely disseminated throughout the cell, whereas β2 adrenoceptor signaling is compartmentalized, partly due to the enrichment of β2 adrenoceptors in caveolae [36, 37]. The positive inotopic effect elicited by β2/Gs signaling is therefore comparatively small [34, 38]. It is possible that translocation of β2 adrenoceptors out of caveolae causes the switch from Gs to Gi association during prolonged activation [39]. Overall, it is clear that the coupling of β-adrenoceptors to their downstream signaling pathways is compartmentalized and time-dependent. Sustained activation of β1 adrenoceptors is harmful, whereas sustained activation of β2 adrenoceptors is cardioprotective, but the latter effect is constrained by the limited compartmentalization of the pathways.

Another consequence of PI3K/Akt activation is stimulation of nitric oxide (NO) production by nitric oxide synthase (NOS), which catalyses the generation of NO is from the terminal guanidine nitrogen atom of the amino acid L-arginine and molecular oxygen. Tetrahydrobiopterin (BH_4) is required as a cofactor; without BH_4, eNOS becomes 'uncoupled' and produces reactive oxygen species such as peroxynitrite. In adult cardiomyocytes, endothelial nitric oxide synthase (eNOS) is constitutively expressed and produces physiological NO signaling in the nanomolar range. Inducible nitric oxide synthase (iNOS) is expressed in response to inflammatory stimuli [40, 41] and produces higher levels of NO that mediate pathophysiological effects [42, 43]. In a well-characterised signaling pathway, soluble guanylyl cyclase is activated following nitrosylation, stimulating the production of cyclic 3′, 5′guanosine monophosphate (cGMP) from guanosine triphosphate [44]. Just as cAMP activates PKA, cGMP activates protein kinase G (PKG) isoforms. In the heart, the NO/cGMP pathway has a negative inotropic effect [40]. β2 adrenoceptor-Gi signaling and β3 adrenoceptor-Gi signaling both stimulate NO production [45, 46].

The effects of diabetes on cardiac β-adrenergic responsiveness have been studied for many years, but the picture remains confusing. Studies have variously a decrease in the cardiac relaxant effects without an effect on heart rate or contractility in rat hearts [47], a decrease in the chronotropic response in rabbit heart *in vivo* [48], an increased chronotropic response and a decreased inotropic response in atria [49] and decreased sensitivity to β-adrenergic stimulation in cardiac tissues [50, 51]. Similar controversy surrounds the effects of diabetes on β-receptor expression and downstream signalling, but an overall picture of a shift away from β1-adrenoceptor signalling seems to be emerging [51–55]. The chronotropic response to noradrenaline was blunted by 14 weeks but not 8 weeks of diabetes, with preservation of the response to fenoterol, a selective β2 agonist, suggesting that β1-mediated responses are selectively blunted [56]. Consistent with this observation, the expression of β1 adrenoceptors is markedly decreased and that of β2 adrenoceptors modestly decreased in the diabetic heart, whereas the expression of β3 adrenoceptors is increased twofold [51]. A similar increase in β3 adrenoceptor expression has also

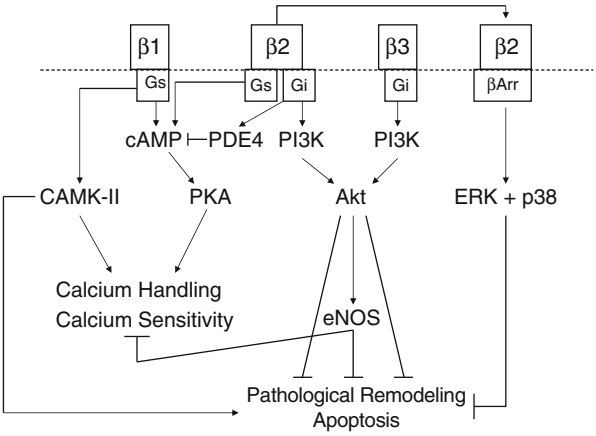

Fig. 2 β-adrenergic signaling pathways. β1-adrenergic receptors activate PKA, which regulates calcium sensitivity and calcium handling. Prolonged activation of this receptor activates a harmful CAMK-II pathway which is pro-apoptotic and induces pathological remodeling. β2-adrenergic receptors also activate PKA, but prolonged activation causes a switch to Gi signaling which activates PDE4, inhibiting cAMP formation, and activates the cardioprotective PI3K/Akt pathway. Desensitization of β2-adrenergic receptors by β-arrestin can recruit p38 and ERK, which protect the cell from apoptosis. β3-adrenergic receptors produce a negative inotopic effect which is mediated by NO produced via the PI3K/Akt pathway (Modified from Figure 2 of [117])

been found in failing human hearts [57]. The significance of this shift in receptor subtypes is unclear; an increase in NO-mediated signalling could produce a harmful negative inotropic effect, but, if β3 adrenoceptor-mediated activation of the PI3K/Akt pathway also prevents apoptosis, the shift could be cardioprotective (Fig. 2).

3 Effects of Metoprolol on Cardiac Function and Metabolism

We have shown that the β1-adrenoceptor-selective β-blocker metoprolol ameliorates the cardiac dysfunction produced by diabetic cardiomyopathy [58]. This improvement is evident both from Starling curves generated by direct left ventricular pressure measurements and from measurements of cardiac output and hydraulic power at constant preload and afterload. However, a robust improvement in function was not seen when we repeated the measurements *in vivo* using echocardiography; although metoprolol improved stroke volume and cardiac output, it also increased end-diastolic volume, so it was unclear whether the underlying cardiac dysfunction was being attenuated or worsened [59].

The effects of β1-adrenoceptor-blockade on *ex vivo* fatty acid and glucose metabolism in the heart are complex and depend on the disease state and the duration of the blockade [58]. Surprisingly, chronic metoprolol treatment increases palmitate

oxidation and decreases glucose oxidation in control hearts [58]. However, in diabetic hearts, chronic metoprolol treatment lowers fatty acid oxidation and increasing glucose oxidation. Short term perfusion with metoprolol inhibits fatty acid oxidation and produces marked stimulation of glucose oxidation in both control and diabetic hearts [58]. Before attempting to resolve this apparent paradox, it is important to note that the main target of metoprolol is in the fatty acid oxidation pathway, because it is preserved in the absence of insulin, whereas the effect on glucose oxidation is not [58]. Furthermore, metoprolol inhibits the activity of pyruvate dehydrogenase, so any stimulatory effect on glucose oxidation would have to be mediated by the Randle cycle. The β-blocker propanolol has been reported to induce an increase in CPT-1 activity in normal Sprague–Dawley rats [60], whereas metoprolol decreases CPT-1 activity in conscious dogs with micro-embolism-induced heart failure [17]. In dogs with pacing-induced heart failure, glucose uptake is improved by carvedilol but not metoprolol [61]. However, in clinical studies, metoprolol, carvedilol and bucindolol have all been shown to inhibit fatty acid oxidation [13, 15, 16].

When diastolic filling increases, cardiac work and oxygen consumption also increase in direct proportion via the Frank-Starling mechanism. However, in the normal heart, ATP supply is maintained regardless of cardiac work or oxygen consumption, indicating that cardiac metabolism is driven by cardiac function [62]. The mechanisms which underlie this exquisite coupling are manifold. However, how cardiac function influences cardiac energy substrate *selection* is less clear. Some of the effects of metoprolol on cardiac metabolism could conceivably be *attributable* to, rather than responsible for, its effects on cardiac function. Normalisation of palmitate and glucose oxidation rates to cardiac function, the does not eliminate the pattern of changes observed, suggesting that the shifts cannot be solely explained on the basis of cardiac function. However, to be definitive, it is important to establish whether the metabolic effects are preserved in isolated cardiomyocytes, in which the effects of cardiac function and the Frank-Starling mechanism do not apply.

4 CPT-1 Activity and Regulation by Malonyl CoA

β1-adrenoceptor-blockade has no effect on the activities of acyl-CoA dehydrogenase or citrate synthase, and does not decrease CD36 translocation [59, 63]. The major target of β1-adrenoceptor-signaling is CPT-1. In the heart, the major mechanism by which CPT-1 is regulated is through modulation of malonyl CoA levels. Isoproterenol has previously been shown to lower malonyl CoA levels by increasing PKA-mediated phosphorylation of acetyl CoA carboxylase (ACC) [64]. Furthermore, a study in isolated cardiomyocytes using activators and inhibitors of cAMP showed that stimulation of fatty acid oxidation by contraction was PKA-dependent [65]. We therefore expected that β-adrenergic blockade could have the opposite effect, preventing ACC phosphorylation and increasing malonyl CoA levels, but this turned out not to be the case. Chronic β1-adrenoceptor-blockade decreases malonyl CoA

levels in control hearts and has no effect in diabetic hearts. The mechanism of this effect is unclear, because there is no effect on ACC and malonyl CoA decarboxylase (MCD) expression, AMP-activated protein kinase (AMPK) or PKA-mediated phosphorylation of ACC. Dobutamine, a non-selective β-agonist, was previously found to decrease malonyl CoA levels without an effect on AMPK, ACC or MCD, so there must be other mechanisms by which malonyl CoA levels are regulated [66, 67]. Malonyl CoA levels are known to be dependent on the cytosolic supply of acetyl CoA, which is derived from peroxisomal β-oxidation, citrate and acetylcarnitine [67–69]. It is interesting to note that acute inhibition of CPT-1 has been shown to produce a fall in malonyl CoA levels independent of ACC and MCD [69], raising the possibility that the fall in malonyl CoA levels observed in control hearts could, therefore, have been *secondary* to the inhibition of CPT-1. Why such a mechanism would only lower malonyl CoA levels in control hearts is unclear. It is possible that fatty acid oxidation rates, and therefore the acetyl CoA/CoA ratio, are higher in the diabetic heart, and the fall in cytosolic acetyl CoA levels produced by CPT-1 inhibition in this context may not be sufficient to decrease malonyl CoA levels. Metoprolol tends to decrease tissue acetyl CoA, but measurements of the cytosolic and mitochondrial acetyl CoA pools are required to confirm these speculations. Overall, however, malonyl CoA levels do not correspond with the observed changes in fatty acid oxidation and cannot, therefore, be used to explain the effects of β1-adrenoceptor-blockade [58]. β1-adrenoceptor-blockade decreases the maximum capacity of CPT-1 activity as measured *in vitro* following both short-term and chronic exposure in both control and diabetic hearts. Surprisingly, metoprolol also decreases the sensitivity of CPT-1 to malonyl CoA. Incubation of metoprolol with CPT-1 *in vitro* has no effect on the maximum activity or malonyl CoA sensitivity of the enzyme.

Overall, the time and disease-dependent changes in fatty acid oxidation can be summarised as follows. In control hearts, acute metoprolol perfusion lowers malonyl CoA levels. The sensitivity of CPT-1 to malonyl CoA falls, and the activity of CPT-1 is markedly reduced. With chronic treatment, malonyl CoA levels remain low but the sensitivity of CPT-1 to malonyl CoA is restored and the inhibition of CPT-1 activity is less marked. Fatty acid oxidation is therefore inhibited following short-term β1-adrenoceptor-blockade, but this effect is lost with time. In diabetic hearts, short-term β1-adrenoceptor-blockade markedly lowers CPT-1 activity. This reduction is sustained with chronic blockade and produces inhibition of fatty acid oxidation despite a concomitant decrease in malonyl CoA sensitivity. The major determinants of the fatty acid oxidation rate are CPT-1 activity and malonyl CoA levels. In our studies, fatty acid oxidation was always inhibited if CPT-1 activity was inhibited by approximately 50 %, which is consistent with previous observations that CPT-1 only becomes rate-limiting when its activity is inhibited by approximately 50 % [70]. The decrease in malonyl CoA sensitivity would be expected to increase flux through CPT-1; however, this may represent a fine tuning mechanism since at no point do changes in malonyl CoA sensitivity hold sway over the overall fatty acid oxidation rate.

Both CPT-1A (the liver isoform) and CPT-1B (the muscle isoform) are present in the heart [71, 72]. We found that the IC_{50} of control hearts was approximately 30 μM

malonyl CoA, which is intermediate between the high sensitivity of CPT-1B and the low sensitivity of CPT-1A [58, 73]. The N- and C- termini of CPT-1 both face the cytosol and are separated by a loop region containing two membrane spanning domains inserted into the outer mitochondrial membrane. The catalytic region is within the C-terminus and residues which regulate malonyl CoA sensitivity have been found within the C-terminus, the N-terminus and the loop region [74–77]. In the liver, regulation of CPT-1A sensitivity is more important than regulation of malonyl CoA levels, and has been attributed to regulation by cytoskeletal elements [78], changes in the membrane environment [79] and direct phosphorylation of CPT-1 [80]. Peroxynitrite-mediated nitration of CPT-1B has been shown to decrease CPT-1B catalytic activity following endotoxemia in the heart [81]. Our own studies have revealed that both changes in expression and covalent modifications of CPT-1 occur within the heart, and provide an explanation for the short and long-term effects on CPT-1 activity and malonyl CoA sensitivity we observed. However, the relationship of these effects to β1-adrenergic signalling remains incompletely characterised.

5 Regulation of CPT-1 Expression

Chronic metoprolol treatment reduces total CPT-1 expression in the diabetic heart only, and this decrease is entirely attributable to a fall in CPT-1B expression. Intringuingly, there is a modest increase in CPT-1A expression which provides a partial explanation for the decrease in CPT-1 malonyl CoA sensitivity. CPT-1 expression is known to be controlled by PPAR-α, but the PPAR-α/RXR complex is only a modest inducer of CPT-1 when acting alone [82–84]. The induction of CPT-1 by PPAR-α is greatly enhanced by PGC1α, but PGC1α can also induce CPT-1 independently by binding to MEF-2A [85]. PGC1α-mediated expression of CPT-1 is repressed in isolated cardiomyocytes by upstream stimulatory factor (USF)-2. Upstream stimulatory factors are transcription factors of the basic helix-loop-helix leucine zipper family which bind to the E-box consensus sequence CANNTG and which, in the heart, respond to sustained increases in electrical stimulation by increasing the expression of sarcomeric genes such as sarcomeric mitochondrial creatine kinase and MHC [86–88]. PGC1α occupancy of the CPT-1 promoter is increased in diabetes. Chronic β1-adrenoceptor-blockade increases the binding of USF-2 to PGC1α, and this is associated with a decrease in the occupancy of the CPT-1 promoter by the PGC1α complex [63]. No such effect is observed in control hearts. We propose that USF-2 maintains a constant level of tonic repression of CPT-1 expression in the normal heart. In the diabetic heart, this tonic repression is clearly lost, because USF expression, and USF activity as indicated by MHC expression, are both decreased. Restoration of USF-2 repression in the diabetic heart produces marked changes in CPT-1 expression (Fig. 3), and we believe that this effect is most likely due to an increase in electrical stimulation produced by the improvement in function; in other words, it is a *consequence* of improved function.

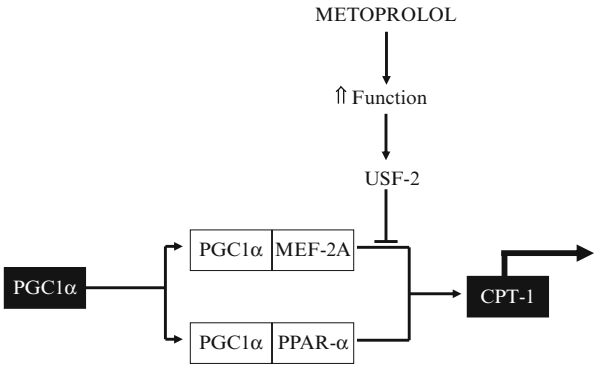

Fig. 3 Repression of CPT-1 by metoprolol. Improved contractile function leads to stimulation of USF-2, which represses PGC1-α transcriptional complex and reduces the expression of CPT-1 (Modified from Figure 3 of [117])

However, β1-adrenoceptor-blockade in the diabetic heart is associated with a more global regulation of the PGC1α transcriptional complex which is not explicable solely on the basis of USF binding. β1-adrenoceptor-blockade produces a decrease in the association of PGC1α with PPAR-α and MEF2A. Although this could be an indirect effect of the acute changes in fatty acid metabolism, it is more likely that active regulation of the complex is occurring. β1-adrenoceptor-blockade, by promoting glucose oxidation, reduces pyruvate levels, leading to a decrease in the binding of the pyruvate-activated deacetylase SIRT-1 to PGC1α. Furthermore, the binding of the acetylase p300 was increased by metoprolol. One would expect both of these changes to increase acetylation state of PGC1α, but we observed the opposite effect; β1-adrenoceptor-blockade decreased PGC1α acetylation! The acetylation site is very close to the USF-2 binding region, so it is possible that binding of USF-2 to PGC1α interferes with the acetylation reaction. This may be another mechanism of the repressive effect of USF-2, and one which could produce a more global repression of PGC1α target genes. Surprisingly, the phosphorylation of PGC1α by PKA, was increased by β1-adrenoceptor-blockade in the diabetic heart. We have no explanation for this. It is conceivable that PGC1α is a low priority target of PKA, and that binding of PGC1α to PKA only occurs when PKA is not interacting with its primary targets. Phosphorylation of p38 mitogen-activated protein kinase (MAPK) increases both PGC1α/PPAR-α coactivation and downstream signaling to PGC1α and PPAR-α targets [89–92]. It has been suggested that phosphorylation by p38 MAPK may serve to integrate and coordinate contractile and metabolic gene expression [85]. Activation of β2-adrenoceptors in the heart has been shown to increase signaling through the p38 MAPK pathway [93]. It is therefore possible that metoprolol decreases p38 phosphorylation by blocking β2-adrenoceptors, leading to a decrease in the association of PGC1α with its coactivators. Whether p38 MAPK-mediated phosphorylation of PGC1α is affected in this way in the diabetic heart remains to be determined.

The association of MEF-2A with the CPT-1 promoter is obliterated by both β1-adrenoceptor-blockade and diabetes. In diabetes, this may reflect generalised repression of MEF-2A targets, which is known to occur. With β1-adrenoceptor-blockade, it may represent sequestration of MEF-2A to higher priority gene targets, covalent modifications of MEF-2A or PGC1α, perhaps mediated by p38, or displacement of MEF-2A from its consensus site by PPAR-α, whose own consensus site overlaps with that of MEF-2A.

α-MHC and SERCA expression are decreased in the diabetic heart as part of the fetal gene program; both effects are reversed by β1-adrenoceptor-blockade [58]. α-MHC is regulated by USF's, while SERCA is regulated by MEF-2A [94] and possibly by PPAR-α [95]. It is therefore conceivable that the PGC1α/PPARα/MEF2A/USF complex can regulate and reverse induction of the fetal gene program. In other words, contractile and metabolic remodelling could be regulated in parallel by the same transcriptional complex.

6 NO/RNS-Induced Covalent Modifications of CPT-1

There is growing interest in the ability of RNS to directly regulate protein function [96–98]. Physiological levels of NO and RNS typically produce the following reversible modifications to thiol groups within critical cysteine residues: S-nitrosylation (addition of NO), glutathiolation (formation of mixed disulphides between the thiol group and glutathione) or oxidation from thiol to sulfenate. Glutathiolation and S-nitrosylation have been most frequently implicated in the regulation of enzyme activity, and the effects can be inhibitory or stimulatory [96]. The unique redox chemistry of protein thiol groups confers specificity and reversibility to these covalent modifications. The specificity is mediated by a consensus sequence, analogous to kinase consensus sequences [99]. However, this consensus sequence is not "recognised" as such; instead, it creates the correct reaction conditions for the covalent modification to occur. Reversibility is conferred by a number of enzymatic and non-enzymatic reactions [100–102]. The list of proteins proposed to be regulated by these modifications is growing, and, in the heart, includes GAPDH and SERCA [103]. Higher pathological levels of RNS induce further oxidation of the sulfenate (one oxygen) to sulfinate (two oxygens) and sulfonate (three oxygens). This causes irreversible loss of function and is therefore toxic. Glutathiolation, by committing the thiol to an alternate reaction pathway, protects critical thiol residues against irreversible oxidation [96] (Fig. 4).

Another covalent modification produced by RNS is tyrosine nitration. Peroxynitrite is the best characterised inducer of tyrosine nitration; indeed, tyrosine nitration is frequently used as a biomarker of peroxynitrite [104, 105]. Although classically described as an inhibitory modification, some proteins are activated by tyrosine nitration including cytochrome C, fibrinogen and PKC [105–108]. Tyrosine nitration, like the thiol modifications, also exhibits site-specificity via a similar mechanism.

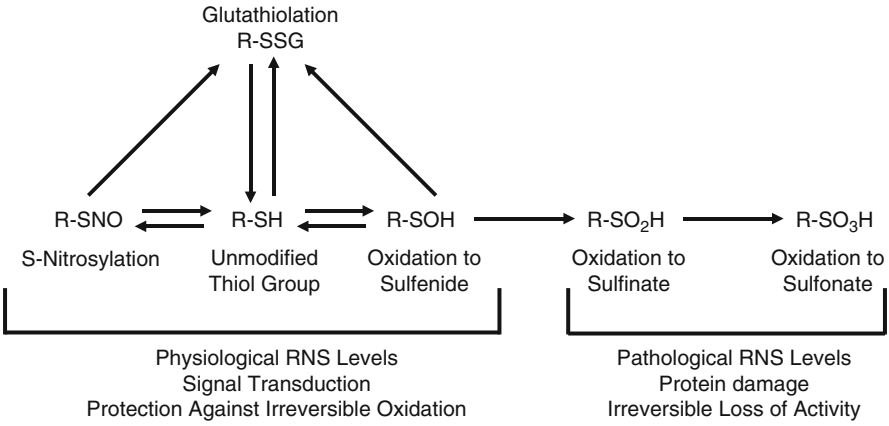

Fig. 4 NO and RNS-mediated modifications of thiol residues. Thiol (SH) residues undergo a series of reversible modifications in response to changes in the redox potential or exposure to physiological levels of reactive nitrogen species or nitric oxide. Oxidation of the thiol to the corresponding sulfenide or the formation of a disulphide bond between the thiol and glutathione (glutathiolation) are reversible either by changes in the equilibrium, or enzymatic restoration of the thiol group by thiol transferases. Further oxidation of a glutathiolated residue is not possible, so glutathiolation confers protection against oxidative damage for as long as it persists. However, exposure of the thiol group or the sulfenide to pathological levels of reactive nitrogen or oxygen species results in the formation of sulfinate and then sulfonate; these are irreversible modifications which result in protein damage and loss of activity (Modified from Figure 4 of [117])

Incubation of CPT-1 with continuous peroxynitrite, NO or hydrogen peroxide producing systems *in vitro* produces a decrease in CPT-1 activity which is associated with tyrosine nitration [109]. Furthermore, endotoxemia produces inhibition and nitration of CPT-1 in suckling rats [81]. Cysteine-scanning mutagenesis of CPT-1 revealed that cysteine 305 is critical for catalytic activity of the enzyme [110]. In our own studies, we incubated isolated mitochondria both with increasing concentrations of peroxynitrite ranging from 100 nM to 1 mM, and with a peroxynitrite-generating system, and dithiothreitol to remove the resulting covalent modifications. We found that CPT-1 was stimulated by peroxynitrite at low physiological levels but inhibited at high levels, and that peroxynitrite could induce tyrosine nitration, cysteine nitrosylation and cysteine glutathiolation. Activation of CPT-1 was most consistently associated with glutathiolation of CPT-1B. We hypothesised that the key residue involved was cysteine 305, but our efforts to confirm this by Mass Spectroscopy were unsuccessful [73].

We also successfully detected cysteine-nitrosylation, glutathiolation and nitration of CPT-1 in whole heart homogenates. Short-term β1-adrenoceptor-blockade increased nitrosylation and glutathiolation, but decreased tyrosine nitration, in diabetic hearts. In control hearts, nitrosylation was low and glutathiolation increased only following chronic treatment. This increase in CPT-1 glutathiolation would be expected to increase CPT-1 activity based on our *in vitro* studies, so CPT-1 glutathiolation does not explain the changes in CPT-1 activity seen following short-term

β1-adrenoceptor-blockade. Furthermore, it is not clear how the changes in CPT-1 nitrosylation, glutathiolation and nitration are linked to β1-adrenoceptor signalling; the patterns in systemic NO/RNS and CPT-1 covalent modifications which we observed do not match. The mitochondrial isoform of NOS (mtNOS) affects targets within the mitochondrial matrix and the inner mitochondrial membrane [111]. CPT-1 predominantly faces the cytosol, so it is likely that regulation of CPT-1 by NO/RNS is mediated by eNOS and possibly iNOS. eNOS has been proposed to translocate to the mitochondria [112, 113], so it is conceivable that mitochondrial eNOS translocation could determine NO/RNS mediated effects on CPT-1.

7 Phosphorylation of CPT-1

Phosphorylation of CPT-1A is known to occur *in vitro* [80] and is prevented by a specific inhibitor of CAMK II [114]. Activation of the sympathetic nervous system centrally by cerulinin stimulates CPT-1B activity in soleus muscle within 3 h [115]. This effect must have been mediated by an as-yet unidentified covalent modification of CPT-1B which could conceivably be phosphorylation. We speculated that the reason phosphorylation of CPT-1B had never been reported is because the kinases involved require other mediators to be present in order to bind their targets. We examined the effects of the main downstream kinases of β1-adrenoceptor signaling on CPT-1 activity, malonyl CoA sensitivity, and on the association of CPT-1 with known scaffolding proteins of these kinases. For example, A-kinase anchoring proteins (AKAPs) are a group of proteins which bind to PKA targets in order to regulate PKA-dependent phosphorylation of those targets [116]. We found that, when PKA was incubated with isolated mitochondria, it bound and phosphorylated CPT-1A, and increased the binding of AKAP-149 to CPT-1A; the functional effect was a decrease in CPT-1 sensitivity without any effect on catalytic activity.

When CAMK-II was incubated with isolated mitochondria, it bound and phosphorylated CPT-1B; however, the functional effect in this case was an increase in CPT-1 sensitivity without any effect on catalytic activity [73]. Serendipitously, our mitochondrial isolation produced a variation in the initial sensitivity of CPT-1, most likely as a result of membrane effects. Thanks to this unintended effect, we found that CAMK-II tended to restore CPT-1 sensitivity to a "set-point" represented approximately by an IC_{25} of 20 μM, an IC_{50} of 100 μM and an IC_{75} of 150 μM malonyl CoA. In this case, the scaffolding protein turned out to be α-actinin, whose binding was decreased by CAMK-II phosphorylation of CPT-1. We believe it unlikely that α-actinin, which is a cytoskeleton protein, is anchored in the mitochondrial membrane; CPT-1B probably forms a point of attachment of the cytoskeleton to the mitochondria. We speculate that AKAP-149 and α-actinin limit access of malonyl CoA to its binding site, and produce changes in malonyl CoA sensitivity by varying the strength of their association with their respective isoforms.

The MAPK p38 bound and phosphorylated CPT-1B via the scaffolding protein JIP-2, stimulating CPT-1 catalytic activity without affecting malonyl CoA

sensitivity [73]. By contrast, Akt did not bind or phosphorylate CPT-1, and had no effect on the activity or sensitivity of the enzyme.

Short and long-term β1-adrenoceptor-blockade increase the total phosphorylation state of CPT-1. β1-adrenoceptor-blockade increases PKA-mediated desensitization of CPT-1 to malonyl CoA in control hearts, and decreases CAMK-II-mediated sensitization in diabetic hearts [117]. Both effects decrease malonyl CoA sensitivity. Short, but not long-term, β1-adrenoceptor-blockade abolishes the association of p38 with CPT-1, providing a partial explanation for the inhibition of CPT-1 activity following short term β1-adrenoceptor-blockade in control hearts. A similar effect on p38 is not seen in diabetic hearts, because diabetes itself abolishes the association of p38 with CPT-1 [117]. The binding of PKA, CAMK-II and p38 to CPT-1 bear no relation to the overall activities of these kinases in the whole heart. It appears that the translocation of these kinases to CPT-1 on the mitochondrial surface is the important mechanism. Precisely how β-adrenoceptors might regulate such a translocation process is not known. Clearly the compartmentalisation and regulation of the signalling pathway are altered in the setting of diabetes.

Overall, we propose that changes in CPT-1 sensitivity produced by short-term β1-adrenoceptor-blockade are due to decreased CAMK-II phosphorylation of CPT-1 (in diabetic hearts) and/or increased phosphorylation of CPT-1 by PKA (in control hearts), mediated by changes in the association of CPT-1 with scaffolding proteins (Fig. 5). Obliteration of the CPT-1/p38 interaction could explain the decrease in CPT-1 activity in control hearts, but not in diabetic hearts, which show an increase in CPT-1 activity despite loss of this interaction. There is no explanation for the acute decrease in CPT-1 catalytic activity seen in the diabetic heart. The association with p38 MAPK is obliterated in the diabetic heart to begin with, and glutathiolation of CPT-1 is increased by short term β1-adrenoceptor blockade. The *in vitro* effects of the covalent modifications we identified may not reflect their effects *in vivo*. Also, it is possible that these covalent modifications produce more complex and varied effects when acting together as opposed to in isolation. There may also be other mechanisms for regulating CPT-1 catalytic activity which have not yet been identified.

8 Pro-Survival Signalling

Diabetes produces a decrease in β1-adrenoceptor expression and a marked increase in β3-adrenoceptor-expression. Long-term β1-adrenoceptor blockade increases the expression of all three adrenoceptor subtypes. In the whole heart, the major effect of short-term β1-adrenoceptor blockade is, unsurprisingly, to decrease classical cAMP/PKA signaling. Long-term blockade, in addition, increases PI3K/Akt signaling, probably due to the marked increase in β3-adrenoceptor-expression. This is associated with pro-survival effects. The pro-apoptotic factors FOXO-3 and Bad are inhibited, and the anti-apoptotic factor BCl-2 is stimulated [59, 118]. Another intriguing pro-survival effect is that long-term β1-adrenoceptor blockade increases

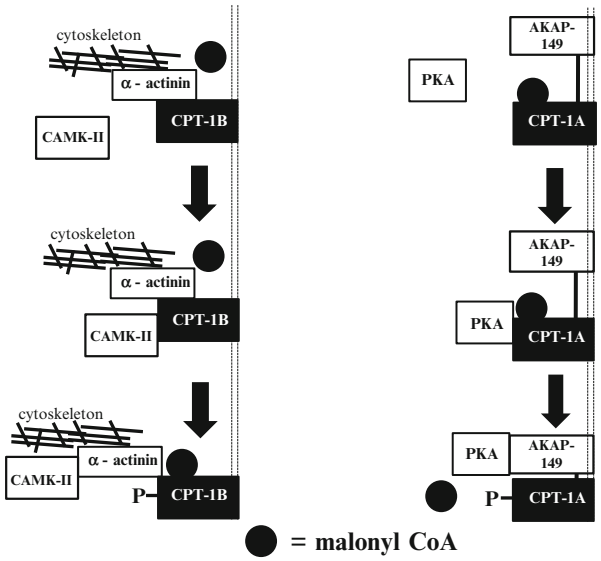

Fig. 5 Proposed model of the actions of PKA and CAMK-II. Left panel: Exogenously applied PKA phosphorylates CPT-1A and is then captured by its scaffolding protein. Phosphorylation produces a conformational change tightening the interaction between AKAP-149 and CPT-1A. As a result, malonyl CoA is denied access to its binding site, and the sensitivity of CPT-1 to malonyl CoA is reduced. Note that CPT-1 and AKAP-149 are both anchored in the mitochondrial membrane. Right panel: Exogenously applied CAMK-II phosphorylates CPT-1B and is then captured by its scaffolding protein α-actinin. Phosphorylation produces a conformational change, loosening the interaction between α-actinin and CPT-1B. As a result, malonyl CoA has improved access to its binding site and the sensitivity of CPT-1 to malonyl CoA is increased. Note that CPT-1B is anchored to the mitochondrial membrane whereas α-actinin is anchored to the cytoskeleton (Modified from Figure 10 of [117])

the sequestration of activated caspase-3 by caveolins [59]. These pro-survival effects provide the key to the beneficial effects of β1-adrenoceptor blockade on cardiac function. Although β1-adrenoceptor blockade inhibits CPT-1, it has no effect on CD-36 translocation and it therefore increases the cytoplasmic accumulation of long-chain acyl CoAs. It also does not prevent oxidative stress or the resulting DNA damage. The stimulus for cell damage therefore remains unaltered. β1-adrenoceptor blockade improves function by preventing the sequelae of this stimulus [59] (Fig. 6).

9 Clinical Significance

The fact that β1-adrenoceptor blockade improves cardiac function in diabetic hearts raises the question as to whether the drug should be used earlier in diabetic patients. However, its *in vivo* effects in this setting are equivocal. There are also clinical

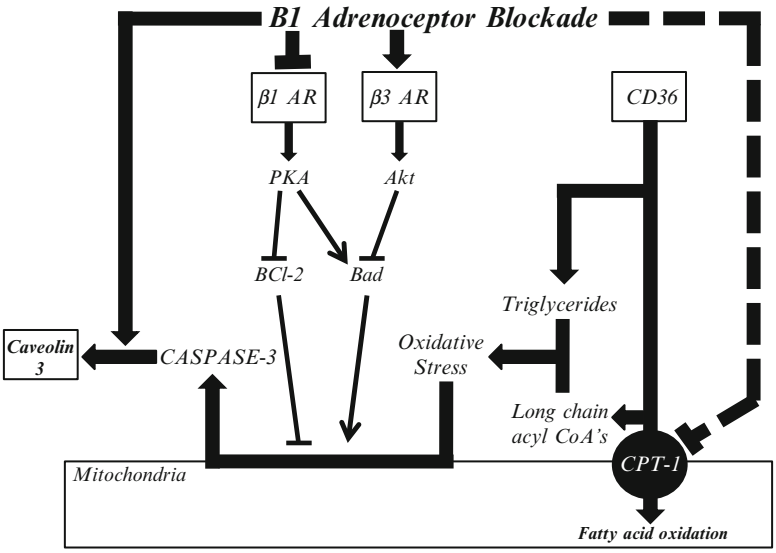

Fig. 6 Mechanisms of action of metoprolol. Metoprolol inhibits fatty acid oxidation by inhibiting carnitine palmitoyltransferase-1 (CPT-1), but has no effect on CD36. Triglyceride and long chain acyl CoA accumulation, and stimulation of oxidative stress, are therefore unaltered. Metoprolol also promotes β3 adrenoceptor signaling, leading to inhibition of Bad and stimulation of BCl-2, and inhibition of caspase-3 activation. Finally, metoprolol stimulates sequestration of caspase-3 by caveolins. The net effect is a prevention of caspase-3 activation (Modified from Figure 7 of [73])

concerns with the use of β-blockers in diabetic patients which need to be carefully weighed against the benefits of introducing the drug so early. First and foremost are concerns about the effects of β-blockers on glycemic control. The use of β-blockers as antihypertensive agents has been associated with an increased risk of new-onset diabetes [119]. Hepatic glucose output is controlled by the β2 adrenoceptor, and blockade of this receptor, which does occur with the β1-selective agents, lowers hepatic glucose output, delaying recovery from hypoglycemia [120, 121]. Attenuation of hypoglycaemic symptoms by β-blockade is no longer considered to be a problem, because sweating and paresthesias are preserved, and these are signs which patients can be taught to recognize [121, 122]. Another concern with chronic β-blockade is the presence of sustained unopposed α1-adrenoceptor stimulation, because activation of the sympathetic nervous system by hypoglycemia can increase unopposed α1-adrenoceptor stimulation to the point where a hypertensive crisis occurs [121]. Also, unopposed α1-adrenoceptor stimulation produces peripheral vasoconstriction which could worsen peripheral vascular disease and, by decreasing muscle flow, also worsen insulin resistance [12, 123]. These concerns are not sufficient to deny β-blockers to patients with systolic heart failure because these drugs are lifesaving in this setting. However, the risks and benefits of earlier β-blocker use must be weighed carefully. There is currently no evidence on which to base these considerations.

10 Conclusion

The range of effects produced by β-adrenergic blockade are broad and illustrate how interconnected the signalling pathways of function and metabolism are in the heart. Our initial hypothesis that inhibition of fatty acid oxidation would be a key mechanism of action was disproved. However, unexpected results have led us to some new and hitherto unexpected regulatory mechanisms of cardiac metabolism. The first is USF-2-mediated repression of PGC-1α, most likely occurring as a consequence of improved function. The second is the identification of covalent modifications which directly regulate CPT-1 at the level of the mitochondria. We also found that β-adrenergic signalling interacts with caveolins, which could be a key mechanism of action of β-adrenergic blockade. Our experience of studying this labyrinthine signalling web illustrates that it is not necessary for initial hypotheses to be correct, and all ends foreseen, in order for valid lines of inquiry to be opened and new information revealed.

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The Myocardial Creatine Kinase System in the Normal, Ischaemic and Failing Heart

Craig A. Lygate and Stefan Neubauer

Abstract The creatine kinase (CK) system is the final step in cardiac energy metabolism providing a direct link between energy production in the mitochondria and energy utilising ATPases. It acts as an energy storage and transport mechanism and maintains favourable local ATP/ADP ratios, thereby supporting further energy production and high levels of free energy from ATP hydrolysis. Down-regulation of CK activity and myocardial creatine levels is a universal finding in chronic heart failure, and the degree of impairment has been shown to be an excellent prognostic indicator in patients. However, it is unclear whether these changes represent epiphenomenon or contribute to disease pathophysiology. This chapter focuses on attempts over the past 20 years to address this question using genetic loss-of-function models in the mouse. Findings from these models have been equivocal and at times contradictory, however, recent evidence suggests that loss of creatine or CK is not detrimental in surgical models of chronic heart failure, providing the clearest evidence to date that such changes do not contribute to dysfunction. Despite this conclusion, over-expression of CK in mouse heart has been found to protect against heart failure and improve survival. In the setting of ischaemia-reperfusion injury, loss of creatine or CK impairs functional recovery and augmentation of either is cardioprotective. We are therefore entering an exciting new era of research in this field aimed at understanding the benefits of CK system augmentation and identifying new mechanisms to achieve this without genetic modification for possible future clinical translation.

