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Energy Metabolism in the Normal and Failing Heart: Potential for Therapeutic Interventions

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Abstract. **The chronically failing heart has been shown to be metabolically abnormal, in both animal models and in patients. Little data are available on the rate of myocardial glucose, lactate and fatty acid metabolism and oxidation in heart failure patients, thus at present, it is not possible to draw definitive conclusions about cardiac substrate preference in the various stages and manifestations of the disease. Normal cardiac function is dependent on a constant resynthesis of ATP by oxidative phosphorylation in the mitochondria. The healthy heart gets 60–90% of its energy for oxidative phosphorylation from fatty acid oxidation, with the balance from lactate and glucose. There is some indication that compensated NYHA Class III heart failure patients have a significantly greater rate of lipid oxidation, and decreased glucose uptake and carbohydrate oxidation compared to healthy age-matched individuals, and that therapies that acutely switch the substrate of the heart away from fatty acids result in improvement in left ventricular function. Clinical studies using long-term therapy with beta-adrenergic receptor antagonists show improved left ventricular function that corresponds with a switch away from fatty acid oxidation towards more carbohydrate oxidation by the heart. These findings suggest that chronic manipulation of myocardial substrate oxidation toward greater carbohydrate oxidation and less fatty acid oxidation may improve ventricular performance and slow the progression of left ventricular dysfunction in heart failure patients. At present, this intriguing hypothesis requires further evaluation.**

Key Words. **cardiac, heart, glucose, lactate, myocardial metabolism, pyruvate dehydrogenase**

Introduction

Heart failure affects an estimated 4.9 million Americans, and approximately 400,000 new cases are diagnosed each year [1,2]. It is the leading cause of hospitalization in adults older than 65 years, and because of its high prevalence and associated high medical resource consumption, heart failure is now the single most costly cardiovascular disorder in the United States, with estimated annual expenditures in excess of $20.2 billion [1,2]. In contrast to recent declines in age-adjusted mortality rates from coronary heart disease and hypertensive cardiovascular disease, the incidence and prevalence of heart failure are increasing, largely owing to the aging of the population, and are expected to escalate well into the 21st century.

Heart failure is characterized by ventricular dilatation, thinning of the left ventricular wall, reductions in left ventricular function (ejection fractions $<35\%$), increased systemic vascular resistance and activation of compensatory neuro-endocrine systems. Current therapeutic interventions include the use of angiotensin converting enzyme (ACE) inhibitors, diuretic agents, beta-adrenergic blocking agents, and digoxin [2]. These therapies are primarily designed to alter haemodynamics through reductions of cardiac preload and afterload, and through the attenuation of neurohormonal activation [3]. Current pharmacotherapies for heart failure slow the progression of the disease, however the prognosis for optimally treated patients is still poor, and there is an opportunity for novel therapies to further improve symptoms and slow or stop the progression of the disease.

It was recently proposed that disturbances in myocardial substrate utilization have adverse effects in the failing myocardium, and that shifting the energy substrate preference of the heart away from fatty acids towards glucose and lactate may ameliorate many of the hemodynamic and biochemical alterations associated with heart failure [3–5]. The optimization of cardiac energy metabolism, without causing any direct negative hemodynamic or inotropic effects, may prove particularly useful for the treatment of congestive heart failure [3,4]. The use of pharmacological agents, know as "metabolic modulators", that act by improving the efficiency of the transduction of biochemical energy to mechanical contractile work, has been restricted to the treatment of acute myocardial infarction and stable angina [5,6]. These agents could serve as effective

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adjuncts to therapeutic regimens aimed at traditional hemodynamic and neurohormonal targets. Therefore, this review will examine the role of myocardial substrate metabolism in the pathophysiology of heart failure, and will discuss the potential for pharmacological interventions designed to optimize myocardial function in the failing heart.

Overview of Myocardial Substrate Metabolism

Before substrate metabolism in the failing heart can be understood, it is important to have an understanding of metabolism in the healthy heart. The reader is referred to several reviews on the subject [5,7,8]. The regulation of myocardial carbohydrate and fatty acid metabolism is complex in that it is linked to arterial substrate and hormone levels, inotropic state and the nutritional status of the tissue. The chemical energy that fuels cardiac contractile work and relaxation is derived from ATP hydrolysis (Fig. 1). The hydrolysis of ATP provides energy for contractile work (actin-myosin interaction and cell shortening), for pumping Ca^{2+} into the sarcoplasmic reticulum to allow for diastolic relaxation, and for maintaining ion gradients (e.g. Na^{+} and K^{+}). Under normal working conditions approximately 2/3 of the ATP hydrolyzed by the heart is used to fuel contractile work, with the remaining 1/3 used for ion pumps [9]. The primary ion pump that consumes ATP is the sarcoplasmic reticulum Ca^{2+} ATPase.

In the healthy heart, the rate of ATP hydrolysis is exquisitely matched to the rate of ATP resynthesis, and the tissue content of ATP is extremely constant, even with large increases in the rate of ATP turnover. The primary means for ATP resynthesis is by oxidative phosphorylation in the mitochondria (>98%) (Fig. 1). Under normal aerobic conditions only a small amount of ATP comes from glycolysis (< 2%). Oxidative phosphorylation is fueled by the reducing equivalents NADH and $FADH_2$, which are generated by beta-oxidation of fatty acids, the oxidation of pyruvate, and the Krebs cycle (Fig. 2). The mechanical power generation of the myocardium sets the rates of ATP breakdown and oxidative phosphorylation, and the rate of electron transfer from carbon fuels to the electron transport chain via NADH and $FADH_2$. The Krebs cycle is fueled by acetyl-CoA formed from decarboxylation of pyruvate and from beta-oxidation of fatty acids (Fig. 3).

In the healthy human heart in the postabsorptive state about 60–90% of the ATP generation in the mitochondria comes from beta-oxidation of fatty acids, and 10–40% comes from pyruvate (formed from lactate and glycolysis) (Fig. 1). Fatty acids are more reduced molecules, and thus they generate more ATP per gram of substrate than lactate or glucose (Table 1). On the other hand fatty acid oxidation generates more $FADH_2$ relative to NADH than does glucose or lactate. The theoretical ATP yield via the

