

# Insulin Signaling and Heart Failure

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**Abstract:** Heart failure is associated with generalized insulin resistance. Moreover, insulin-resistant states such as type 2 diabetes mellitus and obesity increases the risk of heart failure even after adjusting for traditional risk factors. Insulin resistance or type 2 diabetes mellitus alters the systemic and neurohumoral milieu, leading to changes in metabolism and signaling pathways in the heart that may contribute to myocardial dysfunction. In addition, changes in insulin signaling within cardiomyocytes develop in the failing heart. The changes range from activation of proximal insulin signaling pathways that may contribute to adverse left ventricular remodeling and mitochondrial dysfunction to repression of distal elements of insulin signaling pathways such as forkhead box O transcriptional signaling or glucose transport, which may also impair cardiac metabolism, structure, and function. This article will review the complexities of insulin signaling within the myocardium and ways in which these pathways are altered in heart failure or in conditions associated with generalized insulin resistance. The implications of these changes for therapeutic approaches to treating or preventing heart failure will be discussed. (*Circ Res.* 2016;118:1151-1169. DOI: 10.1161/CIRCRESAHA.116.306206.)

**Key Words:** diabetes mellitus ■ heart failure ■ insulin receptor ■ insulin resistance ■ myocardium

Cardiovascular disease remains the leading cause of death globally in developed and developing economies.<sup>1</sup> The morbidity from cardiovascular disease is broad based and includes consequences of atherosclerosis and hypertension leading to ischemic heart disease, stroke, and heart failure. Heart failure is a major cause of death in industrialized nations and exceeds the mortality of most types of cancers. It is estimated that ≈5.1 million patients are affected by heart failure in the United States. The annual incidence is ≈670 000 cases, with about half of these patients dying within 5 years after the initial diagnosis.<sup>2</sup> The associated annual costs are about \$34.4 billion, resulting in an enormous economic burden.<sup>3</sup> Thus, heart failure once it develops has a dismal prognosis that has not significantly improved, despite advances in pharmacological therapies. Much work has been done to examine diverse signaling pathways that contribute to heart failure, pathological cardiac hypertrophy, and adverse left ventricular (LV) remodeling and have been reviewed extensively.<sup>4,5</sup> A relatively understudied signaling pathway that could represent an important contributor to the pathophysiology of heart failure is insulin signaling, which will be the subject of this review.

The connection between abnormal insulin signaling and heart failure arises, in part, from the established epidemiological association between obesity, type 2 diabetes mellitus, insulin resistance, and heart failure. Diabetes mellitus increases the risk for ischemic heart disease as evidenced by a 2- to 4-fold increase in myocardial infarction in diabetic patients compared with nondiabetics and is associated with a poorer

prognosis.<sup>6</sup> In addition, coexistence of ischemic cardiomyopathy and diabetes mellitus accelerate the progression to heart failure.<sup>7</sup> Numerous epidemiological studies identified insulin resistance and diabetes mellitus as risk factors for the development of heart failure independent of myocardial ischemia. The Framingham Heart Study revealed that diabetes mellitus independently increases the risk of heart failure by ≈2-fold in men and ≈5-fold in women compared with age-matched control subjects, independent of blood pressure and serum cholesterol levels.<sup>8,9</sup> Furthermore, a longitudinal study in the same cohort identified obesity as a risk factor for the future incidence of heart failure in both men and women.<sup>10</sup> These data are supported by the United Kingdom Prospective Diabetes Study (UKPDS), which reported that the risk for developing heart failure increases by 16% for every 1% increase in serum hemoglobin A1c concentrations.<sup>11</sup> Similar results reporting an association between diabetes mellitus and the incidence of heart failure have been provided by the Cardiovascular Health Study,<sup>12</sup> the New Haven cohort of the Established Populations for Epidemiological Studies in the Elderly,<sup>13</sup> post hoc analyses of clinical trials<sup>14</sup> and have been reviewed comprehensively.<sup>15</sup>

In contrast, several studies reported a significant increase in survival in obese subjects in the context of heart failure<sup>16,17</sup> and after myocardial infarction after adjusting for age, severity of illness, and sex.<sup>18</sup> This resulted in the term obesity paradox, which has been previously reviewed.<sup>19,20</sup> Although obesity and type 2 diabetes mellitus are well-described and commonly accepted risk factors for the development of cardiovascular

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**Nonstandard Abbreviations and Acronyms**

<b>FA</b>	fatty acids
<b>FOXO</b>	forkhead box O
<b>GRK</b>	G-protein receptor kinase
<b>IGF-1</b>	insulin-like growth factor-1
<b>IRS</b>	insulin receptor substrates
<b>LV</b>	left ventricle
<b>mTOR</b>	mechanistic target of Rapamycin
<b>ROS</b>	reactive oxygen species

disease, these studies suggest a protective role of obesity when cardiac decompensation occurs. Given that cachexia is independently associated with a worse prognosis in heart failure, the possibility exists that the obesity paradox could, in part, reflect the adverse consequences of cachexia, which would lower body weight. These lower body weight subjects with heart failure were reported, for example, to have higher circulating concentrations of tumor necrosis factor  $\alpha$ ,<sup>21</sup> implying an adverse consequence of systemic inflammation in these subjects. In another cohort, the potential benefit of the obesity paradox in heart failure was seen in the elderly and those with acute heart failure and was not present in subject in diabetes mellitus.<sup>21</sup> Thus, many of the interactions between insulin resistance, obesity, and heart failure might differ between acute and chronic heart failure. The neurohumoral changes in obesity that might confer a survival benefit in acute heart failure are currently poorly understood. Moreover, the absence of a benefit in subjects with type 2 diabetes mellitus suggests that long-standing insulin resistance, hyperinsulinemia, or hyperglycemia might offset any survival advantages that can be attributed to the obesity paradox. Thus, the pathophysiology of the increased risk of heart failure in insulin-resistant states is complex and multifactorial, and the potential impact of generalized insulin resistance and hyperinsulinemia per se remains incompletely understood.

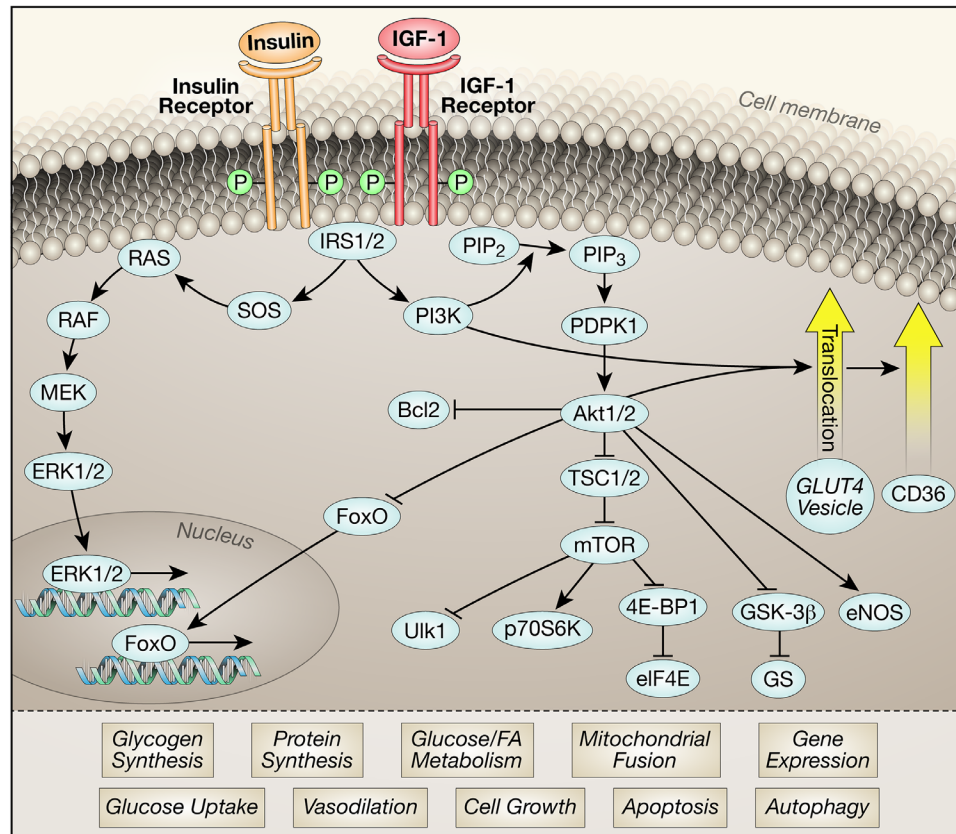
Moreover, studies in humans and animal models have revealed that heart failure is associated with generalized insulin resistance.<sup>22</sup> Metabolic abnormalities observed in patients with advanced heart failure resulting in catabolism, cardiac cachexia, and insulin resistance might correlate with decreased survival.<sup>23</sup> Although it is has not been definitively shown that insulin resistance in the context of heart failure directly contributes to a worse prognosis, a recent report suggested that ventricular unloading leads to improvement in whole-body insulin sensitivity.<sup>24</sup> This review will, therefore, explore the current state of knowledge linking generalized insulin resistance with heart failure with a focus on the physiological and pathophysiological roles of insulin signaling within the heart, the cardiac adaptations to generalized insulin resistance and the interaction between systemic insulin resistance and changes in myocardial insulin signaling, which may inform the pathophysiology of heart failure.

