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Review

Regulation of energy substrate metabolism in the diabetic heart

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1. Introduction

Following a myocardial infarction diabetic patients have almost twice the rate of mortality and 3 times the rate of progression to congestive heart failure when compared to nondiabetic patients [1]. These observations suggest that defects specific to the diabetic myocardium contribute to the greater mortality in diabetic patients [1,2]. Abnormalities in myocardial energy metabolism in the diabetic population are probably an important contributing factor to this greater mortality. Normal cardiac function is dependent on a constant rate of resynthesis of ATP by mitochondrial oxidative phosphorylation and, to a much lesser extent, glycolysis. Oxidation of fatty acids is normally responsible for about 60–90% of the ATP resynthesized [3–7], with the balance coming from the oxidation of pyruvate derived from glycolysis and lactate uptake (Fig. 1). Even in the absence of diabetes myocardial ischemia results in profound derangements in myocardial substrate utilization, particularly impaired pyruvate oxidation and increased lactate production [3–7]. This is particularly evident during reperfusion of ischemic myocardium, where rapid normalization in the rate of oxygen consumption occurs, but pyruvate oxidation is impaired, and rates of fatty acid oxidation are disproportionately high, all of which corresponds with a decrease in contractile work [8–15]. Diabetic patients, even in the absence of ischemia, have decreased myocardial glucose and lactate uptake, and a greater use of free fatty acids and ketone bodies relative to the nondiabetic population. Hearts from animals with experimental diabetes have also been shown to have impaired pyruvate oxidation under normal and post-ischemic conditions [16,17]. The purpose of this review is to examine the abnormalities in substrate metabolism in the diabetic heart, under well-perfused conditions and also during myocardial ischemia and reperfusion.

2. Effects on glucose transport and glycolysis

Myocardial glucose transport is impaired in the diabetic heart [18–21]. With the discovery of the glucose transporter [GLUT) family in the late 1980s [22,23] it was demonstrated that this impairment was at least partially due to a decrease in the myocardial concentration of GLUT 1 and GLUT 4 protein and mRNA levels [24–28]. Myocardial GLUT 1 and GLUT 4 protein levels in diabetic rats can be normalized by insulin treatment [24,25], and partially restored by exercise training [26].

The rate of glycolysis is also significantly reduced in streptozotocin-diabetic isolated working rat hearts [17,29]. This decreased rate of glycolysis is associated with an intracellular accumulation of glucose 6-phosphate, and a decrease in the fructose 1,6-bisphosphate/fructose 6-phosphate ratio [21,30]. Phosphofructokinase (PFK) catalyzes the conversion of fructose 6-phosphate to fructose 1,6-bisphosphate and is inhibited by high levels of citrate, a high ATP/ADP ratio, and increases in the NADH/NAD+ ratio. Increased fatty acid oxidation rates are largely responsible for the increase in citrate concentrations that

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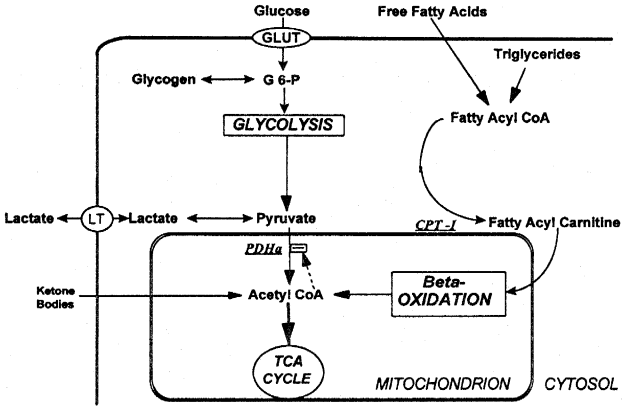

Fig. 1. Schematic depiction of the pathways of myocardial substrate metabolism. GLUT, GLUT 1 and GLUT 4 = glucose transporter isoforms; LT = lactate transporter; G 6-P = glucose 6-phosphate; CPT-I = carnitine palmitoyltransferase I; PDHa = active dephosphorylated pyruvate dehydrogenase; TCA cycle = Krebs tricarboxylic acid cycle.

contribute to the decreased activity of PFK. Myocardial citrate concentration is increased in diabetic animals [30], which may inhibit PFK and lead to increased levels of fructose 6-phosphate and glucose 6-phosphate. It is unclear, however, if citrate regulates PFK activity in vivo due to the apparent low activity of the tricarboxylate carrier [31] in heart mitochondria.

