

## Adaptation and Maladaptation of the Heart in Diabetes: Part II Potential Mechanisms

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The prevailing concept of the heart's response to changes in its environment is a complex network of interconnecting signal transduction cascades.<sup>1</sup> In such a scheme, the focus is on communication of various cell surface receptors, heterotrimeric G-proteins, protein kinases, and transcription factors.<sup>2-4</sup>

Diabetes is a disorder of metabolic dysregulation. At first glance it appears that metabolism and the metabolic consequences of diabetes do not fit into this signal-response coupling scheme. Two questions arise. First, is metabolism simply an "effect" rather than a "cause" of adaptation? Second, is metabolism only a by-product of signal transduction-induced adaptation, allowing equilibrium (and therefore maintenance of function) in the presence of the other adaptational responses?

An alternative is to take a new, less restricted view of metabolism. Beyond its stereotypical function as a provider of ATP, alterations in metabolic flux within the cell create essential signals for the adaptation of the heart to situations such as diabetes. This concept is novel for the heart, but has already been considered in the liver. Like the phosphorylation events occurring in signal transduction cascades, changes in metabolic flux are extremely rapid. For example, translocation of GLUT4 to the cell surface in response to insulin occurs within a second.<sup>5</sup> We have previously found that increases or decreases in workload also change metabolic fluxes in seconds.<sup>6,7</sup> Therefore, changes in metabolites are rapid enough to allow them to act as signaling molecules.

Many of these acute changes in metabolic flux are brought about by the same signal transduction cascades believed to be involved in the adaptation of the heart to changes in its environment. Phosphatidylinositol 3-kinase, Ca<sup>2+</sup>, and protein kinase C, all of which play a role in cardiac adaptation, regulate metabolism in the heart.<sup>8,9</sup> Metabolic signals therefore provide a new dimension to the preexisting concepts of cardiac adaptation, as illustrated in Figure 1.

### Fatty Acid-Regulated Gene Expression

Diabetes is as much a disorder of fatty acid metabolism as it is a disorder of glucose metabolism.<sup>10</sup> In the normal cardiac

myocyte, fatty acids serve many essential functions. These functions include roles as fuels, mediators of signal transduction (eg, activation of various protein kinase C isoforms, initiation of apoptosis), ligands for nuclear transcription factors (eg, peroxisome proliferator-activated receptor  $\alpha$  [PPAR $\alpha$ ]), and essential components of biological membranes.<sup>11-17</sup> Not surprisingly, the levels of intracellular fatty acids and their derivatives are tightly regulated. Loss of this regulation, and subsequent elevation of intracellular fatty acids and lipids (and abnormalities in lipid handling) have been associated with various pathologies, including insulin resistance, pancreatic dysfunction, and cardiotoxicity.<sup>14,18-21</sup> One way in which mammalian organisms respond to elevations in fatty acid levels is by increasing the expression of various proteins involved in fatty acid utilization, and this has been studied in tissues including cardiac and skeletal muscle.<sup>22-24</sup> Fatty acid induced genes known to be involved in cardiac fuel selection and mitochondrial function are listed in Table 1.<sup>22,25-29</sup> The mechanism by which fatty acids activate the transcription of these genes is through activation of the nuclear receptor PPAR $\alpha$ .

On binding of fatty acids to PPAR $\alpha$ , the ligand bound receptor heterodimerizes with 9-cis retinoic acid receptor (RXR).<sup>30</sup> This functional dimer is able to activate the transcription of genes whose promoter contains the PPAR response element (PPRE; also known as fatty acid response element [FARE]) through recruitment of histone acetyltransferases (HATs), thereby increasing access to the transcriptional start site.<sup>31</sup> Cofactors that bind to the PPAR $\alpha$ /RXR heterodimer include CBP/p300, PBP/TRAP220, PGC-1, and SRC-1. Of these, PGC-1 is highly expressed in the heart.<sup>32</sup>

Fatty acids are also able to alter cellular metabolism, function, and gene expression through PPAR $\alpha$ -independent mechanisms. Once transported into the cell, long chain fatty acids are activated by a thioester linkage to coenzyme A (CoA). Long chain fatty acyl-CoAs (LCFACoA) are subsequently transported into the mitochondrion via carnitine palmitoyltransferase I (CPTI), and enter  $\beta$ -oxidation. When the rate of fatty acid transport into the myocyte exceeds that