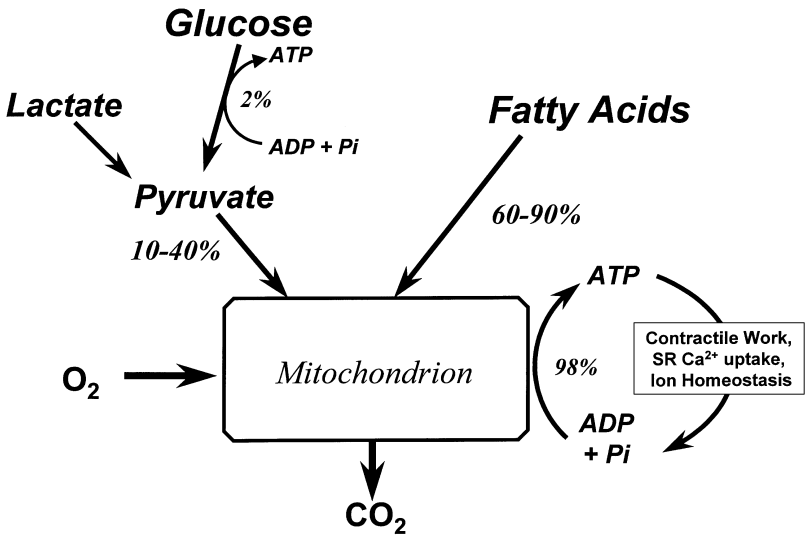

Fig. 1. *Schematic overview of myocardial energy substrate metabolism. In the healthy human heart, about 60–90% of the ATP generation in the mitochondria comes from beta-oxidation of fatty acids, and 10–40% comes from pyruvate (formed from lactate and glycolysis). The primary means for ATP resynthesis is by oxidative phosphorylation in the mitochondria (>98%). Under normal aerobic conditions only a small amount of ATP comes from glycolysis (< 2%).*

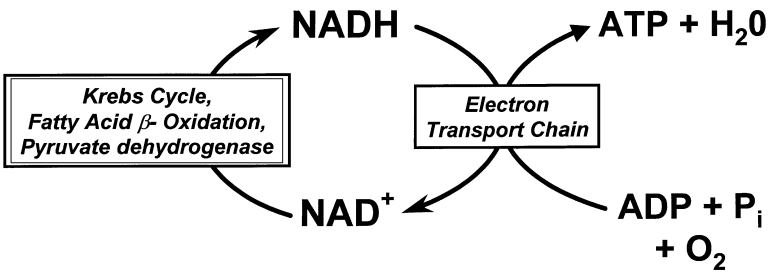

Fig. 2. *ATP production occurs primarily through oxidative phosphorylation which is fueled by the reducing equivalents NADH and $FADH_2$. These reducing equivalents are generated through Krebs cycle, fatty acid beta-oxidation and pyruvate dehydrogenase and are then delivered to the electron transport chain, driving oxidative phosphorylation.*

electron transport chain is 2 for $FADH_2$ and 3 for NADH, which translates into a higher ratio of ATP synthesis to oxygen consumption ratio for glucose and lactate than for fatty acids (Table 1). This translates into an 11% higher molar ratio of ATP to oxygen consumed for glucose and lactate than for fatty acids, thus oxidation of glucose and lactate is slightly more "oxygen efficient" that fatty acids. It is important to note that the amount of ATP synthesized per mole of oxygen consumed is also dependent on the coupling of proton influx into the mitochondrial matrix with oxidative phosphorylation. If the inner mitochondrial membrane is leaky to protons then the ratio of ATP synthesis to oxygen consumption is less than the theoretical values given in Table 1.

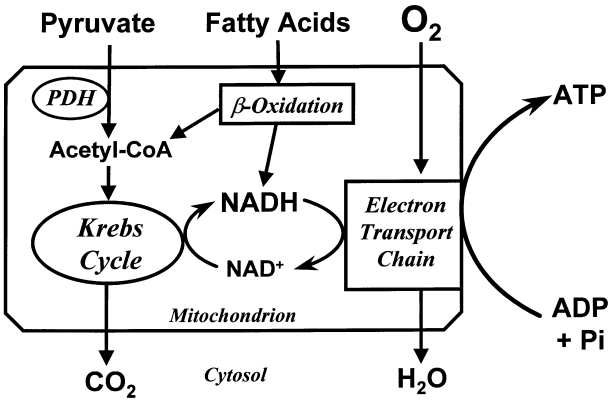

Fig. 3. *Inter-regulation of fatty acid and pyruvate metabolism in the mitochondrion. The Krebs cycle is fueled by acetyl CoA formed from the decarboxylation of pyruvate and from beta oxidation of fatty acids. The reducing equivalents generated through Krebs cycle and beta oxidation fuel the electron transport chain and the production of ATP.*

Fat Metabolism

The heart readily extracts free fatty acids from the plasma and either oxidizes them rapidly, or converts them to triglyceride stores [10]. Studies in humans show that 80% of the free fatty acids taken up by the heart is oxidized rapidly to CO_2 in the mitochondria, with the remaining 20% presumably converted to triglyceride stores [11]. The rate of uptake of free fatty acids is mainly dependent upon the concentration of free fatty acids in the plasma, and the content of a specific fatty acid transport protein in the sarcolemmal membrane [12]. Fatty acids are highly insoluble in aqueous solution, and are bound to albumin in the plasma, and small fatty acid binding proteins in the cytosol of the cardiomyocyte [12]. Free fatty acids are esterified by long-chain fatty acyl-CoA synthetase with coenzyme A to form long-chain fatty acyl-CoA, which is soluble. Long-chain fatty acyl-CoA passes through the β-oxidation pathway system in the mitochondrial matrix, to produce acetyl-CoA. The inner mito-

Table 1. *The theoretical ATP yield and oxygen consumption required for complete oxidation of glucose, lactate, palmitate and oleate. These values assume complete coupling of ATP synthesis to oxygen consumptions in the mitochondria*

Substrate	ATP Yield (moles ATP per mole substrate)	ATP Yield (moles ATP per g substrate)	Oxygen Consumed (moles of O_2 per mole substrate)	ATP/Oxygen (moles ATP per mole atomic O)
Glucose	38	0.2	6	3.17
Lactate	18	0.2	3	3.00
Palmitate	129	0.50	23	2.80
Oleate	146	0.52	25.5	2.86

chondrial membrane, however, is not permeable to long-chain fatty acyl-CoA, and the long-chain fatty acyl moiety must be converted to long-chain fatty acylcarnitine to be transferred across the mitochondrial membrane. The transfer of long-chain fatty acyl moieties into the mitochondrial matrix is catalyzed by three carnitine dependent enzymes. First, carnitine palmitoyltransferase I (CPT-I) catalyzes the formation of long chain acyl-CoA in the compartment between the inner and outer mitochondrial membranes. Second carnitine acylcarnitine translocase, transports long-chain acylcarnitine across the inner mitochondrial membrane, and last CPT-II regenerates long-chain acyl CoA in the mitochondrial matrix. CPT-I serves the key regulatory role in the transmitochondrial membrane transport of long-chain fatty acyl-CoA [10]. The activity of CPT-I is inhibited by malonyl-CoA, which is formed from carboxylation of acetyl-CoA by acetyl-CoA carboxylase (ACC) [10,13–16]. Malonyl-CoA binds to CPT-I and exerts its inhibitory effects on the cytosolic side of the enzyme. There are two isoforms of CPT-I (liver and muscle); both are expressed in the heart; with the muscle isoform being more predominant. The muscle isoform is 30-fold more sensitive to malonyl-CoA inhibition than is the liver isoform [15].

