Akt Signaling Mediates Postnatal Heart Growth in Response to Insulin and Nutritional Status*

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Akt is a serine-threonine kinase that mediates a variety of cellular responses to external stimuli. During postnatal development, Akt signaling in the heart was up-regulated when the heart was rapidly growing and was down-regulated by caloric restriction, suggesting a role of Akt in nutrient-dependent regulation of cardiac growth. Consistent with this notion, reductions in Akt, 70-kDa S6 kinase 1, and eukaryotic initiation factor 4E-binding protein 1 phosphorylation were observed in mice with cardiac-specific deletion of insulin receptor gene, which exhibit a small heart phenotype. In contrast to wild type animals, caloric restriction in these mice had little effect on Akt phosphorylation in the heart. Furthermore, forced expression of Akt1 in these hearts restored 70-kDa S6 kinase 1 and eukaryotic initiation factor 4E-binding protein 1 phosphorylation to normal levels and rescued the small heart phenotype. Collectively, these results indicate that Akt signaling mediates insulin-dependent physiological heart growth during postnatal development and suggest a mechanism by which heart size is coordinated with overall body size as the nutritional status of the organism is varied.

During normal postnatal development there is a linear relationship between the increase in body weight and heart weight (1). The increase in heart weight is largely attributed to the enlargement of myocytes, and there is almost a 3-fold increase in cardiac myocyte diameter in humans during development from infants to adults (2). This process of physiological hypertrophy is proposed to be similar to the heart growth observed in athletes who participate in endurance training (3) and distinct from the pathological forms of hypertrophy that occur in patients with hypertension or valvular heart disease (4). Heart size is also highly responsive to the nutritional state. Heart weight and myocyte fiber size is reduced in starved subjects (5) and in individuals on severe weight loss diets (6). Heart size is markedly reduced in patients with anorexia nervosa (7), and modest weight reduction in normotensive obese subjects is associated with reduced heart size in the absence of changes in blood pressure or other hemodynamic parameters (8). Conversely, heart size is increased in obese subjects without hypertension or other cardiovascular and/or metabolic abnormalities (9). Thus, it is likely that mechanisms exist that couple heart size to nutrient-dependent changes in body size (10). However, compared with our understanding of the regulatory mechanisms that contribute to pathological hypertrophy (11, 12), little is known about the mechanisms that control normal cardiac myocyte growth or how these processes are coordinated with overall changes in the nutritional status of the organism.

One of the potential mediators of intrinsic responses to the nutritional state is insulin. Insulin exerts a wide variety of biological actions including regulation of glucose metabolism and protein synthesis (13, 14). After activation by insulin, the insulin receptor (IR) phosphorylates a number of cellular substrates, leading to the activation of multiple downstream signaling pathways (15, 16). Most notable is the phosphoinositide 3-kinase (PI3K) pathway, which mediates a wide variety of biological actions of insulin (17). Akt is one of the major downstream effectors of PI3K and has been implicated in the regulation of protein synthesis at least in part through the indirect regulatory phosphorylation of eukaryotic initiation factor 4E-binding protein (4E-BP1) and ribosomal S6 kinase (S6K) (18, 19), and insulin was previously shown to stimulate global protein synthesis in the heart (20). Although these findings suggest that insulin positively regulates cardiac muscle growth through the PI3K-Akt pathway, the physiological role of insulin signaling in regulating postnatal heart growth has not been elucidated.