### Insulin Signaling Pathways

Insulin mediates its signaling following ligand binding to the insulin receptor, which is a member of the tyrosine kinase

family of receptors and is closely related to and partially homologous with the receptor for insulin-like growth factor-1 (IGF-1). On ligand binding, insulin receptors are autophosphorylated, which in turn increases its tyrosine kinase activity for other substrates, such as insulin receptor substrates (IRS) proteins (Figure 1). The activated insulin receptor interacts with IRS proteins and other binding partners to activate a network of intracellular signaling pathways, including phosphatidylinositol-3-kinase (PI3K)/Akt and MAP kinases, such as extracellular-regulated kinase.<sup>25,26</sup> As summarized in Figure 1, an essential initial step in IR/IGF-1R receptor signaling is activation of IRS proteins. IRS1 and IRS2 are the 2 most abundant IRS isoforms in the heart and are required for insulin-mediated activation of PI3K. PI3K consists of a p110 catalytic subunit and a p85 regulatory subunit, which catalyzes the generation of the lipid product PIP<sub>3</sub> (phosphatidylinositol (3,4,5)-triphosphate). This results in phosphoinositide-dependent kinase 1-mediated activation of the kinase Akt (protein kinase B). Three members of the Akt family have been identified. Akt1 and Akt2 represent the most abundant Akt isoforms in the heart. Akt mediates a variety of cellular processes by phosphorylating proteins involved in autophagy and members of the forkhead box O transcription factor (FOXO) family, which inhibits nuclear translocation and transcriptional activity<sup>27</sup> of promoters that encode proapoptotic signaling molecules, such as members of the BCL-2 (B-cell lymphoma 2) family. The isoform-specific contribution of Akt on the regulation of these processes is incompletely understood. However, Akt1 but not Akt2 signaling has been identified to mediate somatic growth.<sup>28,29</sup> Furthermore, it has been shown that Akt2 is required for insulin-stimulated glucose uptake and metabolism in isolated cardiomyocytes, and this effect is independent of Akt1.<sup>30</sup> Together, this suggests a predominant role for Akt1 in the regulation of somatic growth and for Akt2 in the regulation of metabolism. Inhibition of glycogen synthase kinase-3 $\beta$  by Akt-mediated phosphorylation promotes cardiac hypertrophy<sup>31</sup> and glycogen synthesis. In addition, Akt mediates phosphorylation of mechanistic target of Rapamycin (mTOR), a protein complex regulating somatic growth and protein synthesis. mTOR consists of 2 distinct complexes, mTORC1 and mTORC2.<sup>32</sup> Among other components, mTOR complex 1 (mTORC1) is composed of Raptor (regulatory-associated protein of mTOR).<sup>33</sup> Rictor (rapamycin-insensitive companion of mTOR) is part of mTOR complex 2 (mTORC2).<sup>34</sup> mTOR-mediated phosphorylation of 70-kDa ribosomal protein S6 kinase (p70S6K) and 4E-binding protein 1<sup>35</sup> results in its dissociation from eukaryotic translation initiation factor 4E and activation of cap-dependent translation,<sup>36</sup> which is attenuated by the mTOR inhibitor Rapamycin.

In addition to activating the Akt/mTOR signaling cascade and mediating GLUT4 translocation, insulin activates the mitogen-activated protein kinase/extracellular-regulated kinase pathway. This pathway involves activation of a cascade including Raf, MEK, and extracellular-regulated kinases, resulting in nuclear translocation of extracellular-regulated kinase and phosphorylation of transcription factors such as p62TCF, initiating a transcriptional program that mediates cellular differentiation and proliferation.<sup>37</sup> A variety of genetically



**Figure 1. Schematic representation of insulin signaling pathway.** Simplified summary of key elements involved in insulin signal transduction that ultimately regulate multiple cellular processes. On binding to its ligand, insulin and insulin-like growth factor-1 (IGF-1) receptors undergo autophosphorylation, which increases their tyrosine kinase activities. Tyrosine phosphorylation and activation of the docking proteins insulin receptor substrates 1 and 2 (IRS1/2), engages regulatory subunits of the phosphatidylinositol-3-kinase (PI3K) that generates phosphatidylinositol (3,4,5)-triphosphate (PIP<sub>3</sub>) from phosphatidylinositol 3,4, bisphosphate (PIP<sub>2</sub>). The serine threonine kinases phosphoinositide-dependent protein kinase 1 (PDK1) and Akt1 or Akt2 bind to PIP<sub>3</sub> by their PH domains. PDK1 phosphorylates Akt on Thr 308, and mechanistic target of Rapamycin complex 2 (mTORC2) phosphorylates Akt1/2 on Ser 473. Once activated, Akt in turn phosphorylates multiple targets of which a subset is shown. Phosphorylation of these targets induces pleiotropic cellular responses: B-cell leukemia/lymphoma 2 (BCL-2) phosphorylation inhibits apoptosis, forkhead box O (FOXO) protein phosphorylation promotes nuclear exclusion, thereby repressing the expression of FOXO-regulated transcripts that mediate autophagy and apoptosis. Tuberous sclerosis complex (TSC) 1/2 phosphorylation promotes mTOR activation, which increases mRNA translation, promotes protein synthesis, cell growth, mitochondrial fusion, and also inhibits autophagy. Phosphorylation of glycogen synthase kinase (GSK) removes the repression of glycogen synthase, which in concert with increased availability of glucose promotes glycogen synthesis. Phosphorylation of endothelial nitric oxide synthase (NOSIII) or eNOS by Akt increases the generation of nitric oxide to promote vasodilation. Akt phosphorylation mediates the translocation of vesicles containing the GLUT4 glucose transporter, in part, by phosphorylating downstream targets such as AS160 (not shown), leading to increased glucose transport after insertion into the plasma membrane. Akt also mediates, in part, translocation of the fatty acid translocase CD36. Insulin signaling also promotes activation of the mitogen-activated protein kinases (extracellular-regulated kinase [ERK]1/2) to increase the expression of various genes.

altered mouse models with perturbed insulin/IGF-1 signaling has been used to investigate insulin signaling in the heart. The table summarizes the characteristics of previously described transgenic models under basal conditions and superimposed pathological stressors.

Insulin receptors are ubiquitously expressed and are highly abundant in tissues of the cardiovascular system, such as the heart and endothelial cells.<sup>89</sup> Given its identification as a hormone that is intimately involved in fuel metabolic homeostasis, much of the work on insulin signaling has focused on its impact on tissues such as adipose, liver, skeletal muscle, and brain and the mechanisms by which insulin regulates glucose uptake, lipogenesis, and hepatic glucose utilization for example.<sup>90–92</sup> Subsequent studies have revealed that insulin signaling by activating cell survival pathways

may play an important role in the regulation of apoptosis and autophagy and cellular growth.<sup>38,49</sup> Thus, a brief review of the physiological role of insulin signaling in the heart and vasculature is warranted to set the stage for a deeper discussion of the pathophysiological consequences of insulin signaling in heart failure.

### Insulin Signaling in the Vasculature

The major cell types in the vasculature in which insulin signaling has been evaluated are endothelial and vascular smooth muscle cells. In endothelial cells, insulin signaling via PI3K and Akt leads to activation of endothelial nitric oxide synthase. Nitric oxide release not only promotes vasorelaxation but may also activate anti-inflammatory and antiatherosclerotic pathways. As such, impaired endothelial insulin signaling has been suggested to contribute to