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1 Introduction

1.1 The Phosphocreatine/Creatine Kinase System in the Healthy Heart

All vertebrates use creatine kinase as a phosphagen system in the heart, the role of which is to ensure continuity of energy supply in the face of fluctuating demands via the buffering and transport of high-energy phosphates. The utility of this system is based on the intracellular compartmentation of the reactants (Fig. 1) [1]. The forward reaction occurs at the mitochondrial membrane where sarcomeric mitochondrial-creatine kinase

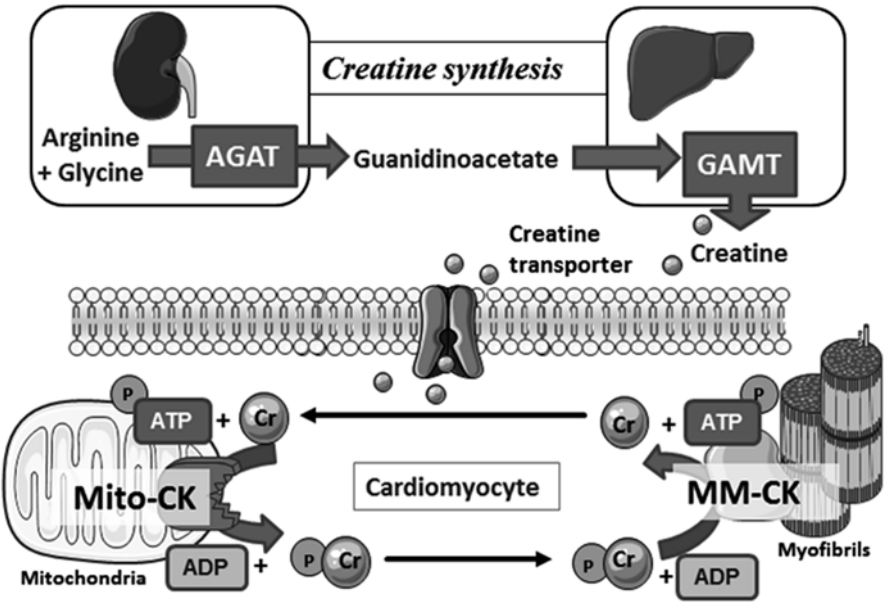

Fig. 1 Schematic showing creatine synthesis pathway and the creatine kinase system in cardiomyocytes. Creatine can be biosynthesised via arginine:glycine amidinotransferase (AGAT), found principally in the kidneys, which catalyses the formation of guanidinoacetate from arginine and glycine. Guanidinoacetate is then methylated, predominately in the liver, by guanidinoacetate methyltransferase (GAMT) to form creatine. Creatine is taken up into the cardiomyocyte by a specific plasma membrane creatine transporter, where mitochondrial creatine kinase (Mito-CK) catalyses the transfer of a phosphoryl group from ATP to form phosphocreatine (PCr). PCr acts as an energy buffer and transport molecule with the reverse reaction catalysed by muscle-CK (MM-CK) to liberate ATP at times of high energy demand. Created using Servier Medical Art by Servier which is licensed under a Creative Commons Attribution 3.0 Unported License http://www.servier.com/slidekit

(Mt-CK) catalyses the transfer of a phosphoryl group from ATP onto creatine to form phosphocreatine (PCr), which accumulates to high levels within the cytosol. Compared to ATP, PCr is relatively small and less polar and therefore more readily diffusible. In this way the CK system acts as both a spatial and temporal energy buffer, with PCr providing a mobile form of high-energy phosphate storage and transport for near instantaneous regeneration of ATP at times of rapidly increasing demand. This reverse reaction is catalysed by cytosolic dimers of CK which are also found closely coupled to ATPases, e.g. the myosin ATPase [2]. In this way local metabolites are maintained at favourable levels, i.e. low [ATP/ADP] ratio at the mitochondria to stimulate oxidative phosphorylation, and relatively high (ATP/ADP) ratio at the ATPases to ensure maximal energy is available from the hydrolysis of ATP (i.e. ΔG_{ATP} is high) [3–5].

1.2 Origin of Creatine and CK in the Heart

The heart expresses four isoenzymes of CK, with Mt-CK and MM-CK by far the most abundant (35 % and 67 % of total CK activity in human heart, respectively) [6]. However, the brain isoform is also expressed and can form homo-dimers or dimerize with the muscle-isoform to give the low abundance cytosolic isoenzymes BB-CK and MB-CK.

Creatine is either obtained from the diet or via a two-step biosynthetic pathway (see Fig. 1) [2]. The proteins required for creatine biosynthesis are not expressed in cardiomyocytes; therefore creatine must be actively taken-up from the bloodstream against a large concentration gradient via a specific creatine transporter located in the plasma membrane (CrT: SLC6A8).

1.3 Scope of this Chapter: Lessons from the Genetic Era

Knockout mouse models have been described for all CK isoforms and also for loss-of-creatine models, CrT, GAMT and AGAT. In this review we seek to bring the story up-to-date by critical appraisal of what has been learnt from these mouse models, with particular reference to the role of CK system impairment as a putative causal factor in chronic heart failure and the potential for CK system augmentation as a novel therapeutic strategy for the treatment of cardiac disease.

2 Observations that Implicate a Role for the PCr/CK System in Heart Failure

The observation that myocardial creatine content and CK activity are significantly lower in the failing heart raises the possibility that such changes may have a direct role in disease progression. These findings occur regardless of disease aetiology or

species, and the examples are numerous (see [7] for a summary). It should be noted that ATP is maintained at near normal levels in the failing heart due to the buffering capacity of PCr and only reduces significantly during end-stage disease as a result of cumulative loss of adenine nucleotides across the plasma membrane [3, 8]. Loss of creatine during heart failure is likely to occur secondary to reduced expression of the CrT [7, 9] (i.e. steady cellular loss but reduced uptake), although the signalling pathways governing this have yet to be established. Similarly, the mechanisms behind loss of CK activity are not yet known and would be a fruitful area for further research.

A significant relationship between maximal CK reaction velocity (the product of CK activity and [Cr]) and ventricular function has been observed in several models of heart failure (e.g. in mice [7] and Syrian cardiomyopathic hamsters [10]). The ratio of PCr to ATP measured by ^{31}P-MRS is often used since absolute values are difficult to obtain and is a sensitive indicator of energetic status. In a murine model of transverse aortic constriction (TAC), low PCr/ATP measured at 3 weeks was predictive of chamber dilatation observed at 6 weeks [11], suggesting that energetic changes precede adverse LV remodelling and therefore could have a causal role. Particularly convincing is the correlative evidence from observations of PCr/ATP ratio in the clinical setting, where low PCr/ATP is associated with more severe heart failure in patients with dilated cardiomyopathy, correlating with New York Heart Association (NYHA) class [12], ejection fraction and wall thickness [13]. In a 2.5 year prospective follow-up study, PCr/ATP was found to be a better prognostic indicator of mortality than ejection fraction [14]. However, the question remains whether these are simply very good biomarkers, or whether loss of creatine and CK activity plays an active role in disease pathophysiology.

3 Loss-of-Function Models

Loss-of-function represent a standard experimental paradigm used to imply causality. For example, the importance of PCr/CK system down-regulation in the progression of heart failure would be inferred by the recapitulation of a failing phenotype when the system is ablated in otherwise normal animals.

3.1 Pharmacological Inhibition Studies

1. A number of creatine analogues have been shown to compete with creatine for cellular uptake at the CrT, but are poor substrates for the CK reaction [2]. Of these, the most commonly used is β-guanidinopropionic acid (β-GPA), see [15] for a recent systematic review. β-GPA can be given in chow or water, however, loss of creatine from the myocardium is a slow process (~2 % of the total creatine pool per day [16]), therefore β-GPA has to be chronically dosed over a period of weeks, which may allow time for substantive compensatory adaptations to develop (for examples see [17, 18]). A further limitation is that creatine loss is

incomplete (residual creatine 10–50 % of starting values). This is an important point since cardiac work is maintained over a wide-range of creatine levels and only drops significantly when total [Cr] falls below a threshold value of ~15 % of control, and even then, only under high workload conditions [19]. This may go some way to explain the variability in findings using this approach. Furthermore, there are potentially confounding systemic effects of creatine inhibition, for example, practically all studies using β-GPA report reduced body weight by ~10–15 %. Despite these limitations, there is general agreement that dysfunction becomes apparent or is exacerbated at higher workloads.

2. Iodoacetamide (IA) is a rapid irreversible inhibitor of CK activity, affecting all CK isoenzymes to an equal extent [20]. It is an alkylating agent that prevents formation of disulphide bonds and is therefore likely to have diverse activity on multiple protein targets, as such, it is too toxic for chronic dosing and has been used in acute studies only (mostly perfused heart). Experimental findings have been highly variable, but, in general, the effects of CK ablation are mostly observed at high workloads. Of particular note is a study by Tian et al. who used different doses of IA in isolated perfused rat hearts to demonstrate dose-dependency between CK inhibition and dysfunction, establishing a role for the CK system in setting contractile reserve [21].

3.2 Evidence from CK Knockout Studies

Mice deficient in creatine kinase (CK) have been described for the myofibril-bound (M-CK$^{-/-}$) [22], the sarcoplasmic mitochondrial isoenzyme (Mt-CK$^{-/-}$) [23], and for the combined double knockout (M/Mt-CK$^{-/-}$) [24]. There is broad agreement in the literature that M-CK$^{-/-}$ mice do not exhibit any discernible cardiac dysfunction or remodelling, either at baseline or at higher workloads, regardless of whether measured in isolated fibres, *ex vivo* perfused heart or *in vivo* [25–29].

Ex vivo—Numerous studies have failed to observe a functional deficit in CK knockout mice *ex vivo* (e.g. [25–27, 30–33]), and it may be that the workloads attainable are simply too low, even under maximally stimulated conditions. This is despite significantly impaired energetics e.g. lower PCr, reduced ATP synthesis rate [34], and low ΔG_{ATP} at high workloads [27].

In vivo findings have produced mixed results. Echocardiography showed impaired contractile reserve in M/Mt-CK$^{-/-}$ mice compared to C57BL/6 controls under one anaesthetic regime, but not another [35]. Lower systemic blood pressure has been reported in Mt-CK$^{-/-}$ when compared to either controls, M-CK$^{-/-}$, or M/Mt-CK$^{-/-}$ [28]. However, subsequent measurements by aortic cannulation have not observed any differences in systolic or diastolic pressures [35, 36]. By 41 weeks of age both Mt-CK$^{-/-}$ and M/Mt-CK$^{-/-}$ mice were found to have significant LV dilatation, hypertrophy, and impaired perfusion using MRI, although with normal ejection fraction [28, 37].

All three CK$^{-/-}$ strains were generated on a mixed genetic background of C57BL/6 and 129/Sv, and it seems likely that much of the variability in the published

literature is related to genetic drift combined with differences in gender and age. Our laboratory has compared mixed and pure genetic backgrounds at 1 year of age under identical experimental conditions. Male M/Mt-CK$^{-/-}$ mice with a mixed background had overt hypertrophy and congestive heart failure, whereas females only had LV dysfunction [29]. In comparison, M/Mt-CK$^{-/-}$ mice on a pure C57BL/6 background had normal LV dimensions, absence of LVH, and only mild functional impairment [29]. These results show that a primary defect in the PCr/CK system (i.e. loss of CK activity) is sufficient to drive the heart into failure. However, it also shows that this deterioration is progressive, occurring over a prolonged period of time, perhaps suggesting that initial adaptive mechanisms are not sustainable in the long run.

3.3 Evidence from "Cr Knockouts"

GAMT knockout mice lack the second essential enzyme in creatine biosynthesis and therefore have a whole body creatine deficiency. This has profound effects on body weight with GAMT$^{-/-}$ mice considerably lighter than controls despite normal activity and food intake [38]. In the initial description, GAMT$^{-/-}$ mice had creatine levels ~27 % of control values in the heart, which was later attributed to coprophagia of wild-type faeces [39]. When housed separately from wild-type mice, myocardial creatine and PCr levels are undetectable, while ATP is unaffected [40]. However, GAMT$^{-/-}$ mice accumulate the creatine pre-cursor guanidinoacetate, which is phosphorylated in the CK reaction to form phospho-guanidinoacetate (P-GA). This may partially compensate for the loss of PCr in an acute setting, since P-GA is apparently used up during ischaemia [39, 40], however, regeneration of P-GA is insufficient to aid recovery [40]. Saturation transfer experiments have since shown that the rate of phospho-transfer from ATP to P-GA is below the limits of detection suggesting that the kinetics of this reaction are too slow to be useful under most conditions [39, 41].

GAMT$^{-/-}$ mice do not develop cardiac hypertrophy and there were no differences in LV structure and function under baseline conditions with the exception of low LV systolic pressure. However, in general agreement with β-GPA feeding studies, contractile reserve was blunted in GAMT$^{-/-}$ mice [40]. Our laboratory has gone to considerable lengths to identify compensatory adaptations, but without success. For example, adenylate kinase, AMPK activation and mitochondrial organisation and respiration are all unaffected [41, 42]. We also took a non-biased approach using 2-D difference in-gel electrophoresis and quantitative mass spectrometry to compare proteomes of GAMT$^{-/-}$ and WT hearts. There were no significant differences in any of the 546 proteins detected [41]. It is not possible to prove the absence of adaptations, however these findings rule out the adaptations previously suggested for CK knockout mice and after β-GPA feeding [17].

AGAT KO—Mice with ablation of the first essential enzyme in creatine biosynthesis have the theoretical advantage that there is no accumulation of the potentially

confounding creatine pre-cursor guanidinoacetate. These mice also have a low body fat phenotype and this was associated with protection against metabolic syndrome when mice were exposed to high-fat feeding [43]. The cardiac phenotype of AGAT$^{-/-}$ has yet to be described.

Comment on loss-of-function models: The current consensus view seems a reasonable one and is supported by the largest body of evidence, i.e. that loss or impairment of the CK system is generally not essential to support normal cardiac function under baseline and low workload conditions, and that the PCr/CK system is only truly important to cardiac function at times of high demand or stress.

4 Consequences of PCr/CK System Ablation During Pathological Stress

4.1 Ischaemia/Reperfusion

A fully-functioning CK system is vitally important for recovery of cardiac pump function following ischaemia. Rat hearts perfused with iodoacetamide prior to hypoxic challenge are incapable of regenerating PCr and subsequently fail to recover [20]. Identical results have been obtained in the genetic ablation models. Both GAMT$^{-/-}$ and M/Mt-CK$^{-/-}$ mice are more susceptible to ischaemia/reperfusion injury than wild-type controls [33, 40]. For example, in M/Mt-CK$^{-/-}$ mice, systolic recovery was prolonged, LVEDP increased, and there were more instances of contracture. This was associated with greater accumulation of diastolic calcium suggesting a role for CK in maintaining calcium homeostasis under stress conditions [33].

4.2 Myocardial Infarction

The results from β-GPA feeding are unequivocal, with two independent studies both suggesting that creatine is obligate for surviving acute MI. Following permanent ligation of the left coronary artery, mortality in creatine-depleted rats ranged from 93 % after 60 min [44] to 100 % mortality at 24 h [45]. In stark contrast, GAMT$^{-/-}$ mice, with zero myocardial creatine, have normal survival in the 24 h following myocardial infarction (~90 %) [41]. This is unlikely to be due to the presence of P-GA in GAMT$^{-/-}$ since β-GPA can also be phosphorylated by CK with similar reaction kinetics [46]. Another explanation might be off-target effects of β-GPA, but mice injected with β-GPA prior to infarction have normal survival [41]. It seems likely that these apparently contradictory results can be explained by species differences in arrhythmogenicity, with ventricular arrhythmia increased post-MI in the β-GPA fed rats [44]. Increased mortality in the β-GPA studies does not therefore necessarily reflect an energetic deficit.

4.3 Chronic Heart Failure

Rats fed β-GPA starting immediately after permanent coronary artery ligation had a trend for more substantial LV hypertrophy at 8 weeks, but cardiac function was indistinguishable from the untreated infarct group despite myocardial ATP levels 18 % lower [45]. A disadvantage of this approach is that creatine levels gradually decline throughout the experiment, reaching 13 % of control values by the end of the study. However, these findings have been recapitulated in the GAMT$^{-/-}$ mouse. Six weeks after MI, knockout mice were indistinguishable from wild-type infarct mice in terms of survival, LV remodelling and cardiac function [41]. Loss of CK activity is also well tolerated in the failing heart. M/Mt-CK$^{-/-}$ mice survive myocardial infarction and the extent of cardiac remodelling and dysfunction was found to be similar to infarcted wild-types when studied 4 weeks post-MI using MRI [28, 37].

Conclusion: Four studies taking three different approaches have all shown that reducing creatine or CK to very low levels in rodent models of heart failure does not have any consequences in terms of survival, LV function or remodelling. This constitutes strong (albeit surprising) evidence that the down-regulation of PCr/CK consistently observed in the failing heart does not make a significant contribution to disease progression.

5 Augmenting the Cr/CK System in Heart Failure

The phenotype of knockout mice may be confounded by physiological redundancy and/or metabolic flexibility, which obscures the full significance of the protein being studied. However, the more clinically interesting question is whether CK system augmentation has promise as a therapeutic intervention, and this requires testing in relevant disease models.

5.1 Elevating [Cr] in Heart Failure

Our laboratory has produced mice constitutively over-expressing the creatine transporter (CrT-OE) resulting in mice with variable levels of creatine and phosphocreatine in the heart [47]. Very high levels of total creatine (>2-fold above wild-type) were found to be detrimental resulting in hypertrophy, dilatation and progressive dysfunction [47]. This was due to an inability to keep the enlarged creatine pool adequately phosphorylated, which adversely affects the energy available from ATP hydrolysis [47]; and reduced expression of enolase resulting in impaired glycolytic capacity [48]. However, levels of creatine up to twofold (<140 nmol/mg protein) are well tolerated even in ageing mice [49], and, using *in vivo* ^{1}H-MRS [50], we therefore pre-selected mice within this range before subjecting them to permanent coronary artery ligation. Six weeks later both WT and CrT-OE groups developed LV

remodelling and chronic heart failure, but there was no benefit associated with maintaining elevated creatine levels throughout. While creatine alone was not beneficial in heart failure, *in silico* modelling suggests that combination with other metabolic approaches could yet prove energetically favourable [51].

5.2 Elevating CK in Heart Failure

Mice overexpressing M-CK have been created using a "tet-off" conditional expression approach (M-CK-OE). With the transgene switched on, total CK activity and flux was significantly increased without altering PCr and ATP concentrations or baseline cardiac function [52]. Following transverse aortic constriction (TAC), *in vivo* CK flux remained elevated in M-CK mice throughout development of heart failure and PCr/ATP ratio was better preserved. This was associated with higher indices of contractile function and significantly improved survival. In an elegant demonstration of cause and effect, the protective effect was lost when the transgene was switched off [52]. This represents the first direct evidence that augmentation of the CK system can be therapeutically beneficial in the failing heart.

6 Augmenting Cr/CK System in Ischaemia/Reperfusion

6.1 Moderately Increasing Myocardial Creatine is Beneficial

Our laboratory tested CrT-OE mice using an *in vivo* model of 45 min ischaemia and 24 h reperfusion. CrT-OE mice had 27 % less myocardial injury than control mice correlating with [Cr] levels suggesting a "dose-dependent" protective effect. CrT-OE mice also exhibited significantly improved functional recovery following 20 min global ischaemia *ex vivo* (Fig. 2) [49]. We have identified the key mechanisms behind this beneficial effect [49]: (1) Baseline myocardial PCr levels were 49 % higher in CrT-OE mice, thereby delaying ischaemic onset, and PCr recovery was more rapid and complete at reperfusion. (2) CrT-OE mice maintained a more favourable ATP/ADP ratio resulting in higher ΔG_{ATP}. (3) CrT-OE mice had baseline levels of myocardial glycogen 2.8-fold above WT, which may in itself be protective [53]. (4) Creatine reduced the opening probability of the mitochondrial permeability transition pore (mPTP) when tested in HL1 cells.

6.2 Over-Expression of M-CK is Beneficial

A similar beneficial effect has recently been described in M-CK-OE [54]. Isolated perfused hearts were subjected to 25 min global no-flow ischaemia and 40 min reperfusion. M-CK-OE hearts had significantly better functional recovery (65 % of

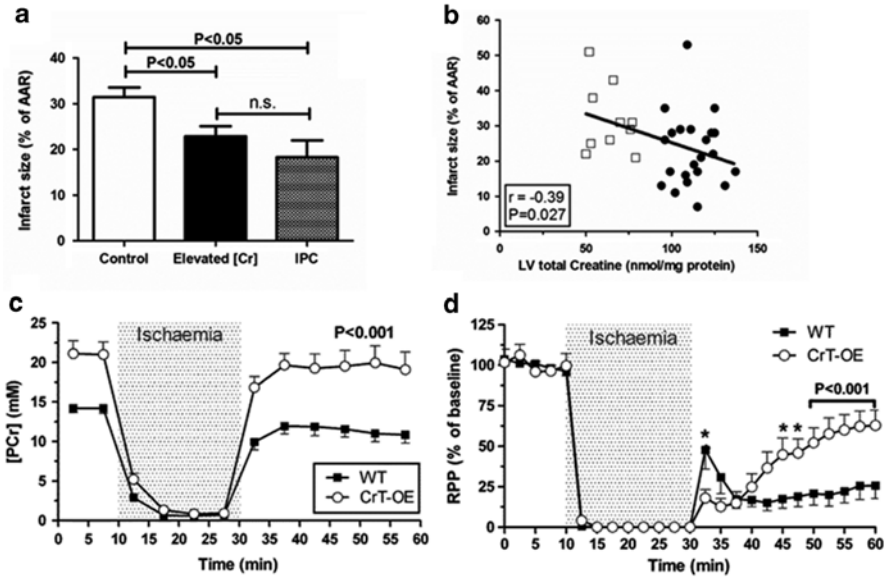

Fig. 2 Creatine transporter overexpressing mice (CrT-OE) are protected from ischaemia-reperfusion injury. *In vivo*: (**a**) Mice with elevated myocardial creatine subjected to 45 min ischaemia and 24 h reperfusion had 27 % smaller infarct size compared to control mice, a reduction comparable in effect to ischaemic post-conditioning (IPC). (**b**) There was a significant inverse correlation between infarct size and myocardial creatine levels. *Ex vivo*: (**c**) Isolated perfused CrT-OE hearts had elevated phosphocreatine at baseline and during reperfusion, which resulted in greatly improved functional recovery (**d**) compared to wild-type (WT). RPP is rate pressure product, i.e. heart rate x developed pressure. Reproduced from [49] with permission from Oxford University Press on behalf of the European Society of Cardiology

baseline vs. 14 %) and reduced LDH release indicating less cellular damage. The rate of ATP synthesis via CK was higher in transgenic mice, both at baseline and during recovery, resulting in reduced acidosis during ischaemia, and greatly improved re-synthesis of PCr.

Comment: Both augmentation of substrate (creatine) and enzyme (M-CK) have now independently shown promise as cardio-protective agents in I/R. Much additional work remains to be done in this area. For example, studies need to be repeated under more clinically relevant conditions, e.g. in older animals with co-morbidities. There are other obvious related targets such as testing the combination of elevated substrate + enzyme and over-expression of other CK isoenzymes, e.g. Mt-CK and B-CK. Mt-CK is of particular interest in I/R injury since creatine has an inhibitory effect on mPTP opening, with mito-CK localisation within the inter-mitochondrial membrane obligate for this effect [55].

Clinical translation remains a long way off and the lack of pharmacological tools for activating CrT and CK activity is a major limiting factor. This will require a much better understanding of the normal physiological control of creatine levels and CK activity in the heart, and non-biased approaches may be particularly useful

in this regard. For example, we recently used gene-expression profiling to identify thioredoxin interacting protein (Txnip) as an endogenous inhibitor of CrT activity, which suggests a potential role for redox regulation [56].

7 Conclusions

Observational studies in animal models and patients clearly suggest a causal role for the CK system in the development of heart failure. However, evidence from knockout mice is equivocal in this regard and difficult to interpret. CK system deficiency probably limits contractile reserve, but on the whole, does not readily recapitulate a heart failure phenotype. The one exception is the M/Mt-CK$^{-/-}$ mouse, but only in ageing male mice with permissive genetic backgrounds. Since equivalent creatine loss is much better tolerated, we should be open to the possibility that the CK proteins may have other, as yet undefined, functions within the cell. The strongest evidence against causality is that loss of CK activity or creatine does not affect outcome in chronic heart failure models. It could be argued that this simply reflects physiological redundancy or the development of compensatory adaptations, but in that case, why don't similar adaptations occur in the failing heart. Ultimately, the argument over causality has been side-lined by the findings that CK or creatine augmentation can be cardio-protective in ischaemia/reperfusion injury and that M-CK overexpression is beneficial in heart failure. This heralds a new and exciting phase of research with the opportunity to focus on translational aspects of creatine and CK augmentation in the heart.

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Fuel Metabolism Plasticity in Pathological Cardiac Hypertrophy and Failure

Stephen C. Kolwicz and Rong Tian

Abstract The cardiac metabolic network is a highly flexible system that adapts to the environment to maintain a high capacity for ATP production. During the development of pathological cardiac hypertrophy, a significant remodeling of metabolic pathways leads to a disruption in energy homeostasis, which contributes to the eventual heart failure. These changes include a shift of substrate preference from fatty acids to glucose, a reduction in the overall oxidative capacity, and a depletion of high energy phosphate content of the heart. Recent studies, using both pharmacological and genetic approaches, have focused on the functional significance of these metabolic changes and have suggested that the loss of metabolic flexibility is a key contributor to the development of cardiac dysfunction. This chapter will focus on advances in the understanding of cardiac metabolic plasticity in pathological cardiac hypertrophy and heart failure as well as therapeutic strategies based on these observations.

Keywords Glucose • Fatty acid • Substrate metabolism • Myocardial energetics • Metabolic flexibility • Metabolic capacity • Mitochondria

1 Introduction: The High Capacity and Flexibility of Cardiac Fuel Metabolism

To maintain its mechanical function, the cardiomyocyte requires a continually high level of energy supply and, since it never stops beating, the heart has the highest oxygen consumption rate in the body. Mitochondria occupy ~30 % of the volume of a cardiac myocyte, consistent with the high oxidative capacity. For humans with

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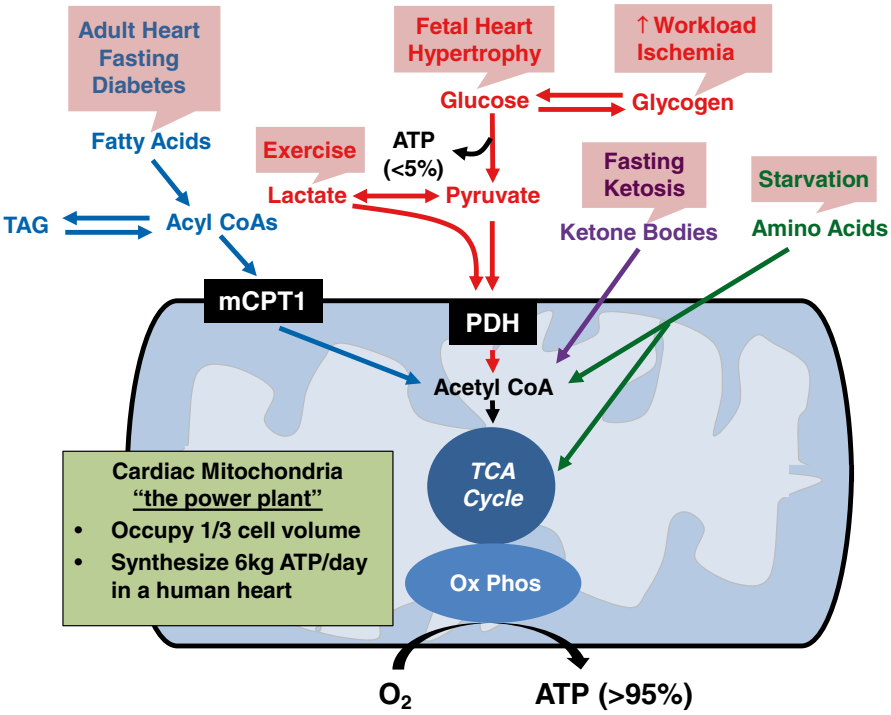

Fig. 1 Overview of the metabolic network. The energy-yielding substrates (fatty acids, glucose, ketones, and amino acids), via specific catabolic pathways, converge on acetyl CoA production with subsequent entry into the tricarboxylic acid (TCA) cycle. The final step of energy transfer is accomplished through oxidative phosphorylation (OxPhos), supplying greater than 95 % of ATP consumed by the heart. The boxes (in pink) above each metabolic pathway indicate the pathological and/or physiological condition in which the specific substrate becomes a predominant contributor to metabolism. *TAG* triacylglycerol, *mCPT1* muscle form of carnitine palmitoyl transferase, *PDH* pyruvate dehydrogenase, O_2 oxygen. F. Reproduced with permission from [86]

average daily activity, ~6 kg of ATP is generated and consumed by the heart each day [1]. Despite the immense ATP turnover, the high energy phosphate storage within the cardiomyocyte is minimal, sufficient to sustain the heart beat for only a few seconds. Therefore, a tight coupling of ATP production and consumption is vital for normal heart function. ATP synthesis involves chemical reactions through complex metabolic pathways that supply and process carbon substrates in the mitochondria for oxidative phosphorylation (Fig. 1). To sustain an uninterrupted high energy supply, the cardiac metabolic network must be extremely versatile, capable of metabolizing all carbon substrates (i.e. lipids, carbohydrates, ketone bodies and amino acids).

A salient feature of cardiac metabolism is its adaptability throughout the life cycle as well as during physiological or pathological stressors (Fig. 1). *In utero*, the fetal heart relies on carbohydrate substrates for ATP generation [2]. Increased oxygen availability after birth, in parallel with increased mitochondrial volume and elevated serum fatty acids (FA) levels, renders fatty acid oxidation (FAO) the dominant energy

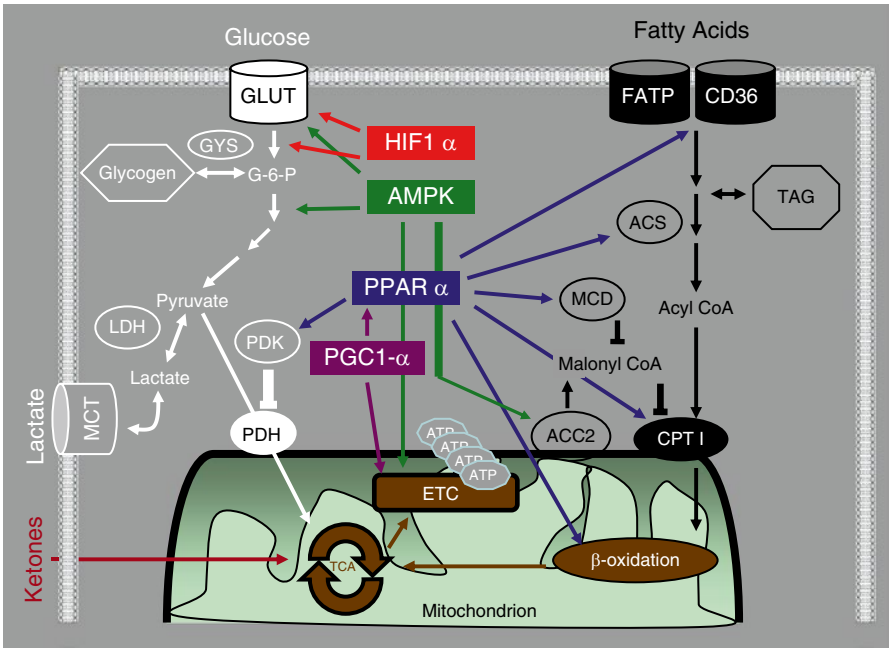

Fig. 2 Regulation of cardiac metabolism. Highlighted in solid colors are key regulatory sites for carbohydrates and lipids metabolism. The molecular targets of several transcriptional and/or signaling pathways in the regulation of substrate selection are also illustrated. *ACC2* acetyl CoA carboxylase 2, *ACS* acyl-coA synthetase, *AMPK* adenosine monophosphate-activated protein kinase, *ATP* adenosine triphosphate, *CD36* cluster of differentiation 36 (fatty acid transporter), *CPT1* carnitine palmitoyl transferase I, *ETC* electron transport chain, *FATP* fatty acid transport protein, *G-6-P* glucose-6-phosphate, *GLUT* glucose transporter, *GYS* glycogen synthase, *HIF1α* hypoxia-inducible factor 1-alpha, *LDH* lactate dehydrogenase, *MCD* malonyl CoA decarboxylase, *MCT* monocarboxylate transporter, *PDH* pyruvate dehydrogenase, *PDK* pyruvate dehydrogenase kinase, *PGC1-α* peroxisome proliferator-activated receptor-gamma coactivator 1 alpha, *PPARα* peroxisome proliferator-activated receptor alpha, *TAG* triacylglycerol, *TCA* tricarboxylic acid cycle. Reproduced with permission from [87]

source [3]. During fasting or diabetic conditions, the adult heart can further increase its dependency on FA [4, 5]. This contrasts the hypoxic or failing heart, where the relative use of carbohydrate, particularly glucose, is increased [6, 7]. Although the focus of cardiac metabolism has centered on the use of glucose and FA the heart can utilize ketone bodies, lactate, and endogenous substrates, i.e. glycogen and triglyceride, as fuel (Fig. 1). For example, oxidation of lactate becomes predominant during exercise as the skeletal muscle lactate production increases [7, 8] while prolonged fasting or ketogenic diets increases serum ketone bodies levels which promotes their utilization by the heart [9]. These observations underscore the flexibility of cardiac metabolism in response to a variety of environmental changes.

A number of mechanisms are responsible for modulating metabolic pathways at the molecular level to promote adaptations (Fig. 2). Transcriptional regulation of proteins involved in FAO by the peroxisome proliferator-activated receptor alpha (PPAR)/

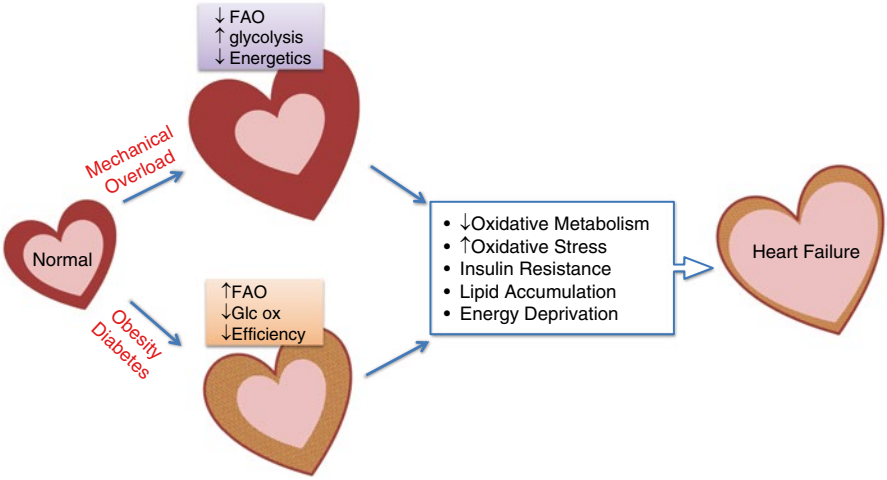

Fig. 3 Metabolic remodeling and the development of heart failure. Pathological hypertrophy in response to mechanical overload, e.g. hypertension, valvular disease or post-MI, is accompanied by metabolic remodeling characterized by decreases in fatty acid oxidation (FAO) and increases in glycolysis. This fetal-like metabolic profile decreases the capacity for ATP synthesis, consistent with the "energy starvation" model. In contrast, the elevated supply of substrates in the heart of obese and/or diabetic individuals leads to an upregulation of FAO with a concomitant decrease in glucose oxidation (Glc ox). This lipid overload condition impairs cardiac efficiency. Regardless of the precipitating factor, the persistent metabolic derangements elicit commonalities of decreased oxidative metabolism, increased oxidative stress, insulin resistance, lipid accumulation, and energy deprivation, all contributing to the progression of heart failure. Reproduced with permission from [86]

estrogen-related receptor (ERR)/peroxisome proliferator-activated receptor gamma co-activator 1 (PGC-1) circuit is a major mechanism responsible for the transition of the glycolysis-dependent fetal heart to oxidative metabolism in the adult heart [10–12]. Likewise, transcriptional regulation by HIF1α governs metabolic adaptations to hypoxia and ischemia [13]. Activation of AMP-activated protein kinase (AMPK) during energy deprivation stimulates, via an array of downstream targets, substrate catabolism for ATP production and inhibits anabolism to reduce energy expenditure [14]. These findings provide an important basis for the consideration of metabolic modulations as a means to maintain or improve cardiac function.

2 The Shift of Fuel Selection in Cardiac Hypertrophy and Failure

It is widely recognized that pathological hypertrophy is associated with the reappearance of the fetal gene expression pattern [15], showing decreased FAO and increased reliance on carbohydrates [6, 7, 16]. The hallmark of increased glucose metabolism in the hypertrophied heart is accelerated glycolysis [7, 17, 18] (Figs. 1 and 3). Consistent with this, several studies observed elevated glucose uptake in animal

models of cardiac hypertrophy [18–21]. Interestingly, alterations in expression or capacity of glycolytic enzymes do not consistently coincide with increased glycolysis [18, 20] suggesting that the altered glycolytic flux is attributable to altered regulation. Despite increased glycolysis many studies have not identified a concomitant increase in glucose oxidation indicating that "uncoupling" of glycolysis and glucose oxidation exists in cardiac hypertrophy [7, 22, 23]. Furthermore, increased activity of lactate dehydrogenase (LDH), the enzyme responsible for the conversion of pyruvate to lactate, and elevated efflux of lactate from the myocardium has been documented [24, 25] without a change in the overall rate of lactate oxidation [7, 26, 27].

