Akt1 Is Required for Physiological Cardiac Growth

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Background—Postnatal growth of the heart chiefly involves nonproliferative cardiomyocyte enlargement. Cardiac hypertrophy exists in a “physiological” form that is an adaptive response to long-term exercise training and as a “pathological” form that often is a maladaptive response to provocative stimuli such as hypertension and aortic valvular stenosis. A signaling cascade that includes the protein kinase Akt regulates the growth and survival of many cell types, but the precise role of Akt1 in either form of cardiac hypertrophy is unknown.

Methods and Results—To evaluate the role of Akt1 in physiological cardiac growth, akt1−/− adult murine cardiac myocytes (AMCMs) were treated with IGF-1, and akt1−/− mice were subjected to exercise training. akt1−/− AMCMs were resistant to insulin-like growth factor-1–stimulated protein synthesis. The akt1−/− mice were found to be resistant to swimming training–induced cardiac hypertrophy. To evaluate the role of Akt in pathological cardiac growth, akt1−/− AMCMs were treated with endothelin-1, and akt1−/− mice were subjected to pressure overload by transverse aortic constriction. Surprisingly, akt1−/− AMCMs were sensitized to endothelin-1–induced protein synthesis, and akt1−/− mice developed an exacerbated form of cardiac hypertrophy in response to transverse aortic constriction.

Conclusions—These results establish Akt1 as a pivotal regulatory switch that promotes physiological cardiac hypertrophy while antagonizing pathological hypertrophy. (Circulation. 2006;113:2097-2104.)

Key Words: heart failure ■ hypertrophy ■ signal transduction

Postnatal mammalian cardiomyocytes respond to mechanical stress, growth factors, hormones, and metabolic and sarcomeric abnormalities by enlarging. Nearly all of these cells, however, are unable to proliferate.1 The clinical consequences of cardiac hypertrophy include the development of cardiac arrhythmia, diastolic dysfunction, and congestive heart failure.2,3 The cardiac hypertrophic response, however, is not universally associated with a poor prognosis. Indeed, cardiac hypertrophy in well-trained athletes does not progress to congestive heart failure.4,5

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The phosphatidylinositol-3-kinase (PI3K)-Akt pathway has been investigated as a participant in the cardiac hypertrophic program. Although the first genetic evidence that this pathway is critical for cellular growth was derived from studies in Drosophila melanogaster,6 this idea was recently confirmed both in vertebrate tissue cultures and in mice. PI3K or activated Akt overexpression in various mouse tissues leads to organ enlargement, and in all cases, increased cell size is a major contributor to the phenotype.

There are 3 members of the Akt family: Akt1, Akt2, and Akt3.7,8 Each member has a highly conserved protein kinase domain and a pleckstrin homology domain, which is required for Akt activation. Activated Akt proteins phosphorylate a variety of intracellular substrates that regulate growth, metabolism, and survival. Akt phosphorylates and inhibits the product of the TSC2 gene, tuberous sclerosis 2 (TSC2), which itself inhibits the mammalian target of rapamycin.9,10 Recent work has emphasized the control of protein synthesis by the mammalian target of rapamycin, an important effector that promotes growth.11 The Foxo family of fork-head transcription factors12 and glycogen synthase kinase–3 (GSK-3)13 also are important negative regulators of protein synthesis that are inhibited by Akt.

PI3K and Akt1 are activated in rodent heart in response to pressure overload and in cultured cardiomyocytes in response to hypertrophic ligands.14 Treatment of cultured cardiomyocytes with PI3K inhibitors blocked ligand-induced hypertrophy.15 Cardiac-specific overexpression of activated PI3K(p110α) in transgenic mice resulted in baseline cardiac hypertrophy without fibrosis.16 Conversely, cardiac-specific expression of a dominant-negative p110α mutant resulted in reduced heart size and weight associated with normal cardiac function.17 Cardiac-specific overexpression of activated mutant (dd)Akt1 resulted in massive cardiac hypertrophy and fibrosis associated with normal contractile function.18
Cardiac-specific membrane-anchored Akt1 (myr-Akt1) overexpression models exhibited a comparable baseline cardiac growth phenotype. Although some investigators have argued for Akt as a major regulator of physiological hypertrophy, some experiments are consistent with Akt also promoting pathological hypertrophy.

