Phosphoinositide 3-Kinase p110α Is a Master Regulator of Exercise-Induced Cardioprotection and PI3K Gene Therapy Rescues Cardiac Dysfunction

Kate L. Weeks, BSc(Hons); Xiaoming Gao, MD, PhD; Xiao-Jun Du, MD, PhD; Esther J.H. Boey, BSc(Hons); Aya Matsumoto, PhD; Bianca C. Bernardo, PhD; Helen Kiriazis, PhD; Nelly Cemerlang, BSc(Hons); Joon Win Tan, BSc(Hons); Yow Keat Tham, BSc(Hons); Thomas F. Franke, MD, PhD; Hongwei Qian, PhD; Marie A. Bogoyevitch, PhD; Elizabeth A. Woodcock, PhD; Mark A. Febbraio, PhD; Paul Gregorevic, PhD; Julie R. McMullen, PhD

Background—Numerous molecular and biochemical changes have been linked with the cardioprotective effects of exercise, including increases in antioxidant enzymes, heat shock proteins, and regulators of cardiac myocyte proliferation. However, a master regulator of exercise-induced protection has yet to be identified. Here, we assess whether phosphoinositide 3-kinase (PI3K) p110α is essential for mediating exercise-induced cardioprotection, and if so, whether its activation independent of exercise can restore function of the failing heart.

Methods and Results—Cardiac-specific transgenic (Tg) mice with elevated or reduced PI3K(p110α) activity (constitutively active PI3K [caPI3K] and dominant negative PI3K, respectively) and non-Tg controls were subjected to 4 weeks of exercise training followed by 1 week of pressure overload (aortic-banding) to induce pathological remodeling. Aortic-banding in untrained non-Tg controls led to pathological cardiac hypertrophy, depressed systolic function, and lung congestion. This phenotype was attenuated in non-Tg controls that had undergone exercise before aortic-banding. Banded caPI3K mice were protected from pathological remodeling independent of exercise status, whereas exercise provided no protection in banded dominant negative PI3K mice, suggesting that PI3K is necessary for exercise-induced cardioprotection. Tg overexpression of heat shock protein 70 did not rescue the phenotype of banded dominant negative PI3K mice, and deletion of heat shock protein 70 from banded caPI3K mice had no effect. Next, we used a gene therapy approach (recombinant adeno-associated viral vector 6) to deliver caPI3K expression cassettes to hearts of mice with established cardiac dysfunction caused by aortic-banding. Mice treated with recombinant adeno-associated viral 6-caPI3K vectors had improved heart function after 10 weeks.

Conclusions—PI3K(p110α) is essential for exercise-induced cardioprotection and delivery of caPI3K vector can improve function of the failing heart. (Circ Heart Fail. 2012;5:523-534.)

Key Words: cardiac hypertrophy ■ exercise ■ gene therapy ■ heart failure

Cardiovascular disease remains the leading cause of death worldwide; thus, identification of signaling pathways that are essential for cardiac protection, and which could be exploited to improve or restore cardiac function, is of great interest. The beneficial effects of regular physical activity on cardiovascular health are well established.1,2 Exercise is one of only a few interventions known to improve cardiac function rather than merely delaying disease progression.1 For example, 6 months of regular exercise training improved ejection fraction in men with stable chronic heart failure.3 However, the critical molecular mechanisms underlying the cardioprotective effects of exercise have not been well defined. This knowledge is essential for the development of novel and viable therapeutic intervention strategies for patients with cardiovascular disease who are either unable or unwilling to undertake regular physical activity intervention programs.

Clinical Perspective on p 534

Changes to skeletal muscle and the vasculature account for some of the beneficial effects of physical activity in patients with heart failure.5 However, exercise also exerts direct, beneficial effects on the heart.6,7 Long-term, high-intensity exercise training can lead to physiological cardiac hypertrophy (an increase in cardiac muscle mass, the athlete’s heart), which enhances cardiac output. Exercise-induced hypertrophy is distinct from pathological cardiac hypertrophy, heart growth...
that occurs as a result of chronic pressure or volume overload in settings of disease. When untreated, pathological cardiac hypertrophy can progress to heart failure, whereas physiological cardiac hypertrophy is reversible and is not associated with adverse remodeling or compromised function. In the last decade, it has become apparent that distinct signaling cascades regulate physiological and pathological growth of the heart. G protein–coupled receptor signaling cascades are required for the induction of pathological cardiac hypertrophy, whereas activation of the insulin-like growth factor 1–phosphoinositide 3-kinase p110α (PI3K[p110α]) signaling pathway is critical for physiological heart growth.

PI3K(p110α) is a lipid kinase that catalyses the phosphorylation of the sarcoplasmic lipid, phosphatidylinositol(4,5)bis phosphate (PI(4,5)P₂), to PI(3,4,5)P₃ to initiate downstream signaling events, particularly activation of protein kinase B (Akt). PI3K(p110α) is activated in the heart during exercise and is critical for postnatal heart growth and exercise-induced physiological hypertrophy. Mice with reduced cardiac PI3K(p110α) activity caused by expression of a dominant negative (dn) PI3K(p110α) mutant or loss of p85 regulatory subunits of p110α displayed smaller hearts under basal conditions and showed an attenuated hypertrophic response to α subunits of p110 negative (dn) PI3K(p110α) activity caused by expression of a dominant PI3K(p110α) activity caused by expression of a constitutively active (ca) PI3K(p110α) mutant, displayed physiological cardiac hypertrophy under basal conditions, and had better cardiac function or lifespan in a setting of myocardial infarction or dilated cardiomyopathy, respectively.

Conversely, transgenic (Tg) mice with elevated cardiac PI3K(p110α) activity caused by expression of a constitutively active (ca) PI3K(p110α) mutant, displayed physiological cardiac hypertrophy under basal conditions, and had better cardiac function or lifespan in a setting of myocardial infarction or dilated cardiomyopathy. However, in each of the disease studies, PI3K activity was regulated before the cardiac insult because transgene expression was switched on largely after birth using the α-myosin heavy chain (MHC) promoter. In a similar context, other genetic and pharmacological interventions have been reported to provide cardiac protection when manipulation occurs before or concurrently with a cardiac insult. However, the key challenge is to identify approaches with the ability to improve cardiac function once dysfunction is already present/diagnosed, mimicking the clinical situation. Because exercise has been shown to improve cardiac function in heart failure patients, we hypothesized that an essential regulator of exercise-induced cardiac protection would have the capacity to improve function of the failing heart.

A complex array of molecular and biochemical changes have been linked with the cardioprotective properties of exercise, including increases in antioxidant enzymes, heat shock proteins (Hsps; eg, Hsp70), NO metabolites, and, more recently, regulators of cardiac myocyte proliferation. The main goal of the present study was to identify a key regulator of exercise-induced protection, and to assess whether its activation could reverse pathological remodeling. Because reduced PI3K(p110α) activity dramatically blunted exercise-induced hypertrophy in mice, we hypothesized that PI3K(p110α) might be the essential master regulator of exercise-induced protection, and that increasing PI3K using a gene therapy approach could also confer benefit in the absence of exercise.