**Table. Rodent Models for Perturbed Insulin/IGF-1 Signaling**

Basal Phenotype						Phenotype After Superimposed Pathological Stressor				
Signaling Molecule	Genetic Manipulation/Treatment	Cardiac Size	Contractile Function	Notes	References	Stressor	Cardiac Size	Contractile Function	Notes	References
<b>IR/IGF-1R</b>										
IR	CKO	↓	=/ ↓	Age-dependent mitochondrial dysfunction	Belke et al, <sup>38</sup> Boudina et al, <sup>39</sup> Kim et al, <sup>40</sup> and Hu et al <sup>41</sup>	ISO	=	↓	Catecholamine-mediated injury ↑	McQueen et al <sup>42</sup>
						AC	=	↓	Susceptibility to heart failure ↑	Hu et al <sup>41</sup>
						MI	=	↓	LV dysfunction post MI ↑	Sena et al <sup>43</sup>
						STZ	↓	=	Mitochondrial dysfunction ↑/cardiac efficiency ↓	Bugger et al <sup>44</sup>
	IPC			Efficacy of IPC to reduce infarct size ↑	Fullmer et al <sup>45</sup>					
	CKO het	=	=		Shimizu et al <sup>46</sup>	AC	↓	↑		Shimizu et al <sup>46</sup>
<b>IGF-1R</b>										
IGF-1R	CKO	=	=		Kim et al <sup>40</sup>					
	MKO	↑	=		Laustsen et al <sup>47</sup>					
IR/IGF-1R DKO	MKO	↓	↓↓		Laustsen et al <sup>47</sup>					
<b>IRS</b>										
IRS1	CKO	↓	=		Riehle et al <sup>48</sup>					
IRS2	CKO	=	=		Riehle et al <sup>48</sup>					
IRS1/2 DKO	CKO	=/ ↓	↓↓		Riehle et al <sup>49</sup> and Qi et al <sup>50</sup>					
	MKO	=	↓↓		Long et al <sup>51</sup>					
	GKO	ND	ND	Embryonic lethal?	Withers et al <sup>52</sup>					
	CKOi	↓	↓↓		Qi et al <sup>53</sup>					
IRS3	GKO	=			Liu et al <sup>54</sup>					
IRS4	GKO	NR			Fantin et al <sup>55</sup>					
<b>PI3K</b>										
p85	p85α MKO p85β GKO	↓	=		Luo et al <sup>56</sup>					
p110α	dn	↓	=		McMullen et al <sup>57</sup> and Shioi et al <sup>58</sup>	AC	=	↓		McMullen et al <sup>57</sup> and McMullen et al <sup>59</sup>
						MI	=	↓		Lin et al <sup>60</sup>
						DCM		↓	Survival after cross ↓	McMullen et al <sup>59</sup>
	ca	↑	=		Shioi et al <sup>58</sup>	AC	=	↑		McMullen et al <sup>59</sup>
						MI	=	↑		Lin et al <sup>60</sup>
						DCM		↑	Survival after cross ↑	McMullen et al <sup>59</sup>
p110γ	GKO	=	↑		Oudit et al <sup>61</sup>	AC		↓		Patrucco et al <sup>62</sup>
						ISO		↑		Oudit et al <sup>61</sup>

(Continued)

Table. Continued

Basal Phenotype						Phenotype After Superimposed Pathological Stressor				
Signaling Molecule	Genetic Manipulation/Treatment	Cardiac Size	Contractile Function	Notes	References	Stressor	Cardiac Size	Contractile Function	Notes	References
PDK1										
PDK1	CKO		↓↓	β1-adrenergic receptor ↓	Ito et al <sup>63</sup> and Di et al <sup>64</sup>					
	MKO	↓	↓↓	Dilated cardiomyopathy	Mora et al <sup>65</sup>					
Akt										
Akt1	GKO	=	=		O'Neill et al <sup>66</sup> and DeBosch et al <sup>67</sup>	AC	↑	↓		DeBosch et al <sup>67</sup>
	GKO het		=		Shimizu et al <sup>46</sup>	AC		↑		Shimizu et al <sup>46</sup>
Akt2	GKO	=/↑	=/↓	Age-dependent hypertrophy and contractile dysfunction	DeBosch et al, <sup>30</sup> O'Neill et al, <sup>66</sup> and Etzion et al <sup>68</sup>	AC	=	=		DeBosch et al <sup>30</sup>
						ISO	↑	=		Etzion et al <sup>68</sup>
						MI		↓	Infarct size ↑	Li et al <sup>69</sup>
Akt3	COE	↑	↓	Age-dependent contractile dysfunction	Taniyama et al <sup>70</sup>					
	GKO	=			Easton et al <sup>71</sup>					
kdAkt	kd	=	=		Shioi et al <sup>72</sup>					
caAkt	ca	↑	↓	Age-dependent contractile dysfunction	Shioi et al <sup>72</sup>					
	myrAkt	↑	=		Matsui et al <sup>73</sup>	MI			Infarct size ↓	Matsui et al <sup>73</sup>
GSK-3β										
GSK-3β	ca	=			Antos et al <sup>74</sup>	AC	↓			Antos et al <sup>74</sup>
	dn	↑	=		Hirotsani et al <sup>75</sup>	AC	=	↑		Hirotsani et al <sup>75</sup>
mTOR										
mTOR	Rapamycin	=	=		Shioi et al <sup>76</sup> and McMullen et al <sup>77</sup>	AC	↓	=/↑		Shioi et al <sup>76</sup> and McMullen et al <sup>77</sup>
						MI	↓	↑	LV remodeling and cell death ↓	Di et al <sup>78</sup>
	CKO (mTOR)	↓	↓↓	Embryonic lethal	Zhu et al <sup>79</sup>					
	CKOi (mTOR)		↓↓		Zhang et al <sup>80</sup>	AC	↓	↓↓	Rapid development of heart failure post AC	Zhang et al <sup>80</sup>
	CKOi (raptor)		↓	Autophagy ↑, Glucose oxidation ↑, Palmitate oxidation ↓	Shende et al <sup>81</sup>	AC	↓	↓↓	Rapid development of heart failure post AC	Shende et al <sup>81</sup>

(Continued)

Table. Continued

Basal Phenotype						Phenotype After Superimposed Pathological Stressor				
Signaling Molecule	Genetic Manipulation/Treatment	Cardiac Size	Contractile Function	Notes	References	Stressor	Cardiac Size	Contractile Function	Notes	References
	CKOi (mTOR)	=	↓	Glucose oxidation ↑, Palmitate oxidation ↓	Zhu et al <sup>82</sup>					
	ca	=	=		Shen et al <sup>83</sup>					
	kd	=	↓		Shen et al <sup>83</sup>	ISO	=	=	Hypertrophic response similar to wildtype	Shen et al <sup>83</sup>
p70S6K										
p70S6K1	TG	↑	=	Mutant with higher basal activity	McMullen et al <sup>84</sup>	AC	=			McMullen et al <sup>84</sup>
	Kd	=	=		McMullen et al <sup>84</sup>					
	GKO	=			McMullen et al <sup>84</sup>	AC	=			McMullen et al <sup>84</sup>
p70S6K2	kd	=	=		McMullen et al <sup>84</sup>					
	GKO	=			McMullen et al <sup>84</sup>	AC	=			McMullen et al <sup>84</sup>
p70S6 K1/2	GKO	=			McMullen et al <sup>84</sup>	AC	=			McMullen et al <sup>84</sup>
GLUT4										
GLUT4	CKO	↑	=		Abel et al <sup>85</sup> and Li et al <sup>86</sup>					
	MKO ( $\alpha$ -actin-Cre)	↑	↓	Age-dependent contractile dysfunction	Kaczmarczyk et al <sup>87</sup> and Huggins et al <sup>88</sup>					

Changes are expressed compared with wildtype controls same treatment. ↑ indicates increased; ↓, decreased; =, no difference observed; AC, aortic constriction; ca, constitutively active mutant; CKO het, cardiac-specific heterozygous knockout ( $\alpha$ MHC-Cre); CKO, cardiac-specific knockout ( $\alpha$ MHC-Cre); CKOi, inducible cardiac-specific knockout; COE, cardiac-specific over expression; DCM, cross to transgenic mouse model of dilated cardiomyopathy; dn, dominant-negative mutant; GKO het, global heterozygous knockout; GKO, global knockout; GSK-3 $\beta$ ; glycogen synthase kinase 3 $\beta$ ; IGF, insulin-like growth factor-1; IPC, ischemic preconditioning; IRS, insulin receptor substrates; ISO, Isoproterenol treatment; kd, kinase-deficient mutant; LV, left ventricle; MI, myocardial infarction; MKO, muscle-specific knockout (MCK-Cre, unless otherwise indicated); mTOR, mechanistic target of Rapamycin; myrAkt, myristoylated Akt; ND, not determined; PI3K, phosphatidylinositol-3-kinase; STZ, streptozotocin treatment; and TG, transgenic model.

obesity and diabetes mellitus-related vascular dysfunction that may contribute to hypertension and accelerated atherosclerosis.<sup>93,94</sup>

It is important to note that although there is broad consensus that impaired nitric oxide availability will contribute to vascular pathology in insulin-resistant states, impairment of eNOS activity may occur independently of defects in insulin signaling per se.<sup>95-97</sup>

Another important consideration in understanding impaired endothelial insulin signaling in the context of insulin-resistant states is the concept of imbalanced insulin signaling, whereby peripheral hyperinsulinemia, leads to activation of certain branches of the insulin signaling pathway, whereas others remain inhibited. For example, in certain circumstances, although insulin signaling to PI3K-Akt and eNOS might be impaired in endothelial cells, activation of mitogen-activated protein kinase signaling pathways leading to induction of endothelin1 expression might remain active and indeed be activated by ambient hyperinsulinemia, thereby leading to increased vasoconstriction.<sup>98-100</sup> Similarly, in vascular

smooth muscle cells, the hyperinsulinemia that accompanies insulin-resistant states has been suggested to be a contributor to vascular smooth muscle cell hyperplasia, which may promote intimal injury and vascular stenosis.<sup>101</sup> The role of impaired insulin signaling in the coronary vasculature and the pathophysiology of heart failure in insulin-resistant states is incompletely understood. However, subjects with insulin resistance and type 2 diabetes mellitus were shown to have significantly impaired endothelial-dependent coronary vasodilation.<sup>102</sup> Thus, the possibility exists that impaired coronary vasodilation in insulin-resistant states could contribute to impaired myocardial contractile reserve in heart failure, particularly in subjects with insulin resistance.