The role of Akt signaling in the control of organ and cell size

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1 The abbreviations used are: IR, insulin receptor; PI3K, phosphoinositide 3-kinase; 4E-BP1, eukaryotic initiation factor 4E-binding protein 1; S6K1, 70-kDa S6 kinase 1; CIRKO, cardiac-specific insulin receptor knockout; Ad-myrAkt1, adenoviral vector expressing constitutively active Akt1; Ad-β-gal, adenoviral vector expressing β-galactosidase; Ad-Akt1AAA, adenoviral vector expressing dominant-negative Akt1; PDK1, phosphoinositide-dependent protein kinase 1; mTOR, mammalian target of rapamycin; TRITC, tetramethylrhodamine isothiocyanate; m.o.i., multiplicity of infection; ANP, atrial natriuretic peptide; IGF, insulin-like growth factor; PTEN, phosphatase and tensin homolog on chromosome 10.
Akt Mediates Insulin-dependent Postnatal Heart Growth

Akt has been gaining considerable attention. Akt has been shown to be an important mediator of growth in Drosophila (21) and mice (22, 23) and to control cellular hypertrophy in cardiac, skeletal, and smooth muscle cells (24–28). Recently, it has also been reported that heart size is increased in transgenic mice that express constitutively active forms of Akt from a cardiogenic-specific promoter (29, 30). However, overexpression of a kinase-deficient form of Akt did not reduce heart size (29). Therefore, although it is clear that overexpression of Akt can promote heart growth, the role of this signaling step in normal postnatal growth in response to insulin or nutritional status is not clear.

In the present study, we examined the physiological role of Akt signaling during nutrient-dependent postnatal cardiac growth. Akt signaling in the heart was up-regulated during early postnatal developmental stage when the heart was rapidly growing and down-regulated by short-term fasting, suggesting a role of Akt in regulating postnatal heart growth in response to nutritional status. Consistent with this notion, cardiac-specific IR knockout (CIRKO) mice, which have a small heart phenotype (31), exhibited reductions in Akt, S6K1, and 4E-BP1 phosphorylation in the heart, and caloric restriction had little effects on cardiac Akt activity in CIRKO mice. Expression of Akt1 induced cell hypertrophy and phosphorylation of S6K1 and 4E-BP1 in cultured cardiac myocytes, and expression of dominant-negative Akt1 inhibited insulin-induced phosphorylation of S6K1 and 4E-BP1 and cardiomyocyte hypertrophy. Furthermore, ectopic expression of Akt1 in the hearts of CIRKO mice restored the phosphorylation of Akt, S6K1, and 4E-BP1 to the normal levels and rescued the small heart phenotype. These results collectively indicate that insulin-dependent postnatal heart growth is mediated by Akt signaling. This mechanism of regulatory control may account for how heart size is coordinated with overall body size during postnatal development and as the nutritional status of the organism is varied.

EXPERIMENTAL PROCEDURES

Overnight Fast and Insulin Stimulation of Mice—For overnight fast, 6-week-old mice were deprived of food overnight. For insulin stimulation, 12-week-old wild-type mice were starved overnight, and 1 mg/kg of insulin treatment, acute stimulation of mice with insulin resulted in a robust activation of Akt signaling, and serum insulin concentration to the basal level (38), we speculated that insulin might be an upstream activator of cardiac Akt signaling. Supporting this notion, acute insulin treatment with insulin 24 h after infection and assessed for cell surface area 48 h after insulin treatment. Ad-myrAkt1-infected cells were used as control. For analysis of protein synthesis, myocytes were cultured in 24-well plates, and 3Hleucine incorporation was measured. For Ad-myrAkt1-infected cells, [3H]leucine (1µCi/ml) was added 24 h after infection and incubated for an additional 4 h. For Ad-Akt1AAA-infected cells, cells were treated with vehicle or insulin 24 h after infection. The [3H]leucine was added 20 h after insulin treatment, and cells were incubated for an additional 4 h. After a 4-h incubation with [3H]leucine, radioactivity of trichloroacetic acid-insoluble fraction was counted.

Stimulated Heart Growth—For S6 kinase assay, 200 µg of cell lysate was immunoprecipitated with anti-S6K1 antibody, and in vitro kinase assay was performed using S6 kinase assay kit (Upstate Biotechnology) according to the manufacturer's instructions.