Methods

Experimental Animals

All aspects of animal care and experimentation were approved by the Alfred Medical Research and Education Precinct Animal Ethics Committee. Cardiac-specific Tg mice with increased or decreased PI3K(p110α) activity (caPI3K and dnPI3K mice, respectively; FVB/N background) were originally generated as described. To assess the impact of increasing Hsp70 expression in hearts of dnPI3K mice, dnPI3K-Hsp70 Tg mice were generated by breeding heterozygous dnPI3K mice with heterozygous Hsp70 Tg mice (overexpression of Hsp70 in heart and skeletal muscle using β-actin promoter and a cytomegalovirus enhancer; BALB/c background). To examine the effect of deleting Hsp70 from hearts of caPI3K mice, caPI3K-Hsp70 knockout (KO) mice were generated by breeding caPI3K mice with Hsp70 KO mice (C57BL/6 background). Recombinant adeno-associated viral 6 (rAAV6) vectors carrying a caPI3K expression cassette were delivered to wild-type mice on FVB/N or C57BL/6 backgrounds.

Experimental Protocols

Protocol 1

We first established a protocol in which chronic exercise training protected the mouse heart against a subsequent cardiac insult (ascending aortic constriction [AAC]). Adult (8–12 weeks) female non-Tg (Ntg/control) mice were subjected to swim training for 4 weeks, as described. One day after the last training session, AAC or a sham operation was performed as reported. No further exercise training was undertaken after AAC. Cardiac function was assessed 1 week postsurgery, before tissue collection. The AAC model was used because it causes significant left ventricular (LV) remodeling within 1 week and is associated with signs of heart failure including fluid in the chest, lung congestion, and atrial enlargement.

We confirmed the exercise training protocol induced significant physiological cardiac hypertrophy in a subset of Ntg mice (≈43% increase in heart weight [HW] standardized to tibial length [TL]; P<0.0001; n=8 nonswim; n=5 swim), as previously shown. Heart size returned to baseline within a week of cessation of exercise, with no difference in HW/TL ratio between trained and untrained sham-operated mice 1 week postsurgery (Figure 1B: comparison of untrained and trained sham mice). Thus, any increase in HW observed in trained Ntg mice subjected to pressure overload was considered indicative of pathological cardiac hypertrophy induced by aortic-banding rather than remnant physiological hypertrophy from exercise training.

Protocol 2

To examine whether PI3K(p110α) is critical for mediating exercise-induced cardiac protection, adult (8–12 weeks) female caPI3K, dnPI3K, and Ntg mice were subjected to the same procedures described in protocol 1.

Protocol 3

To assess whether Hsp70 plays a role in PI3K-mediated cardiac protection, AAC was performed in dnPI3K mice overexpressing Hsp70 (dnPI3K-Hsp70 Tg) and caPI3K mice deficient for Hsp70 (caPI3K-Hsp70 KO). Cardiac function was assessed 1 week postsurgery.

Protocol 4

To determine whether delivery of an rAAV vector containing the ca mutant of PI3K(p110α) could mimic the effects of the caPI3K transgene in mice (ie, induce physiological heart growth), mice were administered rAAV6-caPI3K or a control vector (rAAV6-null). Adult (10–12 weeks) male C57BL/6 mice received 6×10¹⁰ or 2×10¹ⁱ vector genomes via a tail vein injection. Systolic function was assessed before administration of vector and 8 weeks after delivery.

Protocol 5

To assess whether rAAV6-caPI3K could restore function in a mouse model with preexisting cardiac dysfunction, 2×10¹⁰ vector genomes were administered via a tail vein injection to adult male FVB/N mice with pressure overload–induced cardiac dysfunction. Because
vector transduction has been shown to take ≈11 days, the slower developing transverse aortic constriction (TAC) model of pressure overload rather than AAC was used. TAC was performed as described and is associated with cardiac dysfunction within 4 weeks. Cardiac function was measured 4 weeks post-TAC, and mice were randomized to receive single administration of rAAV6-caPI3K, rAAV6-null, or saline. Cardiac function was assessed 4, 8, and 10 weeks post–AAV delivery.

AAV Generation and Administration

A PI3K construct that encodes a ca protein (caPI3K) is a chimeric molecule that contains the iSH2 domain of p85 fused to the N-terminus of bovine p11031) was cloned into an AAV vector plasmid with a cytomegalovirus promoter and packaged into pseudotype 6 capsids (further details presented in online-only Data Supplement Material). Systemic delivery of rAAV vectors was previously shown to preferentially transduce cardiac and skeletal muscle. Approximately 6x1010 or 2x1011 vector genomes of rAAV6-caPI3K or a transgene-null vector (rAAV6-null; control) were administered via a tail vein injection.

Figure 1. Swim training protects mice from pathological remodeling and signs of heart failure in response to aortic-banding. A, Fractional shortening, (B) heart weight/tibia length, and (C) lung weight/tibia length ratios of female nontransgenic mice after 1 week of ascending aortic constriction (band) or sham surgery. Trained mice underwent 4 weeks of swim training before surgery. *P<0.05 vs sham of the same exercise group (untrained/trained); n=3 to 6 per group. D, Northern blotting (top) and quantification of gene expression. ANP indicates atrial natriuretic peptide; BNP, B-type natriuretic peptide; αMHC, α-myosin heavy chain; SERCA2a, sarcoplasmic/endoplasmic reticulum Ca2+-ATPase 2a; GAPDH, glyceraldehyde 3-phosphate dehydrogenase. *P<0.05 vs sham of the same exercise group (untrained/trained); n=3 to 4 per group.

Histological Analyses

Ventricle samples were frozen in cryoprotectant compound or fixed in 4% paraformaldehyde, dehydrated, and embedded in paraffin. Assessment of fibrosis with Masson trichrome, apoptosis by terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick end labeling, immunostaining for quantification of capillary density, and the detection of Myc-tagged caPI3K in mice administered rAAV6-caPI3K is described in online-only Data Supplement Material.

Gene, Protein, and Biochemical Activity Analyses

A detailed description is presented in online-only Data Supplement Material. In brief, protein and RNA were extracted from tissue as previously described. Gene expression was determined by Northern blotting, microarray, or real-time quantitative polymerase chain reaction, and protein expression was assessed by Western blotting. Citrate synthase activity in mixed gastrocnemius of untrained and trained Ntg and caPI3K mice was measured as reported.15-3
Statistical Analyses
Results are presented as means±SEM. All variables in the analysis were continuous. Differences between groups were identified using 1-way ANOVA, or repeated-measures ANOVA if serial echocardiography measurements were taken. Fisher least significant difference post hoc pairwise t tests were performed when the ANOVA was significant (P<0.05). All pairwise P values are 2-sided. Unpaired t tests were performed when the ANOVA was not significant and when comparing 2 groups for single measure. Given the nature of the study, no adjustment for multiple comparisons was made. All relative units are expressed as a fold change with the relevant control group normalized to 1.

Results
Exercise Training Attenuated Pressure Overload–Induced Pathological Remodeling and Cardiac Dysfunction
AAC induced pathological cardiac hypertrophy associated with an increase in LV wall thickness, depressed systolic function, and lung congestion (see untrained banded mice; Figure 1A–1C; online-only Data Supplement Table I). Despite similar degrees of aortic stenosis in untrained and trained banded groups (aortic pressure gradient; online-only Data Supplement Table I), systolic function was not depressed in trained banded mice and, notably, was not different from sham-operated mice (Figure 1A; online-only Data Supplement Table I). HW/TL and LV wall thicknesses were lower in trained banded mice compared with untrained banded mice (Figure 1B; online-only Data Supplement Table I), and there was no evidence of lung congestion in trained banded mice (Figure 1C). Furthermore, markers associated with pathological hypertrophy (atrial natriuretic peptide [ANP] and B-type natriuretic peptide) were lower in banded hearts from trained mice compared with untrained mice, and specific genes important for contractile function (sarcomplasmic/endoplasmic reticulum Ca2+-ATPase 2a [SERCA2a] and αMHC) were depressed in hearts of untrained banded mice but not in trained banded mice (Figure 1D).