Studies in isolated rat hearts suggest that the diabetes-induced impairment in glycolysis may be advantageous during and after no-flow ischemia, when the heart has a high rate of anaerobic glycolysis, resulting in the formation of lactate [17]. The ensuing lactic acidosis is detrimental to postischemic recovery and myocyte survival. Thus despite impaired pyruvate oxidation, the lower rate of anaerobic glycolysis during and after ischemia may be beneficial. In contrast, during less severe ischemia, these low glycolytic rates may not be beneficial [32].

3. Role of circulating substrate and insulin levels

Insulin-dependent diabetes results in hypoinsulinemia, hyperglycemia, elevated plasma ketone bodies (β-hydroxybutyrate and acetoacetate) and elevated free fatty acid levels. These alterations in substrate levels in the blood result in dramatic changes in myocardial metabolism in nondiabetic myocardium [3–7], and thus make it inherently difficult to study the metabolic effects of diabetes *per se*, especially in vivo [27]. In general, when comparing nondiabetic hearts to diabetic hearts, it is desirable to know how they compare under normoglycemic and hyperglycemic conditions, as well as under hypo-, normo- and hyperinsulinemic conditions, and with normal and high free fatty acid concentrations. When one takes into consideration all of these factors, then all studies are limited to a greater or lesser extent in their experimental design.

The hyperglycemia that occurs with diabetes helps compensate for the impaired capacity for myocardial glucose transport. Hyperglycemia *per se* results in an increase in myocardial glucose uptake in nondiabetic myocardium under either normal or ischemic conditions [33,34]. This is due to an increase in the extracellular glucose concentration and an increase in the gradient for glucose across the sarcolemmal membrane. When myocardial glucose uptake in nondiabetic normoglycemic swine is compared to diabetic hyperglycemic animals, the rates of glucose uptake are not different under either normal aerobic conditions or when myocardial blood flow is reduced by 60% (Fig. 2). This occurs even though the diabetic animals has significantly reduced levels of GLUT 1 and GLUT 4 protein [26–28]. When nondiabetic animals are infused with somatostatin to prevent insulin release and made acutely hyperglycemic to match the diabetic animals, they have a significantly greater rate of glucose uptake when compared to hyperglycemic diabetic animals [28] (Fig. 2). Thus it could be argued that hyperglycemia compensates for the decreased capacity for glucose transport across the sarcolemmal membrane in diabetic animals.

The low concentration of insulin in untreated or poorly controlled diabetes results in removal of the normal insulin inhibition of lipolysis in adipocytes, resulting in an increase in plasma free fatty acid concentration. Plasma free fatty acids feed back and inhibit myocardial glucose and lactate uptake and oxidation [3–7]. Conversely, lowering the rate of fatty acid oxidation by either lowering the plasma free fatty acid concentration or by pharmacologically inhibiting carnitine palmitoyl transferase-I (CPT-I, Fig. 1) will result in an increase in cardiac glucose and lactate oxidation. Several studies have demonstrated that stimulation of lipolysis due to low insulin levels is largely responsible for the impaired glucose uptake and oxidation observed with diabetes [35]. The inhibition of glucose and lactate oxidation by elevated fatty acid levels is primarily the result of inhibition of flux through pyruvate dehydrogenase (PDH).

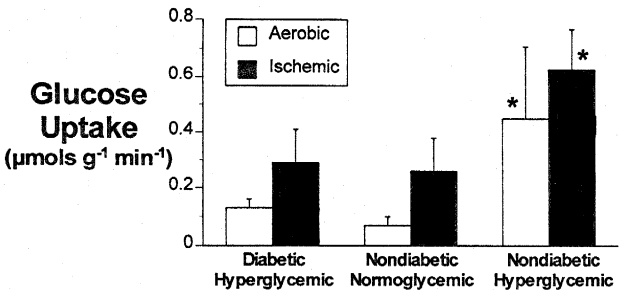

Fig. 2. Effects of hyperglycemia (12 mM) on glucose uptake in healthy and diabetic swine. From Refs. [26,27]. * Significantly different from both nondiabetic normoglycemic and diabetic hyperglycemic groups.