Pyruvate can also contribute to the tricarboxcylic acid (TCA) cycle through accessory pathways termed "anaplerosis" [28]. Specifically, carboxylation of pyruvate by pyruvate carboxylase (PC) or malic enzyme (ME) yields oxaloacetate or malate, respectively. In hypertrophied rat hearts, an 80–90 % increase in anaplerotic flux was observed and supported with increased tissue content of malate [29, 30]. A significant increase in the gene expression of malic enzyme without a change in pyruvate carboxylase [30] supported the idea of increased anaplerosis through the pyruvate-malate pathway. Although increased anaplerotic flux in hypertrophied myocardium is sufficient to maintain overall TCA cycle flux, it reduces the efficiency of ATP production from pyruvate.

Cardiac hypertrophy and dysfunction have been increasingly noted in individuals with diabetes and obesity, initiating the term "metabolic cardiomyopathy". The metabolic profile of this cardiomyopathy is characterized by increased FA uptake and oxidation associated with reduced glucose oxidation [4, 31, 32] (Figs. 1 and 3). One undesirable effect of high FAO is the lower oxygen efficiency [33, 34], as well as the increased presence of FA derivatives that may further reduce efficiency by promoting mitochondrial uncoupling, resulting in increased myocardial oxygen consumption and increased oxidative stress [4, 35, 36]. A unique aspect of cardiac metabolism in obesity and diabetes is that substrate supply exceeds the need for ATP synthesis. Despite elevated FAO, hearts of obese and diabetic individuals accumulate a significant amount of lipid (Fig. 3). A positive correlation of cardiac lipid accumulation and cardiac dysfunction exists and gives rise to the term "lipotoxic cardiomyopathy" [37–39]. Studies show that increases of lipid supply in animal models of cardiac lipotoxicity exceed the increases in the rate of oxidation, which eventually leads to downregulation of FAO, accumulation of toxic lipid intermediates, and contractile failure [39, 40]. Thus, regardless of the etiology, metabolic derangements in heart failure converge on the impairment of oxidative metabolism, increased oxidative stress, and energy starvation (Fig. 3).

3 Potential Mechanisms for Substrate Preference Switch in Pathological Cardiac Hypertrophy

Transcriptional regulation of genes involved in mitochondrial oxidative metabolism changes significantly during the development of pathological hypertrophy and heart failure (HF) [41]. Downregulation of PPARα and PGC-1α, master regulators of

genes involved in FAO and mitochondrial biogenesis, have been observed in rodent models of cardiac hypertrophy and failure [26, 42–44]. The PPARα target, carnitine-palmitoyl transferase 1 (CPT1), which facilitates FA transport into the mitochondria (Figs. 1 and 2), is also down-regulated [20, 30, 44, 45]. Moreover, it has been shown that the liver isoform of CPT1 (LPCT1), which is highly expressed in the fetal heart, increases in pressure-overload hypertrophy [30]. Medium chain acyl-CoA dehydrogenase (MCAD), an enzyme important for beta-oxidation, is also reduced [20, 26, 44]. Decreases in plasma membrane bound fatty acid transporters [46, 47] and carnitine, which is necessary for transport of fatty acyl CoAs into the mitochondria have been noted [22]. Therefore, an overall reduction in the supply of cytosolic and mitochondrial FA may be responsible for hampered FA metabolism in cardiac hypertrophy.

Another potential regulatory mechanism is the activation of the intracellular energy-sensor, AMPK, which is triggered by impaired myocardial energetics in pathological hypertrophy [18, 20, 48]. Increased AMPK activity promotes translocation of glucose transporters to the plasma membrane and enhances glucose uptake (Fig. 2) [18]. In addition, AMPK stimulates glycolysis by phosphorylation and activation of phosphofructokinase 2 (PFK2), which generates fructose-2,6-diphosphate that allosterically stimulates the rate limiting enzyme of glycolysis, PFK1 [49]. The AMPK mechanism is consistent with the observation that increased glucose uptake and glycolysis is insulin-independent in cardiac hypertrophy.

Thus far, our understanding of metabolic adaptations in hypertrophied or failing hearts has been garnered from animal models. In this regard, translation from animal studies to the human population remains a serious challenge. For example, downregulation of PPARα is not evident in human HF and the expression level of PGC-1 is variable with the most recent reports showing a down-regulation of ERRα rather than PGC-1 [50, 51]. Although evidence that the human failing heart is energy "starved" suggests a strong link to mitochondrial dysfunction [52], mechanisms contributing to altered substrate metabolism in human HF is much more complex than in animal models. Assessment of myocardial glucose and FA uptake and utilization is limited to positron emission tomography (PET). The PET study is not routinely used clinically and results are influenced by myocardial perfusion, plasma FA levels, and insulin sensitivity. A number of conflicting results have been reported in HF patients depending on etiology and co-morbidity [32, 53–55]. Therefore, further experimentation elucidating the differences in animal and human models is warranted.

4 The Functional Consequence of Altered Substrate Preference in Hypertrophied Hearts

Past studies have repeatedly questioned whether a switch in substrate preference is adaptive or maladaptive in pathological hypertrophy. Subsequent investigations of metabolic cardiomyopathy generated the observations of "glucotoxicity",

"lipotoxicity" and "glucolipotoxicity" suggesting that excessive substrate is potentially harmful to the heart [56]. The availability of bioengineered mouse models with up- and down-regulations of specific pathways for substrate metabolism has provided powerful tools to decipher the underlying mechanisms for these observations. It also facilitated recent studies addressing metabolic flexibility and the balance between substrate supply and utilization.

4.1 The Shift of Preference: Is there a Better Substrate?

The benefit of increased glucose utilization has been attributed to its higher oxygen efficiency for ATP synthesis compared to FAO [33, 34]. However, there are concerns whether increased glucose uptake and utilization in the adult heart impairs cardiac function since cardiomyocytes cultured in high glucose media develop "glucotoxicity" [57, 58]. Results from bioengineered mouse models with enhanced or reduced glucose utilization demonstrated that glucose reliance is not harmful while reduced glucose utilization is detrimental in cardiac hypertrophy and failure. For example, mice overexpressing the insulin-independent glucose transporter GLUT1 in the heart have increased glucose uptake, high glycolytic flux partially uncoupled with glucose oxidation, and a concomitant decrease in FAO [59–61]. These mice lived a normal life span with unaltered cardiac function despite a fetal-like metabolic profile [60]. When subjected to pressure overload by aortic constriction, the mice were protected against the development of cardiac dysfunction and left ventricular dilation [59].

Enhancing glucose oxidation, via partial inhibition of FAO with pharmacological inhibitors of CPT-1, demonstrated positive outcomes in animal models of heart failure. Oxfenicine in a canine model of HF resulted in an attenuation of LV dilation and wall thinning while maintaining gene expression of enzymes involved in cardiac energy metabolism [62]. In rodent models of HF, etomoxir enhanced myocardial performance through partial normalization of myosin isozymes [63] and improvement of sarcoplasmic reticulum calcium uptake [64]. In patients with chronic HF short-term treatment with perhexiline, in conjunction with standard therapeutic interventions, sufficiently improved cardiac function [65]. Trimetazidine, another partial inhibitor of FAO, led to improved LV systolic and diastolic function in elderly patients with ischemic cardiomyopathy [66].

Despite overwhelming evidence suggesting that increasing glucose utilization is beneficial for the failing heart, recent studies indicate that FAO remains essential for cardiac function. Acipimox, a nicotinic acid derivative with profound anti-lipolytic effects, was used to acutely lower serum fatty acid levels and hence the rate of FA uptake in the heart. In patients with dilated cardiomyopathy, acipimox promoted glucose oxidation but significantly lowered cardiac work and efficiency [67]. In a mouse model deficient of cardiac lipoprotein-lipase, cardiac dysfunction was observed despite the upregulation of myocardial glucose utilization suggesting a critical role of lipase-derived FA for cardiac metabolism [68].

4.2 The Metabolic Capacity

Fatty acids are a very effective carbon source for the high volume of ATP production in the heart. They are abundantly available and each molecule of long chain fatty acids provides ~4 times more carbon than a glucose molecule. This raises the question whether increased reliance on glucose meets the demand for ATP synthesis in the adult heart? PPARα-null hearts, which had permissive increases in glucose oxidation as a result of impaired FAO, failed to maintain myocardial energetics and function during a high workload challenge [69] and developed cardiomyopathy at an old age [70]. Interestingly, the loss of energetic and contractile reserves in PPARα-null hearts could be rescued by overexpressing GLUT1, which markedly expanded the capacity for glucose uptake and utilization [69]. These findings suggest that the inherent capacity for glucose utilization in an adult heart, when FAO is severely impaired, is insufficient for sustaining normal energy supply under stress. Overexpression of GLUT1 under these conditions expanded the capacity and provided an optimal substrate when FAO was limited.

There is significant evidence suggesting that the failing heart is insulin resistant [71, 72]. Since the capacity for glucose uptake and utilization in an adult heart is highly insulin-dependent, impaired insulin signaling in combination with decreased FAO can severely limit substrate oxidation in HF. The benefit of overexpressing GLUT1, an insulin-independent glucose 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. Pharmacological compounds that improve insulin signaling, such as glucagon-like peptide (GLP) that decreases circulating FA levels and increases myocardial glucose uptake, have been beneficial in the short-term treatment of HF in both animal experiments and clinical studies [23, 73–75]. It is also important to recognize that these approaches have changed both the relative contribution and the capacity of glucose utilization. Therefore, the benefit could be attributed to improved oxidative ATP production.

4.3 The Fatty Acid Paradox: A Lesson of Flexibility

Since FAO is responsible for the majority of energy supply in the normal heart, strategies focusing on preventing the deficiency of FAO in the failing heart seem reasonable. However, enhancing FAO by targeting PPARα has not provided convincing conclusions. Overexpression of PPARα resulted in contractile dysfunction while reactivation of PPARα with an agonist in a model of pressure overload hypertrophy resulted in impaired response to myocardial ischemia [39, 76]. Chronic activation of PPARα with fenofibrate in rats post MI or in dogs with pacing-induced HF maintained FAO gene expression but modestly affected the

development of HF [77, 78]. Recent studies suggested that high fat diet (HFD) protected against the development of HF in rat models [79–81]. Of note, this coincides with the clinical observation of the "Obesity Paradox" in which patients with a high body mass index (BMI) have reduced morality from HF [82, 83]. Although mechanisms underlying the benefits of HFD in rats and/or obese HF patients are unknown, the observations clearly offer an opposing view to the dogma that FA is detrimental to the failing heart.

To directly manipulate FAO in the mitochondria genetic approaches were used to target pyruvate dehydrogenase kinase (PDK) or acetyl-CoA carboxylase 2 (ACC2) [84, 85]. PDK phosphorylates and inactivates the pyruvate dehydrogenase (PDH) complex and, as a consequence, inhibits pyruvate oxidation and promotes FAO (Fig. 2). As expected, cardiac-specific overexpression of PDK4, a major isoform in the heart, led to increased cardiac FAO with decreased glucose oxidation in mice [85]. Surprisingly, these hearts demonstrated similar functional recovery from ischemia, indicating that enhanced FAO is not exclusively detrimental during cardiac stress. Similarly, cardiac-specific deletion of ACC2, the enzyme responsible for the production of malonyl CoA, a key inhibitor of FA transport into the mitochondria (Fig. 2), causes increased FAO and decreased utilization of glucose. Despite high FAO, at a level comparable to that reported in diabetic hearts, ACC2-deficient hearts maintain normal cardiac function [84]. When these mice were exposed to pressure-overload hypertrophy, the hearts were resistant to metabolic remodeling and demonstrated improved myocardial energetics and function. Results of these studies indicate that increases in FAO observed in metabolic cardiomyopathy are not the sole culprit mechanism in cardiac stress and, more importantly, highlight the importance of therapeutic strategies that maintain the inherent metabolic profile in cardiac pathologies.

A recent study exploring the effects of a HFD in the cardiac-specific GLUT1 transgenic mouse provided additional insights into cardiac metabolic flexibility [61]. Control animals on HFD displayed the expected increase in cardiac FAO, while GLUT1 transgenic hearts maintained high glucose oxidation despite comparable levels of obesity and insulin resistance in both genotypes. The ability to sustain glucose oxidation in the GLUT1-TG heart during HFD was attributed to glucose-dependent changes in gene expression that restricted FAO and promoted glucose oxidation. The resistance to increased FAO in GLUT1 transgenic hearts was however, associated with elevated oxidative stress and cardiac dysfunction. This was unexpected, given the mounting evidence suggesting that a switch of substrate utilization towards glucose would be beneficial. An important point raised by this study is that molecular remodeling caused by excessive reliance on one substrate (glucose in the case of GLUT1 mice) compromises the flexibility of the metabolic network and prevents the heart from utilizing the most available substrate. Under conditions of increased lipid supply, i.e. HFD induced obesity, FAO becomes critical for achieving a balance of substrate supply and utilization. The benefit of upregulating FAO outweighs the benefit of sustaining glucose oxidation.

5 Conclusion

Genetic and pharmacological 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 to sustain the flexibility of the network and not concentrate on the shift of substrate utilization towards one end of the spectrum. Potential targets for metabolic therapy in the future should also include treatments that improve insulin sensitivity and sustain mitochondrial function, thereby satisfying the enormous energy requirement of the heart.

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Defects in Mitochondrial Oxidative Phosphorylation in Hearts Subjected to Ischemia-Reperfusion Injury

Vijayan Elimban, Paramjit S. Tappia, and Naranjan S. Dhalla

Abstract Although several studies have shown impairment of mitochondrial respiratory and oxidative phosphorylation activities in the ischemic-reperfused (I/R) hearts, the mechanisms of these alterations are not fully understood. Recently, we have shown that the I/R-induced changes in mitochondrial function were attenuated by an oxyradical scavenging system or antioxidants such as N-acetyl-L-cysteine and N-(2-mercaptopropionyl)-glycine. On the other hand, perfusion of hearts with an oxyradical generating system or H_2O_2, a well known oxidant, simulated the I/R induced mitochondrial dysfunction. Furthermore, intracellular Ca^{2+}-overload induced by perfusing the Ca^{2+}-depleted hearts with Ca^{2+}-containing medium produced changes in mitochondrial respiratory and oxidative phosphorylation activities similar to those seen due to I/R injury. Mitochondrial dysfunction was also observed upon incubating these organelles with high concentrations of Ca^{2+}. These observations support the view that mitochondrial dysfunction with respect to energy production due to I/R injury, occurs as a result of oxidative stress and intracellular Ca^{2+}-overload. It is also evident that mitochondrial alterations in the I/R hearts lead to the depletion of high-energy phosphate stores and thus may play a critical role in the development of cardiac dysfunction.

Keywords Mitochondrial respiration • Oxidative phosphorylation • Mitochondrial energy production • Ischemia-reperfusion injury • Oxidative stress • Intracellular Ca^{2+}-overload • Ca^{2+}-Paradoxic hearts • High energy phosphate stress • Cardiac dysfunction

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1 Introduction

It is well known that the mitochondria are the major power house in cardiomyocytes as these organelles are involved in the production of energy in the form of ATP. The production of ATP in mitochondria is carried out by oxidative phosphorylation and electron transport, which processes are intimately associated with the maintenance of high energy phosphate stores in the myocardium [1–4]. In view of the critical role of the high energy phosphate stores in the processes of cardiac contraction and relaxation, as well as for the maintenance of myocardial cell integrity and ultrastructure, it is evident that any defect in mitochondrial oxidative phosphorylation and electron transport activities would result in cardiac dysfunction [3–10]. Mitochondria are also known to accumulate a large amount of Ca^{2+} and are thus considered to serve as a reservoir to maintain the intracellular concentration of Ca^{2+} within certain limits [4, 11, 12]. However, it should be noted that an excessive amount of Ca^{2+} in the cytoplasm results in mitochondrial Ca^{2+}-overload, which is considered to impair energy production by mitochondria under various pathological situations [4, 12, 13]. Mitochondria have also been demonstrated to produce reactive oxygen species such as superoxide, hydroxyl radicals and H_2O_2 (an oxidant molecule) and result in the development of oxidative stress [14–16]. Some studies have shown the role of mitochondrial pores in the leakage of cytochrome C and the induction of apoptosis [17, 18], whereas others have implicated these organelles in cardioprotection because of the presence of mitochondrial K-ATP channels [19]. Thus mitochondria are multifunctional organelles and their roles in both adaptive and pathogenic processes for the maintenance of cardiac cell integrity as well as the development of cardiac dysfunction have also been well recognized.

In spite of the extensive work which has been carried out to understand mitochondrial abnormalities in the failing heart [12, 20–30], the role of these organelles in cardiac dysfunction associated with ischemic heart disease is poorly understood. It is now well known that myocardial ischemia, as a consequence of reduction in blood flow due to atherosclerosis or blockade of blood flow due to thrombosis as well as coronary spasm, results in the impairment of mitochondrial function and defects in cardiac performance. Reperfusion of the ischemic myocardium by procedures such as angioplasty, coronary artery bypass surgery or thombolytic therapy is essential for the recovery of cardiac function. However, if the reperfusion is not carried out within a certain period after the occurrence of ischemic episode, cardiac function is further deteriorated as marked ultrastructural damage to the myocardium becomes evident. This phenomenon is called ischemia-reperfusion (I/R) injury [9, 14, 20, 24]. Different subcellular organelles including mitochondria have been demonstrated to exhibit abnormal function in the ischemic-reperfused hearts [30–35].

Since the development of oxidative stress and the occurrence of intracellular Ca^{2+}-overload due to I/R injury have been shown to induce alterations in myocardial function and cellular structure [20, 30, 36–49], this article is focussed to explore the mechanisms of mitochondrial abnormalities with respect to changes in energy production in the I/R-hearts. These experiments have been conducted by using isolated

rat hearts and the I/R injury was induced by subjecting to global ischemia for 30 min followed by reperfusion for a period of 30 min [27]. Furthermore, the experimental model of intracellular Ca^{2+}-overload (Ca^{2+}-paradox) was employed by using isolated rat hearts and perfusion with Ca^{2+}-free medium for 5 min followed by 5 min of reperfusion [28]. Mitochondria from the hearts were isolated and the respiration rate as well as oxidative phosphorylation activities were determined [27, 28]. In some experiments, mitochondria isolated from some unperfused hearts were incubated in the presence of different concentrations of Ca^{2+} before determining their respiratory and oxidative phosphorylation activities [28].

2 Relationship of I/R-Induced Mitochondrial Dysfunction with Changes in Energy Stores, Ultrastructure and Cardiac Performance

Time-dependent changes in the energy stores, indicating impairment of mitochondrial oxidative phosphorylation activity, and ultrastructure upon reperfusing the ischemic dog heart were seen under in vivo conditions [9]. Subjecting the isolated rat hearts to global ischemic for 30 min was also found to depress the left ventricular developed pressure (LVDP) and increase the left ventricular end diastolic pressure (LVEDP) (Fig. 1). These alterations in cardiac function were associated with a decrease in the high energy phosphate (both creatine phosphate and ATP) content of the ischemic myocardium. Reperfusion of these ischemic hearts further depressed the high energy stores and increased the LVEDP, but LVDP was slightly recovered (Fig. 1). Since myofibrillar Ca^{2+}-stimulated ATPase activity, which is intimately involved in energy utilization, was decreased under similar conditions of I/R injury [34], it appears that the ability of mitochondria to produce energy is impaired in the ischemic hearts. This is exactly what was seen in the 30 min ischemic hearts as different mitochondrial parameters such as state 3 respiration, respiratory control index (RCI), ADP/O ratio and oxidative phosphorylation rate (OPR) were significantly depressed (Fig. 2). Although these observations suggest a good relationship between changes in mitochondrial function and cardiac performance in 30 min ischemic hearts, no changes in mitochondrial sate 3 respiration, RCI, ADP/O ratio or OPR were seen in hearts subjected to 20 min global ischemia (Fig. 2). It should be emphasized that there occurs a complete loss of contractile activity in 20 min ischemic hearts [27]. On the other hand, depressions in cardiac performance and mitochondrial function were observed in 20 or 30 min ischemic hearts upon reperfusion [27]. Accordingly, it has been suggested that changes in mitochondrial function are dependent upon the duration of ischemia whereas both cardiac performance and mitochondrial function in the heart become depressed upon inducing I/R injury. Nonetheless, a good correlation between changes in the high energy phosphate content and contractile activity has been shown to occur upon perfusing the heart with hypoxic medium or substrate-free medium [7, 8].

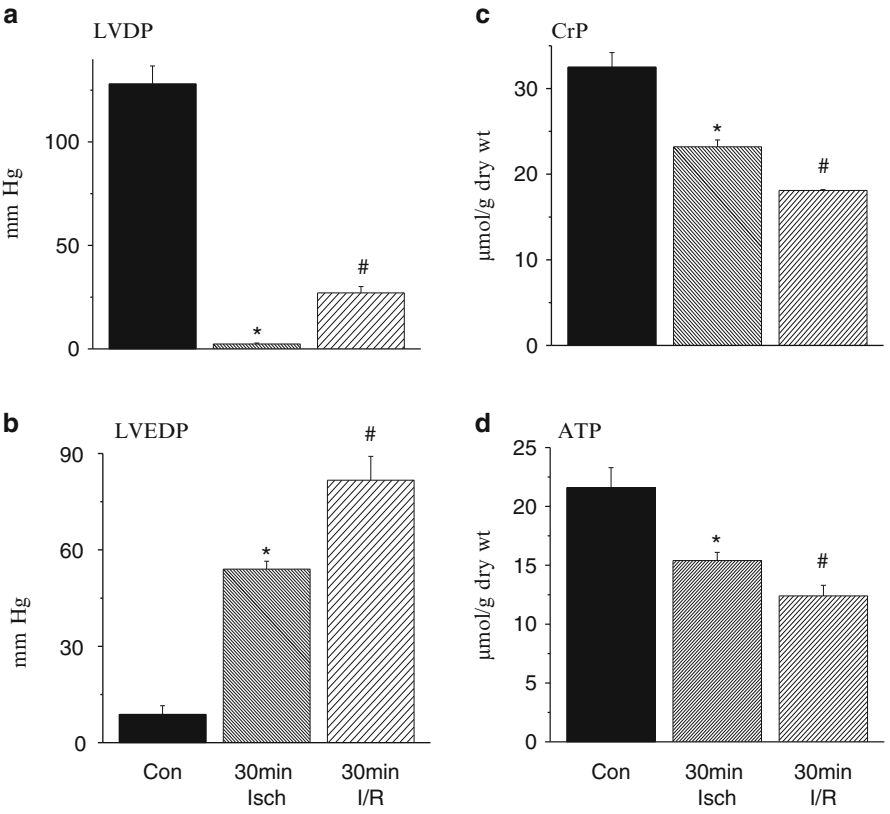

Fig. 1 Cardiac performance and high energy phosphate content of isolated rat hearts subjected to 30 min of global ischemia (Isch) or 30 min ischemia followed by 30 min reperfusion (I/R). *LVDP* left ventricle developed pressure, *LVEDP* left ventricle end-diastolic pressure, *CrP* creatine phosphate, *ATP* adensosine triphosphate. *Significantly different ($P<0.05$) in comparison to control (Con); #Significantly different ($P<0.05$) in comparison to Isch. The data are based on results from our papers [10, 45]

3 Role of Oxidative Stress in I/R-Induced Mitochondrial Defects

Several studies have shown that oxidative stress is a major mechanism for the occurrence of I/R-induced defects in cardiac performance, sarcolemma, sarcoplasmic reticulum and myofibrils [14, 15, 30–34, 50–52]. Although mitochondria are known to participate in the generation of oxidative stress in cardiomyocytes [14, 15, 30], the energy production by these organelles has also been demonstrated to be impaired due to the formation of superoxide anion, hydroxyl radicals and H_2O_2 during the development of I/R injury [3–5, 11–15, 27]. In fact perfusion of the hearts with

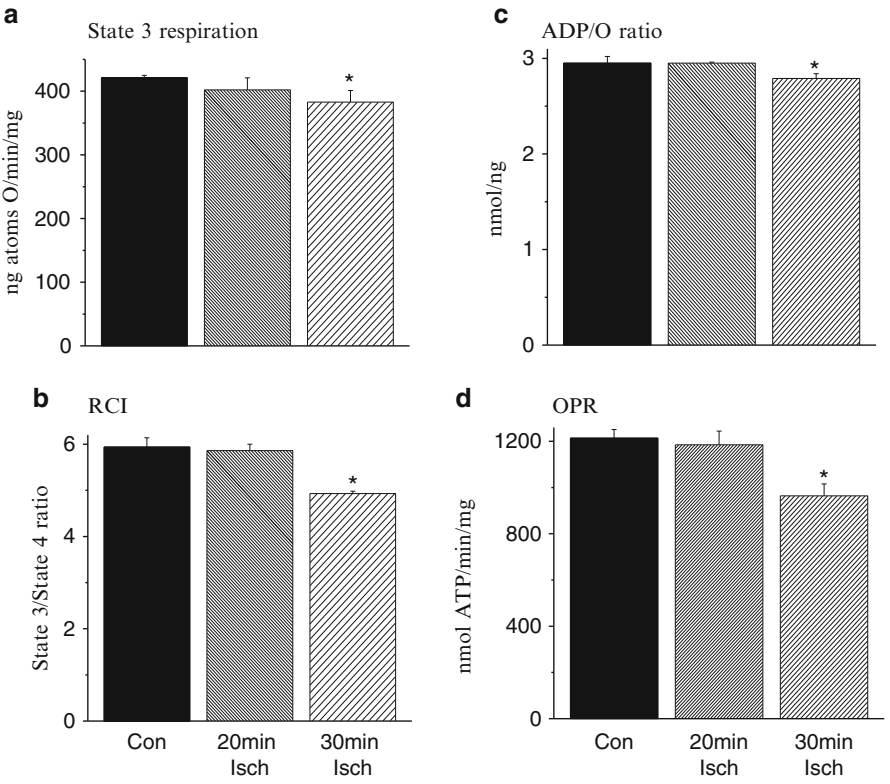

Fig. 2 Mitochondrial respiration rate and oxidative phosphorylation activity in hearts subjected to 20 min or 30 min of global ischemia (Isch). *RCI* respiratory control index, *OPR* oxidative phosphorylation rate. *Significantly different ($P<0.05$) in comparison to control (Con). The data are based on results in our paper [27]

an oxyradical scavenging mixture containing superoxide dismutase (SOD) and catalase (CAT) was found to attenuate the I/R-induced depressions in both the level of reduced glutathione and the ratio of reduced/oxidized glutathione in mitochondrial preparations [27]. Likewise, pretreatment of hearts with SOD and CAT mixture, attenuated the I/R-induced depressions in mitochondrial state 3 respiration, RCI, ADP/O ratio and OPR activities (Fig. 3). Furthermore, the I/R-induced depressions in mitochondrial respiratory and oxidative phosphorylation activities were attenuated by well known antioxidants, namely N-acetyl-L-cysteine (Fig. 4) and N-2-mercaptopropionyl glycine (Fig. 5). These observations support the view that the I/R-induced changes in energy production by mitochondria are mediated through the generation of oxidative stress in the myocardium.

In order to provide further evidence that the I/R-induced alterations in mitochondrial function are mediated by the development of oxidative stress, hearts were perfused

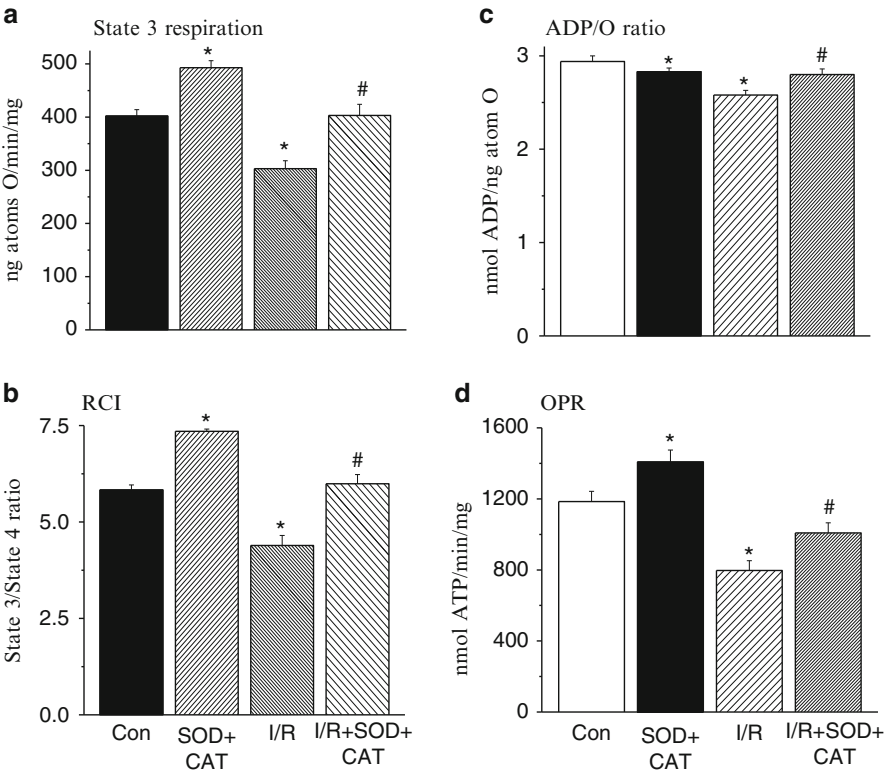

Fig. 3 Effects of superoxide dismutase (SOD) and catalase (CAT) mixture on mitochondrial respiration rate and oxidative phosphorylation activity in hearts subjected to 30 min of ischemia followed by 30 min of reperfusion (I/R). *Significantly different ($P<0.05$) in comparison to control (Con); #Significantly different ($P<0.05$) from I/R. *RCI* respiratory control index, *OPR* oxidative phosphorylation rate. The data are based on results in our paper [27]

with an oxyradical generating mixture containing xanthine plus xanthine oxidase, or an oxidant, H_2O_2, for 30 min and the mitochondrial respiration and oxidative phosphorylation activities were monitored upon the isolation of these organelles [27]. The data in Fig. 6 show that the mitochondrial state 3 respiration, RCI, ADP/O ratio and OPR were depressed in hearts perfused with xanthine plus xanthine oxidase mixture or H_2O_2. Similar results regarding depressed mitochondrial function were obtained when mitochondria isolated from control nonperfused hearts were incubated for 10 min with xanthine plus xanthine oxidase mixture or H_2O_2 under in vitro conditions. These effects of oxyradical generating system or of H_2O_2 on mitochondrial respiratory and oxidative phosphorylation activities under in vitro conditions were prevented by SOD plus CAT mixture indicating the direct nature of oxidative stress on mitochondrial function. Oxidative inactivation of mitochondrial electron transport chain and other components has been demonstrated previously [2].

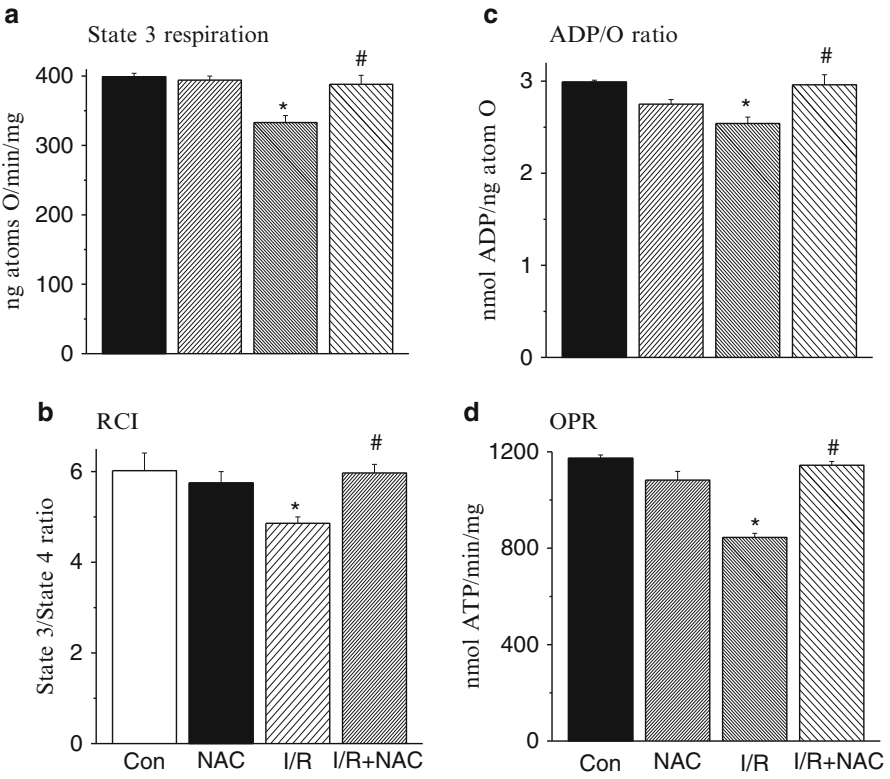

Fig. 4 Effects of N-acetyl-L-cysteine (NAC) on mitochondrial respiration rate and oxidative phosphorylation activity in hearts subjected to 30 min of ischemia followed by 30 min of reperfusion (I/R). *Significantly different ($P<0.05$) in comparison to control (Con); #Significantly different ($P<0.05$) from I/R. *RCI* respiratory control index, *OPR* oxidative phosphorylation rate. The data are based on results in our paper [27]

4 Role of Intracellular Ca^{2+}-Overload in I/R-Induced Mitochondrial Defects

It is now clear that an excessive amount of Ca^{2+} enters the cardiomyocyte during the induction of I/R injury and results in the development of intracellular Ca^{2+}-overload in the myocardium [14, 15, 30, 45, 53]. The possibility of I/R-induced depression in mitochondrial function due to intracellular Ca^{2+}-overload was tested by reperfusing the 30 min ischemic hearts with a medium containing different concentrations of Ca^{2+} because the magnitude of intracellular Ca^{2+}-overload is dependent upon the extracellular concentration of Ca^{2+} during the reperfusion phase [27]. Although varying degrees of depressions in different parameters of mitochondrial respiratory and oxidative phosphorylation activities were seen in I/R hearts reperfused at different

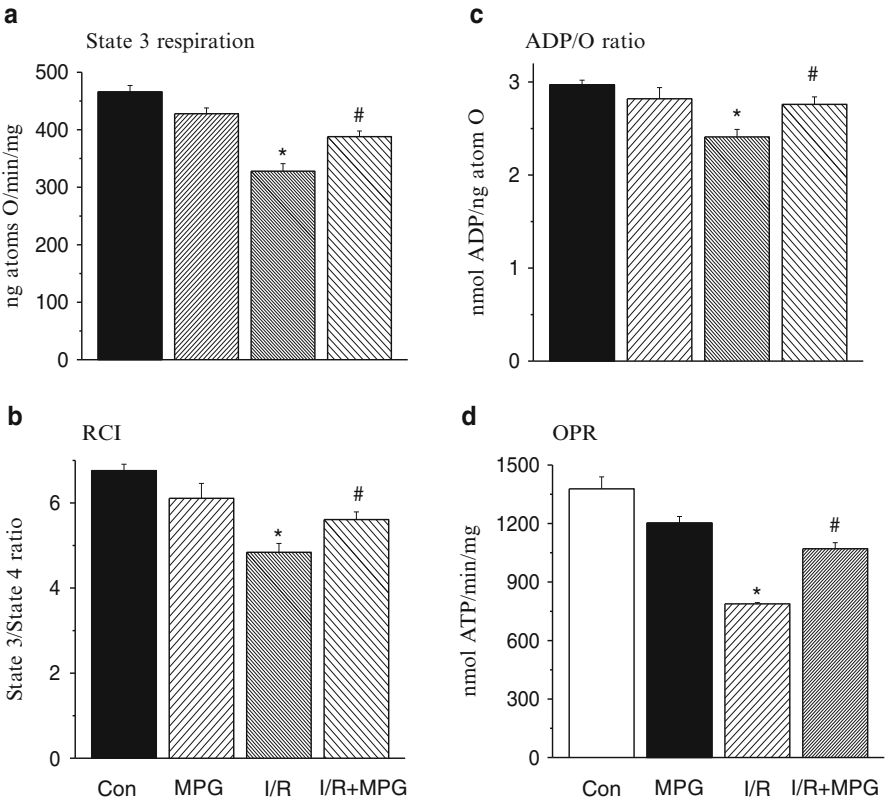

Fig. 5 Effects of N-2-mercaptopropionyl glycine (MPG) on mitochondrial respiration rate and oxidative phosphorylation activity in hearts subjected to 30 min of ischemia followed by 30 min of reperfusion (I/R). *Significantly different ($P<0.05$) in comparison to control (Con); #Significantly different ($P<0.05$) from I/R. *RCI* respiratory control index, *OPR* oxidative phosphorylation rate. The data are based on results in our paper [27]

concentrations of Ca^{2+}, the depressions of state 3 respiration, ADP/O ratio and OPR, except RCI, at 2.5 mM Ca^{2+} were not greater than at 1.25 mM Ca^{2+} (Fig. 7). On the contrary, the I/R-induced depressions in state 3 respiration, RCI and OPR, unlike ADP/O ratio, at 0.1 mM Ca^{2+} were greater than that at 1.25 mM Ca^{2+} (Fig. 7). Our inability to demonstrate Ca^{2+}-dependency of the I/R-induced depressions in mitochondrial function may be due to the fact that we did not monitor the mitochondrial function at initial phases of reperfusion of the ischemic hearts. It is also possible that a sufficient amount of Ca^{2+} may not be retained in mitochondria as varying amounts of Ca^{2+} may have leaked out during the isolation procedure. It should be noted that other investigators have reported depressions in both respiratory and oxidative activities upon loading of mitochondria with Ca^{2+} [4, 48] and mitochondrial Ca^{2+}-loading has been observed at initial phases of I/R injury [4]. Attenuation of I/R-induced depressions in mitochondrial respiratory and oxidative phosphorylation

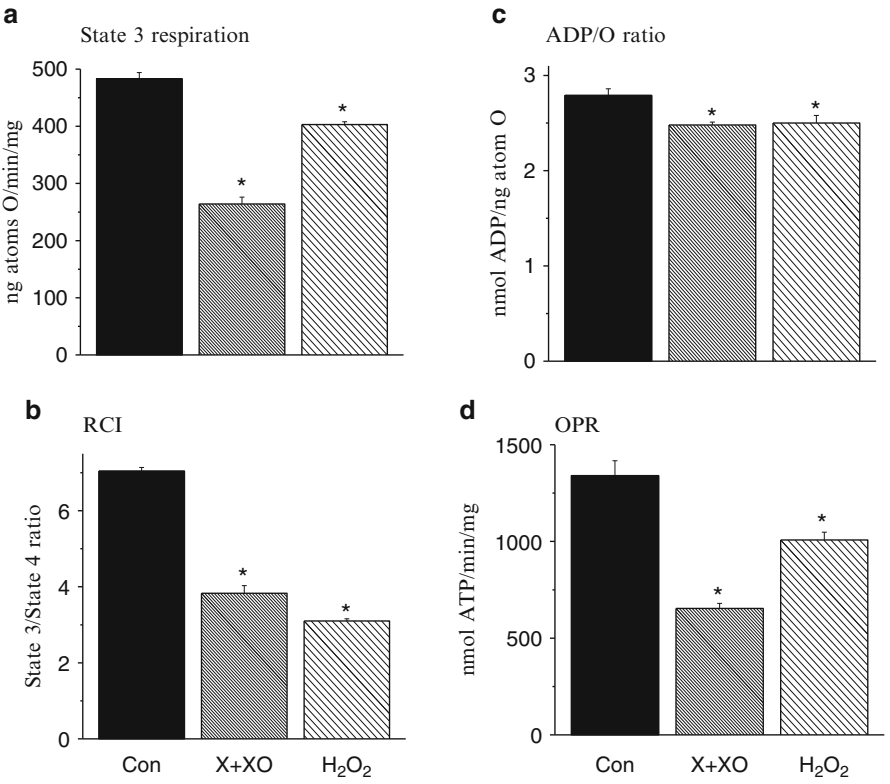

Fig. 6 Mitochondrial respiration rate and oxidative phosphorylation activity in hearts perfused with xanthine plus xanthine oxidase (X + XO) or H_2O_2 for 30 min. *Significantly different ($P < 0.05$) in comparison to control (Con). *RCI* respiratory control index; *OPR* oxidative phosphorylation rate. The data are based on results in our paper [27]

activities was also seen upon treating the hearts with ruthenium red, which is known to prevent Ca^{2+}-uptake by mitochondria [6].