Fatty acid beta-oxidation takes place in the mitochondrial matrix, and produces an acetyl-CoA, one NADH and one $FADH_2$ with each successive turn of the spiral. The cleaving of each two carbon acetyl-CoA occurs in a four step process that is catalyzed by four classes of enzymes: acyl-CoA dehydrogenase, enoyl-CoA hydratase, β-OH-acyl CoA dehydrogenase, and keto acylthiolase. There are separate isoforms of each of these enyzmes for long, medium, and short-chain fatty intermediates. Once long-chain acyl-CoA enters the beta-oxidation pathway it is oxidized entirely to acetyl-CoA by rapid repeated spins through the spiral. Acetyl-CoA is oxidized to CO_2 in the Krebs cycle, resulting in further generation of NADH and $FADH_2$. The NADH and $FADH_2$ formed from beta-oxidation and Krebs cycle delivers reducing equivalents to the electron transport chain, driving oxidative phosphorylation (Fig. 2).

Several lines of evidence suggest that the contractile performance of the heart at a given rate of oxygen consumption is worse when the heart is oxidizing more fatty acids in lieu of glucose and lactate. On a theoretical basis, fatty acid oxidation requires a greater rate of oxygen consumption for a given rate of ATP synthesis than does carbohydrate oxidation (Table 1). The actual values in vivo may be much lower due to the leakage of protons across the inner mitochondrial membrane [17]. It was demonstrated in the 1950s and 60s that fatty acids uncouple oxidative phosphorylation [18] and cause a wasting of ATP by mitochondria [19], which would further decrease the amount of ATP produced at a given rate of oxidative phosphorylation when fatty acids are the substrate. Studies in the isolated rat heart have demonstrated that the mechanical work of the left ventricle is less at a given rate of oxygen consumption when fatty acids rather than glucose are the sole substrate [20]. Classic studies by Mjøs in closed-chest dogs demonstrate that increasing the rate of fatty acid uptake by the heart with an infusion of heparin and triglyceride emulsion to raise arterial fatty acid concentration to 3 mM resulted in a 26% increase in myocardial oxygen consumption without changing the mechanical power of the left ventricle [21]. A similar decrease in cardiac mechanical efficiency with elevated plasma free fatty acid concentration was observed during moderate severity ischemia in dogs [22] and pigs [23], and during post-ischemic reperfusion in pigs [24] and isolated rat hearts [25]. Furthermore, inhibition of fatty acid beta-oxidation by 4-bromocrotonic acid results in a decrease in the rate of oxygen consumption and an increase in mechanical efficiency of the left ventricle of the working rat heart [26]. This principle has been applied to the treatment of stable angina pectoris with the introduction of agents (e.g. trimetazidine and ranolazine) that directly suppress beta-oxidation of fatty acids, stimulate flux through PDH, and improve the contractile efficiency of the heart [5,6,27]. These agents appear to successfully reduce the anginal symptoms in coronary artery disease patients without any direct hemodynamic effects [5,6].

Carbohydrate Metabolism

The healthy heart consumes glucose and lactate, which are converted to pyruvate in the cytosol and subsequently oxidize to CO_2 in the mitochondria. The uptake of extracellular glucose is regulated by the transmembrane glucose gradient and the concentration and activity of glucose transporters in the plasma membrane. Two isoforms from the glucose transporter family have been identified in the myocardium, GLUT 1 and GLUT 4, with GLUT 4 being predominant in the heart. Both transporters are located in the sarcolemmal membrane and in intracellular microsomal vesicles. The capacity of the cell to take up glucose is dependent upon the fraction of the glucose transporters that reside in the plasma membrane. Insulin results in translocation of GLUT 1 and GLUT 4 from the intracellular site into the plasma membrane, which results in an increase in the membrane capacitance for glucose trans-

port [28,29]. A similar translocation occurs when the heart is subjected to ischemia, and the effects of insulin and ischemia are additive [29]. The rate of glucose uptake by the heart is also very dependent upon the interstitial glucose concentration and the transmembrane glucose gradient, and thus the driving force for glucose transport across the plasma membrane [5]. Upon entering the cell, free glucose is rapidly phosphorylated by hexokinase to form glucose 6-phosphate, thus rendering the carbon skeleton of glucose impermeable to the cell membrane. From here, the glucose unit can either be converted to glycogen for storage, or enter the glycolytic pathway.

The overall rates of glucose uptake, glycogen synthesis and breakdown, and the rate of glycolysis are controlled by multiple steps distributed along these pathways, and not subject to control at discrete points [30]. Given a constant supply of glucose 6-phosphate, the primary regulators of glycolytic rate are the activity of phosphofructokinase and the ability to form reduced NADH [7,30–33]. The activity of phosphofructokinase is inhibited by H^+, citrate and ATP, and stimulated by ADP, Ca^{2+}, and fructose 2,6-diphosphate. NAD^+ is reduced to NADH by the conversion of glyceraldehyde 3-phosphate to 1,3-diphosphoglycerate by the enzyme glyceraldehyde 3-phosphate dehydrogenase. Cytosolic NADH is shuttled into the mitochondria via the malate-asparate shuttle, and oxidized to NAD^+ by NADH reductase on the electron transport chain. Under conditions of very high mitochondrial NADH/NAD^+ ratio, or when the electron transport chain flux is reduced (as during ischemia), the cytosolic NADH is converted back to NAD^+ by the reduction of pyruvate to lactate by lactate dehydrogenase. The glyceraldehyde 3-phosphate dehydrogenase reaction appears to be the major rate-controlling step for glycolysis with high rates of contractile work [33], or during myocardial ischemia [34].

In the healthy heart under resting conditions lactate uptake from the blood is a major source of pyruvate formation, supplying approximately 50% of the pyruvate oxidized by the heart *in vivo* [35,36]. Lactate is taken up and rapidly oxidized by lactate dehydrogenase, decarboxylated by pyruvate dehydrogenase (PDH) and oxidized to CO_2 in the Krebs cycle. Lactate is transported across the sarcolemmal membrane and is mediated by at least one inhibitable, stereoselective transport protein. The primary isoform of the lactate transporter in the heart is the monocarboxylate transporter-1 [37]. During ischemia, when intracellular lactate concentrations are high, the rate of lactate efflux is limited by the capacity of the carrier [38]. The main determinant of lactate uptake by the healthy human heart is the arterial lactate concentration, as seen in the strong positive correlation between arterial lactate concentration and the arterial-coronary sinus lactate difference [39]. During physical exercise, when arterial lactate levels rise due to lactate production by exercising skeletal muscle, lactate can become the predominant fuel for the heart [9,36].

