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Editorial

Metabolic profile in heart disease

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The practice of cardiology today is largely dominated by a mechanical approach to the treatment of cardiac diseases. Both percutaneous and surgical procedures have made dramatic advances over the past 20 years, mostly as a result of a constant improvement in the technology and equipment available. Yet, despite these advances, there remain important unmet medical needs in the treatment of patients with cardiac disease.

Procedures have become progressively more widely applicable, and their use has been extended to a widening range of disease and patient subgroups. Worldwide, the number of procedures, including revascularization for ischemic heart disease or implantation of a cardioverter-defibrillator with or without randomized clinical trial for heart failure, has increased year after year. However, the overall impact of this mechanical approach on mortality and morbidity for cardiac disease remains highly controversial. Even when a significant reduction in mortality has been reported, this often translates, from the patient's perspective, into no more than a limited survival gain.

As far as symptoms are concerned, revascularization procedures in chronic ischemic heart disease are followed by the relief of angina in the majority of patients. Nonetheless, persistence of angina after ''successful'' revascularization procedures has been reported in up to 67% of patients, and recurrence of angina during follow-up does occur, after an initial pain-free interval, in the majority of patients – so much so that, after 3 years, there is no significant difference in the prevalence of angina between patients who have undergone revascularization and those treated medically.

Overall, the limitations of the mechanical approaches underscore the need for a better understanding of the metabolic mechanisms underlying cardiac diseases as a prerequisite for the discovery of more effective treatments.

Cardiac energy metabolism may be altered in many forms of cardiac disease, including ischemic heart disease and heart failure. Some changes occurring in disease conditions may be beneficial and compensate, at least in part, for the underlying abnormalities. Others may be detrimental and worsen the cardiac condition. Because of the importance of cardiac energy metabolism in several cardiac diseases, pharmacologic interventions that optimize cardiac energetics are emerging as an exciting and promising alternative in the treatment of cardiac disease. Therapeutic modalities that can increase cardiac efficiency by modulating cardiac energy metabolism are effectively and extensively discussed by Drs Ussher and Lopashuck in the main clinical paper of this issue of *Heart and Metabolism*.

Understanding these concepts is critical to understanding the potential of metabolic interventions for heart disease. Transgenic mice may be of great help in defining the causal role of metabolic remodeling in the pathogenesis and progression of heart disease, including heart failure. This is clearly documented in the Basic Article by Dr Tian, in which he considers animal models in a discussion of the functional consequences of altered substrate metabolism and sheds light on an innovative, phenotype-based, therapeutic concept.

Magnetic resonance spectroscopy (MRS) is a versatile and powerful tool for the non invasive study of cardiac metabolism. Drs Hove and Neubauer offer a brief overview of the possible applications of cardiac MRS, providing a pathophysiological insight into the evaluation of the metabolic changes associated with heart diseases. To date, MRS has been applied as a research tool; however, technical improvements allowing a better matching of voxels to the shape of the heart and a more favorable signal-to-noise ratio promise to bring MRS into the clinical arena in the near future.

Editorial

Mario Marzilli

The hypothesis that fatty acids may regulate gene expression at the nuclear level is discussed by Drs Sampath and Ntambi, opening novel, exciting alternatives to the treatment and prevention of heart disease.

Metabolic profiling as a new type of diagnostic test is proposed by Dr Grainger. By using nuclear magnetic resonance spectroscopy or chromatographic separation, a ''molecular fingerprint'' can be generated that could be used to identify metabolic profiles associated with atherosclerotic lesions, moving from pathogenesis to risk stratification.

The current issue of *Heart and Metabolism*, true to its name, provides strong evidence that cardiac metabolism is a major player in a number of cardiac conditions, both on the pathogenetic side and on the therapeutic side. Agents such as trimetazidine, which can optimize cardiac energy metabolism, offer an innovative and extremely promising alternative for the treatment of heart diseases. Large-scale studies, based on a better understanding of the possible beneficial effects of metabolic modulation, are urgently needed to establish the role of these agents in clinical practice.

Understanding the metabolic phenotype of heart disease

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Abstract

Substantial changes in cardiac energy metabolism have been observed in a variety of heart diseases. Understanding the functional role of these changes is critical for developing the concept of metabolic intervention for heart disease. The use of genetically engineered mice in recent studies has made it possible to alter cardiac metabolism independently of secondary influence by disease and, thus, allow the causal role of metabolic remodeling in the pathogenesis and progression of heart disease – in particular, heart failure – to be tested. Results from these studies also shed light on the potential tactics for targeting energy metabolism in heart failure.
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Keywords: energetics, heart failure, transgenic mice, fatty acid oxidation, glucose metabolism, metabolic therapy

Introduction

Cardiovascular disease is the leading cause of death in developed countries. Improved survival after an acute ischemic episode in recent years has, paradoxically, resulted in an increased diagnosis of heart failure in patients post-myocardial infarction. Despite the success of neurohormonal inhibition, many patients with heart failure still experience progression of their disease, and heart failure remains the number one killer in the USA [1]. The recent failure of trials of treatment with endothelin-1 receptor blockers and cytokine antibodies has fueled the search for alternative options in chronic therapy in order to achieve further improvement in the limited prognosis of patients with heart failure.

Targeting energy metabolism for heart failure therapy is worth considering, not only because the heart is an organ with high energy consumption, but also because failing hearts are energy-deprived. Studies in animal models and in patients have shown a significant decrease in myocardial content of the energy reserve compound, phosphocreatine, which can rapidly regenerate ATP in case of abrupt increases in energy demand [2–4], followed by the eventual depletion of myocardial ATP content at the end-stage of heart failure [4]. Long-term follow-up studies in patients with idiopathic dilated cardiomyopathy have demonstrated that decreased phosphocreatine is an independent predictor of mortality [3], suggesting that impaired myocardial energetics contributes significantly to the progression of heart failure. This notion is further supported by consistent observations in clinical trials in patients with heart failure showing that energy-costly treatments such as positive inotropic agents (β-receptor mimetic drugs, phosphodiesterase inhibitors) increase mortality, whereas energy-sparing treatments such as angiotensin-converting enzyme inhibitors, angiotensin II blockers or β-receptor blockers reduce mortality. These observations collectively suggest that myocardial energy metabolism plays an important part in the progression of heart failure. Thus a better understanding of the metabolic phenotype may offer new opportunities to mend the failing heart.

Basic article
Rong Tian

Energy metabolism in animal models of heart failure

An important change in energy metabolism observed in animal models of cardiac hypertrophy and failure is a shift in the preference of substrates for energy generation. Although the heart is able to utilize a variety of substrates, preference in substrate utilization has been documented and it can change in response to altered substrate availability or altered regulation of metabolic pathways. As illustrated in *Figure 1*, substrate preference of the heart changes under several physiological and pathological situations. For example, glucose and lactate (collectively referred to as carbohydrates) are the primary carbon substrates for fetal hearts, whereas fatty acids become the predominant fuel in adult hearts, supporting more than 67% of the total ATP synthesized [5–7]. In contrast, hypertrophied and failing hearts demonstrate increased reliance on glucose as a fuel while decreasing its fatty acid utilization, an apparent recurrence of the fetal metabolic profile [8–10].

Studies using animal models of heart failure show that the shift of substrate preference is associated with downregulation of peroxisome proliferator-activated receptor α (PPARα), a transcription factor controlling the expression of key enzymes for fatty acid oxidation [11,12]. Subsequent studies using transgenic mouse hearts deficient in PPARα have demonstrated a similar shift in substrate selection, supporting a causal role of PPARα in altered substrate utilization in cardiac hypertrophy and failure [13]. Furthermore, impaired myocardial energetics also leads to activation of AMP-activated protein kinase, a cellular energy sensor [14]. Increased AMP-activated protein kinase activity promotes glucose uptake and glycolysis and thus enhances the shift of substrate utilization toward glucose [14,15].
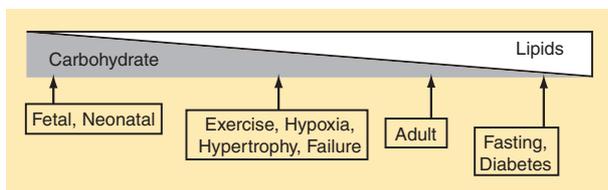

Figure 1. Substrate preference of the heart. Although the heart is able to use several substrates, the contribution of each class of substrate to ATP synthesis varies depending on the developmental stage and the (patho)physiological conditions. Fatty acids are the predominant fuel for the adult heart, whereas carbohydrates are the preferred substrates for fetal hearts. Furthermore, the adult heart can shift its substrate utilization profile in response to altered substrate availability such as during fasting and exercise or in response to altered regulatory mechanisms such as in heart failure.

Functional consequences of the altered substrate metabolism

Is the shift in substrate preference beneficial or detrimental, or of no functional significance for the heart? To address such a question, it is necessary to alter substrate preference by a mechanism that is independent of heart failure. This is made possible by studying transgenic mouse hearts in which substrate preference has been altered by genetic manipulations. In mouse hearts deficient in PPARα, increased glucose utilization has been observed and it was sufficient to compensate for the decrease in fatty acid oxidation at baseline workload [13,16,17]. However, these hearts showed impaired energetics and contractile function when challenged with high workloads [17,18]. In contrast, myocardial glucose utilization increased by overexpression of an insulin-independent glucose transporter, GLUT 1, was not associated with an adverse phenotype [17,19], and the overexpression of GLUT 1 was able to correct the energetic and contractile defects in PPARα-deficient hearts [17]. Analysis of substrate oxidation profiles using a carbon-13 nuclear magnetic resonance technique showed that the compensatory increases in glucose oxidation in hearts that were deficient in PPARα at baseline had exhausted the reserve for a further increase at high workload, thus depleting the metabolic reserve and consequently the contractile reserve of the heart. Subsequent overexpression of GLUT 1 restored the ability to achieve a further increase in the contribution of glucose to oxidative metabolism [17].

An important lesson learned from these studies is that increased reliance on glucose *per se* is not harmful for the heart. However, the intrinsic adaptation to impaired fatty acid oxidation through an increase in glucose utilization is limited in the adult heart and comes with the cost of depleting the functional reserve of the heart. Such a scenario is clearly unfavorable for failing hearts that constantly struggle to accomplish their workload. This is even more problematic when one considers that heart failure is often associated with insulin insensitivity [20,21], thus further compromising the glucose utilization of the heart. Therefore, altered substrate metabolism, which is part of the myocardial remodeling process after an initial pathological event such as myocardial infarction or pressure or volume overload of the heart, may ultimately fail to satisfy the high energy demand of the heart coping with mechanical overload caused by the underlying diseases. The mismatch of energy supply and demand thus drives a vicious cycle that contributes to the ultimate failure of the heart.

Developing therapeutic concepts based on phenotyping

How do we restore the supply of energy to the overloaded heart? Several hypotheses derived from the metabolic phenotype discussed above have been tested (*Figure 2*). One obvious approach is to restore substrate preference by reactivating PPARα. Although systemic benefits of the lipid-decreasing and anti-inflammatory effects of PPARα agonists have been noted in several studies [22,23], increased myocardial fatty acid oxidation that results from reactivation of PPARα is apparently detrimental to hypertrophied hearts [24,25]. Decreased fatty acid oxidation is probably beneficial for hearts with compromised perfusion, because of its oxygen-sparing effect [26]. Furthermore, a recent study showed that decreased PPARα activity in ischemic cardiomyopathy protected the heart from lipotoxicity by shifting the fuel preference away from fatty acids [25]. This is consistent with earlier observations that partial inhibition of fatty acid utilization is cardioprotective [27].

Therefore, strategies for improving the energy supply of the failing heart without increasing fatty acid utilization would be highly desirable. The rescue of PPARα-deficient hearts by overexpressing GLUT 1 suggests that improving myocardial glucose utilization can be an effective approach to increasing the capacity of the failing heart for ATP synthesis. Supportive of this concept, mouse hearts overexpressing GLUT 1 showed increased tolerance to chronic pressure overload, with delayed progression to heart failure and reduced mortality [19]. Similarly, in a large animal model of heart failure caused by dilated cardiomyopathy, enhancement of myocardial glucose uptake and utilization by recombinant glucagon-like peptide-1 improved left ventricular performance [28].
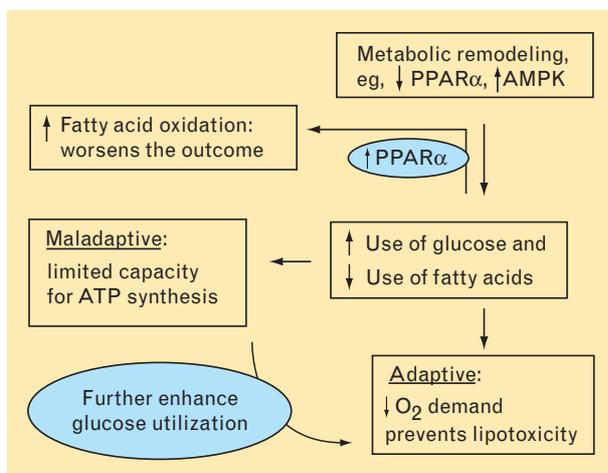

Figure 2. Working hypotheses regarding the adaptive and maladaptive aspects of the shift in substrate preference in heart failure, and proposed strategies for sustaining energy homeostasis.

Summary

The use of animal models as ''proofs-of-concept'' in understanding of the metabolic phenotype has generated valuable evidence for the therapeutic potential of manipulating cardiac metabolism. We anticipate that the concept will be further tested by clinical studies and eventually in large-scale clinical trials; however, in addition, basic research leading to the development of novel compounds that have high efficacy in myocardial metabolism and few side effects is urgently needed to advance the practice of metabolic therapy. ■

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Clinical implications of energetic problems in cardiovascular disease

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Abstract

Cardiac energy metabolism can be altered in many forms of heart disease, including ischemic heart disease, cardiac hypertrophy, heart failure, and cardiac arrhythmias. Some of these energy metabolic changes are beneficial and help the heart adapt to the presence of the underlying cardiac pathology. However, some of the changes can also be maladaptive and actually contribute to the severity of cardiovascular disease. Because of the importance of energy metabolism in mediating cardiovascular disease, optimization of cardiac energetics has recently emerged as a novel approach to treat cardiovascular disease. This includes increasing the efficiency of oxygen utilization by the heart, which can be achieved by shifting cardiac metabolism to favor the use of carbohydrates rather than fatty acids as a metabolic fuel. This can be attained by reducing the circulating concentrations of fatty acids to which the heart is exposed, by inhibiting the uptake of fatty acids into the mitochondria, by directly inhibiting the enzymes of fatty acid oxidation, or by directly stimulating glucose metabolism. Clinical studies using these approaches have shown promise in treating various cardiovascular diseases, including ischemic heart disease, acute myocardial infarction, cardiac surgery, and heart failure. One agent that uses this approach is trimetazidine, which directly inhibits cardiac fatty acid oxidation and has been shown to be clinically effective in treating ischemic heart disease and heart failure. The paper will review those alterations in energy metabolism that occur in ischemic heart disease and heart failure, and the promising clinical approach of switching energy metabolism from fatty acid to glucose oxidation as a therapy in heart disease.
■ *Heart Metab.* 2006;32:9–17.