In order to investigate if the I/R-induced depressions in mitochondrial function are simulated under conditions of intracellular Ca^{2+}-overload, we examined mitochondrial respiratory and oxidative phosphorylation activities in hearts subjected to Ca^{2+}-depletion followed by Ca^{2+}-repletion (Ca^{2+}-paradox) [28]. Perfusion of the heart with Ca^{2+}-free medium for 5 min was found to decrease mitochondrial state 3 respiration, RCI, ADP/O ratio and OPR (Fig. 8); these mitochondrial changes were further augmented upon reperfusing the Ca^{2+}-depleted hearts with medium containing Ca^{2+}, a condition which is known to produce marked intracellular Ca^{2+}-overload [28]. Indeed, the alterations in mitochondrial function due to Ca^{2+}-depletion followed by Ca^{2+}-repletion are similar to those observed in I/R hearts (Fig. 8). To show if these changes were due to excessive amount of Ca^{2+}-accumulation, mitochondria isolated from nonperfused hearts were incubated with different concentrations of

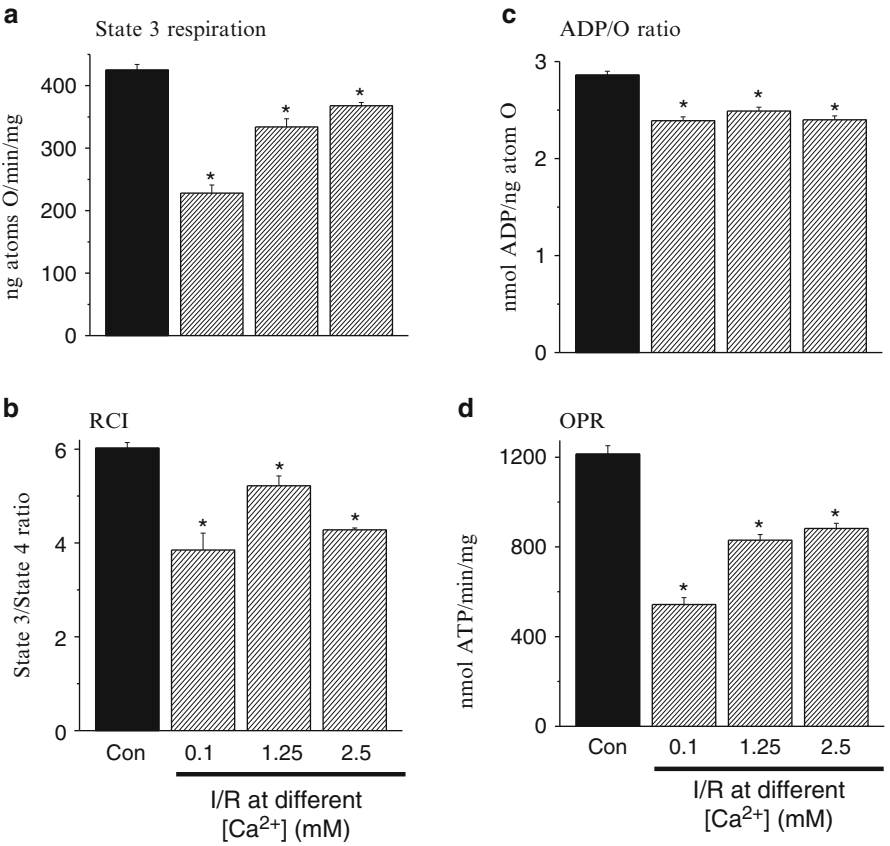

Fig. 7 Mitochondrial respiration rate and oxidative phosphorylation activity in hearts subjected to 30 min of ischemia followed by 30 min of reperfusion (I/R) in presence of different concentrations of Ca^{2+}. *Significantly different ($P < 0.05$) from control (Con). Control hearts were perfused in the presence of 1.25 mM Ca^{2+}. *RCI* respiratory control index, *OPR* oxidative phosphorylation rate. The data are based on results in our paper [27]

Ca^{2+} as described earlier and their energy producing function was monitored [28]. The data in Fig. 9 indicate varying degrees of depressions in mitochondrial state 3, RCI, ADP/O ratio and OPR upon increasing Ca^{2+} concentration in incubation medium. Although the exact mechanisms of depressions in mitochondrial function by high concentrations of Ca^{2+} are not clear, it has been suggested that depletion of cardiolipin from mitochondria due to Ca^{2+}-overloading may depress mitochondrial respiratory and oxidative phosphorylation activities [40, 54]. Since intracellular Ca^{2+}-overload is also known to activate different phospholipases and proteases [30, 55], it is likely that the depressed mitochondrial function in I/R injury may also be due to alterations in the composition of these organelles with respect to both phospholipids and proteins.

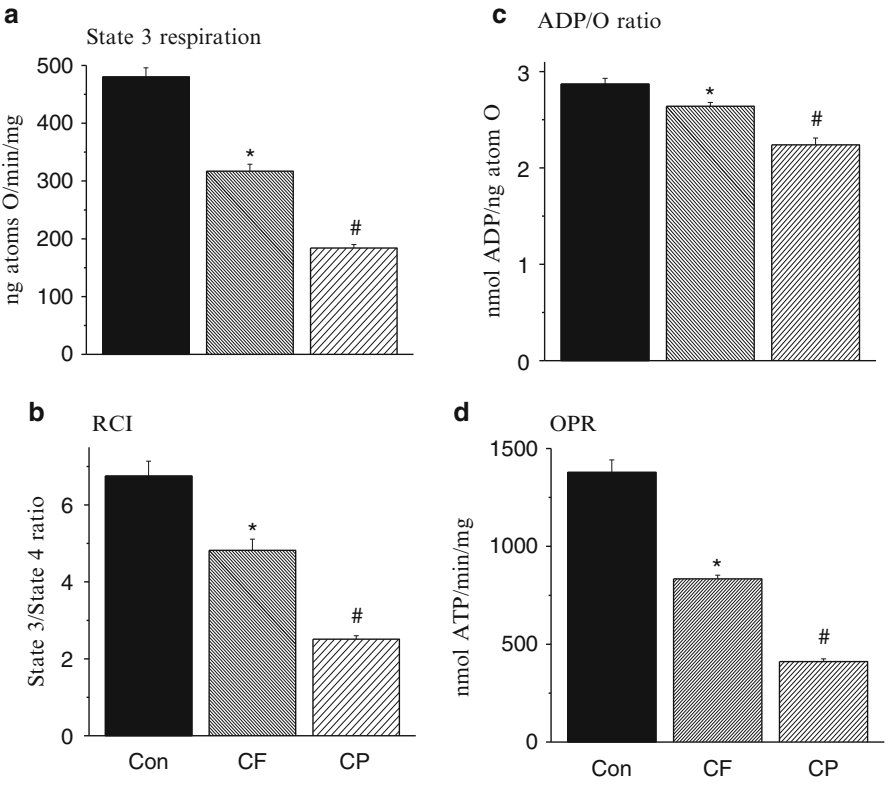

Fig. 8 Mitochondrial respiration rate and oxidative phosphorylation activity in hearts perfused with Ca^{2+}-free medium for 5 min (CF) or Ca^{2+}-free medium for 5 min followed by perfusion with medium containing 1.25 mM Ca^{2+} (Ca^{2+}-paradox, CP). *Significantly different ($P<0.05$) in comparison to control (Con); #Significantly different ($P<0.05$) from CF. *RCI* respiratory control index, *OPR* oxidative phosphorylation rate. The data are based on results in our paper [28]

5 Conclusions

From the foregoing discussion, it is evident that the ability of mitochondria to produce energy, as monitored by state 3 respiration, RCI, ADP/O ratio and OPR, becomes impaired upon induction of I/R injury in the heart. These defects in the mitochondrial respiratory and oxidative phosphorylation activities due to I/R injury appears to be a consequence of the development of oxidative stress and the occurrence of intracellular Ca^{2+}-overload. Although the exact role and contribution of mitochondrial dysfunction in the genesis of depressed cardiac performance due to I/R injury cannot be stated with certainty, it seems that defects in mitochondrial respiratory and oxidative phosphorylation activities may produce depletion of high energy phosphate stores and thus result in the development of cardiac dysfunction. Since I/R injury has been recognized as an essential component involved in the

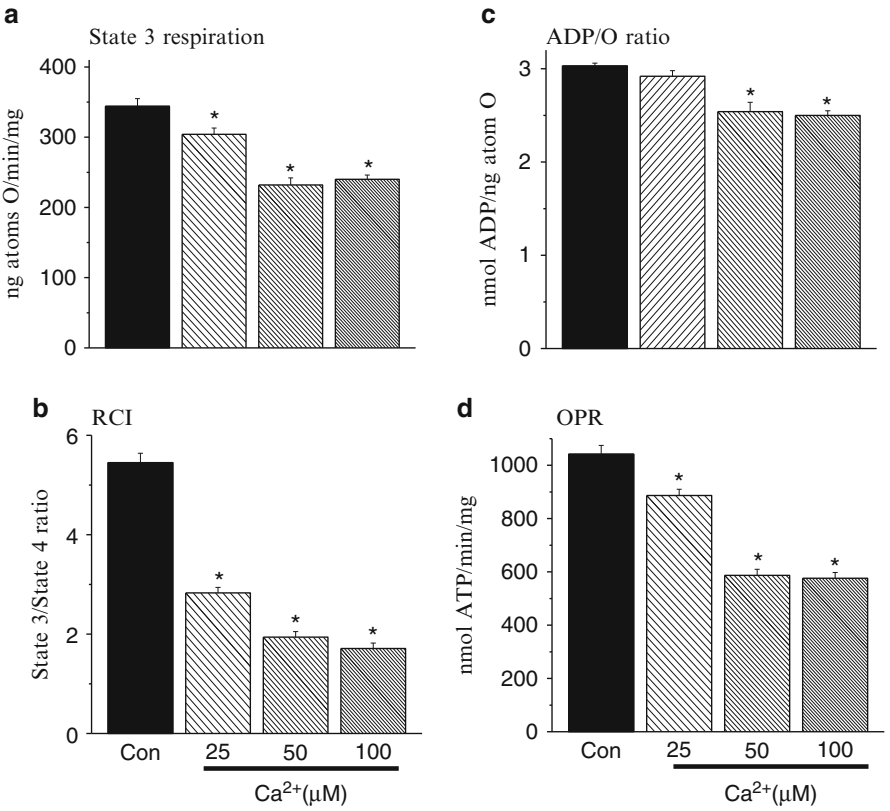

Fig. 9 Mitochondrial respiration rate and oxidative phosphorylation activity measured in mitochondria (isolated from non perfused hearts) upon incubation with different concentrations of Ca^{2+}. *Significantly different ($P<0.05$) from control (Con) values. *RCI* respiratory control index, *OPR* oxidative phosphorylation rate. The data are based on results in our paper [28]

genesis of ischemic heart disease [4, 14, 24, 36, 44, 56], it is suggested that some novel metabolic interventions be developed to prevent the I/R-induced defects in the myocardium if we have to improve the therapy of ischemic heart disease.

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The Role of AMPK in the Control of Cardiac Hypertrophy

Nikole J. Byrne, Miranda M. Sung, and Jason R.B. Dyck

Abstract The development of cardiac hypertrophy in response to sustained cardiac stress causes considerable structural and metabolic remodeling of the heart that can have profound detrimental consequences. AMP-activated protein kinase (AMPK), a well-studied mediator of cellular energy homeostasis, has been shown to be a regulator of cardiac hypertrophy via its influence on several key signaling pathways involved in cardiomyocyte growth control. Although the ability of activated AMPK to inhibit protein synthesis has been a major focus of the anti-hypertrophic effects of AMPK, alterations in other cellular processes such as cardiac energy metabolism and cytoskeletal remodeling have also emerged as complimentary pathways by which AMPK is thought to inhibit the development of cardiac hypertrophy. Consistent with this, increasing evidence supports the use of pharmacological activators of AMPK to prevent the progression of cardiac hypertrophy. Despite these findings, this concept is not universally accepted as AMPK has also been shown to be elevated in hypertrophic hearts, suggesting that AMPK plays a role in promoting rather than inhibiting cardiomyocyte growth. This chapter reviews some of the published literature that focuses on the role of AMPK in the control of cardiomyocyte growth and discusses the potential benefits and pitfalls that may accompany the approach of pharmacologically activating AMPK to control the pathogenesis of cardiac hypertrophy.

Keywords Cardiac hypertrophy • AMPK • Energy metabolism • Protein synthesis • prkag2 mutation

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1 Introduction

In response to hemodynamic stress such as an increase in aortic pressure, the heart undergoes both structural and metabolic remodeling. Early structural remodeling often involves thickening of the walls of the left ventricle (LV), which leads to a physiological condition known as cardiac hypertrophy [1]. There are several processes necessary for cardiac hypertrophy including enhanced protein synthesis [2], cell enlargement, expansion of the cytoskeleton and a higher degree of sarcomeric organization [3]. Although early morphological changes involved in LV hypertrophy (LVH) are initially adaptive in order to normalize wall stress and maintain cardiac output [4], prolonged pathological stimuli such as hypertension or valvular heart disease often induce changes in the cardiomyocyte that become maladaptive. This condition can worsen over time and transition from a compensated hypertrophy to a decompensated hypertrophy and eventually heart failure [3, 5].

Although much of the pathogenesis to decompensated hypertrophy can be attributed to structural remodeling of the cardiomyocyte and the surrounding extracellular matrix, these changes in cardiac morphology also impact other parameters including cardiac energy metabolism. Indeed, it has been well documented that pathological LVH results in a genetic "reprograming" of the metabolic regulatory circuits of the cardiomyocyte such that rates of fatty acid oxidation (FAO) are reduced while rates of glycolysis are increased [6–11]. This reprograming to a more fetal metabolic profile ultimately results in impaired cardiac energetics and contributes to impaired cardiac performance [11–14]. As such, interventions that would lessen cardiomyocyte growth as well as improve cardiac energetics could be of great benefit in the treatment of pathological LVH. Importantly, the energy sensing kinase, AMP-activated protein kinase (AMPK) has emerged as a key player in these processes (i.e. cell growth control and cardiac energetics) providing a potential therapeutic target in the prevention and/or treatment of cardiac hypertrophy.

LVH is recognized as a significant independent risk factor for cardiac-related morbidity and mortality [15, 16], and a growing body of evidence shows that regression of LVH greatly improves outcomes [17, 18]. Therefore, there has been a great deal of research aimed at understanding the mechanisms involved in the development of cardiac hypertrophy and developing new therapeutic strategies for the treatment of LVH. Our current understanding of the pathophysiology of LVH with a focus on the role of AMPK in the hypertrophic process will be discussed herein, as well as the use of AMPK modulators as a potential anti-hypertrophic therapy.

2 AMP-Activated Protein Kinase (AMPK)

AMPK is known to be a major regulator of cellular substrate metabolism, which is necessary to maintain an adequate energy level for cell survival during both normal and pathological conditions [19–21]. AMPK is a serine-threonine kinase that responds to metabolic stresses by sensing changes in the intracellular AMP:ATP

ratio and turning on catabolic pathways to generate ATP while turning off anabolic processes, such as protein synthesis, that consume ATP [21–23]. This has led to AMPK being termed the "fuel gauge" of the cell and it plays an important role in the heart, which has the highest energy demand of any organ in the body.

AMPK is a heterotrimeric protein comprised of a catalytic α subunit as well as β and γ regulatory subunits. While the α and β subunits each have two isoforms [24–26], both of which are found in the mammalian heart, only two of three isoforms of the γ subunit are expressed in the heart [27]. Of the two isoforms of the α subunit, the α2 subunit is found predominantly in the murine heart [27, 28], whereas both α1 and α2 are expressed equally in the human heart [27]. Activating phosphorylation of Thr172 residue in the activation loop of the α subunit by upstream kinases (AMPKKs) is a key mechanism by which AMPK is activated [29]. The regulatory γ subunit senses the metabolic status of the cell upon allosteric binding of two molecules of either AMP or ATP in one of its two competing binding sites [27, 30], thereby regulating phosphorylation of Thr172 under varying energy conditions. Lastly, the regulatory β subunit has a central region that binds glycogen, as well as serves as a scaffolding protein that binds both the α- and γ-subunits at their C-terminal region to hold the holoenzyme together as one complex [28]. As mentioned earlier, activation of AMPK is dependent upon both allosteric activation by AMP and most importantly phosphorylation at Thr172 of the α1 and α2 subunit by upstream AMPKKs [31]. In addition, hormones such as adiponectin, insulin and leptin have also been shown to alter AMPK phosphorylation and activity [32]. Studies have also shown that AMPK can undergo inhibitory phosphorylation at AMPK α1 on its Ser485 residue and AMPK α2 on its Ser491 residue by Akt or protein kinase A, respectively [33, 34].

3 Activation of AMPK

To date, three upstream kinases of AMPK have been identified; including calcium/malmodulin-dependent protein kinase kinase (CaMKK) [35, 36], the tumor suppressor LKB1 [37] and transforming growth factor-beta-activating kinase 1 TAK1 (Fig. 1). However, LKB1 has been recognized as the major AMPKK in the heart [31] and more work on the role of LKB1 in the heart has been performed compared to the other two known AMPKKs. While LKB1 was originally thought to be constitutively active [37], studies in cancer cells have shown that LKB1 can be inhibited by covalent modification [38], suggesting that LKB1 activity may be regulated in a similar manner in other cell types such as the cardiomyocyte. Consistent with this, more recent studies have shown that inhibition of cardiac LKB1 occurs upon formation of covalent adducts with 4-hydroxy-2-nonenal (HNE) [39, 40], which subsequently decreases the activity of AMPK. As the LKB1/AMPK signaling axis has been shown to act to suppress cardiomyocyte cell growth [39, 41], decreased LKB1 activity by HNE adduct formation leads to a permissive environment for increased protein synthesis and hypertrophic growth [39]. In agreement with impaired LKB1

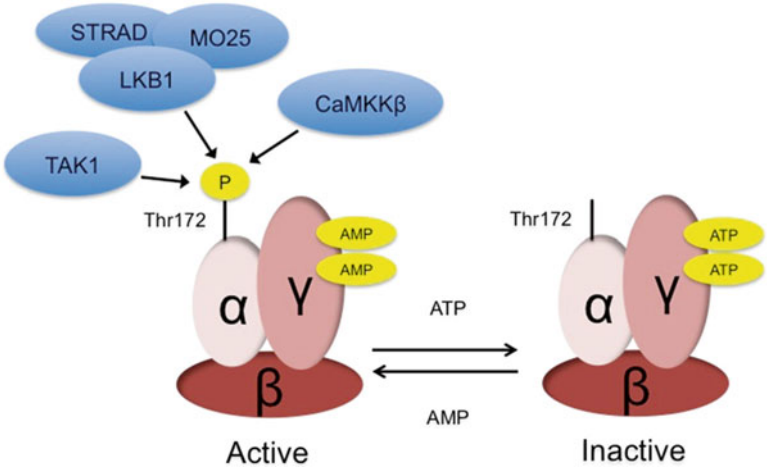

Fig. 1 Structure and activation of AMPK. AMPK is made up of three subunits: α, β, and γ. Activation of AMPK requires binding of AMP to the γ subunit during times of cellular stress, which promotes phosphorylation of threonine 172 of the α subunit by upstream kinases (AMPKKs). Known upstream kinases include the calcium-dependent protein kinase kinase (CaMKK), transforming growth factor-β-activated kinase-1 (TAK1), and most importantly in the heart, LKB1 in a complex with two accessory subunits (STRAD and MO25). In the presence of low AMP:ATP ratio, ATP molecules bind to AMPK thus inhibiting the allosteric activation of the molecule

activity allowing increased cellular growth, mice with a cardiomyocyte-specific deletion of LKB1 have reduced AMPK activity and develop LVH [41]. Although LKB1 is known to regulate at least 13 different downstream kinases [37, 42], it has been shown that cardiomyocyte cell growth induced by LKB1 suppression can be rescued via activation of AMPK [41], demonstrating that AMPK and not another LKB1 target protein is the mediator of this specific cellular process. Together, these findings support the concept that an intact LKB1/AMPK signaling pathway is necessary to prevent abnormal cardiomyocyte cell growth.

4 The Role of AMPK in Cardiac Metabolism and Cell Growth

The healthy heart has a high energy demand and as a result must produce a considerable amount of energy in the form of ATP (estimated 3.5–5 kg of ATP in the human heart) in order to support normal contractile function and ionic homeostasis [43]. Under normal physiological conditions, the healthy adult heart derives >95 % of its ATP from mitochondrial oxidative phosphorylation, while the remainder is generated from glycolysis [44–47]. Although the heart can utilize a variety of substrates to produce energy, the normal healthy adult heart preferably uses fatty acids as a fuel substrate and obtains 50–70 % of its ATP from the oxidation of fatty acids.

Cardiac AMPK plays a pivotal role in regulating cardiac energy metabolism and increasing net ATP production [48]. Indeed, AMPK increases FA utilization in response to increased energy demand by enhancing the availability [49] and uptake of fatty acids [50, 51] as well as via direct effects on acetyl CoA carboxylase (ACC) to promote FAO [52]. In addition to its actions on the regulation of FAO, AMPK also regulates glucose metabolism by inducing expression and promoting translocation of the glucose-transporter GLUT4 to the plasma membrane, thus enhancing glucose uptake [53] and subsequently stimulating glycolysis [54]. Moreover, AMPK is partially responsible for turning off energy consuming processes, such as protein synthesis, in times of metabolic stress in order to help conserve ATP [55, 56]. More recent evidence supports the role of AMPK in regulating the expansion of microtubules [57], which is a key component of the cytoskeleton. This is especially important in modulating cell growth and proliferation in the context of preventing and/or treating cardiac hypertrophy. Therefore, it is now recognized that AMPK targets a wide variety of signaling pathways involved in controlling cardiac energy metabolism as well as cell growth, which will each be further discussed throughout this review.

As mentioned earlier, energy production from the oxidation of FAs is significantly reduced in the setting of pressure-overload induced cardiac hypertrophy [6, 58–60]. Reduced expression of both oxidative enzymes [9, 61] and fatty acid transport proteins [45, 62] following chronically increased cardiac load have been noted as possible explanations. Furthermore, hypertrophied hearts display a significant down-regulation of peroxisome proliferator-activated receptor-alpha (PPARα) and PPAR gamma coactivator 1alpha (PGC-1α), which are critical regulators of genes involved in cellular energy metabolism, in particular FAO [63, 64]. Deactivation of PPARα has previously been linked to the development of cardiac hypertrophy [63]. In fact, both transcription and protein levels of PPARα in the heart were found to be reduced in rats subjected to transverse aortic constriction (TAC) [65], an experimental model of pressure-overload induced hypertrophy.

Although the exact mechanism responsible for decreased PPARα levels remains unclear, it has been shown that AMPK has the ability to inhibit cardiac hypertrophy by enhancing the activity of PPARα [66] and that it mediates this effect by reducing the activity of extracellular signal regulated protein kinase (ERK1/2) [65]. This finding correlates with previous studies showing that inhibition of ERK1/2 may play a role in regulating hypertrophy [67, 68]. In fact, pharmacological activation of AMPK by 5-aminoimidazole-4-carboxamide 1-β-D-ribofuranoside (AICAR) prevented the development of cardiac hypertrophy and increased the transcriptional level and activity of PPARα *in vitro* and *in vivo* [65]. Importantly, this effect of AMPK activation on PPARα and ERK1/2 was abolished in the presence of EGF, an activator of ERK1/2 [65]. Thus, AMPK is thought to restore PPARα activity by reducing the phosphorylation of ERK1/2, thereby promoting FAO and leading to improved myocardial energy metabolism [66]. As well, these findings suggest that AMPK activation may be beneficial to alleviate pressure-overload induced cardiac hypertrophy. However, it should be noted that not all evidence supports PPARα as an effective target for treatment of cardiac hypertrophy as overexpression of PPARα can cause contractile dysfunction in the hypertrophied rat heart [69].

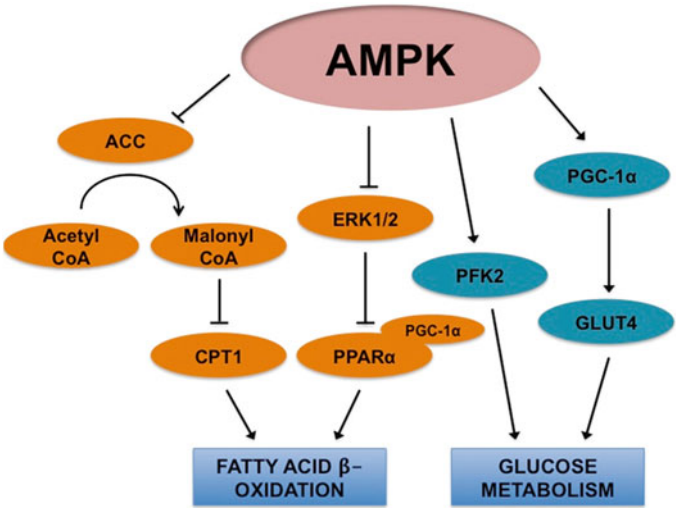

Fig. 2 Regulation of cardiac metabolism by AMPK. Once activated, AMPK is shown to act on numerous signaling cascades that alter energy metabolism of the cardiomyocyte. For example, AMPK is thought to reduce phosphorylation of extracellular signal regulated protein kinase (ERK1/2), thus restoring activity of PPARα which then upregulates genes that encode oxidative enzymes involved in fatty acid β oxidation. PPAR gamma coactivator 1alpha (PGC-1α) acts as a coactivator with PPARα to promote mitochondrial biogenesis, thereby improving myocardial oxidative metabolism. FAO is also increased upon phosphorylation of acetyl-CoA carboxylase (ACC) by AMPK. This inactivation of ACC reduces the conversion of acetyl-CoA to malonyl-CoA, thus permitting carnitine palmitoyltransferase I (CPT1) to transport fatty acids into the mitochondria for subsequent oxidation. AMPK also enhances glucose metabolism by inducing expression of the glucose-transporter GLUT4 via PGC-1α as well as promoting translocation of vesicles containing GLUT4 to the plasma membrane. This together with the activation of phosphofructokinase 2 (PFK2) by AMPK ultimately stimulates glycolysis

Therefore it remains to be determined whether targeting this pathway as a potential treatment for LVH is an appropriate strategy.

The heart displays tremendous metabolic flexibility and the subsequent increase in glucose metabolism in the hypertrophied heart is thought to be a compensatory mechanism to generate ATP in response to decreased rates of FAO [6]. Indeed, both humans and animal models with cardiac hypertrophy demonstrate an increase in glucose utilization [70, 71]. AMPK acts on PGC-1α to promote expression of the glucose transporter GLUT4 [72] and has also been shown to indirectly promote the translocation of vesicles containing GLUT4 to the plasma membrane [53]. In addition, AMPK also accelerates rates of glycolysis via its effects on phosphofructokinase 2 (PFK2) [54] (Fig. 2). Together, these effects may provide benefit to the hypertrophying heart by increasing the production of ATP via glycolysis [73, 74] and the ability of AMPK to increase glycolytic flux may play a critical role in the heart's response to stress. However, this shift towards accelerated rates of glycolysis in the hypertrophied heart does not fully compensate for the reduced energy output resulting

from diminished FAO [6, 10, 75]. In fact, despite increased glucose uptake and accelerated rates of glycolysis, most studies show either no change or a reduction in mitochondrial glucose oxidation in the hypertrophied heart [6, 76–78]. As a result, by-products of incomplete glucose metabolism such as protons and lactate, are shown to accumulate in cardiomyocytes and may divert ATP towards clearance of these by-products, thereby reducing efficiency of myocardial contraction [79, 80].

5 AMPK Inhibits Protein Synthesis

Cell growth is a complex and energetically costly process that is highly regulated at several levels. Along with the energy status of the cell, gene transcription and protein synthesis are key requirements for cell enlargement. As mentioned earlier, there is increasing evidence showing that AMPK acts as a negative regulator of LVH by down-regulating protein synthesis in cardiomyocytes [81]. Indeed, AMPK has been shown to influence several pathways involved in protein synthesis through both direct and indirect control of multiple mediators. For example, eukaryotic elongation factor-2 (eEF2) functions in mediating the translocation of the ribosome along mRNA during peptide-chain elongation [82]. Phosphorylation of eEF2 (Thr56) by its upstream kinase eEF2 kinase (eEF2K) results in the inactivation of eEF2 [83]. Interestingly, AMPK is able to directly phosphorylate eEF2K at Ser398 (and subsequently activate eEF2K) [56], and thus has the ability to regulate the activity of eEF2 [2, 19, 84, 85]. Indeed, activation of AMPK by AICAR in adult rat ventricular myocytes results in increased phosphorylation of eEF2 (thus reducing its activity) and subsequent inhibition of protein synthesis [85]. Furthermore, pharmacological activation of AMPK by metformin and AICAR was shown to inhibit protein synthesis and cardiac hypertrophy induced by phenylephrine treatment or activated Akt, and this was mediated by an increase in phosphorylation of eEF2 [2, 84]. Therefore, increased AMPK activity is shown to negatively regulate protein synthesis and cardiac hypertrophy through the eEF2 kinase/eEF2 signaling pathway and may be a key pathway by which cell growth can be controlled.

In addition to controlling peptide-chain elongation, AMPK also regulates protein synthesis through indirect regulation of the pro-hypertrophic mammalian target of rapamycin (mTOR)/p70S6 kinase signaling cascade [55, 86]. mTOR is a key regulator of myocardial protein synthesis [87] and can regulate cell growth and proliferation by coordinating a response to availability of amino acids and nutritional requirements [87, 88]. Assembly of mTOR with numerous adaptor proteins forms a distinct complex named mTOR complex 1 (mTORC1), and its activation leads to increased cardiac growth [89]. Phosphorylation of mTOR at Ser2448 by Akt leads to activation of this kinase [90], which in turn activates p70S6K by phosphorylation of this protein at multiple sites [91]. Activation of p70S6K occurs via phosphorylation of Ser411, Ser418, Thr421 and Ser424 residues [91, 92]; which is then followed by phosphorylation of the catalytic domain [93] and the linker region [94] to promote activity of the kinase. Through phosphorylation of the 40S ribosomal

protein S6, p70S6K promotes translation of mRNAs specific for ribosomal proteins as well as initiation and elongation factors [95]. Importantly, AMPK is able to inhibit protein synthesis and cardiac hypertrophy by directly phosphorylating and inactivating mTOR at Thr 2446 [96]. Indeed, several studies suggest that mTOR signaling is involved in regulating cardiac hypertrophy [89, 97–99]. Rapamycin, a specific inhibitor of mTOR, attenuates pressure-overload induced hypertrophy and prevents the activation p70S6K, a target of mTOR [89, 99]. Furthermore, the AMPK activator metformin prevented the development of cardiac hypertrophy induced by pressure overload as well as blunted mTOR activation. However this effect of metformin was abolished in AMPKα2-deficient mice [99], suggesting that inhibition of mTOR by metformin is dependent upon activation of the AMPK pathway. Moreover, AMPKα2-deficient mice are shown to have increased phosphorylation of cardiac p70S6K and are more prone to developing cardiac hypertrophy in response to isoproterenol or TAC [100]. Lastly, spontaneously hypertensive rats with impaired cardiac LKB1/AMPK signaling display enhanced mTOR/p70S6K signaling, which is consistent with the profound cardiac hypertrophy observed in these animals [39]. Interestingly, restoration of the LKB1/AMPK signaling pathway using resveratrol, a known AMPK activator, decreases the activation of p70S6K and lessens the development of cardiac hypertrophy in these rats [39]. Similarly, pharmacological activation of AMPK in neonatal rat cardiomyocytes has been shown to result in a significant decrease in p70S6K activity and subsequently reduced rates of protein synthesis [56, 84]. Taken together, there is strong evidence supporting the role of activated AMPK in controlling mTOR/p70s6K activity and preventing protein synthesis and hypertrophic growth in the cardiomyocyte.

Another possible mechanism by which AMPK regulates protein synthesis is via TSC2 [55, 86], a tumor suppressor gene shown to inhibit mTORC1 activity [101, 102] and reduce cell growth [103]. The ability of AMPK to promote TSC2 activity, which is normally active under unstressed conditions [104, 105], was first observed in HEK293 cells where it was shown to phosphorylate the enzyme at two residues (Thr1227 and Ser1345), leading to increased activity [55]. The heterodimeric complex that forms between TSC1 and TSC2 [106] goes on to inhibit the mTOR/p70S6K signaling cascade described above [55]. Consistent with this, mice embryos carrying TSC1/2 homozygous mutations display excessive cardiac cell growth during maturation and die prematurely [107]. In addition, inhibition of AMPK in neonatal rat cardiomyocytes is thought to prevent activation of TSC2, thus allowing for stimulation of the mTOR/p70S6K pathway to up-regulate protein synthesis, resulting in increased cell size [108]. Taken together, these studies suggest that AMPK also down-regulates protein synthesis and the development of cardiac hypertrophy through TSC2, which lies upstream of the mTOR/p70S6K signaling axis (Fig. 3).

Although somewhat controversial, the serine/threonine protein kinase Akt appears to oppose the effects of AMPK on the mTOR/p70S6K pathway [109, 110]. In numerous studies, activation of Akt has been found to be involved in promoting cardiac growth [109, 111] as well as cardiac hypertrophy [101, 106, 112]. More specifically, overexpression of Akt in neonatal rat cardiomyocytes results in increased activity of p70S6K, increased protein synthesis and increased myocardial

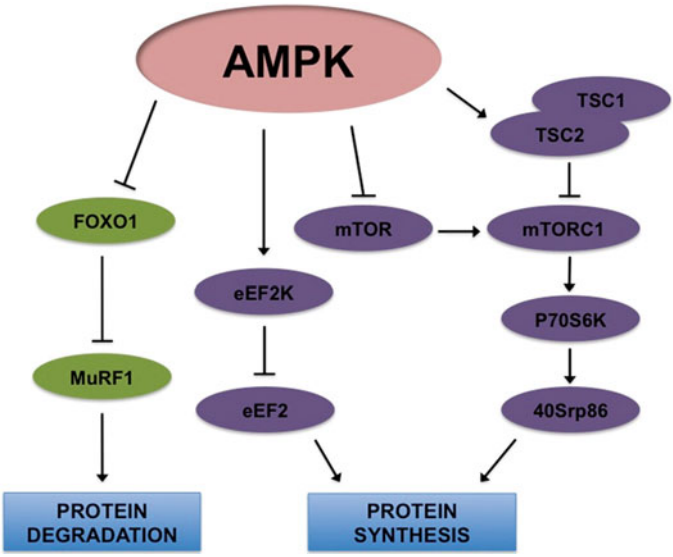

Fig. 3 Regulation of cellular growth by AMPK. AMPK is also shown to act on several signaling cascades that limit cell growth. Primarily, AMPK activates the tuberous sclerosis complex 2-gene product (TSC2), which forms a complex with TSC1 to inhibit the mammalian target of rapamycin (mTOR)—p70S6 kinase (p70S6K) signaling cascade and thus reduces protein synthesis. AMPK also inhibits protein synthesis via activation of eEF2 kinase which phosphorylates/deactivates the eukaryotic elongation factor-2 (eEF2). In addition to these effects, AMPK may also contribute to increased degradation of unnecessary bulk proteins via inhibition of the FOXO/MuRF1 signaling pathway

cell size [84, 110]. Furthermore, Akt is known to reduce activation of AMPK by phosphorylating Ser485/491, which subsequently impedes phosphorylation of AMPK at Thr172 by LKB1 [106, 109, 111, 113, 114]. In agreement with this, LKB1 was unable to activate AMPK in cardiomyocytes expressing a constitutively active form of Akt1 [115], providing further evidence that Akt may promote the development of cardiac hypertrophy in part by preventing AMPK activation [84]. Furthermore, pharmacological activation of AMPK was shown to inhibit Akt-induced protein synthesis in neonatal rat cardiomyocytes, likely through its regulation of both p70S6K and eEF2 signaling pathways [84]. Therefore, pharmacological activation of AMPK may be an approach to counteract the pro-hypertrophic actions of Akt.