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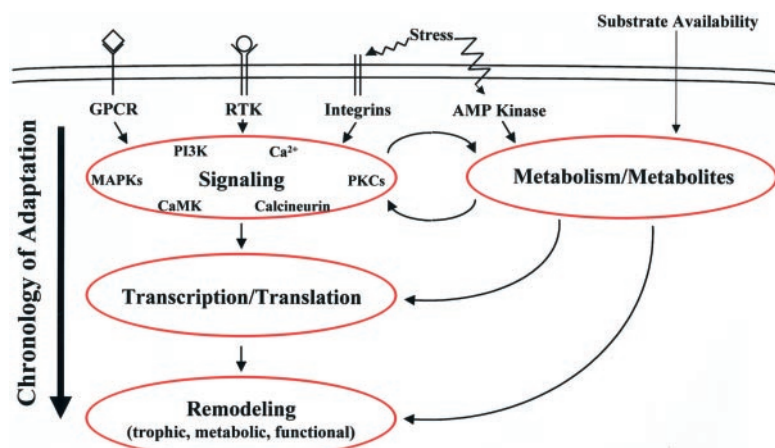
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**Figure 1.** Hypothetical involvement of metabolism in cardiac adaptation. The central dogma for the adaptation of the myocardium to various stimuli is depicted on the left side of the figure. Activation of G-protein coupled receptors (GPCR), receptor tyrosine kinases (RTK), and integrins in response to multiple stimuli result in the activation of numerous signaling cascades, such as those employing calcium ( $\text{Ca}^{2+}$ ), protein kinase C (PKC) isoforms, calcineurin,  $\text{Ca}^{2+}$ /calmodulin kinase (CaMK), mitogen-activated protein kinases (MAPKs), and phosphatidylinositol 3-kinase (PI3K). The latter affect transcription and translation, processes essential for the chronic adaptation of the heart. The current review hypothesizes that alterations in metabolism generate essential components involved in this adaptation (right).

of transport into the mitochondrion (eg, because of excessive fatty acid availability and/or CPTI inhibition by malonyl-CoA), cytosolic LCFA-CoA levels increase. The latter are utilized in the synthesis of both diacylglycerol (DAG) and ceramide. DAG is an allosteric activator of several PKC isoforms. Increased DAG levels and PKC activity are observed in myocytes isolated from diabetic animals.<sup>19,33</sup> Chronically activated PKC has been suggested to play a role in the development of insulin resistance.<sup>34</sup> Ceramide, whose levels are elevated in the Zucker Diabetic Fatty (ZDF) rat heart, can initiate apoptosis and cardiac dysfunction.<sup>20,35</sup> In addition, fatty acids may affect cellular processes through reactive oxygen species.<sup>36</sup>

### Glucose-Regulated Gene Expression

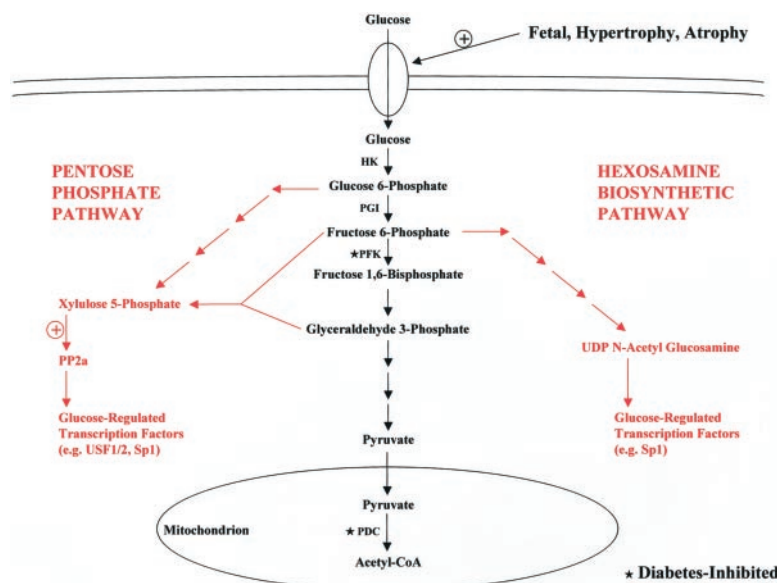
As is true for fatty acids, glucose has multiple functions in the cardiac myocyte, extending far beyond its use as an energy source. Once transported into the cardiomyocyte via specific regulatory transporters (glucose transporters [GLUTs] 1, 4, and 8), glucose becomes phosphorylated by hexokinase to generate glucose 6-phosphate. The latter has multiple potential fates. These include flux through the glycolytic pathway and full oxidation via the Krebs cycle (or conversion to lactate), storage as glycogen, entry into the pentose phosphate pathway (PPP) with concomitant ribose formation, carbon

utilization for the generation of alternative cellular components, and entry into the hexosamine biosynthetic pathway. Increased flux through the latter pathway leads to increased O-linked glycosylation of proteins, affecting various processes such as transcription, translation, and protein stability.<sup>37–39</sup> As exemplified in Figure 2, glucose is more than just an energy source for the heart. Failure to adequately control intracellular glucose levels (glucotoxicity) has also been implicated in the development of insulin resistance and in the generation of reactive oxygen species (ROS) in various tissues.<sup>40,41</sup>

Compared with fatty acids, relatively little is known about the effects of glucose metabolism on cardiac gene expression. It has been known for some time that glucose availability affects the expression of several specific genes in the liver.<sup>42–44</sup> These genes include those encoding for pyruvate kinase (PK), acetyl-CoA carboxylase  $\alpha$  ( $\text{ACC}\alpha$ ), fatty acid synthase (FAS), and Spot 14 (S14). Through investigations concentrating on the glucose/carbohydrate responsive elements (GIRE/ChoRE) in the promoter regions of these various glucose regulated genes, a number of candidate transcription factors have been identified that are believed to be involved in glucose mediated gene expression. Upstream stimulatory factor (USF), stimulatory protein 1 (Sp1), and sterol regulatory element binding protein 1 (SREBP1) have