Pyruvate decarboxylation is catalyzed by PDH, and is the key irreversible step in carbohydrate oxidation [40]. PDH is located in the mitochondrial matrix, and is regulated by inactivation by phosphorylation of the enzyme complex by a specific PDH kinase (PDHK), and is activated by dephosphorylation by PDH phosphatase [41–44] (Fig. 4). There are four isoforms of PDHK. Type IV PDHK is the predominate form in heart, and is rapidly inducible by starvation and diabetes [45,46]. The rate of pyruvate oxidation is dependent on the degree of phosphorylation of PDH, as well as on the concentrations of its substrates and products in the mitochondria as these control the rate of flux through the active dephosphorylated form of the enzyme [40,47]. The activity of PDH phosphatase is increased by Ca^{2+} and Mg^{2+} [48], while PDH kinase is inhibited by pyruvate and ADP, and activated by increases in the ratios of acetyl-CoA/CoA and NADH/NAD^+ [40,44] (Fig. 4). Pyruvate oxidation and the activity of PDH in the heart are decreased by elevated rates of fatty acid oxidation caused by increased plasma levels of free fatty acids, and are enhanced by suppression of free fatty acid oxidation induced by a decrease in plasma free fatty acid levels [40], or by inhibition of CPT-I [10,48–50].

Mitochondrial Fuel Selection

Acetyl-CoA is the confluence of fat and carbohydrate metabolism. High rates of fatty acid β-oxidation result in an increase in content of NADH and acetyl-CoA and the NADH/NAD^+ and acetyl-CoA/CoA ratios in the mitochondrial matrix. Thus at any given activity of PDH the rate of flux through the enzyme falls as the ratios of NADH/NAD^+ and acetyl-CoA/CoA rise, resulting in less glucose and lactate oxidation (Fig. 4). Elevated mitochondrial levels of acetyl-CoA and NADH also activate PDH kinase, which phosphorylates and inhibits PDH [10,40] (Fig. 4). Pharmacological inhibition of CPT-I (with etomoxir or oxfenicine) inhibits fatty-acyl CoA transport into the mitochondria and thus its subsequent oxidation, and results in greater glucose and lactate oxidation, presumably by lowering acetyl-CoA and/or NADH levels in the mitochondrial matrix and relieving the inhibition of PDH [10,48,49].

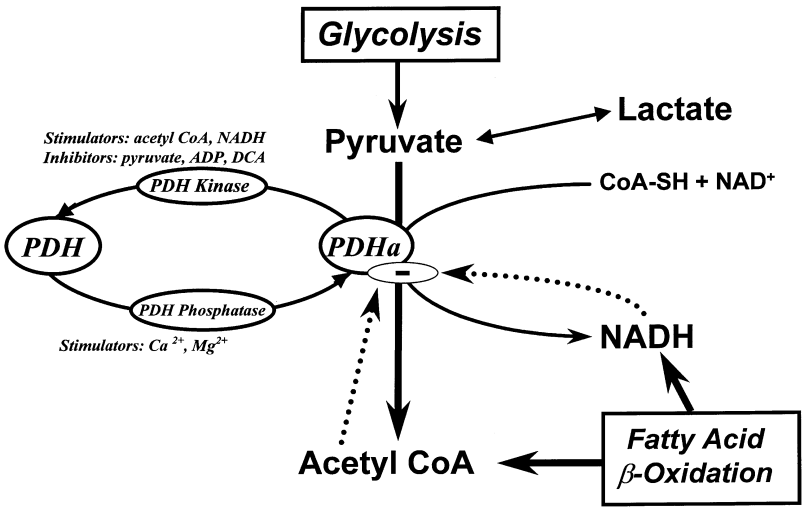

Fig. 4. Regulation of pyruvate dehydrogenase (PDH) activity. PDH is located in the mitochondrial matrix, and is regulated or inactivated by phosphorylation of the enzyme complex by a specific PDH kinase (PDHK). Alternatively, the enzyme complex is activated (PDHa) by dephosphorylation by PDH phosphatase. Elevated mitochondrial levels of acetyl-CoA and NADH also activates PDH kinase, which phosphorylates and inhibits PDH (product inhibition is illustrated by the dotted arrows).

Reciprocal regulation of myocardial pyruvate and free fatty acid metabolism are linked through changes in cytosolic malonyl-CoA concentration. Malonyl-CoA is a potent physiological inhibitor of CPT-I [10,13–16], and is produced by the carboxylation of acetyl-CoA by acetyl-CoA carboxylase [51]. Malonyl-CoA is degraded back to acetyl-CoA by malonyl-CoA decarboxylase [52,53]. Treatment of isolated rat hearts or intact swine hearts with dichloroacetate, an inhibitor of PDH kinase, increases the levels of active PDH [54], acetyl-CoA and malonyl-CoA, causing a decrease in fatty acid oxidation [55,56]. Mitochondrial acetyl-CoA can be transferred to the cytosol by the formation of acetylcarnitine via carnitine acetyl transferase (CAT), transported into the cytosol by carnitine acetylcarnitine translocase [57], and converted back to acetyl-CoA by cytosolic CAT. Consequently, an increase in intramitochondrial acetyl-CoA production due to DCA stimulation of PDH increases cytosolic acetyl-CoA concentration and provides more substrate to ACC; this results in an increase in malonyl-CoA production and a subsequent inhibition of CPT-I activity and fatty acid oxidation [55,56]. Thus activation of carbohydrate oxidation at the level of PDH results in inhibition of CPT-I via elevated malonyl-CoA concentrations.

Metabolism during Myocardial Ischemia and Reperfusion

Insights relevant to the biochemical mechanisms responsible for the abnormal metabolism in the failing heart can be gained from recent studies examining substrate metabolism during and following myocardial ischemia. Myocardial energy metabolism during ischemia is very dependent upon the duration and severity of ischaemia [7,24,58]. Complete elimination of flow results in a rapid depletion of ATP and creatine phosphate, glycogen depletion, lactate accumulation, and contractile akinesis, which over time evolves into tissue necrosis and myocardial infarction. On the other hand, a more modest reduction in flow (40–60%) causes a decrease in myocardial oxygen consumption ($\sim$l0–50%), a transient increased dependence on anaerobic glycolysis (glycogen depletion and lactate production), oxidation of free fatty acids at a reduced rate, and modest to more severe contractile dysfunction. These moderate reductions in blood flow do not immediately lead to irreversible tissue damage. Despite contractile dysfunction and transient lactate production during moderate ischemia, the primary oxidative fuel is fatty acids [23,59]. Studies in pigs with a 60% reduction in regional coronary flow have shown that oxidation of exogenous ^{14}C-labeled fatty acids supply most of the energy for ATP synthesis during ischaemia even under conditions of severe contractile dysfunction, reduced myocardial oxygen consumption, and net lactate production [23,60,61]. Similar results were more recently obtained using ^{13}C-glucose and NMR analysis in dogs subjected to a 30% reduction in coronary blood flow [59]. Thus non-oxidative glycolysis and net lactate

production occur during moderate ischaemia even though the majority of the acetyl CoA is derived from fatty acid beta-oxidation.