6 The Role of AMPK in Transcriptional Remodeling and Cell Growth

In addition to the role of AMPK in regulating protein synthesis, AMPK has also been shown to regulate the calcineurin/nuclear factor of activated T cells (NFAT) pathway that is responsible for mediating transcription of several pro-hypertrophic

genes [116]. Calcineurin is a calcium-calmodulin-dependent protein phosphatase that dephosphorylates the transcription factor NFAT causing it to translocate into the nucleus and promote transcription of its target genes [117]. Of importance, calcineurin/NFAT signaling is thought to play an important role in pathologic hypertrophic signaling. Indeed, calcineurin transgenic mice display significant enlargement of the LV when compared to their non-transgenic littermates [118]. However, activation of AMPK has been shown to reduce the degree of NFAT translocation [2, 119] and thus may contribute to the anti-hypertrophic effect of AMPK in the heart. Indeed, pharmacological activation of AMPK using resveratrol was associated with suppression and inhibition of calcineurin [2], as well as its downstream target NFAT [119]. Although the precise molecular signaling events involved in this regulatory circuit have not yet been fully investigated, blocking the calcineurin/NFAT pathway clearly reduces the hypertrophic response, providing some insight into signaling mechanisms involved in pathological hypertrophy as opposed to physiological hypertrophy.

As cardiac hypertrophy is characterized by cardiomyocyte enlargement in the absence of cellular division [57], cytoskeletal remodeling is a very important aspect of this process. An imbalance between protein synthesis and turnover can lead to enhanced accumulation of contractile myofibers and other proteins that is also characteristic of cell hypertrophy [120]. As such, preventing the accumulation of contractile myofibers may be another approach that could be used to lessen the development of LVH [113]. Importantly, it has been hypothesized that part of AMPK's anti-hypertrophic effects also involves inhibiting the atrophy-related FOXO/MuRF1 signaling pathway [113]. Muscle RING finger 1 (MuRF1), a ubiquitin ligase, is thought to both degrade unnecessary bulk proteins (that would otherwise augment cell growth) [121] and impede pro-hypertrophic stimuli (i.e. ERK1/2) [122]. Interestingly, activation of AMPK by AICAR in neonatal rat cardiac myocytes prevents phenylephrine-induced hypertrophy and upregulates MuRF1 via the FOXO1 transcription factor [113]. Although the exact mechanism through which this AMPK-mediated activation occurs is unknown, MuRF1 is thought to regulate pressure-overload induced cardiac hypertrophy via interaction with several proteins and transcription factors [123]. Therefore, a better of understanding of how/if AMPK regulates MuRF1 to prevent cardiac hypertrophy is needed.

Independent of protein synthesis and transcription events, AMPK has also been shown to limit cellular expansion of cultured cells by reducing the proliferation of microtubules [57]. Microtubules play a key role in determining cellular size and organization and contribute to both structure and transport within the cell. As microtubules accumulate they are known to contribute to the development of pressure-overload induced hypertrophy leading to contractile dysfunction and thus may play a significant role in the development of heart failure [124, 125]. Interestingly, AMPK has been shown to change the binding of the microtubule-associated protein tau to microtubules in neurons [126]. However, whether or not this occurs in the cardiomyocyte has not been firmly established. That said, AMPK is closely related to the MAP-microtubule affinity-regulating kinases (MARK) subfamily, which is responsible for phosphorylating microtubule-associated proteins (MAPs) [127].

In fact, AMPK is thought to be able to phosphorylate and thus deactivate MAP4 [57], which would otherwise promote assembly and stabilization of microtubules [128, 129] in response to pressure overload [130]. Following pharmacological activation of AMPK, phosphorylation of MAP4 was increased and this was associated with reduced stability of microtubules and more importantly limited cell expansion and microtubule growth [57]. However, MAP4-deficient cells demonstrate similar reduction in microtubule stability [57], thus it has yet to be shown to what extent this microtubule instability depends on MAP4 alone as opposed to in conjunction with other kinases. Furthermore AMPK deficient mice showed increased levels of total tubulin in the heart following TAC [57]. As increased levels of total tubulin is known to be required for microtubule growth, these findings strongly suggest that AMPK may also play a role in the regulation of microtubule levels and may represent an alternative mechanism whereby AMPK regulates the development of cardiac hypertrophy [57].

7 PRKAG2 Mutations

In addition to changes in AMPK activity via phosphorylation and/or pharmacological activation, perturbations in the AMPK γ subunits have also been associated with the development of cardiac hypertrophy. Indeed, mutations in the *prkag2* gene that encodes for the γ2 subunit result in the decreased ability of AMPK to bind ATP [131]. This disruption of the ability to sense AMP:ATP homeostasis has been shown to result in changes in AMPK activity [131] and may be responsible for excessive cellular glycogen storage that is characteristic of these mutations [58]. In addition to glycogen accumulation in hearts of humans with *prkag2* mutations, ventricular pre-excitation can develop in these patients and they display symptoms similar to Wolff-Parkinson-White syndrome (WPW) [132]. Ventricular pre-excitation is caused by glycogen accumulation in cardiomyocytes that leads to functional bypass tracts which connect the atria and ventricles [131]. These abnormal conduction pathways allow electrical impulses to bypass the atrioventricular node, resulting in a defective cardiac conduction system [132–134]. In addition, up to 80 % of individuals affected with this naturally occurring mutation also exhibit left ventricular hypertrophy [132], supporting the concept that alterations in AMPK signalling may be causative in the development of ventricular pre-excitation and/or LVH.

In order to further elucidate the mechanisms by which *prkag2* mutations produce LVH and electrophysiological abnormalities characteristic of this condition, several transgenic mouse models have been generated. Data from these *in vivo* models as well as *in vitro* models have suggested that the PRKAG2 cardiac syndrome arises as a result of alterations in AMPK activity [135, 136]. However, it is unclear if PRKAG2 mutations are AMPK activating or inactivating mutations. While N488I and T400N mutations of the *prkag2* gene have been reported to increase activity of AMPK [136, 137], both R302Q and R531G mutations result in inhibition of AMPK activity [138]. In the past, these variations in the activity of AMPK have made it

difficult to distinguish between compensatory alterations and changes which are a direct product of the mutation itself [131]. Nonetheless, the cardiac phenotype of glycogen accumulation is consistent in all murine models exhibiting a *prkag2* missense mutation [136, 139].

Comparing the activity of cardiac AMPK in early and later stages of the disease in the various transgenic mouse models with *prkag2* mutations has allowed for a better understanding of the role of AMPK in this disease. Transgenic mouse models with heart-specific R302Q mutations revealed significantly higher activity of AMPK at 7 days than at 2–5 months of age [139]. Although not shown directly, it is speculated that this latter decrease in AMPK activity is due to a feedback mechanism in which the accumulation of glycogen found as a result of AMPK activation eventually inhibits AMPK [139]. Contrary to this, the N488I mutation has been shown to be an AMPK activating mutation in both early development and in adulthood of the mice [136, 140, 141]. Therefore, regardless of the mechanism responsible for glycogen accumulation, the development of ventricular pre-excitation commonly observed in patients with *prkag2* mutations is now known to be a result of excessive deposition of glycogen and not directly attributed to alterations in AMPK activity [142]. This condition highlights the important role that AMPK has in normal cellular physiology and how alterations in AMPK activity can contribute to glycogen storage cardiomyopathy and cardiac hypertrophy. In addition, the phenotype induced by the activating *prkag2* mutations also raise concerns about whether pharmacological activation of AMPK for the treatment of LVH would cause additional cellular growth or glycogen deposition.

8 AMPK in the Hypertrophied Heart

The precise role of AMPK in the hypertrophied heart remains somewhat controversial, as various studies have implicated AMPK as both contributing to and inhibiting the development of cardiac hypertrophy. For instance, an early study by Tian et al. [59] showed that both expression and activity of AMPK are elevated in hearts subjected to chronic pressure overload. While this finding appears to be in direct contrast with the concept that AMPK activation prevents LVH, it is also possible that AMPK activation occurs at a much later stage of LVH development when growth has already occurred and energetic deficiency is driving the activation of AMPK. In agreement with this, decreased AMPK signaling in adiponectin-deficient mice was found to permit hypertrophic growth in response to pressure overload [143, 144], thus supporting the concept that AMPK activation may, indeed, be beneficial in the treatment of LVH.

Although the question of whether harmful or beneficial results arise from the activation of AMPK in cardiac hypertrophy remains, these seemingly opposite results may, in fact, be explained by the stage of hypertrophy studied. The aforementioned studies showing increased activity of AMPK in the hypertrophied heart

were performed in animal models with advanced stages of hypertrophy [59], at a point when activation of AMPK may be an adaptive response of the heart in response to ATP depletion. Contrary to this, numerous pharmacological activators of AMPK have been shown to prevent cardiac hypertrophy in a number of models, which support the idea that AMPK is a negative regulator of cardiac hypertrophy. Therefore, it is likely that AMPK activation early on during the development of hypertrophy may be able to prevent cardiac hypertrophy. However, activation of AMPK during later stages of pathological hypertrophy may be an adaptive response to an energetic deficiency and thus pharmacological activation at this stage may also be of benefit, albeit for ATP supply and not cellular growth control.

9 AMPK as a Pharmacological Target to Prevent Cardiac Hypertrophy

The discovery of an association between AMPK and the development of LVH has led to numerous studies investigating pharmacological agents that may be able to activate AMPK in order to clinically treat this pathological condition. Due to its role in maintenance of glucose and lipid homeostasis, AMPK is a well-known target in the treatment of type 2 diabetes and has more recently emerged as a potential target in the treatment of the metabolic syndrome [145]. Similarly, the antidiabetic drug, metformin, was found capable of activating AMPK [146] and is under investigation to determine its potential role in attenuating LVH [147]. However, more recent evidence also supports the potential effectiveness of the polyphenol resveratrol, an active ingredient of green tea and red wine, in preventing LVH [2, 39, 148, 149]. Lastly, the AMP-analog AICAR has long been used as an AMPK activator following the finding of its ability to stimulate glucose uptake in skeletal muscle [150]. Studies providing evidence that indirect pharmacological activators of AMPK (such as metformin, resveratrol and AICAR) as well as specific AMPK activators (such as A-769662) may prevent and/or reverse the development of cardiac hypertrophy will be further outlined below.

Metformin has been found to lessen the hypertrophic effects of pressure overload in TAC mice [99] through indirect activation of AMPK [22, 151]. Moreover, administration of metformin following occlusion of the left coronary artery of a mouse model was associated with increased phosphorylation of AMPK but also a reduced heart to body weight ratio [152]. Metformin has been shown to not only reduce protein synthesis in the cardiomyocyte [84], but is also capable of suppressing oxidative stress and associated cardiac hypertrophy [22]. The ability of metformin to attenuate cardiac hypertrophy appears to rely on activation of AMPK as this effect of metformin is lost in AMPKα2–/– mice [99]. Lastly, long-term metformin treatment shows promise in preventing pressure overload-induced LVH via activation of AMPK and downstream endothelial nitric oxide synthase (eNOS) [147]. Although the exact mechanism through which metformin activates AMPK is not

fully understood, there is speculation that it may influence the AMP:ATP ratio or perhaps activate an upstream kinase (AMPKK) [153].

The naturally-occurring polyphenol resveratrol has also been used to activate AMPK in hopes of developing a strategy to treat LVH. Evidence from studies with resveratrol suggests that resveratrol-mediated AMPK activation attenuates cardiac hypertrophy via a direct effect on pathways controlling protein synthesis and cell growth including eEF2 and p70S6 kinase [154]. Resveratrol treatment was also found to reduce hypertrophy in the spontaneously hypertensive rat upon reactivation of the LKB1/AMPK signaling pathway [39]. Resveratrol treatment prevented LVH in hypertensive rodents in both the presence or absence of changes in systolic blood pressure, suggesting that resveratrol may have direct effects on the heart to prevent LVH independent of changes in cardiac load [39, 149, 155–157]. Although preliminary data supports the use of resveratrol as a potential adjunct therapy in the treatment of LVH, future studies are required to show whether its anti-hypertrophic effects do, in fact, depend on AMPK.

Another activator of AMPK is AICAR, an analog of adenosine that mimics the effects of AMP by allosterically activating AMPK [158, 159]. Following treatment with AICAR, rats subjected to TAC demonstrated both elevated AMPK activation and reduced cardiac hypertrophy [119]. Pharmacological activation of AMPK via AICAR is also associated with regression of cardiac hypertrophy [2] as a result of reduced protein synthesis and growth of cardiac fibroblasts [2, 19, 139]. Neonatal cardiomyocytes treated with AICAR to activate AMPK demonstrated reduced free tubulin and therefore a significant decrease in stability of microtubules, as well as a weakened hypertrophic response to phenylephrine [57]. However, it should be noted that mimicking the stimulatory effects of adenosine is reported to have other consequences on cardiac function, and thus requires further understanding prior to any pharmacological use of AICAR [160, 161]. While these studies provide evidence that the use of AICAR can reduce or eliminate the development of cardiac hypertrophy, a better understanding of AICAR's mode of action, both in activating AMPK in the heart as well as potential side effects is necessary.

Since the compounds discussed above are indirect AMPK activators, treatment with these compounds may cause undesirable off-target effects resulting from activation of non-AMPK signaling cascades. Therefore, many arguments favor the use of specific AMPK activators such as A-769662, a thienopyridone compound developed by Abbott Laboratories [162]. The compound has shown to be effective in activating and maintaining phosphorylation of AMPK in cell-free assays [163, 164], likely through binding to a novel site on the β subunit of AMPK [163, 164]. Recent studies have demonstrated the cardioprotective effect of the small molecule A-769662 on the ischemic heart [164]. Although A-769662 activates AMPK independent of alterations in the AMP:ATP ratio, due to its poor oral availability the drug may not be ideal for pharmacological use in patients [163]. Nonetheless, this small molecule is a useful experimental tool that offers insight into the effectiveness of targeted AMPK activation as a therapeutic approach to treat different cardiac diseases.

10 Conclusions

AMPK is known to phosphorylate multiple downstream targets and has a critical role in modulating metabolic activities such as glucose transport and fatty acid oxidation. In addition, AMPK has emerged as a key player in protein synthesis and cell remodeling. Since alterations in both metabolism and cell growth occur in the development and pathogenesis of LVH, AMPK agonists may prove to be useful in the treatment of cardiac hypertrophy. However, genetic mutations in the AMPK γ2 subunit gene (*prkag2*) cause inappropriate activation of AMPK and a glycogen storage myopathy that ultimately leads to ventricular pre-excitation. Therefore, whether or not pharmacological activation of AMPK by compounds such as metformin, resveratrol, AICAR, and A-769662 (or newly developed AMPK agonists) is beneficial or harmful in the setting of LVH is still being investigated. Thus, despite exciting preclinical findings, additional research in this area is necessary before AMPK agonism can be considered for further development with the aim of treating LVH in humans.

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The Role of Incomplete Fatty Acid β-Oxidation in the Development of Cardiac Insulin Resistance

John R. Ussher

Abstract As obesity is a significant risk factor for cardiovascular disease, there is a growing need to understand the precise mechanisms by which obesity and its associated dyslipidemia negatively affect the myocardium and lead to cardiac dysfunction. Current dogma suggests that obesity-associated dyslipidemia increases fatty acid delivery to the heart, which contributes to both excessive fatty acid uptake and subsequent fatty acid oxidation rates that ultimately result in the development of cardiac lipotoxicity and insulin resistance. However, recent evidence demonstrates that increased rates of incomplete fatty acid oxidation (mismatch between mitochondrial β-oxidation rates and tricarboxylic acid cycle activity) may also contribute to the progression of cardiac insulin resistance through inhibition of insulin-sensitive glucose oxidation, which will be the topic of discussion in this specific chapter.

Keywords Incomplete fatty acid β-oxidation • Fatty acid β-oxidation • Insulin resistance • Cardiac lipotoxicity • Cardiomyopathy • Type 2 diabetes • Obesity • Triacylglycerol • Diacylglycerol • Ceramide

1 Introduction

Cardiovascular disease is a major cause of morbidity and death in the world today. With recent advancements in evidence-based medicine, the overall management of patients with cardiovascular diseases has significantly improved. However, there are still a large number of patients who are ineligible or refractory to conventional treatment, and thus novel approaches to treat these patients are necessary.

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With the heart being the most metabolically demanding organ in the body, the optimization of cardiac energy metabolism appears to be one such novel approach. Indeed, alterations in fatty acid oxidation have been demonstrated in numerous cardiovascular pathologies, including ischemic heart disease, diabetic cardiomyopathy, and heart failure [1–3]. Furthermore, a multitude of studies in the past 20 years have illustrated that reversing these alterations in myocardial fatty acid metabolism can improve cardiac function and alleviate disease pathology [4–10]. As the individual chapters in this textbook will delineate in great detail the regulation of cardiac energy metabolism, it's contribution to the development and progression of various cardiovascular diseases such as ischemic heart disease and heart failure, this specific chapter aims to focus on the potential role of incomplete fatty acid β-oxidation in the heart as a mediator of cardiac insulin resistance.

2 Cardiac Energetics

In the healthy heart, virtually all (~95 %) ATP generated arises from mitochondrial oxidative phosphorylation, with the remainder derived from glycolysis. In cardiac muscle fatty acids account for the majority of oxidative energy metabolism (~60–80 %), while carbohydrate metabolism accounts for the remaining 20–40 %. The following section will briefly describe the processes of glucose and fatty acid metabolism in the myocardium.

2.1 Carbohydrate Metabolism

Glucose and lactate are the primary carbohydrates metabolized by the heart (*for in-depth review of the regulation of cardiac carbohydrate metabolism, please refer to Jaswal et al.* [2]). Glucose transporters (i.e. GLUT1/4) are responsible for glucose uptake into the cardiac myocyte, whereby glucose either undergoes anabolism to produce glycogen, or is catabolized for ATP production through both glycolysis and glucose oxidation. Glycolysis produces pyruvate and accounts for less than 10 % of the total ATP generated by the aerobic adult heart [1]. If glycolysis is coupled to glucose oxidation, the pyruvate produced from glycolysis will be transported into the mitochondria via the mitochondrial pyruvate carrier and subsequently converted into acetyl CoA by the enzymatic activity of pyruvate dehydrogenase (PDH).

2.2 Fatty Acid Metabolism

Fatty acids enter the cardiac myocyte via either passive diffusion or through a number of protein transporters such as the fatty acid translocase, CD36, or fatty acid transport proteins. Prior to either storage as triacylglycerol (TAG) or mitochondrial

fatty acid β-oxidation for energy production, these fatty acids must first be activated into fatty acyl CoA via fatty acyl CoA synthase, which converts a fatty acid into a fatty acyl CoA ester in an ATP-dependent manner (*for in-depth review of the regulation of cardiac fatty acid metabolism, please refer to Lopaschuk et al.* [3]). Because the inner mitochondrial membrane is impermeable to CoA esters, mitochondrial uptake of fatty acyl CoA requires a complex of proteins that relies on a carnitine-dependent shuttle system [11]. Carnitine palmitoyl-transferase 1 (CPT-1), which is the rate-limiting enzyme for mitochondrial fatty acid uptake and subsequent fatty acid β-oxidation, resides on the outer mitochondrial membrane and converts fatty acyl CoA esters into their corresponding fatty acylcarnitine moieties [12, 13]. The acylcarnitine is then transported into the mitochondrial matrix by carnitine translocase, and reconverted back into its corresponding fatty acyl CoA ester by CPT-2, which resides on the inner mitochondrial membrane's inner leaflet [3]. Fatty acyl CoA esters are sequentially oxidized within the mitochondrial matrix by the successive enzymatic actions of acyl CoA dehydrogenase, enoyl CoA hydratase, 3-hydroxyacyl CoA dehydrogenase, and 3-ketoacyl CoA thiolase. The process of fatty acid β-oxidation results in the progressive shortening of a fatty acyl CoA ester by two carbon units through liberation of acetyl CoA, while also producing the reducing equivalents, NADH and $FADH_2$, which act as electron donors for the electron transport chain, in order to fuel ATP synthesis via oxidative phosphorylation [14].

3 Insulin's Effect on Myocardial Metabolism

The heart is an insulin sensitive organ whereby internal GLUT4 transporters are translocated to the sarcolemmal membrane in response to insulin to facilitate glucose uptake (refer to Allard [15] for an extensive review on the effects of insulin on myocardial metabolism). Therefore, one of the primary functions of insulin in the heart is to stimulate glucose metabolism, which includes an increase in glucose uptake, glycogen synthesis, glycolysis and glucose oxidation (Fig. 1). Insulin's effects on glycolysis are due to activation of 6-phosphofructo-2-kinase (PFK-2), which increases fructose 2,6-bisphosphate, a potent stimulator of 6-phosphofructo-1-kinase (PFK-1), the rate-limiting enzyme of glycolysis [16]. Insulin also has positive effects on PDH activation, possibly via PDH phosphatase, which contributes to its overall effect to enhance glucose oxidation [17]. Moreover, as other chapters have highlighted the role of the Randle Cycle and the inverse relationship between fatty acid and glucose oxidation, insulin's ability to reduce fatty acid mobilization from adipose tissue reduces the delivery of fatty acids to the heart. This results in a subsequent reduction in myocardial fatty acid β-oxidation rates and decreases the inhibitory effects of fatty acid oxidation on PDH activity and glucose oxidation.

Although the heart's glycogen stores are not as large as those present in the skeletal muscle or liver, the mechanisms driving glycogen synthesis in the myocardium are very similar. Perfusion of rat hearts in the presence of insulin leads to the rapid dephosphorylation and activation of glycogen synthase [18]. This may be explained by the activation of phosphatidylinositide 3-kinase, which increases

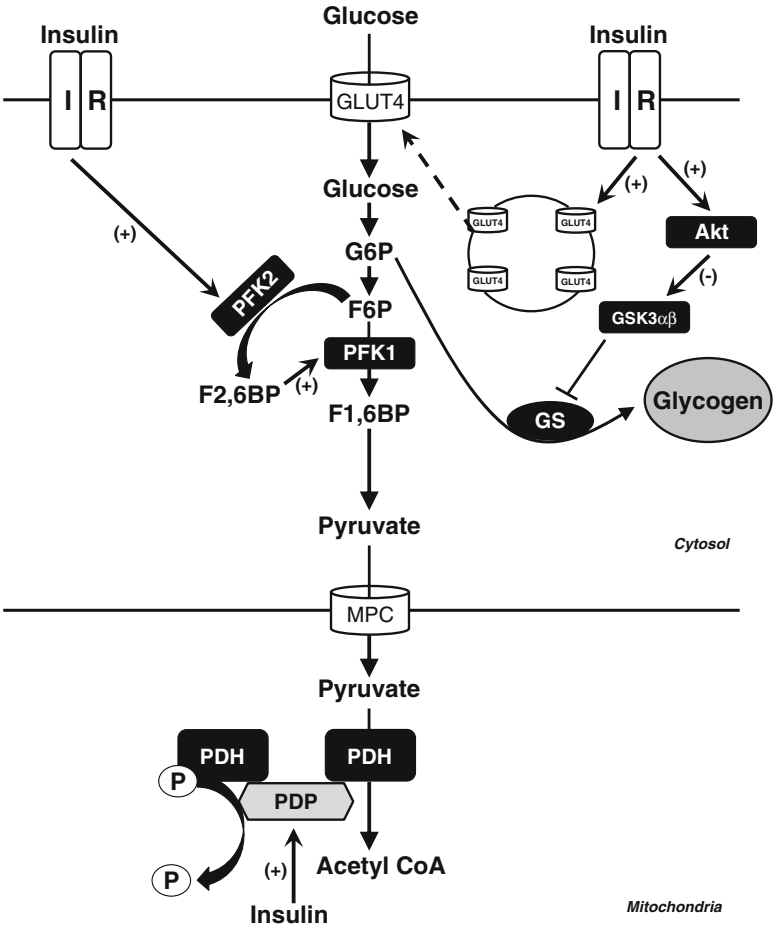

Fig. 1 Insulin and cardiac glucose metabolism. In the heart, insulin increases glucose uptake, glycolysis, glycogen synthesis, and glucose oxidation. Upon binding to its receptor at the sarcolemmal membrane, insulin activates Akt, which through a number of downstream mediators, ultimately results in the translocation of GLUT4 transporters from internalized vesicles to the membrane, thereby facilitating cellular glucose uptake. At the same time, activation of Akt relieves GSK3β-mediated inhibition of glycogen synthase, allowing glycogenesis to take place. Furthermore, insulin receptor activation results in the activation of PFK2, which increases fructose 2,6-bisphosphate generation, thereby stimulating 6-phosphofructo-1-kinase and subsequently increasing glycolysis rates. Last, insulin has also been postulated to increase PDH activity and subsequent glucose oxidation rates, possibly by direct actions to increase PDH phosphatase activity, which relieves phosphorylation-mediated inhibition of PDH activity. *IR* insulin receptor, *G6P* glucose-6-phosphate, *F6P* fructose-6-phosphate, *F1,6BP* fructose-1,6-bisphosphate, *F2,6BP* fructose-2,6-bisphosphate, *PFK1* 6-phosphofructo-1-kinase, *PFK2* 6-phosphofructo-2-kinase, *GS* glycogen synthase, *GSK3* glycogen synthase kinase 3, *MPC* mitochondrial pyruvate carrier, *PDH* pyruvate dehydrogenase, *PDP* PDH phosphatse

phosphatidylinositol (3,4,5) trisphosphate production, leading to activation of phosphoinositide-dependent kinase 1 and the subsequent activation of Akt. Active Akt phosphorylates and inactivates glycogen synthase kinase α/β (GSK3α/β), allowing glycogen synthase to remain in an active dephosphorylated state, resulting in increased glycogen synthesis [19].

In muscle, insulin can inhibit CPT-1 and subsequent fatty acid β-oxidation rates [20], and it has been hypothesized that this mechanism also occurs in the heart, though conflicting opinions exist in regards to this manner [15, 21, 22]. Direct changes in malonyl CoA (a potent endogenous inhibitor of CPT-1) levels have been proposed to account for the rapid effect of insulin on CPT-1 activity, which would likely arise from increased acetyl CoA carboxylase (ACC) activity, the only source of malonyl CoA in mammalian cells. In support of this, isolated working heart perfusions in the absence of insulin demonstrate a lack of effect of citrate on ACC activity [23]. On the other hand, isolated working heart perfusions in the presence of insulin show a positive allosteric effect of citrate on ACC activity [24]. Insulin has also been shown to inhibit AMPK, a major kinase whose activation leads to increased rates of fatty acid oxidation in both heart and skeletal muscle [25, 26]. However, this effect of insulin is unlikely to play a role in reducing fatty acid β-oxidation rates in the insulin resistant heart, as studies have demonstrated a loss of insulin-dependent AMPK inhibition in the presence of high free fatty acid levels in the perfusate, which would mimic circulating free fatty acid levels seen in insulin resistant and type 2 diabetic patients [27].

4 Insulin Resistance

Insulin resistance is the failure of insulin to mediate a normal insulin-type response from insulin-sensitive tissues, resulting in dysregulated blood glucose homeostasis. It involves decreased glucose uptake into the skeletal muscle, heart, and adipose tissue, and increased hepatic glucose production, all of which contribute to the elevation in glycemia observed in insulin resistant patients. As the skeletal muscle serves as the "sink" for glucose disposal in response to insulin, numerous studies investigating the molecular mechanisms responsible for causing insulin resistance have focused their efforts in this tissue. Although there has been no true discovery to date as to what specific factor causes insulin resistance, there has been vast progress in regards to potential culprits of this devastating condition. Some of these include inflammatory responses associated with increased circulating tumor necrosis factor alpha and other cytokines [28–30], increased intramyocellular ceramide production [31, 32], oxidative stress [33, 34], intramyocellular lipid metabolite accumulation (TAG, long chain acyl CoA, and DAG) [35, 36], and hyperlipidemia [37]. While there are many different proposed candidates for what initially causes insulin resistance, common cellular mechanisms do exist, primarily involving a failure of insulin to increase insulin receptor substrate-associated phosphatidylinositide

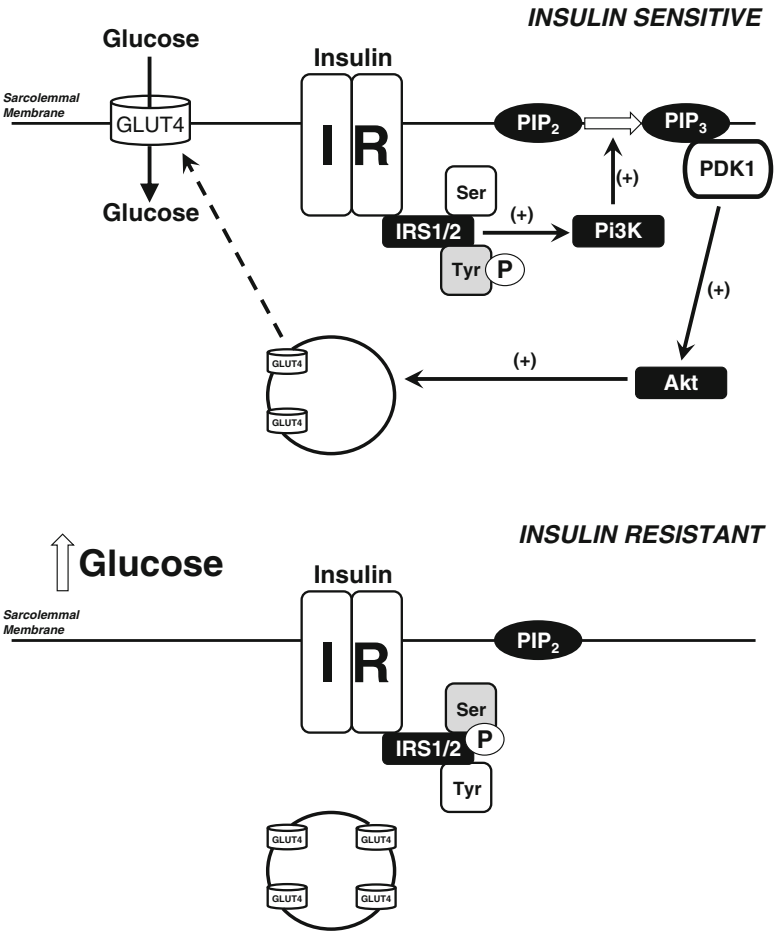

Fig. 2 Defects in insulin signaling during insulin resistance. During normal insulin signaling, insulin receptor activation results in tyrosine phosphorylation of IRS1/2, which results in activation of PI3K. A number of downstream signaling events ultimately result in activation of Akt and subsequent translocation of GLUT4 transporters from internalized vesicles to the membrane, thereby facilitating cellular glucose uptake. It has been proposed that during insulin resistance, serine phosphorylation of IRS1/2 somehow interferes with tyrosine phosphorylation of IRS1/2, which inhibits IRS1/2's ability to recruit and activate PI3K, preventing GLUT4 translocation and subsequent glucose uptake. *IR* insulin receptor, *PDK1* phosphoinositide-dependent kinase 1, *PI3K* phosphatidylinositide 3-kinase, PIP_2 phosphatidylinositol 3,5-bisphosphate, PIP_2 phosphatidylinositide 3-phosphate, *Ser* serine, *Tyr* tyrosine

3-kinase activity [30, 35, 38]. This results in reduced generation of phosphatidylinositol (3,4,5) trisphosphate and thereby prevents activation of phosphoinositide-dependent kinase 1, which impairs insulin-stimulated GLUT4 translocation to the sarcolemmal membrane (Fig. 2).

While insulin resistance is primarily discussed in relation to defects in skeletal muscle signaling, one must not forget that the heart is also a muscle, and many of

the proposed factors that cause insulin resistance also inflict many negative effects on cardiac muscle [39]. However, there is mounting evidence that there is little to no loss of insulin sensitivity in the hearts from patients with type 2 diabetes [3]. Studies measuring the effects of hyperinsulinemia on myocardial glucose uptake demonstrate either minor or a lack of insulin resistance in type 2 diabetic patients compared with nondiabetic control subjects [40, 41]. This is particularly evident when circulating free fatty acid levels are matched [42]. Similarly, in animal studies, utilization of a genetic mouse model of type 2 diabetes supports the concept that myocardial insulin responsiveness is relatively intact in type 2 diabetes [43, 44]. This is in clear contrast to skeletal muscle and adipose tissue, where insulin resistance is a significant contributor to the observed elevations in circulating glucose and free fatty acid concentrations. The constant exposure of the heart to high free fatty acid and glucose levels could exert toxic effects (glucolipotoxicity) from the generation of harmful derivatives of glucose and lipid metabolism [45, 46]. Thus, although the heart may be less vulnerable to insulin resistance than skeletal muscle, systemic insulin resistance may still exert profound negative effects on the myocardium through the toxic effects of excess substrate [45, 46].

On the other hand, with regards to cardiac glucose metabolism, it is becoming clear that the most robust alterations take place at the level of glucose oxidation in the insulin resistant heart, and not at the level of glucose uptake and glycolysis [9, 47]. The following section will discuss in depth how the incomplete β-oxidation of fatty acids may contribute towards the pathogenesis of cardiac insulin resistance at the level of glucose oxidation, and whether this may represent a novel target to improve altered cardiac energetics in the setting of obesity and type 2 diabetes.

5 Incomplete Fatty Acid β-Oxidation

The complete oxidation of a fatty acyl CoA ester encompasses the production of acetyl CoA continuously until the fatty acyl chain is catabolized into its final four carbons, with the final β-oxidation spiral liberating the last two acetyl CoA molecules. As mentioned in the previous section, during the complete oxidation of a fatty acyl CoA ester, these acetyl CoA molecules are subsequently metabolized via the tricarboxylic acid (TCA) Cycle, generating the reducing equivalents NADH and $FADH_2$, which are utilized by the electron transport chain to fuel ATP synthesis via oxidative phosphorylation. If the TCA cycle cannot keep up with the incoming acetyl CoA being generated via mitochondrial fatty acid β-oxidation, the fatty acyl CoA esters will end up being incompletely oxidized (Fig. 3). As total CoA and carnitine levels are similar in the matrix space, and with CPT-2's transferase reaction maintained at a thermodynamic equilibrium of 0.45, as fatty acyl CoA esters are incompletely oxidized and various mitochondrial acyl CoAs accumulate, their respective acylcarnitine counterparts will also accumulate in the mitochondria [48]. Therefore, the use of targeted lipid profiling metabolomics to quantify various carbon chain length acylcarnitines has become a powerful tool to assess the status of incomplete fatty acid β-oxidation in peripheral tissues such as the muscle and heart [49].

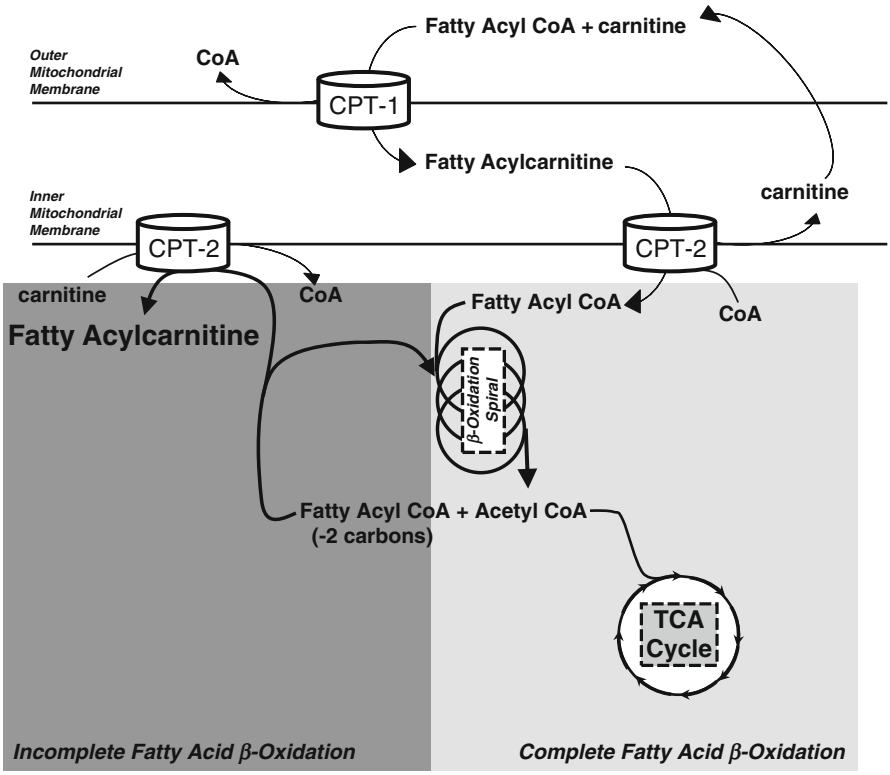

Fig. 3 Incomplete fatty acid β-oxidation. If the TCA cycle cannot sustain metabolism of the acetyl CoA being generated via mitochondrial fatty acid β-oxidation, the fatty acyl CoA esters will end up being incompletely oxidized. Because CoA and carnitine concentrations are similar in the mitochondrial matrix space, and with CPT-2's transferase reaction maintained at a thermodynamic equilibrium of 0.45, if a fatty acyl CoA ester is incompletely oxidized and mitochondrial fatty acyl CoA esters accumulate, CPT-2 will catalyze the reconversion of the accumulated acyl CoAs back into their respective acylcarnitine moieties

5.1 Incomplete Fatty Acid β-Oxidation and Skeletal Muscle Insulin Resistance

Elegant studies by Muoio and colleagues have demonstrated a significant role for excessive incomplete fatty acid β-oxidation rates in the development of skeletal muscle insulin resistance in response to underlying obesity. In cultured L6 rat skeletal muscle myotubes, increasing fatty acid concentrations increases incomplete fatty acid β-oxidation rates, which can be overcome via overexpression of peroxisome proliferator-activated receptor-γ-coactivator 1α [50]. Moreover, obese rats, mice, and Zucker diabetic fatty rats exhibit significant insulin resistance that is associated with a marked increase in the accumulation of a number of long chain

acylcarnitines in gastrocnemius muscle, combined with a reduction in a number of intermediates of the TCA cycle [51]. These findings are consistent with elevated incomplete fatty acid β-oxidation rates in insulin resistant skeletal muscle. Of interest, mice deficient for malonyl CoA decarboxylase (MCD−/−) exhibit reduced incomplete fatty acid β-oxidation rates due to reduced CPT-1 activity and subsequent mitochondrial fatty acid uptake, and are protected against obesity-induced skeletal muscle insulin resistance [51]. Similarly, treatment of diet-induced obese mice with the CPT-1 inhibitor, oxfenicine, protects mice against skeletal muscle insulin resistance and glucose intolerance [52], and this is associated with a reduction in myocyte incomplete fatty acid oxidation rates (unpublished observations). On the contrary, mice with a muscle-specific deficiency of carnitine acetyltransferase (CrAT) demonstrate an elevation in long chain acylcarnitines in gastrocnemius muscle and are glucose intolerant [53]. Furthermore, muscle-specific CrAT deficient mice exhibit metabolic inflexibility, as they are unable to switch to carbohydrate oxidation during the transition from fasting to feeding [53]. These findings appear clinically relevant, as studies in obese and type 2 diabetic humans have recapitulated observations of acylcarnitine accumulation and incomplete fatty acid β-oxidation in muscle [54, 55]. Furthermore, obese patient-derived primary human skeletal muscle myocytes cultured in the presence of lipolytically active adipocytes demonstrate a metabolic signature consistent with incomplete fatty acid β-oxidation, as medium and long chain acylcarnitines accumulated compared to myocytes cultured in the absence of adipocytes [56]. Intriguingly, treatment with the CPT-1 inhibitor, etomoxir, reduced the detrimental effects of lipolytically active adipocytes on glucose oxidation rates in obese patient-derived primary human skeletal muscle myocytes [56].