**TABLE 1. Fatty Acid-Induced Genes and Their Role in Fuel Selection and Mitochondrial Metabolism**

Gene Encoding for	Protein Function	References
Fatty acid translocase (FAT/CD36)	Fatty acid transport into the cell	113, 114
Fatty acid transport protein (FATP)		
Fatty acid binding protein (FABP)	Binding to fatty acids in cytosol	115
Malonyl-CoA decarboxylase (MCD)	Removal of malonyl-CoA, an inhibitor of mCPTI	27, 116
Acyl-CoA synthetase (ACS)	Activation of fatty acids	117
Muscle-specific carnitine palmitoyltransferase I (mCPTI)	Long chain fatty acyl-CoA transport into the mitochondrial matrix	25
Acyl-CoA dehydrogenases	$\beta$ -Oxidation of fatty acyl-CoAs in the mitochondrial matrix	22, 26
3-hydroxyacyl-CoA dehydrogenases		
Pyruvate dehydrogenase kinase 4 (PDK4)	Phosphorylation and inhibition of pyruvate dehydrogenase	29
Uncoupling protein 3 (UCP3)	Regulator of mitochondrial membrane potential (?), fatty acid metabolism (?), ROS generation (?)	28



**Figure 2.** Pathways of glucose sensing. An uncoupling of pyruvate oxidation from glycolysis, either due to an acceleration of glucose transport (fetal, hypertrophied, and atrophied hearts) or inhibition of pyruvate oxidation (diabetes), will result in an accumulation of glycolytic intermediates. Increased intracellular levels of xylulose 5-phosphate from either the oxidative or non-oxidative branches of the pentose phosphate pathway will result in activation of protein phosphatase 2a (PP2a). The latter dephosphorylates and activates glucose sensing transcription factors. Increased flux through the hexosamine biosynthetic pathway results in increased intracellular levels of UDP-N-acetylglucosamine, which is used directly in O-linked glycosylation of target proteins (eg, Sp1, c-myc).

all been shown to play roles in liver and fat cell glucose sensing.<sup>42–44</sup>

There are two known isoforms of USF, designated USF1 and USF2. They are encoded by different genes. These ubiquitously expressed transcription factors are members of the basic helix loop helix/leucine zipper (bHLH/LZ) family, that recognize E-boxes within promoters of target genes, such as PK and S14. USF binds to E-boxes as dimers (either homo- or heterodimers). Amphipathic  $\alpha$ -helices located C-terminal to the basic domain allow dimerization through projection of hydrophobic residues on the exterior of the protein, thereby promoting protein-protein interaction.

Sp1 is a ubiquitous transcription factor, able to bind to the promoter region of multiple target genes. The consensus is that Sp1 maintains basal rates of transcription of constitutive genes. However, Sp1 appears to be regulated at multiple levels, suggesting a role that extends beyond the initial image of basal transcription. Of the stimuli known to affect Sp1 DNA binding activity, glucose availability is one. Work investigating glucose-induced ACC $\alpha$  expression was the first to suggest the involvement of Sp1 in glucose sensing.<sup>45</sup>

Recent work on the *FAS* gene promoter has suggested a role of SREBP1 in glucose sensing.<sup>46</sup> SREBP, also known as ADD (adipocyte determination and differentiation factor), is encoded by 2 genes, SREBP1/ADD1 and SREBP2/ADD2. The encoded proteins, of which splice variants exist, are bHLH/LZ capable of binding to E-boxes, reminiscent of USF. In adipocytes, the E-box in the promoter for the *FAS* gene has been shown to be essential for transcriptional induction.<sup>47</sup> Classically, SREBP is regulated by proteolytic cleavage, releasing this transcription factor from its endoplasmic reticulum anchor when intracellular cholesterol levels are depleted.<sup>48</sup>

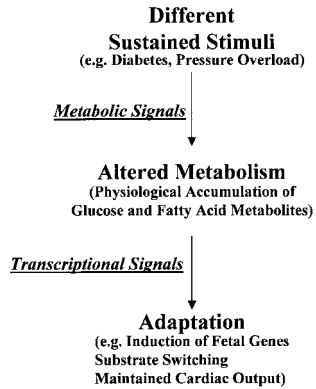
The mechanism(s) by which glucose availability affects the DNA binding activity of these transcription factors is (are) not known precisely. Extensive evidence from studies on glucose sensing mechanisms in the liver strongly suggests that reversible phosphorylation is involved. Protein phosphatase