### Insulin Signaling and the Regulation of Substrate Metabolism in Cardiomyocytes

Like skeletal muscle, activation of insulin signaling pathways in cardiomyocytes leads to an increase in glucose uptake by

promoting the translocation of GLUT4-containing vesicles to the plasma membrane.<sup>103</sup> These vesicles ultimately fuse with the plasma membrane and expose GLUT4 proteins on the cellular surface. This process involves the modulation of filamentous actin networks, soluble NSF attachment protein receptor (SNARE) proteins, and small GTPases of the Rab family.<sup>104</sup> Insulin signaling mediates GLUT4 translocation at multiple checkpoints. Recently identified mechanisms include Akt-mediated phosphorylation of Akt substrate of 160 kDa (AS160), a GTPase-activating protein for the Rab family of small G proteins. Phosphorylation of AS160 in response to insulin regulates its interaction with 14-3-3 and inhibits AS160 GTPase activity. This results in increased Rab activity and translocation of GLUT4 vesicles to the plasma membrane.<sup>105,106</sup> It has also been shown that selective activation of Akt might be sufficient to stimulate GLUT4 translocation to an extent which is similar to insulin.<sup>107</sup> However, Akt-independent mechanisms have also been identified.<sup>106</sup> In perfused hearts or in vivo, the increase in glucose utilization (uptake and oxidation) that is mediated by insulin signaling is associated with reduced fatty acid (FA) oxidation on the basis of the Randle phenomenon.<sup>38,108</sup>

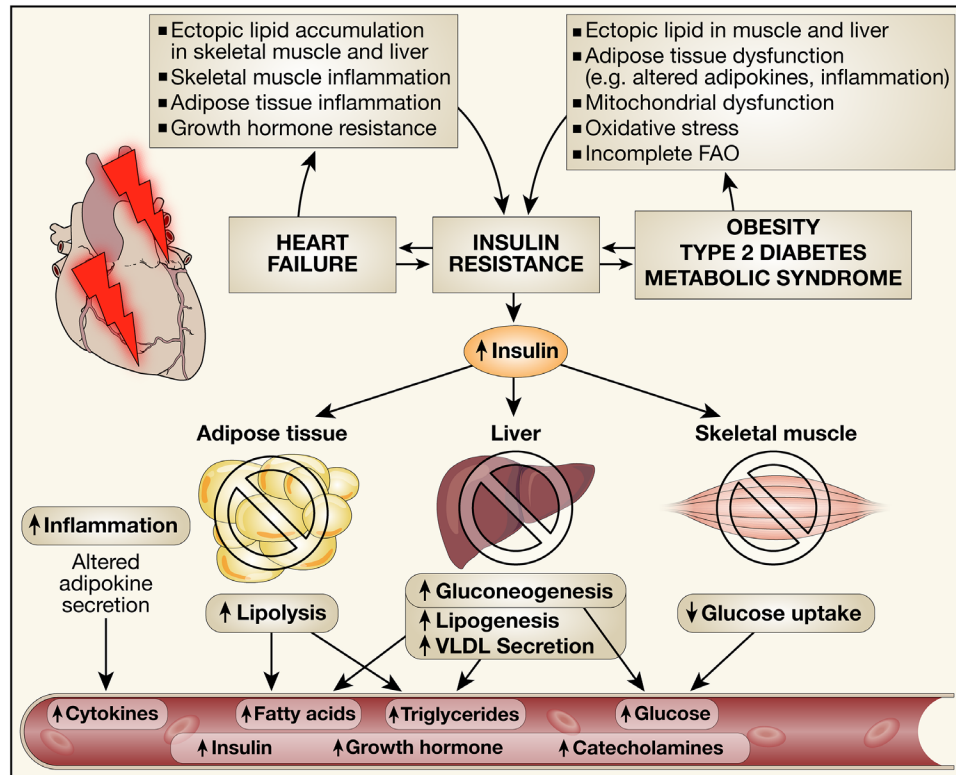
Transmembrane uptake of FAs in cardiomyocytes is mediated, in part, by fatty acyl translocases of the FA transporter family and CD36.<sup>109</sup> FAs are then converted to FA-CoA by the enzyme Acyl-CoA synthase and may be stored in the form of triglycerides. However, excess lipids may also be shunted into nonoxidative pathways, which results in the generation of toxic lipid intermediates. This eventually results in mitochondrial dysfunction,<sup>110</sup> perturbed cellular signaling,<sup>111</sup> incomplete FA oxidation, and increased apoptosis,<sup>112</sup> a phenomenon called lipotoxicity.<sup>113</sup> Lipotoxicity has been extensively studied in animal models of obesity and type 2 diabetes mellitus, including *ob/ob*, *db/db* mice (models for obesity and insulin resistance based on leptin deficiency or resistance, respectively), and Zucker fatty rats.<sup>114</sup> Importantly though, insulin also promotes the translocation of the FA translocase CD36, which may increase FA uptake that may be partitioned toward lipid synthesis and storage.<sup>109</sup> This insulin-mediated increase and translocation of CD36 is transduced by PI3K/Akt signaling pathways<sup>115</sup> and may contribute to increased FA oxidation and increased myocardial oxygen consumption ( $mVO_2$ ) in hyperinsulinemic states that is often associated with increased availability of triglycerides and FA to the heart. Increased FA utilization is associated with decreased cardiac efficiency (cardiac work/ $mVO_2$ ). Studies in *ob/ob* and *db/db* mice suggest that mitochondrial uncoupling and increased reactive oxygen species (ROS) parallel the increase in FA oxidation.<sup>116,117</sup> Mitochondria-derived ROS can activate uncoupling proteins<sup>118</sup> allowing protons to bypass the ATP synthase of the electron transport chain and decrease coupling of mitochondrial ATP production to  $O_2$  consumption. As a consequence, FA oxidation further increases, which subsequently decreases cardiac efficiency. Similar to these studies performed in animal models, a recent study using human myocardial samples from type 2 diabetics showed mitochondrial dysfunction and increased oxidative stress.<sup>119</sup> Furthermore, increased  $mVO_2$  along with increased FA oxidation was reported for young women with obesity, insulin resistance, and increased body

mass index.<sup>120</sup> Increased ROS production and mitochondrial uncoupling were not observed in rodent models of type 1 diabetes mellitus.<sup>121</sup> This suggests that ROS-mediated mitochondrial uncoupling might not be attributable to hyperglycemia per se, but is more likely a consequence of generalized insulin resistance and type 2 diabetes mellitus. Interestingly, hearts from cardiomyocyte-specific insulin receptor knockout mice show increased ROS generation and mitochondrial uncoupling, in the absence of hyperglycemia.<sup>39</sup>

Insulin may also directly regulate mitochondrial metabolism by promoting mitochondrial fusion via a mechanism that involves the induction of OPA1 (optic atrophy 1).<sup>122</sup> Short-term insulin-mediated Akt activation may also activate prosurvival signaling pathways related to Akt inhibition of apoptotic signaling.<sup>123</sup> Studies in gene-targeted mice with cardiomyocyte-restricted loss of insulin receptors or IRS1 proteins have also suggested that insulin signaling may play an important trophic role within the heart.<sup>38</sup> Thus, loss of insulin receptors or IRS1 is associated with a reduction in cardiomyocyte size with little hemodynamic consequences in nonstressed animals.<sup>38,48</sup> In addition, insulin/IGF-1 or IRS1 or IRS2 signaling pathways seem to be required for physiological cardiac hypertrophy in response to exercise.<sup>40,48,124</sup> Insulin signaling also regulates mitochondrial oxidative capacity. Thus, mice with genetic deletion of the insulin receptor or IRS proteins develop reduced mitochondrial oxidative capacity via partially understood mechanisms that may involve repressed expression of nuclear-encoded mitochondrial genes.<sup>39,48,49</sup> We have also shown that activation of PI3K signaling is responsible for increasing mitochondrial oxidative capacity in response to physiological cardiac hypertrophy.<sup>48,66,125</sup> However, this effect of PI3K seems to be independent of changes in Akt signaling and as will be discussed later, constitutive activation of Akt seems to repress mitochondrial oxidative capacity.