Akt1 and Akt2 appear to have distinct biological functions. akt2−/− mice exhibit defective insulin-stimulated glucose uptake in muscle and fat, implicating Akt2 in glucose homeostasis. In contrast, akt1−/− mice exhibit normal glucose homeostasis, but their body and organ sizes are proportionally 20% less than that of wild-type (WT) littersmates throughout their lifespan. In the present study, we analyzed the ability of Akt1-deficient mice to develop cardiac hypertrophy in response to provocative stimuli.

Methods
akt1+/− and akt1−/− Mice
Mice with targeted disruption of the akt1 gene in the C57Bl/6 genetic background were generated as previously described. Mice were repeatedly backcrossed (>6 times) with WT C57Bl/6 mice obtained from the Jackson Laboratory (Bar Harbor, Me) before experimentation. Pregeny were screened by tail-prep PCR. All animal procedures were approved by the Committee for Handling and Care of Laboratory Animals before experimentation. Experiments with mouse models were performed in strict accordance with the Committee for Handling and Care of Laboratory Animals protocols (Washington University approval 20030049).

Adult Murine Cardiomyocyte Cultures
Adult murine cardiomyocytes (AMCMs) were prepared according to published AFCS protocols. Briefly, 8- to 12-week-old mice were killed, and their hearts were cannulated on a 16-gauge needle. Collagenase (1 mg/mL) maintained at 37°C was circulated through the coronary arterial system for ~20 minutes. Hearts were minced, and dissolved calcium was introduced into the medium. AMCMs were then plated onto laminin-coated tissue culture vessels and grown in a humidified incubator with 5% CO2 to confluence.

Swimming Exercise
Eight-week-old akt1+/−, akt1−/−, and WT sex- and age-matched mice were subjected to a forced swimming program for 20 days as previously reported. Mice swim twice daily; each session lasted up to 90 minutes. Constant monitoring ensured the safety of the mice and prevented them from floating or holding their breath under water. After completion of the training, the mice were subjected to transthoracic echocardiography. The mice were killed, and the hearts were dissected and weighed. Left ventricular (LV) weight, tibial length (TL), and body weight (BW) were measured so that LV/TTL could be calculated. Because both genotypes lost weight during the swim training period, we used LV/TTL as the index of comparison.

Histology
Ventricular tissue was fixed in formalin, embedded in paraffin, microtome sectioned, and stained with hematoxylin and eosin. Myocyte cross-sectional areas from randomly selected high-power fields were calculated on an Axioskop microscope (Carl Zeiss, Inc, Chester, Va) using Axiosvision 4.0 software.

Transverse Aortic Constriction Procedures
Anesthetized akt1−/−, akt1+/−, and WT mice were subjected to pressure overload by transverse aortic constriction (TAC). Sham-operated akt1−/− and akt1+/− mice and their WT littermates were used as controls. Seven days after surgery, mice were evaluated by echocardiography. The mice were killed, and postmortem and histological studies were performed as described above.

Echocardiography
Mice were imaged in the left lateral decubitus position on a Sequoia cardic echocardiography machine (Acuson Co, Malvern, Pa) equipped with a 15-MHz linear transducer (15–L8). Two-dimensional parasternal long- and short-axis views were recorded, as was 2D targeted M-mode tracings throughout the anterior and posterior LV walls.

Protein Analysis
Immunoblotting was performed using standard techniques. Phospho-Akt(S473), phospho-GSK-3(S9), phospho-ERK, phospho-JNK, JNK, phospho-p70 S6K(T389), and phospho-S6(S235/236) antisera were obtained from Cell Signaling Technology (Danvers, Mass). Akt1 and ERK1/2 anti-sera were obtained from Santa Cruz Biotechnology, Inc (Santa Cruz, Calif). Blots were visualized with an ECL kit (Amersham Biosciences Inc, Piscataway, NJ). Scanned blot densitometry was performed with Scion Image densitometry software.

Leucine Incorporation Assays
AMCMs were cultured in 12-well tissue culture plates and serum deprived for 4 hours before ligand addition in the presence of 1 μCi/mL [3H]-leucine (16 to 24 hours). Cultures were washed in PBS before 5% trichloroacetic acid precipitation. Acid-precipitable counts were lysed in 0.2N NaOH/0.1% SDS buffer and counted in a Beckman scintillation counter in 5 mL Ultima Gold (Packard Instruments, Meridian, Conn) liquid scintillation counting fluid.