inhibitors, such as okadaic acid, block the induction of glucose regulated genes without effecting glucose metabolism.<sup>49</sup> An interesting question is how glucose availability affects protein phosphorylation. A suggested mechanism involves AMP-activated protein kinase (AMPK). AMPK has been termed the fuel gauge of the cell.<sup>50</sup> When the “energy charge” of the cell decreases (decreased ATP/AMP and PCr/Cr ratios), AMPK becomes activated.<sup>51</sup> AMPK compensates for the energetic imbalance by increasing metabolic substrate availability (increased glucose transport by increased translocation of GLUT4 to the cell surface and increased lipolysis through activation of lipases) and metabolism (increased  $\beta$ -oxidation by lowering intracellular malonyl-CoA levels and increased glycolysis by increasing intracellular fructose 2,6-bisphosphate).<sup>52,53</sup> Long-term activation of AMPK affects gene expression in both skeletal muscle and liver.<sup>54</sup> Treatment of hepatocytes with a pharmacological activator of AMPK, 5-aminoimidazole-4-carboxamide ribonucleoside (AICAR), blocks glucose-induced changes in gene expression.<sup>55</sup> Thus, when glucose levels are low, a combination of reduced glucose metabolites and increased AMPK activity inactivate glucose sensing transcription factors. In contrast, increased glucose availability, and therefore decreased AMPK activity, prevents phosphorylation of components of glucose sensing.

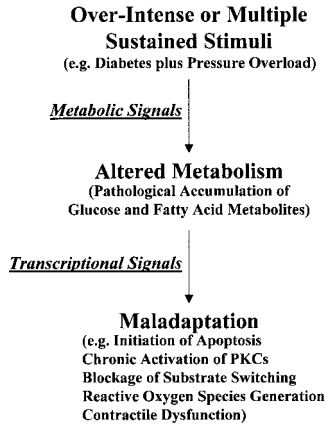
Protein phosphatase 2a (PP2a) appears to be involved in glucose sensing, as suggested by inhibitor studies in the liver (Figure 2, left). PP2a is allosterically activated by xylulose 5-phosphate, a PPP intermediate,<sup>56,57</sup> that accumulates during increased glucose uptake and subsequent increased flux through the PPP (both the oxidative branch and the non-oxidative branch, through glucose 6-phosphate dehydrogenase and transketolase respectively). Xylulose 5-phosphate activates PP2a and the dephosphorylation of glucose sensing components, either the transcription factors themselves (eg, Sp1) or associated proteins (eg, glucose regulated factor [GRF] binding with USF1/2).

A second mechanism of glucose sensing involves reversible covalent modification by O-linked glycosylation of

**Metabolic Adaptation**



**Metabolic Maladaptation**



**Figure 3.** Metabolic adaptation and maladaptation of the heart. Various stimuli can result in rapid alterations in fatty acid and glucose metabolites within the cardiomyocyte. These metabolites then act as essential signaling molecules for the adaptation process. However, if the intensity of the stimulus is too great, or multiple stimuli act simultaneously (eg, pressure overload plus diabetes), pathological accumulation of metabolites results in metabolic maladaptation.

proteins. Such O-linked glycosylation of signaling proteins occurs at serine and threonine residues otherwise used for regulatory phosphorylation.<sup>58</sup> The level of cytosolic UDP-N-acetyl glucosamine, the principal end product of the hexosamine biosynthetic pathway and the donor substrate used for protein glycosylation, is rate limiting for the O-glycosylation of proteins (Figure 2, right).<sup>59</sup> Levels of UDP-N-acetyl glucosamine are in turn dependent on the uptake and metabolism of glucose, as well as the activity of glutamine:fructose 6-phosphate amidotransferase (GFAT), the rate limiting enzyme in hexosamine biosynthesis.<sup>60,61</sup> Sp1 is regulated by both reversible phosphorylation and reversible glycosylation. Dephosphorylation of Sp1 in response to increased glucose availability promotes DNA binding.<sup>62</sup> Glycosylation greatly increases the stability of the Sp1 protein, thereby enabling promotion of transcriptional initiation.<sup>38</sup>

Lastly, glucose sensing components might themselves be under transcriptional control. Insulin has been shown to increase the expression of the SREBP1 in adipocytes.<sup>47</sup> Evidence therefore exists that glucose, through increased insulin secretion, is able to increase the transcription of SREBP1.

**Metabolic Adaptation**

Metabolic adaptation appears essential for the maintenance of contractile function of the heart under different stresses. How can metabolic adaptation, which occurs relatively rapidly, play a role in the transcriptional adaptation of the myocardium? The following section provides a novel hypothesis for a role of metabolism in the adaptation of the heart. This hypothesis is summarized in Figures 2 and 3 (left).

**Metabolism as a Common Mechanism of Adaptation**

At the level of contractile protein gene expression, rodent models of both diabetes and pressure overload result in cardiac re-expression of fetal genes, with concomitant adult gene repression (Table 2). This adaptation is believed to be essential for the maintenance of contractile function of the adapted heart. We have found that the unloaded, atrophied

heart also reverts to a fetal pattern of gene expression.<sup>63</sup> Therefore, one or more common signals must be present in the fetal, diabetic, hypertrophied, and unloaded heart. This common factor is most likely glucose. The evidence is as follows (Figure 2).

