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Insulin Signaling Pathways and Cardiac Growth

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Abstract

The development, growth, function and metabolism of the heart are regulated by extracellular growth factors, cytokines and ligands. In this review, the role of insulin and insulin-like growth factors in the regulation of cardiac growth will be discussed. In addition, the role of insulin- and insulin-like growth factor-stimulated intracellular signaling proteins in cardiac growth will be reviewed.

INTRODUCTION

The insulin signal transduction pathway is a highly conserved pathway that regulates several aspects of cellular physiology, including most notably the regulation of cellular growth and of glucose uptake and utilization. Evolutionarily, it is clear that insulin mediates the close relationship between an organism's size and nutritional status by coupling nutrient availability sensing with substrate uptake and protein synthesis. The advantage of coupling nutrient delivery to cellular growth is that the energetic expense of cell growth is only undertaken in the presence of abundant environmental substrate, whereas in the absence of substrate, efficiency and economy of smaller size is favored. Indeed, this phenomenon appears to occur in yeast [1], drosophila [2] and humans [3].

Role of Insulin in Coupling Cardiac Growth and Nutrient Status

Numerous clinical observations support the hypothesis that cardiac growth is coupled to nutrient status. It is well-known that a dynamic linear relationship exists between heart weight and body weight, and this remains constant throughout childhood development [4]. Perturbations in body weight indeed result in predictable alterations in heart weight. For example, starvation [5], pharmacologic weight loss [6], severe lifestyle modification diets [7], anorexia nervosa [8] and bariatric surgery [9] result in significant reductions in LV mass. That this phenomenon exists in the absence of influences on ventricular afterload is supported by the lack of significant reductions in systolic or diastolic blood pressures after weight loss intervention. However, weight loss in morbidly obese subjects is associated with improved insulin sensitivity, glycemic control and lower serum insulin levels. Decreased cardiac mass after weight loss was directly associated with significant reductions in serum insulin levels in at least one recent study [10]. These clinical observations raise the possibility that heart size is linked to body mass and nutritional status, at least in part, through serum insulin levels.

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A corollary of the above clinical observations is that alterations in cellular insulin signaling are associated with alterations in body size. This is clearly demonstrated in the context of Donohue's syndrome or in Rabson-Mendenhall syndrome. Both syndromes occur as a result of mutations in the regulatory or coding domains of the insulin receptor, which give rise to a functionally null receptor [reviewed in 11–13]. Patients with Donohue's syndrome exhibit severe intrauterine growth retardation as well as dysmorphia. Patients with Rabson-Mendenhall syndrome also display poor growth, but have an overall milder dysmorphic phenotype.

Poor growth and development in the context of impaired insulin signaling predicts the outcomes in patients in which insulin signaling is likely to be elevated, such as in the setting of gestational diabetes mellitus (GDM). One of the hallmarks of GDM is in utero exposure to elevated maternal serum insulin levels. Indeed, infants born to mothers with GDM exhibited an increased incidence of large-for-gestational age fetal status, an increased incidence of fetal macrosomia and an increased mean birth weight [14,15]. Thus, exposure to elevated insulin levels in utero correlates with enlarged fetal dimensions. Together, these clinical data support the idea that insulin signaling regulates body size during development.

While it is clear that insulin signaling coordinately regulates organ and body size, this evolutionarily conserved phenomenon can become maladaptive in an environment where excessive nutrition is a chronic state, such as in the context of type II diabetes mellitus. This is illustrated in cases of diabetic cardiomyopathy (DCM), cardiac dysfunction in the context of diabetes mellitus that occurs independently of hypertension and coronary artery disease [16,17]. DCM is associated with an increased frequency of heart failure even when adjusting relative risk for such factors as age, blood pressure, obesity, hypercholesterolemia and coronary artery disease. Indeed, the presence of left ventricular hypertrophy is a powerful independent risk factor for heart failure, and insulin resistance and hyperinsulinemia are thought to be the driving forces toward cardiac hypertrophy in the setting of type II diabetes mellitus [18–20]. Thus, cardiac enlargement in the setting of diabetes mellitus is one maladaptive manifestation of nutrition and organ growth coupling mediated by insulin.

The GH-IGF Axis: Cardiac Issues

Growth hormone (GH), as its name suggests, is a key regulator of body and organ size in animals. Perhaps the most important effector of growth hormone-mediated growth stimulation is activation of insulin-like growth factor-1 (IGF-1) secretion in local tissues. IGF-1 is locally secreted and acts in an autocrine, paracrine and endocrine fashion to stimulate protein synthesis. While it is well known that GH is secreted during organismal development to stimulate protein synthesis, GH is also secreted in response to vigorous exercise [21]. Given the metabolic and growth-promoting actions of IGF-1, teleologically, GH-IGF-1 activation after exercise may be another means by which organisms deliver nutrients and synthetic substrate to tissues that may have been damaged and nutrient-depleted.