4. Effects on pyruvate oxidation

The rates of cardiac glucose and lactate oxidation are significantly reduced in diabetic animals in vitro [16,17,29,30] and in vivo [36,37]. Pyruvate decarboxylation is the key irreversible step in carbohydrate oxidation and is catalysed by PDH (Fig. 3). The decreased rates of myocardial glucose uptake and oxidation correspond with decreased PDH activity in diabetic rodents and swine [35,37–40]. The amount of active dephosphorylated PDH is reduced due to phosphorylation of PDH by PDH kinase [35], and the enzyme can be reactivated by dephosphorylation by PDH phosphatase [39]. The activity of PDH phosphatase is increased by Ca^{2+} and Mg^{2+} [42], while PDH kinase is inhibited by pyruvate and ADP, and activated by increases in acetyl CoA/CoA and $NADH/NAD^{+}$ [35,39–43]. The rate of pyruvate oxidation is very dependent on the degree of phosphorylation of PDH, as well as on the intramitochondrial concentrations of its substrates (pyruvate, NAD^{+}, and CoA) and products (NADH and acetyl CoA) as these control flux through the active dephosphorylated form of the enzyme [41]. Thus the elevated levels of plasma free fatty acids and ketone bodies, and resultant increase in acetyl CoA levels in the mitochondrial matrix inhibits flux through PDH by stimulating PDH kinase and phosphorylation of PDH, and by direct product inhibition of PDH by acetyl CoA. It is unclear how much of the decrease in glucose uptake in the diabetic heart is due to impaired pyruvate oxidation and feedback inhibition on the glycolytic pathway.

The fraction of myocardial PDH in the dephosphorylated active form is significantly reduced in experimentally induced diabetic rodents [35,38,40,43–45] and swine [37]. Lack of insulin and high circulating free fatty acid levels are both contributing factors to the decreased PDH activity in diabetic rats [40]. The diabetes-induced elevation in plasma free fatty acid levels and rate of fatty acid oxidation results in an elevation of intramitochondrial acetyl CoA levels, which likely results in activation of PDH kinase and phosphorylation of PDH to its inactive form. In addition, acetyl CoA inhibits the rate of flux through active dephosphorylated PDH [41] However, the percent active dephosphorylated PDH is decreased even when substrate and insulin levels are matched [45]. Stimulation of PDH with the PDH kinase inhibitor, dichloroacetate (DCA), results in an increase in myocardial glucose oxidation and contractile function in diabetic rat hearts perfused with fatty acids, suggesting that the diabetes-induced inhibition of PDH is responsible for impaired contractile function [17,46]. However, DCA stimulated glucose oxidation only 2-fold in diabetic hearts compared to 5-fold in control hearts during aerobic perfusion [17], which suggests that PDH activity in diabetic hearts is not as responsive to stimulation, possibly due to the increased use of free fatty acids resulting in increased intramitochondrial $NADH/NAD^{+}$ and acetyl CoA/CoA. Other data would suggest that the intrinsic ability of the enzyme to decarboxylate pyruvate is unaffected [47], and that this decreased ability to activate PDH is due to a chronic increase in PDH kinase in the diabetic heart [43]. A similar up-regulation of PDH kinase occurs in long-term starvation [43,46].

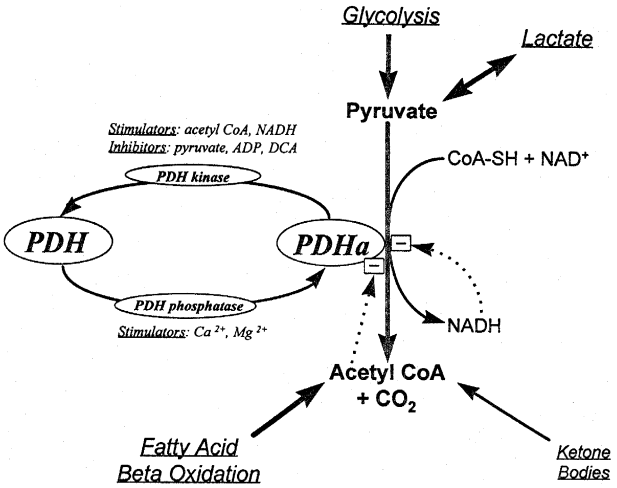

Fig. 3. Regulation of pyruvate dehydrogenase activity and flux rate by phosphorylation and product inhibition. PDH = pyruvate dehydrogenase; PDHa = active dephosphorylated PDH; DCA = dichloroacetate.