Northern Blotting—Total RNA was extracted from cultured cardiomyocytes by RNeasy Mini Kit (Qiagen), and samples (10 µg each) were resolved on a 1.2% formaldehyde agarose gel and transferred to a nylon membrane. Blots were hybridized with a 32P-labeled cDNA probe for mouse atrial natriuretic peptide (ANP) (35).

Adenovirus Injection into Newborn Mouse Hearts—Adenovirus vectors were injected into the ventricles of newborn mice as described previously (36) with 1 × 1010 plaque-forming units of Ad-β-gal or Ad-myrAkt1 for each mouse. Animals were sacrificed at the age of 6 weeks old.

Histology—Heart sections were prepared as described (34) and stained with hematoxylin and eosin. Transverse diameter of myofibers was measured as previously described (37).

RESULTS

Cardiac Akt Activity Is Regulated by Developmental Stage and Nutritional Status—To examine the regulation of Akt signaling during postnatal cardiac growth, Akt phosphorylation status was examined in hearts of mice harvested at 6 weeks of age, when animals are in a rapid growth phase, and at 12 weeks of age, when the heart grows more slowly and nears a plateau as it approaches its full adult size. The level of Akt phosphorylation was markedly higher in the hearts of 6-week-old mice than in the 12-week-old mice (Fig. 1A), indicating that Akt signaling is up-regulated in the postnatal heart under conditions of rapid growth. To gain insight about the upstream activator of Akt signaling, the effects of fasting on cardiac Akt activation was assessed in 6-week-old mice. Animals were deprived of food overnight, and Akt phosphorylation levels in the heart were compared with those of randomly fed mice of the same age. An overnight fast dramatically reduced the phosphorylation of Akt without changing total Akt expression level (Fig. 1B). Fasting also reduced the phosphorylation of S6K1 and 4E-BP1 (Fig. 1B), as shown by the increase in mobility on SDS-PAGE gel, consistent with diminished Akt signaling in the heart under these conditions. Because an overnight fast leads to the reduction of serum insulin concentration to the basal level (38), we speculated that insulin might be an upstream activator of cardiac Akt signaling. Supporting this notion, acute insulin treatment with insulin 24 h after infection and assessed for cell surface area 48 h after insulin treatment. Ad-Akt1AAA-infected cells were used as control. For analysis of protein synthesis, myocytes were cultured in 24-well plates, and 3Hleucine incorporation was measured. For Ad-myrAkt1-infected cells, [3H]leucine (1µCi/ml) was added 24 h after infection and incubated for an additional 4 h. For Ad-Akt1AAA-infected cells, cells were treated with vehicle or insulin 24 h after infection. The [3H]leucine was added 20 h after insulin treatment, and cells were incubated for an additional 4 h. After a 4-h incubation with [3H]leucine, radioactivity of trichloroacetic acid-insoluble fraction was counted.

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Histology—Heart sections were prepared as described (34) and stained with hematoxylin and eosin. Transverse diameter of myofibers was measured as previously described (37).

Statistical Analysis—Data are shown as the means ± S.E. of mean. All data were evaluated with a two-tailed, unpaired Student's t test or compared with one-way analysis of variance.
examine Akt signaling in the heart of CIRKO mice, in which insulin receptor gene is disrupted specifically in cardiac muscle cells (31). Consistent with the previous report (31), CIRKO mice displayed a small heart phenotype with heart weight/body weight ratio ~20% smaller than those of wild type littermates at 6 weeks of age (Fig. 2A). Western blot analysis indicated a decrease in Akt signaling in the hearts of CIRKO mice. Akt phosphorylation was reduced in the hearts of 6-week-old CIRKO when compared with age-matched controls despite similar levels of total Akt protein (Fig. 2B). Consistent with a decrease in Akt signaling, the phosphorylation of S6K1 and 4E-BP1 were also reduced. We also performed short term fasting experiments on CIRKO mice at 6 weeks old. In contrast to the dramatic down-regulation of cardiac Akt activity in wild type animals, overnight fasting had little effect on cardiac Akt phosphorylation in CIRKO animals (Fig. 2C). Quantitative analysis of Akt phosphorylation levels indicated that the relative reduction of Akt phosphorylation by IR deletion and short term fasting were almost identical (Fig. 2D). Taken together, these results suggest the possibility that insulin-dependent cardiac growth is mediated by Akt signaling in the heart.