Given the importance of GH-IGF-1 action in cardiac growth and metabolism, patients with defects in the GH-IGF-1 axis expectedly have profound cardiac manifestations. Experimental and clinical studies provide evidence that GH and IGF-1 regulate cardiac development [22]. A very specific cardiomyopathy develops in the majority of patients with acromegaly, characterized by biventricular concentric myocardial hypertrophy with interstitial fibrosis, lympho-mononuclear infiltration and areas of monocyte necrosis. The left ventricular hypertrophy in acromegaly is reversible, as measured echocardiographically, after blocking the GH pathway with the drugs lanreotide, octreotide or octreotide-LAR [reviewed in 23]. Conversely, patients with childhood or adulthood-onset GH deficiency (GHD) may exhibit both structural and functional cardiac abnormalities, such as narrowed ventricular walls and impaired left ventricular peak exercise responses [22].

GH is one of the hormones secreted by the anterior pituitary gland that is markedly undersecreted in the context of clinical hypopituitarism. Epidemiological data suggest that adults with hypopituitarism have reduced life expectancy and a greater than two-fold increase in cardiovascular disease mortality when compared with healthy controls [24]. Moreover, various GH replacement trials in patients with GH deficiency consistently demonstrated increased LV function (LVEF) with somewhat inconsistent improvements in diastolic function, LV mass and exercise capacity [25–28].

GH - IGF-1 axis manipulation also shows promise for therapeutic benefit in other clinical conditions, such as heart failure. In both experimental rodent models of heart failure [29–33] and in human subjects with heart failure [34–35], GH / IGF-1 activation appear to augment cardiovascular function and prolong survival. The above clinical observations thus support the involvement of GH-IGF-1 signaling in development and maintenance of cardiac structure and function.

Because defects in insulin and IGF-1 signaling are increasingly prevalent as a result of the obesity and type II diabetes mellitus pandemic, impaired insulin and IGF-1 signaling are associated with significant morbidity and mortality. Therefore, the mechanisms by which insulin and its related ligands modulate cardiac growth are a target of intense study. Understanding these mechanisms may lead to the development of molecular therapeutics in the treatment of diseases related to insulin and IGF-1 signaling. This review will explore primarily in vivo models that have been utilized to elucidate such mechanisms.

INSULIN AND IGF FAMILY MEMBERS

Insulin and insulin-like growth factors (IGFs) are secreted polypeptides that circulate in the bloodstream and extracellular space of mammals where they act by binding to cell surface receptors that possess intrinsic tyrosine kinase activity [36]. Insulin and IGFs are ancient factors since they are found in many invertebrate animals, such as insects and worms. Indeed, the worm *Caenorhabditis elegans* has 10 insulin-like peptides in its genome [37].

Insulin-related proteins are all characterized by a signal peptide, a B-chain, a C-peptide, and an A-chain. The C-peptide of the propeptide is proteolytically cleaved and two disulfide bonds between the A- and B-chains, and a third disulfide bond within the A-chain are present in the mature peptide. In mammalian IGF-1 and IGF-2, the C-peptide is retained in the mature peptide [37].

In mammals, insulin is made in the pancreatic β cells, and acts in an endocrine fashion, while IGFs are made throughout the body, especially in the liver, skeletal muscle and heart, and they act in both an endocrine and a paracrine fashion. Therefore, while serum insulin levels are informative, serum IGF levels are not necessarily reflective of ligand available to bind to receptors in the heart. In addition, regulation of effective IGF concentrations by a multitude of IGF binding proteins is an additional layer of IGF signal modification [38].

RECEPTORS FOR INSULIN IN THE HEART

The insulin receptor (IR) is a heterotetrameric receptor consisting of two α and two β subunits [39,40]. These subunits are disulphide-linked in a β - α - α - β configuration. The α subunits lie extracellularly and are the ligand binding subunits of the receptor, while the β subunits contain a membrane-spanning domain and an intracellular domain. It is the intracellular domain of the β subunit that possesses intrinsic tyrosine kinase activity involved in signal transduction [40–42].

The IGF-1 receptor (IGF-1R) is assembled in a β - α - α - β structure that is identical to that of the insulin receptor [43]. When comparing the IR with the IGF-1R, the β subunit kinase domains share greater than 80% homology [42]. The point of greatest divergence between the IR and IGF-1R is in the extracellular α subunit, which thus controls the ligand-binding specificity of the receptor, such that each receptor significantly binds only its own ligand at physiological concentrations [43].