Gene Expression Analysis
Quantitative real-time RT-PCR analysis on RNA extracted from heart lysates with Trizol reagent (Invitrogen Corp, Carlsbad, Calif) was carried out with the Taqman master mix kit (Applied Biosystems, Foster City, Calif) according to the manufacturer’s specifications. The measured abundances of atrial natriuretic factor (ANF) and β-MHC mRNA were normalized to GAPDH in each sample as an internal loading control.

Statistical Analysis
Normality and equal-variance assumptions were assessed by Levene’s test using the SigmaStat Statistical Analysis Package (version 3.1). Mann-Whitney rank-sum tests with Bonferroni’s post hoc correction were used in comparisons for which normality or equal-variance assumptions were invalid. When indicated, data sets were compared by 2-sample, 2-tailed homoscedastic t tests with Bonferroni’s post-hoc correction. In other instances, 1-way ANOVA, followed by Tukey’s honestly significant difference post-hoc correction, was calculated by SigmaStat (version 3.1) or with VassarStats Statistical Software.

The authors had full access to the data and take full responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results
Normal Gross Anatomic and Molecular Baseline Cardiac Characteristics in Akt1-Deficient Mice
The akt1−/− mice were recently generated and are live born, are fertile, and have a lifespan of normal duration. Unperturbed WT and Akt−1-deficient mice were examined by transthoracic echocardiography to determine whether akt1 deletion disrupts normal cardiac anatomic dimensions or cardiac function. Each of the LV wall dimensions measured—including the diastolic LV posterior wall thickness, diastolic interventricular septum thickness, and most notably LV mass index (LVMI)—were statistically identical in WT, akt1−/−, and akt1−/− mice (Table 1). The LV internal dimen-
Therefore, [3H]-leucine incorporation was a marker of cardiomyocyte hypertrophy at the cellular level. Therefore, [3H]-leucine incorporation was

**TABLE 1. Echocardiographic Analysis of Mice at Baseline**

<table>
<thead>
<tr>
<th></th>
<th>WT (n=20)</th>
<th>akt1++/− (n=8)</th>
<th>akt1++/− (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, min⁻¹</td>
<td>684±12</td>
<td>706±8.67</td>
<td>707±7.23</td>
</tr>
<tr>
<td>LV PWd, mm</td>
<td>0.62±0.02</td>
<td>0.67±0.02</td>
<td>0.62±0.03</td>
</tr>
<tr>
<td>IVSd, mm</td>
<td>0.64±0.02</td>
<td>0.67±0.03</td>
<td>0.64±0.03</td>
</tr>
<tr>
<td>LV Idd, mm</td>
<td>3.36±0.07</td>
<td>3.59±0.06</td>
<td>3.34±0.10</td>
</tr>
<tr>
<td>LVMi, mg/g</td>
<td>2.85±0.11</td>
<td>3.04±0.20</td>
<td>3.08±0.24</td>
</tr>
<tr>
<td>LV Ids, mm</td>
<td>1.40±0.07</td>
<td>1.78±0.05*</td>
<td>1.44±0.03</td>
</tr>
<tr>
<td>FS, %</td>
<td>58.6±1.65</td>
<td>50.37±1.19*</td>
<td>56.88±1.32</td>
</tr>
</tbody>
</table>

*n* indicates the number of mice analyzed in each group; LV PWd, diastolic left ventricular posterior wall thickness; and IVSd, diastolic interventricular septal thickness. Eight- to twelve-week-old mice subjected to no intervention underwent thoracoscopic echocardiography. Values are mean±SE.

**Figure 1.** Akt1 is required for IGF-1–stimulated signal transduction during cardiac angiogenesis. A, AMCMs from WT and akt1++/− mice were serum deprived (4 hours) and incubated with IGF-1 (10 nmol/L) for 0, 5, and 10 minutes. Lysates were analyzed by phosphospecific Akt (S473), GSK-3β (S9), p70 S6K (T389), and p70 S6K (T389) immunoblotting. Total S6 (bottom) was probed to control for protein loading. B, Radiolabeled leucine incorporation was measured in WT and akt1++/− AMCMs stimulated with vehicle or with IGF-1 (10 nmol/L) for 18 hours in the presence of [3H]-leucine. Data are expressed as mean tri- chloroacetic acid–precipitable cpm/μg protein±SD. Sample sizes analyzed in each experiment (n) are shown in parentheses. This experiment was performed twice, and each experiment showed similar results. Shown are the mean±SD from a single, representative experiment. One-way ANOVA with Tukey’s post hoc correction was performed to simultaneously compare WT unstimulated, IGF-1–unstimulated, IGF-1–stimulated, akt1++/− unstimulated, and akt1++/− stimulated. *P<0.05 vs WT unstimulated controls; **P<0.05 vs IGF-1–treated WT cultures.