### Insulin Resistance

Insulin resistance has been classically defined as the inability of insulin to promote its metabolic actions in organs, such as adipose tissue, skeletal muscle, and liver (Figure 2). Insulin resistance is a hallmark of obesity and type 2 diabetes mellitus and also is a characteristic of heart failure. Thus, in insulin-resistant states there is impaired insulin-mediated glucose uptake in muscle and adipocytes, impaired suppression of hepatic glucose production and impaired suppression of lipolysis.<sup>126</sup> Paradoxically, triglyceride synthesis and hepatic secretion of very low-density lipoprotein is increased on the basis of increased insulin-mediated activation of lipogenesis.<sup>127</sup> This underscores the important concept of selective insulin resistance, indicating that although certain targets of insulin signaling might be repressed in insulin-resistant states, other targets may retain their sensitivity.<sup>128</sup> The metabolic milieu in insulin-resistant individuals is, therefore, characterized by increased circulating concentrations of free fatty acids and triglycerides, and variable increases in glucose concentrations. Moreover,  $\beta$  cells adapt to the insulin-resistant environment by increasing insulin release, leading to hyperinsulinemia. In addition to these systemic metabolic abnormalities, insulin resistance is associated with additional changes to the systemic metabolic



**Figure 2. Summary of mechanisms that lead to insulin resistance in heart failure or in the metabolic syndrome.** Signaling events in skeletal muscle, liver, adipose tissue, and brain (not shown) impair insulin signaling in each respective organ leading to metabolic perturbations as illustrated, which alter the systemic milieu in ways that may adversely affect cardiac structure and function.

milieu, including low-grade inflammation, increasing circulating concentrations of various cytokines, and altered secretion of adipokines, such as leptin, resistin, and adiponectin.<sup>129,130</sup> Mechanisms for insulin resistance include ectopic accumulation of lipid intermediates in skeletal muscle and liver. In the liver, a proposed mechanism for steatosis is hyperinsulinemia-driven de novo lipogenesis in the face of excess caloric intake. The molecular mechanisms for hepatic insulin resistance are incompletely understood; however, increased endoplasmic reticulum stress, ROS generation, and mitochondrial dysfunction have been suggested to contribute to the development of insulin resistance.<sup>131</sup> Ectopic lipid accumulation and oxidative stress have been proposed to contribute to skeletal muscle insulin resistance.<sup>132</sup> Additional mechanisms contributing to insulin resistance include adipose tissue dysfunction that impairs the release of insulin sensitizing adipokines, such as adiponectin, mitochondrial dysfunction, incomplete FA oxidation, and activation of inflammatory responses leading to increased promulgation of inflammatory cytokines such as tumor necrosis factor  $\alpha$  and interleukins. Mechanistically, tumor necrosis factor  $\alpha$ , interleukin-6, and free fatty acids activate intracellular kinases that induce serine phosphorylation of IRS proteins, thereby attenuating insulin signaling and inducing insulin resistance.<sup>133</sup> This complex pathophysiology of insulin resistance has been classically associated with obesity and a sedentary lifestyle. However, in heart failure generalized insulin resistance develops in patients with cardiac cachexia. Most studies seeking to explore the mechanisms for insulin resistance have been performed in subjects with obesity or type 2

diabetes mellitus. Fewer studies have been conducted in patients with heart failure.

Insulin resistance in heart failure also correlates with increased serum concentrations of proinflammatory cytokines, catecholamines, catabolic steroids, and growth hormone.<sup>134–138</sup> In addition, decreased physical activity, loss of skeletal muscle mass, and sympathetic overactivity increases lipolysis, thereby increasing the circulating concentrations of free fatty acids, which may further exacerbate insulin resistance in skeletal muscle and liver by promoting the accumulation of bioactive lipids. Free fatty acids may further increase the activity of the sympathetic nervous system,<sup>139</sup> which may independently attenuate peripheral insulin signaling and reduce glucose utilization in skeletal muscle.<sup>140</sup> Furthermore, increased catecholamine concentrations stimulate hepatic glycogenolysis and gluconeogenesis, leading to increased circulating glucose. Heart failure is also associated with hyperactivation of the renin–angiotensin–aldosterone system, which has been implicated in the pathophysiology of insulin resistance.<sup>141</sup> In addition to increasing circulating concentrations of angiotensinogen (originating from adipose tissue),<sup>142</sup> chronic hyperinsulinemia may also increase the expression of angiotensin II receptors, which may further exacerbate adverse LV remodeling.<sup>143</sup> Recent studies have also indicated that heart failure patients with insulin resistance also exhibit evidence of ectopic accumulation of bioactive lipids such as ceramide in their skeletal muscles, adipose tissue inflammation, and hypoadiponectinemia, all of which may contribute to generalized insulin resistance.<sup>144,145</sup> Moreover, skeletal



muscle hypoperfusion impairs skeletal muscle oxidative capacity. This induces growth hormone resistance and a state of low-grade inflammation, which might also contribute to insulin resistance.<sup>146–148</sup> Growth hormone resistance described in patients with heart failure results in decreased hepatic IGF-1 production in response to growth hormone, but circulating growth hormone concentrations increase.<sup>137</sup> Increased growth hormone receptor signaling has been shown to induce insulin resistance in multiple tissues including the heart,<sup>149–151</sup> thereby exacerbating peripheral insulin resistance, which might further impair cardiac function (Figure 2).

The changes in the metabolic milieu summarized above may contribute to the myocardial maladaptations that have been described in insulin-resistant states, often referred to as diabetic cardiomyopathy and which have been extensively reviewed.<sup>152,153</sup> In brief and as outlined above, multiple mechanisms have been proposed to contribute to the increased vulnerability of the heart in insulin-resistant states, such as increased FA utilization, impaired mitochondrial oxidative capacity, mitochondrial dysfunction, decreased cardiac efficiency, oxidative stress, accumulation of bioactive lipids, inflammation, increased apoptosis, altered calcium metabolism and signaling, increased apoptosis and myocardial fibrosis. In addition, expansion of local adipose tissue depots might compromise cardiac function under conditions of obesity and type 2 diabetes mellitus. Epicardial adipose tissue is a visceral-like adipose depot that is located on the surface of the ventricles, along the coronary arteries, and at the apex of the heart. Perivascular adipose tissue surrounds the blood vessels. Epicardial adipose tissue mass and diameter are increased in obesity,<sup>154,155</sup> and the volume of perivascular adipose tissue is associated with visceral obesity.<sup>156</sup> Neither epicardial adipose tissue nor perivascular adipose tissue are separated by a fascial layer from the surrounding tissue. Therefore, it has been suggested that adipokines and possibly FAs secreted from epicardial adipose tissue and perivascular adipose tissue might directly contribute to the development of cardiovascular dysfunction in type 2 diabetes mellitus.<sup>157</sup> The induction of generalized insulin resistance and hyperinsulinemia in the context of pressure overload accelerates LV remodeling.<sup>46,158</sup> Thus, it is plausible that insulin resistance that accompanies heart failure could further exacerbate myocardial dysfunction by similar mechanisms. There is a paucity of studies that have directly addressed the possible beneficial effects of therapeutic manipulations that might increase insulin sensitivity in patients with heart failure to determine if such changes will meaningfully impact LV function or alter long-term prognosis. In a cohort of patients with advanced heart failure, the Schulze group<sup>24</sup> recently reported that mechanical unloading resulted in a significant reduction in indices of systemic insulin resistance that occurred in concert with improvement in hemodynamic indices. Their study design did not address the question of whether the improvement in insulin sensitivity directly contributed to improved LV function. However, the authors reported that improvement in insulin sensitivity did correlate with reversal of certain metabolic and signaling abnormalities in the heart. An important question that these observations raise is: what are the contributions of altered insulin

signaling and insulin resistance per se in cardiomyocytes and the potential contribution of these changes to heart failure and LV remodeling?

### Cardiac Muscle Insulin Signaling in Insulin-Resistant States or in Heart Failure

Before summarizing important studies in the field, it is important to discuss the various ways in which myocardial insulin resistance has been defined in the literature. In most instances, insulin resistance has been defined as a reduction in insulin-mediated myocardial glucose uptake. This definition is advantageous in the sense that it enables the inclusion of noninvasive studies that evaluate myocardial glucose utilization. The limitation, however, is that this definition of myocardial insulin resistance does not account for changes in the activation of signaling intermediates that are downstream of the insulin receptor. For example, a reduction in GLUT4 protein could account for decreased insulin-mediated glucose uptake in the face of an otherwise intact insulin signaling pathway, whereas impaired intracellular signaling could also reduce insulin-mediated glucose uptake even when GLUT4 protein levels are normal. As will be discussed, intact versus impaired intracellular signaling might influence LV remodeling in distinct ways.