Insulin-induced Cardiomyocyte Hypertrophy Is Akt-dependent—The role of Akt signaling in insulin-dependent heart growth was investigated in cultured cardiac myocytes in vitro. Myocytes were infected with control virus (Ad-β-gal) or adenovirus expressing a dominant-negative mutant of Akt1 (Ad-Akt1AAA) and stimulated with insulin 24 h after infection. The insulin-induced increase in cell surface area and [3H]leucine incorporation were reduced by the expression of dominant-negative Akt1 (Fig. 3, A and B), and these effects on cell size and protein synthesis were not observed when Akt1AA (in which Thr-308 and Ser-473 are converted to alanine) was used as a dominant-negative Akt mutant (data not shown). Insulin-induced phosphorylation of S6K1 and 4E-BP1 as well as S6K1 kinase activity were also partly reduced by Akt1AAA (Fig. 3C). Inhibition of endogenous Akt by Akt1AAA was indicated by the attenuation of insulin-induced glycogen synthase kinase-3β phosphorylation. Because Akt is phosphorylated by phospho-

![Fig. 1. Cardiac Akt activity is regulated by developmental stage and nutritional status. A, protein extracts were prepared from 6- and 12-week-old wild type mouse hearts and subjected to Western blot analysis (40 μg/lane). B, protein extracts were prepared from the hearts of random-fed or overnight-fasted 6-week-old wild type mice and subjected to Western blot analysis (40 μg/lane). S6K1 (αI) (85 kDa) and S6K1 (αII) (70 kDa) are alternatively spliced variants. C, protein extracts were prepared from the hearts of 12-week-old wild type mice with or without insulin stimulation (1 mg/kg body weight, 5 min) and subjected to Western blot analysis (40 μg/lane).](image1)

![Fig. 2. Cardiac Akt signaling is down-regulated in CIRKO mice. A, body weight (BW) and heart weight (HW)/body weight ratio were measured in wild type (WT) and CIRKO mice at 6 weeks of age. Data represent the mean ± S.E. (*, p < 0.01, n = 4 in each group). B, protein extracts were prepared from the hearts of wild type or CIRKO mice at 6 weeks of age and subjected to Western blot analysis (40 μg/lane). C, protein extracts were prepared from the hearts of random-fed or overnight-fasted 6-week-old CIRKO mice and subjected to Western blot analysis (40 μg/lane). D, the intensities of phospho-Akt bands on Western blots from Figs. 1B, 2B, and 2C were measured by NIH Image, and relative Akt phosphorylation levels were indicated with the mean value of random-fed wild type animals as 1.0. The data from Fig. 2C were adjusted so that the relative intensities of random-fed CIRKO mice in B and C become equal.](image2)
inositol-depndent protein kinase 1 (PDK1) at Thr-308, it is possible that overexpression of Akt1AAA competitively interferes the regulatory phosphorylation of other PDK1 targets. However, insulin-induced phosphorylation of protein kinase C, a direct substrate of PDK1 (39), was not attenuated by Akt1AAA, suggesting that the effects of dominant-negative Akt1 overexpression is not because of nonspecific interference of PDK1. These results suggest that insulin promotes cardiomyocyte growth through Akt-dependent signaling, although Akt-independent regulation of S6K1 and 4E-BP1 phosphorylation is indicated because the inhibition by dominant-negative Akt is partial.

Akt Can Rescue the Small Heart Phenotype of CIRKO Mice—Because ablation of insulin receptor in the heart also disrupts insulin-dependent signaling other than PI3K-Akt pathway, it is possible that the lack of insulin-dependent but Akt-independent signaling pathways is the primary cause of small heart phenotype and down-regulation of Akt-dependent pathways is a para-phenomenon. To test whether the small heart phenotype of CIRKO mice is due to down-regulation of the IR-PI3K-Akt pathway, we examined whether this phenotype could be rescued by overexpressing Akt.