The post-ischemic heart has increased fatty acid oxidation and decreased flux through PDH. Reperfusion following myocardial ischemia results in a return to normal rates of myocardial oxygen consumption and mitochondrial respiratory function, and a gradual decline in myocardial lactate levels. Contractile power output is impaired, thus there is a significant decrease in the mechanical efficiency of the left ventricle (mechanical power output/myocardial oxygen consumption) in the reperfused segment of the left ventricle (i.e. "stunning"). Studies in isolated rat hearts or intact swine or dogs using ^{14}C-labeled substrates with $^{14}CO_2$ measurements [62–64], or ^{13}C-labeled substrates and isotopomer analysis of Krebs cycle intermediates [65,66], demonstrate that there is an overshoot in the rate of fatty acid oxidation after ischemia. Increasing plasma free fatty acid concentration during ischemia/reperfusion impairs contractile function in swine hearts [24] and isolated rat hearts [10,62]. Thus there is impaired pyruvate oxidation during reperfusion that appears to result in sub-optimal oxidation of lactate that accumulated during ischemia.

Pharmacological interventions that activate PDH or suppress fatty acid oxidation (etomoxir or ranolazine) result in improved functional recovery from ischemia [5]. Stimulation of pyruvate oxidation during reperfusion with the PDH kinase inhibitor DCA results in improved contractile recovery and an increase in the mechanical efficiency of the left ventricle [5, 62,66]. Improved post-ischemic function has also been observed with inhibition of CPT-I [48,49], or with partial inhibition of fatty acid beta-oxidation with the anti-anginal drug ranolazine [5,6].

The reason for impaired pyruvate oxidation during post-ischemic reperfusion appears to be at least partially due to low tissue malonyl-CoA levels, resulting in reduced inhibition of CPT-I and thus, an increase in fatty acid oxidation. Kudo et al. [53] recently observed a 40% decrease in malonyl-CoA concentration in isolated hearts subjected to 30 minutes of no-flow ischemia. Malonyl-CoA concentration fell after 30 minutes of reperfusion to only 2% of control values and the rate of fatty acid oxidation was significantly elevated. This 98% decrease in malonyl-CoA levels corresponded to a fall in ACC activity and an increase in AMP protein kinase (AMPK) activity, with no change in MCD activity. We observed a nonsignificant 25% decrease in malonyl-CoA with a 60% reduction in coronary blood flow in open-chest swine [56]. These results suggest that reduced malonyl-CoA concentration lessens the inhibition on CPT-I and causes an overshoot in fatty acid oxidation during reperfusion, and thus is responsible for inhibition of PDH activity and carbohydrate oxidation observed under these conditions.

Metabolism in the Failing Heart

Substrate Flux

The only published study of myocardial substrate metabolism in heart failure patients reveal that there is an increase in the rate of fatty acid oxidation and a decrease in carbohydrate oxidation. Paolisso et al. [67] found increased extraction and rate of uptake of plasma free fatty acids, and decreased glucose uptake in congestive heart failure patients ($n = 10$; NYHA Class II and III) compared to age-matched healthy individuals (Fig. 5). In addition, the rate of myocardial lipid oxidation, as estimated from the transmyocardial respiratory quotient, increased by 50% in CHF patients. There was a corresponding 60% decrease in carbohydrate oxidation by the heart in CHF patients compared to healthy controls. It should be noted that these indirect measurements of substrate oxidation do not differentiate between lactate, glucose or glycogen oxidation. CHF patients had increased plasma norepinephrine (5.2 ± 0.2 vs. 1.4 ± 0.3 pmol/mL) that corresponded to increased plasma free fatty acid concentrations (1.0 ± 0.1 vs. 0.66 ± 0.08 mM). The higher free fatty acid levels thus were attributed to greater beta-adrenergic stimulation. However the CHF patients also had significantly higher plasma insulin levels, which would likely stimulate glucose uptake by the heart through direct mechanisms [5]. These confounding effects make it difficult to draw conclusions from this study regarding the effects of CHF on substrate oxidation in the heart. In any case, there was a decrease in carbohydrate oxidation and flux though PDH, and greater fatty acid oxidation.

A recent study by Recchia et al. [68] showed that during the decompensation period of pacing-induced heart failure in dogs there is a switch in myocardial substrate use away from free fatty acids towards glucose, as determined from the respiratory quotient and the arterial-venous difference for substrates. The increase in carbohydrate oxidation was not observed during the compensated period of LV dysfunction, but only when LV end-diastolic pressures rose. These results run counter to the findings of Paolisso et al. in patients with more moderate heart failure [67], but are supported by the work of Sack et al., which found a down-regulation of key enzymes of fatty acid oxidation in patient with severe heart failure undergoing transplantation [69].

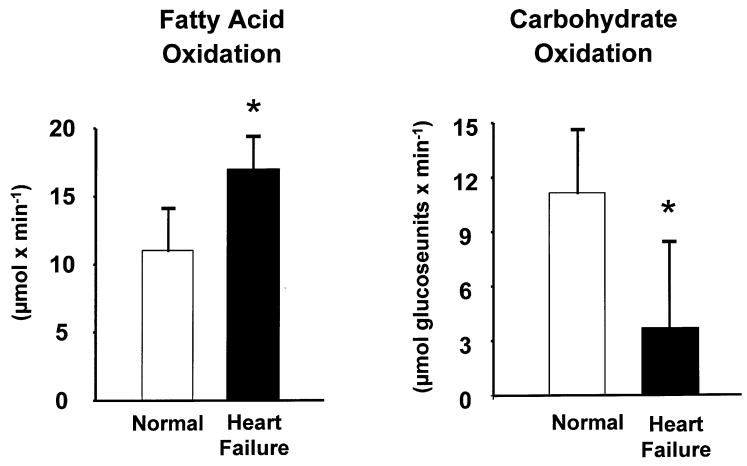

Fig. 5. *Myocardial fatty acid and carbohydrate oxidation in NYHA class III and IV heart failure patients and age-matched controls. Substrate oxidation was estimated from the myocardial oxygen consumption and the respiratory quotient, n = 10 per group. Redrawn from Paolisso et al., Metabolism 43:174, 1994.*

Mitochondrial Metabolism in the Failing Heart

Studies on mitochondrial function in cardiac tissue from the failing heart demonstrated that the capacity of the mitochondria for oxygen consumption and oxidative phosphorylation are significantly reduced compared to the normal heart [70]. Studies in the rat infarct model of CHF have shown that there is a decrease in myocardial ATP (32%) and creatine phosphate (38%) and in mitochondrial state III respiration (39%) with glutamate and malate as substrates [71]. Chronic treatment with ACE inhibitors resulted in significant increases in mitochondrial respiratory capacity and cardiac ATP and CP contents. Studies by Sabbah et al. in dogs with coronary microembolism-induced heart failure show disrupted mitochondria and a lower rate of mitochondrial respiration [72,73]. Most recently, Jarreta et al. showed in both idiopathic dilated cardiomyopathy and heart failure patients with a history of ischaemic coronary disease that the activity of complex III of the electron transport chain is reduced by 35%, without changes in the activities of complexes I, II or IV, or in the mitochondrial marker enzyme citrate synthase [74]. Evidence for a causal link between impaired mitochondrial metabolic function and cardiac failure was found in several cases of inherited cardiomyopathies [75], presenting as classic idiopathic dilated cardiomyopathy. Surprisingly little is known about the molecular aspects of mitochondrial pathology in the failing heart. While point mutations in key mitochondrial enzymes could significantly impair the ability of the mitochondria to generate ATP, Jarreta et al. found only neutral polymorphisms in the mitochondrial cytochrome b gene, suggesting that the complex III defect is not a primary mitochondrial disease [74]. This is further supported by the observation that cardiomyopathy is not a presentation in patients with a congenital complex III defect [76]. The metabolic consequences of a lower capacity for mitochondrial respiration in heart failure remain to be understood.