Keywords: Fatty acid oxidation, trimetazidine, glucose oxidation, energy metabolism

Introduction

Cardiovascular disease is a major health problem worldwide, and by 2010 could be the leading cause of death worldwide [1]. It is therefore important to develop new therapeutic approaches to lessen the burden of this major health problem. One such approach is to optimize energy metabolism in the heart. The heart has a very high energy demand, and most forms of cardiovascular disease are accompanied by alterations in cardiac energy metabolism. An obvious example of this is ischemic heart disease, in which the myocardium is deprived of the necessary oxygen and energy needed to sustain contractile function. However, independent of ischemia, cardiac energy metabolism can also be altered in other forms of heart disease, including cardiac hypertrophy, heart failure, and cardiac arrhythmias.

Some of the metabolic changes that occur in heart disease are beneficial, and help the heart adapt to the presence of the underlying cardiac pathology. However, it is also clear that some of the energy metabolic changes can be maladaptive, and can contribute to the severity of cardiovascular disease (this subject has

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been reviewed elsewhere [2–5]). Mutations in genes involved in energy metabolism can also be the actual precipitating cause of the heart disease [2]. Because of the importance of energy metabolism in cardiovascular disease, it is reasonable to expect that optimization of cardiac energetics may be a suitable therapeutic approach to the management of cardiovascular disease. One such approach involves shifting cardiac metabolism to favor the use of carbohydrates rather than fatty acids as a metabolic fuel, thereby allowing the heart to use oxygen and produce energy more efficiently [2–5].

The aim of this review will be to discuss the potential clinical implications for optimizing cardiac metabolism in heart disease. It will examine the clinical trials aimed at optimizing cardiac metabolism for the treatment of angina, acute myocardial infarction, cardiac surgery, and heart failure. This will be preceded by a brief discussion of how cardiac metabolism is regulated and what alterations occur in various forms of cardiovascular disease.

Cardiac energy metabolism

In order to meet the high energy demands of contraction and ionic homeostasis, the heart must produce an abundant supply of ATP [6]. In the normal healthy heart, almost all (>95%) ATP generated in the heart comes from mitochondrial oxidative phosphorylation, with the remainder derived from glycolysis [2,6]. The contribution of fatty acids and carbohydrates to oxidative generation of ATP in the heart is influenced by a number of conditions, which include alterations in hormonal control, workload, energy substrate supply, and oxygen supply to the heart. Mitochondrial metabolism of fatty acids accounts for approximately 60–90% of total energy production (in the form of ATP), with carbohydrates contributing the remaining 10–40%. However, despite producing more ATP than carbohydrates, fatty acids are not as oxygen-efficient, requiring approximately 10% more oxygen to produce an equivalent amount of ATP [6]. This is of particular importance when oxygen becomes a limiting factor for oxidative metabolism.

The rates of flux through the various metabolic pathways are controlled by both the degree of expression of key metabolic proteins (enzymes and transporters) and the complex pathway regulation including both allosteric regulation of enzymes and substrate–product relationships. As will be discussed, one of the main clinical approaches used to optimize cardiac energetics involves manipulating a number of these enzymes/transporters to inhibit the oxidation of fatty acids, or to increase the oxidation of carbohydrates, thereby making oxygen utilization and energy production more efficient.

Carbohydrate metabolism

Glucose and lactate are the primary carbohydrates metabolized by the heart. The majority of glucose that the heart metabolizes is derived from the blood, with its uptake being facilitated by glucose transporters (GLUT) (*Figure 1*). GLUT 1 is responsible for maintaining basal glucose uptake, whereas GLUT 4 translocates from an intracellular pool to the sarcolemmal membrane in response to insulin, increased work demand, or ischemia [7,8].

Alternatively, the mobilization of endogenous glycogen stores can generate glucose-6-phosphate, which is the first intermediate in the metabolic pathway of glucose. Subsequent glucose metabolism can be separated into two major components: glycolysis and glucose oxidation (*Figure 1*). Glycolysis results in the production of pyruvate and accounts for less than 10% of the total ATP produced by the non ischemic heart [6]. If glycolysis is coupled to glucose oxidation, the pyruvate generated from glycolysis will be converted to acetyl coenzyme A (CoA) (which can be subsequently oxidized in the tricarboxylic acid cycle) by the enzymatic action of the multienzyme complex, pyruvate dehydrogenase (PDH). The other major source of pyruvate for PDH is lactate, which, after uptake by the heart, is converted to pyruvate by lactate dehydrogenase.

The PDH complex itself is under tight regulation by an upstream kinase, PDH kinase, which acts to phosphorylate and inhibit the activity of the PDH complex [9]. This PDH kinase is positively regulated by acetyl CoA and NADH. Because the oxidation of fatty acids generates acetyl CoA and NADH, the oxidation of fatty acids is a potent inhibitor of PDH and glucose oxidation. This can ''uncouple'' glycolysis from glucose oxidation, resulting in the production of lactate and protons [2,10]. The oxidation of fat uses approximately 10% more oxygen than carbohydrates, but the uncoupling of glycolysis from glucose oxidation can also cause a substantial decrease in cardiac efficiency. The decreased coupling of glycolysis to glucose oxidation caused by increased fatty acid oxidation can cause myocardial tissue acidosis from the hydrolysis of glycolytic ATP and build-up of lactate and protons [10]. Accumulation of protons can lead to the accumulation of sodium and calcium, requiring the use of ATP to maintain ion homeostasis. This redirection of ATP from contractile function to ion homeostasis can dramatically decrease cardiac efficiency [2,10]. Thus a number of drugs designed for the optimization of cardiac energetics either inhibit fatty acid oxidation to give indirect improvement in the coupling of glycolysis to glucose oxidation, or
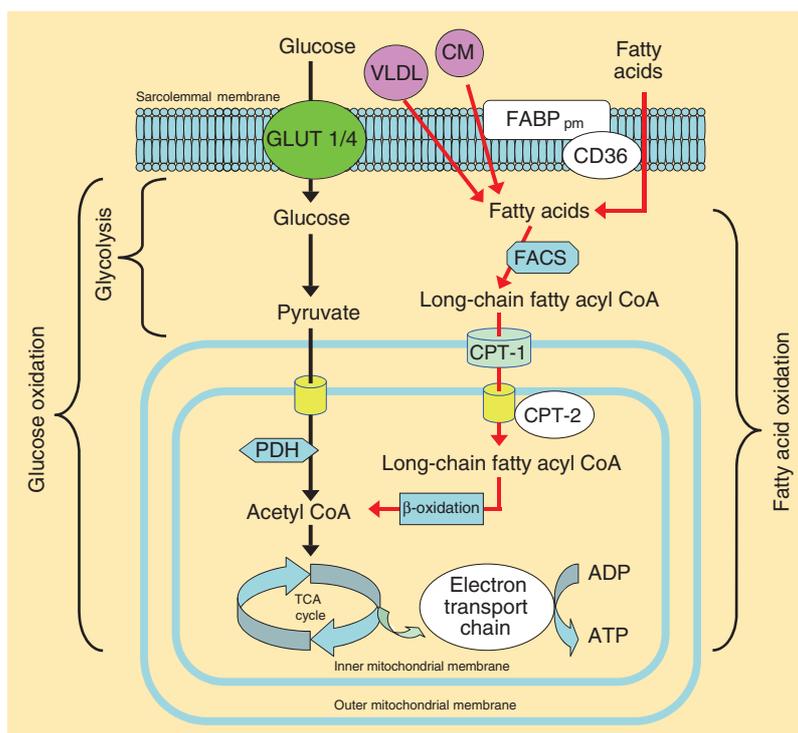
Figure 1. Glucose and fatty acid metabolism. Glucose enters the cardiomyocyte through glucose transporter (GLUT) 1 or 4 and undergoes the first phase of glucose metabolism, glycolysis. The end product of glycolysis, pyruvate, can be further oxidized in the mitochondria to acetyl coenzyme A (CoA) by the actions of pyruvate dehydrogenase. Acetyl CoA then enters the tricarboxylic acid (TCA) cycle to generate reducing equivalents for the production of ATP in the electron transport chain. Fatty acids enter the cardiomyocyte by either passive diffusion or protein-mediated transport through the sarcolemma via plasma membrane fatty acid binding protein (FABP$_{pm}$) or CD36/fatty acid translocase. The fatty acids are then esterified by the actions of fatty acyl CoA synthase (FACS) and subsequently taken up via carnitine palmityl transferase (CPT)-1 into the mitochondria, where they can be oxidized to generate acetyl CoA for the TCA cycle. CM, chylomicron; PDH, pyruvate dehydrogenase; VLDL, very low density lipoprotein.

increase glucose oxidation, giving direct improvement in its coupling to glycolysis.

Fatty acid metabolism

Long-chain fatty acids are supplied to the heart either as triglycerides in chylomicrons and very low density lipoproteins, or as fatty acids in the non esterified form bound to albumin [2]. The rate of fatty acid uptake by the heart is primarily determined by the concentration of fatty acids in the blood, which can vary over a 4-fold range in healthy humans during the course of a day (from about 0.2 to 0.8 mmol/L) [3,6,11]. Under conditions of metabolic stress such as ischemia, diabetes, or starvation, plasma free fatty acid concentrations can increase to much greater values (>1.0 mmol/L) [2,12].

Fatty acids enter the cardiomyocyte by either passive diffusion or protein-mediated transport across the sarcolemma (*Figure 1*) [13]. Once transported across the sarcolemma, the fatty acids are subsequently activated by esterification to fatty acyl CoA by fatty acyl CoA synthase. This acyl CoA can either be esterified to intracellular lipids or converted to long-chain fatty acyl carnitine by carnitine palmi-

tyltransferase (CPT)-1 [2]. Studies have demonstrated in humans that 70–90% of fatty acids taken up by the heart are immediately oxidized, and the remaining 10–30% probably become part of the intracardiac triglyceride pool [11].

Fatty acid β-oxidation (*Figure 1*) occurs predominantly in the mitochondria [14]. Before mitochondrial β-oxidation of fatty acids can begin, the cytoplasmic long-chain fatty acyl CoA must first be transported into the mitochondrial matrix. A key enzyme in this process is CPT-1 [15]. As will be discussed later in this review, one way to optimize cardiac energetics in the patient with heart disease is to inhibit CPT-1 (*Figure 2*). This indirectly leads to decreased cardiac mitochondrial β-oxidation of fatty acids by preventing the uptake of fatty acids into the mitochondria, which indirectly increases glucose oxidation and improves its coupling to glycolysis, thereby reducing proton production. Thus benefit is provided, not only by making ATP production more fuel-efficient, but also by reducing myocardial tissue acidosis.

Once fatty acids are taken up by the mitochondria, they undergo β-oxidation, a process that repeatedly cleaves off two carbon acetyl CoA units, generating

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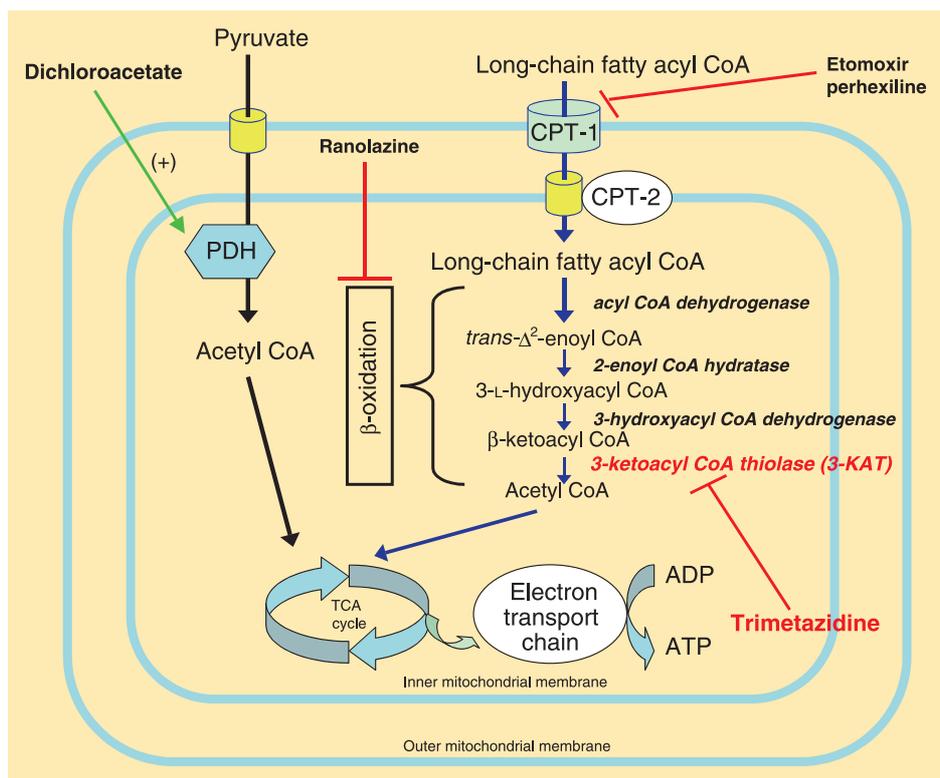
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Figure 2. Targets for optimization of cardiac energetics. Before fatty acids can be oxidized, they must first be taken up into the mitochondria via carnitine palmityl transferase (CPT-1). Therefore, inhibition of CPT-1 with etomoxir or perhexiline will optimize cardiac energetics by indirectly inhibiting the oxidation of fatty acids, which thereby increases glucose oxidation and its coupling to glycolysis. Direct inhibition of fatty acid oxidation represents another approach to optimizing cardiac energetics. This can be achieved with trimetazidine, which inhibits 3-ketoacyl CoA thiolase (3-KAT), or ranolazine, a partial inhibitor of fatty acid oxidation. One other approach to optimize cardiac energetics is to increase glucose oxidation and its coupling to glycolysis. This can be achieved directly with dichloroacetate, which inhibits pyruvate dehydrogenase kinase (PDH) kinase to stimulate PDH, resulting in increased oxidation of pyruvate to acetyl CoA. TCA, tricarboxylic acid.

NADH and reduced flavine adenine dinucleotide in the process. The β-oxidation process involves four enzymatically catalyzed reactions, starting with acyl CoA dehydrogenase, followed by 2-enyl CoA hydratase, and then 3-hydroxyacyl CoA dehydrogenase. The last reaction is catalyzed by 3-ketoacyl CoA thiolase (3-KAT), which regenerates acyl CoA for another round of β-oxidation and releases acetyl CoA for the citric acid cycle. As mentioned earlier, the oxidation of a fatty acids generates acetyl CoA, which inhibits PDH and glucose oxidation. Therefore, potential targets for optimizing cardiac energetics in heart disease include the enzymes of the β-oxidation pathway. Targeting such enzymes (*Figure 2*) can directly inhibit fatty acid oxidation, leading to a secondary increase in glucose oxidation. Examples of agents that do this include the 3-KAT inhibitor, trimetazidine.