In addition to the α - β heterodimeric receptors for insulin and for IGF-1, a growing body of literature supports the existence of hybrid insulin / IGF-1 hybrid receptors. Hybrid receptors are heterologous, covalently linked IGF-1R and IR α - β hemireceptors that maintain trans-autophosphorylation activity upon ligand binding [44]. IR / IGF-1R hybrids are present in numerous mammalian tissue types, including the heart [45]. Although IGF-1 is thought to bind to hybrid receptors with greater affinity in comparison to insulin [46–47], both insulin and IGF-1 ultimately have the capacity to activate hybrid receptor signaling.

The physiological significance of hybrid receptor function is incompletely understood, although one group observed that brown preadipocytes cell lines that normally lack either IGF-1 or insulin receptor retain the ability to phosphorylate and activate growth-promoting targets Akt and MAPK in response to *both* insulin *and* IGF-1 [48]. Therefore, hybrid receptors may play an important role in both IGF-1 and insulin-mediated cardiac growth. While this hypothesis remains to be studied in greater detail, the availability of mouse models deficient in either or both receptor type in the heart makes this an approachable and attractive hypothesis to investigate.

INSULIN AND IGF-1 SIGNAL TRANSDUCTION

Insulin binding to its cell-surface receptor activates a complex signal transduction network that regulates numerous cellular functions [reviewed in 49]. As described above, the IR and IGF-1R are β - α - α - β heterotetramers. Upon ligand binding to the extracellular α -subunits, a cascade of intracellular events is initiated. The IR β -subunits are trans-autophosphorylated on multiple tyrosine residues. The activated insulin receptor recruits and phosphorylates insulin receptor substrate (IRS) proteins at the cell surface. IRS phosphorylation at multiple tyrosine residues generates Src homology 2 (SH2)-domain binding sites for numerous effectors such as the regulatory p85 subunit of the lipid kinase, phosphatidylinositol 3-kinase (PI3K) [36,40,49].

Binding of the p85 subunit of PI3K allosterically activates the p110 catalytic subunit to generate the phosphatidylinositol 3,4,5-trisphosphate (PIP3) from the substrate phosphatidylinositol 4,5-bisphosphate at the cell surface. PIP3 recruits the 3-phosphoinositide-dependent protein kinase-1 (PDK-1), and this ultimately results in PDK-1 phosphorylation and activation. Activated PDK-1 subsequently phosphorylates and activates downstream insulin actions via serine-threonine kinases including Akt and atypical protein kinase C (PKC) isoforms.

In addition to PI3K-dependent insulin signaling, another major insulin signaling branch involves tyrosine-phosphorylated insulin receptor substrate-1 (IRS-1) or SH2 domain-containing transforming protein (Shc) binding to the SH2 domain of the growth factor receptor-bound protein-2 (Grb-2) that results in activation of the pre-associated GTP exchange factor Sos [reviewed in 36]. This activates the small GTPase Ras, which then activates Raf kinase, the MAP kinase kinase (MKK1) and the extracellular regulated kinases-1 and -2 (ERK1/2). This MAPK-dependent branch of insulin signaling pathways modulates biological actions such as growth, mitogenesis, and differentiation [49].

Important negative regulators of insulin-signal transduction include both protein tyrosine phosphatases that dephosphorylate the IR and IRS-1 as well as lipid phosphatases that dephosphorylate PIP3. Key phosphatases regulating insulin signaling include PTEN, SHIP2

and SHP2 [50,51]. Insulin signaling pathways are arranged in complex networks that include multiple feedback loops, cross-talk between major signaling branches and cross-talk from signaling pathways of heterologous receptors, which contribute to the specificity of insulin signaling and insulin action [36,49].

Perturbation of Insulin Signaling: Effects on Cardiac Growth

Rodent models have been utilized to observe the effects of IGF-1 or insulin stimulation on cardiac growth and function (Table I and Figure 1). Acute infusion of either insulin or IGF-1 enhanced cardiac protein synthesis [52]. Similar to hyperinsulinemic human disease states such as type II diabetes mellitus, chronic insulin infusion in mice increased LV mass and relative wall thickness and reduced stroke volume and cardiac output [53]. Histochemical examination of these mice demonstrated myocyte hypertrophy and increased interstitial fibrosis without cardiomyocyte proliferation.