It is important to note that mitochondria in the heart exist as two populations, interfibrillar and subsarcolemmal, which might respond differently to heart failure. Subsarcolemmal mitochondria cluster beneath the sarcolemmal membrane, while interfibrillar mitochondria reside between the myofibrils [77]. Hoppel and coworkers have found defects selective to one population in ischaemic injury [78], and aging [79]. The cardiomyopathic Syrian hamster, a model of heart failure, had a significant decrease in the capacity for oxidative phosphorylation in the interfibrillar mitochondria but not the sarcolemmal mitochondria [80]. Thus it is important to distinguish between these two populations when assessing mitochondrial function and enzyme activity in the heart.

Studies in two strains of cardiomyopathic Syrian hamsters found a decrease in the activity of PDH that was linked to impaired contractile performance of the left ventricle [81]. Systolic performance of the left ventricle was depressed in isolated glucose-perfused hearts from cardiomyopathic animals, and PDH activity was 16% and 32% of values obtained from normal hearts (Fig. 6). When the hearts were perfused with

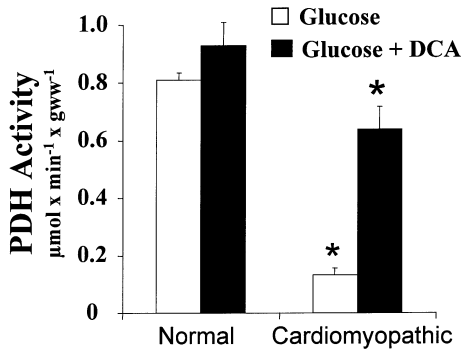

Fig. 6. *The activity of pyruvate dehydrogenase in perfused heart from normal and cardiomyopathic Syrian hamsters. Systolic performance of the left ventricle was depressed in isolated glucose-perfused hearts from cardiomyopathic animals, and PDH activity was 16% of the value obtained from normal hearts. When the hearts were perfused with 5 mM DCA (to inhibit PDH kinase and convert the enzyme to the dephosphorylated active form) there was an marked increase in PDH activity (increased by 392%). (Redrawn from Di Lisa et al., Am J Physiol 264: H2188–2197, 1993.)*

5 mM DCA (to inhibit PDH kinase and convert the enzyme to the dephosphorylated active form) (Fig. 4) there was an increase in PDH activity and a significant increase in systolic function of the heart. Despite the significant increase in PDH activity with DCA, the "maximal" activity of PDH was significantly less in these animals (68 and 62% of values obtained from normal hamsters). These results suggest that in this model of heart failure, there is both less total PDH enzyme present and less activation of the enzyme. The expression of the isozymes of PDH kinase in failing hearts is not known. Furthermore, the rates of substrate flux in the cardiomyopathic Syrian hamster have not been reported.

Severe heart failure results in downregulation of the enzymes of the fatty acid oxidation pathway. Biopsies from NYHA Class IV heart failure patients with severe LV dysfunction and undergoing heart transplantation had a down-regulation in the mRNA for the fatty acid oxidation enzymes long chain acyl-CoA dehydrogenase (LCAD) and medium chain acyl-CoA dehydrogenase (MCAD), and the protein levels of MCAD [69]. It is not known if this down regulation occurs in patients with less severe Class I–III heart failure, or compensated Class IV failure. The same authors studied Spontaneously Hypertensive Heart Failure Rats (SHHF) and observed a down-regulation in MCAD enzyme, protein and enzyme activity, however CPT-I activity was not examined. It is important to note that the SHHF rats have other metabolic and hemodynamic problems including obesity, diabetes, hyperglycemia and hypertension that could influence these metabolic observations.

We recently observed that neither CPT-I nor MCAD activities were reduced in dogs with moderate severity heart failure (ejection fraction = 26%) induced by multiple coronary microembolizations [82]. This leads us to believe that there are not major heart failure-induced changes in the expression of the enzymes of the beta-oxidation pathway during the period of hemodynamic compensation. It is important to note that a large increase in flux through the beta-oxidation pathway in the heart does not require an increase in the expression and maximal activity of these enzymes. We [56,83,84] and others [10,13,53,55] have shown that the rate of fatty acid oxidation in the heart is dependent on the tissue content of malonyl-CoA, which is a potent inhibitor of CPT-I activity, as discussed above. It is not known if heart failure affects the tissue content of malonyl-CoA, or the activity of the enzymes that regulate its production and degradation.

The rate of fatty acid oxidation is largely regulated by the activity of CPT-I. Both the muscle and liver isoforms of CPT-I are expressed in the heart, with the muscle form predominating [15,85,86]. The liver isoform is not as sensitive to malonyl-CoA inhibition as is the muscle isoform [15]. Depre et al. recently showed that cardiac hypertrophy in the rat results in a significant reduction in the mRNA for the muscle isoform, but no change in the mRNA for the liver isoform [85]. A similar response was observed in rat heart subjected to chronic ventricular unloading by heterotopic transplantation of the heart into an isogenic recipient [85]. Assuming that these mRNA levels reflect enzyme protein levels, this shift toward the malonyl-CoA insensitive liver isoform would result in less regulation of CPT-I activity and the rate of fatty acid oxidation. Interestingly, Xia et al. [87] observed that electrical stimulation of neonatal rat cardiac myocytes in culture resulted in a CPT-I isozyme shift away from the liver isoform and toward the muscle isoform. It is not known if the failing heart up-regulates the liver isoform and down-regulates the muscle isoform, resulting in less sensitivity to malonyl-CoA inhibition on CPT-I.

Severity of Heart Failure as a Determinant of Myocardial Energy Metabolism

It is important to note that there may be differential alterations in myocardial metabolism in response to heart failure that are dependent on the particular stage of the disease. Paolisso et al. found increased fatty acid oxidation and

decreased carbohydrate oxidation in Class II and III heart failure patients [67]. Substrate metabolism has not been examined in patients with more severe heart failure, however Recchia et al. [68] showed that during the decompensation period of pacing-induced heart failure in dogs there is a switch in myocardial substrate use away from free fatty acids towards glucose, as determined from the transmyocardial respiratory quotient and the arterial-venous difference for substrates. Sack et al. found that explanted myocardium from heart failure patients undergoing heart transplantation had a down-regulation in the mRNA and activity of several fatty acid oxidation enzymes [69] suggesting that there is a metabolic phenotype switch in severe end stage heart failure. It is also important to note that substrate use has not been assessed in Class IV heart failure patients, or in end-stage decompensated failure. Taken together, it appears that the story of substrate utilization in heart failure patients is incomplete, and further human studies are required to fully describe the metabolic aspects of the natural history of the disease.