Exercise-Induced Protection Was Mimicked by Enhanced PI3K(p110α) Activity and Abolished by Reduced PI3K(p110α) Activity
To assess the role of PI3K(p110α) in mediating exercise-induced protection of the heart, cardiac-specific Tg mice with elevated or reduced PI3K(p110α) activity (caPI3K and dnPI3K mice, respectively) were subjected to exercise training and aortic-banding. Hearts of caPI3K mice (nonswim sham/basal state) displayed normal fractional shortening and were ≈20% larger than Ntg hearts (Figure 2A and 2B; reflecting physiological heart growth), and hearts of dnPI3K mice were ≈20% smaller (Figure 2B), consistent with previous reports.17 There was a significant pressure gradient between the aorta and ventricle of all banded mice included in the study, and this was not different between untrained and trained mice of the same genotype (online-only Data Supplement Table II).

As observed in the pilot study (protocol 1), swim-trained banded Ntg mice were protected against cardiac dysfunction, pathological hypertrophy, and lung congestion (Figure 2A–2C; online-only Data Supplement Table III). Trained banded Ntg mice also displayed less LV fibrosis, reduced ANP expression, and increased SERCA2a and αMHC expression compared with untrained banded Ntg mice (Figure 2D and 2E). caPI3K mice, regardless of exercise status, displayed comparable protection against pressure overload–induced remodeling to that observed in trained Ntg mice. Both untrained and trained caPI3K mice displayed minimal hypertrophy in response to aortic-banding (≈9–12% increase in HW/TL; Figure 2B; no significant increase in LV wall thicknesses; online-only Data Supplement Table II), showed no signs of cardiac dysfunction, no lung congestion, no ventricular fibrosis, and no significant increase in ANP gene expression (Figure 2A–2E). In contrast, exercise-induced protection was completely absent in dnPI3K mice. Trained and untrained dnPI3K mice showed a greater degree of cardiac dysfunction than untrained banded Ntg mice (Figure 2A). Furthermore, exercise training was unable to attenuate lung congestion, LV fibrosis, the increase in ANP expression, or the decrease in SERCA2a and αMHC expression in banded dnPI3K mice (Figure 2C–2E).

It is important to note that differential responses between Tg mice were not due to reduced exercise capacity in dnPI3K mice or increased exercise capacity in caPI3K mice. Transgene expression is restricted to the heart, and exercise capacity has been shown to be similar, as assessed by measurement of citrate synthase activity in skeletal muscle (see Reference 15; online-only Data Supplement Figure I) and treadmill exhaustion and endurance tests.

In summary, banded caPI3K mice were protected from LV remodeling and signs of heart failure independent of exercise status, whereas exercise did not provide protection in banded dnPI3K mice, suggesting that PI3K(p110α) is a key regulator of exercise-induced cardiac protection.

PI3K(p110α) Regulates the Expression of Genes and Proteins Previously Linked With Exercise-Induced Protection
Increased expression of antioxidant enzymes, Hsps (Hsp27, Hsp70/72, Hsp90) and regulation of transcription factors associated with cardiomyocyte proliferation (decrease in CCAAT/enhancer binding protein β [C/EBPβ] and increase in Cbp/p300-interacting transactivator with ED-rich carboxy-terminal domain 4 [CITED4]) have been implicated in mediating exercise-induced protection. Gene expression of catalase, Hsp27, and Hsp90 was increased in hearts of caPI3K mice and reduced in hearts of dnPI3K mice (Figure 3A). C/EBPβ expression was also reduced in hearts of caPI3K mice, and CITED4 expression tended to be elevated (Figure 3B and 3C). In contrast, there was a trend for higher C/EBPβ and lower CITED4 expression in the dnPI3K mice (Figure 3B and 3C).

Of the mechanisms linked with exercise-induced cardiac protection, Hsp70 has been the most extensively studied, possibly because genetic mouse models of Hsp70 loss- and gain-of-function clearly demonstrate that Hsp70 protects the heart in settings of stress.26,36,37 In the present study, Hsp70 protein expression was elevated in hearts from Ntg mice after 4 weeks of swim training, as well as trained and untrained caPI3K mice, but not dnPI3K mice (Figure 4A). Thus, it was of interest to assess whether Hsp70 was important for PI3K-induced protection.
To determine whether Hsp70 was important for PI3K-induced cardiac protection, 2 independent studies were performed: (1) dnPI3K mice were bred with Hsp70 Tg mice to generate dnPI3K-Hsp70 Tg mice and (2) caPI3K mice were bred with Hsp70 KO mice to generate caPI3K-Hsp70 KO mice. Increasing Hsp70 in the dnPI3K heart was predicted to improve outcome, whereas deleting Hsp70 from the caPI3K heart was predicted to have an adverse impact in settings of cardiac stress.

Hearts of dnPI3K-Hsp70 Tg mice expressed similar levels of Hsp70 protein to Hsp70 Tg mice (Figure 4B). Under basal conditions, there were no significant differences in systolic function between caPI3K and caPI3K-Hsp70 KO mice (Figure 4H; online-only Data Supplement Table V). In response to AAC, caPI3K and caPI3K-Hsp70 KO mice were protected to a similar degree, as assessed by systolic function (Figure 4H; online-only Data Supplement Table V). The degree of pathological hypertrophy was also similar, and there was no evidence of lung congestion (Figure 4I) or LV fibrosis (Figure 4F) and enlargement of the left atrium, as well as atrial thrombi (data not shown).

Hsp70 protein was not expressed in hearts of caPI3K-Hsp70 KO mice (Figure 4G). Under basal conditions, there were no significant differences in systolic function between caPI3K and caPI3K-Hsp70 KO mice (Figure 4H; online-only Data Supplement Table V). In response to AAC, caPI3K and caPI3K-Hsp70 KO mice were protected to a similar degree, as assessed by systolic function (Figure 4H; online-only Data Supplement Table V). The degree of pathological hypertrophy was also similar, and there was no evidence of lung congestion (Figure 4I) or LV fibrosis (Figure 4F). It is interesting to note that phosphorylated Akt/total Akt was elevated in hearts from caPI3K-Hsp70 KO compared with caPI3K under basal conditions and in response to AAC (online-only Data Supplement Figure II).
rAAV6-caPI3K Induced Physiological Heart Growth in Adult Mice

Having demonstrated that PI3K(p110α) was essential for mediating exercise-induced cardiac protection, and regulated genes previously linked with the cardioprotective properties of exercise, we next assessed whether a rAAV vector containing a caPI3K expression cassette could (i) induce physiological hypertrophy in the normal adult mouse heart and (ii) restore function of the failing heart.