Similarly, a genetic model of chronic IGF-1 hyperstimulation was generated in which the IGF-1 gene was specifically overexpressed in cardiac muscle. However, IGF-1 overexpression resulted in cardiomegaly mediated uniquely by increased myocyte cell number without changes in cell volume. This was associated with enhanced cardiac function, as measured by myocyte shortening velocity and cellular compliance [54].

To more specifically probe the role of insulin and IGF-1 signaling in cardiac growth, several groups have generated mouse models with either global, cardiac-specific or muscle-specific receptor deletions [reviewed in 55]. Cardiac insulin receptor knockout mice (CIRKO) exhibited a reduction in histologically-determined myocyte size, resulting in a 22% and 28% reduction in female and male cardiac weight, respectively [56]. This reduction in heart cardiac weight was associated with diminished Akt, S6K1 and 4E-BP1 phosphorylation. The reduced heart weight / body weight ratio (HW/BW), the reduced myofiber diameter and the Akt substrate hypophosphorylation phenotypes of the CIRKO mouse were completely normalized upon forced cardiac expression of a constitutively active myristoylated Akt in vivo, indicating that Akt signaling downstream of the insulin receptor is sufficient for normal heart and myofiber growth [57].

In concordance with the CIRKO phenotype, muscle specific IGF-1 and IR double-knockout mice (MI2RKO) also have reduced heart size and reduced cardiomyocyte size measured at postnatal days 8 and 20. Interestingly, muscle-specific IGF-1 receptor knockout mice (MIGF-1RKO) exhibit a dilated cardiomyopathy resulting in a 50% baseline elevation in HW/BW without changes in myofiber cross-sectional circumference when compared with wild-type (WT) mice [58]. The concomitantly severe systolic dysfunction and 100% mortality at ~4 weeks of age in MI2RKO mice suggests that a critical basal level of IGF-1 or IR signaling is required for normal cardiac development and function, and that the IGF-1R and IR exhibit some overlapping functions with respect to cardiac growth. One particularly attractive hypothesis is that the PI3K – Akt pathway – a well-known effector pathway downstream of both IR and IGF-1R – might be sufficient to compensate for the absence of both receptors in the heart. One might hypothesize a normalization of cardiac growth, function and overall mortality upon restoration of Akt signaling in MI2RKO mice via forced muscle-specific expression of a constitutively active PI3K or Akt mutant in cardiac muscle.

Perturbations in IRS Protein Function – Effects on Cardiac Growth

Downstream of both IR and IGF-1R activation are the IRS proteins. At least 4 IRS family members are known to be present in the heart – IRS-1, IRS-2, IRS-3 and IRS-4. Targeted deletion of the IRS-1 gene results in significantly reduced HW/BW and an impaired response to IGF-1 ligand-induced heart growth that was not rescued by the IGF-1 transgene [59].

Therefore, IGF-1 cannot cross-react at the IR to induce completely normal heart growth. While IRS-3 mRNA is present in heart tissue [59], whole-body deletion of IRS-3 has a grossly normal heart with no changes in histologic morphology or HW/BW [60]. IRS-4 protein was detected in heart [61], although no gross heart defects were reported upon whole-body knockout of this gene [62]. Similarly, IRS-2-deficient mice displayed no gross cardiac phenotype in IRS-2-deficient mice despite a profound type II diabetic phenotype secondary to liver insulin resistance and absent beta cell compensation [63]. Therefore, IRS-1 may be the most important IRS involved in IGF-1 and IR-mediated cardiac growth. However, more subtle cardiac phenotypes may remain to be elucidated in IRS-2-4 mice under stressed conditions such as in pressure overload, chronic exercise or chronic insulin or IGF-1 ligand infusion.

Perturbation of Regulatory Lipid and Protein Phosphatases: Effects on Cardiac Growth
Negative modulators of IR and IGF-1 signaling include the phosphatase and tensin homolog (PTEN), the SH2 domain-containing protein tyrosine phosphatase (SHP2) and the SH2 domain-containing inositol 5-phosphatase-2 (SHIP2) [50,51]. SHP2 is a 70kDa protein tyrosine phosphatase that interacts with and dephosphorylates IRS-1 and the IR [64,65]. In contrast, PTEN and SHIP2 block Akt activation by converting PIP3 back to PIP2 via 3' and 5' phosphatase activity, respectively [66,67]. Therefore, the enzymatic activities of SHP2, SHIP2 and PTEN would be expected to blunt cardiac growth via suppression of the PI3K-Akt pathway. Perturbations in SHP2, SHIP2 and PTEN might be predicted to enhance cardiac growth.