Alterations in energy metabolism in cardiovascular disease

Mitochondrial oxidative metabolism is critically dependent on oxygen supply to the heart, and any decrease in oxygen supply to the myocardium results in a decrease in the production of mitochondrial ATP. An initial adaptive response is to increase glycolysis, because glycolysis can produce ATP in the absence of oxygen [6]. However, during myocardial ischemia there are a number of additional subcellular changes occurring that alter cardiac metabolism, which can further exacerbate the deleterious effects of an imbalance between oxygen supply and demand (reviewed in [16]). In particular, the heart is exposed to high concentrations of fatty acids [2,12], and alterations in the subcellular control of fatty acid oxidation result in fatty acid oxidation becoming the main residual source of mitochondrial oxidative metabolism. This results in low rates of glucose oxidation during ischemia. The high glycolysis coupled to low glucose oxidation results in the production of lactate and protons [10], which in turn leads to a decrease in cardiac efficiency. Clinical therapy that improves this coupling of glycolysis to glucose oxidation can reduce myocardial tissue acidosis and the build-up of lactate.

If the myocardium is reperfused after ischemia (such as during thrombolysis, angioplasty, or reperfusion after cardiac bypass surgery), mitochondrial oxidative metabolism recovers as oxygen is reintroduced to the heart [2]. However, during reperfusion, fatty acid

oxidation dominates as a source of ATP production, primarily as a result of the high circulating concentrations of fatty acids to which the heart is exposed, but also as a result of decreased subcellular control of fatty acid oxidation (reviewed in [2]). This results in low rates of glucose oxidation, and a continued coupling of glycolysis to glucose oxidation. Consequently, the continued production of lactate and protons contributes to a decrease in cardiac efficiency [10]. Promising experimental and clinical studies, however, have shown that inhibiting fatty acid oxidation or stimulating glucose oxidation, or both, can increase cardiac efficiency during this critical period of reperfusion (as discussed in the next section).

Heart failure is a complex clinical syndrome that can result from any structural or functional cardiac disorder that impairs the ability of the ventricle to fill with or eject blood [17]. Despite the growing incidence of heart failure in our society, there is not a consensus as to the effects of heart failure on myocardial energy metabolism and fuel selection (reviewed in [15,16]). In general, it appears that energetic reserve is compromised in heart failure, as the consequence of an impaired mitochondrial function (reviewed in [18]). This results in a compensatory increase in glycolysis, similar to the adaptive increase in glycolysis seen in the ischemic heart. It appears that, in the early stages of heart failure, rates of fatty acid oxidation are normal or increased, whereas rates of glucose oxidation are low [18]. In late-stage heart failure, overall mitochondrial oxidative metabolism can be markedly impaired (with both fatty acid and glucose oxidation being decreased), while glycolysis becomes a more important source of energy. The increase in glycolysis in relation to glucose oxidation can result in an uncoupling of glycolysis from glucose oxidation similar to that observed in the ischemic heart. This can cause a decrease in efficiency as a result of lactate and proton production, and suggests that switching mitochondrial oxidative metabolism from fatty acid to glucose oxidation may also be a valid approach to the treatment of heart failure. The clinical evidence to support this concept will be discussed in the following section.

Optimization of cardiac energetics for the treatment of heart disease

Treatment of angina pectoris

Inhibition of fatty acid oxidation and stimulation of glucose oxidation can improve cardiac efficiency and cardiac function in the ischemic heart. Trimetazidine, a piperazine derivative that inhibits 3-KAT in the β-oxidation pathway [19], offers one such approach to switching the heart from fatty acid to glucose oxidation. Trimetazidine is available in more than 80 countries for the treatment of angina pectoris, and has been the subject of a large number of clinical studies in patients with angina (reviewed in [16]). A meta-analysis of human clinical trials of trimetazidine was recently undertaken by the Cochrane Collaboration [20], to determine its efficacy and tolerability in patients with stable angina. A total of 23 studies encompassing 1378 patients were analyzed, and it was concluded that trimetazidine is an effective treatment for stable when angina compared with placebo (approximately 40% reduction in mean number of angina attacks per week), alone or combined with conventional antianginal agents. The authors of the meta-analysis also concluded that the use of trimetazidine may result in fewer patients withdrawing from trials as a result of adverse events. It has also been pointed out that, although there are insufficient data to permit analysis of the effect of trimetazidine on mortality or major adverse cardiovascular events, the intensity of anginal symptoms consistently predicts total mortality among outpatients with ischemic heart disease [21], and their quality of life [22]. Overall mortality has never served as a primary endpoint in a trial assessing the effects of a drug on stable angina. Time to onset of angina, exercise duration, and time to 1 mm ST-segment depression are often the primary/secondary endpoints analyzed in trials in stable angina, as they are determinants of quality of life. A patient who experiences improvements in these endpoints is likely to enjoy a better quality of life and it would, therefore, be safe to conclude that trimetazidine represents an exciting novel treatment for ischemic heart disease.

Ranolazine is another piperazine derivative that acts as a partial inhibitor of fatty acid β-oxidation [22]. This drug has recently been proposed to act as a slow sodium channel modifier, but the concentrations known to inhibit fatty acid oxidation [22] are also consistent with the plasma concentrations observed in clinical trials that demonstrated its efficacy in angina pectoris [23,24]. Like trimetazidine, ranolazine has the potential to provide benefit during ischemia by making ATP production more fuel-efficient, although ranolazine is substantially less potent then trimetazidine. Ranolazine may also benefit the patient with ischemic heart disease by decreasing myocardial tissue acidosis, because it improves coupling of glycolysis to glucose oxidation by indirectly stimulating PDH [25]. It was recently introduced into the US market for the treatment of angina, and can be used either as monotherapy or in combination with other antianginal agents [23,24].

Treatment of acute myocardial infarction

Despite promising experimental evidence that optimizing energy metabolism can be beneficial after

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severe ischemia (reviewed in [16]), few clinical studies have addressed the potential of altering energy metabolism as an approach to treating acute myocardial infarction. Some studies have addressed the concept of improving metabolism with infusions of glucose–potassium–insulin (GIK): the exogenous insulin suppresses circulating concentrations and myocardial uptake of free fatty acids, and the high-dose glucose can make glucose the preferred fuel for the heart. The actions of both result in improved overall efficiency of energy production by the heart. A meta-analysis of GIK trials, published in 1997 [26], covered the findings of nine trials with a total of 1932 patients; its authors concluded that GIK treatment may have an important role in reducing in-hospital mortality after acute myocardial infarction (154 deaths among 956 patients in the GIK groups [16.1%], compared with 205 deaths among 976 patients in the placebo group [21%]). A more recent analysis [27] also demonstrated that mortality was reduced by 18% with GIK therapy. In line with similar findings in another meta-analysis that examined 13 studies involving 4992 patients [28], benefit with GIK appeared to be greatest when a high-dose infusion of GIK was administered.

Recently, the results from the merged Clinical Trial of Reviparin and Metabolic Modulation in Acute Myocardial Infarction Treatment Evaluation (CREATE) and Estudios Cardiologicas LatinoAmerica (ECLA) Study Group 2 GIK Full Scale Trial were published [29]. A total of 20 201 patients were allocated randomly to groups to receive either usual care alone (10 110 patients) or an infusion of GIK plus usual care (10 091 patients). Unfortunately, the results of this trial demonstrated equal mortality rates between the two groups, and the authors ultimately concluded that infusion of GIK in patients with acute myocardial infarction has no impact on mortality, and is unlikely to be of any real value in these patients.

As mentioned earlier, a key physiological change that takes place during reperfusion of previously ischemic myocardium is an increase in the concentration of circulating fatty acids, which is one of the primary causes of the enhanced rates of fatty acid oxidation observed during reperfusion. Such an observation suggests that an infusion of GIK for the treatment of acute myocardial infarction should take place immediately at the onset of reperfusion with either thrombolytic therapy or primary percutaneous coronary intervention (PCI). This would provide the best therapeutic window in which to allow insulin to block the myocardial uptake of fatty acids. In the CREATE–ECLA trial, patients received an infusion of GIK immediately after random allocation to groups. However, the median time from onset of symptoms to reperfusion therapy (in patients receiving thrombolysis or primary PCI) was 3.9 h in the GIK infusion group, and the median time from onset of symptoms to allocation to groups was 4.7 h. Moreover, GIK infusion was started within 1 h of the allocation to groups in more than 90% of patients. Hence, the majority of patients receiving the GIK infusion were receiving it at least 1 h after they received reperfusion therapy. Because the optimal therapeutic window for the actions of insulin is within the first few minutes immediately after the onset of reperfusion, the authors' negative conclusions on the use of GIK infusions for treating acute myocardial infarction are misleading.

A few trials have also investigated the effect of trimetazidine in the treatment of acute myocardial infarction. A small study of 81 patients [30] demonstrated that pretreatment with trimetazidine before thrombolytic therapy reduces reperfusion damage, or infarct size, or both, in patients with anterior acute myocardial infarction. In addition, another small study involving 94 patients demonstrated that treatment with trimetazidine before primary PCI on the infarct vessel was safe and led to earlier resolution of ST-segment elevation in the patients [31]. A larger multicenter trial has also been performed [32], involving 17 169 patients in whom the effects of trimetazidine (in conjunction with or without thrombolysis) on acute myocardial infarction mortality were investigated. As with the findings of the CREATE–ECLA trial, the multicenter trial showed no significant reduction in mortality with trimetazidine. Interestingly, there was a non significant trend to increased short- and long-term mortality with trimetazidine in patients receiving thrombolysis; a significant short-term decrease in mortality in patients not receiving thrombolysis was also observed. Perhaps the beneficial effects of trimetazidine in conjunction with thrombolysis were masked by reperfusion being established at so late a time that the myocardial area at risk could no longer be salvaged. This may also explain why there was a significant trend to decreased mortality in patients not receiving thrombolysis, as these patients probably have improved collateral circulation, and thus a longer therapeutic window for myocardial salvage. It could also simply be that trimetazidine is not beneficial in conjunction with thrombolysis, and may be more suited as a therapy in conjunction with primary PCI. A previous small trial demonstrated that trimetazidine delivered intracoronarily during percutaneous transluminal coronary angioplasty (PTCA) at 10% of the dose used in the large acute myocardial infarction trial had direct anti-ischemic effects in man [33]. The results of this small trial by no means imply that trimetazidine is beneficial in conjunction with primary PCI for treating acute myocardial infarction, but suggests that trimetazidine does warrant further investigation for use in this setting.

Primary cardiac surgery techniques to reperfuse the heart involve coronary artery bypass grafting (CABG) surgery and PTCA. Because circulating concentrations of fatty acids increase during reperfusion [12], the optimization of cardiac energetics during CABG and PTCA has been explored to future enhance cardiac recovery further. In a small trial of 19 patients, pretreatment for 3 weeks with trimetazidine and the addition of trimetazidine to the cardioplegic solution were shown to have cardioprotective effects in patients undergoing CABG surgery [34]. Furthermore, trimetazidine has also been shown to have cardioprotective effects during PTCA [33]. More recent PTCA trials have demonstrated an earlier resolution of ST-segment elevation in patients treated with trimetazidine during recanalization of the infarct vessel [35], and that pretreatment with trimetazidine results in a shorter time to pain relief [36]. Larger trials are necessary to determine whether the optimization of cardiac energetics during cardiac surgery should be implemented in routine practice.

Treatment of heart failure

Although clinical data are limited, the findings of a number of small clinical trials suggest that inhibiting fatty acid oxidation and stimulating glucose oxidation may also improve heart function and increase cardiac efficiency in heart failure. Bersin and co-workers [36,37] observed improved contractile function in 10 patients with heart failure (New York Heart Association [NYHA] classes III and IV) treated with intravenous dichloroacetate. Dichloroacetate inhibits PDH kinase to activate PDH, thereby increasing pyruvate oxidation and the coupling of glycolysis to glucose oxidation [38], and reducing myocardial tissue acidosis and making ATP production more fuel-efficient. Consistent with a stimulation of PDH, during treatment with dichloroacetate there was a significant increase in stroke volume and stroke work, and an increase in left ventricular mechanical efficiency from 15.2 to 20.6%. These results suggest that dichloroacetate increases pyruvate oxidation and mechanical efficiency by switching the preference of the heart to the more efficient fuel. However, caution should be taken when interpreting these results, as the study lacked a vehicle-treated control group, and because rates of glucose and free fatty acid uptake and oxidation were not measured.

Hermann et al [38,39] evaluated the effects of an acute intracoronary infusion of sodium pyruvate on left ventricular function in patients with NYHA class III heart failure with dilated cardiomyopathy (ejection fraction <25%). Infusion of pyruvate resulted in rapid increases in left ventricular peak dP/dt, ejection fraction, and cardiac output that immediately reversed upon cessation of the infusion [38,39]. Unfortunately, from a practical standpoint, it would not be feasible to infuse sodium pyruvate intravenously to attain high arterial pyruvate concentrations, because of the high sodium load that accompanies infusion of the sodium salt.

Because the optimization of cardiac energetics could be effective in the early stages of heart failure, chronic inhibition of myocardial fatty acid oxidation may slow down the progression of the heart failure and improve cardiac function. This has been investigated in a few trials with trimetazidine [40–42]. One of these [40] demonstrated that, compared with placebo, 2 months of treatment with trimetazidine resulted in a significant improvement in left ventricular ejection fraction at rest and enhanced left ventricular wall motion during a dobutamine stress test in patients with NYHA class II/III heart failure. Furthermore, two small clinical trials [41,42] demonstrated that, compared with placebo, treatment with trimetazidine improved systolic left ventricular function in patients with diabetes and ischemic cardiomyopathy. More recently, another trial involving 200 patients with ischemic cardiomyopathy demonstrated that patients treated with trimetazidine experienced a reduction in the insult from of an ischemic attack as determined by gated single photon emission computed tomography [43]. This finding was not accompanied by changes in hemodynamics. In addition, trimetazidine was able to improve functional NYHA heart failure class and left ventricular function in a double-blind, crossover study involving 12 patients [44]. To date, trimetazidine has not been evaluated in patients with heart failure from other than an ischemic origin, and no large-scale clinical trials have been conducted to investigate clinically relevant outcomes such as overall mortality.

As mentioned earlier, another approach to optimizing cardiac energetics is to prevent the uptake of fatty acids into the mitochondria through CPT-1. Such an approach provides benefit by making ATP production in the heart more fuel-efficient, and also reduces myocardial tissue acidosis by improving the coupling of glycolysis to glucose oxidation. Etomoxir is one agent that acts in this way by inhibiting CPT-1 [45], and in an open-label pilot study in patients with NYHA class II/III heart failure was shown to improve left ventricular function and exercise performance after 3 months of treatment. The first controlled trial of a CPT-1 inhibitor for treating heart failure was recently published; perhexiline was the agent used [46]. The authors demonstrated improved maximal oxygen consumption, left ventricular ejection fraction, resting and peak stress myocardial function, and skeletal muscle energetics, in patients with NYHA class II/III heart failure. They concluded that perhexiline may represent a novel treatment for heart failure,

provided that the dosage is adjusted according to plasma concentrations.

Long-term β-adrenergic receptor antagonists reduce mortality and improve left ventricular function in patients with heart failure [47,48]. Interestingly, in clinical studies, this improvement has been associated with a switch in myocardial metabolism away from fatty acid uptake and oxidation towards more glucose uptake and carbohydrate oxidation [49,50], and greater lactate uptake [51]. However, the mechanisms for these effects are not known, and they have not been demonstrated in large-scale clinical trials.