The diabetic heart appears to have a greatly impaired ability to oxidize pyruvate under conditions of normal work, and when contractile work is increased with a β-adrenergic agonist. We recently showed that anesthetized streptozotocin diabetic swine have impaired myocardial lactate uptake and a 20–30% decrease in PDH activity under conditions where plasma glucose and free fatty acids are acutely matched to diabetic conditions [37]. When the rate of contractile work and myocardial oxygen consumption are increased by infusing dobutamine, the healthy heart responds by increasing the rate of lactate uptake in proportion to the increase in cardiac work, while the diabetic heart shows impaired lactate uptake relative to nondiabetic animals, and no significant increase in lactate uptake with increased work (Fig. 4). It is interesting to note that the rate of contractile work and glucose uptake are not impaired in the diabetic animals during dobutamine infusion, suggesting that the major impairment with increased cardiac work in the diabetic swine heart is not in glycolysis, but rather in the ability of the heart to oxidize pyruvate.

5. Fatty acid oxidation and the tricarboxylic acid cycle

5.1. Fatty acid supply and myocardial uptake

The main source of fatty acids for the heart are supplied by free fatty acids bound to albumin, and fatty esters present in chylomicrons and very-low-density lipoproteins, both of which are elevated in the diabetic condition [32,48].

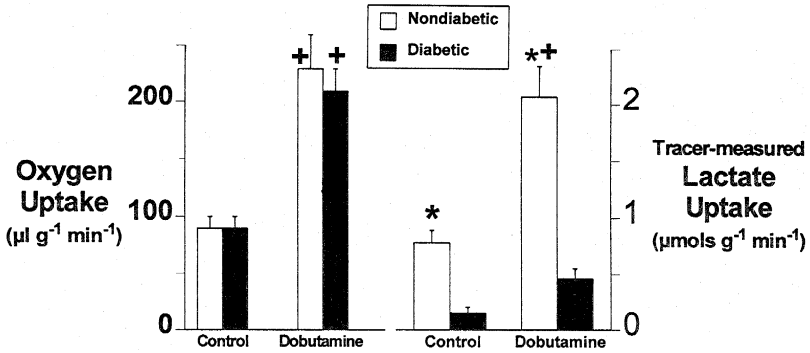

Fig. 4. The effects of diabetes on the rate of myocardial oxygen consumption and lactate uptake in healthy and streptozotocin-diabetic swine under basal conditions, and with β-adrenergic receptor stimulation with dobutamine. * Significantly different from the diabetic group. + Different from the control period within the same group. From Hall et al. [37].

The effects of elevated levels of lipoproteins on myocardial fatty acid metabolism are not clear. Studies by Kreisberg have shown that oxidation of chylomicron fatty acids is decreased in diabetic rat hearts [49]. This may be due to a diabetes-induced reduction in heart lipoprotein lipase activity and a decrease in heparin-releasable lipoprotein lipase activity [50–52]. As a result, it appears that while metabolism of free fatty acids is high in the diabetic heart, the metabolism of fatty acids esterified to lipoproteins is decreased.

In addition to the changes in circulating fatty acids, diabetes can cause a dramatic increase in myocardial triacylglycerol content [53–55]. These high myocardial triacylglycerol levels are in part related to the high concentrations of plasma free fatty acids [54,55] and elevated myocardial CoA levels [56] seen in diabetes. These triacylglycerols can be rapidly mobilized in diabetic rats in both the absence and presence of a high concentration of exogenous fatty acid, and triacylglycerol lipolysis is significantly enhanced in diabetic rat hearts [57].