While the aforementioned studies support the notion that incomplete fatty acid β-oxidation negatively impacts skeletal muscle insulin sensitivity and subsequent carbohydrate utilization, the mechanism(s) responsible for this effect remain to be elucidated. Moreover, whether the accumulation of long chain acylcarnitines, which are often used as an index to identify that an increase in incomplete fatty acid β-oxidation rates is taking place in the muscle, can actually have direct cellular effects to inhibit insulin sensitivity is unknown. It has been suggested that acylcarnitine accumulation does not directly mediate insulin resistance, but rather may reflect a failed attempt to alleviate oxidative stress caused by mitochondrial lipid overload [57]. On the other hand, in recent years our appreciation of protein acylation/acetylation has grown considerably, with alterations in protein acylation/acetylation now thought to play a significant role in regulating mitochondrial function in the setting of obesity-related metabolic diseases [58]. Indeed, results from mass spectrometry-based proteomics have suggested that at least 20 % of the mitochondrial proteome is acetylated. Furthermore, the NAD^+-dependent deacetylase, sirtuin 3 (Sirt3), has been demonstrated to acetylate PDH, resulting in an inhibition of PDH activity [59]. In mice deficient for Sirt3, PDH in muscle is hyperacetylated, which results in a subsequent reduction in glucose oxidation and impairs metabolic flexibility during the fasting to feeding transition [59]. Thus, it is possible that the accumulation of acylcarnitines in the mitochondria may provide surplus acetyl residues

and contribute to the elevated acetylation of PDH and reduced glucose oxidation rates observed in the muscle in response to obesity-associated insulin resistance. As mentioned previously, muscle-specific CrAT deficient mice exhibit long chain acylcarnitine accumulation in muscle and are markedly insulin resistant, which is associated with a reduction in PDH activity [53]. Moreover, overexpression of CrAT in primary cultured human skeletal myocytes protects against fatty acid-induced PDH inhibition and improves glucose uptake [60]. Hence, CrAT activity may play a critical role in protecting PDH activity against incomplete fatty acid β-oxidation and subsequent mitochondrial lipid stress. Whether CrAT activity is altered in response to overnutrition and obesity remains to be determined.

5.2 Incomplete Fatty Acid β-Oxidation and Cardiac Insulin Resistance

Unlike the muscle, the heart is not subject to voluntary contraction and must continuously beat in order to deliver blood, oxygen, and nutrients to the rest of the body's organs for proper function. Thus, incomplete fatty acid β-oxidation is less likely to occur in the heart than the skeletal muscle, as there is constant demand on the TCA cycle to generate NADH and $FADH_2$ for oxidative phosphorylation to fuel ventricular contraction. Indeed, studies in the intact, isolated working heart demonstrate equivalent palmitate oxidation rates when palmitate is labeled with either [U-^{14}C], [1-^{14}C], or [9,10-^{3}H] palmitate [61–63]. However, these studies in the intact isolated heart are only measuring the metabolism of a single, exogenously supplied fatty acid substrate, and do not account for oxidation of endogenously supplied fatty acids arising from TAG mobilization. Despite palmitic acid being the most abundant plasma saturated fatty acid, oleic acid circulates at even higher concentrations in the plasma. Regardless, at equivalent and noncompeting concentrations, palmitate and oleate are oxidized at similar rates by the isolated working aerobic rat heart [3]. Whether there are alterations in the complete oxidation of fatty acids in the heart during the development and progression of various pathologies such as angina pectoris remains to be determined. Of interest, isolated working heart studies demonstrate similar palmitate oxidation rates between hearts from lean and obese (12 weeks of unrestricted access to a 60 % lard diet) mice [9]. In contrast, using gas chromatography–mass spectrometry targeted metabolomics to obtain a snap shot of in vivo fatty acid myocardial metabolism through quantification of acylcarnitines revealed more comprehensive information, as a number of long chain acylcarnitines accumulated in hearts from obese mice compared to their lean counterparts [9]. These findings are consistent with an elevation in myocardial incomplete fatty acid β-oxidation rates in the setting of obesity.

As alluded to in previous sections, the effect of insulin resistance on the myocadium appears to be most apparent at the level of insulin-stimulated glucose oxidation, and not at the level of glucose uptake and glycolysis [9, 47]. Indeed, studies in MCD−/− mice, a mouse model that demonstrates reduced incomplete fatty acid

β-oxidation rates in skeletal muscle due to reduced CPT-1 activity and subsequent mitochondrial fatty acid uptake [64], are resistant to the effects of obesity to reduce myocardial PDH activity and impair insulin-stimulated glucose oxidation [9]. Paralleling findings in skeletal muscle, metabolic profiling in hearts from obese MCD−/− mice demonstrate a marked reduction in the accumulation of a number of long chain acylcarnitines, indicative of reduced incomplete fatty acid β-oxidation rates [9]. These findings suggest that MCD inhibition or reducing mitochondrial fatty acid uptake to limit incomplete fatty acid β-oxidation rates may have clinical utility for reversing the effects of obesity on cardiac glucose oxidation.

It is well established that obesity increases one's risk for heart failure [65]. With heart failure being associated with impaired energetics and reductions in mitochondrial function [2], it will be important to ascertain whether elevations in myocardial incomplete fatty acid β-oxidation rates contribute to the high prevalence of heart failure in obese patients. Indeed, studies investigating chronic ischemia-induced heart failure demonstrate a significant protection against adverse left ventricular remodeling and improved left ventricular ejection fraction in MCD−/− mice compared to their wild type littermates at 4 weeks following permanent left anterior descending coronary artery occlusion [7]. However, whether the reduced incomplete fatty acid β-oxidation rates observed in hearts from MCD−/− mice plays a role in this cardioprotection against heart failure remains to be determined.

6 Conclusions

Studies exploring the alterations in myocardial fatty acid metabolism in cardiovascular disease have experienced a significant resurgence in the past decade. This is due in part to the obesity epidemic that now plagues our population, as obesity is a significant risk factor for cardiovascular diseases such as heart failure, and one of the most immediate effects of obesity on the heart involves increased delivery of fatty acids to the myocardium. Although studies suggest that the healthy heart for most part will completely oxidize the fatty acids it extracts for the purposes of energy production to sustain contractile function, recent findings illustrate at least in obesity, that the heart begins to exhibit increased rates of incomplete fatty acid β-oxidation, and this is associated with reductions in insulin-stimulated glucose oxidation rates. As the most potent effects of obesity on cardiac insulin sensitivity appear to take place at the level of glucose oxidation and not glucose uptake or glycolysis, it will be important for future research to delineate the mechanism(s) by which incomplete fatty acid β-oxidation impairs glucose oxidation. Such findings may lead to the development of new targets to alleviate obesity-associated cardiac insulin resistance.

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Part III
Optimization of Energy Metabolism

Metabolic Therapy for the Ischemic Heart

Giacinta Guarini, Alda Huqi, and Mario Marzilli

Abstract Myocardial ischemia is universally accepted to be the result of an imbalance between oxygen supply and requirements to the myocardium. The presence of flow limiting coronary stenosis is the main recognized pathological mechanism underlying this condition. While revascularization procedures are performed with the aim to remove the flow limiting stenosis, traditional medical therapy with hemodynamic agents aim at reducing oxygen demand of the myocardium. However, although effective, none of these treatment strategies or their combination confers symptomatic relief in all patients, in this way underlying the need for further research in this area.

Metabolic derangement is critical in patients who presents with ischemic heart disease (IHD). Under normal conditions the heart derives most of its energy from β-oxidation of free fatty acids (FA). However, the healthy heart is able to easily switch from one substrate to another according to substrate availability, nutritional status, and exercise level. Paradoxically, during prolonged and severe ischemia the myocardium continues to derive most of its energy (50–70 %) from β-oxidation, despite a high rate of lactate production. At this stage it is believed that FA oxidation can turn to be detrimental in that, while requiring more oxygen, it produces less ATP. Given such metabolic derangements, pharmacological approaches aimed at rebalancing myocardial metabolism may play a key role in treatment of patients with IHD. In this scenario, therapeutic interventions aiming at a shift of myocardial substrate utilization towards glucose metabolism would particularly benefit cardiac efficiency and IHD symptoms. In the next session principal metabolic agents will be discussed to further address their role in IHD.

Keywords Ischemic heart disease • Cardiac metabolism • Metabolic modulation

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1 Introduction

In the last thirty years mortality from cardiovascular disease has shown a global reduction. Such results have been mainly attributed to improved pharmacological and mechanical treatments, as well as efficacious educational programs. However, ischemic heart disease (IHD) remains the most important cause of death and morbidity in the western countries [1, 2]. Myocardial ischemia is commonly attributed to obstructive coronary artery disease (CAD) that, by limiting coronary blood flow, cause an unbalance between myocardial metabolic demands and blood supply. Based on this concept, anti-ischemic therapy has been mainly focused on the mechanical removal of the coronary obstructions, and/or pharmacological modulation of cardiac work and coronary blood flow, or both. These strategies are expected to restore an adequate supply/demand balance, improve symptoms and prolong survival. Unfortunately, an objective evaluation of available evidence does not confirm such expectations. Actually, several trials have reported that over 30 % of patients still experience angina despite "successful" coronary revascularization, by percutaneous coronary intervention (PCI) or by coronary artery bypass grafting (CABG) [3, 4]. Although with a certain degree of inertia, factors other than epicardial stenosis, such as microvascular dysfunction and metabolic derangement have been suggested as the underlying physiopathological mechanisms for persistent symptoms. Given the above mentioned incomplete success with current treatment, therapeutic strategies that target these "alternative physiopathological mechanisms" (i.e. metabolic modulation) have gained augmented interest.

Indeed, studies have shown that metabolic modulation therapy plays a critical role in the acute phase of ischemic events, impacting on the results of acute interventions, in the subsequent development of heart failure (HF)-stunned and hybernated myocardium- as well as for those who experience chronic stable angina [5]. A better understanding of the metabolic changes occurring at the time of ischemic events and following reperfusion are translating into new therapeutic opportunities.

2 Energy Metabolism and Myocardial Ischemia

As described in details in the previous chapters, the elevated energy demands of the heart are met by the hydrolysis of adenosine triphosphate (ATP), which is derived from a number of different metabolic pathways, including glycolysis and the mitochondrial oxidation of glucose, lactate, fatty acids (FA), and ketones. Under normal, aerobic conditions, 50–70 % of ATP requirements are derived from fatty acid ß-oxidation, with the remainder being mainly derived from carbohydrates oxidation, primarily glucose and lactate [6–8].

Importantly, the healthy heart is able to easily switch from one substrate to another according to substrate availability, nutritional status, and exercise level. For example, during increased workload (i.e. inotropic challenge) energy demands

are primarily met with increase in the glycolytic rates and carbohydrate oxidation, whereas exogenous fatty acid oxidation largely remains unchanged [9, 10].

On the other hand, in conditions of chronic neuro-hormonal activation (i.e. heart failure, HF) there is an enhanced catecholamine induced activation of lipolysis and up-regulation of genes associated with FA use via peroxisome proliferator-activated receptor (PPAR) which ultimately lead to increased FA delivery to the heart. In addition, FA promote their own uptake and oxidation and antagonize the uptake of glucose, lactate, and pyruvate, in part through direct inhibition of pyruvate dehydrogenase. Mitochondrial effects of FA include uncoupling of cellular respiration, resulting in decreased ATP production and oxygen wasting. Thus, elevated blood levels of FA augment lactate and proton accumulation, decrease cellular pH, and disrupt cellular function. Other consequences of excessive FA concentration in the blood stream include impaired calcium handling, oxidative stress, reduced activity of the glucose transporter (GLUT)-4 and myocytes apoptosis [11].

Similarly, energy substrate utilization is a determinant factor of the capability of the heart to sustain ischemic insults [6, 12]. During mild to moderate ischemia, myocardial cells enhance glucose uptake in order to generate sufficient ATP for maintaining appropriate ionic gradients and calcium homeostasis. Paradoxically, during prolonged and severe ischemia, myocardium continues to derive most of its energy from FA β-oxidation, despite a high rate of lactate production. In this conditions, acute allosteric and/or covalent modulation of cardiac energy metabolism occur and high rates of FA oxidation further inhibit glucose oxidation due to competitive interaction (Randle phenomenon). Although the complete oxidation of FA yields more adenosine triphosphate (ATP) than does complete oxidation of glucose, it requires a greater amount of oxygen. Therefore, for a given amount of oxygen consumed, metabolism of glucose is more "energy efficient," producing about 15 % more ATP. During sustained ischemia, when O_2 availability is very low and metabolic supply is impeded, FA oxidation can turn to be detrimental. By requiring more oxygen, it produces less ATP and more reactive oxygen species (ROS), all events that concur in depressing mitochondrial respiratory efficiency. However, absolute FA and carbohydrates oxidation is limited by the lack of an adequate supply of oxygen to the muscle during ischemia. In the post-ischemic period, rates of FA oxidation rapidly recover in the face of depressed contractile function, whereas rates of glucose oxidation remain low [6, 13, 14].

In conclusion, alterations in the availability of oxygen and circulating energy substrates, as well as alterations in the mechanisms regulating substrate metabolism contribute to the metabolic phenotype during ischemia, and can be responsible for disruption of cell homeostasis, alterations in cellular and mitochondrial membrane structure, and altered cardiac contractility and efficiency.

Based on this rationale, pharmacological agents and strategies that shift the balance of oxidative ATP production from FA β-oxidation towards glucose oxidation [15–21] increase cardiac post-ischemic recovery function and efficiency during chronic ischemia [22, 23]. Figures 1 and 2 encompass normal energy generation in the healthy heart and metabolic derangement in the ischemic heart, respectively.

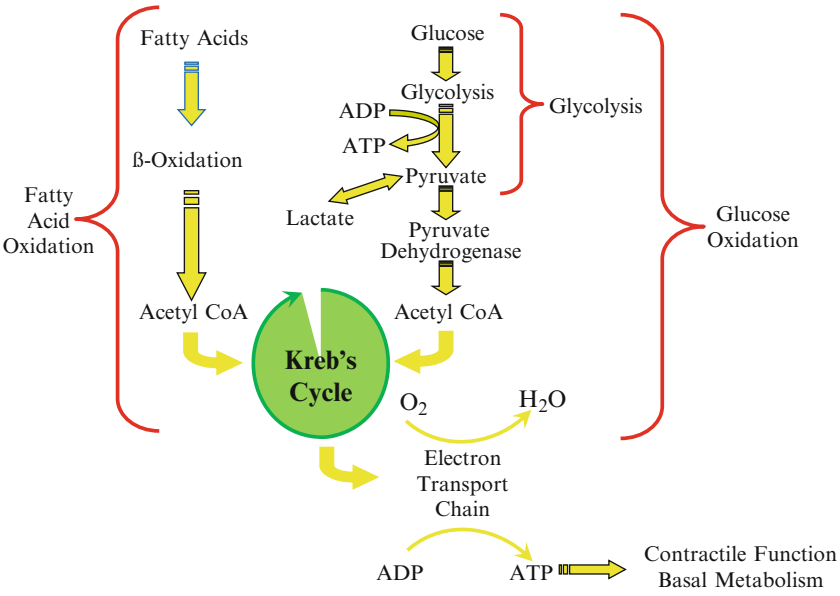

Fig. 1 Schematic representation of cardiac metabolism in normal condition. The heart derives most of its energy by fatty acids oxidation, but nutritional status (fasting, substrate availability) as well as exercise and hormones can modify fuel selection (see text for details)

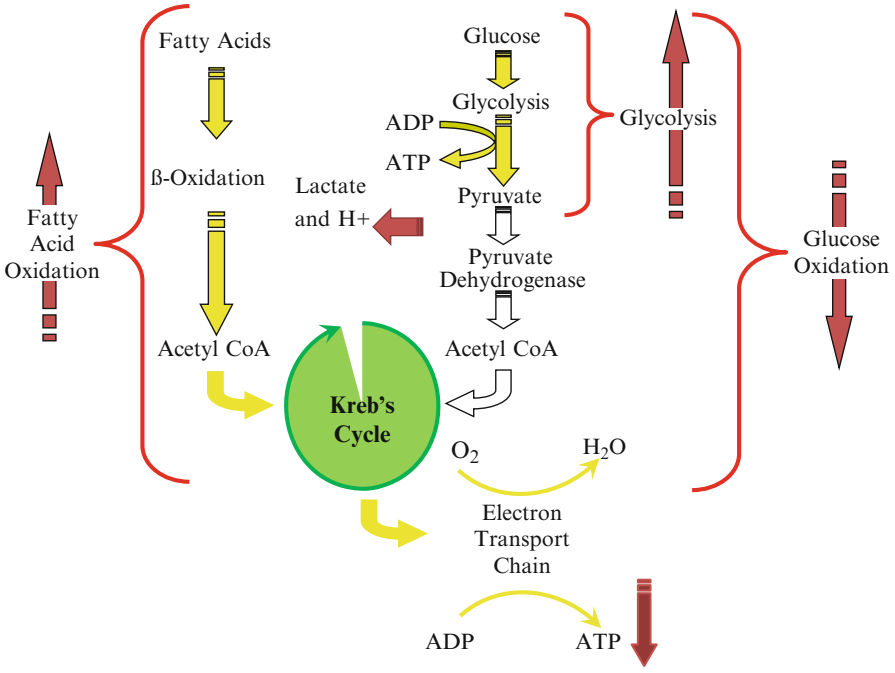

Fig. 2 Metabolic alterations during sustained period of ischemia. Glycolysis is the main catabolic pathway during ischemia, whereas fatty acid oxidation is enhanced in this period. As consequence less ATP is generated at the expenses of more protons production, which are ultimately responsible for myocardial acidosis and ions (mainly Calcium) accumulation (see text for more details). *Red arrows*: enhanced pathway; *white arrows*: almost abolished pathways

3 Metabolic Modulation Therapy

Given the interdependence between FA and glucose oxidation, metabolic modulation therapy with optimization of energy substrate utilization can be achieved by either inhibiting FA oxidation or stimulating glucose oxidation. This purpose can be achieved through three major strategies: (1) directly enhancing glucose oxidation; (2) decreasing the circulating levels of FA and/or their uptake by cardiac myocytes or mitochondrion; (3) directly inhibiting the enzymes that participate in FA oxidation.

3.1 Strategies to Enhance Glucose Oxidation

3.1.1 Dicholoroacetate (DCA)

The rate-limiting step for glucose oxidation is catalyzed by the pyruvate dehydrogenase (PDH) complex, which consists of PDH, PDH kinase (PDK), and PDH phosphatase (PDHP) enzymes [24]. Flux through PDH is regulated both by substrate/product ratios and by covalent modification. PDH flux is increased in response to increases in glycolysis and hence an increased generation of pyruvate, while PDH flux is decreased by increased ratios of mitochondrial NADH/NAD^+ and acetyl-CoA/CoA [25]. With regards to covalent modification, PDHP dephosphorylates and activates PDH, whereas PDK, in response to acetyl-CoA and NADH (produced primarily from FA oxidation) phosphorylates and inhibits PDH; and thus restricts the oxidation of glycolytically derived pyruvate [24]. Dichloroacetate (DCA) inhibits PDK activity, thus stimulating the mitochondrial PDH with improved glycolysis and glucose oxidation coupling and decreased proton production [26].

Despite the promising experimental evidence, a contemporary PubMed search displays only two small studies of DCA, conducted on a total of less than 50 ischemic heart disease patients. No definitive conclusions can therefore be derived for clinical purposes. In addition, when used in other pathological conditions, DCA treatment has been associated with relevant neurotoxicity, thus further compromising the expectations for any future clinical utilization.

3.2 Strategies to Reduce Cellular/Mitochondrial FA Uptake

Many years ago, Oliver and coworkers developed the concept that suppression of circulating plasma non-esterified FA, and thus myocardial FA uptake and oxidation, reduced ischemic damage and ventricular arrhythmias during acute myocardial infarction (AMI) or exercise-induced angina. This concept was further developed in the 1980s with the demonstration that direct inhibition of FA transport into the mitochondria with oxfenicine increased glucose oxidation and decreases lactate production, which resulted in symptom relief in patients with stable angina.

3.2.1 Glucose–Insulin–Potassium (GIK) Solution

The rationale for this pharmacological approach originates from the early nineteenth century, when it was observed that chest pain could be relieved by administration of sugar. Later on, it was found that patients with AMI present high plasma levels of FA, within 30 minutes of the onset of chest pain, and such levels could be reduced by infusion of glucose, insulin and potassium (GIK) in experimental models of AMI [27]. Such a property becomes particularly relevant in patients with diabetes, who have blunted baseline glucose utilization capability due to insulin resistance. Indeed, GIK solutions have been shown to augment glucose utilization at the expense of FA uptake through increased GLUT-1 and GLUT-4 translocation to the sarcolemmal membrane. In animal models of AMI, early GIK administration was associated with reduced myocardial oxygen extraction, malignant arrhythmias, and myocardial infarct size and ultrastructural damage and improved systolic function. Despite these promising results, over the years there has been only one clinical study, the Immediate Myocardial Metabolic Enhancement During Initial Assessment and Treatment in Emergency care (IMMEDIATE) Trial, confirming the benefits with administration of GIK solution in the first critical hours after the onset of symptoms of AMI, when FA levels are elevated. This study showed that pre-hospital administration of intravenous GIK, compared with glucose placebo, was associated with lower rates of the composite outcome of cardiac arrest or in-hospital mortality [28].

However, subsequent trials designed to support the beneficial effects of GIK solution in preserving cardiac tissue during AMI did not confirm these results. In this regard, a meta-analysis, including a total of 16 randomized trials which investigated the role of GIK solution in 28,374 AMI patients from 1966 to 2008, was recently conducted. There were a total of 1,367 deaths (9.6 %) in the GIK group and 1,351 deaths (9.6 %) in the control group. The lack of benefit was also confirmed in the subgroup analysis of patients given high-dose GIK and in those whom reperfusion was not performed [29].

Overall, there is no definitive evidence to support the use of the GIK solution in clinical practice. Such controversies have been attributed to the possible opposed effects of hyperglycemia with glucose toxicity and insulin infusion, with hyperglycemia neutralizing the benefits of insulin [30]. Indeed the beneficial effects, if any, have been related to the various properties of insulin (i.e. vasodilator [31], anti-inflammatory [32], antioxidative [33], positive inotropic [34], prosurvival [35] and antifibrinolytic [36]). Therefore, studies designed to separate out the effects of hyperglycemia and glucose toxicity are needed in order to confirm these broad spectrum beneficial effects of insulin and its utility in the AMI setting.

3.2.2 Carnitine Palmitoyl Transferase Inhibitors

A strategy to inhibit mitochondrial uptake of FA is to suppress rate-limiting enzyme for the mitochondrial uptake of fatty acids like carnitine palmitoyl transferase (CPT) I or II. Perhexiline, a reversible CPT-1, and to a lesser extent, CPT-2 inhibitor has been shown to ameliorate angina [37], and, more recently to attenuate the increase

in diastolic tension associated with myocardial ischemia, thereby improving myocardial efficiency [38]. Inhibition of CPT-1/CPT-2 by perhexiline increases myocardial oxygen utilization efficiency by at least 13 %. However, following perhexiline administration, cardiac efficiency increases by approximately 30 %, therefore suggesting additional mechanisms of effect [39]. However, interest in the long term administration of perhexiline has been diminished due to association with infrequent but serious hepatotoxicity and neuropathy that necessitates regular monitoring of plasma levels and makes perhexiline relatively contraindicated in patients with hepatic or renal dysfunction [40].

3.2.3 Malonyl-CoA Decarboxylase (MCD) Inhibitors

Malonyl-CoA is another potent, endogenous inhibitor of CPT-1 which decreases the uptake of FA into the mitochondria, thereby reducing mitochondrial FA β-oxidation. Malonyl-CoA decarboxylase (MCD) degrades malonyl-CoA and this effects leads to an increased fatty acid oxidation. Inhibition of MCD significantly increases malonyl-CoA levels, therefore causing a significant decrease in FA oxidation rates and a subsequent increase in glucose oxidation rates. In line with such pharmacodynamic properties, animal models of inhibition of MCD have shown a significant improvement in cardiac functional recovery of aerobically reperfused ischaemic hearts [15]. Inhibition of MCD in the heart appears to be a safe and a very promising therapeutic target for IHD but unfortunately has not yet been introduced for testing in a clinical setting.

3.3 Strategies to Reduce FA Oxidation

The concept of metabolic protection of the ischemic myocardium is in constant evolution and has recently been supported by clinical studies. The beneficial effect of coupling glycolysis to glucose oxidation explains the efficacy of anti-ischemic FA oxidation inhibitors such as trimetazidine and ranolazine. This is supported by the sub-cellular linkage between key glycolytic enzymes and the activity of two survival-promoting membrane-bound pumps, namely the sodium–potassium ATPase, and the calcium uptake pump of the sarcoplasmic reticulum (SERCA). Indeed, it has been demonstrated that ischemia induced disruption of cardiac metabolism can be minimized by metabolic agents that decrease oxidation of FA and increase the rate of combustion of glucose and lactate. In line with these considerations, the greatest progress in the use of metabolic therapy came with the advent of the direct inhibitors of myocardial FA oxidation, specifically TMZ and ranolazine [41].

3.3.1 Trimetazine

The anti-anginal efficacy of TMZ was established prior to the discovery that the drug acts via partial inhibition of myocardial FA oxidation (Fig. 3) [41, 42]. Initial preclinical studies demonstrated that it was cytoprotective in several models of

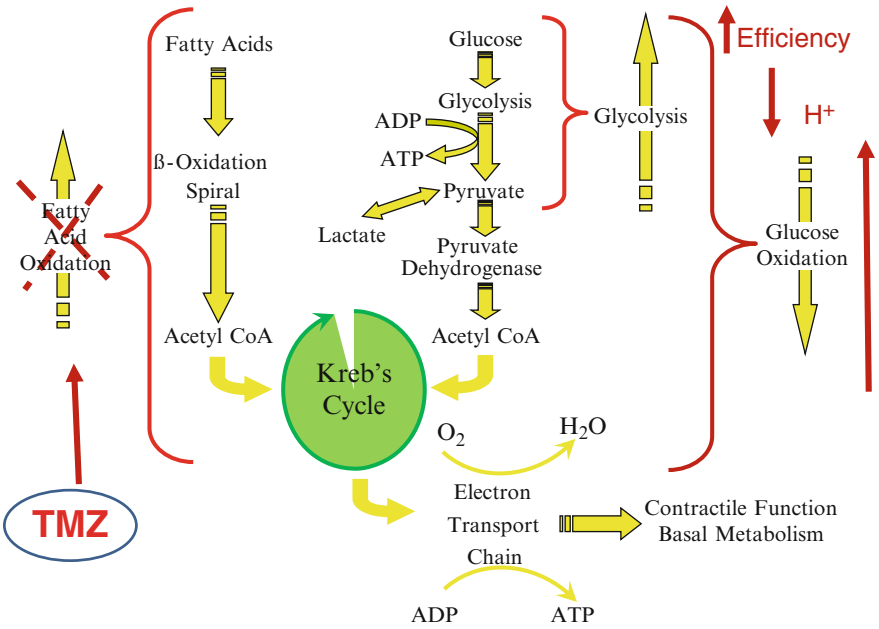

Fig. 3 TMZ inhibits LC-3KAT, one of the enzymes of fatty acid oxidation. In this way there is a reduced production of acetyl-CoA originating from fatty acid oxidation and therefore a relief of PDH activity. *ATP* adenosine triphosphate, *TMZ* trimetazidine

myocardial ischaemia and reperfusion [43]. More recently, Kantor et al. showed that TMZ specifically inhibits the long-chain activity of the enzyme 3-ketoacyl-CoA thiolase [17]. This enzyme, commonly referred to as '3-KAT', catalyzes the terminal reaction of FA β-oxidation, using long-chain 3-ketoacyl-CoA as a substrate, to generate acetyl-CoA. At clinically relevant concentrations TMZ does not inhibit the short or medium chain activity of 3-KAT. These results suggest that TMZ acts via inhibition of 3-KAT to reduce the NADH/NAD^+ and acetyl-CoA/free CoA ratios in the mitochondrial matrix. This would remove inhibition on PDH, and increase the rate of glucose oxidation. Indeed, in the working rat heart, TMZ significantly increased the rate of glucose oxidation despite only modestly reducing the rate of FA oxidation [12, 17]. A recent meta-analysis proved that TMZ was as effective as Ca-channel blockers, long-acting nitrates, micorandil or ranolazine to improve exercise tolerance and/or clinical parameters, whether used as monotherapy or in combination therapy [44]. Clinical trials have also proved the efficacy of this metabolic agent in angina refractory to Ca^{2+}-channel blockers, and have supported the superior benefit associated to the addition of this metabolic agent to classic hemodynamic agent, such as β-blockers or nitrates [45]. Furthermore, other studies have supported the use of TMZ to improve clinical manifestation in patients with stable IHD. Indeed, following chronic administration of TMZ a decrease of

average number of attacks per week, reduction of mean weekly consumption of short acting nitrates, improvement of quality of life, lessening of severity of main clinical manifestations of chronic HF, and lowering of its functional class have been observed [44, 46–48]. Moreover, similar efficacy of TMZ has been demonstrated both in men and women, which allows recommendation of this metabolic myocardial cytoprotector to patients with IHD irrespective of gender [49, 50]. TMZ has been provided to confer cardioprotection also in patients undergoing CABG and PCI [44, 51].

Beneficial effects of TMZ with reduction in left ventricular end-systolic volume, improved NYHA class, increased exercise duration and, more importantly, reduced all-cause mortality and cardiovascular events have been documented also in HF patients [52]. Unfortunately, although its clinical benefits have been documented since early eighties, trimetazidine still lacks a widespread clinical use and has only lately been included in the European guidelines (with a class IIb recommendation) for the management of chronic stable angina patients [53].

3.3.2 Ranolazine

In 2006, ranolazine (RNZ), a piperazine structurally related to TMZ was approved in the U.S. for the relief of angina in patients who remained symptomatic on BBs, CCB, or nitrates [54]. RNZ was shown to display antischemic properties through promotion of glucose oxidation at the expense of FA oxidation since early nineties [19]. However, additional properties such as reduction in intracellular calcium overload through inhibition of the late sodium channels have recently gained more attention [55]. These effects have been associated with a preserved mitochondrial structure, decreased intracellular calcium content and, finally, decreased post-ischemic ventricular fibrillation, myocardial stunning and infarct size. For this reason RNZ is currently considered a first generation of a new drug category (i.e. inhibitor of late sodium currents). Nonetheless, it is important to note that therapeutic concentrations at which a reduction in calcium overload is observed are similar to those at which an increase in glucose oxidation has been documented [19]. However, regardless of the action mechanism, RNZ has been shown to confer significant clinical benefit in angina patients. Its use is associated with a significant prolongation in exercise duration to angina and to ST-segment depression (1 mm), either in comparison or on top of defined anti-angina drugs [56]. Nonetheless, its effects on the morbidity of angina patients remain still to be determined. Despite QTc-prolonging action, clinical data have not shown a predisposition to torsades de pointes, and the medication has shown a reasonable safety profile even in those with structural heart disease. Other common side effects include dizziness and constipation.

Despite the “younger” age, as compared to TMZ, RNZ has evoked a major success and has gained a class IIa recommendation for angina relief in both European and American guidelines on management of stable ischemic heart disease [57].

4 Conclusions

Metabolic agents improve effectiveness of energy production, decrease the oxygen debt, and protect myocardial cells from the effects of ischemia. In this way they provide a valid alternative action mechanism as compared to classical hemodynamic agents which induce changes such as reduction in systemic vascular resistance, coronary vasodilatation, or negative inotropism. These effects offer particular advantage in patients in whom conventional treatment has proven insufficient or in those patients in whom conventional hemodynamic agents induce symptomatic hypotension, inappropriate bradycardia, or worsening HF. Unfortunately, a consistent part of the enthusiasm achieved from bench side research gets lost in translation when findings are applied into the clinical setting. For this reason, research efforts should be centered into the careful selection of the treatment agent as well as proper designation and support of clinical trials.

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Inhibition of Fatty Acid Oxidation to Treat Heart Failure in Patients

Rui Yan, Jin Wei, and Dengfeng Gao

Abstract Heart failure is a major cause of morbidity and mortality in the world. Cardiac energy metabolism, specifically fatty acid and glucose metabolism, is altered in heart failure and has been considered a contributing factor in the impaired heart function observed in patients with heart failure. Emerging evidence demonstrates that correcting these changes in energy metabolism by modulating mitochondrial oxidative metabolism may be effective treatment for heart failure. Promising strategies include the downregulation of fatty acid oxidation and increased coupling of glycolysis to glucose oxidation. Fatty acid β-oxidation enzymes carnitine palmitoyl transferase I and pyruvate dehydrogenase kinase are examples of metabolic targets for the treatment of heart failure. This article reviews the existing evidence for inhibition of fatty acid oxidation to treat heart failure. Further studies are needed to confirm the potential benefit of modulating these metabolic targets as an approach to treating heart failure in clinical settings.

Keywords Fatty acid • Oxidation • Heart failure • Treatment

1 Introduction

To preserve normal cardiac ejection, the myocardium requires more energy than do other tissues. Under the normal state, the major source of adenosine triphosphate (ATP) production is fatty acid oxidation (FAO), and others come from glucose and some amino acids. When heart failure occurs, the myocardial energy metabolism appears abnormal, which includes the downregulation of glucose and FAO,

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dependent more on glucose as its preferential substrate [1, 2]. In the early stage of heart failure, the abnormality may be inconspicuous; an animal experiment found that moderate or severe heart failure exhibited normal myocardial FAO [3]. This situation may be caused by elevated fatty acid concentration in heart failure. As failure progresses, mitochondrial oxidative metabolism is reduced and glycolysis is increased with the downregulation of glucose and FAO, and the shift of the preferential substrate is more significant. Considering that fatty acids are an inefficient energy substrate as compared with glucose, theoretically requiring 11–12 % more oxygen for identical ATP produced as carbohydrates [1], the change may be an important compensatory or protective mechanism for heart failure [4], which would improve "energy starvation". The decrease in FAO and increase in glycolysis observed in pressure-overload hypertrophied hearts is accompanied by changes in expression and activity of metabolic enzymes involved in these pathways.

Because of insufficient energy metabolism in heart failure, the modulation of cardiac metabolism may be a new approach to the treatment of heart failure. One important strategy is inhibition of FAO, which modifies fatty acid β-oxidation, decreases levels of circulating fatty acids, and increases the utilization of glucose. The pathway of fatty acid metabolism includes many enzymes, with gene transcription perhaps controlled by peroxisome proliferator-activated receptor (PPAR) and regulated by the sympathetic nerve. So FAO can be inhibited in many ways and used to treat heart failure (Fig. 1).