Because PI3K(p110α) has tumorigenic properties in other cell types, muscle-specific delivery is critical. We used a vector configuration that had previously been shown to preferentially transduce cardiac muscle, that is, rAAV6 with expression mediated by a cytomegalovirus promoter. Modification of PI3K to render it ca results in a protein that is ≈40 kDa larger than p110α (ie, 150 kDa versus 110 kDa). The caPI3K protein was expressed in cardiac myocytes (myc tag within caPI3K colocalized with α-sarcomeric actin; Figure 5A) in a dose-dependent manner after administration of 6×10¹⁰ and 2×10¹¹ vectors, respectively (Figure 5B). As expected, the caPI3K protein was strongly expressed in the heart, and to a considerably reduced extent in skeletal muscle (Figure 5C). No transgene expression was observed in liver, lung, kidney, or spleen (Figure 5C). In subsequent studies, 2×10¹¹ vector genomes were administered.

Adult male mice examined 8 weeks after a bolus administration of rAAV6-caPI3K exhibited increased heart size (Figure 5D and 5E) that was characteristic of physiological hypertrophy, that is, associated with larger cardiac myocytes (Figure 5F), increased angiogenesis (Figure 5H), and increased activation of the Akt-mammalian target of rapamycin-p70S6K pathway (Figure 5I). rAAV6-caPI3K did not have an effect on markers associated with cardiomyocyte proliferation (C/EBPβ and CITED4; online-only Data Supplement Figure IIIA). Consistent with rAAV6-caPI3K inducing physiological rather than pathological hypertrophy, there was no increase in lung weight (Figure 5J) or LV ANP gene expression (Figure 5K).

rAAV6-caPI3K Improved Function in a Model of Pressure Overload–Induced Cardiac Dysfunction

Adult male mice were subjected to TAC for 4 weeks to induce systolic dysfunction before delivery of rAAV6-caPI3K. Four weeks post-TAC, LV posterior wall thickness was increased by ≈27% and systolic function was reduced from ≈42% to ≈34% (online-only Data Supplement Table VII). After the echocardiographic assessment, mice were randomly assigned to receive rAAV6-caPI3K, a control vector, or saline. Expression of caPI3K protein in heart tissue of mice that received rAAV6-caPI3K was confirmed by Western blotting (Figure 6A). Four weeks postvector delivery, PI3K-treated mice showed a trend for an improvement in heart function, which was significant by 10 weeks posttreatment (Figure 6B). No improvement was observed in the cohorts of TAC mice receiving either control vector or saline. Improved systolic function in rAAV6-caPI3K–treated TAC mice was associated with a trend for an increase in Akt phosphorylation (Figure 6C). Control mice subjected to TAC displayed a significant increase in βMHC expression and decreases in SERCA2a expression and the αMHC/βMHC ratio compared with sham-operated mice (Figure 6D). These alterations in gene expression were not as pronounced in the mice treated with rAAV6-caPI3K vectors. Apoptosis was relatively low in TAC hearts (≈0.03%), and rAAV6-caPI3K had no effect on this parameter (online-only Data Supplement Figure IV). C/EBPβ and CITED4 gene expression were also unaltered by rAAV6-caPI3K (online-only...

Figure 3. Phosphoinositide 3-kinase (PI3K) p110α regulates cardioprotective genes (A) catalase, heat shock protein 27 (Hsp27), and Hsp90 mRNA expression by microarray (n=4 per group). B, Representative Northern blotting and quantification of CCAAT/enhancer binding protein β (C/EBPβ) expression, standardized to GAPDH (n=3 to 4 per group). C, Real-time quantitative polymerase chain reaction analysis of CREB-binding protein/p300-interacting transactivator with ED 4 (CITED4) expression, standardized to 18S (n=5 per group). *P<0.05 vs nontransgenic (Ntg); †P<0.05 vs constitutively active PI3K (caPI3K). dnPI3K indicates dominant negative PI3K; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.
Figure 4. Heat shock protein 70 (Hsp70) is not required for phosphoinositide 3-kinase (PI3K) p110α–induced cardiac protection.

A, Representative Western blotting and quantification of Hsp70 expression in nontransgenic (Ntg), caPI3K (caP), and dnPI3K (dnP) mice after 4 weeks of swim training. ns indicates nonswim; sw, swim; *P<0.05 vs Ntg nonswim by unpaired t test; n=3 to 4 per group.

B, Western blotting showing expression of Hsp70 in Hsp70 transgenic (Tg) and dnPI3K-Hsp70 Tg mice.

C, Heart weight/tibia length ratio and fractional shortening of male dnPI3K and dnPI3K-Hsp70 Tg mice at 10 weeks of age. *P<0.05 vs Ntg; n=3 to 4 per group.

D, Fractional shortening of male dnPI3K and dnPI3K-Hsp70 Tg mice after 1 week of ascending aortic constriction (AAC; band) or sham surgery. *P<0.05 vs dnPI3K sham; n=3 to 4 per group.

E, Heart weight/tibia length ratio and lung weight/tibia length ratios of male dnPI3K and dnPI3K-Hsp70 Tg mice after 1 week of AAC (band) or sham surgery. Unpaired t tests. *P<0.05 vs dnPI3K sham; ns, not significant; n=3 to 4 per group.

F, Left ventricular (LV) cross-sections stained with Masson trichrome. Magnification ×200; scale bar=100 µm.

G, Western blotting showing loss of Hsp70 in hearts of caPI3K-Hsp70 knockout (KO) mice.

H, Fractional shortening at baseline and after 1 week of AAC in male caPI3K and caPI3K-Hsp70 KO mice; n=3 to 4 per group.

I, Heart weight/tibia length and lung weight/tibia length ratios of male caPI3K and caPI3K-Hsp70 KO mice after 1 week of AAC; n=3 to 4 per group.

J, LV cross-sections stained with Masson trichrome. Magnification ×200; scale bar=100 µm. caPI3K indicates constitutively active PI3K; dnPI3K, dominant negative PI3K; GAPDH, glyceraldehyde 3-phosphate dehydrogenase.
Figure 5. Recombinant adeno-associated viral 6 (rAAV6)–constitutively active phosphoinositide 3-kinase (caPI3K) vectors induce physiological cardiac hypertrophy in mice. Male C57BL/6 mice were dissected 8 weeks after single administration of rAAV6-caPI3K or a control vector (CON). A, Immunofluorescence of ventricular sections. α-sarcomeric actin stains cardiac myocyte actins (red); myc stains the myc tag within the caPI3K construct (green; magnification ×1000; scale bar=20 µm). B, Western blotting showing expression of caPI3K and endogenous PI3K(p110α) in heart tissue. C, Expression of the caPI3K transgene is restricted to heart (H) and skeletal muscle (Sk). Li indicates liver; Lu, lung; Ki, kidney; Sp, spleen. D, rAAV6-caPI3K induces cardiac hypertrophy in mice. Representative heart pictures (D, scale bar=0.5 cm) and (E) heart weight/tibia length. *P<0.05 vs control; n=4 to 5 per group. F, Left ventricular cross-sections stained with hematoxylin and eosin (magnification ×200; scale bar=50 µm). G, Fractional shortening, n=6 per group. H, Capillary density by immunofluorescence of ventricular sections. Wheat germ agglutinin (WGA) stains myocyte membranes (green), isolectin stains capillaries (red); magnification ×400; scale bar=50 µm). I, Representative Western blotting and quantification of phosphorylation of Akt (S473), mammalian target of rapamycin (mTOR, S2448), and p70S6K (Thr389) in heart tissue. *P<0.05 vs control; n=4 to 8 per group. J, Lung weight/tibia length ratio. n=4 to 5 per group. K, Atrial natriuretic peptide (ANP) is not increased in hearts of mice treated with rAAV6-caPI3K. +Positive control (mouse subjected to myocardial infarction). GAPDH indicates glyceraldehyde 3-phosphate dehydrogenase.
Exercise activates a complex network of molecular events that can protect the heart in settings of stress. Regular physical activity is also known to improve function in heart failure patients. However, exercise as a treatment modality may not always be feasible for patients with heart disease. Thus, understanding the key molecular mechanisms responsible for the beneficial properties of exercise is of significant importance to identify novel therapeutic targets. The major goal of the present study was to determine whether PI3K is a master regulator of exercise-induced protection that could then be targeted to improve function of the failing heart. We hypothesized that PI3K might represent a critical nodal regulator of exercise-induced protection because it lies downstream of receptor tyrosine kinases, upstream of multiple divergent signaling pathways, and was previously shown to be a critical regulator of exercise-induced hypertrophy.