Initially, we characterized the activities of an adenovirus vector expressing a constitutively active form of Akt1 (Ad-myrAkt1) in cultured cardiac myocytes. Overexpression of Akt1 induced cardiomyocyte hypertrophy as evidenced by the increase in cell surface area (Fig. 4, A and B) and "H]leucine incorporation (Fig. 4C) by 48 h post-transduction, consistent with a previous report (24). Western blot analysis revealed that the increased expression and phosphorylation of Akt is associated with an increase in phosphorylation levels of S6K1 and 4E-BP1, as shown by the decrease in mobility during SDS-PAGE, from as early as 4 h after transduction, when the activation status of Akt is still modest (Fig. 4D). It has been reported that expression of constitutively active Akt1 transactivates the promoter of ANP gene in transient transfection assay (24). However, induction of the endogenous ANP gene and the organization of sarcomere, which are generally associated with hypertrophic agonist-induced cardiomyocyte hypertrophy (40), were not observed despite marked overexpression of Akt1 (Fig. 4, E and F).
Fig. 4. Akt overexpression induces cardiomyocyte hypertrophy in vitro. A, cultured cardiomyocytes were infected with Ad-β-gal (control) or Ad-myrAkt1 (Akt) at the m.o.i. of 50 and stained with anti-tropomyosin antibody 48 h after infection. B, cell surface area of cardiomyocytes was measured 48 h after infection with Ad-β-gal (control) or Ad-myrAkt1 (Akt) at the m.o.i. of 50. Data represent the mean ± S.E. (*, p < 0.01). The experiments were repeated three times, and essentially the same results were obtained. The representative data are shown (n = 10 in each group). C, [3H]leucine incorporation into cardiomyocytes was measured between 44 and 48 h after infection with Ad-β-gal (control) or Ad-myrAkt1 (Akt) at the m.o.i. of 50. Data represent the mean ± S.E. (*, p < 0.01). The experiments were repeated three times, and essentially the same results were obtained. Representative data are shown (n = 3 in each group). D, cell lysates were prepared from Ad-myrAkt1-infected cardiomyocytes (m.o.i. = 50) at the indicated time points after infection and subjected to Western blot analysis (10 μg/lane). E, Cardiomyocytes were infected with Ad-β-gal (control) or Ad-myrAkt1 (Akt) at the m.o.i. of 50 and stained with TRITC-conjugated phalloidin 48 h after infection. Phenylephrine treatment (PE; 100 μM for 24 h) was used as a positive control for sarcomeric organization. F, total RNA was extracted from Ad-myrAkt1-infected cardiomyocytes (m.o.i. = 50) at the indicated time points after infection and subjected to Northern blot analysis (10 μg/lane) with α-32P-labeled mouse ANP cDNA as a probe. The lower panel shows ethidium bromide staining of ribosomal RNA.