**Insulin-Like Growth Factor-1 Signal Transduction and Protein Synthesis Is Akt1 Dependent**

Swimming training of rodents increases myocardial IGF-1 gene expression, and insulin-like growth factor-1 (IGF-1) treatment of AMCMs is an in vitro model of physiological hypertrophy. To test whether IGF-1 activates downstream growth-promoting signaling intermediates via Akt, AMCMs from adult WT and akt1++/− mice were incubated with IGF-1 (10 nmol/L) for 0 to 10 minutes. Lysates derived from IGF-1–treated AMCMs were analyzed by phosphospecific Akt, GSK-3β, p70 S6 kinase (S6K), and S6 immunoblotting (Figure IA). Although IGF-1 induced robust phosphorylation of each signaling intermediate in WT AMCMs, IGF-1–stimulated akt1++/− AMCMs exhibited attenuated IGF-1 signaling.

The requirement for Akt1 in IGF-1–stimulated cardiac Akt pathway signaling prompted us to determine whether IGF-1–stimulated cardiac growth is Akt1 dependent. Enhanced protein synthesis is a marker of cardiomyocyte hypertrophy at the cellular level. Therefore, [3H]-leucine incorporation was measured in serum-deprived AMCMs stimulated with or without IGF-1 (10 nmol/L) for 18 hours (Figure IB). WT AMCMs responded to IGF-1 by incorporating an average of 1.49±0.03 versus only 1.25±0.05 cpm/μg protein in unstimulated control cultures. akt1++/− AMCMs did not exhibit statistically different baseline protein synthesis (1.17±0.15 cpm/μg protein). After 18 hours of induction by IGF-1 (10 nmol/L), akt1++/− AMCMs incorporated markedly less [3H]-leucine compared with similarly treated WT cultures (1.28±0.007 cpm/μg protein; *P<0.05 vs IGF-1–induced WT cultures).

**Impaired Cardiac Growth Response to Swimming Exercise in akt1++/− Mice**

The impaired protein synthesis in akt1++/− cardiomyocytes after IGF-1 stimulation led us to evaluate the ability of Akt1-deficient mice to develop cardiac hypertrophy in response to involuntary swimming training. WT and Akt1-
deficient mice were trained in 90-minute sessions twice daily for 20 days, after which cardiac function was assessed by transthoracic echocardiography in trained and untrained mice (Table 2). The LVMI increased significantly in swim-trained WT mice compared with sedentary WT mice. In contrast, the LVMI did not significantly increase in swim-trained akt1+/− mice compared with congenic sedentary controls. Furthermore, the LV internal dimension at diastole (LVIDd) and LVIDs in akt1+/− mice were significantly elevated after swimming training, indicating ventricular dilatation in akt1+/− mice. In comparison, LVIDd and LVIDs did not increase in WT mice after swimming training. Cardiac function, as indicated by FS, was modestly but statistically lower in trained akt1+/− mice compared with sedentary akt1+/− mice. Ventricular dilatation in trained Akt1-deficient mice may represent a compensatory mechanism to maintain cardiac output in the absence of an adequate LV hypertrophic response.

Cardiac hypertrophy also was assessed by measurement of the LV/TL ratio. WT mice developed significant cardiac hypertrophy in response to swimming training compared with sex-, age-, and weight-matched littermate control animals (Figure 2A). LV/TL increased by 19.4% in WT animals after 20 days rest (R) or swimming training (S). LV/TL was determined by morphometry. Number of animals analyzed in each group (n) is indicated in parentheses. To evaluate whether swimming training differentially affected WT, akt1+/−, or akt1−/− mouse LV/TL, the following 2-tailed, 2-sample homoscedastic t tests were performed with Bonferroni’s post hoc correction: WT resting vs WT swimming and akt1−/− resting vs akt1−/− swimming. *P<0.05 vs resting congenic control mice.