We first demonstrated that our exercise protocol provided subsequent protection against pressure overload–induced pathological cardiac hypertrophy. Exercise-trained Ntg mice had better systolic function and less pathological hypertrophy and lung congestion than untrained controls after aortic-banding, indicating that chronic exercise can provide sustained protection against a cardiac insult. Next, we used PI3K Tg mice to determine whether PI3K was important for the observed exercise-induced protection, and the magnitude of any protection. Here, we identify PI3K as an essential regulator of exercise-induced cardiac protection.
Chronic exercise training provided protection against pressure overload–induced pathological hypertrophy and cardiac dysfunction in Ntg, but this was completely ablated in mice with reduced cardiac PI3K activity, that is, dnPI3K mice. It is important to note that increased cardiac PI3K activity in untrained caPI3K mice recapitulated the protection observed with exercise training.

Consistent with PI3K being obligatory for exercise-induced protection, expression levels of the antioxidant catalase, Hsps, and markers of cell proliferation were beneficially altered in caPI3K mice under basal conditions. Because numerous studies have demonstrated that Hsp70 is upregulated in the heart in response to exercise,24,35,41 and Hsp70 has been linked with cardiac protection,26,36 we investigated the importance of Hsp70 in mediating PI3K-induced cardiac protection. Tg overexpression of Hsp70 could not rescue the phenotype of banded dnPI3K mice, and deletion of Hsp70 from banded caPI3K mice had no effect. Collectively, these data suggest that Hsp70 is neither necessary nor sufficient for PI3K–induced protection. Changes in PI3K activity may override or compensate for changes in Hsp70 via activation of parallel pathways. Consistent with this premise and the general hypothesis that PI3K represents a master regulator, Akt activation, which is elevated in caPI3K hearts compared with Ntg, was elevated even further in caPI3K–Hsp70 KO hearts. The ability of PI3K to further augment Akt activation in the absence of Hsp70 may explain why caPI3K–Hsp70 KO hearts remained protected in response to AAC. Akt1 is a critical mediator of cardiac protection in a setting of pressure overload.42

An unavoidable complication of the present study is that PI3K activity was decreased in the dnPI3K heart during exercise training and the subsequent period of pressure overload. The more optimal experimental design would have been one in which PI3K was only inhibited in the heart during exercise training but not during aortic-banding. However, there are currently no available genetic models or tools that would allow inducible reduction of PI3K during the exercise bouts, followed by complete restoration the following day, before AAC. Despite this limitation, the data suggesting PI3K is an essential master regulator of exercise-induced protection are still compelling for the following reasons. First, if exercise training provided any significant protection via mechanisms other than that provided by PI3K, some protection would have been observed in trained banded dnPI3K mice compared with untrained banded dnPI3K mice. Based on cardiac function, fibrosis, and molecular markers (eg, ANP gene expression), no protection was observed in exercise-trained banded dnPI3K mice. Second, a decrease in PI3K cardiac activity under basal conditions (ie, dnPI3K mice without the stimulus of exercise or pressure overload) was associated with changes in genes previously linked with exercise-induced cardiac protection, for example, Hsps, catalase, and markers of proliferation.

Having demonstrated that PI3K was an essential regulator of exercise-induced protection, we next used a gene therapy approach to determine whether delivery of an rAAV6-caPI3K vector in a mouse model with preexisting cardiac dysfunction could restore function. First, we assessed the impact of rAAV6-caPI3K in normal adult mice under basal conditions. Administration of rAAV6-caPI3K induced physiological heart growth that was reminiscent of that observed in caPI3K Tg mice. We also confirmed that gene delivery was restricted largely to the heart. We then administered rAAV6-caPI3K to mice with pathological cardiac hypertrophy and dysfunction caused by 4 weeks of TAC. Examination of TAC mice, 10 weeks after single injection of rAAV6-caPI3K, demonstrated that systolic function was improved compared with pretreatment and control mice. Improved cardiac function in rAAV6-caPI3K–treated TAC mice was associated with more favorable levels of Akt phosphorylation, SERCA2a and αMHC/βMHC ratio than control vector–treated mice, and increased angiogenesis. rAAV6-caPI3K had no significant effect on apoptosis or markers of proliferation in TAC mice; however, apoptosis was low in this model (≤0.04%). Whether rAAV6-caPI3K can inhibit apoptosis and promote myocyte proliferation in cardiac stress models associated with higher levels of apoptosis, remains to be elucidated.

Numerous genetic interventions and pharmacological approaches have been shown to protect against pathological insults when present or administered before, or simultaneously with, a cardiac insult. Only a limited number of studies have specifically targeted the heart and improved outcome in a setting of established cardiac dysfunction. Rengo et al43 demonstrated that 12 weeks of rAAV6-β-adrenergic receptor kinase-1 C terminus (βARKct) gene delivery to rats with cryoinfarction–induced heart failure improved cardiac function. Here we demonstrate, for the first time, that gene delivery of caPI3K also has the capacity to restore cardiac function. Akt1, a well-characterized downstream regulator of PI3K, has also been shown to provide protection against pressure overload–induced dysfunction and increase angiogenesis in a paracrine manner.42,44,45 However, whereas chronic activation of PI3K has no reported adverse effects,17,20,13,17,20 the impact of Akt seems to be dependent on subcellular localization and the degree of Akt activation and cardiac growth.45

A recognized challenge in the cardiac field is the similarities between signaling pathways that drive tumorigenesis and those that regulate protection in the heart.46 Amplification and mutation of PI3K(p110α) has been associated with cancer,46,47 To prevent this complication, we used an rAAV vector that preferentially increases PI3K in the heart. The translation of effective gene therapies into the clinic has also been challenging. However, enthusiasm regarding AAV strategies entering the clinic for many diseases has grown recently, with significant developments in relation to optimization of vector design and improved manufacturing methods.48 Furthermore, an AAV1-based intervention recently entered a phase 2 trial in patients with advanced heart failure.49 In our study, single administration of rAAV6-caPI3K resulted in improvement in heart function 8 to 10 weeks after delivery. Of note, transgene expression was shown to be sustained for >1 year in mice.50

In summary, the present study provides the first demonstration that PI3K(p110α) is indispensable for exercise-induced cardiac protection, and that rAAV6-caPI3K has the potential to improve cardiac function in mice with preexisting pressure overload–induced remodeling and cardiac dysfunction. This represents a novel approach to recapitulate some of the
positive effects of exercise, using a modality that is potentially applicable to heart failure patients who are unable to undertake regular exercise.