Nearly, all studies that have examined insulin-stimulated glucose uptake in humans or in animals with generalized insulin resistance, usually induced by diet-induced obesity, have demonstrated impaired insulin-mediated glucose uptake.<sup>159</sup> Studies of myocardial glucose uptake in subjects with heart failure need to be interpreted in light of whether measurements of glucose uptake were performed under basal conditions or in response to physiological or pharmacological hyperinsulinemia as would occur during a euglycemic hyperinsulinemic glucose clamp. In many animal studies in which heart failure is induced by aortic banding (thoracic or abdominal) or by coronary artery ligation, basal glucose uptake has been reported to be increased, and this has been attributable to an increase in the GLUT1 transporter.<sup>160–163</sup> However, when the augmentation of glucose uptake in response to insulin was evaluated, the fold increase in insulin-mediated glucose uptake was impaired relative to nonfailing control groups.<sup>164</sup> Importantly, in contrast to GLUT1, GLUT4 protein levels in the hearts of experimental models of heart failure or pathological cardiac hypertrophy are variable<sup>161,162,165,166</sup> and the impact of GLUT4 expression and translocation in the context of heart failure is incompletely understood. Studies in transgenic mouse lines revealed that GLUT4 expression is dispensable for contractile function under basal conditions,<sup>85</sup> whereas these mice exhibited increased fibrosis<sup>167</sup> and a poor response to ischemic injury.<sup>168</sup> In contrast, deletion of GLUT1 in the hearts of mice did not exacerbate heart failure progression after transverse aortic constriction.<sup>161</sup> Thus, it is likely that GLUT1- and GLUT4-mediated glucose transport may play divergent as well as overlapping roles in the modulation of myocardial glucose uptake in heart failure. It is important to note that although myocardial glucose utilization might be increased in compensated stages of LV hypertrophy and early in the course of heart

failure, glucose utilization may be reduced in the hearts of patients and animals with advanced heart failure.<sup>169</sup>

Insulin stimulation of the heart activates PI3K and Akt, which are requisite upstream signaling steps that promote GLUT4 translocation and increased glucose uptake. Studies of insulin-mediated activation of Akt in the heart in rodent models of generalized insulin resistance have yielded variable and potentially divergent results, which might be explicable by differences in the models used and differences in experimental conditions, such as dietary lipid composition. Studies based on 45% fat diets of varying durations have suggested that the ability of insulin to stimulate Akt might be augmented or preserved at a time when insulin-mediated glucose uptake is impaired.<sup>170</sup> In contrast, studies in more severe models of insulin resistance such as ob/ob mice or after a more aggressive high-fat protocol with 60% fat leads to impaired insulin-mediated activation of Akt and with FOXO1 dephosphorylation and nuclear localization.<sup>171</sup> It is important to put in context that the usual rodent diet is significantly lower in fat than human diets. Thus, caution is warranted in extrapolating findings from rodent studies in which a large increase in dietary fat is imposed for variable periods of time, given that species-specific differences may exist in the myocardial adaptations to a pathological lipid load, relative to those that might already be partially adapted to diets that are higher in fat content. In studies of failing hearts in experimental models, usually induced by transverse aortic constriction (on normal chow), Akt phosphorylation is increased in concert with increased IRS1 phosphorylation.<sup>46,172</sup> In 1 study, genetically reducing the expression of insulin receptors, Akt1 or inducing systemic hypoinsulinemia reduced Akt phosphorylation and interestingly attenuated cardiac hypertrophy, and prevented adverse LV remodeling and heart failure.<sup>46</sup>

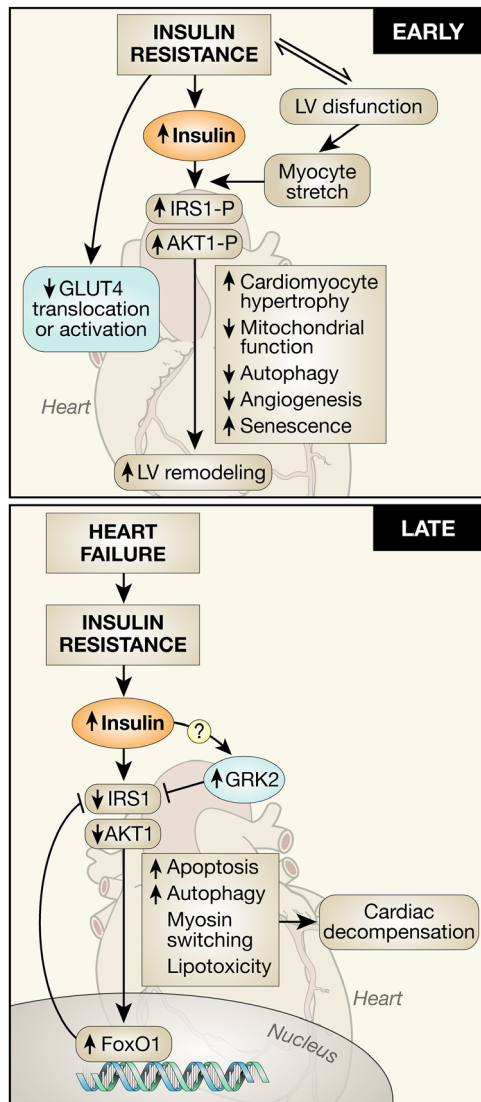
Limited availability of human heart tissue has hampered the ability of investigators to evaluate changes in insulin signaling pathways in heart failure or insulin-resistant states. Early studies suggested dynamic regulation of uncoupling protein and GLUT4 content in human heart atrial appendages in response to increasing circulating FA concentrations, indicating that increased myocardial lipid delivery could repress myocardial GLUT4.<sup>173</sup> Subsequently in an elegant study combining LV biopsy with metabolic analyses, Cook et al<sup>174</sup> reported that a significant reduction in plasma membrane GLUT4 accounted for the reduction in myocardial glucose utilization in humans with heart failure (average ejection fraction, 27%) or in subjects with type 2 diabetes mellitus without heart failure, despite minimal changes in overall GLUT4 content. The depletion of sarcolemmal GLUT4 was corroborated by animal studies that revealed that only 2 weeks of high-fat feeding significantly reduced myocardial GLUT4 content and sarcolemmal translocation even before any weight gain and at a time when myocardial insulin signaling was preserved.<sup>170</sup> Importantly, the human studies by Cook et al<sup>174</sup> also showed that PI3K and Akt signaling was substantially increased in the hearts of subjects with type 2 diabetes mellitus or heart failure and positively correlated with circulating insulin concentrations and with indices of insulin resistance. These studies suggest a paradigm whereby insulin-mediated glucose uptake in the heart in

insulin-resistant states could be dissociated from activation of insulin signaling to PI3K and Akt. The mechanisms contributing to reduced GLUT4 expression and translocation in the heart in obesity are incompletely understood, but could be due, in part, to altered regulation of SNARE proteins.<sup>174</sup>

In contrast, in an analysis of LV samples obtained from subjects with advanced heart failure at the time of left ventricular assist device implantation, Chokshi et al<sup>24</sup> reported a significant repression in myocardial Akt phosphorylation that occurred in concert with evidence of accumulated bioactive lipids such as diacyl glycerol and ceramides, leading to activation of protein kinase C isoforms that have been implicated as mediators of impaired insulin signaling. In materials obtained after mechanical unloading and after evidence of recovery of LV function, these signaling changes were reversed, leading to the conclusion that in end-stage heart failure there is significant repression of insulin signaling pathways and that recovery correlated with improvement in insulin action. In a study from a different group, mechanical unloading was associated with a decrease in the phosphorylation of the Akt target FOXO3 in human hearts.<sup>175</sup> This study did not measure Akt phosphorylation, but in light of other studies that have reported activation of Akt signaling in human failing hearts, the implications of this observation is that in some cases ventricular unloading could potentially reduce myocardial Akt signaling.

The complexity of the Akt–FOXO interactions is further underscored by studies showing that 6 months of treatment with a 60% high-fat diet leads to heart failure, which is associated with reduced Akt phosphorylation and dephosphorylation and nuclear localization of FOXO1.<sup>171</sup> Genetic deletion of FOXO1 prevented the development of heart failure and the associated metabolic abnormalities that was induced by high-fat feeding. Interestingly, genetic deletion of FOXO1 also restored insulin sensitivity by reducing serine phosphorylation of IRS1 proteins and restoring Akt phosphorylation in cardiomyocytes in response to insulin stimulation. Thus FOXO activation could represent an important mechanism leading to impaired insulin signaling in heart failure that is induced by lipotoxic cardiomyopathy. It is noteworthy that additional post-translational modifications of FOXO proteins could potentially drive nuclear localization<sup>176</sup>; therefore, it will be of interest to determine if nuclear FOXO localization precedes impaired insulin–IRS1–Akt signaling in metabolic cardiomyopathy or occurs as a consequence of it.