Effects of Pharmacologically Altering Myocardial Substrate Metabolism in Heart Failure

Rationale for the Metabolic Approach

Abnormalities in myocardial energy metabolism have been cited as key contributors to contractile dysfunction and the progressive worsening of left ventricle function in the heart failure state [3,4,67,88,89]. Several lines of evidence suggest that the contractile performance of the heart at a given rate of oxygen consumption is impaired when the heart is oxidizing high rates of fatty acids and little glucose and lactate [11,20,22–24,26,62]. The general principle of suppressing fatty acid oxidation and increasing carbohydrate oxidation has been applied to the treatment of stable angina pectoris. The partial fatty acid oxidation inhibitors (PFOX inhibitors) ranolazine and trimetazidine reduce the rate of fatty acid oxidation by the heart, and remove fatty acid induced inhibition of PDH in the mitochondria [5,6,27,90–92]. Increasing the flux through PDH during ischemic conditions results in less lactate production and H^+ accumulation, and higher ATP content [5,40]. These agents significantly reduce exercise-induced anginal symptoms in coronary artery disease patients, without eliciting any of the classic anti-ischemic effect of traditional therapies (e.g. decrease heart rate, coronary vasodilation, decreased arterial blood pressure) [6,93,94]. As discussed above, fatty acid oxidation is slightly less efficient than carbohydrate oxidation in terms of ATP yield per O_2 consumed. In addition, high intracellular concentrations of fatty acids may uncouple oxidative phosphorylation and cause wasting of O_2 consumption by mitochondria [18]. Thus pharmacologically switching the oxidative fuel of the heart away from fatty acids toward carbohydrate (glucose and lactate) may correct the abnormal energy metabolism and improve contractile function in the failing heart. If in the failing heart an overly high rate of fatty acid oxidation or impaired pyruvate oxidation contributes to the pathophysiology of left ventricular dysfunction and progression of the disease, then treatment with agents that switch substrate preference toward carbohydrate oxidation may improve contractile function and clinical outcome.

Acute Effects of Metabolic Agents

Increasing the flux through PDH has been shown to acutely improve left ventricular contractile performance in the failing heart. Bersin et al. [95] treated ten CHF patients (NYHA Class III and IV) with intravenous dichloroacetate to activate PDH and increase pyruvate oxidation. Dichloroacetate was infused at $1.67\,mg\,kg^{-1}\,min^{-1}$ for 30 minutes, and the patients were monitored for 2 hours. Cardiovascular measurements made during the treatment period were compared to values obtained immediately prior to infusing dichloroacetate. The results showed an increase in lactate uptake during dichloroacetate infusion despite a significant fall in arterial lactate concentration. 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%. The rates of glucose or free fatty acid uptake and oxidation were not measured. These results suggest that dichloroacetate increases pyruvate oxidation and mechanical efficiency by switching the heart towards the more efficient fuels. One should use caution in interpreting these results because of the lack of a vehicle-treated control group. In order to establish the mechanism of action of dichloroacetate in improving ventricular function in heart failure patients, it is important to show that dichloroacetate increases the rates of glucose and lactate oxidation and decreases fatty acid oxidation.

We observed a similar response to acute treatment with the partial fatty acid oxidation inhibitor ranolazine [6] in dogs with microembolism-induced heart failure [96]. Ranolazine improved LV systolic function as evidenced by a significant increase in left ventricular ejection fraction (from 27 ± 2 to $35 \pm 2\%$) and stroke volume (20 ± 2 to $27 \pm 1\,mL$), without any effects on heart rate or systemic blood pressure. Ranolazine had no

effects on left ventricular function in normal dogs, suggesting that the drug acts by optimizing metabolism in the chronically failing heart.

Supraphysiological arterial concentrations of pyruvate have been shown to acutely improve left ventricular function in Class III heart failure patients (ejection fraction $<25\%$) [97]. Hermann et al. infused pyruvate directly into the left main coronary artery at 0.9 mmols/min for 15 minutes, followed by 1.8 mmols/min for 15 minutes, and finally 15 minutes of saline infusion. It was estimated that these infusion rates result in coronary arterial pyruvate concentrations of 3 and 6 mM, which is well above the normal arterial concentration of ~ 0.1 mM. Both doses of pyruvate resulted in a significant improvement in stroke volume and cardiac index, which was rapidly reversed by saline infusion (Fig. 7). It is important to note that sodium pyruvate is infused, making it impossible to attain such high arterial pyruvate concentrations with a systemic infusion of pyruvate due to the high sodium load that accompanies infusion of the sodium salt. A similar improvement in mechanical performance with high pyruvate concentrations has been demonstrated in isolated rodent hearts during post-ischemic reperfusion [98]. The beneficial effect of pyruvate may be due to a greater cytosolic ATP phosphorylation potential, as well as a greater rate of pyruvate oxidation and less fatty acid oxidation [98].

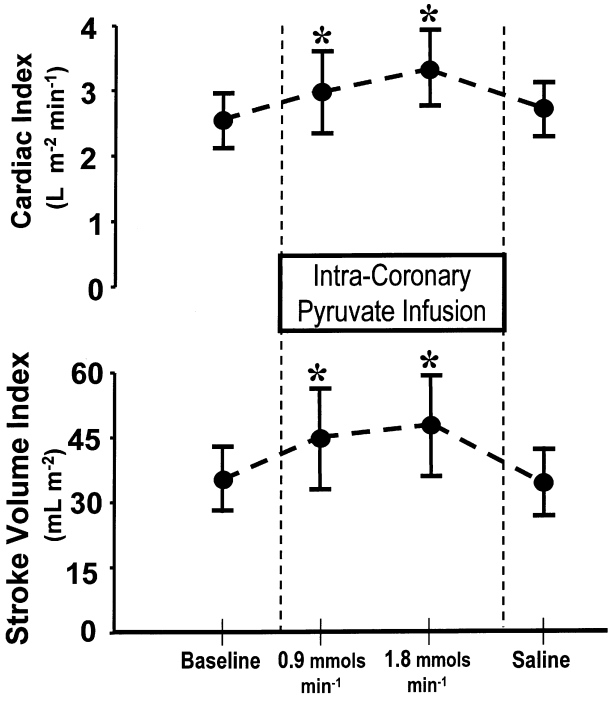

Fig. 7. *The effects of an infusion of pyruvate into the left main coronary artery on cardiac index and stroke volume in NYHA Class III heart failure patients (n = 8). (Redrawn from Hermann et al., Lancet 353:1321–1323, 1999.)*

Effects of Chronic Treatment

There is indirect evidence to suggest that long-term partial inhibition of fatty acid oxidation and stimulation of pyruvate oxidation improves contractile function in heart failure. The cardiomyopathic Syrian hamsters, has a significant increase in left ventricular systolic function when PDH is activated with dichloroacetate. Chronic treatment of cardiomyopathic Syrian hamsters (a heart failure model with severely decreased PDH activity) with the partial fatty acid oxidation inhibitor trimetazidine results in a significant 57% increase is survival time from 364 to 560 days (Fig. 8) [99]. These results suggest that inhibition of fatty acid oxidation can slow the progression of heart failure, and that therapies that partially inhibit fatty acid oxidation and stimulate carbohydrate oxidation in the heart could result in a long-term improvement in clinical outcome.