Glucose is the primary fuel used by the fetal heart. At birth, increased dietary fatty acids result not only in increased

**TABLE 2. Transcriptional Alterations During Pressure Overload and Diabetes**

	Hypertrophy	Diabetes
Contractile proteins		
α-MHC	↓	↓
β-MHC	↑	↑
Cardiac α-actin	↓	↓
Skeletal α-actin	↑	↑
Ion pumps		
α <sub>2</sub> Na/K-ATPase	↓	?
SERCA 2a	↓	↓
Metabolic proteins		
GLUT1	=	↓
GLUT4	↓	↓
Muscle CPT-1	↓	=
Liver CPT-1	=	=
mCK	↓	↓
PPARα	↓	↓
PDK4	↓	↑
MCD	↓	↑
UCP2	↓	=
UCP3	↓	↑
Protooncogenes		
c-fos	↑	↓

Summary of transcriptional changes in the heart in animal models of hypertrophy (pressure overload-induced) and diabetes (streptozotocin-induced). With the exception of genes encoding for fatty acid metabolizing proteins, the transcriptional adaptation of the heart is similar in response to these two situations.



availability of fatty acids as a fuel, but also result in activation of the genes involved in fatty acid metabolism, an effect which is mediated in part by PPAR $\alpha$ .<sup>64–66</sup> During hypertrophy, the heart mimics the fetal situation by increasing reliance on glucose as a fuel while depressing fatty acid utilization.<sup>67,68</sup> Like the hypertrophied heart, the atrophied heart increases reliance on glucose as its fuel for respiration.<sup>69</sup> Therefore, in fetal, hypertrophied, and atrophied hearts, glucose uptake and metabolism are increased. In addition, this high rate of glucose uptake exceeds the rate of pyruvate oxidation. The dissociation of glycolysis and pyruvate oxidation results in increased levels of glucose metabolites.

The diabetic heart increases reliance on fatty acids as a fuel. Fatty acids inhibit glucose oxidation at the level of the pyruvate dehydrogenase complex (PDC). PDC catalyzes the committed step for carbohydrate oxidation. Increased mitochondrial acetyl-CoA levels (due to increased fatty acid and ketone body utilization) and phosphorylation by PDK4 (due to PPAR $\alpha$ -mediated induction) both inhibit PDC.<sup>29,70</sup> In addition, increased citrate levels in the diabetic heart due to increased fatty acid utilization result in potentiation of inhibition of phosphofructokinase (PFK) by ATP. Such an inhibition will prevent further the full oxidation of glucose. Despite decreased glucose transporter expression and decreased insulin-mediated glucose transport, the rates of glucose uptake by the diabetic heart, within the diabetic environment, are comparable to those observed in normal hearts because of the hyperglycemia.<sup>71,72</sup> Thus, with normal glucose influx into the cardiomyocyte, and the blocks at PFK and PDC, glucose metabolites accumulate. This sequence is consistent with Randle's hypothesis<sup>73</sup> that fatty acids inhibit glucose oxidation more than glycolysis, and glycolysis more than glucose uptake. Intracellular concentrations of glucose, glucose 6-phosphate, fructose 6-phosphate, glycogen, pyruvate, and lactate have all been shown to be increased in the diabetic heart.<sup>74,75</sup> Glycolytic intermediates therefore accumulate when the glycolytic flux exceeds the rate of glucose oxidation.

By what mechanism can glycolytic intermediate accumulation play a role in the adaptation of the heart? Here we refer again to the liver as an organ in which glucose regulated gene expression has already been investigated. The 2 major pathways of transcription factor activation by glucose metabolites are reversible phosphorylation and glycosylation (see above). There is considerable circumstantial evidence that these pathways exist in the heart as well. When glucose flux increases in the hepatocyte, so too does flux through the PPP, resulting in increased xylulose 5-phosphate levels that activate PP2a.<sup>56,57</sup> Appreciable flux through the non-oxidative PPP occurs in the heart.<sup>76</sup> In addition, pressure overload induces glucose 6-phosphate dehydrogenase (G6PDH), the enzyme catalyzing the flux generating step in the oxidative PPP.<sup>77</sup> It is therefore reasonable to assume that xylulose 5-phosphate levels increase in the hypertrophied heart due to the combined increase in glucose flux into the cardiomyocyte and increased G6PDH activity.

We have recently found that O-linked glycosylation may be increased in the hypertrophied heart (M.E. Young, DPhil, et al, unpublished data, 2002). Two isoforms of GFAT are

expressed in the rodent heart. Of these 2 isoforms, GFAT2 is induced in the hypertrophied heart. UDP-N-acetyl glucosamine levels are also elevated in the hypertrophied heart, likely because of a combination of increased glucose influx into the cardiomyocyte and induction of GFAT2, which catalyzes the flux generating step of the hexosamine biosynthetic pathway (M.E. Young, DPhil, et al, unpublished data, 2002). As glycolytic intermediates accumulate in the diabetic heart, it is likely that O-linked glycosylation increases as well.