Even if diabetic rat hearts are perfused in the absence of fatty acids, glucose oxidation rates are very low and provide less than 20% of the heart's ATP requirements [29,57]. This suggests that high levels of circulating fatty acids are not completely responsible for the decrease in glucose metabolism in diabetic rat hearts, suggesting that

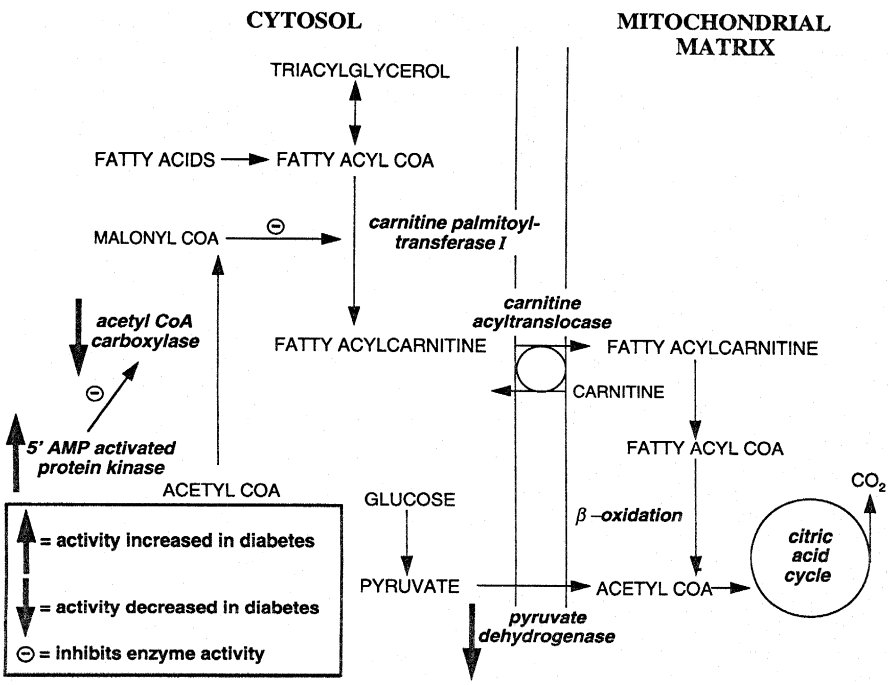

Fig. 5. Scheme depicting the pathway by which 5′-AMP-activated protein kinase and acetyl CoA carboxylase control fatty acid uptake and the changes that occur with diabetes.

up-regulation of PDH kinase plays a role, as discussed above. Thus the inhibition of flux through PDH with diabetes appears to be partially due to intrinsic changes in PDH activity, and is not solely the result of enhanced fatty acid oxidation. Our recent studies have implicated alterations in the regulation of mitochondrial fatty acid uptake as an important contributory factor to the high fatty acid oxidation rate.

5.2. Mitochondrial fatty acid uptake

Esterified fatty acids (long-chain acyl-CoA esters) are transferred into the mitochondrial matrix by the concerted efforts of three carnitine-dependent enzymes (Fig. 5): carnitine palmitoyltransferase I (CPT-I) (which catalyzes the formation of long-chain acylcarnitine from long-chain acyl-CoA in the compartment between the inner and outer mitochondrial membrane), carnitine:acylcarnitine translocase (which transports long-chain acylcarnitine across the inner mitochondrial membrane), and CPT-II (which regenerates long-chain acyl-CoA in the mitochondrial matrix) (see Fig. 1). CPT-I is the key regulatory enzyme involved in the mitochondrial uptake of fatty acids. This enzyme transfers the fatty acid moiety from acyl-CoA to carnitine to form long-chain acylcarnitine, which is then transported into the mitochondria (see Ref. [58] for review). Malonyl-CoA, which is produced in the cytosol by acetyl-CoA carboxylase (ACC), is a potent inhibitor of CPT-I [59,60], and acts at a site distinct from the catalytic site of CPT-I. Studies in swine hearts have shown that streptozotocin-diabetes results in a decrease in malonyl CoA, suggesting less malonyl CoA inhibition of CPT-I and a greater rate of fatty acid oxidation [32,37]. Studies in rat hearts have demonstrated that control of malonyl CoA production can be markedly altered in diabetes (see below).

In heart, two isoforms of ACC are expressed: a 280 kDa isoform (which predominates) and a 265 kDa isoform. Studies from our laboratory [48,59,61–66] and others [67] suggest that ACC in the heart functions primarily as a regulator of fatty acid oxidation, due to inhibition of CPT-I by malonyl-CoA (Fig. 5). In lipogenetic tissues where the 265 kDa isoform predominates, the main role of ACC is to provide malonyl CoA for fatty acid synthesis. The molecular differences between the 280 and 265 kDa isoform of ACC have not been well characterized, although both isoforms appear to be under phosphorylation control (see below). It also appears that the cytoplasmic supply of acetyl CoA is an important determinant of the activity of the 280 kDa isoform of ACC [48]. The shuttling of acetyl groups out of the mitochondria by a short-chain carnitine carrier may be a key source of acetyl CoA for the cardiac isoform of ACC [61,64,65]. Whether the cytoplasmic supply of acetyl CoA is an important determinant of ACC activity in the diabetic heart has yet to be determined.