In general, decreasing the rate of fatty acid oxidation and stimulating carbohydrate (pyruvate) oxidation appears to be beneficial to the heart under conditions of stress. Treatment with the CPT-I inhibitor etomoxir results in inhibition of fatty acid oxidation and activation of PDH in the heart [100], and improves cardiac

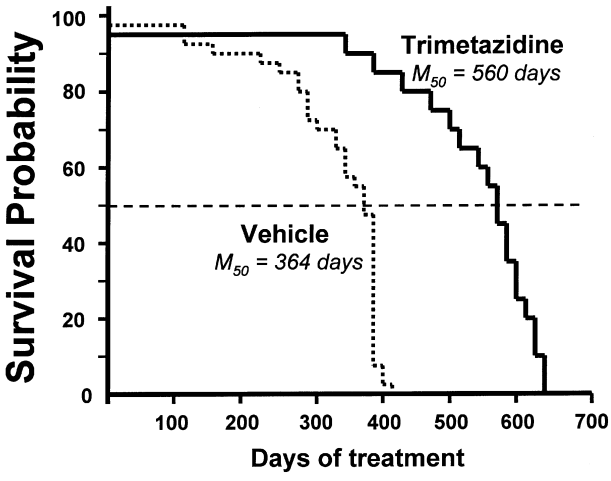

Fig. 8. *Effects of trimetazidine, the partial fatty acid oxidation inhibitor, on survival in cardiomyopathic hamsters. Chronic treatment of cardiomyopathic Syrian hamsters (a heart failure model with severely decreased PDH activity) with trimetazidine results in a significant 57% increase is the average survival time from 364 to 560 days. (From D'hahan et al., Euro J Pharmacol 328:163–174, 1997.)*

contractile function of diabetic rats [101] or of normal rats following myocardial ischemia [5]. Studies by Rupp et al. showed that chronic treatment with etomoxir prevents the deterioration in ventricular function and sarcoplasmic reticulum Ca^{2+} handling, and the shift in myosin isozymes toward the fetal type [99–102] in rats with left ventricular hypertrophy due to aortic banding. The cellular mechanisms for these effects are not understood. A pilot study with etomoxir therapy in Class II–III heart failure patients showed a significant improvement in maximal cardiac output and stroke volume during exercise following three months of oral therapy (80 mg/day), however this study was not blinded and did not include a placebo group of patients [106].

Acute and chronic administration of carnitine stimulated pyruvate oxidation in the heart by increasing the acetylcarnitine concentration and decreasing the acetyl-CoA concentration, and thus relieving acetyl-CoA inhibition on PDH (see Fig. 4) [107]. Oral carnitine therapy significantly slows the expansion of left ventricular end diastolic volume in patients following a myocardial infarction [108]. Interestingly, a recent clinical trial with 80 NYHA Class III and IV heart failure patients found a significant ($p < 0.04$) decrease in mortality with three years of oral carnitine therapy (2 g/day) [109]. A properly powered mortality trial has not been performed. The mechanisms for these beneficial effects of carnitine therapy are not well understood, but could be due to carnitine stimulation of glucose oxidation [5].

Long-term therapy with beta-adrenergic receptor antagonist significantly reduces mortality in heart failure patients, though the biochemical mechanism for this improvement is unclear [110–114]. Improvement in left ventricular function with chronic beta-blockers therapy may be due, in part, to a switch away from fatty acid oxidation toward more carbohydrate oxidation by the heart. Studies with both metoprolol and carvedilol suggest that three months of treatment result in a significant decrease in fatty acid oxidation and greater lactate uptake and carbohydrate oxidation by the myocardium. Andersson et al. demonstrated that 12 weeks of treatment of patients in severe heart failure (mean ejection fraction of 22%) resulted in a significant increase in myocardial lactate uptake (Fig. 9) [115]. Eichhorn et al. showed that CHF patients have improved cardiac function and a decrease in fatty acid oxidation and increased carbohydrate oxidation by the heart (as measured indirectly from the transmyocardial respiratory quotient) following 12 weeks of treatment with metoprolol [116,117]. Similar findings were observed with 12 weeks of carvedilol therapy in NYHA Class III patients, showing a decrease in fatty acid uptake,

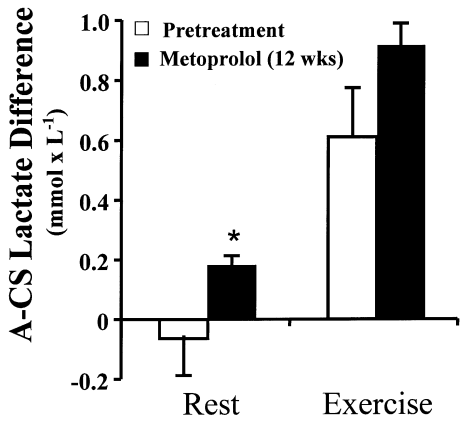

Fig. 9. *Three months of treatment with the beta-adrenergic receptor antagonist metoprolol in patients with severe heart failure (mean ejection fraction of 22%) resulted in a significant increase in the arterial (A)—coronary sinus (CS) lactate difference, suggesting greater carbohydrate oxidation by the myocardium. Improvement in left ventricular function with chronic beta-blockers therapy may be due, in part, to a switch away from fatty acid oxidation towards more carbohydrate oxidation by the heart. (Redrawn from Andersson B et al., J Am Coll Cardiol 1991;18:1059–1066.)*

as assessed using positron emission tomography and [^{18}F]FTHA tracer [118]. Studies using the coronary microembolism-induced heart failure model in dogs showed that 12 weeks with metoprolol therapy improved ventricular function and resulted in a 28% decrease in CPT-I activity [82]. These results suggest that the improved function observed with beta-blockers in heart failure patients may be due, in part, to a decrease in CPT-I activity, and less fatty acid and more carbohydrate oxidation by the heart [3].

Summary

The chronically failing heart has been shown to be metabolically abnormal, in both animal models and in patients. Little data are available on the rate of myocardial glucose, lactate and fatty acid metabolism and oxidation in heart failure patients, thus at present it is not possible to draw definitive conclusions about cardiac substrate preference in the various stages and manifestations of the disease. There is some indication that compensated NYHA Class III heart failure patients have impaired carbohydrate oxidation, and that therapies that partially inhibit fatty acid oxidation and increase carbohydrate oxidation may result in acute and chronic improvement in left ventricular function and slow the progression of the disease. At present, this is an intriguing hypothesis awaiting further evaluation.

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