SHP2-deficient mice die during embryogenesis. However, haploinsufficiency of SHP2 is associated with defective semilunar valvulogenesis with no gross cardiac growth abnormalities reported [68]. Conversely, PTEN-deficient hearts expectedly exhibit spontaneously increased cardiomyocyte cross-sectional area and HW/BW as early as 10 weeks of age [69]. This hypertrophic phenotype was reversed by forced transgenic expression of a dominant negative mutant of the PI3K p110 α subunit, suggesting that PTEN suppresses PI3K signaling downstream of the IR / IGF-1R. Although whole-body SHIP2-deficient have been generated [70,71], no heart growth phenotype in these mice was reported.

PI3K Perturbations: Effects on Cardiac Growth

The above studies suggested the importance of PI3K signaling in cardiac growth. Briefly, there are three classes of PI3Ks, based upon the enzyme's preferred substrate [72]. Class I PI3Ks consist of a regulatory subunit and a catalytic subunit that phosphorylates phosphoinositide-(4,5)-bisphosphate (PIP2). Class II PI3Ks phosphorylate phosphoinositol and phosphoinositide-4-phosphate to generate PI and PIP2, respectively. They are 170–210 kDa single-subunit proteins containing a catalytic domain that shares approximately 50% homology with the class I PI3Ks. Class III PI3Ks phosphorylate PI to generate PI3P, and are thought primarily to regulate vesicular transport. Because the Class I PI3Ks are postulated to be the class responsive to extracellular stimuli, they are by far the most well-characterized PI3K class (For an extensive review on this topic, please refer to [73,74]).

The role of various PI3K subunits was also directly studied in vivo. Luo, et al [75] generated mice with cardiac-directed deletion of both isoforms of the catalytic PI3K p85 subunit thought to be involved in growth factor receptor signaling – p85 α and p85 β . Deletion of both p85 subunits caused attenuated Akt and Akt substrate phosphorylation, associated with reduced HW/BW, reduced exercise-induced cardiac hypertrophy while cardiac contractility and cardiac histology were unaffected in these mice [76].

Similarly, cardiac-directed dominant negative inhibition of the regulatory PI3K subunit p110 α resulted decreased baseline HW/BW [76–78, reviewed in 79], while forced expression of constitutively active mutant p110 α in cardiac myocytes increased HW/BW [76]. Cardiac

function in both mouse models expressing p110 α subunit mutants was preserved. Preserved baseline HW/BW with attenuated isoproterenol-induced cardiac hypertrophy was observed in mice with a global deletion of the p110 γ subunit [80]. Together, these data suggest that PI3K signaling involving the p85 α/β catalytic subunits and the p110 α regulatory subunits are critical effectors in physiologic and developmental cardiac growth, whereas p110 γ is not required for cardiac development downstream of the IR and IGF-1R.

PDK1 – Akt1/2/3 – S6K1/2 Perturbation: Effects on Cardiac Growth

The phosphoinositide-dependent kinase, PDK1, is recruited to the plasma membrane via its pleckstrin homology domain in response to PI3K-mediated PIP3 generation, thus, it is a key intermediate that links IR and IGF-1R – PI3K activation with downstream Akt signaling. Accordingly, mice engineered with a muscle-specific deletion of PDK1 exhibited a complete inability to activate Akt and S6K1 in response to insulin infusion in vivo [81]. Moreover, muscle-specific PDK1-deficient mice uniformly died between 5 and 11 weeks of age with severe systolic dysfunction, decreased cardiomyocyte volume and decreased cardiac mass without changes in myocyte number.

Akt family members are key effectors downstream of PDK1. Each of the three known Akt family members – Akt1, Akt2 and Akt3 – is expressed in the heart, however, Akt1 and Akt2 are most abundantly expressed [82]. Global deletion of Akt1 resulted in reduced cardiac mass that was proportional to body size, thus both HW/BW and cardiomyocyte cross-sectional area were similar to that in WT littermates at baseline. However, Akt1-deficient mice were completely resistant to exercise-induced cardiac hypertrophy in vivo and IGF-1-stimulated hypertrophy in cultured adult mouse cardiomyocytes [82]. Conversely, mice with a global deletion of the Akt2 gene exhibited normal HW/BW and LV mass indices at baseline, as well as a normal response to IGF-1-stimulated cardiomyocyte growth in culture [83]. Additionally, although cardiac-specific Akt3 overexpression was associated with baseline hypertrophy and progression to heart failure in mice [84], Akt3 deletion had no grossly detectable baseline cardiac abnormalities, and HW/BW was normal in these mice [85]. Therefore, Akt1 appears to be the critical Akt isoform that mediates physiological cardiac hypertrophy in mammals.