**Figure 2.** Akt1 is required for exercise-induced cardiac hypertrophy. A, Morphometric analysis of WT, akt1+/+, and akt1−/− mice after 20 days rest (R) or swimming training (S). LV/TL was determined by morphometry. Number of animals analyzed in each group (n) is indicated in parentheses. To evaluate whether swimming training differentially affected WT, akt1+/+, or akt1−/− mouse LV/TL, the following 2-tailed, 2-sample homoscedastic t tests were performed with Bonferroni’s post hoc correction: WT resting vs WT swimming, akt1−/− swimming vs WT swimming, and akt1−/− swimming vs WT swimming. *P<0.05 vs resting WT mice; **P<0.05 vs WT swimming group. B, Histological analysis of cardiomyocyte cross-sectional area of WT, akt1+/+, and akt1−/− mice after rest (R) or swimming training (S) for 20 days: low-power photomicrographic images of cardiac tissue sections stained with hematoxylin and eosin. Scale bar=20 μm. C, Average myocyte cross-sectional areas from transverse cardiac sections from WT, akt1+/+, and akt1−/− mice subjected to 20 days of rest or swim training were determined by computerized photomicrographic analysis. Numbers of cells (n) measured in random fields in at least 3 mice per treatment group are shown in parentheses. To evaluate whether swimming training differentially affected WT, akt1+/+, or akt1−/− mouse cardiomyocyte cross-sectional area, statistical comparisons were performed with Bonferroni's post hoc correction: WT resting vs WT swimming and akt1−/− swimming vs WT swimming (Mann Whitney), and akt1+/+ swimming vs WT swimming (2-sample homoscedastic t test). *P<0.05 vs resting WT mice; **P<0.05 vs WT swimming group.
ocyte cross-sectional area was significantly lower in both akt1<sup>+/−</sup> and akt1<sup>−/−</sup> mice after swimming training compared with sedentary congenic mice (Figure 2C).

**Normal G Protein–Coupled Receptor-Mediated Akt Pathway Signaling in Endothelin 1–Stimulated akt1<sup>−/−</sup> AMCMs**

Recent evidence indicates that the adaptive, physiological cardiac growth profile is mediated by signaling pathways distinct from those that mediate pathological or maladaptive cardiac growth. Whereas physiological hypertrophy develops in response to stimuli such as IGF-1 stimulation or exercise training, pressure overload and chronic G protein–coupled receptor agonism are known to stimulate cardiac growth associated with interstitial fibrosis, abnormal gene expression, and progression to heart failure. The role of Akt1 in G protein–coupled receptor–mediated cardiac signal transduction was first probed in akt1<sup>−/−</sup> AMCMs stimulated with or without the GPCR ligand endothelin 1 (ET1; 200 mol/L). Surprisingly, ET1-stimulated Akt pathway signal transduction was indistinguishable in WT and Akt1-deficient AMCMs (Figure 3A). Indeed, the steady-state phosphorylation of downstream Akt targets GSK3β(S9), p70 S6 kinase (T389), and the S6 ribosomal subunit (S235/236) was unaltered in the presence or absence of Akt1.

**Enhanced Protein Synthesis in Response to ET1 Treatment in akt1<sup>−/−</sup> AMCMs**

We examined whether Akt1 was required for ET1-induced protein synthesis by measuring trichloroacetic acid–precipitable counts after a 24-hour pulse period with [3H]-leucine (Figure 3B). WT cardiomyocytes incorporated more [3H]-leucine in response to ET1 compared with WT vehicle-treated controls (15.7 ± 2.6%), although this trend did not reach statistical significance. In striking contrast, ET1-treated akt1<sup>−/−</sup> cardiomyocytes exhibited an enhanced leucine incorporation response compared with vehicle-treated congenic controls (40.4 ± 15.3% versus vehicle-treated akt1<sup>−/−</sup> cultures). The observed stimulation in protein synthesis was significantly greater than that observed in ET1-treated WT cardiomyocytes (P<0.05). The enhanced AMCM growth response to ET1 was corrected by acute wild-type Akt1 reconstitution by adenoviral transduction (Data Supplement Figure III).