What is the evidence that altered metabolism plays a role in the adaptation of the heart to diabetes and/or other sustained stimuli? The first question to be answered is whether altered metabolism is required at all to modulate gene expression. Work investigating the role of substrate switching in the hypertrophied heart suggests that alterations in metabolic flux are essential for the adaptation of the heart to pressure overload.<sup>78</sup> As mentioned, the nuclear receptor PPAR $\alpha$  is an essential component in cardiac substrate switching. Reactivation of PPAR $\alpha$  in the hypertrophied heart prevents substrate switching, and causes contractile dysfunction, suggesting that altered metabolism is essential for the adaptation of the heart.<sup>78</sup> In addition, these studies found that the induction of the fetal isoform of  $\alpha$ -actin by pressure overload was completely blocked when PPAR $\alpha$  was reactivated. The induction of skeletal  $\alpha$ -actin has previously been shown to be dependent on the activation of Sp1 in response to pressure overload.<sup>79</sup> It is therefore possible that the prevention of skeletal  $\alpha$ -actin induction by PPAR $\alpha$  reactivation in the hypertrophied heart is due to prevention of Sp1 activation by glucose metabolites. Whether diabetes activates Sp1 in the heart is not known.

Previously we addressed the question of whether substrate switching is essential for the adaptation of the diabetic heart. Induction of diabetes in an animal model in which PPAR $\alpha$  is inactivated is an obvious way to test such a hypothesis. Although such an experiment has not yet been performed, much of the metabolic adaptation in the diabetic heart is akin to that during the adaptation of the heart to fasting. When PPAR $\alpha$  knockout mice are fasted, the animals develop contractile dysfunction and die.<sup>24</sup> Fatal cardiac dysfunction in this model appears to be due to the accumulation of lipids within the cardiomyocytes (so-called lipotoxicity).<sup>24</sup> Thus, a failure of the myocardium to respond to increased fatty acid availability, through activation of PPAR $\alpha$ -regulated genes, results in heart failure.

### Transcription of Genes Encoding for Contractile Proteins and SERCA2a are Regulated by Metabolism in the Heart

As mentioned above, the contractile protein skeletal  $\alpha$ -actin, which is induced in the heart with pressure overload, mechanical unloading and diabetes, appears to be a glucose-regulated gene. The expression of myosin heavy chain  $\alpha$  (MHC $\alpha$ ), the adult isoform in the rodent, decreases in response to either pressure overload, unloading, or diabetes, whereas the expression of the fetal isoform, MHC $\beta$ , increases in response to these stresses.<sup>63,80,81</sup> Further evidence to support the hypothesis that glucose regulates sarcomeric gene

expression in the heart came from studies in which substrate switching was prevented in response to pressure overload through control of dietary intake. Feeding the animals with an isocaloric, low carbohydrate, high fat diet, thereby forcing the heart to utilize fatty acids, abolishes this MHC isoform switching in response to pressure overload (M.E. Young, DPhil, et al, unpublished data, 2002). Likewise, when metabolic adaptation is blocked in the diabetic heart, through pharmacological inhibition of fatty acid oxidation by either methyl palmoixir or etomoxir, MHC isoform switching is attenuated.<sup>82,83</sup>

The above experimental strategies provide further evidence for glucose regulated gene expression in the heart. Although the exact mechanism by which glucose affects MHC isoform expression is presently unknown, examination of the promoters of both MHC $\alpha$  and MHC $\beta$  show potential Sp1 and USF binding sites.<sup>84,85</sup> Taken together, these observations suggest that the MHC isoforms, in addition to skeletal  $\alpha$ -actin, are glucose regulated genes. It is not surprising that genes encoding for proteins directly involved in energy consumption are influenced by the metabolic status of the cell.

Because heart muscle from diabetic animals exhibits a slow rate of relaxation (diastolic dysfunction), the sarco-(endo-)plasmic reticulum Ca<sup>2+</sup> ATPase (SERCA) 2 has been considered a major site for contractile dysfunction.<sup>86</sup> SERCA2 is regulated transcriptionally by the metabolic status of the cardiomyocyte. SERCA2a, the major splice variant in the heart, mRNA, and activity both decrease in response to pressure overload and diabetes.<sup>80,87–89</sup> During both of these situations we have hypothesized that the discordance between the influx of glucose into the cell and the rate of pyruvate oxidation will result in increased glucose metabolites, thereby activating glucose-regulated transcription factors. Etomoxir, an irreversible inhibitor of long chain fatty acid oxidation, increases the rate of glucose oxidation by relieving the inhibition of PDC.<sup>90</sup> In doing so, etomoxir would be expected to decrease the levels of glucose metabolites in the cell. Treatment of rats with etomoxir blocks the decrease in SERCA2a in response to either pressure overload or diabetes.<sup>91–93</sup> Indeed, the promoter of SERCA2a contains both E-boxes and Sp1 binding sites, consensus sequences on which known glucose sensing transcription factors to bind.<sup>94</sup> Consistent with a role of glucose metabolites in the regulation of SERCA2a gene expression is a recent study which reported that perfusion of hearts with glucose lowered SERCA2a mRNA levels.<sup>95</sup> An understanding of the mechanisms regulating SERCA2a transcription and activity are of particular interest in light of reports showing that the activity of this Ca<sup>2+</sup>-pump, which is closely related to its level of expression, affects the contractile function of the heart.<sup>96</sup>