Overall, the optimization of cardiac energetics for the treatment of heart failure does show promise in the small pilot studies and trials that have been completed to date. Larger, long-term multicenter trials looking at clinically relevant outcomes such as overall mortality are both warranted and needed.

Conclusions

The optimization of cardiac energetics represents an exciting new approach to the treatment of heart disease. This form of optimization involves switching the fuel preference of the heart to become carbohydrate-dependent rather than fatty-acid-dependent. This can be achieved with agents that (i) directly inhibit mitochondrial fatty acid oxidation (trimetazidine and ranolazine), (ii) prevent the mitochondrial uptake of fatty acids (etomoxir), (iii) reduce the circulating concentrations of free fatty acids (GIK infusion), or (iv) directly stimulate glucose oxidation and improve its coupling to glycolysis (dichloroacetate). The initial results of trials investigating the optimization of cardiac energetics to treat angina, acute myocardial infarction, cardiac surgery, or heart failure have demonstrated more exciting promise than negative findings. However, larger long-term studies evaluating this form of treatment against first-line treatments, while assessing other clinically relevant outcomes such as overall mortality, are necessary to establish a role for the optimization of cardiac energetics in routine clinical practice. ■

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Evaluating metabolic changes in heart disease by magnetic resonance spectroscopy

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Abstract

Magnetic resonance spectroscopy (MRS) is a versatile and powerful tool for the non invasive study of cardiac metabolism and can be used to measure myocardial concentrations of many different metabolites. The most widely studied nucleus, phosphorus-31, allows for the detection of phosphocreatine, ATP, intracellular pH, and flux through the creatine kinase reaction. Carbon-13-MRS has a low sensitivity, but several metabolites can be measured to facilitate study of substrate utilization and flux. Finally, hydrogen-1 (proton)-MRS can be used to study myocardial oxygenation and creatine concentrations. Here, we give a brief overview of the different applications of cardiac MRS and the pathophysiological insights derived from such studies.
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Keywords: ^{31}P-Magnetic resonance spectroscopy, ^{1}H-magnetic resonance spectroscopy, ^{13}C-magnetic resonance spectroscopy, cardiac metabolism

Magnetic resonance imaging (MRI) has become a routine diagnostic tool in clinical cardiology, but magnetic resonance spectroscopy (MRS) has to date been applied only in research. In contrast to MRI, which uses signal derived from protons (^{1}H) in water and fat, MRS uses signals from many different nuclei. The major challenge when applying MRS is the intrinsic low resolution. However, MRS can be used to measure concentrations of many different compounds, intracellular pH, substrate selection, and even enzyme kinetics in the intact heart. MRS, like MRI, is truly non invasive and non destructive, and does not require radioactive tracers or harmful ionizing radiation.

Most cardiac MRS studies have been performed on the isolated heart model or in vivo in humans. Using isolated hearts has the advantage that signal from the entire heart is acquired, increasing sensitivity and making absolute quantification relatively straightforward. In-vivo MRS requires localization techniques and is complicated by the fact that movement necessitates cardiac and, possibly, respiratory gating, all of this inevitably leading to signal loss. Absolute quantification of metabolite concentrations is also difficult when in-vivo MRS is being used. Therefore, results from in-vivo phosphorus-31 (^{31}P)-MRS are often presented as phosphocreatine (PCr) : ATP ratios, although absolute quantification is preferable where possible.

Phosphorus-31 is the nucleus most widely used to study cardiac metabolism and allows for the detection of phosphocreatine, ATP, inorganic phosphate (Pi), and intracellular pH. ATP is the main substrate for all energy-consuming reactions in the cell, whereas phosphocreatine buffers ATP concentrations and transports energy within the cell via the creatine

kinase reaction:

$$ATP + Cr \leftrightarrow PCr + ADP + H^+$$

where Cr is creatine. Flux through this reaction can also be measured with ^{31}P-MRS.

Magnetic resonance spectroscopy can easily be used in a longitudinal fashion, allowing for monitoring of changes resulting from various pathologies over time in the same heart. Metabolic changes during ischemia and reperfusion have been studied extensively both in isolated hearts and in vivo. During global ischemia, phosphocreatine rapidly decreases to near undetectable concentrations, whereas ATP decreases much more slowly [1], illustrating the buffering capacity of (phospho)creatine. Heart failure has also been extensively studied using MRS. It was shown that, in intact residual myocardium of rat hearts with chronic myocardial infarction, phosphocreatine concentrations and flux through the creatine kinase reaction are both reduced [2]. Similar results were obtained in other animal models of cardiac hypertrophy and failure, such as dogs with volume-overload hypertrophy as a result of severe mitral regurgitation [3] and Syrian cardiomyopathic hamsters with advanced heart failure [4].

Results from studies on human heart failure have confirmed findings in animal models: the myocardial PCr : ATP ratio is reduced in symptomatic patients with dilated cardiomyopathy [5] (*Figure 1*). This reduction correlates with left ventricular ejection fraction [6]. The myocardial PCr : ATP ratio was even found to be a better predictor of long-term survival of patients with dilated cardiomyopathy than either left ventricular ejection fraction or the New York Heart Association class [7]. Measuring absolute concentrations revealed that, in patients with severe heart failure, ATP concentrations per se are also reduced [8], implying that PCr : ATP ratios underestimate the

metabolic derangement in these patients. Recently, flux through the creatine kinase reaction has been measured in human hearts, and showed that creatine kinase flux is reduced in patients with heart failure [9]. Interestingly, PCr : ATP ratios were also found to be reduced in patients with type 2 diabetes but no evidence of coronary artery disease or impaired cardiac function [10]. Overall, such studies suggest altered energetics as a key mechanism in heart failure.

Over the past decade, ^{31}P-MRS has been extensively applied in transgenic mouse models targeting cardiac metabolism; it is a particularly useful way in which to study models targeting the creatine/creatine kinase system. Measuring creatine kinase flux in hearts from creatine kinase knockout mice elucidated the relative contributions of the different isoenzymes [11]. Guanidinoacetate-*N*-methyltransferase (GAMT) knockout mice, which lack cardiac creatine, show a mild cardiac phenotype. Using ^{31}P-MRS, we were able to explain this finding, showing that the precursor of creatine, guanidino-acetate, takes over the role of creatine in these mice [1] (*Figure 2*). Recently, we found that the presence of supranormal creatine concentrations resulting from overexpression of the cardiac creatine transporter leads to heart failure [12]. ^{31}P-MRS revealed that the free energy change of ATP hydrolysis is reduced in these hearts, explaining contractile dysfunction. Using ^{31}P-MRS, abnormal cardiac energetics have been characterized in many other transgenic animal models; for example, mice lacking glucose transporter 4 [13] or peroxisome proliferator-activated receptor-α [14]. In mice with a mutation in the myosin heavy chain, the metabolic consequence of the mutation (reduced phosphocreatine) has been detected with ^{31}P-MRS [15].

In contrast to ^{31}P-MRS, carbon-13 (^{13}C)-MRS can be used to study a much larger number of compounds,
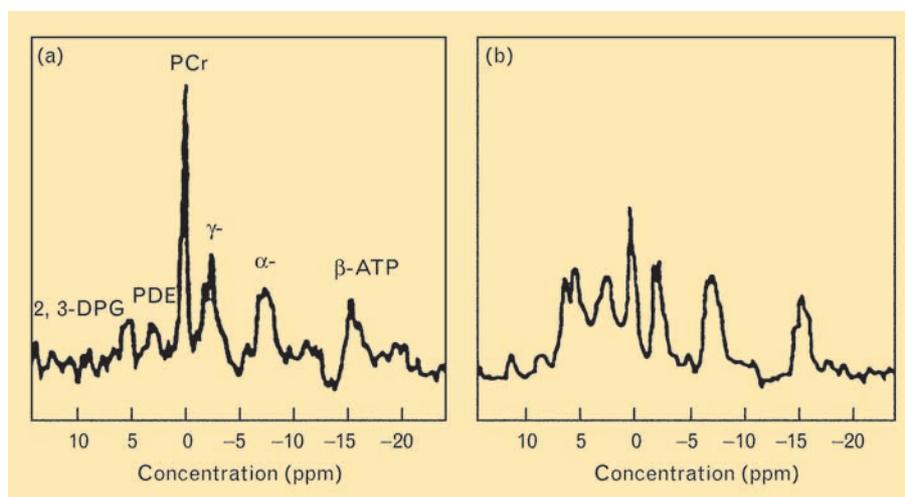

Figure 1. Typical phosphorus-31 magnetic resonance spectra (a) from a volunteer and (b) from a patient with severe dilated cardiomyopathy. In the patient, the phosphocreatine (PCr) : ATP ratio is markedly reduced. 2,3-DPG, 2,3-diphosphoglycreate; PDE, phosphodiesters; ppm, parts per million. (Modified from Neubauer et al [6], with permission).

Metabolic imaging
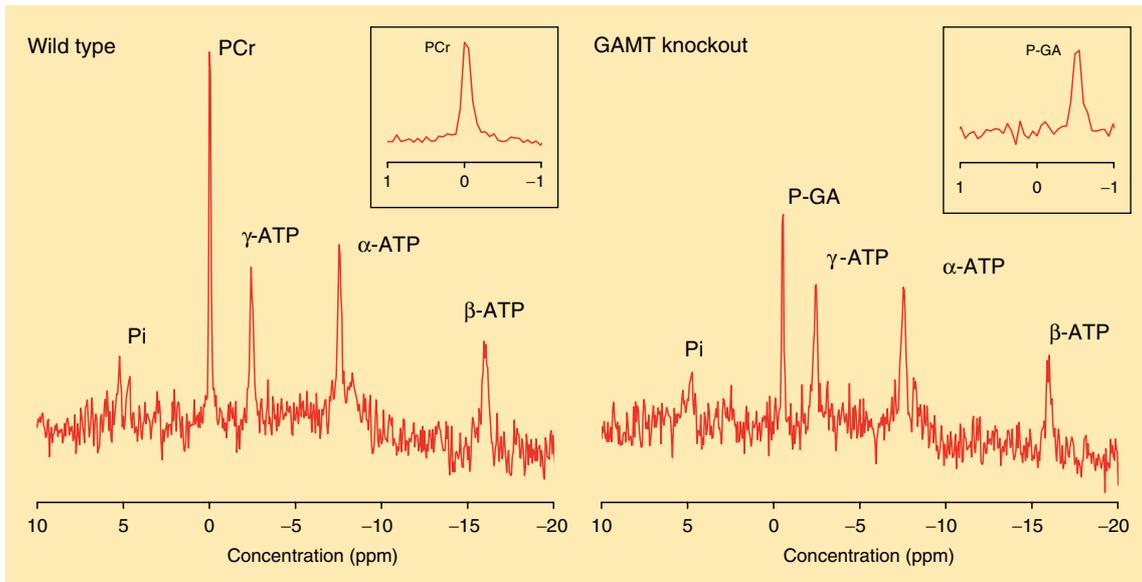## Michiel ten Hove and Stefan Neubauer

Figure 2. Typical phosphorus-31 magnetic resonance spectra of isolated perfused hearts from (a) a wild-type and (b) a guanidinoacetate-N-methyltransferase (GAMT) knockout mouse. GAMT knockout mice completely lack creatine and therefore no phosphocreatine (PCr) is visible in the spectrum. Instead, the phosphorylated form of guanidino-acetate (P-GA), the precursor of creatine, is visible. The P-GA peak appears just to the right of the position in which PCr appears in normal hearts (see inset). α-ATP, β-ATP and γ-ATP = α-, β- and γ-P-atom of ATP; Pi, inorganic phosphate; ppm, parts per million.

including many intermediates of metabolic pathways; it can thus be an extremely powerful tool. However, the sensitivity of this technique is particularly low, because of the low ''nuclear magnetic resonance (NMR) visibility'' and the low natural abundance of ^{13}C (approximately 1%). Therefore, ^{13}C-MRS of the intact heart is usually performed while supplying compounds enriched with ^{13}C, making it an expensive technique. However, using enriched compounds has

advantages. For example, isolated hearts have been perfused with ^{13}C-labeled substrates to study tricarboxylic acid cycle kinetics [16]. The use of intravenous infusion of labeled substrates in rats enabled identification of the substrates that the heart uses preferentially [17]. Recently, triacylglycerol storage and turnover rates measured with ^{13}C-MRS were found to be profoundly different in isolated hearts from diabetic rats compared with those from control
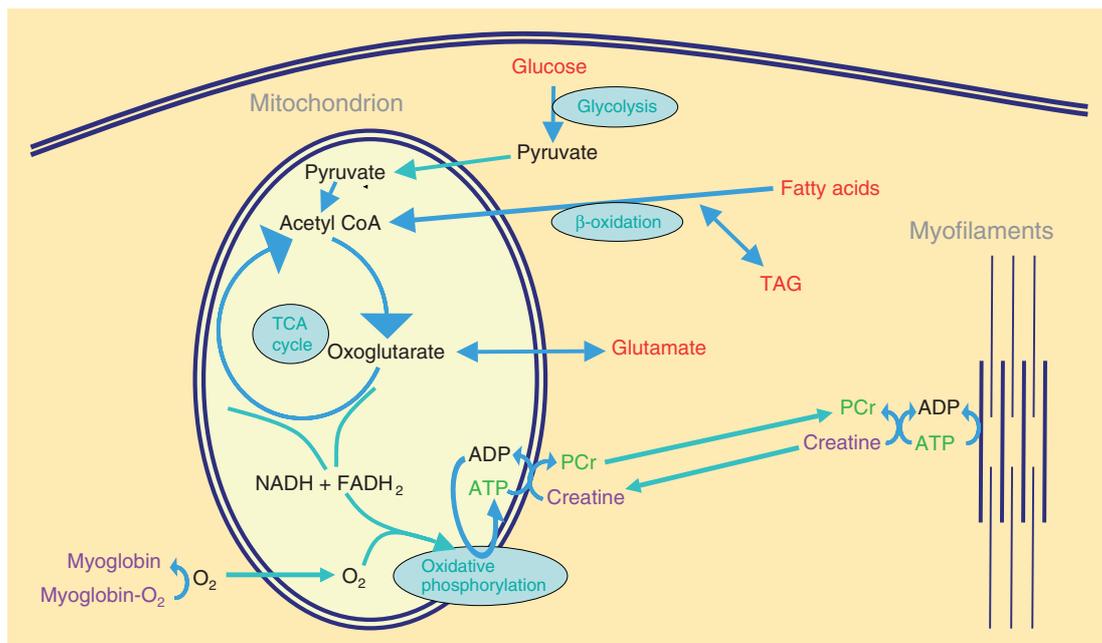

Figure 3. Schematic representation of myocardial metabolism. Metabolites that have been measured with magnetic resonance spectroscopy (MRS) in the intact heart are marked in green (phosphorus-31-MRS), red (carbon-13-MRS) and purple (hydrogen-1[proton]-MRS). CoA, coenzyme A; P-Cr, phosphocreatine; TAG, triacylglycerol; TCA, tricarboxylic acid.

rats [18]. However, because of the low sensitivity associated with ^{13}C-MRS, in many studies perchloric acid extractions of the heart are used for a more detailed study of cardiac metabolism. With this technique it has been shown that, in myoglobin knockout mice, cardiac substrate metabolism is switched from fatty acid to glucose oxidation [19].