The ribosomal S6 kinases are well-characterized downstream effectors of Akt kinases involved in cellular protein synthesis. Overexpression of S6K1, but not S6K2, resulted in a baseline cardiac hypertrophic phenotype [86]. In contrast, however, S6K1 and S6K2-deficient mice and S6K1/2 double-knockout mice all demonstrated completely normal responses to transverse aortic constriction, IGF-1R overexpression and to constitutively active p110 α overexpression in the myocardium. These data provide genetic evidence that the S6Ks are not required for pathological or physiological cardiac hypertrophy downstream of the IGF-1R.

GLUT4 Perturbations – Effects on Cardiac Growth

One of the most well-characterized effector function of insulin in various tissues is GLUT4 translocation and glucose uptake. Two tissue-directed mouse models engineered with a deletion of the gene encoding GLUT4 in heart muscle were generated [87,88]. GLUT4 deficiency in both skeletal muscle and heart caused profound cardiac hypertrophy at baseline associated with whole-body glucose intolerance [87]. That these cardiac abnormalities were due to intrinsic loss of GLUT4 – and not secondary to whole-body changes in glucose homeostasis – is supported by the compensated hypertrophic phenotype of the cardiac-specific GLUT4 knockout (G4HKO) mouse [88]. In the absence of functional or fibrous cardiac changes in G4HKO mice, wet HW/BW at baseline in G4HKO male and female mice were 39% and 17% greater, respectively, versus WT littermate mice. This increased HW/BW corresponded with increased myocyte size assessed histologically [88].

Parallel pathways in insulin signaling – Role in Cardiac Growth

In addition to a PI3-kinase-dependent pathway downstream of insulin binding at its receptor, a parallel, insulin-independent signaling cascade has been shown in numerous cell cultures models to be required for GLUT4 translocation to the plasma membrane [reviewed in 89]. Insulin receptor tyrosine autophosphorylation in the juxtamembrane regions and intracellular tail activates the receptor, which then tyrosine phosphorylates intracellular adaptor proteins, including the IRS-1 through -4, IRS-5/DOK4, IRS/DOK5, Gab-1 and Shc isoforms.

A distinct pool of insulin receptors localized to lipid raft microdomains can also phosphorylate the adaptor protein with a PH and SH2 domain (APS). APS is a non-enzymatic scaffolding protein that facilitates recruitment of three other scaffolding proteins – Cbl [90], Cbl-associated protein (CAP) and CrkII [91,92]. The APS/Cbl/CAP complex binds Crk, which is bound constitutively to the guanine nucleotide exchange factor C3G. C3G catalyzes activation via GDP-GTP exchange on the lipid-raft-associated protein TC10 [93]. Upon activation, TC10 binds with a number of potential effector molecules involved in translocation of GLUT4-containing vesicles, including CIP4, Exo70, and the Par6/Par3/PKC λ multimeric complex [89].

The involvement of this alternative pathway in cellular metabolism raises the possibility that it is involved in the substrate delivery – cell growth coupling action of insulin and IGF-1 as well. However, the physiological relevance of this parallel insulin signaling pathway remains poorly understood. Indeed, one mouse model deficient in APS (APSKO) demonstrated that energy intake, energy expenditure, fat content, body weight and plasma insulin, leptin, glucose and lipid levels were similar between APSKO and WT littermates fed either normal chow or a high-fat diet [94]. Furthermore, deletion of APS did not perturb whole-body insulin tolerance or glucose homeostasis. In contrast, APSKO mice generated through an independent gene targeting strategy [95] exhibited increased sensitivity to insulin. Insulin-stimulated glucose transport in isolated adipocytes of APSKO mice was increased over that of WT mice, and APSKO mice also showed increased serum levels of leptin and adiponectin. No gross alterations in cardiac growth or in whole-body growth were reported in either study, however.

Similarly, mice deficient in c-Cbl exhibited unimpaired insulin action [96], and mice expressing only c-Cbl with a deletion in the RING finger ubiquitin ligase domain (c-Cbl A/-) was sufficient to recapitulate this phenotype [97]. In contrast, c-Cbl F/F mice, which express c-Cbl with a mutated PI3K binding domain, have no metabolic phenotype, indicating that c-Cbl ubiquitin ligase activity plays a key role in the regulation of whole-body energy metabolism. Cbl-b knockout mice (Cbl-bKO) exhibit impaired macrophage infiltration and activation and macrophage activation [98]. Elderly Cbl-bKO mice became peripherally insulin resistant and glucose intolerant, and this was due to cytokine secretion from Cbl-b-deficient macrophages. None of the Cbl-deficient mice, however, had grossly abnormal growth or cardiac phenotypes reported.