Indeed, the hemagglutinin-tagged myrAkt1 could be detected by Western blot analysis of Akt immunoprecipitates prepared from Ad-myrAkt1-injected CIRKO hearts at 6 weeks of age (Fig. 5A). Although this level of transgene expression did not appreciably increase total Akt levels, activation of this signaling step was clearly indicated by an increase in the level of phosphorylated Akt, boosting it to the level detected in wild type mice. Injection of Ad-myrAkt1 in the heart of CIRKO mice also reversed the down-regulation of S6K1 and 4E-BP1 phosphorylation, indicative of enhanced Akt signaling. The restoration of Akt activity in the hearts of Ad-myrAkt1-injected CIRKO mice significantly increased heart weight/body weight ratio when compared with Ad-β-gal-injected CIRKO mice and was comparable with that of wild type mice (Fig. 5B). Histological analysis of the heart indicated that the transverse diameter of myofibers is increased in Akt1-injected hearts to comparable levels with those of wild type animals (Fig. 5, C and D), suggesting that the increase in heart weight/body weight ratio induced by Akt1 overexpression is due to the hypertrophy of myocytes. Liver weights were also measured because it has previously been shown that transgene expression by this method is observed in this organ. Although there seems to be a slight increase in liver weight/body weight ratio in Akt1-injected CIRKO mice compared with β-gal-injected CIRKO mice (liver weight (mg)/body weight (g) = 45.8 ± 2.3 (CIRKO) versus 48.6 ± 1.7 (CIRKO + Akt)), it was not statistically significant. Also, there was no difference in body weight between these two groups (body weight (g) = 19.5 ± 0.43 (CIRKO) versus 19.9 ± 0.22 (CIRKO + Akt)). Thus, the effects of adenovirus dissemination on other organs appear to be minimal, suggesting that the contribution of peripheral effects on heart size in Akt1-injected CIRKO mice is very small, if any. Altogether, these results support the hypothesis that the small heart phenotype of CIRKO mice is mediated by down-regulation of the IR-PI3K-Akt pathway in the heart.

DISCUSSION

Body size and organ growth is tightly coupled in multicellular organisms. Studies in Drosophila have implicated members of the insulin signaling pathway, such as the IR, insulin receptor substrate, PI3K, Akt, and S6K, in the regulation of organ growth and body size (21, 41). The role of this pathway in vertebrate growth control is indicated by the growth retardation observed after targeted disruption of insulin-like growth factor (IGF)-I, IGF-II, type1 IGF receptor, insulin receptor substrate-1, Akt1, or S6K1 in mice (22, 23, 42–45). In cultured cardiac myocytes in vitro, it has been shown that overexpres-
sion of Akt or inactive mutant of PTEN (a negative regulator of Akt) induces cardiomyocyte hypertrophy (24, 46). In transgenic studies in mice, it has also been shown that overexpression of constitutively active or dominant-negative mutants of PI3K in the heart increases or decreases heart size, respectively (47), and chronic Akt overexpression in the heart has recently been shown to increase heart size (29, 30). In the present study, we examined the role of Akt signaling in insulin-dependent heart growth during postnatal development. Specifically we show that Akt signaling is down-regulated in CIRKO mice, which have a small heart phenotype. Transduction of a constitutively active form of Akt1 into these hearts rescued the small heart phenotype, indicating that Akt signaling is a regulator of insulin-dependent postnatal heart growth. Although overexpression of dominant-negative Akt did not lead to a reduction in heart size in transgenic mice (29), this is probably due to the relatively weak nature of the dominant-negative Akt construct used in that study. In support of this hypothesis, our in vitro experiments were only able to detect an inhibition of cardiomyocyte growth when cells were transduced with the Akt1AAA construct, which functions as a more potent dominant-negative regulator than other mutant forms of Akt (33). Our study also showed that an overnight fast markedly decreased Akt signaling in wild type mouse heart, whereas caloric restriction had no effect on the low level of Akt signaling in CIRKO mouse heart. Taken together these data indicate that the IR-PI3K-Akt pathway may function as a link between heart size and the nutritional state of the organism. Studies in Caenorhabditis elegans are consistent with this notion. In response to nutritional deprivation, these organisms arrest in the dauer stage, where feeding and reproduction stops and metabolism shifts from energy utilization to storage (48, 49). This adaptive response to adverse environmental conditions is mediated by the down-regulation of the IR-PI3K-Akt pathway.