Hydrogen-1 (proton)-MRS has the advantage of relatively high "NMR sensitivity", but ^{1}H-MRS of biological tissue is dominated by signal from protons in water. Additional water suppression techniques are therefore required, leading to signal loss. Although ^{1}H-MRS can be used to measure many different compounds, its application to study of the metabolism of the intact heart has been limited. Total creatine (phosphocreatine plus free creatine) has been measured in perfused rat hearts [20] and in vivo in humans [21] and, more recently, in the mouse [22]. In patients with heart failure, total creatine concentrations, like phosphocreatine concentrations, were reduced [21]. ^{1}H-MRS has also been applied to measure oxygenation of myoglobin in isolated mouse hearts [23], and cardiac deoxymyoglobin has been measured in vivo in infarcted swine heart [3]. More recently, ^{1}H-MRS examination of perchloric acid extracts from hearts has been used for so called "metabolomics", an approach to measure changes in a large number of metabolites [24].

Figure 3 summarizes myocardial metabolism and identifies the metabolites that have been measured in the intact heart with MRS.

To date, human MRS has been limited to ^{31}P-MRS and ^{1}H-MRS, and has been applied only as a research tool. Better coils and magnets with greater field strength, in addition to application of a localization technique called spectral localization with optimum pointspread function (SLOOP), which allows for better matching of voxels to the curved shape of the heart, should ultimately bring clinical MRS closer to reality. In addition, hyperpolarized ^{13}C-MRI and/or MRS has been reported [25]. With this technique, the signal-to-noise ratio is enhanced by a factor of 10^5 and metabolic ^{13}C imaging may become a possibility. ■

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Metabolic profiling in heart disease

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Abstract

Identifying the individuals who will suffer a myocardial infarction as a result of coronary artery disease (CAD), in order to direct available therapeutic resources efficiently, remains an important limiting factor in the delivery of clinical care in the cardiovascular arena. Metabolite profiling is an example of a new type of diagnostic test that shows considerable promise. Nuclear magnetic resonance spectroscopy or chromatographic separations can be used to generate a ''molecular fingerprint'' of a serum sample, and cutting-edge pattern recognition techniques can then be applied to identify molecular signatures associated with the presence of occlusive atherosclerotic lesions and, perhaps in the future, those associated with increased risk of myocardial infarction. Such approaches may improve clinical management of CAD and at the same time provide novel insights into the metabolic disturbances that underlie the disease.
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Keywords: Metabonomics, atherosclerosis, myocardial infarction, pronostics

The need for improved diagnostic capability

Coronary heart disease remains the single biggest cause of morbidity and mortality in the UK, with almost 40 000 premature deaths (defined as death before age 75 years) attributed to it each year [1]. Over the past two decades, we have seen considerable improvements in therapeutic options (particularly with the widespread use of cholesterol-lowering drugs of the statin class [2]). However, the maximum benefits of these improved therapies are difficult to achieve in practice because of difficulties in selecting targets for preventative interventions (whether pharmaceutical in nature, or public health interventions such as dietary modification).

Existing diagnostic tests are generally focused on detecting cardiac ischemia (resulting, for example, in chest pain, shortness of breath or edema) or coronary artery stenosis, and as a result do not necessarily direct preventative treatments to the majority of individuals who would otherwise go on to suffer a myocardial infarction. Optimizing strategies to make sure both that the majority of those who will suffer a myocardial infarction are receiving the most aggressive treatment, and that the minimum number of people who would not otherwise suffer an infarction are treated, has therefore become a major task facing the health-care profession. Any steps that improve identification of these individuals will pay a large public health dividend.

The aim of any diagnostic strategy is to identify the smallest subgroup of the population under study that still contains all the individuals who will go on to suffer myocardial infarction. Unfortunately, this definition is often clouded by the idea of searching for ''high-risk'' individuals. If you mistakenly optimize your diagnostic strategy simply to identify those at greatest risk (without concern for the number of such people you can find), the size of the ''high-risk'' group will shrink as the extent of the risk is increased, resulting in the majority of myocardial infarction events occurring in people outside the high-risk category. As a result, much of the benefit of aggressive treatment will be missed.

New therapeutic approaches
Metabolic profiling in heart disease

"Pronostics": profiling diagnostic technology

Over the past decade, it has become increasingly clear that improved diagnostic and prognostic capability can be achieved by combining simple risk factors. For example, the risk-scoring methods derived from the Prospective Cardiovascular Münster Study (PROCAM) or Framingham studies are considerably superior to the individual measurements that compose them ([3] and references therein).

Taking this idea further, it should be possible to collect very much larger profiles (containing many thousands of data elements related to an individual) and search within those profiles for signatures that predict future myocardial infarction events with high sensitivity and specificity. Recent rapid advances in genomics, proteomics, and "metabonomics" have made such profiling diagnostics (or "pronostics") technically feasible. The key to such approaches is to measure the concentrations of as many different molecules as possible (whether they are mRNA, protein, or low molecular weight metabolites), ideally choosing the analytes at random rather than through any pre-existing idea that they may be associated with the disease endpoint. Mathematical modeling tools can then be applied to trawl through the resulting mountain of data to extract any molecular signature that reliably associates with the endpoint under investigation.

Metabolite profiling

Using techniques such as nuclear magnetic resonance (NMR) spectroscopy or gas chromatography followed by mass spectrometry (GS–MS), it is possible to generate such a profile of low molecular weight metabolites. Neither NMR nor GC–MS can generate an "ideal" profile (with every metabolite represented, identified, and quantitated), and guidelines on selecting the most appropriate method depending on the question under study have been published elsewhere [4]. For disease diagnosis (which does not depend on identifying any specific metabolites in the profile, but merely on reproducible differences in the profile between cases and controls), NMR spectroscopy may be the superior approach, but too few studies have yet been published to permit definitive conclusions to be drawn.

Profiling metabolites is a particularly attractive approach to developing a pronostic test for coronary artery disease (CAD). In general, metabolite profiling is more straightforward than genomic or proteomic profiling, because of the high reproducibility of the tools for chemical analysis that are used to generate the profile. For example, NMR spectroscopy yields a replicate reproducibility of about 1% across the information-dense region of the spectrum [5], which is superior to conventional biochemical assays such as enzyme-linked immunosorbent assay (in which the reproducibility is typically 5–10%) and more than 10-fold better than gene array methodologies or labour-intensive proteomic profiling based on differential gel electrophoresis and mass spectrometry.

More specifically, there is already a well-known metabolic component to the pathogenesis of CAD. Dysregulated lipid metabolism and increased plasma cholesterol concentrations play an important part in disease development, as demonstrated by numerous genetically modified mouse models of atherosclerosis in which enzymes regulating lipid metabolism have been disrupted [6]. Similarly, the constellation of factors that compose the metabolic syndrome are believed to represent a risk factor for CAD in the human population [7]. On this basis, it seems inherently plausible that even a simple or imperfect metabolite profile might be able to offer improved clinical diagnosis of CAD.

Metabolic signatures of occlusive atherosclerosis

To test the above proposal, we performed a small-scale study [8], comparing NMR-derived serum metabolite profiles of individuals with severe angiographically-defined CAD and those from individuals apparently free from stenotic lesions. For each group, serum samples were prepared from approximately 40 individuals who presented at Papworth Hospital, Cambridge, UK, and were subjected to 600 MHz one-dimensional proton NMR spectroscopy, using previously published conditions [9]. The resulting spectra were phased, baseline-corrected and processed into 207 integral regions representing the average intensity across small regions of the spectra (*Figure 1*).

Simple inspection of the resulting profiles highlights the exceptional degree of similarity in the serum metabolome between individuals. However, even cursory analysis reveals differences between samples (yellow inset boxes in *Figure 1*). The important question is whether any of these differences represents a reproducible signature that distinguishes the cases from the controls. To test this rigorously, we used a range of pattern-recognition methodologies (reviewed in depth elsewhere [10]) to identify any signature within the profile that was reproducibly associated with CAD. Encouragingly, this yield a clear separation between the two groups [8], based largely on subtle compositional differences between the lipoprotein particles [5,8].

These findings suggested that an NMR-derived metabolic profile could usefully distinguish severe

New therapeutic approaches
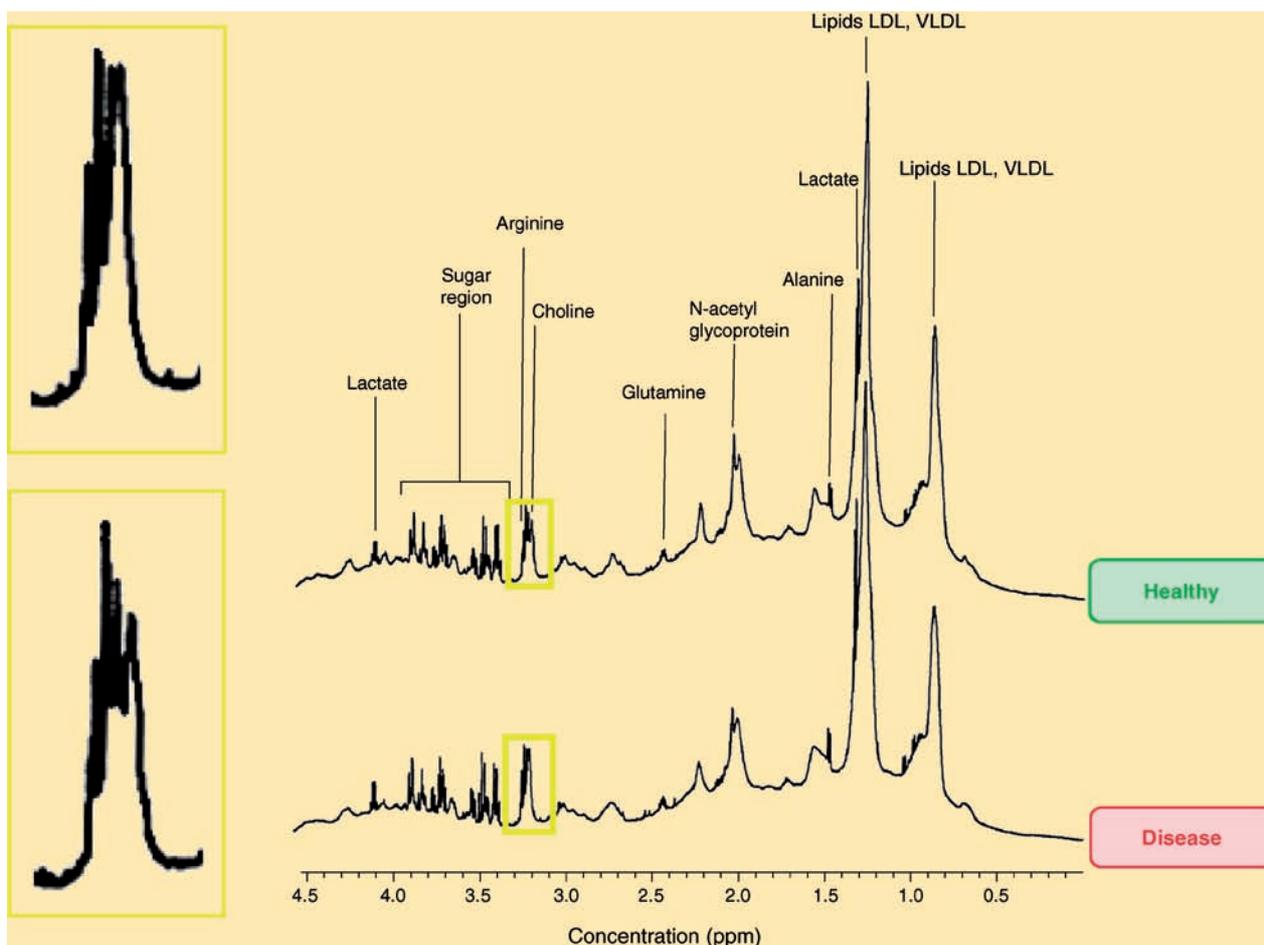*David J. Grainger*

Figure 1. Serum metabolic profiles. Two metabolic profiles, derived by one-dimensional 600 MHz proton nuclear magnetic resonance spectroscopy of serum samples as previously described [9], are shown. The lower profile represents serum from an individual with no angiographic evidence of heart disease, and the upper profile represents serum from an individual with severe (at least 70% stenosis of all three major coronary arteries) heart disease. Grossly the profiles are similar, but careful examination reveals potentially important differences (insets).

disease from normal coronary arteries. At the same time, we compared the profiles from individuals with varying degrees of disease severity, and the results suggested that some information regarding severity was encoded within the profile [8], although considerably larger studies will be required to establish the extent to which any clinically useful discrimination could be achieved.

Unfortunately, the one-dimensional proton NMR procedure that we adopted for these studies, which was chosen to improve our diagnostic capability, provides little indication about the precise molecular nature of the differences in lipid composition. Further studies are now under way using profiles derived from GC–MS, and altered concentrations of particular fatty acids are now being identified which may shed new light on the pathogenesis of CAD.

Moving to a physiological endpoint

There is little doubt that clinical management of CAD could be improved by the existence of a serum-based

test that reliably predicted the outcome of coronary angiography (allowing the available angiography resource to be more efficiently targeted); however, ultimately, it is prediction of cardiovascular events (stroke and acute myocardial infarction, for example) that is most urgently required. Angiographic stenosis may predict chronic angina, but is only a small part of the story regarding unstable disease, in which plaque composition rather than size or location may be the biggest determinant of the risk of myocardial infarction [11,12]. None of the studies to date have provided any indication as to whether metabolic profiling can provide improved prediction of such events, and addressing such questions will be an important component of future research in this area.

Encouragingly, however, we have already demonstrated the existence of a metabolic signature associated with hypertension (a physiological rather than anatomical endpoint) [13], and Sabatine and colleagues recently reported a metabolic signature associated with myocardial ischemia [14]. On the

basis of these early results, there is reason to be optimistic about the use of metabolic profiling to provide clinically useful prognostic information about hard cardiovascular endpoints.

The future for metabolic profiling in heart disease

Metabolic profiling (and, indeed, profiling diagnostics in general) is an emerging field, and much remains to be discovered. The results of pilot-scale studies have provided an impetus to further investigations. A dedicated, large-scale clinical study of metabolite profiling for the diagnosis of heart disease, the Metabonomics and Genomics in Coronary Artery Disease (MaGiCAD) study [15,16], recently completed recruitment of more than 1300 individuals and will considerably expand our understanding of metabolic and genetic factors in CAD, with early results expected by the end of 2006. Similarly, the very large Human Serum Metabolome (HUSERMET) project [17] has included cardiovascular disease as one of its component studies. Over the coming years, it will become evident whether and to what extent metabolite profiling can provide clinically useful diagnostic capability in the management of CAD.

Almost as important will be comparison of different profiling methods. Today, it seems very likely that a pronostic test for CAD will be developed over the coming years, but it is unclear whether such a test will include a metabolic, genetic, or even an immunological profile. Ultimately, such questions may depend as much on the relative cost of capturing the various profiles as on the scientific rationale for their inclusion. The simplest (and least expensive) profile that contains sufficient information to yield a reliable diagnosis will probably form the basis of any clinical pronostic test; other, more costly, methods will be restricted to the research laboratory, where they seem likely to yield exciting new insights into the pathogenesis of cardiovascular diseases.