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Disclosures
None.

References
32. Huyah K, McMullen JR, Julius TL, Tan JW, Love JE, Cemlerfan N, Kiriazis H, Du XJ, Ritchie RH. Cardiac-specific IGF-1 receptor transgenic expression protects against cardiac fibrosis and diastolic


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**CLINICAL PERSPECTIVE**

The beneficial effects of regular physical activity on cardiovascular health are well established. However, the key molecular mechanisms underlying the cardioprotective effects of exercise are not well defined. Identification of critical pathways that are activated by exercise may lead to novel and viable therapeutic intervention strategies. Although activation of phosphoinositide 3-kinase (PI3K) p110α was previously shown to be critical for physiological heart growth, its contribution and importance in exercise-induced cardiac protection were unknown. Here, we demonstrate that PI3K(p110α) is essential for swim-exercise–induced cardiac protection. Exercise was unable to mediate any protection when PI3K(p110α) activity was reduced in hearts of mice subjected to pressure overload. Furthermore, by using a gene therapy approach to increase PI3K in hearts of mice with preexisting cardiac dysfunction, we showed improved heart function over time. Because the tumorigenic properties of PI3K(p110α) are known in other cell types, we achieved muscle-specific delivery with a recombinant adenovirus–associated viral pseudotype 6 vector that selectively transduces cardiac muscle. Significant developments in vector design and manufacturing, together with the recent entry of an adenovirus–based intervention in a phase 2 trial in heart failure patients, highlight the potential of establishing adenovirus–based delivery of PI3K(p110α) as a potential therapeutic gene delivery strategy in the clinic.
Phosphoinositide 3-Kinase p110α Is a Master Regulator of Exercise-Induced Cardioprotection and PI3K Gene Therapy Rescues Cardiac Dysfunction

Kate L. Weeks, Xiaoming Gao, Xiao-Jun Du, Esther J.H. Boey, Aya Matsumoto, Bianca C. Bernardo, Helen Kiriazis, Nelly Cemerlang, Joon Win Tan, Yow Keat Tham, Thomas F. Franke, Hongwei Qian, Marie A. Bogoyevitch, Elizabeth A. Woodcock, Mark A. Febbraio, Paul Gregorevic and Julie R. McMullen

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SUPPLEMENTAL MATERIAL

Supplemental Methods

Generation of rAAV6-caPI3K

The caPI3K construct which is present in caPI3K transgenic mice (iSH2p1101, 2) was cloned into an adeno-associated viral vector plasmid3 using standard cloning techniques. The plasmid (hereon called pAAV6:caPI3K) contained a CMV promoter and an SV40 polyadenylation signal, which was subsequently replaced with a synthetic poly(A)4 to reduce the size of the expression cassette. The rAAV6-caPI3K vector was produced by co-transfecting HEK293T cells with pAAV6:caPI3K and pDGM6, a packaging plasmid which provides the additional genes required for AAV vector production.3 Purification of vectors from transfected cells was achieved by standard methods, as reported previously.3 The titre of purified vector stocks was determined by RT-qPCR using primers for the CMV promoter: forward 5’-gcgttagcggcgtgtacggtg-3’, reverse 5’-cgctgatggcgtctccaggg-3’.

Histological analyses

Ventricle samples were frozen in cryoprotectant compound (Tissue-Tek OCT, Sakura) or fixed in 4% paraformaldehyde, dehydrated and embedded in paraffin.

Fibrosis: 6 µm sections were deparaffinized and stained with Masson’s trichrome. Percentage fibrosis was calculated by dividing the total area of blue staining (collagen) by the total area of the LV.

Angiogenesis: 5 µm sections were deparaffinized, blocked in PBA (Thermo Shandon 407210) and co-stained with Alexa Fluor 568-conjugated isoelectin B4 (Invitrogen I21412) and FITC-conjugated wheat germ agglutinin (WGA; Vector Labs FL1021). Images were taken at 400x magnification. Capillary density was measured by dividing the number of capillaries by the number of cardiomyocytes per image, for 7-10 images per heart.
Immunofluorescence of Myc-tagged caPI3K: 8 µm cryosections were fixed in 4% PFA, permeabilized and blocked using kits from Vector Labs (Sp-2001 and BMK-2202) according to the manufacturer’s instructions. Sections were stained with anti-c-Myc (Santa Cruz sc-789) and anti-α-sarcomeric actin (Sigma-Aldrich A2172), incubated with biotinylated anti-rabbit IgG, then incubated with Cy3-conjugated goat anti-mouse IgG (Jackson ImmunoResearch Laboratories, 115-165-003) and Alexa Fluor 488 streptavidin (Invitrogen S32354). Images were taken at 1000x magnification.

Apoptosis: 5 µm sections were deparaffinized prior to TUNEL staining using a CardioTACs In Situ Apoptosis Detection Kit (Trevigen 4827-30-K), according to the manufacturer’s instructions. The percentage apoptosis was calculated by dividing the number of apoptotic nuclei (stained blue) by the total number of nuclei per section and multiplying by 100%. Average number of nuclei counted and used for each heart was 13,400.

**Microarray analysis**

Catalase, Hsp27 and Hsp90 mRNA expression data were obtained from a previously published microarray data set.⁵

**RT-qPCR**

CITED4 expression was measured relative to 18S using the SYBR Green method (Applied Biosystems). Primers for CITED4 were designed using Roche Applied Science’s Assay Design tool: forward 5’ ccgagaacacctgccttg-3’, reverse 5’-agegagacccaactgtcatc-3’. 18S primer sequences have been previously described.⁶ We verified that the primer sets yielded a single peak for the melt curves before proceeding with our analyses.
**Northern hybridisation**

Northern blotting was performed as previously described except that 10 μg total RNA was used. Probes for ANP, BNP, SERCA2a, αMHC, βMHC and GAPDH were generated as previously described. Probes for procollagen III, C/EBPβ and CITED4 were generated by PCR using mouse cDNA as a template and the following primers: procollagen III forward 5’-ccaccccgaactcaagagtgg-3’, reverse 5’-ccatcctctagaactgtgtaagtg-3’; C/EBPβ forward 5’-geaacacacgtgtaactgtc-3’, reverse 5’-cggaaaggttetcacaata-3’; CITED4 forward 5’ttcctcagcacactaaca-3’, reverse 5’ttgtaagcaacccagttt-3’.

**Western blotting**

Western blotting was performed using 100 μg protein from heart lysates as previously described. The following concentrations of primary antibodies were used: 1:500 phospho-Akt (Cell Signaling 9271), 1:2500-1:5000 Akt (Cell Signaling 9272), 1:1000 Hsp70 (Stressgen, 810B), 1:1000 PI3K(p110α) (Cell Signaling 4249), 1:1000 phospho-mTOR (Cell Signaling 2971), 1:2000 mTOR (Cell Signaling 2972), 1:500 phospho-p70S6K (Cell Signaling 9205), 1:500 p70S6K (Cell Signaling 9202), 1:1000 α-tubulin (Cell Signaling 2144).
**Supplementary Table I.** Echocardiography data of untrained and exercise-trained non-transgenic (control) mice subjected to pressure overload.