### Glucose Downregulates Fatty Acid Utilization at the Level of Gene Expression

As discussed previously, glucose and fatty acid oxidation are closely interrelated. Fatty acids are able to inhibit the utilization of glucose acutely, as described by Randle et al.<sup>73</sup> Conversely, glucose is able to inhibit fatty acid oxidation in the heart (and skeletal muscle), most likely through elevation of intracellular levels of malonyl-CoA.<sup>12,97</sup> Evidence also

exists suggesting that long-term elevations in glucose availability can block fatty acid utilization at the level of gene expression. Recent work in islet cells have shown that glucose exposure decreases the expression of PPAR $\alpha$ , as well as several PPAR $\alpha$  regulated genes involved in fatty acid metabolism.<sup>98</sup> If this glucose repression of PPAR $\alpha$  expression also occurs in the heart, it may be the mechanism by which PPAR $\alpha$  expression is low in fetal, hypertrophied, atrophied, and diabetic hearts.<sup>28,32,99</sup> In addition, MCAD expression is directly repressed by Sp1 activation in the hypertrophied heart.<sup>100</sup> Glucose can therefore decrease fatty acid utilization not only through repression of PPAR $\alpha$  expression, but also through activation of glucose sensing transcription factors directly binding to the promoter of fatty acid metabolizing genes.

### Metabolic Adaptation of the Heart in Diabetes

We offer the following hypothesis for the metabolic adaptation of the heart in diabetes. Type I (insulin-dependent) diabetes mellitus is characterized by hypoinsulinemia, hyperglycemia and hyperlipidemia, whereas type II (insulin-resistant) diabetes mellitus is characterized by initial hyperinsulinemia, hyperglycemia, and hyperlipidemia. The elevation of plasma nonesterified fatty acid levels in diabetes results in the activation of PPAR $\alpha$ . This activation induces the expression of PPAR $\alpha$  regulated genes, such as FAT, mCPTI, MCAD, LCAD, PDK4, MCD, and UCP3.<sup>22,25–29</sup> The induction of fatty acid metabolizing genes, in combination with increased fatty acid availability, is associated with increased fatty acid utilization by the heart in diabetes.<sup>71</sup> Increased intramitochondrial acetyl-CoA levels (due to increased fatty acid and ketone body utilization), along with induction of PDK4, severely inhibits pyruvate oxidation. The uncoupling of glycolysis and pyruvate oxidation leads to an accumulation of glycolytic intermediates (in the face of hyperglycemia), resulting in the activation of glucose-sensing transcription factors and subsequent transcriptional adaptation (eg, induction of MHC $\beta$  and skeletal  $\alpha$ -actin, with concomitant repression of MHC $\alpha$  and SERCA2a). It is therefore obvious that metabolism is not an innocent bystander when it comes to gene expression in the heart.

### Metabolic Maladaptation

How is it possible that the heart is energy-starved in the midst of excess substrate supply in diabetes? Figure 3 summarizes the hypothetical models of both metabolic adaptation and metabolic maladaptation. Three major mechanisms of metabolic maladaptation are lipotoxicity, glucotoxicity, and a combination of the two that we would like to call glucolipotoxicity in the heart. Each will be discussed in turn.

### Lipotoxicity

In diabetes, the heart is exposed to a hyperglycemic and hyperlipidemic environment. The heart initially adapts to this environment by increasing the expression of fatty acid metabolizing proteins, thereby increasing the reliance on fatty acids as a fuel. This adapted heart is able to maintain cardiac output under these conditions. It is our hypothesis that continued exposure of the heart to this metabolic environment

eventually results in contractile dysfunction. We propose the following explanation for this sequence of events. As diabetes progresses, the excessive availability of lipids and fatty acids (as well as their uptake) may exceed the rate of their use by the heart, resulting in lipid accumulation within the cardiomyocyte. We have previously shown that progression of diabetes is associated with a dramatic decrease in the expression of PPAR $\alpha$  (and the PPAR $\alpha$  regulated gene, *UCP3*) within the rat heart, through an unknown mechanism.<sup>28</sup> Thus, continued exposure to high fatty acid levels, accompanied by limiting PPAR $\alpha$  activity, will accelerate lipid accumulation. Lipid accumulation within cells, such as the pancreas and heart, is associated with a phenomenon termed lipotoxicity.<sup>20,101</sup> It has been hypothesized that excessive lipids and fatty acids, through long chain acyl-CoAs, result in increased intracellular ceramide levels. The latter can then induce ROS accumulation, iNOS, and apoptosis.<sup>35,102</sup> Contractile dysfunction of the heart may ensue. There is evidence for this hypothesis, in the insulin-resistant, ZDF rat. Hearts isolated from these animals possess increased lipid deposition within the cardiomyocytes, increased ceramide levels, DNA laddering indicative of apoptosis, and contractile dysfunction.<sup>20</sup> Treatment of rats with the thiazolidinedione, troglitazone, reduced the hyperlipidemia, decreased intracellular lipid deposition and ceramide levels, reduced the incidence of apoptosis, and normalized contractile function.<sup>20</sup> Lipotoxicity may occur independently from ceramide, as recently suggested by Schaffer and colleagues.<sup>36</sup> Additional mechanisms by which lipid deposition may be involved in contractile dysfunction include chronic activation of PKCs. Indeed, targeted overexpression of PKC $\beta$ 2 in the myocardium causes cardiomyopathy.<sup>103</sup>