In recent experiments we have investigated diabetes-induced changes in ACC expression and activity in hearts obtained from streptozotocin diabetic rats [Gamble and Lopaschuk, unpublished data]. Despite the presence of severe diabetes, neither the levels of mRNA for the 280 kDa isoform of ACC nor the protein expression of either the 280 kDa or 265 kDa isoform were altered. However, a significant depression of ACC activity was seen in diabetic hearts. This decrease occurred even in the presence of saturating concentrations of acetyl CoA. A decrease in cardiac ACC activity is also seen in hearts from insulin-resistant diabetic rats [Gamble and Lopaschuk, unpublished data]. These results suggest that the primary change in ACC activity in diabetic rat hearts occurs due to an alteration in either phosphorylation control or allosteric control of the enzyme, as opposed to an alteration in expression of ACC. This contrasts with the liver and adipose tissue, in which we observed a dramatic decrease in ACC expression in diabetic animals.

Although a number of different kinases will phosphorylate ACC in vitro, it is now apparent that cAMP-dependent protein kinase and a recently characterized 5′-AMP-activated protein kinase (AMPK) are the kinases important in regulating ACC activity [66]. In diabetes, the activity of AMPK is increased (possibly due to an activation of the kinase that phosphorylates AMPK) resulting in a phosphorylation and inhibition of ACC (Fig. 5). Malonyl CoA levels decrease, resulting in a greater activity of CPT-I and enhanced mitochondrial fatty acid uptake and oxidation in the diabetic heart, with a parallel decrease in carbohydrate oxidation. To date, the phosphorylation control of cardiac ACC in the diabetic is still poorly understood. In addition to AMPK, it has also been shown that ACC in myocytes is controlled by cAMP-dependent protein kinase [67]. Whether this pathway is altered in the diabetic heart has not been determined. It has also yet to be established whether an increased phosphorylation of ACC acts synergistically with a decrease in acetyl CoA supply to decrease malonyl CoA production in the diabetic heart.

5.3. Mitochondrial fatty acid β-oxidation

Once in the mitochondrial matrix long-chain acyl-CoA passes through the β-oxidation enzyme system (or spiral) to produce acetyl-CoA. Each successive cycle of the β-oxidation spiral results in a 2-carbon shortening of the fatty acid (as acetyl CoA) and formation of 1 NADH and 1 $FADH_2$. The activity of β-hydroxyacyl-CoA dehydrogenase (a key enzyme of β-oxidation) has been shown to be normal [68] or high [69] in diabetic rat mitochondria. The combination of high circulating levels of fatty acids, a decreased regulation of fatty acid uptake by the mitochondria, and a normal or accelerated β-oxidative pathway results in a large proportion of acetyl CoA for the TCA cycle being derived from fatty acid oxidation. The accumulation of acetyl CoA and reducing equivalents from fatty acid β-oxidation is primarily responsible for the dramatic inhibition of PDH that can be seen in diabetic rats, as discussed above.

Following the production of acetyl CoA, the pathways for fatty acid oxidation and glucose oxidation merge (Fig. 1). For the purposes of the present discussion we shall regard the oxidation of ketone bodies as leading to similar endpoints as fatty acid oxidation. Acetyl-CoA derived from both β-oxidation and PDH enters the tricarboxylic acid (TCA) cycle. The myocardial TCA cycle does not appear to be altered in diabetes. If overall TCA cycle CO_2 production from glucose oxidation and both exogenous and endogenous fatty acid oxidation is measured, no difference in rates are observed in diabetic versus control rat hearts at the same rate of myocardial oxygen consumption [70]. Measurement of TCA cycle enzyme activities also do not differ in diabetic rats [30,68]. As a result, the primary change that occurs in diabetic hearts is not a change in TCA cycle activity, but rather the source of acetyl CoA for the TCA cycle.