Embryos lacking both Crk isoforms, CrkI and CrkII (CrkDKO), exhibited developmental cardiac defects in addition to craniofacial and vascular abnormalities. Importantly, the muscular wall of the CrkDKO heart was thinner and contained fewer, loosely packed cells. Moreover, ventricles in the CrkDKO embryonic hearts were severely dilated, potentially due to the extremely thin and poorly developed muscular wall [99]. Whether these profound abnormalities are due to intrinsic defects in cardiomyocyte growth or whether they are secondary to vascular abnormalities remains to be investigated. In contrast, the CrkII knockout mouse generated by insertional mutagenesis displayed no obvious growth or cardiac phenotype [100]. Taken together, these data suggest that CrkI is required for normal ventricular growth during cardiac development.

Deletion of the gene encoding CAP (gene name: Sorbs1) protected against high-fat diet (HFD)-induced insulin resistance in mice while having an insulin-sensitizing effect [101]. This phenotype was also associated with reduced tissue markers of inflammation. The insulin-sensitive phenotype was transferred to WT mice by transplantation of Sorbs1-null bone marrow, indicating that macrophages are an important cell type in the induction of insulin resistance and that CAP has a modulatory role in this function. Importantly, the authors reported no significant differences in body mass or organ weights in CAP-deficient mice versus WT littermates, although the heart was not included in these organ weight analyses.

Glucose transport was markedly impaired in heart muscle of mice in which PKC λ was specifically deleted in muscle (PKC λ KO mice). This defect in muscle glucose transport was associated with systemic insulin resistance, glucose intolerance, abdominal obesity, hyperlipidemia and hepatosteatosis. The weights of the heart muscles, vastus lateralis and livers, however, were not significantly altered in PKC λ KO mice, suggesting that PKC λ is uninvolved in developmental cardiac growth [102].

Precluding immediate investigation of C3G and TC10 in cardiac growth is the fact that mice with homozygous deletion of the guanine nucleotide exchange factor C3G died before embryonic day 7.5 [103], while the existence of TC10-deficient mice, to our knowledge, is not reported in the literature. Overall, the PI3K-independent arm of insulin-signaling is poorly defined with respect to its participation in heart growth. The lack of readily apparent cardiac growth phenotypes in existing mouse models in this alternative pathway suggest several possibilities: 1) the PI3K-dependent pathway adequately compensates for lesions in the PI3K-independent insulin signaling pathway, 2) the PI3K-independent pathway is uninvolved in cardiac growth or 3) the PI3K-independent insulin signaling pathway is important in post-developmental cardiac growth in the context of exercise training or pressure overload. Further experimentation using available mouse models will be required to distinguish amongst these possibilities.

CONCLUSION

Insulin and IGF-1 regulate diverse cellular functions, perhaps the most critical being regulation of glucose homeostasis and cellular growth. Insulin and IGF-1 act through their cognate receptors as well as through hybrid receptors and through cross reactivity to activate at least two major signaling pathways – a PI3K-dependent and a PI3K-independent pathway. While the importance of IRS-1-p85 α /p110 PI3K α and Akt1 in insulin and IGF-1 mediated cardiac growth are becoming increasingly well characterized, the precise purpose of the PI3K-independent pathway remains to be fully elucidated, particularly under stressed cardiac conditions such as exercise, chronic ligand infusion and pressure overload. Moreover, the cardiac phenotype of mouse models that are genetically deficient of downstream Akt effectors have not been thoroughly investigated. As the downstream targets of the Akt pathway specifically involved in IGF-1- and insulin-induced growth are more clearly defined, and as the *in vivo* role of the PI3K-independent pathway is better understood, the opportunities for preventing morbidity and mortality in the contexts of diabetic cardiomyopathy and in the treatment of heart failure will continue to evolve.

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Figure 1. Schematic overview of phenotypes observed in mouse models with perturbed insulin / IGF-1 signaling