<table>
<thead>
<tr>
<th></th>
<th>Untrained</th>
<th></th>
<th>Trained</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>Band</td>
<td>Sham</td>
<td>Band</td>
</tr>
<tr>
<td>No. of animals</td>
<td>3</td>
<td>6</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>491 ± 20</td>
<td>458 ± 9</td>
<td>503 ± 22</td>
<td>516 ± 25</td>
</tr>
<tr>
<td>LVPW, mm</td>
<td>0.7 ± 0.0</td>
<td>1.0 ± 0.1 *</td>
<td>0.6 ± 0.1</td>
<td>0.7 ± 0.0 †</td>
</tr>
<tr>
<td>IVS, mm</td>
<td>0.7 ± 0.1</td>
<td>1.2 ± 0.0 *</td>
<td>0.7 ± 0.1</td>
<td>0.8 ± 0.1 †</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>3.6 ± 0.1</td>
<td>3.7 ± 0.2</td>
<td>3.8 ± 0.2</td>
<td>3.6 ± 0.2</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>1.5 ± 0.2</td>
<td>2.1 ± 0.2</td>
<td>1.6 ± 0.2</td>
<td>1.6 ± 0.1</td>
</tr>
<tr>
<td>FS, %</td>
<td>59 ± 4</td>
<td>43 ± 3 *</td>
<td>58 ± 4</td>
<td>56 ± 2 †</td>
</tr>
<tr>
<td>AoPg, mmHg</td>
<td>3 ± 0</td>
<td>28 ± 2 *</td>
<td>3 ± 0</td>
<td>27 ± 1 *</td>
</tr>
</tbody>
</table>

LV, left ventricular; LVPW, LV posterior wall thickness; IVS, interventricular septum thickness; LVEDD, LV end-diastolic dimension; LVESD, LV end-systolic dimension; FS, fractional shortening; AoPg, aortic pressure gradient. Data are shown as mean ± SEM. One-way ANOVA across 4 groups (HR: NS, LVPW: *P*<0.0001, IVS: *P*<0.0001, LVEDD: NS, LVESD: *P*=0.07, FS: *P*=0.008, AoPg: *P*<0.0001) followed by Fisher’s least significant difference post-hoc pairwise t-tests. *P*<0.05 compared with sham from the same group. †*P*<0.05 compared with untrained aortic-band.
**Supplementary Table II.** Echocardiography data for non-transgenic (Ntg), caPI3K and dnPI3K mice following one week of pressure overload induced by ascending aortic constriction. Trained mice underwent swim training twice daily for four weeks prior to surgery.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>HR (bpm)</th>
<th>IVS (mm)</th>
<th>LVPW (mm)</th>
<th>LVEDD (mm)</th>
<th>LVESD (mm)</th>
<th>AoPg (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ntg non-swim sh</td>
<td>6</td>
<td>569±24</td>
<td>0.7±0.0</td>
<td>0.7±0.0</td>
<td>3.6±0.1</td>
<td>1.3±0.1</td>
<td>3±0</td>
</tr>
<tr>
<td>Ntg non-swim b</td>
<td>7</td>
<td>531±20</td>
<td>1.0±0.1</td>
<td>1.0±0.1</td>
<td>3.5±0.1</td>
<td>2.0±0.2</td>
<td>32±2</td>
</tr>
<tr>
<td>Ntg swim sh</td>
<td>6</td>
<td>494±23§</td>
<td>0.7±0.0</td>
<td>0.7±0.0</td>
<td>3.8±0.1</td>
<td>1.4±0.1</td>
<td>7±1</td>
</tr>
<tr>
<td>Ntg swim b</td>
<td>5</td>
<td>482±19</td>
<td>0.8±0.1</td>
<td>1.0±0.1</td>
<td>3.7±0.1</td>
<td>1.6±0.1</td>
<td>35±3</td>
</tr>
<tr>
<td>caPI3K non-swim sh</td>
<td>6</td>
<td>528±23</td>
<td>0.7±0.1</td>
<td>0.8±0.0</td>
<td>3.5±0.1</td>
<td>1.3±0.1</td>
<td>4±0</td>
</tr>
<tr>
<td>caPI3K non-swim b</td>
<td>7</td>
<td>541±26</td>
<td>0.8±0.0</td>
<td>0.9±0.0</td>
<td>3.4±0.1</td>
<td>1.4±0.1</td>
<td>33±4</td>
</tr>
<tr>
<td>caPI3K swim sh</td>
<td>6</td>
<td>489±13</td>
<td>0.8±0.0</td>
<td>0.8±0.0</td>
<td>3.5±0.1</td>
<td>1.5±0.1</td>
<td>3±0</td>
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<tr>
<td>caPI3K swim b</td>
<td>8</td>
<td>528±13</td>
<td>0.9±0.1</td>
<td>0.8±0.0</td>
<td>3.7±0.1</td>
<td>1.6±0.1</td>
<td>37±3</td>
</tr>
<tr>
<td>dnPI3K non-swim sh</td>
<td>6</td>
<td>543±14</td>
<td>0.5±0.0</td>
<td>0.5±0.0</td>
<td>3.3±0.1</td>
<td>1.4±0.1</td>
<td>5±0</td>
</tr>
<tr>
<td>dnPI3K non-swim b</td>
<td>6</td>
<td>528±22</td>
<td>0.8±0.1</td>
<td>0.8±0.0</td>
<td>3.7±0.1</td>
<td>2.4±0.1</td>
<td>18±3</td>
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<tr>
<td>dnPI3K swim sh</td>
<td>5</td>
<td>463±20§</td>
<td>0.5±0.0</td>
<td>0.5±0.0</td>
<td>3.7±0.1</td>
<td>1.6±0.1</td>
<td>3±1</td>
</tr>
<tr>
<td>dnPI3K swim b</td>
<td>4</td>
<td>480±13</td>
<td>0.8±0.0</td>
<td>0.9±0.1</td>
<td>3.6±0.1</td>
<td>2.5±0.1</td>
<td>20±4</td>
</tr>
</tbody>
</table>