Conclusion

Early pilot-scale studies suggest that metabolite profiling may provide clinically useful information about the existence and extent of CAD, although any relationship to cardiovascular events remains entirely unknown. Novel metabolic markers of CAD are now emerging from such studies, and may yield important new insights into the pathogenesis of the disease. However, research in this area is still at an early stage,

and larger studies are required to obtain a proper definition of both the clinical and scientific utility of metabolite profiling in cardiovascular disease.

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Trimetazidine and the metabolic profile of ischemia

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Abstract

Trimetazidine, a metabolic agent, acts at the cellular level to improve myocardial metabolism at the time of ischemia. A recent Cochrane Collaboration review of its use in stable angina has confirmed its effectiveness in reducing anginal attacks and nitrate consumption, at the same time as improving exercise performance with minimal adverse events. Additional reports identifying improved left ventricular function in cardiac failure suggest a potential prognostic role.
■ *Heart Metab.* 2006;32:26–27.

Keywords: Heart disease, left ventricular function, myocardial metabolism, prognosis, trimetazidine

The conventional management of stable angina focuses on reducing myocardial demand by means of drugs that reduce heart rate, blood pressure, and contractility [1]. There is no doubt that these drugs are very effective but, once the hemodynamic option has been maximized, the addition of agents with a similar mode of action has never been shown to improve symptoms. Indeed, adverse effects are often the consequence, and it is adverse effects that may limit the usefulness of hemodynamic agents; however, this should not be over emphasized. The most common dose-limiting adverse effects are cold peripheries, lethargy and ''heavy legs'' or a ''zombie'' feeling in patients receiving β-blockers, fluid retention in those receiving calcium antagonists, and headaches in individuals receiving oral nitrates or nicorandil. To circumvent these effects, suboptimal combination treatment is often used – for example, atenolol 50 mg plus amlodipine 5 mg daily, rather than atenolol 100 mg daily.

An alternative strategy is to address the metabolic causes or consequences of ischemia [2]. The normal heart derives 60–90% of its energy from the consumption of free fatty acids (FFAs), and the remainder from glucose and lactate. FFA metabolism yields more adenosine triphosphate (ATP) per gram, but requires more oxygen consumption to do so. When ischemia develops and there is a deficiency in oxygen delivery to the myocardium relative to the demand for ATP, glycolysis is activated in an attempt to generate ATP anaerobically. Glycogen stores are broken down, glucose uptake is increased, and instead of lactate consumption there is lactate production, which can be measured in the coronary sinus. As a consequence, the pH in the cell is reduced and calcium overload occurs, leading to contractile dysfunction [3].

Trimetazidine inhibits the enzyme of fatty acid β-oxidation, long-chain 3-ketoacyl coenzyme A thiolase (known as 3-KAT). Through the inhibition of myocardial fatty acid oxidation, glucose and pyruvate oxidation is increased (pyruvate dehydrogenase activity is increased) and lactate production is decreased at the time of effort- (or emotionally) induced ischaemia – that is, the supply–demand imbalance is restored, independently of hemodynamic actions. The evidence base for the effectiveness of trimetazidine in stable angina has been the subject of a recent Cochrane Collaboration review, but we also have increasing reports of its beneficial actions in patients with heart failure, who show improvements in symptoms, exercise performance, ejection fraction and, possibly, prognosis [4,5].

In the Cochrane review, the objective was to determine the efficacy and tolerability of trimetazidine in

Table 1. Summary of the effectiveness of trimetazidine in stable angina.

Comparison between trimetazidine and	Outcome	Superiority
Placebo	Angina: events per week	Trimetazidine
	Glyceryl trinitrate: use per week	Trimetazidine
	Time to 1-mm ST-segment depression on exertion	Trimetazidine
	Number of adverse events	Trimetazidine
Hemodynamic agent	Angina: events per week	Equal
	Glyceryl trinitrate: use per week	Equal
	Time to 1-mm ST-segment depression on exertion	Equal
	Number of adverse events	Trimetazidine

patients with stable angina. Selection criteria were for randomized trials comparing trimetazidine with placebo or with other antianginal drugs, in adults with stable angina. A total of 23 studies, involving 1378 patients, met the inclusion criteria. The findings are summarized in *Table I*. Compared with placebo, trimetazidine reduced the weekly angina attack rate by 40% (mean difference −1.44, 95% confidence interval [CI] −2.10 to −0.79; $P < 0.0001$) and nitrate consumption (−0.73, 95% CI −1.47 to −2.20; $P < 0.0001$). Objectively, trimetazidine improved exercise time to 1-mm ST-segment depression ($P = 0.0002$). The benefits occurred with trimetazidine as monotherapy and in combination. An important feature of all the studies was the low incidence of adverse effects – fewer than placebo!

This report demonstrated the efficacy of trimetazidine versus placebo and in addition to conventional haemodynamic agents in the treatment of stable angina. Trimetazidine is therefore an effective antianginal drug that with its minimal side effect profile it has an important role to play when side effects limit haemodynamic agents and in those vulnerable to adverse effects such as one elderly. It is therefore, at present, a drug for relief of symptoms and, with its minimal side-effect profile, it has an important part to play when side effects limit the usefulness of hemodynamic agents and in those patients who are vulnerable to adverse effects, such as the elderly [6].

The question of whether there are prognostic benefits needs to be addressed, given the evidence of an improved ejection fraction in patients with cardiac failure. In one study of 200 patients with ischemic left ventricular dysfunction as a result of multivessel coronary artery disease, 100 patients were placed on a regimen of trimetazidine in addition to conventional treatment and 100 received placebo [7]. Trimetazidine improved ischemic attacks clinically and also improved both exercise performance and perfusion judged using single photon emission computed tomography. Survival at 2 years was 92% among patients treated with trimetazidine and 62% among those treated with placebo. Clearly, this observation is of significance, given the poor prognosis in cardiac failure even when all the evidence-based medicine has been deployed.

Trimetazidine is therefore an effective, well tolerated treatment for stable angina, with a fascinating and potentially exciting potential in cardiac failure in which there is evidence of systolic dysfunction. ■

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Altered AMP-activated protein kinase activity and pathologic cardiac disease

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Abstract

AMP-activated protein kinase (AMPK) regulates vital metabolic pathways in the cell. Although downstream substrates are abundant, a major role of AMPK is to preserve ''energy homeostasis'', particularly in highly metabolically active cells such as skeletal and cardiac myocytes. The biologic importance of AMPK in regulating cellular metabolism is confirmed by the observation that genetic defects in the gamma regulatory subunit of this heterotrimeric protein lead to pathologic skeletal and cardiac disease.
■ *Heart Metab.* 2006;32:28–31.

Keywords: AMP-activated protein kinase (or AMPK), Wolff–Parkinson–White, arrhythmia, glycogen storage disease, hypertrophy, genetics

Case report

In 1985, a 23-year-old man experienced a sudden loss of consciousness, with a spontaneous recovery and without adverse clinical sequelae. Cardiovascular examinations identified ventricular pre-excitation (Wolff–Parkinson–White) on the 12-lead electrocardiogram (*Figure 1*). Two-dimensional echocardiography demonstrated significant left ventricular hypertrophy (septal thickness 20 mm) and a diagnosis of hypertrophic obstructive cardiomyopathy was made.

Three years later, the patient experienced a recurrent loss of consciousness. Emergency personnel documented severe bradycardia (heart rate 20–30 beats/min). Following this event, a permanent pacemaker was implanted. At the age of 31 years, the patient was pacemaker-dependent, and had persistent atrial flutter progressing to permanent atrial fibrillation.

In recent years, the patient has developed progressive and severe left ventricular dilatation and dysfunction. A multigated nuclear scan has documented a left ventricular ejection fraction of 15% (normal >50%). His clinical course has been further complicated by renal and splenic infarcts, despite a therapeutic international normalized ratio for the coumadin that he was receiving as treatment for permanent atrial fibrillation. Transesophageal echocardiography confirmed a left atrial appendage thrombus. Surgical excision of the left atrial appendage was performed and histologic examination of atrial tissue was completed (*Figure 2*). At the age of 43 years, the patient is awaiting cardiac transplantation. Genetic testing had previously confirmed that he patient harbors an Arg302Gln amino acid substitution in the gamma-2 regulatory subunit (PRKAG2) of AMPK [1].

Discussion

The case presented exemplifies the characteristic features of the PRKAG2 cardiac syndrome, which we have described previously [2]. Typically, affected
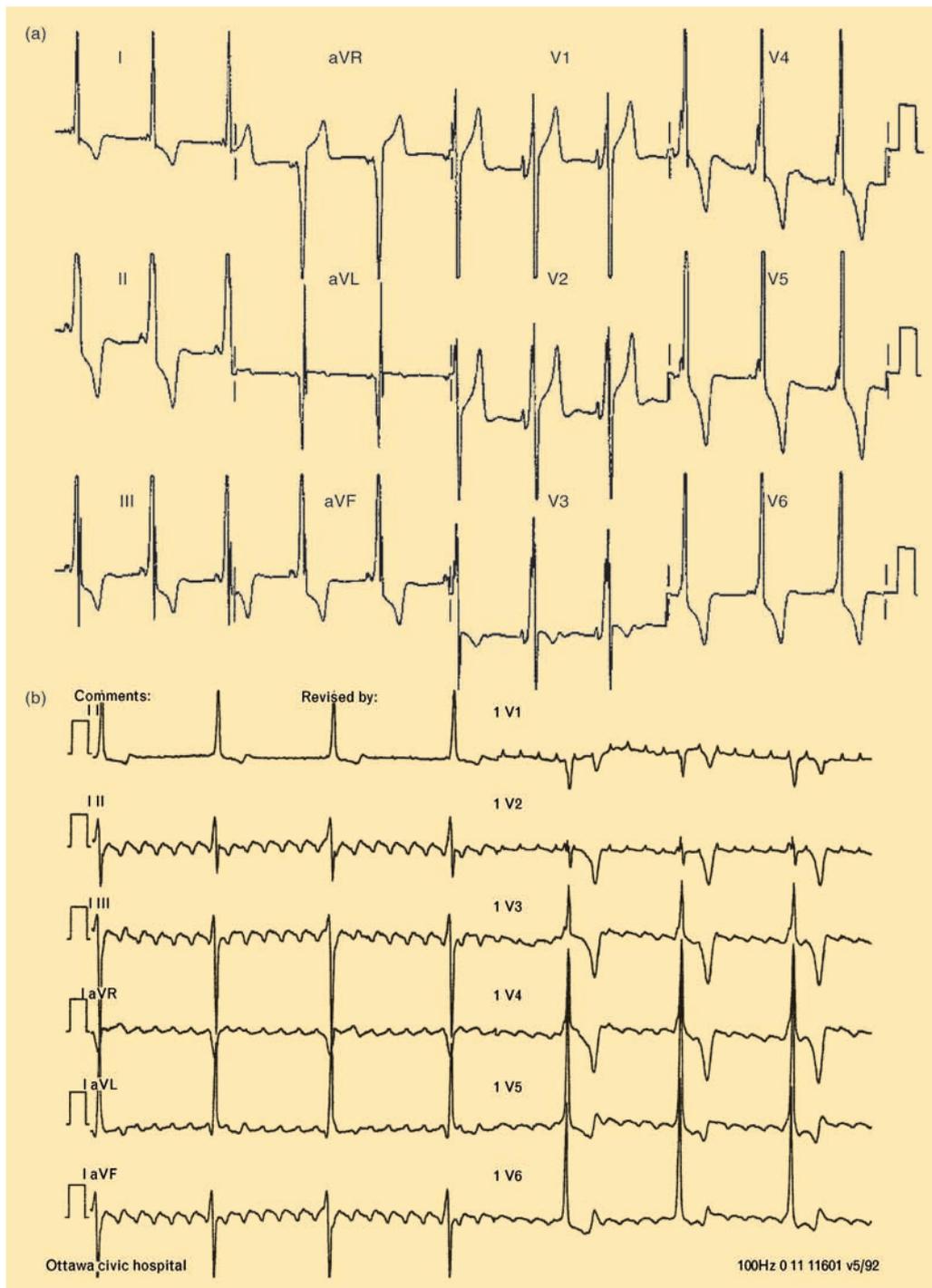
Figure 1. Progressive 12-lead electrocardiographic changes in the PRKAG2 (gamma-2 regulatory subunit of AMPK) cardiac syndrome. (a) Baseline 12-lead electrocardiogram (ECG) of the patient at age 23 years, demonstrating a short P–R interval and broad QRS with slurred upstroke, consistent with ventricular pre-excitation. (b) ECG at age 26 years, showing atrial flutter with a slow ventricular response as a result of poor atrioventricular node conduction. The patient was not receiving any medications.

patients present in late adolescence with symptomatic palpitations and documented supraventricular tachycardias. Progressive disease is usual, with the paradoxical development of cardiac conduction system disease, including sinus node dysfunction and impaired atrioventricular node conduction, leading to the onset of bradycardic heart rhythms.

Presumably, the impaired cellular metabolism caused by altered AMPK activity over decades is progressively toxic to cardiac myocytes and conductive tissue.

More than 70% of our patients harboring the Arg302Gln mutation have required a permanent pacemaker during or before their 4th decade of life. In excess of 80% of patients develop either

Case report
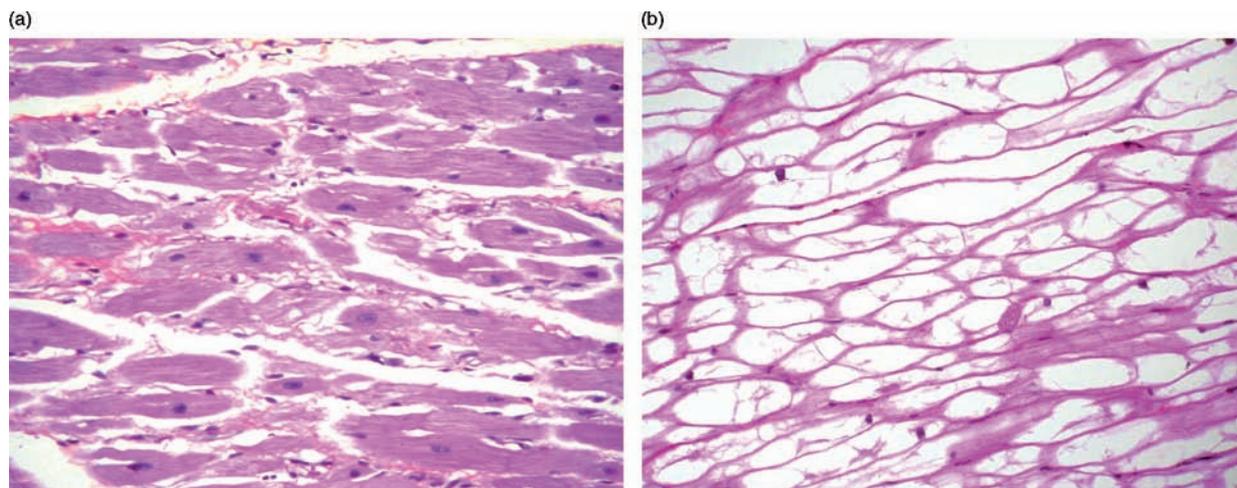*Michael H. Gollob, Martin S. Green, and John P. Veinot*

Figure 2. Tissue pathology in the PRKAG2 (gamma-2 regulatory subunit of AMPK) cardiac syndrome. (a) Normal left atrial myocardium, with normal myocyte myofiber staining and content, from a patient without cardiomyopathy. (b) Grossly abnormal vacuolated, cleared myocytes, glycogen-filled and with scarce myofiber contents, in the PRKAG2 cardiac syndrome.

paroxysmal or permanent atrial fibrillation, commonly during their 20s. Although our patient described developed severe cardiomyopathy, evidence of cardiac hypertrophy, dilatation, or dysfunction is observed in only 40% of patients harboring the Arg302Gln mutation. Clinical penetrance of this autosomal dominant genetic disease is 100%, as we have never observed carriers of a clinically silent mutation in adulthood.