The PI3K-dependent (Left) and PI3K-independent (Right) arms of insulin signaling are depicted. **Left.** Ligand activation of the IGF-1R / IR results in tyrosine autophosphorylation at multiple residues on the IGF-1R / IR β -subunits. IRS-1 binds the phosphorylated IGF-1R / IR and recruits p85 and p110 α . p110 α generates PIP3 at the plasma membrane, which recruits and tethers PDK1 and Akt1/2/3 into close proximity. Akt1 appears to phosphorylate downstream targets involved in protein synthesis and cardiac hypertrophy, while Akt2 activates GLUT4 translocation to the plasma membrane. Akt3-specific function is poorly understood. PTEN, SHIP2 and SHP2 inhibit insulin and IGF-1 signaling at various steps shown above. PTEN and SHIP2 are lipid phosphatases that convert PIP3 back to PIP2, while SHP2 is a protein tyrosine phosphatase that dephosphorylates IRS-1 and the beta subunits of the IGF-1R / IR. **Right.** A separate pool of insulin receptor heterodimers are localized to lipid raft domains. The insulin receptor is trans-autophosphorylated on β -subunits in response to ligand activation, which recruits a scaffolding complex containing APS, Cbl, CAP. This complex recruits the adaptor protein Crk I and its constitutive binding partner C3G. C3G catalyzes GDP – GTP exchange on TC10, which then activates the PKC λ /par3/par6 complex. This complex is involved in GLUT4 translocation. Symbols: * decreased HW/BW at baseline. ***increased HW/BW at baseline. Grey-shaded molecules denote a growth phenotype under cardiac stress conditions. Absence of notation indicates the lack of a reported phenotype in a mouse model deficient in the molecule shown.

Table 1
Summary of phenotypes observed in mouse models with perturbed insulin / IGF-1 signaling.

Signaling Molecule	Genetic Manipulation	HW/BW	Cardiomyocytes	Notes	Reference
IGF-1	COE	↑	Increased number		54
IR	IR CKO	↑	Atrophic		56
	IR MKO	↓	Atrophic		58
IGFIR	IGFIR MKO	↑	↔		58
IR / IGFIR	IR / IGFIR MKO	↑	Atrophic		58
IRS-1	IRS1 GKO	↔	↔	Impaired heart growth in females upon widespread IGF-1 overexpression	59
IRS-2	IRS2 GKO	NR	NR		63
IRS-3	IRS3 GKO	↔	↔		60
IRS-4	IRS4 GKO	NR	NR		62
SHP2	Ptpn11 GHet	NR	NR	Defective semilunar valvulogenesis	68
SHIP2	SHIP2 GKO	NR	NR		70,71
PTEN	PTEN CKO	↑	Hypertrophic	Cardiac DN-P110α overexpression blocked baseline hypertrophy	69
p85	p85 CKO	↓	Atrophic	Reduced exercise-induced cardiac hypertrophy, normal contractility and myocardial histology.	75
P110α	DNp110α COE	↓	Atrophic	DNp110α COE blocked IGF-1-induced and exercise-induced cardiac hypertrophy	76,77,78
	CAP110α COE	↑	Hypertrophic		76
p110γ	p110γ GKO	↔	↔	p110γ GKO blocked isoproterenol-induced hypertrophy and heart failure	80
PKD1	PKD1 MKO	↓	Atrophic	Elevated markers of heart failure.	81
Akt1	Akt1 GKO	↔	↔	Akt1 GKO blocked exercise-induced hypertrophy while TAC-induced hypertrophy was enhanced.	82
Akt2	Akt2 GKO	↔	↔	Normal cardiac phenotype in response to TAC. NC in myocyte protein synthesis in response to IGF-1	83
Akt3	Akt3 COE	↑	Hypertrophic		84
	Akt3 GKO	↔	↔		85
S6K1	S6K1 COE	↑	Hypertrophic		86
	S6K1 CKO	↔	↔		86
	S6K2 COE	↔	↔		86
	S6K2 CKO	↔	↔		86
	S6K1/2 CKO	↔	↔		86
GLUT4	GLUT4 MKO	↑	NR	Resistant to TAC-, IGF-1R- and CAP110α-induced cardiac growth	87
	GLUT4 CKO	↑	Hypertrophic		88
APS	APS GKO	NR	NR		94,95
c-cbl	c-cbl GKO	NR	NR		96
Chl-b	Chl-b GKO	NR	NR	Impaired macrophage activation	98
Crk I / II	Crk I / II GKO	↓	Decreased number	Dilated cardiomyopathy	99
Crk II	Crk II GKO	NR	NR		100
Cap	Sorbs1 GKO	NR	NR		101
PKCα	PKCα MKO	↔	NR		102
C3G	C3G GKO	NR	NR	Embryonic lethal before day 7.5	103

Abbreviations: COE, cardiac-specific overexpression; CKO, cardiac-specific knockout; MKO, muscle-specific knockout; GKO, global knockout; Ghet, global heterozygote; DN, dominant-negative mutant; CA, constitutively active mutant; ↑ increased versus control animals; ↓ decreased versus control animals; ↔, no change versus control animals; NR, phenotype not explicitly reported.