**Enhanced Cardiac Growth Response to Pressure Overload in akt1<sup>−/−</sup> Mice**

To determine the role of Akt1 in pathological cardiac hypertrophy in response to pressure overload, TAC was performed on 8- to 12-week old akt1<sup>+/−</sup>, akt1<sup>−/−</sup> mice, and their WT littermates. A survival rate of 100% in all genotypes was ascertained by TUNEL and cleaved caspase-3 immunohistochemical analysis (not shown) of LV transverse sections. Echocardiographic analysis of LVMI confirmed that both akt1<sup>−/−</sup> and WT mice developed LV hypertrophy to a similar extent 7 days after TAC. In contrast, akt1<sup>−/−</sup> mice developed a trend toward more profound cardiac hypertrophy after TAC that did not reach statistical significance compared with WT littermate controls (Table 3). The mean±SE LVMI determined by echocardiography was 5.55±0.66 mg/g in akt1<sup>−/−</sup> mice 7 days after TAC versus 3.95±0.23 and 3.96±0.24 mg/g in WT and heterozygous littermates, respectively. The Doppler flow gradient achieved by TAC was nearly identical for all 3 genotypes; therefore, the enhanced hypertrophic response observed in akt1<sup>−/−</sup> mice was not due to more stringent constriction of the transverse aorta. In addition to profound cardiac hypertrophy, akt1<sup>−/−</sup> mice developed cardiac dysfunction in response to TAC. Echocardiography of akt1<sup>−/−</sup> mice 7 days after TAC revealed LV dilatation and systolic dysfunction (Table 3). Mean LVIDs after TAC in akt1<sup>−/−</sup> mice was 2.30±0.24 versus 1.52±0.10 mm in TAC-operated WT mice (P=0.033). Mean FS was 54.9±2.4% in TAC-operated WT mice versus 36.3±4.2% in TAC-operated akt1<sup>−/−</sup> mice (P=0.003).
TABLE 3. Echocardiographic Analysis of Mice After TAC or Sham Operation

<table>
<thead>
<tr>
<th></th>
<th>Sham Operation</th>
<th>TAC Operation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WT akt1+/−</td>
<td>akt1−/−</td>
</tr>
<tr>
<td></td>
<td>(n=10)</td>
<td>(n=3)</td>
</tr>
<tr>
<td>Heart rate, min⁻¹</td>
<td>705±11</td>
<td>717±11</td>
</tr>
<tr>
<td>LVWd, mm</td>
<td>0.65±0.01</td>
<td>0.73±0.03</td>
</tr>
<tr>
<td>LVDd, mm</td>
<td>0.70±0.02</td>
<td>0.73±0.06</td>
</tr>
<tr>
<td>LVMI, mg/g</td>
<td>3.48±0.05</td>
<td>3.44±0.08</td>
</tr>
<tr>
<td>LVIDs, mm</td>
<td>3.16±0.10</td>
<td>3.39±0.52</td>
</tr>
<tr>
<td>FS, %</td>
<td>55.1±1.2</td>
<td>48.2±2.8</td>
</tr>
<tr>
<td>Doppler velocity, m/s</td>
<td>...</td>
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</table>

Abbreviations as in Table 1. Eight- to twelve-week-old mice underwent transthoracic echocardiography 7 days after TAC or sham operation. Doppler velocity was measured at the site of TAC. To evaluate differences in the magnitude of TAC effects on cardiac parameters, the following statistical comparisons were performed with Bonferroni's post-hoc correction: WT sham vs WT TAC, akt1+/− TAC vs WT TAC, akt1−/− TAC vs WT TAC. Values are mean±SE.

*P<0.05 vs WT mice after sham operation; †P<0.05 vs WT after TAC operation.

The mean±SE LV/BW in WT animals increased from 3.40±0.03 mg/g after a sham operation to 4.21±0.13 mg/g after TAC. This represents a 23.8% increase relative to sham-operated WT mice. The cardiac hypertrophic response of akt1−/− mice to pressure overload was indistinguishable from that in WT mice (Figure 4A). akt1−/− mice, however, developed more profound hypertrophy after TAC compared with TAC-operated WT mice. The LV/BW in TAC-operated akt1−/− mice was 5.30±0.367 mg/g, which represents a 51% increase in LV/BW compared with sham-operated akt1−/− mice. The LV/BW in akt1−/− mice was significantly greater than that observed in TAC-operated WT mice (Figure 4B), but the mean cross-sectional area was 41% greater in TAC-operated akt1−/− mice compared with TAC-operated WT mice (P<0.001; Figure 4C).