Induction of diabetes in rats with pressure overload-induced hypertrophy results in rapid cardiac failure.<sup>104</sup> This outcome is similar to the observation that reactivation of PPAR $\alpha$  in the hypertrophied heart results in contractile dysfunction.<sup>78</sup> With pressure overload, substrate switching by the hypertrophied heart is essential for maintenance of function; the hypertrophied heart must utilize glucose as a fuel. However, the decreased reliance on fatty acids as a substrate of the hypertrophied heart in the diabetic milieu will accelerate lipid deposition within the cardiomyocyte, thereby accelerating lipotoxicity. A compromise will therefore be set, balancing the need for glucose metabolism with that of the utilization of fatty acids to reduce the rate of lipid deposition, at the expense of contractile function. This may actually be the case in the ZDF rat heart, which is a pressure overload (hypertension) induced hypertrophied heart.<sup>105</sup> Therefore, forcing a hypertrophied heart to utilize fatty acids, in the diabetic milieu will result in a maladapted heart exhibiting contractile dysfunction.

### Glucotoxicity

Just as excessive intracellular lipid accumulation is detrimental, excessive glucose metabolite accumulation is associated with various pathologies. Excessive glucose uptake is known to induce insulin resistance in multiple organs, including skeletal muscle, liver, and adipose.<sup>40</sup> Consistent with glucose induced insulin resistance, both the hypertrophied and dia-

betic heart possess decreased insulin sensitivity.<sup>69,106,107</sup> One current hypothesis for glucose-induced insulin resistance is increased flux through the hexosamine biosynthetic pathway, resulting in increased O-linked glycosylation of specific proteins involved in insulin signal transduction, such as the insulin receptor substrates.<sup>108</sup>

Chronic hyperglycemia is associated with advanced glycation end-product (AGE)-induced ROS generation.<sup>41</sup> Excessive free radical generation can affect ion channels, Ca<sup>2+</sup> homeostasis, mitochondrial function, transcription factor DNA binding activity, growth, and even initiation of apoptosis.<sup>109–112</sup> Many of these studies have been performed in vascular tissue and await full investigation in cardiomyocytes.

### Glucolipotoxicity

We propose that glucolipotoxicity of the cardiac myocyte is an extension of lipotoxicity, in which both glucose and fatty acid availability is high, as seen in the diabetic environment. As mentioned previously, glucose appears to downregulate the expression of fatty acid metabolizing genes, through PPAR $\alpha$  repression, as well as activation of Sp1. If this glucose-induced inhibition of fatty acid metabolism occurs in an environment in which fatty acids are in excess, then lipid deposition within the cardiomyocyte will be accelerated, resulting in cardiac dysfunction. Such a phenomenon, if proven, should be termed glucolipotoxicity.<sup>113–117</sup>

### Conclusions

The initial adaptation and subsequent maladaptation of the heart to a diabetic environment can be traced to a complex system of metabolic signals. Elevated circulating fatty acids during diabetes result in activation of PPAR $\alpha$  within the cardiomyocyte. The subsequent induction of enzymes involved in fatty acid oxidation, in addition to increased fatty acid availability, result in increased fatty acid oxidation. Inhibition of pyruvate dehydrogenase (due to the combined effects of PDK4 induction and fatty acid and ketone body derived acetyl-CoA) limits pyruvate oxidation. The dissociation of glycolysis and pyruvate oxidation in the diabetic heart results in the accumulation of glycolytic intermediates. We hypothesize that the latter activate glucose sensing transcription factors as part of the adaptation process. However, if the diabetes progresses or additional stresses are placed on the heart (eg, hypertension), metabolic maladaptation will occur. Decreased PPAR $\alpha$  expression (due to pressure overload and/or prolonged exposure to hyperglycemia and/or hyperlipidemia) will limit the fatty acid oxidation capacity of the heart. When fatty acid availability exceeds fatty acid oxidation rates, intramyocardial lipids will accumulate. The subsequent lipotoxicity plays a role in the development of contractile dysfunction observed in the diabetic heart.

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KEY WORDS: diabetes mellitus ■ heart failure ■ cardiovascular diseases

## Adaptation and Maladaptation of the Heart in Diabetes: Part II: Potential Mechanisms Martin E. Young, Patrick McNulty and Heinrich Taegtmeier

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