The next step in ATP production is the entry of NADH and $FADH_2$ into the electron transport chain, the extrusion of protons from the mitochondrial matrix, and the generation of H_2O and ATP by the F_1 ATPase. Biochemical evidence to date suggests that this process may be impaired in the diabetic. Isolated mitochondrial studies have demonstrated that state 3 respiration and oxidative phosphorylation rates are depressed in the diabetic heart [71–73]. How these relate to overall ATP production in the intact heart is still not clear. Although respiration has been shown to be depressed in diabetic heart mitochondria, overall ATP production in the intact heart does not appear to be decreased [29,57]. The main difference in mitochondrial metabolism appears to be in a shift in the source of acetyl CoA for the TCA cycle, though one cannot rule out the potential negative effects of high rates of ketone body oxidation and the impaired rate of pyruvate carboxylation and anaplerosis, as recently noted in nondiabetic hearts [74]. Whether the capacity of mitochondria to produce adequate ATP is compromised at high workloads remains to be determined.

6. Contribution of substrate metabolism to contractile dysfunction in the diabetic heart

It has been recognized for many years that diabetic patients have a significantly greater incidence and severity of angina, acute myocardial infarction (AMI), congestive heart failure, and other manifestations of atherosclerosis compared to the nondiabetic population [75–79]. More recently, it has been determined that ventricular performance can be impaired (diabetic cardiomyopathies) even in the absence of ischemic heart disease [80–88]. Although an increased incidence of atherosclerosis in diabetics contributes to these complications, population-based studies have shown that noncoronary factors are also important contributing factors [2]. For instance, the incidence and severity of complications associated with AMI are greater in the diabetic population even though the size of the infarct is not significantly different, and may even be smaller, compared to the nondiabetic population [89,90]. Diabetes-induced changes within the heart appear to be important contributing factors to injury during and following an AMI [1,91,92]. Both heart failure following an AMI and diabetic cardiomyopathies have been correlated with the degree of glycemic control in the patient [93–95]. Furthermore, cardiomyopathies in the absence of ischemic heart disease can be improved by correction of hyperglycemia [95]. Accumulating evidence has implicated changes in myocardial energy substrate use as contributing to diabetic cardiomyopathies [96–102].

Several lines of evidence, both clinical and experimental, suggest that high plasma levels of free fatty acids and high rates of fatty acid oxidation in the myocardium result in impaired contractile function and more arrhythmias during and after ischemia in both the normal and diabetic heart [32,103]. Accumulation of fatty acids and their toxic intermediates have been associated with mechanical dysfunction and cell damage in diabetic hearts subjected to ischemia [104] and to depressed sarcoplasmic reticulum Ca^{2+} pump and myofibrillar ATPase activities and myosin isozymes [105]. The best evidence for a causative link between high fatty acid oxidation and impaired cardiac function comes from studies in isolated rat hearts where either fatty acid oxidation was inhibited (with CPT-I inhibitors) or PDH activity stimulated (with DCA), and contractile recovery from ischemia improved. However, to date, results of clinical trials aimed at suppressing fatty acid oxidation during AMI in diabetics have not been reported. Several pharmacological approaches are available that suppress fatty acid oxidation and increase flux through PDH, such as suppression of peripheral lipolytic rate and plasma free fatty acid levels with insulin, direct inhibition of β-oxidation [106,107], inhibition of CPT-I [108], or decreasing intramitochondrial acetyl CoA levels with carnitine [109,110]. As a result, there is a clear rationale for using this approach to improve contractile function and decrease irreversible damage following AMI.

7. Summary and conclusions

The effects of diabetes on myocardial metabolism are complex in that they are tied to the systemic metabolic abnormalities of the disease (hyperglycemia and elevated levels of free fatty acid and ketone bodies), and changes in cardiomyocyte phenotype (e.g., down-regulation of glucose transporters and PDH activity). The cardiac adaptations appear to be driven by the severity of the systemic abnormalities of the disease. The diabetes-induced changes in the plasma milieu and cardiac phenotype both cause impaired glycolysis, pyruvate oxidation, and lactate uptake, and a greater dependency on fatty acids as a source of acetyl CoA. Studies in isolated hearts suggest that

therapies aimed at decreasing fatty acid oxidation, or directly stimulating pyruvate oxidation would be of benefit to the diabetic heart during and following myocardial ischemia.

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