2 Inhibitors of FA Beta-Oxidation

2.1 Trimetazidine

Trimetazidine (TMZ) is a piperazine derivative (1-(2,3,4-trimethoxybenzyl)-piperazine dihydrochloride) that optimizes energy metabolism presumably by partial inhibition of long-chain 3-ketoacyl CoA thiolase (3-KAT), with subsequent decrease in FAO and stimulation of glucose oxidation [5]. TMZ can relieve angina pectoris in patients with coronary artery disease [6–11], and these benefits do not depend on the change in heart rate, blood pressure [10], and rate-pressure product at rest, during submaximal and peak exercise [9, 11]. Some clinical trials have demonstrated the effect of TMZ in heart failure, including ischemia or nonischemia. One trial of 50 patients with ischemic cardiomyopathy indicated that in addition to conventional treatment, TMZ could improve exercise tolerance, reduce the plasma bone natriuretic protein (BNP) and cardiac troponin T levels without improving New York Heart Association (NYHA) class and left ventricular ejection fraction (LVEF) after 6-month follow-up [12]. Some other studies demonstrated that TMZ could improve NHYA class, LVEF, endothelium-dependent relaxation, and quality of life; prolong 6-min walk distance; increase cardiac phosphocreatine and adenosine triphosphate (PCr/ATP) ratio; and even reduce all-cause mortality and heart-failure hospitalization mortality [13–18]. In patients with diabetes and idiopathic dilated

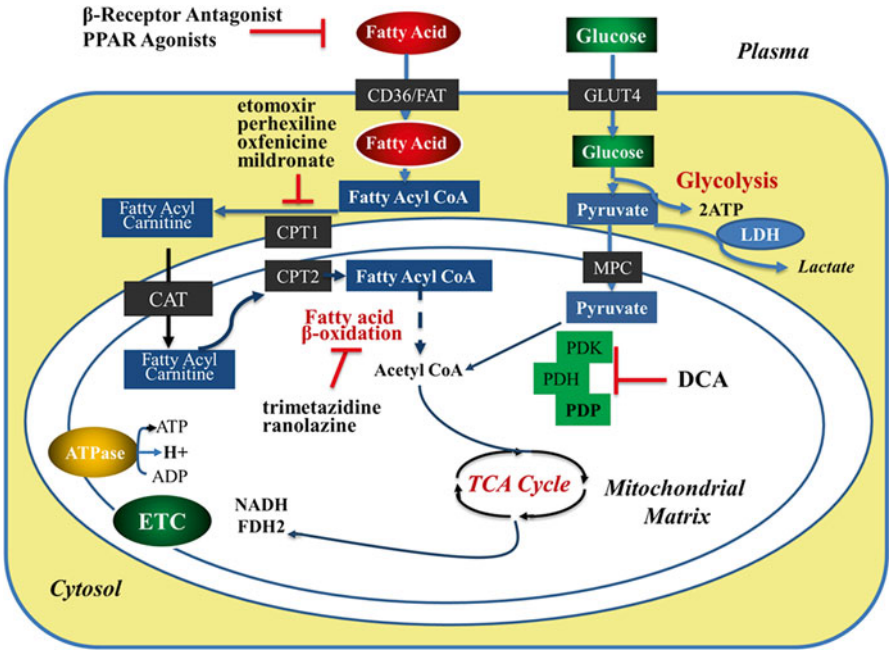

Fig. 1 Inhibition of fatty acid oxidation for the treatment of heart failure. β-Adrenoreceptor antagonists and peroxisome proliferator-activated receptor (PPAR) agonists decrease the level of circulating fatty acids. Carnitine palmitoyltransferase I (CPT-1) inhibitors decrease fatty acid β-oxidation and increase glucose oxidation by decreasing fatty acid transport into the mitochondria. Trimetazidine and ranolazine can directly inhibit fatty acid β-oxidation. Dichloroacetate (DCA) increases glucose oxidation by inhibiting pyruvate dehydrogenase kinase (PDK) activity, thereby stimulating pyruvate dehydrogenase (PDH)

cardiomyopathy, TMZ could also significantly improve cardiac function and physical tolerance [19–21]. At the same time, in patients with TMZ, inflammatory response was decreased [21]. Especially, LVEF was greater in diabetic than nondiabetic patients [22]. Although some trials provided encouraging results, some controversies remained. To solve the problem of inconsistent results of different clinical trials, Gao et al. and Zhang et al. contributed to meta-analyses in 2010 and 2012, respectively [23, 24]. The two integrated analyses suggested that TMZ improves LVEF, improves subjective and objective measures of functional status in heart failure, simultaneously ameliorates LV remodeling, and reduces hospitalization. Zhang et al. noted a significant association of gender and LVEF improvement. Otherwise, there were some disputes on all-cause mortality.

TMZ may contribute to the shift of energy production from free fatty acids (FFAs) to glucose oxidation, preservation of intracellular levels of phosphocreatine and ATP; reduction of cell acidosis, calcium overload and free radical-induced injury, and cardiac fibrosis; and improvement of endothelial function, which benefit heart failure. However, direct measurement of cardiac FAO in patients with chronic

non-ischemic heart failure revealed no changes in FFA uptake and only a 10 % decrease in FAO with TMZ. This finding challenges the concept that the beneficial effect of TMZ is mediated primarily by FAO inhibition [25]. Otherwise, researchers demonstrated that TMZ did not inhibit any component of β-oxidation in an isolated human cardiomyocyte cell line in 2003 [26]. Recently, some evidence indicated that the benefits of TMZ also came from its effect on the whole metabolism modulation. According to Fragasso and Tuunanen [18, 19], TMZ reduced the whole-body resting energy expenditure, regardless of the etiology and diabetic status of heart failure, reduced the cardiac FFA oxidation and improved whole-body insulin sensitivity in heart failure without diabetes. These data suggest that the metabolic effect of TMZ may also take place in other organs and tissues, to reduce the whole-body energy demand. Then, the improvement in insulin resistance, which contributes to the development of LV dysfunction by reducing cardiac efficiency through a metabolic shift toward fatty acid utilization would also decrease whole-body FFA oxidation.

An increasing number of studies have indicated that the cardioprotective effect of TMZ may occur via different mechanisms possibly involving regulation of mitochondrial function. In hypertrophied hearts, TMZ normalized post-ischemic function and fractional glucose oxidation via inhibiting glycolysis, possibly in response to reduced energy reserve and/or low rates of FAO. In ischemia-reperfusion, TMZ could decrease the infarct size of myocardium as with ischemia preconditioning, whose cardioprotective effect was represented by inhibiting mitochondria permeability transition pore (mPTP) opening, a critical event leading to cell death [27]. In myocytes of the failing heart, mPTP opening could also be antagonized by TMZ, which benefitted heart failure related to reduced complex II- and uncoupled oxidative stress mediated by mitochondrial nitric oxide synthase [25]. In a rat model *of ex vivo* perfusion with glucose, TMZ had a positive impact on mitochondrial homeostasis, which significantly increased the respiration control rate and respiratory chain complex I activity, thus leading to decreased reactive oxygen species (ROS) production, maintained mitochondrial electrical potential, and improved mitochondrial membrane integrity [28]. These effects may be attributed to the antioxidation of TMZ. When acute ischemia occurs, the positive effects of TMZ occur by increasing complex I activity, with decreased futile O_2 consumption and reduced ROS production, rather than depending on an increase in ATP production.

2.2 Ranolazine

Ranolazine is similar to TMZ, a piperazine derivative, ((±)-N-(2,6-dimethyl-phenyl-4-[2-hydroxy-3-(2-methoxy-phenoxy)-propyl]-1-pi-perazine acetamide; RS-43285)) [37]. It is an anti-ischemia drug and approved in the United States and some European countries for treatment, combined with amlodipine, beta-blockers or nitrates, of chronic stable angina in patients [29]. Some clinical evidence has demonstrated that ranolazine could improve ischemia symptoms and quality of life of patients with chronic stable or severe angina; it increased the exercise capacity,

time to angina and time to ≥1-mm ST-segment depression, and reduced angina frequency without clinically meaningful hemodynamic effects [30–34]. In non–ST-elevated acute coronary syndrome, ranolazine could increase exercise duration, reduce worsening angina and the incidence of arrhythmias, and thus decrease the risk of cardiovascular death, myocardial infarction and recurrent ischemia with elevated BNP accompanied by acute coronary syndrome [34–39]. Ranolazine has benefits in anti-ischemia, but the mechanism is not completely clear. One proposed mechanism is metabolic modulation. *In vitro* studies suggested that ranolazine inhibits FAO in skeletal muscle, and in isolated working rat hearts, ranolazine stimulated glucose oxidation, which may be a primary effect and concomitantly decrease FAO. This metabolic modulation would have some advantages for cells exposed to conditions of oxygen limitation or heart failure, including increased efficiency of ATP production, reduced production of lactate and H+, and reduction of other adverse effects from increased fatty acid metabolites. In guinea-pig hearts during low-flow ischaemia, ranolazine could improve pyruvate dehydrogenase activity, which was inhibited by global low-flow ischaemia [40]. Subsequent studies indicated no effects of ranolazine on pyruvate dehydrogenase kinase or phosphatase or on pyruvate dehydrogenase catalytic activity, which suggests that ranolazine activates pyruvate dehydrogenase indirectly [41]. Some evidence supported ranolazine attenuating calcium overload and ROS generation, thus preserving mitochondrial function [42, 43]. Therefore, ranolazine should partially inhibit β-oxidation, but the main mechanism does not appear to be through inhibition of myocardial FAO.

A few reports have described the effects of ranolazine on human metabolism. In the MERLIN TIMI-36 trial, ranolazine improved hyperglycemia control, with lower haemoglobin A1c level and fasting plasma glucose in diabetic patients [44, 45]. A few registered clinical trials have studied the effect of ranolazine in heart-failure patients, but most are not completed, and the results of the complete RALI-DHF trial have not been published [46]. However, animal experiments have given some inspiring results. Studies of acute intravenous infusion of ranolazine previously indicated improved LVEF, LV end-diastolic pressure (LVEDP), LV end-systolic volume, stroke volume, and cardiac output without an increase in myocardial oxygen consumption and thus improve mechanical efficiency in dogs with chronic heart failure [47, 48]. Another study of long-term ranolazine monotherapy in dogs with heart failure demonstrated significantly decreased LVEDP, accompanied by increased LVEF, stroke volume, and cardiac index [49].

2.3 Dichloroacetate

Dichloroacetate (DCA) inhibits pyruvate dehydrogenase (PDH) kinase activity, thereby stimulating PDH and carbohydrate oxidation, the rate-limiting enzyme of glucose oxidation. DCA treatment could increase glucose uptake and cardiac energy reserves and ameliorate chronic heart failure [50]. When DCA was administered intravenously for 30 min, myocardial lactate consumption and forward stroke

volume were elevated, myocardial oxygen consumption was reduced, and LV mechanical efficiency was improved in heart failure patients with NYHA functional class III–IV [51]. However, another clinical trial demonstrated that intravenous infusion of DCA with the same dose over 15 min did not significantly protect heart-failure patients with LVEF ≤ 40 %, which increased LV diastolic and systolic volumes and did not increase LVEF and stroke volume significantly [52]. Recently, some data suggested that DCA may be useful for treating pulmonary hypertension by increasing the mitochondria-dependent apoptosis of pulmonary artery smooth muscle cells and right ventricular failure.

3 Carnitine Palmitoyltransferase/Carnitine System Inhibitors

The rate-limiting enzyme for fatty acid β-oxidation and thus a potential drug target for regulating mitochondrial fatty acid uptake is carnitine palmitoyltransferase (CPT). Drugs targeting CPT-1 include etomoxir, an irreversible CPT-1 inhibitor; perhexiline; oxfenicine; and mildronate.

3.1 Etomoxir

Carnitine palmitoyltransferase 1(CPT-1) is the first limiting-rate enzyme for mitochondrial β-oxidation of long-chain fatty acids, which is located on the outer membrane of mitochondria. Etomoxir is a CPT-1 inhibitor.. This inhibition of mitochondrial CPT-1 is common to a number of oxirane carboxylic acid derivatives and is both irreversible and stereospecific. Etomoxir has been developed for treating diabetes mellitus type 2 but has been rarely explored for heart failure. A small clinical trial of heart-failure patients with NYHA class II-III indicated that etomoxir had neither a positive inotropic effect nor vasodilatory properties in acute studies; however, long-term treatment with etomoxir reduced resting heart rate, elevated LVEF, with increased CO and stroke volume during exercise [53]. However, in the ERGO study, etomoxir induced unacceptably high liver transaminase levels, which caused premature study termination. Then, according to the ERGO data, etomoxir produced no improvement of 6-min corridor walk test or echocardiographical values [54]. Long-term etomoxir treatment improved the performance of the hypertrophied ventricle in rats with ascending aorta constriction, including increased maximal developed pressure, LV pressure-volume area, and ±dP/dt(max) [55]. Yet, in a similar animal experiment with shorter treatment time and smaller drug dose, etomoxir could not affect cardiac function *in vivo* but improved function associated with a substrate switch in the isolated heart [56]. This evidence suggested that the effects of etomoxir on heart failure were still indistinct, and the effectiveness and safety required further investigation. In isolated working hearts with ischemia-reperfusion,

low-dose etomoxir decreased long-chain acylcarnitine and long-chain acyl-coenzyme A (CoA) levels but did not prevent depressed function. In contrast, a high dose of etomoxir prevented the palmitate-induced depression of function but did not decrease myocardial long-chain acylcarnitine or long-chain acyl-CoA levels, accompanied by decreased oxygen consumption per unit work during reperfusion recovery and increased ATP and creatine-phosphate levels. Thus, the potential protection of etomoxir was unrelated to changes in levels of long-chain acylcarnitines but might be due to increased glucose use by the reperfused heart. Moreover, this CPT-1 inhibitor increased the sarcoplasmic reticulum (SR) Ca2+ uptake rate. In rats with hypertrophied hearts by aortic constriction, etomoxir could prevent the reduced SR Ca2+-ATPase (SERCA2) gene expression and thus may prevent the transition of cardiac hypertrophy into heart failure [57, 58].

3.2 Perhexiline

Perhexiline is an antianginal agent that inhibits rat cardiac mitochondrial CPT-1 and CPT-2 levels [59]. Perhexiline inhibits FAO by reducing mitochondrial fatty acid uptake. However, in working rat hearts, perhexiline increased the efficiency of myocardial oxygen utilization by approximately 30 % without significant effects on palmitate oxidation, which suggested that the mechanism of perhexiline with improvement of myocardial efficiency was largely or entirely independent of its effects on CPT [60]. The use of this agent decreased because of the acute and chronic potential toxicity. Recently, some investigators conducted a re-evaluation of perhexiline. They collated the retrospective clinical data for patients with chronic heart failure and/or refractory angina and found that perhexiline therapy offered symptom relief for most patients, with only a small minority showing any side effects or abnormal liver function test results, and patients with refractory angina were more likely to be responders [61]. With careful plasma drug level monitoring for dose titration, perhexiline was effective and safe, with admirable results in chronic heart failure and especially refractory angina. A short-term, placebo-controlled clinical study of 56 patients with ischemic or nonischemic heart failure reported that perhexiline improved the quality of life, peak exercise oxygen consumption and LVEF [62]. However, perhexiline therapy in LV dysfunction after myocardial infarction failed to demonstrate an improvement in echocardiographical parameters and function of viable segments by dobutamine echocardiography [63]. Another clinical trial indicated that in symptomatic patients with hypertrophic cardiomyopathy, perhexiline improved diastolic dysfunction and exercise capacity and increased the myocardial ratio of phosphocreatine to ATP as measured by nuclear magnetic resonance, which is consistent with a metabolic mechanism of action [64]. Although these small clinical studies suggest that perhexiline should have beneficial effects in heart failure patients, more large-scale pivotal trials should be conducted in patients with heart failure or hypertrophic cardiomyopathy to further confirm the findings.

3.3 Oxfenicine

Oxfenicine (S-4-hydroxyphenyl glycine) is a selective inhibitor of cardiac mitochondrial CPT-1. It has not been used in human clinical studies, but some animal experiments have explored its effects on metabolism, myocardiac ischemia and heart failure. Oxfenicine could increase glucose metabolism and insulin sensitivity in mice with diet-induced obesity and insulin resistance via reduced FAO [65]. As well, early and sustained treatment with this CPTI inhibitor prevented by almost 1 week LV chamber dilation, wall thinning and delayed onset of decompensation in dogs with pacing-induced heart failure [66]. This finding is consistent with the drug's prevention of various changes in protein phenotype, especially metalloproteinase-2 and -9. In rats with diet-induced obesity, chronic oxfenicine treatment improved post-ischemic cardiac function and reduced myocardial infarct size by approximately 40 % after ischemia-reperfusion [67]. However, oxfenicine also affected myocardial hypertrophy in dogs and rats after 1 or 2 years, which might be caused by inhibition of FAO. However, 8-week administration of oxfenicine did not result in cardiac hypertrophy or contractile dysfunction in normal rats, even combined with a high-fat diet [68]. Otherwise, oxfenicine significantly blocked cell death induced by the combination of palmitate and carnitine, which suggests that oxfenicine could reduce palmitate-induced cardiac apoptosis [69].

3.4 Mildronate

Mildronate (3-(2,2,2-trimethylhydrazine)) propionate, also named THP and MET-88, could inhibit the uptake of long-chain fatty acids into mitochondria by reducing the level of carnitine biosynthesis, which is required by CPT-1, and inhibit FAO ultimately. In the mouse, long-term mildronate treatment significantly decreased carnitine concentration in plasma and heart tissue and increased the rate of insulin-stimulated glucose uptake by 35 %, accompanied by upregulated expression of related genes, including glucose transporter 4, hexokinase II, and insulin receptor proteins. These findings suggested that mildronate has benefits by decreasing FAO but also increasing glucose oxidation, prompting the shift of substrate preference. Mildronate has benefits on cardioprotection. In ischemia-reperfusion, mildronate could reduce the infarct size significantly, improve the recovery of cardiac function and decrease the incidence of ventricular fibrillation, thus preventing the accumulation of long-chain acylcarnitine induced by ischemia [70, 71]. Interestingly, mildronate did not affect cardiac function and energy metabolism without ischemia. In addition, long-term mildronate treatment attenuated the development of atherosclerosis in apoE/low-density-lipoprotein receptor double-deficient mice [72]. Mildronate prolonged survival with a median 50 % survival of 103 days as compared with 79 days for rats with heart failure following myocardial infarction as compared with control rats and attenuated the dilatation of the left ventricle and

decreased ATP level, thus preventing the increase in right atrial pressure [73]. The mildronate improvement in cardiac function in rats with heart failure induced by coronary artery ligation was attributed to increased SR Ca2+ pump activity. In LV myocardial homogenates, SERCA2 protein content was 32 % lower in the myocardial infarction group than the sham-operated group. However, in the mildronate group with myocardial infarction, SERCA2 content was the same as in the sham-operated group [74]. In another similar model, mildronate significantly prevented the decrease in Vmax for SR Ca2+ uptake activity and improved myocardial high-energy phosphate [75]. Mildronate prevented the cardiopathologic changes and reduced the increased expression of nuclear factor kBp65 induced by azidothymidine. Subsequent experiments indicated that mildronate acted at the level of complex I, mainly by reducing H_2O_2 to protect mitochondria in liver [76]. However, mildronate pre-treatment of rats at 100 or 200 mg/kg/day for 1 or 2 weeks did not prevent ischemia-reperfusion–induced mitochondrial dysfunction and liver injury [77]. Recently, mildronate was found to have beneficial effects in diabetes and neuroprotective properties. In conclusion, mildronate has greater protective action than inhibition of carnitine biosynthesis and thus would be an extremely potential drug.

4 Beta-Blocker

When heart failure occurs, β-adrenergic overdrive increases the level of circulating fatty acids, caused by enhanced fatty acid mobilization, inhibited myocardial uptake of glucose, and promoted onset of insulin resistance. Then, excess plasma fatty acid and insulin resistance could result in abnormalities of myocardial function. Therefore, an indirect therapeutic approach to inhibit FAO in the failing heart would be to reduce the circulating levels of fatty acids via a β-blocker. Long-term therapy with b-adrenergic receptor antagonists (metoprolol and carvedilol) can improve cardiac performance and survival in patients with heart failure. In cultured mouse C2C12 cells, carvedilol could inhibite palmitate oxidation and increase glycolysis by nearly 50 %. A few small clinical trials assessed the metabolic modulation of β-blockers. Carvedilol or bisoprolol treatment in heart failure patients could improve NYHA class and LVEF and increase mean cardiac phosphocreatine and ATP (PCr/ATP) ratio by 33 % on *in vivo* 31P-magnetic resonance spectroscopy [78]. Carvedilol treatment for 6 months for heart failure also reduced total body resting energy production rate and increased glucose oxidation. In another trial of nine patients with ischemic cardiomyopathy, carvedilol treatment in patients with heart failure resulted in a 57 % decrease in myocardial FFA use without a significant change in glucose use [79]. Metoprolol also caused a significant decrease in basal plasma FFA levels in patients with heart failure and decreased CPTI activity and increase in triglyceride content in dogs with coronary microembolism-induced heart failure [80]. A model of dogs with pacing-induced dilated cardiomyopathy was used for evaluating the effects of carvedilol and metoprolol on myocardial metabolism. Short-term treatment with carvedilol had superior hemodynamic and

metabolic effects as compared with metoprolol and included increasing plasma insulin levels and suppressing nonesterified fatty acids and glucagon levels [81]. These findings suggested that carvedilol had a more pronounced ability of shifting the substrate preference from FFAs to glucose. Not unsurprisingly, carvedilol treatment caused a 20 % reduction in myocardial FFA extraction, whereas metoprolol had a neutral effect in patients with chronic heart failure after 4 months of therapy [82]. Meanwhile, carvedilol treatment tended to increase cardiac lactate extraction, so it caused a shift from FFA utilization to lactate utilization in heart failure. The differences in these two agents on cardiac sympathetic activity and energy metabolism may be related to the differential effects of these drugs on clinical outcomes.

5 Conclusions

Although some small clinical trials showed surprisingly efficacious effects of inhibition of fatty acid metabolism on heart failure, some other studies did not support the approach for heart failure. For example, in a review published in 1994, cardiomyopathy often develops in children with genetic defects in FAO enzymes, which forces the heart to rely on glucose. This finding suggested that FAO may be indispensable for myocytes. Acipimox, a nicotinic-acid derivative that could decrease the FFA level of plasma, was used for heart failure, without profound benefits in cardiac efficiency [83]. The peroxisome proliferator-activated receptor alpha (PPARα) agonist, which would prevent changes in myocardial substrate metabolism in the failing heart treated with fenofibrate in pigs with pacing-induced heart failure, increased the expression of PPARα-regulated genes, prevented LV hypertrophy, and delayed the development of LV dilation and dysfunction [84]. In addition, new evidence supports that metabolic abnormalities in the failing heart post-infarction revealed that the heart was not energetically starved but rather inefficient in energy utilization for mechanical function [85]. Thus, we regret that we cannot state clearly that inhibition of FAO is an adaptive or a maladaptive process based on existing evidence. In conclusion, although energy metabolic modulation is an important and effective approach in heart failure, some questions remain but are worth more efforts to search for evidence to verify further mechanisms and clinical effects.

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Cardiac Metabolic Protection for the Newborn Heart

J. Carter Ralphe and Thomas D. Scholz

Abstract As the newborn heart transitions into the extrauterine environment, it emerges from a relatively hypoxic state, restricted predominately to glucose for energy, into an oxygen abundant environment in which a wider selection of substrates is available. Exposed to the unique milieu *in utero*, the fetal cardiac myocyte developed metabolic pathways that will subsequently adapt to postnatal life. Prior to the transition to a more adult-like metabolism, the newborn heart can take advantage of the metabolic profile developed *in utero* to protect itself during times of stress such as global ischemia and abrupt hypoxia. This relative tolerance to low oxygen levels and robust coronary perfusion of the neonatal heart have been exploited by surgeons to allow prolonged periods of bypass or cardiac arrest, facilitating repair of complex congenital heart defects. The ability of the newborn heart to utilize a variety of energy substrates has generated great interest in defining the optimal composition of cardioplegia solutions to enhance the ability of newborn myocardium to tolerate open heart surgery.

Keywords Cardioplegia • Extracorporeal membrane oxygenation • ECMO • Preservation • Cardioplumonary bypass • Preconditioning

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1 Introduction

1.1 Neonatal Cardiac Metabolism

In utero, circulating fetal levels of substrates are largely determined by the placenta, which while providing substantial amounts of glucose and lactate, serves as a barrier to fatty acids [1]. Thus, the growth of the fetal heart occurs in a substrate environment largely restricted to glucose and lactate.

Uptake of glucose in the fetal heart is mainly dependent on the glucose transporters, GLUT1 and GLUT4. During fetal developmental, the amount of both transporters increases, although GLUT1 is thought to play the primary role in bringing glucose into the cardiac myocyte [2–4]. Postnatally, GLUT1 levels decline and GLUT4 levels increase so that the adult heart has a substantial store of the insulin-recruitable glucose transporter GLUT4. Interestingly, this maturational process can be accelerated if the fetal heart is stressed (Fig. 1). In chronically anemic fetal sheep, Ralphe et al. found that GLUT1 protein levels decreased while GLUT4 myocardial protein levels were greater in the stressed animals [5]. Further, GLUT4 was found on the myocyte cell membrane where it could be actively involved in glucose uptake, likely facilitating glucose uptake in the stressed fetal heart.

The exact amount of glucose that enters a cardiac myocyte and is completely oxidized varies depending on what other substrates are made available to the heart [6]. Using isolated, working fetal porcine hearts, Werner and Sicard found that these hearts readily take up and oxidize both glucose and lactate, but the addition of lactate to the perfusate greatly inhibited glucose uptake [7]. Postnatally, similar results are found. Lopaschuk et al. studied isolated, working rabbit hearts at 1 and 7 days of age perfused with glucose, lactate, and palmitate [8]. Careful measurements of substrate uptake and oxidation revealed glycolytic rates nearly five times greater in one-day old hearts compared to 7 days. Low glucose oxidation rates were found at both ages, with glycolytic rates 46 and 25 times glucose oxidation rates at one- and seven-days, respectively. Comparing perfusates with and without lactate found that the addition of lactate did not affect glycolysis in the one-day hearts, but significantly decreased glucose oxidation. The mechanism of the imbalance between glycolytic

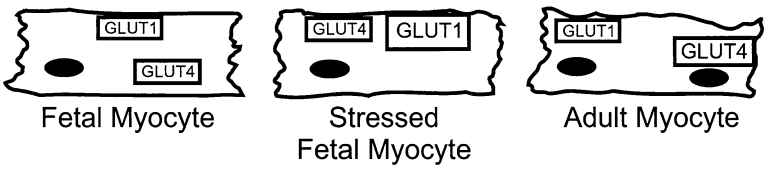

Fig. 1 Glucose Uptake by the Fetal and Postnatal Cardiac Myocyte. In the fetal myocyte, the glucose transporter GLUT1 is the dominant glucose transporter with lesser amounts of GLUT4. If stressed, GLUT4 levels in the fetal cardiac myocyte increase and translocate to the sarcolemma membrane while GLUT1 levels decrease. In the adult, the insulin-recruitable GLUT4 is the dominant glucose transporter, although GLUT1 is constitutively expressed on the cell membrane

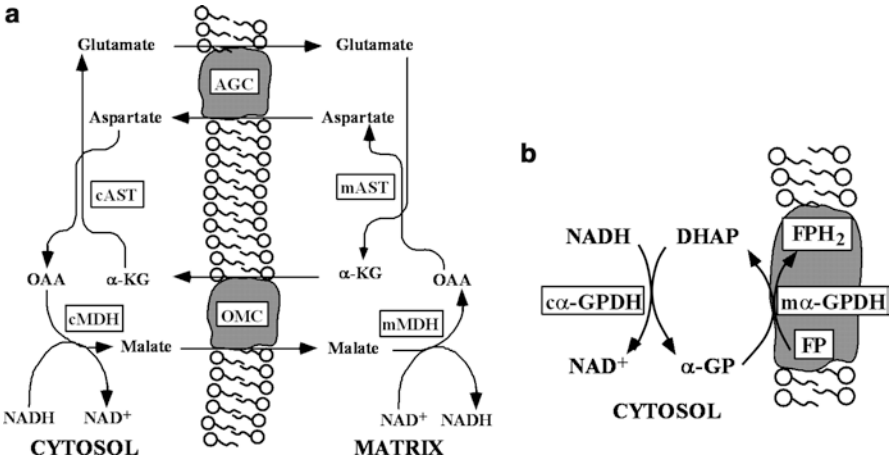

Fig. 2 The NADH Shuttles. (**a**) Schematic of the malate/aspartate shuttle. A sect. of the inner mitochondrial membrane is shown. *cAST* cytosolic aspartate aminotransferase (AST), *cMDH* cytosolic malate dehydrogenase (MDH), *OAA* oxaloacetate, *alpha-KG* alpha-ketoglutarate or oxoglutarate, *NADH* reduced nicotinamide adenine dinucleotide, *NAD+* oxidized nicotinamide adenine dinucleotide, *OMC* oxoglutarate/malate carrier, *AGC* aspartate/glutamate carrier, *mMDH* mitochondrial MDH, *mAST* mitochondrial AST. (**b**) Schematic of the alpha-glycerophosphate shuttle. *alpha-GPDH* cytosolic alpha-glycerophosphate dehydrogenase (alpha-GPDH), *DHAP* dihydroxyacetone phosphate, *alpha-GP* alpha-glycerophosphate, *m-alpha-GPDH* mitochondrial alpha-GPDH, *FP* flavoproteinm, *FPH2* reduced flavoprotein

rates in the cytosol and glucose oxidation by the mitochondria remains uncertain, although flux limitation at the pyruvate dehydrogenase complex does not appear to play a primary role.

The two moles of NADH that are produced for each mole of glucose oxidized by glycolysis must traverse the inner mitochondrial membrane to enter the electron transport chain. The demonstration by Lehninger and others [9, 10] that the inner mitochondrial membrane was impermeable to NADH led to the description of various "shuttle" mechanisms (Fig. 2). The two primary NADH shuttle systems in heart are the α-glycerophosphate (α-GP) and malate/aspartate shuttles [11–13]. The α-GP shuttle is a relatively uncomplicated reaction sequence with maximal activity primarily regulated at the mitochondrial α-glycerophosphate dehydrogenase [14]. The malate/aspartate shuttle is the dominant NADH shuttle in cardiac myocytes with a capacity at least an order of magnitude greater than the α-GP shuttle [12]. The regulation of the malate/aspartate shuttle is complex [15–19]. It has been demonstrated, however, that malate/aspartate shuttle capacity (per mg mitochondria) is nearly three times greater in newborn mitochondria compared to adult mitochondria [12].

Postnatally, the ability of the newborn heart to utilize fatty acids develops rapidly and fatty acid oxidation becomes the preferred source for ATP production. In studies using isolated, perfused working rabbit hearts, Lopaschuk et al. found that palmitate oxidation provided 43 % of ATP production in hearts from one-day animals and 90 %

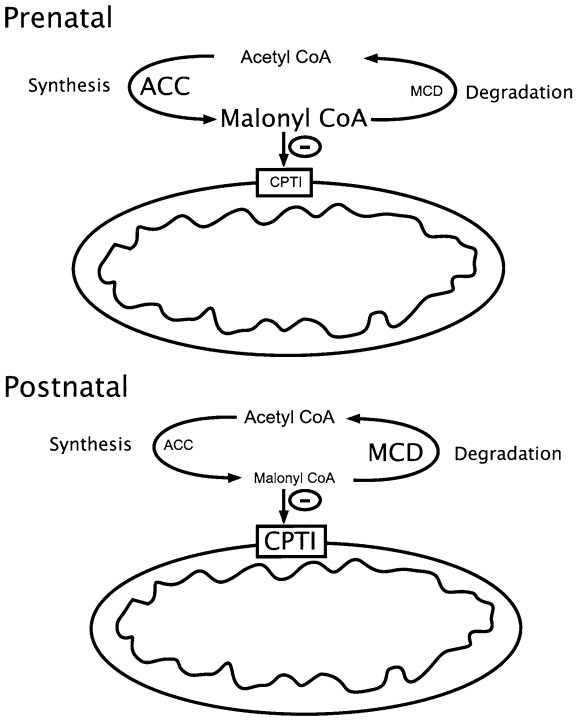

Fig. 3 Developmental Regulation of Fatty Acid Metabolism. Malonyl coenzyme A (Malonyl CoA) directly inhibits activity of carnitine palmitoyltransferase I (CPT I). Malonyl CoA levels are impacted by rates of synthesis by acetyl CoA carboxylase (ACC) and degradation rates by malonyl CoA decarboxylase (MCD). ACC activity decreases postnatally due to decreased levels and inactivation by AMP kinase while MCD levels increase postnatally. The decreased postnatal levels of malonyl CoA result in greater CPT I activity

of ATP in seven-day animals [8]. This corresponded to a decrease in ATP supplied from glucose oxidation from 57 to 10 % at 1 and 7 days, respectively. Lopaschuk's laboratory has extensively investigated the mechanisms by which the rapid change in fatty acid metabolism occurs with important control occurring at CPT I (Fig. 3). Malonyl CoA has been shown to be an effective inhibitor of CPT I and there is a rapid decline in malonyl CoA postnatally [20–22]. Malonyl CoA levels are altered by postnatal changes in both its synthesis via acetyl CoA carboxylase (ACC) and its degradation via malonyl CoA decarboxylase (MCD) [20]. Synthesis of malonyl CoA is decreased due to a decline ACC activity following birth as well as through inactivation of ACC by AMP kinase, which increases postnatally [23]. Activity of MCD, which decarboxylates malonyl CoA to acetyl CoA, also rapidly increases after birth [24]. Thus, there are several factors that impact malonyl CoA levels in the late fetal and postnatal period that play a prominent role in regulating fatty acid utilization in the neonatal heart. The mechanisms controlling these enzymes of fatty acid metabolism are not entirely clear but may be related to substrate supply, hormonal milieu, and alterations in cellular protein expression.

The postnatal period is accompanied by marked increases in gene expression encoding myocardial enzymes in the fatty acid oxidation pathway. Lavrentyev et al. have shown in the rat that mRNA expression of fatty acid translocase, fatty acid transporter protein and liver and muscle CPT I isoforms increase immediately

after birth [25]. Myocardial expression of CPT I and other nuclear genes encoding mitochondrial fatty acid oxidation enzymes are regulated by the transcription factor peroxisome proliferator-activated receptor α (PPARα) [26]. PPARα, a member of the extended nuclear hormone receptor superfamily, is in turn activated by medium and long chain fatty acid ligands. Not surprisingly, expression of PPARα and it coactivator PGC-1α mRNA increase 1.5–2.5 fold during the first week of life [25].

2 Metabolic Changes in Pathologic States

The adaptation of specific metabolic pathways of the newborn heart to pathologic conditions, such as hypoxemia or ischemia, has not been extensively studied. As reviewed by Ascuitto and Ross-Ascuitto [27], the immature myocardium has an extraordinary capacity to maintain glycolysis during times of oxygen deficiency through an increase in glucose uptake and acceleration of glycogenolysis. During such periods, studies reveal that oxidative metabolism of glucose and fatty acids is depressed and anaerobic glycolysis becomes the major pathway of ATP production. Hypoxic newborn animals display increased expression of LDH5, the LDH isoform facilitating the reduction of pyruvate to lactate, reflecting anaerobic glycolysis [28]. In newborn piglets subjected to prolonged moderate hypoxia, myocardial substrate utilization shifts towards glucose and lactate, whereas palmitate oxidation is significantly decreased [29]. With prolonged hypoxia end products of glycolysis, including lactate, accumulate exacerbating intracellular acidosis leading to inhibition of anaerobic glycolysis. The immature heart appears to have protective mechanisms to limit energy demand during periods of diminished energy production, resulting in part from less myocyte myofibrillar content [27] and the isoform expression patterns of sarcomere proteins including myosin, titin, and troponin I [30, 31]. There is also delayed depletion of ATP in isolated myocytes from neonatal hearts previously subjected to chronic hypoxia compared to controls. The adaptive metabolic changes in neonatal myocardium to chronic oxygen depletion serve to maintain cardiac function and offer protection from ischemic injury.

Whereas a lack of oxygen to the heart is accompanied by maintained or increased coronary flow allowing washout of metabolic intermediates, ischemia due to inadequate coronary flow results in decreased clearance of metabolites [27, 29]. Many of these metabolites, including NADH, lactate and AMP, feedback upon metabolic pathways to regulate pathway activity. Here again, under adverse conditions, the neonatal heart appears to exhibit better tolerance than adult hearts. The more rapid improvement in contractile function upon reperfusion is likely related to the improved ability of the neonatal heart to maintain intracellular pH and ATP compared to the adult heart [32].

Extracorporeal membrane oxygenation (ECMO) represents a state of prolonged cardiopulmonary support in which the heart and lungs are rested to allow recovery from a major insult. A shorter-term version, cardiopulmonary bypass, is used for infants undergoing open-heart surgery. Recent studies by Portman et al. using an

immature swine model have explored some of the metabolic responses the heart undergoes when placed on ECMO. In contrast to the newborn heart subjected to hypoxia, his studies demonstrated that the unloaded swine heart increased the utilization of fatty acids [33]. Up-regulation of pyruvate dehydrogenase kinase 4 and subsequent phosphorylation of pyruvate dehydrogenase was thought to decrease pyruvate conversion to acetyl-CoA and reciprocally increase fatty acid oxidation. No information about how the human heart responds metabolically to ECMO is yet available.

3 Protective Role of Thyroid Hormone

The active form of thyroid hormone, 3.5.3′-triiodothyronine (T3), has a broad role in regulating myocardial function and metabolism through both gene dependent and gene independent mechanisms. T3 can directly impact cardiac function during recovery from an ischemic insult by increasing heart rate, ejection fraction and cardiac output [34]. By binding to thyroid response elements in the nucleus, thyroid hormone can modulate metabolic activity through activation of a number of dehydrogenases including succinate dehydrogenase, NADH deyhydrogenase, and alpha-glycerophosphate dehydrogenase [19, 35]. There are also genes encoding metabolic proteins that are regulated by T3 that do not have thyroid response elements. Expression of these metabolic genes is indirectly impacted by thyroid hormone through transcription factors and coactivators that are regulated by T3 [36]. Weitzel and coworkers speculated that T3 target genes that increase their expression within hours of exposure to T3 contain thyroid response elements while those whose expression increases 12–24 h after administration of thyroid hormone are regulated by indirect means [36]. However, changes in protein content of metabolic enzymes have been seen in as little as 60 min following exposure to T3 suggesting that posttranscriptional processes are activated and contribute to greater protein levels [37].