The growth of specific organs during embryonic and postnatal development is mainly achieved by the increase in cell number. However, cardiomyocytes exhibit a limited capacity to replicate soon after birth, and heart growth at this developmental stage is largely achieved by increasing cardiomyocyte size in response to growth-promoting stimuli (4). A similar type of growth response is observed in the hearts of athletes and is associated with normal or augmented contractile function and increased capillary density (50). In contrast, pathological cardiomyocyte hypertrophy occurs in patients with hypertension or valvular heart disease, and it ultimately leads to depressed contractility, decreased capillary density, and interstitial fibrosis (50). Many signaling pathways have been implicated in the development of pathological cardiac hypertrophy (11, 12), but relatively little is known about the mechanisms that control physiological cardiac growth during postnatal development. Because a reduction in post-natal heart growth in CIRKO mice is associated with diminished Akt signaling, we speculate that this signaling pathway is a constituent of normal postnatal heart development. In support of this hypothesis, overexpression of Akt1 in cultured cardiomyocytes promoted hypertrophy in the absence of endogenous ANP induction or sarcomere organization. Furthermore, it has recently been reported that Akt-mediated skeletal muscle hypertrophy is coupled to vascular endothelial cell growth factor synthesis and blood vessel recruitment (26), and similar mechanisms may function during normal postnatal heart growth. On the other hand, Akt is also activated in pressure overload-induced hypertrophy (data not shown) (51). Thus, it is tempting to speculate that although Akt may control cardiomyocyte cell size during pathological hyper-

![Fig. 5. Akt can rescue the small heart phenotype of CIRKO mice. A, Ad-myrAkt1 or Ad-β-gal (1×10⁶ plaque-forming units/animal) was injected into ventricles of newborn CIRKO mice. Ad-β-gal-injected wild type mice (WT) were used as control. Protein extracts were prepared from the hearts of these mice at 6 weeks of age and subjected to Western blot analysis. In blots indicated as IP:Akt, samples (400 μg each) were immunoprecipitated with anti-Akt1 antibody before Western blot analysis. In other blots, 40 μg of protein extract was analyzed. B, wild type or CIRKO animals were treated as in A. Heart weight (HW/body weight (BW) ratio was measured at 6 weeks old. Data represent the mean ± S.E. (*, p < 0.01, n = 4 in each group). C, wild type or CIRKO animals were treated as in A, and heart sections were prepared and stained with hematoxylin and eosin. D, transverse diameter of myofibers was measured in each group. Data represent the mean ± S.E. (*, p < 0.01).]
Akt Mediates Insulin-dependent Postnatal Heart Growth

The coordinated regulation of heart and body size suggests that they are both responsible for the organism's external nutritional condition. However, the growth regulatory mechanism shown here for the heart may not apply to the control of growth in other organs. Muscle-specific or neuron-specific deletion of the IR gene has little effect on muscle mass or brain size, respectively (65, 66). Pancreatic β cell-specific IR knockout mice also show normal islet size at 2 months of age (67). On the other hand, liver-specific deletion of the IR results in marked reduction of liver size (60% of control) (38). These findings indicate that the relative contribution of insulin signaling to growth varies between different organs and tissues. In humans, both acute and chronic starvation results in a marked reduction in weights of the heart, liver, and spleen, but brain, spinal cord, and lung weights are spared (5); these results are in good agreement with the phenotypes of tissue-specific IR knockout mice. In marked contrast, IGF-I knockout mice display increased heart, liver, and spleen size relative to the degree of reduction in body weight, whereas lung weights are significantly decreased relative to the reduction in body weight (68). These studies suggest that postnatal organ growth is coordinately regulated by both insulin and IGF-I signaling, the former primarily reflecting the external nutritional condition, whereas the latter reflects the intrinsic activity of hypothalamo-pituitary axis. Collectively, these findings indicate the existence of as-yet-undefined regulatory mechanisms that determine the differential contribution of insulin signaling to postnatal growth in each organ or tissue.