The known role of AMPK in regulating key cellular metabolic pathways, particularly glucose metabolism, led to our original hypothesis that the cellular basis for the observed cardiac hypertrophy in many cases was secondary to abnormal glycogen storage in myocytes [2,3]. This hypothesis was supported after the development of transgenic mouse models expressing known disease-causing PRKAG2 mutations [4,5]. The histology presented in *Figure 2* in this report confirms that the cellular enlargement and cardiac hypertrophy in affected humans are secondary to glycogen-filled vacuoles, and not a result of the presence of an increase in sarcomeric units, as occurs in the more common form of hypertrophic cardiomyopathy seen in adulthood. The mechanism by which altered AMPK activity results in accessory atrioventricular connections, the anatomic substrate for ventricular pre-excitation, remains unclear. Nevertheless, these accessory atrioventricular connections behave in a fashion similar to that observed in patients with Wolff–Parkinson–White syndrome but without this genetic disease. We have documented atrioventricular re-entrant tachycardia in patients with the Arg302Gln PRKAG2 mutation and confirmed the use of the accessory atrioventricular connection in the tachycardia circuit. In addition, we have performed successful radiofrequency ablation on accessory atrioventricular connections in such patients.

The propensity for frequent atrial arrhythmias in patients implies that AMPK may have a direct role in regulating cardiac ion channels, perhaps through phosphorylation. Evidence suggests that cardiac sodium channels may be a substrate of AMPK [6].

Although the genetic basis of this complex cardiac phenotype has been elucidated, the precise biochemical mechanism of the disease remains controversial. The biochemical effects on AMPK activity of known disease-causing mutations in PRKAG2 has been studied by various methodologies. Mutant AMPK activity has been measured by in-vitro assays, ex-vivo assays of transgenic mice heart extracts, and after expression in various mammalian cell types. Directly conflicting results have been reported, suggesting either a constitutive gain-of-function induced by PRKAG2 mutations, or a significant loss-of-function [4,5]. Recently, Burwinkel et al [7] obtained data suggesting that both a loss-of-function and a gain-of-function may be imposed by PRKAG2 mutations. The presence of the mutation abolished AMP-induced activation of AMPK, hence loss of function. However, the basal state of phosphorylation and activity of AMPK were increased in the presence of the mutation studied, resulting in a constitutive gain-of-function in the cell, independent of the need of AMP for activation [7]. These opposing data sets illustrate the challenges in understanding biochemical phenomena in experimental systems. It is hoped that future studies, perhaps through novel methodologies, will resolve the current controversy.

Conclusion

In view of the significant function of AMPK under conditions of myocardial ischemia and in regulating

glucose metabolism, this enzyme has been considered as a potential target for drugs. However, the observation that genetically induced perturbations in AMPK activity may lead to a severe cardiac pathology must be considered in the context of the future development of drugs targeting this protein. ■

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Regulation of gene expression by polyunsaturated fatty acids

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Abstract

Consumption of polyunsaturated fatty acids (PUFAs) has been shown to be beneficial in the prevention of several human diseases, including obesity, diabetes, heart disease, and stroke. It has become clear that linolenic (n-3) and linoleic (n-6) PUFAs can act at the nuclear level to affect expression of genes involved in diverse metabolic pathways. PUFAs act via nuclear receptors such as peroxisome proliferator activated receptor α and liver X receptor α, and through the transcription factor, sterol regulatory element binding protein-1c, to elicit a favorable hypolipidemic phenotype. Further understanding of the molecular effects of PUFAs will be key to devising novel approaches to the treatment and prevention of disease.
■ *Heart Metab.* 2006;32:32–35.

Keywords: Polyunsaturated fatty acids, SREBP1c, PPARα, LXRα

Introduction

Linoleic (n-6) and linolenic (n-3) acids are polyunsaturated fatty acids (PUFAs) that cannot be synthesized de novo by mammals and are hence considered to be essential to the diet. n-3 (or omega-3) PUFAs, including eicosapentaenoic acid and docosahexanoic acid, are concentrated in marine mammals and high-fat fish, whereas the main sources of dietary n-6 PUFAs are vegetable oils and organ meats. n-3 PUFAs have been shown to promote fatty acid oxidation while decreasing the rates of lipid synthesis [1]. They have also been shown to decrease plasma lipid concentrations [2] and enhance insulin sensitivity [3]. In addition, they are believed to be preventive in various chronic diseases, including rheumatoid arthritis [4], coronary heart disease [5], and stroke [6], and certain types of cancer, including breast, prostate, and colorectal cancers [7,8]. These beneficial effects of PUFAs

are of obvious therapeutic interest; however, there has also been some concern over excess consumption of n-6 PUFAs, because of their proinflammatory and proaggregatory effects [9]. Thus understanding the mechanisms by which these fatty acids exert their effects will be key to understanding whether and how PUFAs can help promote optimal health and in establishing a much-needed healthy dietary n-3 : n-6 ratio.

Regulation of genes by polyunsaturated fatty acids

Once fatty acids enter the cell, they are rapidly converted to fatty acyl coenzyme A (CoA) thioesters by an acyl CoA synthetase [10] (*Figure 1*). This reaction is essential to the further partitioning of fatty acids into various pathways, including complex lipid synthesis, β-oxidation, elongation/desaturation, and production of secondary signaling intermediates such as prostaglandins, thromboxanes, and leukotrienes

Refresher corner

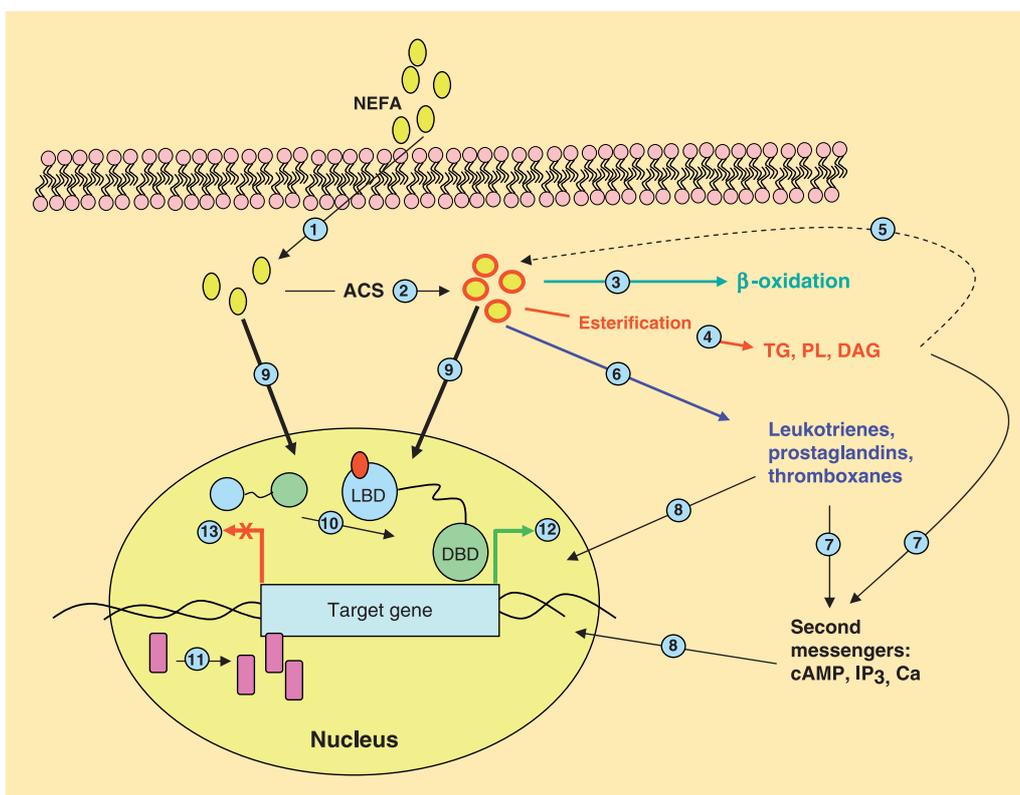*Regulation of gene expression by PUFAs*

Figure 1. Regulation of gene expression by fatty acids and their metabolites. Non esterified fatty acids (NEFA) are transported into the cell (1) and are rapidly converted to acyl coenzyme A (CoA) by acyl CoA synthetase (ACS) (2). The acyl CoA can be oxidized (3) or can be esterified into complex lipids (4) such as triglycerides (TG), phospholipids (PL), or diacylglycerols (DAG). These complex lipids can also replenish the cellular fatty acid stores as necessary (5). Alternatively, fatty acyl CoAs can give rise to leukotrienes, prostaglandins, and thromboxanes (6). These secondary metabolites, in addition to complex lipids such as DAG, can increase cellular concentrations of second messengers such as cyclic AMP (cAMP), inositol triphosphate (IP_3), and calcium (Ca) (7). These second messengers or their lipid precursors can all have effects on gene expression (8). Alternatively, free fatty acids and fatty acyl CoAs can act directly at the nuclear level (9). In the nucleus, signaling through fatty acids or their metabolites can lead to changes in nuclear receptor activation (10), as in the case of peroxisome proliferator activated receptors and liver X receptors, or to changes in transcription factor abundance (11), as in the case of sterol regulatory element binding protein-1c, leading to upregulation (12) or downregulation (13) of target genes. DBD, DNA binding domain; LBD, ligand binding domain.

(*Figure 1*), which can in turn lead to changes in production of cellular second messengers such as inositol triphosphate, cyclic AMP (cAMP) and calcium (*Figure 1*). Because of the rapid nature of the acyl CoA synthetase reaction and the several fates of cellular fatty acids, the free fatty acid concentration within the cell is generally maintained at very low values. Thus the molecular effects of fatty acids within cells are likely to be mediated, not only by free fatty acids, but also by fatty acyl CoAs and second messengers (*Figure 1*).

It is now clear that PUFAs do not regulate gene expression exclusively through changes in membrane composition or through production of secondary signaling intermediates. The discovery by Gottlicher et al [11] of a nuclear receptor capable of binding fatty acids established a direct role for PUFAs in gene regulation. PUFAs have been shown to exert their effects on gene transcription very rapidly [12]. Within hours of animals being fed diets rich in

PUFAs, there is coordinated induction of expression of genes involved in hepatic and skeletal muscle fatty acid oxidation, and repression of genes that encode lipogenic, glycolytic, and cholesterolgenic enzymes [12]. This dual action results in a hypolipidemic phenotype [1,2].

Regulation through nuclear receptors and transcription factors

Among other mechanisms, PUFAs have been shown to exert their effects on gene transcription via nuclear receptors such as peroxisome proliferator-activated receptors (PPARs) and liver X receptors (LXRs), and through the transcription factor, sterol regulatory element binding protein (SREBP).

Nuclear receptors are found only in metazoan organisms and consist of two domains: the ligand binding domain and the DNA binding domain.

Binding of a ligand causes the receptor to bind to a nuclear receptor response element on target genes (*Figure 1*) and regulate transcription of the target gene [13].

PPARs are a family of nuclear receptors consisting of three isoforms: PPARα, PPARβ/δ, and PPARγ. PPARα is strongly activated by the fibrate class of drugs used in the management of high plasma cholesterol, whereas PPARγ is a target of the thiazolidinediones used in the clinical management of diabetes and insulin resistance [14]. In general, both n-3 and n-6 PUFAs have been shown to function via PPARα to upregulate transcription of genes involved in β-oxidation, such as carnitine palmitoyl transferase-1 (*CPT-1*), acyl CoA oxidase and *CYP4A2* [1,15].

Another set of nuclear receptors shown to mediate the hypolipidemic effects of PUFAs are the liver X receptors. LXRs α and β bind oxysterols as endogenous ligands and function to regulate genes involved in fatty acid and cholesterol metabolism [16], including *SREBP-1c*, lipoprotein lipase, fatty acid synthase (*FAS*), acetyl CoA carboxylase (*ACC*), and stearyl CoA desaturase-1 (*SCD1*). LXRs also regulate genes involved in bile acid synthesis, such as 7-α hydroxylase [17]. Studies in established cell lines have suggested that PUFAs may inhibit the hyperlipidemic effects of LXRs in a variety of ways [18,19]. However, there is also evidence that, although the administration of PUFA in vivo does decrease the expression of lipogenic genes, this is not accompanied by changes in classical LXRα target genes [20]. Thus further research is needed to clarify whether PUFAs have a role in modulating LXRα activity in vivo [21].

One of the best-characterized modes of regulation of gene expression by PUFAs is through the lipogenic transcription factor, SREBP. SREBP-1c is the predominant SREBP isoform in human and rodent liver, and regulates genes of fatty acid and triglyceride synthesis [22]. PUFAs have been shown to inhibit expression of the *SREBP-1c* gene [23] and proteolytic maturation [24], resulting in decreased transcription of SREBP-1c target genes such as *ACC*, *FAS*, glycerol phosphate acyl transferase, *SCD1*, and *SREBP-1* itself.

Conclusion

Research undertaken over the past few decades has certainly made it clear that fat is more than just an inert storage form of energy. Even in the face of the growing obesity epidemic that brings with it a host of secondary lipid-related conditions, there is growing understanding, not only that is fat an essential nutrient, but also that the type and amount of fat ingested can have dramatic effects on health. At the same time, there is some controversy regarding the use of n-3 PUFAs to improve health. The findings of a recent meta-analysis suggested that n-3 fatty acids may offer no added protection against cardiovascular disease or cancer as previously believed [25]. Rather, the risk of exposure to toxic chemicals such as methylmercury dioxins and polychlorinated biphenyls, which are also concentrated in fatty fish high in n-3 fatty acids, may negate any beneficial effects of n-3 PUFAs [25]. The emergence of such conflicting reports on the possible effects of an essential nutrient makes further research on the topic all the more essential. Understanding the site-specific molecular effects of particular fatty acids will no doubt be key both to establishing valid dietary recommendations and to formulating new approaches to combat growing medical issues. ■

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Featured research

Abstracts and commentaries

Insulin resistance, abnormal energy metabolism and increased ischemic damage in the chronically infarcted rat heart

Murray AJ, Lygate CA, Cole MA, et al. *Cardiovasc Res.* 2006; 71:149–157.