The mechanisms by which T3 regulates expression of metabolic proteins in the heart are relevant given the clear evidence that thyroid hormone plays a protective role in the immature heart both in humans and in experimental models [38, 39]. Using a piglet model of cardiopulmonary bypass, Olson et al. investigated the role short-term exposure to T3 had on both recovery of the immature heart and flux of pyruvate into the citric acid cycle [38]. Fifty minutes of exposure to thyroid hormone resulted in improved functional recovery following cardiopulmonary bypass. In addition, pyruvate flux through pyruvate decarboxylase and pyruvate carboxylase was increased nearly fourfold. Pyruvate also served as an anapleurotic substrate to supply intermediary metabolites in the citric acid cycle, which was enhanced with thyroid hormone treatment. These studies may provide the mechanism for the beneficial effects of thyroid hormone seen in children undergoing cardiopulmonary bypass for correction of congenital heart disease [39]. In a multicenter study in children under the age of two years (the TRICC trial), T3 was given just prior to cardiopulmonary bypass, immediately after bypass, then at 3 h intervals for up to

9 h post-bypass. A number of clinical parameters were measured. Administration of thyroid hormone shortened postoperative mechanical ventilation time, improved ejection fraction, and reduced the need for inotropic support. Although assessment of T3-induced changes in myocardial metabolism was not possible in this study, the rapid response to the T3 suggested that enhancements in cardiac metabolic performance contributed to the positive findings.

4 Role of Calcium in Neonatal Myocardial Protection

Intracellular calcium overload has long been appreciated as both a consequence of ischemia-reperfusion and a trigger for cardiomyocyte dysfunction after periods of hypoxia [40]. The effects of calcium overload are widespread resulting in a depletion of ATP in the face of decreased mitochondrial ATP generation, deceased contractility, and ultimately cell death. The vulnerability of immature myocardium to fluctuations in extracellular calcium stem from its relatively under-developed sarcoplasmic reticulum with resulting decreased intracellular calcium stores and increased reliance on extracellular calcium to maintain optimal excitation-contraction coupling [41]. Hypoxia disrupts ATP-dependent transport processes and results in intracellular accumulation of calcium, which is thought to be a triggering event in ischemia/reperfusion injury [40]. One of the most studied downstream effects of calcium overload is the opening of the mitochondrial permeability transition pore (MPT) and resulting loss of mitochondrial membrane potential. This leads to further derangement of calcium homeostasis, energy balance, and cell death through apoptosis. A variety of therapeutic interventions have been investigated to mitigate opening of the MPT including ischemic preconditioning with and without concurrent administration of thiopental [42] and glycogen synthase kinase-3 inhibitors [43] without wide acceptance.

One approach to reduce cardiopulmonary bypass-related myocardial injury that has shown some benefit is hypoxic pre- and post-conditioning using short duration hypoxic exposure prior to, and after, cardiopulmonary bypass [44]. The mechanisms leading to enhanced post-ischemic contractile function reflect the impact both within the cardiomyocyte as well as attenuation of the well-known bypass-related systemic inflammatory activation. Within the myocardium there is evidence of reduced oxidative stress, enhanced calcium handling, and preserved mitochondrial function. Recent studies in children undergoing repair of congenital heart defects indicates a role for remote postconditioning in preservation of function. In this approach, repeated brief distal limb ischemia resulted in a reduction of circulating inflammatory mediators and reduced release of cardiac troponin and creatine kinase-MB, indicating an attenuation of myocardial injury [45]. Interestingly, this remote postconditioning effect on preservation of function can even be transferred from one preconditioned heart to a naïve heart subsequently subjected to ischemia, an effect that appeared to be mediated through adenosine receptors [46]. A randomized controlled study in children undergoing heart surgery also suggested protective

effects of remote postconditioning, with a reduction in troponin release, reduced need for post-operative inotrope support, and improved lung function [47]. However, the small size of this study precluded drawing definitive conclusions about the mechanisms underlying the observed effects.

While the goal of conditioning is to reduce the myocardial insult that leads to intracellular calcium overload, a complementary approach has centered on reducing the concentration of calcium in the cardioplegia solutions perfused during the cardiopulmonary bypass surgery. The theory behind the hypocalcemic cardioplegia is the recognized sensitivity of the immature cardiomyocyte to extracellular calcium and the relative reduced calcium-sequestering capacity of the sarcoplasmic reticulum. Studies in animal models have produced conflicting results as to whether calcium concentration impacted post-ischemic functional recovery. An elegant study by Bolling et.al, using intact neonatal piglets simulating operative courses showed that while the calcium concentration had little impact on normoxic myocardium, the impact was profound in myocardium that was hypoxic prior to surgery, much like the hypoxic neonate with congenital heart disease [48]. In the hypoxic group, the reduced cardioplegia calcium resulted in preserved systolic function, normal left ventricle compliance, and normal coronary resistance. These data stood in marked contrast to the hypoxic animals exposed to the standard normal calcium concentration which developed significant systolic dysfunction, stiff left ventricles, and elevated coronary vascular resistance. A reduced-calcium cardioplegia solution (del Nido solution) has been shown to reduce intracellular calcium overload in neonatal rat cardiomyocytes, improve myocardial performance post-bypass, and reduce release of troponin I in young children undergoing heart surgery [49]. This beneficial effect was also seen in vulnerable aged human hearts [50].

5 Metabolic Approaches to Myocardial Protection in the Neonate

A major obstacle in the development of surgical treatment of complex congenital heart defects has been the need to subject the neonatal heart to prolonged periods of ischemia. While the immature heart tolerates this condition better than adult hearts, the impact of prolonged periods of hypoxia and ischemia on the newborn heart often resulted in severely depressed ventricular function. Cardiac surgeons have devoted considerable effort to identifying ways to reduce the requirements of the myocardium intra-operatively for both oxygen and metabolic substrates through the use of hypothermia and provide better metabolic support for the heart during the post-operative recovery period. For those focused on the care of the immature heart, the possibility that the neonatal heart may have unique energetics that might be exploited has been tantalizing, though practical applications remain elusive.

The impact of surgically induced hypoxia and ischemia is multifactorial with acute injury to the myocardium secondary to forced anaerobic metabolism, depletion of phospo-intermediates, development of intracellular acidosis, and disruption of calcium homeostasis as discussed above. The immediate result is severe

contractile dysfunction and cell damage. A longer-term issue stemming from these insults is compromised mitochondrial integrity that stimulates the cascade leading to apoptotic cell death during the post-operative recovery period [51]. With these negative outcomes in mind, efforts have focused on reducing energetic demand (various degrees of intraoperative myocardial cooling), increased oxygen delivery (blood-based cardioplegia solutions), prevention of intracellular calcium overload, and finally manipulation of substrate availability and utilization in an effort to preserve the energetic balance within the stressed myocyte.

Numerous animal studies have shown promise in identifying substrates that can be added to cardioplegia solutions to enhance functional recovery after ischemia. Amino acids that easily enter the cell and function as intermediates of the Krebs cycle are particularly attractive. Fumarate is a Krebs' cycle intermediate that is converted to succinate, generating adenosone triphosphate and oxidizing the reduced form of nicotinamide-adenine nucleotide. Additionally, fumarate may also function as a free-radical scavenger and is involved in calcium transport. When fumarate-supplemented blood cardiplegia was administered to neonatal piglets at induction and recovery from ischemia, the fumarate treated hearts demonstrated complete functional recovery, while the unsupplemented hearts showed a 30–40 % reduction in functional recovery [52]. A previous study in young rabbits had found better preservation of intracellular energy stores and similar improvement in functional recovery when L-glutamate was provided in the cardioplegia [53]. Translation of these insights from animal studies to neonatal human cardiac surgery has been slow with only 37 % of surgeons reporting use of cardioplegia supplemented with metabolic substrates [54].

Cardioplegia composition and how it is administered during surgery evolved over the years from both experimental studies in laboratories as well as the practical experience of surgeons. In a recent survey of 56 congenital heart surgeons across North America, 86 % used a blood-based cardioplegia versus a crystalloid-only solution [54]. While one of several standard solutions were used by the majority of surgeons, over 30 % use their own custom formulations. These custom cardioplegia solutions shared similarities with standard solutions but were more likely to contain additional substrates such as glutamate or aspartate. An additional variable that appears to impact outcomes is the temperature and timing/frequency of cardioplegia administration. These factors are thought to impact the immature heart more significantly than the mature heart indicating that they are either more vulnerable or more amenable to manipulations.

6 Conclusions

As discussed above and in other sections of this book, modification of metabolic substrate uptake or utilization has been shown to influence myocardial performance during, and after, ischemia. Targets for pharmacologic manipulation including fatty acid and glucose uptake, substrate transport within the cell, and myocardial ATP utilization have strong theoretical appeal and experimental merit from animal

studies (see [55] for review). Unfortunately, data on metabolic manipulation in human neonates remain unavailable.

With the steady decline in post-surgical mortality and improved post-operative function, the collective cumulative procedural experience of practicing pediatric heart surgeons is clearly having a positive impact. Unfortunately there have not been any well-designed prospective assessments of the relative merits of specific cardioplegia composition or administration practices to allow informed advancement of the field. Taken together, optimal strategies to enhance cardiac energy balance, preserve mitochondrial integrity, and allow for robust post-ischemic restoration of cardiac function will likely rely upon several simultaneously employed interventions. Testing and validating the growing animal data in the human neonate will be a challenge since conducting randomized controlled studies in this population faces multiple obstacles, including a reluctance to modify individual approaches that are working well enough in the hopes of achieving something better.

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Targeting Transcriptional Control of Fatty Acid Oxidation to Treat Heart Disease

Michael A. Portman and Aaron K. Olson

Abstract The role of fatty acid oxidation in influencing clinical outcome in congestive heart failure still requires determination. However, the impact of fatty acid oxidation on contractile function likely depends on the specific form and on the etiology of the heart disease. Modifications in transcription of genes regulating fatty acid metabolism represent strategies for clarifying this impact, and may provide treatment options. The nuclear receptors, which regulate pathways controlling substrate metabolism, provide targets for transcriptional regulation. To date there have been few if any clinical trials examining the role of these receptors in modifying transcriptional regulation of fatty acid oxidation in heart failure. We will review the perturbations of these receptors as they occur with heart failure and their potential as therapeutic targets.

Keywords PPAR • PGC-1 • Thyroid hormone • Nuclear receptor

1 Introduction

Heart failure continues as a major cause of cardiovascular mortality and morbidity world wide. Regular use of ACE inhibitors and beta-blockers for specific indications of heart failure has improved outcomes. However, current pharmacological therapy does not fully address all forms of heart failure. New strategies to further optimize treatment outcome, as well as refinements for specific indications are needed. Modulation of cardiac metabolism represents one strategy, which has not been fully accessed for clinical improvement in heart failure [1]. Studies in animal models

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suggest that targeting metabolic pathways may alter the course of the pathological ventricular remodeling. Reponses in these animal models, particularly in mice are variable and depend on the specific etiology of heart failure. Some data suggest that enhanced efficiency for myocardial ATP production plays an important role in improving cardiac function [2]. The impact of altering metabolic pathways extends beyond energy balance, as these pathways integrate with those responsible for cardiac growth.

2 Fatty Acid Metabolism and Heart Failure

Fatty acid metabolism undergoes alterations during many conditions causing heart failure. As described by Randle, carbohydrate oxidative flux occurs in a reciprocal arrangement with fatty acid oxidation [3]. In most models, pressure overload associated hypertrophy leading to heart failure induces a shift in metabolic preference from fatty acids to carbohydrates [4, 5]. Some have suggested that this shift represents a mechanism for improving oxidative efficiency, as carbohydrates produce more ATP per oxygen consumed than do fatty acids. However, this concept would only have relevance for oxygen deprivation conditions, such as coronary ischemia. In fact, free fatty acids generate approximately 20 % more ATP per carbon atom than glucose under fully aerobic conditions [6, 7]. Other experimental models of hypertrophy such as c-myc induction show a shift in oxidative preference towards free fatty acids [8]. Thus, it remains unclear if these metabolic shifts represent a response to specific stimuli causing heart failure, or play an integral role in the progression of pathological remodeling and cardiac contractile dysfunction. Regardless, this issue will stay unresolved until experimental and clinical research determine if modulation of fatty acid oxidation alters contractile function, and more importantly clinical outcome for heart failure.

In this chapter, we will review transcriptional modulation of fatty acid oxidation as a potential therapy for heart failure. This control occurs through modulation of transcript factor production and then their binding to promoter or repressor regions, which regulate expression for target genes. Efficiency of translation of mRNA to protein also plays an important role in these regulatory patterns.

3 Nuclear Co-Factor PGC-1 and Heart Failure

The peroxisome proliferator-activated receptor gamma coactivator-1 (PGC-1) orchestrates activation of the nuclear receptor network in coordination with mitochondrial biogenesis. The PGC-1 family also contains two other members, PGC-1β and PGC-1-related coactivator (PRC) [9, 10]. PGC-1α is an inducible transcriptional coregulator activated by stimuli which increase mitochondrial ATP production. This co-regulator directly binds with multiple nuclear receptors including the PPARs,

and thyroid hormone receptors and activates transcription of their target genes. Working with nuclear receptors PGC-1α promotes mitochondrial biogenesis, thereby enhancing myocardial oxidative capacity. In various cell lines PGC-1 also coactivates nuclear receptors such as PPARα and promotes transcription of genes encoding enzymes responsible for fatty acid oxidation. Transcriptional upregulation for the fatty acid oxidation genes in these cell lines increases fatty oxidation rates [11]. Mice deficient in PGC-1α show reductions in gene expression for enzymes responsible for fatty acid oxidation [12]. Ex-vivo isolated heart experiments from these mice demonstrate reduced palmitate oxidation and increased reliance on glucose oxidation. These hearts have limited capacity to recruit triglyceride as a source for lipid oxidation during adrenergic challenge [12]. The metabolic changes consistent with fatty acid oxidation impairment occur concomitantly with both mitochondrial and contractile dysfunction. Thus, the impaired fatty acid oxidation associated with decreased PGC-1α activity may contribute to congestive heart failure caused by specific conditions. However, this relationship between heart failure and PGC-1 has not really been established in humans. Some studies show no difference in PGC-1α mRNA between controls and failing human hearts [13], although at least one study shows increased PGC-1α protein [14]. Considerable variability occurs in human patients with heart failure including treatment modes, which might alter expression for this co-factor. These studies do seem to be significant differences in PGC-1α expression when comparing heart failing from different etiologies, e.g. dilated versus ischemic cardiomyopathy.

Variabilities in PGC-1α expression in heart failure may relate to upstream endocrine control. Forced expression studies in mice suggest that PGC-1 in coordination with PPARα regulate the postnatal metabolic shift from primarily carbohydrate to fatty acid oxidation [11]. However, studies in other mouse models and other mammalian species suggest that this shift depends more on thyroid hormone stimulation [15–18]. Removal of the thyroid hormone postnatal surge inhibits PGC-1 upregulation of mitochondrial pool expansion and respiratory control maturation. The relationship between thyroid hormone and PGC-1 in regards to control of fatty acid oxidation requires clarification. However, shifts in thyroid hormone homeostasis, which occur in heart failure patients, may be responsible for the variability in PGC-1 expression.

4 Nuclear Receptors and Heart Failure

Ligand-activated nuclear receptors regulate close coupling of substrate flux and demand. The nuclear receptors can rapidly respond to changing substrate levels by activating or inhibiting transcription of their target genes. Thyroid receptors (TR) and peroxisome proliferator-activated receptors (PPARs), which are tightly involved in the regulation of cardiac metabolism, are type 2 receptors in a nuclear receptor super family with 48 members in humans [19]. These receptors bind small lipophilic ligands and have the ability to form heterodimers with retinoid X receptor (RXR),

other receptor partners or with each other and bind to response elements in the promoter or repressor regions of target genes. Both PPARs and TRs have also been shown to bind to a wide variety of structurally diverse response elements as monomers and homodimers [20, 21]. In addition to ligand and cofactor binding, dimerization and gene activation, these receptors are regulated posttranscriptionally by phosphorylation, acetylation and other modes. Their ability to transcriptionally activate is also controlled by epigenetic type modifications on their binding sites in promoter regions of target genes [22].

4.1 PPARs

The peroxisome proliferator-activated receptors have been the principal focus for pharmacological modulation of substrate flux. PPARα in particular is considered by many a primary target for transcriptional regulation of fatty acid oxidation during heart failure. Much of what we know regarding the role PPARα in control of fatty acid oxidation stems from studies performed in transgenic gain-of-function and loss-of function mouse models. Mice displaying cardiac specific PPARα (MHC-PPARα) overexpression show increased fatty acid oxidation and reciprocally decreased glucose utilization, which results in myocardial triglyceride accumulation and a diabetic-like phenotype [23, 24]. These animals exhibit left ventricular hypertrophy and cardiac dysfunction that can be inhibited by deletion of CD36, a cellular fatty acid import protein. Mice lacking PPARα demonstrate a decrease in fatty acid oxidation and increased glucose utilization [25]. They show contractile dysfunction and inability to adapt to high workload, which can be reversed slightly by increasing glucose oxidation [25, 26].

Evidence for PPARα dysregulation, as a target for transcriptional based therapy for human heart failure, remains sparse. Many presumptions related to PPARα regulation during heart failure even in animal models are based solely on gene expression data. However, several studies in rodents and other animals have shown dissociation between gene and protein expression for PPARα and its target genes [15, 27]. In several experiments, changes in gene expression induced by heart failure or other conditions are actually opposite to protein expression. Thus, standard expression studies may not be the key to determining PPARs role in the development of heart failure.

Expression data for PPARα and its' metabolic targets during human heart failure are highly variable and often conflicting. The most recent studies showed no difference in PPARα mRNA expression between control donor hearts and explanted hearts from either dilated or ischemic cardiomyopathy patients [13, 14]. However, in the absence of diabetes mellitus, the dilated group did show significantly higher PPARα mRNA and protein levels than did the ischemic group [13]. Parallel differences in expression for PPARα target genes involved in myocardial fat transport, CD36 and fatty acid binding protein, also occurred between these two groups. Thus, these data suggest that PPARα transcriptional regulation in heart may be altered in a disease specific manner.

Results obtained from studies in human hearts, suggest that modifications in PPARα mRNA or protein play a role in regulation of fatty acid metabolism during heart failure. For instance, studies using from endomyocardial septal biopsies from hypertensive patients reveal that alterations in PPARα isoform expression caused by alternative splicing occur and progress with left ventricular disease severity [28]. The expression of native PPARα protein decreased with progressive left ventricular hypertrophy leading to failure, while expression for a truncated PPARα mRNA and protein product increased. The truncated PPARa mRNA gives rise to a form of PPARa protein (30 kDa) lacking the ligand-binding domain [29]. Upon nuclear translocation, the truncated isoform of PPARa exerts repressive action on the native PPARa function, probably through competition for essential coactivators. Additionally, a progressive decrease in PPARα target genes (carnitine palmitoyl transferase-I (CPTI) and long-chain 3-hydroxyacyl-CoA dehydrogenase) mRNA expression was observed among hypertensive, hypertensive with LVH, and hypertensive with LVH and heart failure, although PPARα total mRNA and protein expression does not differ among hearts from these groups [28]. Thus, PPAR functionality appears to be a primary factor for regulating transcription for these target genes.

PPARβ/δ target activation overlaps PPARα and includes the fatty acid oxidation enzymes. In contrast PPARδ activates myocardial glucose utilization [30]. Mice overexpressing PPARδ resist diet induced myocardial lipotoxicity, while those with cardiac specific deletion for PPARβ/δ exhibit reduced fatty acid and glucose oxidation in conjunction with decreased expression for genes controlling these metabolic pathways [31]. In transgenic mice both PPARδ deletion and overexpression lead to cardiac dysfunction [30, 31]. However, similar to PPARα, the role of PPARδ in human heart failure remains unclear.

4.2 PPAR Agonists

Given the relationships between the PGC-1 network, fatty acid oxidation, and contractile function, it seems reasonable to test strategies to increase activity along this transcriptional network in order to improve outcomes for congestive heart failure. There are several potential strategies that can and in part have been pursued including pharmacological modulation, endocrine manipulation and/or nutritional supplementation. To date there are no known small molecules available to directly modulate PGC-1 activity [22]. However, current strategies do exist to promote transcription by PPARs.

Fibrates are PPARα agonists which increase transcriptional activity at fatty acid oxidation genes. They are a well-established class of drugs that have been utilized in the management of hyperlipidemia and metabolic syndrome [7]. The fibrates increase lipoprotein lipase activity, which decreases circulating triglyceride levels. Additionally, fibrates increase hepatic synthesis of apolipoprotein AI and AII leading to increased high density lipid levels [7]. Sarma et al. have extensively reviewed animal studies in which PPARα was tested for therapeutic affect in a heart failure model [7]. The results are highly variable and dependent on the species and

model for heart failure. Many studies do not actually link fatty acid oxidation rates or substrate switching to PPARα activity. Young et al. showed that the specific PPARα agonist WY,14,643 did not affect trophic response, but prevented substrate switching to glucose, and caused severe contractile depression (measured ex vivo) in rat hearts with pressure overload hypertrophy [32]. In contrast, chronic administration of fenofibrate, a PPARα agonist, prevented depression in fatty acid oxidation caused by 3 weeks of pacing induced heart failure in canines, while delaying the increase in LV-end diastolic pressure [33]. This beneficial effect was only transient and did not further affect the progression toward end-stage failure. However, another study testing WY14, 643 also showed benefit for symptomatic outcome and left ventricular function and remodeling indices in a porcine model of pacing induced heart failure [34].

The potential of the same PPARα agonists for treatment of human heart failure has been extensively considered in the literature [7]. However, currently clinicaltrials.gov shows no record of a clinical trial testing these agonists for primarily for outcome in congestive heart failure. The impact of fibrates on congestive heart failure is difficult to determine because of confounding factors in patients enrolled in clinical trials evaluating treatment for lipid abnormalities. There have been multiple trials evaluating these drugs for lipid management and there are some safety concerns [35, 36]. Furthermore, as these trials did not primarily evaluate modulation of fatty acid oxidation for treatment of congestive heart failure, we will not extensively address their results. The reader is referred to the numerous reviews regarding fibrates and prevention of cardiovascular disease. Two large clinical trials studied fibrates as treatments of dyslipidemia in patients with diabetes or metabolic syndrome and found that gemfibrozil reduced the combined end points of death from coronary heart disease and non-fatal MI [35, 37]. Subgroup analyses in the Veterans Affair HDL Intervention Trial (VA-HIT) suggested that suggested that gemfibrozil compared to placebo prevented CHF hospitalizations [37]. The mechanism for this reduction in hospitalization is unclear as gemfibrozil showed only modest affect on lipid levels. The effects of fibrates on cardiac fatty acid oxidation in heart failure patients have not been studied.

The thiazolidinediones (TZDs) provide another form of therapy which transcriptionally activates PPARγ. Considerable debate has occurred regarding safety concerns of this class of drug, particularly as rosiglitazone therapy is associated with increased risk for MI although not for cardiovascular or all cause mortality in patients with diabetes or other metabolic abnormalities [38, 39]. A relatively small study evaluated the effect the PPARγ pioglitazone on myocardial metabolism and insulin sensitivity in HIV patients with metabolic abnormalities [40]. Myocardial fatty acid oxidation as assessed by fatty acid extraction fraction, utilization, oxidation, and esterification after 1-11C–palmitate injection did not change significantly in patients after 4 months of pioglitazone treatment. The authors indicated that the study was not adequately powered to detect differences.

4.3 Thyroid Hormone and Thyroid Hormone Receptors

Thyroid hormones exert ubiquitous actions on cellular and organ level functions. Thus, it is difficult to suggest that thyroid influence on substrate metabolism is a primary mechanism of action in heart failure. However, substantial data does suggest that thyroid regulates substrate metabolism both through direct nongenomic mechanisms as well as through transcriptional actions at the nuclear receptor-DNA binding site interface [41–44]. Several studies have confirmed primary regulation of metabolism through transcriptional processes by showing thyroid modulation of mRNA for various genes encoding proteins involved in oxidation of these substrates [45–47]. Some investigators have shown that hyperthyroidism modifies protein content, or specific activity for key enzymes regulating glycolysis and glucose oxidation [48]. Chronic TH supplementation decreases pyruvate dehydrogenase (PDH) complex activity through transcriptional up regulation of pyruvate dehydrogenase kinases (PDK), which phosphorylate and inhibit PDH [48, 49]. A prolonged hyperthyroid state also attenuates insulin initiated glucose uptake by freshly isolated cardiomyocytes [50]. Rat thyroidectomy decreased FFA flux, accounting for the observed decrease in total citric acid cycle (CAC) flux often noted during hypothyroidism [42]. A selective decrease in cardiac FFA flux caused by prolonged TH deficiency occurred coordinately with reduced expression of a key enzyme, Muscle-CPTI [42]. T3 infusion partially reversed this deficit in FFA oxidation, indicating that these fluxes were regulated by transcriptional and translational processes, as well as nongenomic processes. The data imply that over periods of days, TH modifications in protein content likely occurring through transcriptional pathways alter fatty acid flux in the intact heart.

Some studies in humans have demonstrated abnormal thyroid receptor distribution in failing hearts [51]. In particular, myocardial TRα1 mRNA expression is down regulated in relationship to TRα2, a dominant negative isoform which lacks the ligand binding domain. The dominant negative TRs preferentially bind to nuclear binding receptor elements, and sequester cofactors such as the heterodimeric binding partner RXR [52, 53]. Thus the dominant negative TRs not only suppress activation of ligand bound TRα1 and TRβ1 but inhibit PPAR activity, as these nuclear receptors cross react and share binding sites. Mice expressing Δ337T a naturally occurring dominant negative mutation in humans show substantially modified responses to the PPARα agonist WY, 14,643 [16]. In addition to overall decreases in citric acid cycle flux and myocardial oxygen consumption the cardioselective Δ337T hearts show reduced rates for fatty acid and ketone body oxidation, suggesting that alterations in substrate flux are responsible for reduced contractile function observed in this mouse model [43]. Thyroid hormone (T3) regulates expression for TRα1, thus creating a negative feedback loop, which could depress transcriptional activation at TR target genes during a low thyroid states [54].

4.3.1 Thyroid Hormone Homeostasis and Heart Failure

Several population based studies have established the important relationship between thyroid hormone homeostasis and clinical heart failure. The thyroid syndromes are generally separated into two separate classes. Subclinical hypothyroidism refers to elevated circulating thyrotropin or thyroid stimulating hormone (TSH) levels in the presence of normal or low normal triiodothyronine and thyroxine levels. Heart failure patients with subclinical hypothyroidism show increased mortality and cardiac related hospitalizations compared to those with normal TSH levels [55, 56]. Epidemiological data also suggest that subclinical hypothyroidism, particularly in subjects with TSH >10 μIU/mL [57, 58], is the only reversible cause of left ventricular (LV) diastolic dysfunction with slowed myocardial relaxation and impaired filling.

Low T3 syndrome occurs during many chronic disease states including those involving the heart. Depression of T3 levels inherent in this syndrome can occur without primary hypothyroid function. For instance, 20–30 % of dilated cardiomyopathy patients show low T3 levels [58, 59]. Chronic inflammation associated with these disease states causes cytokine elevation which impacts thyroid hormone production at several levels, extending from the hypothalamus to the pituitary, and end-organ thyroid gland response [60, 61]. Additionally, inflammatory cytokines suppress peripheral T4 to T3 conversion. Several clinical studies have shown that presence of impaired T4 to T3 conversion is associated with mortality and morbidity in heart failure patients [62]. Furthermore, T3 levels in plasma have been found to strongly correlate with exercise capacity and oxygen consumption in HF patients [63]. Heart failure patients supported by ventricular assist devices display low T3 and associated low TRα1 expression, which can both resolve somewhat with exercise training [54].

Some evidence suggests that heart failure due to subclinical hypothyroidism or low-T3 syndrome has a metabolic basis. The aging mouse heart exhibits both systolic and diastolic contractile dysfunction, particularly at high workloads ex-vivo. This dysfunction is associated with low protein expression for PPARα and its target gene PDK-4, impaired fatty acid oxidation, and low circulating T3 [64]. Thyroid hormone supplementation removes the impairment in fatty acid oxidation and improves contractile function [65]. These functional and metabolic changes caused by TH supplementation occur without modifications in PPARα expression. Thus these data suggest that T3 modifies fatty acid oxidation through mechanism that does not involve changes in PPARα protein expression. However, mediation of PPARα activity through cross reaction of ligand dependent thyroid hormone receptors remains a possible mechanism.

Thus, thyroid hormone appears to enhance fatty acid oxidation in healthy myocardium or during chronic heart failure. The mechanism is likely transcriptional in part as T3 promotes expression of some nuclear receptors and/or cofactors including the TRs. However, the T3 exerts different short term actions, particularly in unloaded or recently reperfused myocardium [44, 66, 67]. Low T3 syndrome is profound in humans and animals undergoing ventricular unloading and mechanical circulatory support. The low T3 levels inversely relate to plasma inflammatory cytokine levels in infants undergoing cardiopulmonary bypass [68]. Similarly, ventricular unloading

by extracorporeal membrane oxygenation elevates interleukin-6 and suppresses circulating T3 in a juvenile porcine model [67, 69]. T3 supplementation in this porcine model suppresses medium chain fatty acid oxidation but not long chain, while promoting pyruvate oxidation. With ventricular reloading or weaning from ECMO following ischemia-reperfusion injury, T3 supplementation shifts myocardial substrate preference towards carbohydrates and improves cardiac contractile function [66].

These modifications in substrate metabolism by T3 still require clarification of the operative mechanism, which may be transcriptional. T3 supplementation in young infants undergoing cardiopulmonary bypass for cardiac surgery improves clinical outcome and cardiac contractile function [70]. Serial biopsies performed during these surgeries in infants suggest that T3 does promote transcription of genes involved in mitochondrial and substrate metabolism during just a few hours [71]. Thyroid receptor response varies substantially by isoform. Certain thyroid hormone analogues provide specificity for either activation or inhibition of TR isoforms, and may represent a strategy for transcriptional mediation of fatty acid oxidation.

5 Posttranscriptional Modifications of Nuclear Receptors- O-GlcNAcylation

Pharmacological and hormonal manipulations of ligands binding to nuclear receptors provide strategies for modifying transcriptional regulation of fatty acid oxidation in heart failure. Post-translational modifications of the receptors themselves can also modify their activity. Phosphorylation of the nuclear receptors is often discussed. Recent studies suggest a role for post-translational modification of serine/threonine residues by *O*-linked β-N-acetylglucosamine (O-GlcNAc) [8, 72–78]. This process, termed O-GlcNAcylation, is a dynamic post-translational modification directly affecting protein function and transcriptional events [79–84]. The moiety necessary for O-GlcNAcylation is generated by the hexosamine biosynthesis pathway, an accessory pathway in the glycolytic breakdown of glucose to pyruvate. Thus any shift in substrate preference away from glucose consumption could increase O-GlcNAcylation or proteins such as the nuclear receptors. Accordingly, O-GlcNAcylation may represent an important intersection between metabolism and heart disease.

O-GlcNAc generation and turnover is shown in Fig. 1 with enzymes noted in ovals. Glucose metabolized to fructose-6-phosphate enters the hexosamine biosynthesis pathway instead of proceeding through glycolysis and/or oxidation. Uridine diphosphate-β-*N*-acetylglucosamine (UDP-GlcNAc) is the ultimate product of this pathway. The hexosamine biosynthesis pathway receives approximated 2–5 % of glucose under cell culture conditions, although this contribution still requires verification in intact organs [85]. Glutamine-fructose-6-phosphate transaminase (GFAT), the rate-limiting enzyme in this pathway, catalyzes fructose-6-phosphate conversion to glucosamine-6-phosphate [86]. O-GlcNAc transferase (OGT) uses

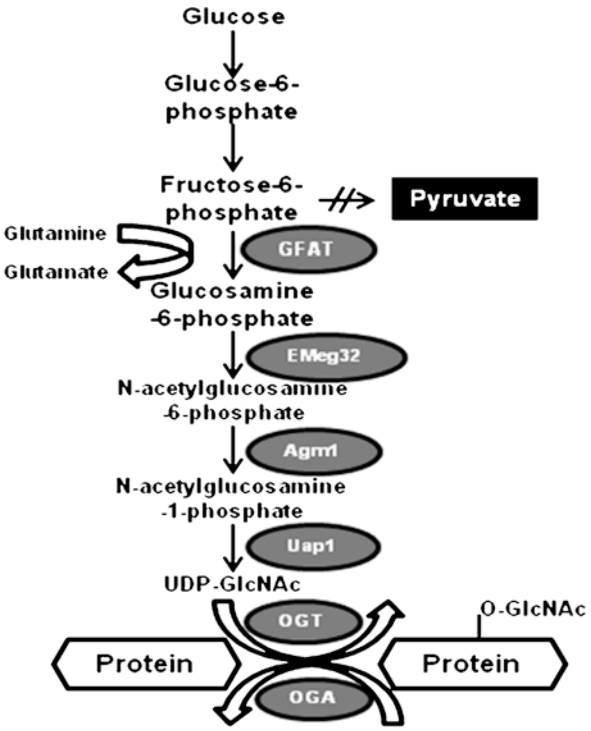

Fig. 1 The metabolic pathway leading to posttranscriptional modification of nuclear receptor protein. Enzyme abbreviations: *GFAT* L-glutamine-D-fructose 6-phosphate amidotransferase, *EMerg32* glucosamine 6-phosphate N-acetyltransferase 1, *Agm1* phosphoacetly-glucosamine mutase, UDP-N-acetylglucosamine pyrophosphorylase, *OGT* O-GlcNAc transferase, *OGA* O-GlcNAcase

UDP-GlcNAc as the monosaccharide donor to attach GlcNAc to target proteins. OGT is highly sensitive to UDP-GlcNAc levels making overall O-GlcNAc levels responsive to changes in hexosamine biosynthesis flux [87]. O-GlcNAc removal is also performed by a single enzyme, O-GlcNAcase (OGA). Emerging evidence suggests that O-GlcNAcylation has important effects on cardiac function during diseases such as hypertrophy and diabetic cardiomyopathy.

Current studies in heart disease reveal both beneficial and deleterious O-GlcNAc effects. In cardiac hypertrophy and heart failure after non-reperfused coronary ligation, inducible inactivation of OGT reduced global O-GlcNAc levels and exacerbated heart failure [74]. OGT inactivation also reduced PGC-1α/β RNA levels and increased myocardial fibrosis and apoptosis, which suggest mechanisms for the reduced cardiac function [74]. Additionally, multiple studies demonstrate a cardioprotective effect from augmenting O-GlcNAcylation during ex vivo global ischemia as well as in vivo ischemia [88–95]. However, other data suggest that increased O-GlcNAcylation is maladaptive. In neonatal rat cardiac myocytes, NFAT-mediated pathologic hypertrophy changes require O-GlcNAcylation [72]. Increased O-GlcNAcylation also contributes to development of cardiomyopathy in diabetic animal models [75–77]. The etiology for this process appears to be from inhibited cardiomyocyte calcium (Ca^{2+}) handling as multiple diabetic models show increased O-GlcNAcylation of proteins regulating Ca^{2+} handling in ways that

reduced contractility [75–78]. A recent study in transgenic mice with inducible cardiac overexpression of glucose transporter 1 demonstrated that altering metabolism during pressure overload hypertrophy also affects O-GlcNAcylation of Ca^{2+} handling proteins [96]. The molecular basis for the incongruent O-GlcNAc functional effects during hypertrophy and other cardiac processes are currently not understood. The potential mechanisms include variations in O-GlcNAc levels, protein specificity, crosstalk with other signaling pathways and/or duration of signal [97]. The identification of specific O-GlcNAcylated proteins and modification sites has proven difficult, which has limited our understanding of the functional effects of this post-translational modification in the heart.

O-GlcNAc may provide feedback regulation of cardiac metabolism through transcriptional and/or direct enzyme modifications. As noted above, OGT inactivation in the early stages of non-reperfused coronary ligation reduced PGC-1α/β RNA levels [74]. This change was associated with suppression of PGC-1α dependent transcripts such as CPT-1 and CPT-2. However, further studies are necessary to prove that this response is from direct modulation of PGC-1 transcription by O-GlcNAcylation, rather than indirect effects. In a separate study, acutely increased O-GlcNAcylation promoted myocardial fatty acid oxidation (specifically palmitate) in isolated perfused rat hearts [98]. This metabolic change was attributed to increased FAT/CD36 membrane translocation and fatty acid cellular uptake. FAT/CD36 immunoprecipitation demonstrated evidence of increased O-GlcNAcylation, suggesting that this modification promoted membrane translocation. Additionally, cardioselective c-myc induction, which produces compensated hypertrophy, promoted myocardial fatty acid oxidation in conjunction with increased O-GlcNAcylation [8]. Thus, data suggest that O-GlcNAcylation yields shifts towards fatty acid oxidation and maintenance of cardiac contractile function.

There are currently no pharmacologic therapies available that directly target O-GlcNAcylation. Theoretically, effective treatment could reduce O-GlcNAcylation during diabetes. Animal models suggest that chronic Western diet ingestion promotes myocardial O-GlcNAcylation [99]. This suggests that dietary interventions could be used to change tissue O-GlcNAcylation. O-GlcNAcylation is considered a therapeutic target for other diseases such as Alzheimer's [100, 101], therefore there is a high potential for the development of drugs targeting O-GlcNAcylation.

6 Conclusions

Fatty acid oxidation represents a potential target for treatment of heart failure. However, considerable debate revolves around the issue and yet whether potentiation or inhibition of fatty acid oxidation will be therapeutic. Likely, the directional change varies according to the etiology of heart failure. We have presented potential modes of altering fatty acid oxidation through transcriptional pathways. To date, none of the modes have been used in human trials linking myocardial fatty acid oxidation with clinical outcome.

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