Taken together, these studies underscore the complex responses of insulin signaling pathways in the myocardium to the changes in the systemic metabolic milieu that characterizes insulin-resistant states or that accompany LV remodeling in the failing heart. Current experimental data and studies in human samples could suggest a model, whereby an early adaptation of the heart to systemic insulin resistance or to heart failure might be the repression of GLUT4 expression and GLUT4-mediated glucose transport. This occurs at a time when proximal insulin signaling to PI3K and Akt might remain intact and could potentially accelerate maladaptive ventricular hypertrophy. Over time, in response to persistent nutrient overload or to ongoing ventricular remodeling, FOXO activation (ie, increased nuclear localization) might accelerate the



**Figure 3. Molecular mechanisms linking increased or decreased insulin signaling with adverse left ventricular (LV) remodeling and heart failure progression.** In early stages of heart failure mediated by pressure overload, or in the early stages of the metabolic syndrome, hyperinsulinemia leads to activation of insulin receptor substrate 1 (IRS1) and Akt1 to promote pathological hypertrophy, mitochondrial dysfunction, and decreased autophagy, which contribute to accelerated LV remodeling. As heart failure progresses, despite systemic hyperinsulinemia insulin signaling pathways may become desensitized leading to loss of cytoprotective consequences of Akt signaling and persistent nuclear localization of forkhead box O (FOXO) proteins that will accelerate heart failure by various mechanisms, such as increased apoptosis and exacerbation of lipotoxicity.

desensitization of proximal insulin signaling leading to loss of Akt signaling and reduction in prosurvival signaling pathways that may further exacerbate cardiomyocyte loss. It has been proposed that myocardial insulin resistance might represent a mechanism by which the heart attempts to protect itself against nutrient overload in insulin-resistant states.<sup>177</sup> Early on, attempts to over-ride myocardial insulin resistance could exacerbate the early phases of adverse ventricular remodeling. However, it is possible that over time, a transition may take

place in which there is subsequent loss of proximal insulin signaling to Akt, which may exacerbate LV dysfunction via additional mechanisms related to further loss of trophic effects of insulin signaling on myocyte mass and mitochondrial capacity and loss of its antiapoptotic actions, all of which could accelerate or exacerbate LV dysfunction (Figure 3). We will next explore potential mechanisms linking changes in myocardial insulin signaling to LV dysfunction and adverse LV remodeling. Given the potential biphasic changes in insulin signaling that occur in the evolution of heart failure or systemic insulin resistance namely an activation of Akt, followed later by loss of insulin-mediated Akt signaling, pathways by which Akt activation may promote heart failure versus mechanisms linking impaired insulin signaling with heart failure will be discussed separately.

### Excessive Insulin Signaling and Heart Failure

As summarized above, there is a clear evidence that hyperactivation of proximal insulin signaling pathways, such as increased IRS1 phosphorylation and Akt hyperactivation, may occur in the context of pathological cardiac hypertrophy and subsequent heart failure, and that reduction of insulin or Akt signaling in this context might reverse LV remodeling and preserve cardiac function.<sup>46</sup> How might persistent activation of Akt lead to pathological hypertrophy? Interesting insights have been obtained from various lines of mutant mice with constitutive activation of various downstream components of the insulin signaling pathway. These models mimic the effects of hyperinsulinemia in type 2 diabetes mellitus and obesity on myocardial insulin signaling. Although constitutive activation of PI3K leads to compensated cardiac hypertrophy, our group recently showed that this leads to desensitization of insulin-mediated glucose uptake followed by impaired ability of insulin to activate Akt.<sup>103</sup> Of note, this impairment in glucose uptake is due, in part, to a defect in activation of glucose transport after insertion of GLUT4 in the plasma membrane.

In mice with inducible transgenic overexpression of Akt, cardiac hypertrophy is reversible and LV function preserved after short-term activation. However, if Akt expression is induced long term, heart failure ensues, in part to a failure of angiogenesis. Importantly, if Akt signaling is subsequently reduced in these failing hearts, the rate of progression to mortality is accelerated.<sup>178</sup> It is tempting to speculate that this temporal relationship between an adverse consequence of Akt activation leading to pathological remodeling that is then exacerbated by reduction of Akt expression might parallel the temporal relationship between an initial increase in Akt signaling in earlier stages of heart failure that subsequently transitions to reduced Akt signaling in end-stage cardiomyopathy. We recently reported an additional mechanism by which persistent Akt signaling impairs mitochondrial energy metabolism that likely contributes to the Akt-mediated LV dysfunction. The mechanism for mitochondrial dysfunction is because of Akt-dependent repression of the expression nuclear-encoded mitochondrial genes via FOXO-dependent and FOXO-independent signaling pathways. We also showed that short-term activation of Akt promotes a metabolic switch characterized by increased glycolysis while impairing mitochondrial FA oxidation and we

speculated that this short-term adaptation might represent a compensatory response to protect mitochondrial integrity and capacity. Importantly, the mitochondrial repression occurs early and is independent of the Akt-induced cardiac hypertrophy.<sup>172</sup> However, in the long term, a mismatch between impaired mitochondrial capacity and the increase in energetic requirements of Akt-mediated cardiac hypertrophy accelerates the onset of heart failure. Partial reduction of insulin or Akt signaling in murine models of transverse aortic constriction-induced cardiac hypertrophy prevents heart failure, which supports a model in which Akt-driven mechanisms might contribute to adverse LV remodeling.<sup>46</sup> It is important to emphasize though, that in these studies Akt signaling was not abolished, but rather maintained at basal levels. In contrast, when Akt1 or insulin receptor signaling is completely abolished, then in response to stressors such as pressure overload, catecholamine toxicity, or myocardial ischemia, all of these models exhibit a more rapid progression to heart failure.<sup>41–43,67</sup> Taken together, these observations suggest that although residual insulin-PI3K–Akt signaling is required to maintain cardiomyocyte viability, excessive signaling by these pathways drives adverse LV remodeling and ventricular dysfunction via multiple mechanisms.

We recently described that hyperinsulinemia may impair myocardial contractility via a mechanism by which the G-protein receptor kinase 2 (GRK2) leads to G<sub>i</sub>-biased  $\beta_2$ AR signaling that inhibits adenylate cyclase, cAMP generation, and cardiac contractility.<sup>179</sup> The inhibition of  $\beta$ AR signaling via GRK2 by hyperinsulinemia requires insulin receptor and IRS1-mediated signaling pathways (Figure 3). Ob/ob mice or mice that have been treated for 12 weeks with a 45% high-fat diet, exhibit increased myocardial GRK2 content.<sup>180</sup> GRK2 overexpression has been shown to reduce myocardial insulin signaling, and in germline GRK2 heterozygous knockout mice, GLUT4 content is increased, insulin signaling was enhanced in aging mice or when these animals were placed on a high-fat diet.<sup>180,181</sup> Moreover, reduced GRK2 signaling promoted a physiological cardiac hypertrophy profile as animals aged. Thus, GRK2 activation might also contribute to impairing myocardial insulin signaling with aging and in response to nutrient overload. GRK2 overactivation may also increase mitochondrial-mediated apoptosis by increasing mitochondrial calcium sensitivity, mitochondrial permeability transition pore opening, and cytochrome C release.<sup>181</sup> Taken together, GRK2 activation could represent another important mechanism linking hyperinsulinemia with adverse LV remodeling. Although increased sympathetic activity is an established mechanism by which GRK2 is induced in the heart, whether hyperinsulinemia induces GRK2 by this mechanism remains to be definitively elucidated.

Suppression of PI3K signaling in the heart was reported to reduce signs of cardiac aging, in part, by sustaining physiological levels of autophagy.<sup>182</sup> Ock et al<sup>183</sup> also recently reported that aging was associated with increasing expression of IGF-1 receptors in cardiomyocytes and in mice with deficiency of myocardial IGF-1R signaling, cardiac senescence, and age-dependent cardiac fibrosis was reduced. Finally, hyperactivation of insulin signaling pathways will lead to autophagy suppression.<sup>184</sup> Autophagy plays a critical role in organellar quality control, thus as has been recently reviewed,

hyperinsulinemic activation of Akt/mTOR signaling could suppress autophagy, thereby contributing to LV dysfunction.<sup>27</sup> In addition to Akt/mTOR-mediated suppression of autophagy, we also reported a novel mechanism that may occur in insulin-resistant states and the metabolic syndrome by which bioactive lipids that accumulate in the heart will activate NOX2 via a protein kinase C–dependent mechanism that ultimately inhibits lysosomal function and autophagic flux.<sup>185</sup> In this model, autophagic flux was impaired despite reduced phosphorylation of mTOR. Recent reports of the time course of autophagy after transverse aortic constriction suggests that although autophagy might be transiently induced, a repression of autophagy coincides with the transition from compensated hypertrophy to heart failure.<sup>186</sup> Consistent with this model are many observations that mouse models with impaired expression of autophagy mediators develop LV dysfunction either at baseline or more rapid decompensation after pressure overload.<sup>187,188</sup> Thus, constitutive repression of myocardial autophagy could represent a plausible mechanism by which hyperinsulinemia and activation of Akt signaling could impair LV function and accelerate adverse LV remodeling. Impaired autophagy might also impair mitochondrial quality control. Whether insulin signaling specifically regulates mitophagy remains incompletely understood, and whether they are linked in heart failure is currently unknown.