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REFERENCES

1. de Simone, G., Daniels, S. R., Devereux, R. B., Meyer, R. A., Roman, M. J., de Divitiis, O., and Alderman, M. H. (1992) J. Am. Coll. Cardiol. 20, 1251–1260
2. Rakusan, K. (1984) in Growth of the Heart in Health and Disease (Zak, R., ed) pp. 131–164, Raven Press, Ltd., New York
3. Shepard, R. J. (1999) in Exercise in the Heart and Health Disease (Shephard, R. J., and Miller, H. S., eds) 2nd Ed, pp. 233–237, Marcel Dekker, Inc., New York
4. Hauflick, O., and Brown, M. D. (1996) J. Vasc. Res. 33, 266–287
5. Rina, A., Bruzek, J., Hensens, R., O’Callahan, M., and Taylor, H. L. (1950) The Biology of Human Starvation, University of Minnesota Press, Minneapolis, MN
6. Isner, J. M., Sours, H. E., Paris, A. L., Ferrans, V. J., and Roberts, W. C. (1979) Circulation 60, 1401–1412
7. de Simone, G., Scafl, L., Galderisi, M., Celentano, A., Di Biase, G., Mannaro, P., Garofalo, M., Marcelli, G. F., de Divitiis, O., and Contaldo, F. (1994) Br. Heart J. 71, 297–299
8. Nimmo, E., Nishino, K., Nakashima, Y., Kuroiwa, A., and Ikeda, M. (1996) Am. Heart J. 131, 313–319
9. Scribani, L., Fats, L., Mannaro, P., Carvalho, C., Silva, F. A., Vieira, M., and Rabelo, A., Jr. (1999) Am. J. Cardiol. 84, 1127–1129
10. McMahon, T. (1973) Science 180, 1201–1204
11. MacLellan, W. R., and Schneider, M. D. (2000) Annu. Rev. Physiol. 62, 289–319
12. Molkentin, J. D., and Jorn, I. G. (2001) Annu. Rev. Physiol. 63, 391–426
13. Kahn, C. R. (1994) Diabetes 43, 1066–1084
14. Pard, G. D., and Dwyer, R. M. (1997) Biochem. J. 328, 329–341
15. White, M. F., and Kahn, C. R. (1994) J. Biol. Chem. 269, 1–4
16. Virkamaki, A., Ueki, K., and Kahn, C. R. (1999) J. Clin. Invest. 103, 931–943
17. Shephard, R. J., and Withers, P. H., and Wethers, J. D., and Siddle, K. (1998) Biochem. J. 333, 471–490
18. Downward, J. (1998)Curr. Opin. Cell Biol. 10, 262–267
19. Shah, O. J., John, C. J., Kimball, S. R., and Jefferson, L. S. (2000) Am. J. Physiol. 279, E715–E723
20. Sugden, P. H., and Fuller, S. J. (1991) Biochem. J. 275, 21–37
21. Vedula, J., Sturmer, W., de, Wilder, E. L., and Birnbaum, M. J. (1999) Nat. Cell Biol. 1, 500–506
22. Chen, W. S., Xu, P. Z., Gottlohb, K., Chen, M. L., Sokol, K., Shiyano, T., Roninison, I., Weng, W., Suzuki, R., Tobe, K., Kadowaki, T., and Hay, N. (2001) Genes Dev. 15, 2203–2208
23. Cho, H., Thorvaldsen, J. L., Chu, Q., Feng, F., and Birnbaum, M. J. (2001) J. Biol. Chem. 276, 38349–38352
24. Ueki, K., and Kahn, C. R. (2001) J. Cell Biol. 153, 2203–2208
25. Morisco, C., Zebrowski, D., Condorelli, G., Tsichlis, P., Vatner, S. F., and Badovinac, J. (2001) J. Biol. Chem. 275, 14468–14475
26. Bodine, C., Stitt, T. N., Gonzalez, M., Klein, W. O., Stover, G. L., Bauerlein, R., Zlotchenko, E., Scrimgeour, A., Lawrence, J. C., Glass, D. J., and Yancopoulos, G. D. (2001) Nat. Cell Biol. 3, 1014–1019
27. Takahashi, A., Kureishi, Y., Yang, J., Luo, Z., Guo, K., Mukhopadhyay, D.,