Previous work demonstrated that the cardiac responses elicited by pressure overload or ET1 stimulation differ greatly from those stimulated by exercise training or IGF-1 infusion. Pressure overload promotes a deleterious form of cardiac hypertrophy characterized by cardiac fibrosis and dysfunction, whereas exercise training and IGF-1 promote a physiological form of cardiac hypertrophy characterized by improved cardiac function without cardiac fibrosis or fetal gene induction. Here, we demonstrate that Akt1 is absolutely required for physiological growth in response to IGF-1 stimulation or to exercise training, whereas Akt1 negatively regulates ET1- and TAC-induced cardiac hypertrophy.

The present study also demonstrates that the absence of Akt1 profoundly exacerbates the TAC-stimulated cardiac hypertrophic program, although the model used in these studies is not without limitations. That developmental, systemic, or other compensatory differences might contribute to the phenotypes observed in the present study remains a possibility. However, transient adenoviral reconstitution of WT Akt1 in cultured adult akt1−/− mouse cardiomyocytes rescued the exacerbated ET1-stimulated cardiomyocyte hypertrophic response as measured by radiolabeled [³H]-leucine incorporation (Data Supplement Figure III). Additionally, the steady-state levels of potentially compensatory kinases (eg, Akt2, ILK, PI3Kα) were indistinguishable from those in WT mice.

The mechanism that explains the negative regulation of pathological hypertrophy by Akt1 remains unclear, although cross-talk through several candidate pathways remains under investigation. Previous studies demonstrate inhibitory effects of Akt signaling on the ERK and JNK MAP kinase pathways, both of which enhance growth in the myocardium. Thus, hyperactivation of the MAPKs in an Akt-deficient model might explain the present observations. Equally promising in the akt1−/− model will be the investigation of calcineurin/NFAT pathway activation, which itself is a critical mediator of pathological, but not physiological, cardiac hypertrophy. Moreover, the residual Akt phosphorylation in akt1−/− AMCMs in response to ET1 stimulation...
the myocardium either are disrupted or are insufficient in the antihypertrophic and cardioprotective effects of ANF in the presence of elevated ANP gene activation suggests that the profound hypertrophy observed in response to TAC even the MAPK or calcineurin signaling pathways. Furthermore, ET1 stimulation in akt1

$$\text{Akt1}$$

lyzed by the Mann-Whitney test in WT sham vs WT TAC and in WT TAC vs akt1

$$\text{Akt1}$$

lack of antihypertrophic signaling may hypersensitize the heart to growth-promoting signal transduction (eg, via the MAPK or calcineurin signaling pathways). Therefore, the profound hypertrophy observed in response to TAC even in the presence of elevated ANP gene activation suggests that the antihypertrophic and cardioprotective effects of ANF in the myocardium either are disrupted or are insufficient in the absence of Akt1. The use of compound genetic murine models to understand the growth-promoting signaling pathways regulated by Akt1 will be imperative in defining candidate pharmacotherapeutic targets for the treatment of pathological cardiac hypertrophy and progression to heart failure.

In conclusion, our studies elucidate for the first time a dual adaptive function of Akt1 to suppress pathological cardiac hypertrophy and promote physiological hypertrophy. Agents that increase Akt1 activity in heart tissue such as IGF-1 are likely to promote the development of adaptive cardiac growth and may therefore be of therapeutic utility.

**Acknowledgments**

This work was supported by grants from the NIH (HL-61567, HL-057278) and the Burroughs Wellcome Fund (A.J.M.). B.J. DeBosch was supported by the Cardiovascular Physiology Training grant T32-HL07873. The akt1

$$\text{Akt1}$$

were a kind gift from Morris Birnbaum, Howard Hughes Medical Institute, University of Pennsylvania School of Medicine. We acknowledge the assistance of the Washington University Digestive Diseases Research Core Center (NIH P30 DK52574) and of Dr William Shannon, Director, Washington University School of Medicine Biostatistical Consulting Center.

**Disclosures**

None.

**References**


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Circulation. 2006;113:2097-2104; originally published online April 24, 2006; doi: 10.1161/CIRCULATIONAHA.105.595231
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0009-7322. Online ISSN: 1524-4539

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