### Decreased Insulin Signaling and LV Remodeling

Mechanisms that are distinct from those associated with hyperactivation of insulin signaling pathways might also contribute to promoting LV dysfunction when insulin-PI3K and Akt signaling ultimately become impaired. In this regard, valuable insights have been obtained from genetic knockouts of components of the insulin signaling pathway (Table). It is important to emphasize that there is significant redundancy in insulin and IGF-1 signal transduction pathways.<sup>124</sup> Thus, there is a clear cross talk between insulin and IGF-1R signaling pathways in the heart, which might reflect the relatively benign phenotypes of single knockouts of the IGF-1R or the IR, respectively.<sup>38,40</sup> This is also true for IRS isoforms in that loss of IRS1 or IRS2 individually do not precipitate LV dysfunction in unstressed hearts and indeed we observed no differences in the ability of insulin to activate Akt or promote glucose uptake.<sup>48</sup> However, combined deletion of IR and IGF-1 receptors or IRS1 and IRS2 leads to catastrophic heart failure due, in part, to unrestrained autophagy, apoptosis, and profound mitochondrial dysfunction.<sup>49,50</sup> Similar phenotypes have been observed in animals with targeted loss of the phosphoinositide-dependent protein kinase 1, which is required for IR- or IGF-1R–dependent activation of Akt.<sup>125</sup>

The second mechanism by which reduced insulin signaling might contribute to LV dysfunction is by persistent nuclear localization of FOXO1. Qi et al<sup>50,53</sup> in mice with cardiomyocyte-restricted deletion of IRS1 and IRS2 showed that they could ameliorate the heart failure, in part, by genetically deleting FOXO1 expression. Similarly, heart failure associated with long-term high-fat feeding could also be prevented when FOXO1 was deleted.<sup>171</sup> It is likely that the mechanism

by which persistent nuclear localization of FOXO proteins might lead to LV dysfunction is multifactorial. Mechanisms by which FOXO may mediate LV remodeling include myosin isoform switching, FOXO-mediated activation of autophagic and atrophic pathways and FOXO-mediated activation of lipid metabolism pathways that would promote lipotoxicity.<sup>53,171,175</sup> The potential biphasic response in insulin signaling is also of particular importance for the design of future studies. Therefore, it is of great interest to perform time course studies, especially in the context heart failure and insulin resistance.

### Implications for Therapy and Future Research

Taken together, this review of the field identifies an important need for uniformity in determining changes in the specific components of the insulin signaling cascade at varying stages in the evolution from compensated cardiac hypertrophy to decompensated cardiac hypertrophy and ultimately to heart failure. Furthermore, a careful analysis of the relationship between altered myocardial insulin signaling in models of generalized insulin resistance in concert with cardiac adaptations to hemodynamic stress will advance our understanding of the complex interactions between generalized insulin resistance, myocardial insulin signaling, and heart failure. To more closely mimic human disease, studies in which heart failure is induced in animals that have developed generalized insulin resistance would be informative. Generalized insulin resistance is usually evident in high-fat fed rodent models after high-fat diets of >10-week duration. We would also suggest that measuring glucose uptake in response to insulin gives only a partial picture of changes in insulin signaling in the heart and as such it is important to evaluate the phosphorylation states of IRS1/2, Akt1/2, and FOXO proteins under conditions of ambient insulinemia and in response to an insulin stimulus. These analyses would be informative if determined in conjunction with assays of signaling defects in skeletal muscle, adipose tissue and liver, as they will not only identify differences in the adaptations of these tissues to insulin resistance but also the potential for crosstalk between these classical insulin signaling targets and the heart in models of heart failure. Moreover, they will test the hypothesis that changes in insulin signaling, either an increase in the early stages followed by impaired signaling in late-stage heart failure, may independently contribute to the pathophysiology of heart failure.

Modulation of myocardial insulin signaling is not currently a consideration in the management of heart failure. As discussed above, there are profound changes in myocardial insulin signaling that develop in the failing heart and are also present in subjects with generalized insulin resistance that may sensitize the heart to LV dysfunction. This raises important questions about whether modulation of generalized insulin resistance, normalization of hyperinsulinemia, and other disturbances in the metabolic milieu will alter the prognosis of heart failure. Second, it remains to be determined if strategies that will directly correct the changes in myocardial insulin signaling described above will reverse heart failure. Oxidative stress is a widely accepted mechanism for cardiac dysfunction in insulin-resistant states, and it was recently reported that targeted antioxidant therapy may partially restore abnormal insulin signaling and

improve cardiac structure and function.<sup>189</sup> Combined treatment with polyphenols resveratrol and S17834 also significantly restored cardiac function in diet-induced heart failure in rodent models via multiple mechanisms, including reducing oxidative stress, reversing oxidative modifications of proteins, reducing hyperinsulinemia, and increasing circulating adiponectin concentrations.<sup>190</sup> As alluded to above, GRK2 could represent an attractive target that could ameliorate pathological LV remodeling via mechanisms that could involve direct modulation of insulin signaling pathways within cardiomyocytes as well as by effects on systemic metabolic homeostasis.<sup>180,181,191</sup> Although ventricular unloading improved myocardial insulin signaling and lipotoxicity, it is not clear if the myocardial changes were primary or secondary to improvement in peripheral insulin resistance.<sup>24</sup> It is probable that ventricular unloading by reversing some of the mechanisms that promote generalized insulin resistance in subjects with heart failure might have contributed to a feed-forward mechanism to increase myocardial recovery.

An important conundrum that has been increasingly recognized is the unique challenges in managing heart failure in the patient with type 2 diabetes mellitus and insulin resistance. Although it is clear that diabetic patients with heart failure will respond to conventional heart failure therapies, many recent clinical trials have revealed a disturbing association between multiple diabetes mellitus therapies and worsening of heart failure or increased heart failure hospitalization. This topic has been the subject of recent reviews.<sup>192,193</sup> However, it is clear that many diabetes mellitus therapies including thiazolidinediones and even insulin might be associated with volume expansion, which may exacerbate heart failure. Similar effects have also been observed with certain DPPIV inhibitors. Specifically, treatment with the DPPIV inhibitor saxagliptin resulted in increased heart failure hospitalization.<sup>194</sup> DPPIV degrades GLP-1 and other vasoactive peptides,<sup>195</sup> including brain natriuretic peptide, which though increased in heart failure could represent an adaptive response.<sup>196</sup> Moreover, the increase in circulating concentrations of insulin that occur as a result of incretin therapy could lead to ligand-dependent activation of insulin signaling pathways. Thus, if hyperactivation of insulin signaling indeed contributes to adverse ventricular remodeling in heart failure, then a plausible mechanism for the relationship between these therapies and heart failure exacerbation could be the increase in myocardial insulin signaling and Akt hyperactivation. In contrast, metformin that acts as an insulin sensitizer and reduces hyperinsulinemia might afford some degree of protection in terms of heart failure.<sup>197</sup> Even though not directly proven, decreased circulating insulin levels after metformin treatment might attenuate cardiac insulin signaling and provide a potential explanation for the cardioprotective effects observed. A recent trial using a novel class of diabetes mellitus therapeutic, an inhibitor of the renal sodium glucose cotransporter 2 had a dramatic impact on subsequent risk of hospitalization for heart failure.<sup>198</sup> It is not known if this beneficial effect was secondary to the mild volume depletion or antihypertensive effects of this agent or to effects on altering the metabolic milieu, such as reducing circulating levels of insulin. Thus, additional studies are required to determine if therapeutic strategies that directly affect the abnormal systemic milieu that characterizes the

insulin-resistant state will affect LV structure and function in heart failure and affect prognosis and survival.

### Concluding Remarks

The heart is an insulin-responsive organ and insulin signaling plays an important role in cardiovascular homeostasis. Cardiac hypertrophy and heart failure are associated with profound changes in myocardial insulin signaling. Moreover, heart failure is an insulin-resistant state that leads to an altered metabolic milieu that will influence myocardial structure and function via mechanisms that are dependent or independent of alterations in myocardial insulin signaling. It is possible that strategies that might correct the systemic metabolic disturbances that are associated with insulin resistance could have an impact on the outcome and prognosis of subjects with heart failure. An important imperative for the future will be to design trials that can rigorously test this hypothesis. Second, an important opportunity for the future will be to identify and evaluate therapeutic strategies that might directly influence changes in myocardial insulin signaling that accompany heart failure. Given the dynamic nature of these changes, it will be important that subjects are carefully stratified or phenotyped to determine which insulin signaling pathways are perturbed and the pathophysiological mechanisms that will most likely benefit from targeted therapies.

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### Disclosures

None.

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