Many patients with heart failure have whole-body insulin resistance and decreased myocardial fluorodeoxyglucose uptake during insulin clamp and decreased glucose transporter activity. Impaired myocardial glucose uptake may contribute to the progression to failure, although the mechanisms for this are not completely understood. Glucose enters heart cells via the facilitative glucose transporters, GLUT 1 and GLUT 4. A large proportion of GLUT 1, the least abundant transporter, resides in the plasma membrane and mediates basal glucose uptake. GLUT 4 resides in intracellular vesicles under basal conditions and translocates to the plasma membrane in response to insulin, ischemia, and exercise. Hence, GLUT 4 translocation represents the major mechanism by which glucose uptake to the cardiomyocyte can be increased. In this study, the authors determined whether there is a link between insulin resistance and ischemic damage in the chronically infarcted rat heart, postulating that the heart would have decreased insulin sensitivity, with lower GLUT 4 glucose transporter protein concentrations as a result of high circulating free fatty acid (FFA) concentrations. A decreased capacity for glucose uptake would decrease glycolytic adenosine triphosphate (ATP) production and thereby increase ischemic injury in the infarcted heart.

Commentary

In-vivo left ventricular ejection fractions, measured using echocardiography, were 40% lower in chronically infarcted rat hearts (10 weeks after coronary artery ligation) than in sham-operated control rats. In response to insulin stimulation, uptake of D-[2-^{3}H]glucose was 42% lower in isolated, perfused, infarcted hearts. Myocardial GLUT 4 glucose transporter protein concentrations were 28% lower in the infarcted hearts. They correlated positively with ejection fractions and negatively with concentrations of plasma FFAs. Compared with controls, chronically infarcted hearts had 46% less total glucose uptake and 3-fold faster ATP hydrolysis, measured using phosphorus-31 nuclear magnetic resonance spectroscopy, during low-flow ischemia (32 min, 0.4 ml/min per gram wet weight). During reperfusion, recovery of left ventricular developed pressure in infarcted hearts was 42% less than that in control hearts. It should be noted that the high circulating concentrations of FFAs in rats with chronically infarcted hearts reflected those found in patients with congestive heart failure; they correlated negatively with cardiac ejection fractions and myocardial GLUT 4 protein concentrations. Decreased myocardial concentrations of GLUT 4 may have contributed to the reduced glucose uptake in response to insulin and during low-flow ischemia. In turn, reduced glucose uptake resulted in lower glycolytic production of ATP and greater loss of ATP during ischemia, and therefore impaired functional recovery during reperfusion. The 28% decrease in GLUT 4 protein may not have been the only reason for the 45% decrease in glucose uptake with insulin stimulation or ischemia. It is possible that there were also changes in expression or activity of other proteins, such as GLUT 1 or proteins of the insulin-signaling pathway, that may have affected glucose uptake in the heart. Further studies might reveal other defects in the ability of the infarcted heart to take up and metabolize glucose.

Danielle Feuvray

Effects of metabolic modulation by trimetazidine on left ventricular function and phosphocreatine/adenosine triphosphate ratio in patients with heart failure

The addition of trimetazidine to standard treatment has been shown to improve left ventricular function in patients with heart failure. The aim of this study was to use in-vivo phosphorus-31 magnetic resonance spectroscopy (^{31}P-MRS) for non invasive assessment of the effects of trimetazidine on left ventricular cardiac phosphocreatine and adenosine triphosphate (PCr : ATP) ratios in patients with heart failure. In a double-blind, crossover study, 12 patients with heart failure were allocated randomly to groups recieving placebo or trimetazidine (20 mg three times a day) for two periods of 90 days. At the end of each period, all patients underwent exercise testing, two-dimensional echocardiography, and MRS. New York Heart Association (NYHA) class, ejection fraction, maximal rate−pressure product, and metabolic equivalent of task units (METS) were evaluated. Relative concentrations of phosphocreatine and ATP were determined by cardiac ^{31}P-MRS. With trimetazidine, NYHA class decreased from 3.04 ± 0.26 to 2.45 ± 0.52 ($P = 0.005$), whereas ejection fraction increased from $34 \pm 10\%$ to $39 \pm 10\%$ ($P = 0.03$) and METS increased from 7.44 ± 1.84 to 8.78 ± 2.72 ($P = 0.03$). The mean cardiac PCr : ATP ratio was 1.35 ± 0.33 with placebo, but was increased by 33%, to 1.80 ± 0.50 ($P = 0.03$), with trimetazidine. It was concluded that trimetazidine improved functional class and left ventricular function in patients with heart failure. These effects are associated with the observed trimetazidine-induced increase in the PCr : ATP ratio, indicating preservation of the myocardial high-energy phosphate concentrations.

Commentary

Evidence that a metabolic approach with trimetazidine improves left ventricular function in patients with heart failure continues to accumulate. This randomized double-blind crossover study comparing trimetazidine 20 mg three times daily with placebo in patients whose heart failure was well-controlled demonstrated a beneficial effect of trimetazidine on functional class, ejection fraction, and exercise time as a result of its metabolic actions. The prognosis for patients with heart failure remains poor, even when all evidence-based medicine is in place (angiotensin converting enzyme inhibitors, β-blockade, antiplatelet or anticoagulant therapy, and diuretics, including spironolactone), so that the possibility of additional benefit, however small, from metabolic manipulation of the ischemic process remains an interesting and potentially exciting option. This paper therefore adds to the scientific need for a prognostic and symptomatic longer-term study of trimetazidine in addition to conventional treatment in patients with heart failure and left ventricular dysfunction. *Heart and Metabolism* issue 27 reviewed the metabolic approach to heart failure and provided a comprehensive review, to which the present issue (32) and this paper now add.

Graham Jackson

AMP-activated protein kinase activates p38 mitogen-activated protein kinase by increasing recruitment of p38 MAPK to TAB1 in the ischemic heart

AMP-activated protein kinase (AMPK) promotes glucose transport, maintains ATP stores, and prevents injury and apoptosis during ischemia. AMPK has several direct molecular targets in the heart, but also may interact with other stress-signaling pathways. This study examined the role of AMPK in the activation of the p38 mitogen-activated protein kinase (MAPK). In isolated heart muscles, the AMPK activator, 5-aminoimidazole-4-carboxy-amide-1-β-D-ribofuranoside (AICAR) increased p38 MAPK activation. In AMPK-deficient mouse hearts, expressing a kinase-dead α_2 catalytic subunit, p38 MAPK activation was markedly reduced during low-flow ischemia (2.3-fold, compared with 7-fold in wild-type hearts; $P < 0.01$) and was similarly reduced during severe no-flow ischemia in kinase-dead hearts ($P < 0.01$ compared with ischemic wild-type). Knockout of the p38 MAPK upstream kinase, MAPK kinase 3 (MKK3), did not affect ischemic activation of either AMPK or p38 MAPK in transgenic mkk3$^{-/-}$ mouse hearts. Ischemia increased the recruitment of p38 MAPK to transforming growth factor-β-activated protein kinase 1 binding protein 1 (TAB1), a scaffold protein that promotes p38 MAPK autophosphorylation. Moreover, TAB1 was associated with the α_2 catalytic subunit of AMPK. Recruitment of p38 MAPK to TAB1−AMPK complexes required the activation of AMPK and was reduced in ischemic AMPK-deficient transgenic mouse hearts. The potential role of p38 MAPK in mediating the downstream action of AMPK to promote glucose transport was also assessed. The p38 MAPK inhibitor, SB203580, partially

inhibited both AICAR- and hypoxia-stimulated uptake of glucose and translocation of glucose transporter 4. Activation of p38 MAPK by anisomycin also increased glucose transport in heart muscles. Thus AMPK has an important role in promoting the activation of p38 MAPK in the ischemic heart by inducing the autophosphorylation of p38 MAPK through interaction with the scaffold protein, TAB1.

Commentary

Myocardial ischemia is complex! A multitude of changes occur within the myocardium that appear to be energy-dependent yet occur when the high-energy phosphate "charge" is low. One such change is the phosphorylation of a mitogen-activated protein kinase known as p38 (p38 MAPK) [1]. This kinase is activated during ischemia by the phosphorylation of two amino acids on a loop within the enzyme that normally prevents substrates from binding. The ATP-dependent dual phosphorylation therefore activates p38 MAPK. The activation of p38 MAPK has a number of consequences – some good; many bad [2].

Although p38 MAPK is known to become dually phosphorylated during ischemia, the underlying mechanisms are poorly understood and rather strange. For example, a compound known as SB203580, which inhibits the kinase activity of p38 MAPK, inhibits the dual phosphorylation step [3]. This and other observations suggest that, in fact p38 MAPK activates itself by autophosphorylating it own activation loop [3]. Superficially, this seems rather odd. How can a kinase normally regulated by dual phosphorylation manage to perform this crucial step on its own, yet still be regulated physiologically? The article by Li et al may seem a little *recherché*, but does address this fundamental question. They show that mouse hearts containing an abnormal form of AMPK do not dual-phosphorylate p38 MAPK in a normal way during ischemia. Moreover, compounds that activate AMPK seem to dual-phosphorylate p38 MAPK. In keeping with the findings of other studies [1,3], the activation of AMPK seemed to promote the association of p38 MAPK with a scaffold protein known as TAB1. This protein scaffold is believed to bind p38 MAPK and cause the change in conformation that allows it to autophosphorylate its own activation loop. Thus AMPK senses the metabolic milieu, activates, and in turn promotes the association between p38 MAPK and TAB1 that causes p38 MAPK autoactivation. What makes the story even more interesting is that this form of p38 MAPK activation, under this circumstance, seems to contribute to some of the changes associated with the activation of AMPK, including a translocation of the proteins of glucose uptake to the cell-surface membrane. These proteins are involved in the enhanced utilization of glucose needed for glycolysis to support myocardial anaerobic metabolism. The ultimate question is whether this is something that should be promoted, or inhibited, during ischemia? Unfortunately, this is a difficult question to answer; glycolysis without matched glucose oxidation could aggravate the accumulation of lactate and intracellular acidosis. This scenario is believed to lead to cellular accumulation of sodium and calcium, and to increase the probability of mitochondria self-destructing at reperfusion. Thus, although this paper adds credence to the central importance of AMPK in orchestrating responses to metabolic stress, it remains unclear whether all such responses are truly adaptive.

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M.S. Marber

Glossary

Glossary

Gary D. Lopaschuk

Acetyl CoA Carboxylase (ACC)

Acetyl CoA carboxylase (ACC) is a key enzyme involved in both synthesis and metabolism of fatty acids. ACC produces malonyl CoA, which is both a substrate for fatty acid biosyntheis, and is a potent inhibitor of mitochondrial fatty acid uptake. In lipogenic tissues like the liver, ACC is the rate-limiting enzyme for fatty acid biosynthesis. In muscle, ACC is a key regulator of fatty acid oxidation, secondary to the production of malonyl CoA.

Allosteric regulation of enzymes

Allosteric regulation of an enzyme involves the change in the shape and activity of an enzyme that results from molecular binding with a regulatory substance at a site other than the enzymatically active one. An example of this is binding of 5-adenosine monophosphate (AMP) to a regulatory site on AMP-activated protein kinase that results in an activation of the enzyme.

AMP-activated protein kinase (AMPK)

AMPK is a key kinase that controls many cellular processes, particularly pathways involved in cellular energy status. AMPK is activated during metabolic stress, where it then can either activate energy producing metabolic pathways or inhibit energy consuming pathways. For these reasons it has been termed a ''fuel gauge'' of the cell.

Arg302Gln amino acid substitution in the gamma-2 regulatory subunit (PRKAG2) of AMPK

PRKAG2 is the gene that encodes the gamma2 subunit of AMP-activated protein kinase (AMPK). Mutations in PRKAG2 have recently been shown to cause cardiac hypertrophy, cardiac glycogen accumulation, Wolff-Parkinson White syndrome and conduction system disease causing pre-excitation. One of these mutations involves the substitution of arginine for glutamine at the 302 amino acid position of the gamma2 subunit of AMPK. It is thought that this mutation decreases the activity of AMPK in the heart.

Eicosapentanoic acid and docosahexanoic acid

Eicosapentanoic acid (EPA) and docosahexanoic acid (DHA) are polyunsaturated fatty acids that are found in abundance in fish oils. EPA and DHA are thought to be effective in treating a number of disorders, many involving inflammation. EPA and DHA can also reduce the level of blood triglycerides in humans, which may reduce the risk of heart disease. Dietary consumption of EPA and DHA have been demonstrated to reduced total mortality, cardiovascular mortality, and morbidity.

Fatty acid synthase (FAS)

FAS is an important enzyme in the synthesis of fatty acids, primarily in liver cells. In eukaryotes, synthesis of fatty acids takes place on a large, multifunctional FAS enzyme complex formed from a single polypeptide chain. Malonyl CoA serves as a substrate for the synthesis of fatty acids.

GAMT knock out mice

Creatine is an important molecule involved in energy storage and in transmission of phosphate-bound energy substrates. One of the creatine synthetic enzymes is S-adenosyl-L-methionine:N-guanidinoacetate methyltransferase (GAMT). GAMT knockout mice lack GAMT, and therefore have a defect in creatine synthesis. They are therefore useful in looking at the role of creatine deficiency in energy homeostasis.

GLUT1

GLUT 1 is a protein that transports glucose across cell membranes. GLUT 1 primarily reside in the plasma membrane, and unlike GLUT 4 it is not responsive to insulin.

Glossary

Gary D. Lopaschuk

Lipoprotein lipase

Lipoprotein lipase is an enzyme that cleaves fatty acids from triacylglycerol contained with lipoproteins

Liver X receptors (LXRs)

Liver X receptors (LXRs) alpha and beta are responsible for the transcriptional regulation of a number of genes involved in cholesterol efflux from cells. LXRs limit cholesterol accumulation by regulating expression of genes involved in cholesterol efflux and storage. As a result, pharmacological activation of LXRs may be a molecular target for the treatment of cardiovascular disease.

Oxysterols

Oxyterols are oxygenated derivatives of cholesterol. They have diverse biological activities, including binding to SREBP-1c and LXRs.

Peroxisome proliferator-activated receptor a (PPARa)

Peroxisomal proliferators-activated receptors are nuclear receptors involved in the transcriptional regulation of proteins. One of these nuclear receptors is peroxisomal proliferatore-activate receptor a (PPARα). PPARα has many functions, including regulating enzymes involved in the control of fatty acid oxidation in muscle.

Prostaglandins, thromboxanes and leukotrienes

Prostaglandins, thromboxanes and leukotrienes are derivatives of arachidonic acid, and belong to a class of biologically active lipids called eicosanoids. They have diverse effects in the body, including vasodilation, vasconstriction, clot formation and mediation of inflammation.

SREBP-1c

Sterol regulatory element-binding protein (SREBP)-1c is a key regulator of fatty acid metabolism and plays a pivotal role in the transcriptional regulation of different lipogenic genes mediating lipid synthesis. Emerging evidence suggests that insulin resistance and its associated metabolic dyslipidemia result from perturbations in the expression of SREBP-1c, inducing lipogensis and production of VLDL particles.

Stearoyl-CoA desaturase-1 (SCD1)

Stearoyl-CoA desaturase (SCD)1 catalyzes the rate-limiting reaction of monounsaturated fatty acid synthesis and plays an important role in the development of obesity. SCD1 is suppressed by leptin but induced by insulin. SCD-1 expression is also under the control of SREBP-1c. Studies have shown that inhibition of SCD1 can prevent the development of high-fat diet-induced obesity and hepatic steatosis.