LV, left ventricular; HR, heart rate; IVS, interventricular septum thickness; LVPW, LV posterior wall thickness; LVEDD, LV end-diastolic dimension; LVESD, LV end-systolic dimension; AoPg, aortic pressure gradient; sh, sham; b, band. Data are shown as mean ± SEM. One-way ANOVA across 12 groups (HR: $P<0.02$, IVS: $P<0.0001$, LVPW: $P<0.0001$, LVEDD: $P=0.06$, LVESD: $P<0.0001$, AoPg: $P<0.0001$) followed by Fisher’s least significant difference post-hoc pairwise t-tests. *$P<0.05$ vs sham of the same exercise group (non-swim/swim) and genotype. †$P<0.05$ vs
non-swim band of the same genotype. ‡P<0.05 vs Ntg non-swim sham. §P<0.05 vs non-swim sham of the same genotype. ||P<0.05 vs caPI3K of the same surgical (sham/band) and exercise (non-swim/swim) group.
**Supplementary Table III.** Morphological data for Ntg, caPI3K and dnPI3K mice following one week of pressure overload induced by ascending aortic constriction. Trained mice underwent swim training twice daily for four weeks prior to surgery.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>BW (g)</th>
<th>HW (mg)</th>
<th>AW (mg)</th>
<th>LW (mg)</th>
<th>TL (mm)</th>
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<tr>
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<td>6</td>
<td>25.9±0.9</td>
<td>106.5±3.7</td>
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<td>126.4±3.7</td>
<td>16.6±0.1</td>
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<td>Ntg non-swim b</td>
<td>7</td>
<td>22.3±1.0 *</td>
<td>156.0±8.6 *</td>
<td>14.5±1.8 *</td>
<td>227.6±32.3 *</td>
<td>16.4±0.1</td>
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<td>Ntg swim sh</td>
<td>6</td>
<td>21.7±0.7</td>
<td>102.6±3.8</td>
<td>7.1±0.2</td>
<td>125.0±1.6</td>
<td>16.1±0.1</td>
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<tr>
<td>Ntg swim b</td>
<td>5</td>
<td>23.9±0.9</td>
<td>136.8±9.7 *†</td>
<td>12.5±1.6 *</td>
<td>144.4±9.4 †</td>
<td>16.3±0.1</td>
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<tr>
<td>caPI3K non-swim sh</td>
<td>6</td>
<td>22.2±1.0</td>
<td>122.3±3.4 ‡</td>
<td>6.4±0.3</td>
<td>122.7±2.9</td>
<td>16.3±0.1</td>
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<td>21.5±0.5</td>
<td>137.0±3.3 *§</td>
<td>9.4±0.6 §</td>
<td>123.8±3.1 §</td>
<td>16.2±0.1</td>
</tr>
<tr>
<td>caPI3K swim sh</td>
<td>6</td>
<td>20.4±0.5</td>
<td>123.3±2.1</td>
<td>7.6±0.5</td>
<td>116.3±1.7</td>
<td>15.7±0.0</td>
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<td>caPI3K swim b</td>
<td>8</td>
<td>21.6±0.6</td>
<td>138.5±3.5 *</td>
<td>10.2±1.6</td>
<td>121.3±4.2</td>
<td>16.2±0.1 *</td>
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<td>25.9±1.4</td>
<td>82.1±4.1 ‡</td>
<td></td>
<td>6.4±0.5</td>
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<td>24.2±1.5</td>
<td>142.6±4.8 *</td>
<td>19.6±1.2 *§</td>
<td></td>
<td>303.6±28.7 *§</td>
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<td>80.1±5.8</td>
<td></td>
<td>6.4±0.8</td>
<td>114.3±6.2</td>
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<td>4</td>
<td>21.2±0.8 †</td>
<td>133.0±8.6 *</td>
<td>18.0±3.3 *§</td>
<td></td>
<td>230.7±42.4 *†§</td>
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</tbody>
</table>

BW: body weight, HW: heart weight, AW: atria weight, LW: lung weight, TL: tibia length, sh: sham, b: band. Data are shown as mean ± SEM. One-way ANOVA across 12 groups (BW: \( P=0.0003 \), HW: \( P<0.0001 \), AW: \( P<0.0001 \), LW: \( P<0.0001 \), TL: \( P=0.006 \)) followed by Fisher’s least significant difference post-hoc pairwise t-tests. *\( P<0.05 \) vs sham of the same exercise group (non-swim/swim) and genotype. †\( P<0.05 \) vs non-swim band of the same genotype. ‡\( P<0.05 \) vs Ntg non-swim sham. §\( P<0.05 \) vs Ntg band of the same exercise group (non-...
swim/swim). \(P<0.05\) vs caPI3K of the same exercise (non-swim/swim) and surgical (sham/band) group.
**Supplementary Table IV.** Echocardiography data of dnPI3K and dnPI3K-Hsp70 Tg mice following one week of ascending aortic constriction.

<table>
<thead>
<tr>
<th></th>
<th>dnPI3K sham</th>
<th>dnPI3K band</th>
<th>dnPI3K-Hsp70 Tg band</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of animals</td>
<td>3</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>530 ± 29</td>
<td>551 ± 23</td>
<td>522 ± 37</td>
</tr>
<tr>
<td>LVPW, mm</td>
<td>0.5 ± 0.0</td>
<td>1.0 ± 0.1 *</td>
<td>1.1 ± 0.1 *</td>
</tr>
<tr>
<td>IVS, mm</td>
<td>0.5 ± 0.1</td>
<td>1.0 ± 0.1 *</td>
<td>1.1 ± 0.1 *</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>3.9 ± 0.2</td>
<td>3.6 ± 0.1</td>
<td>3.9 ± 0.1</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>1.7 ± 0.1</td>
<td>2.1 ± 0.2</td>
<td>2.7 ± 0.2 *</td>
</tr>
<tr>
<td>FS, %</td>
<td>57 ± 3</td>
<td>41 ± 4 *</td>
<td>31 ± 5 *</td>
</tr>
<tr>
<td>AoPg, mmHg</td>
<td>5 ± 1</td>
<td>23 ± 1 *</td>
<td>15 ± 4 *†</td>
</tr>
</tbody>
</table>

LV, left ventricular; LVPW, LV posterior wall thickness; IVS, interventricular septum thickness; LVEDD, LV end-diastolic dimension; LVESD, LV end-systolic dimension; FS, fractional shortening; AoPg, aortic pressure gradient. Data are shown as mean ± SEM. One-way ANOVA (HR: NS, LVPW: $P=0.005$, IVS: $P=0.005$, LVEDD: NS, LVESD: $P=0.03$, FS: $P=0.009$, AoPg: $P=0.002$) followed by Fisher’s least significant difference post-hoc pairwise t-tests. *$P<0.05$ compared with sham. †$P<0.05$ compared with dnPI3K band.
**Supplementary Table V.** Echocardiography data of caPI3K and caPI3K-Hsp70 KO mice at baseline and after one week of ascending aortic constriction (AAC).

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Baseline</th>
<th>Post-AAC</th>
<th>Post-AAC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>caPI3K</td>
<td>caPI3K-Hsp70 KO</td>
<td>caPI3K</td>
<td>caPI3K-Hsp70 KO</td>
</tr>
<tr>
<td>No. of animals</td>
<td>3</td>
<td>4</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>555 ± 69</td>
<td>540 ± 35</td>
<td>491 ± 12</td>
<td>527 ± 29</td>
</tr>
<tr>
<td>LVPW, mm</td>
<td>1.2 ± 0.1</td>
<td>1.2 ± 0.1</td>
<td>1.2 ± 0.2</td>
<td>1.2 ± 0.1</td>
</tr>
<tr>
<td>IVS, mm</td>
<td>1.4 ± 0.2</td>
<td>1.3 ± 0.1</td>
<td>1.4 ± 0.3</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>3.7 ± 0.3</td>
<td>4.0 ± 0.1</td>
<td>4.0 ± 0.3</td>
<td>4.1 ± 0.1</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>2.0 ± 0.2</td>
<td>2.3 ± 0.1</td>
<td>2.4 ± 0.3</td>
<td>2.3 ± 0.1</td>
</tr>
<tr>
<td>FS, %</td>
<td>45 ± 2</td>
<td>43 ± 1</td>
<td>39 ± 6</td>
<td>43 ± 2</td>
</tr>
<tr>
<td>AoPg, mmHg</td>
<td>-</td>
<td>-</td>
<td>25 ± 3</td>
<td>28 ± 3</td>
</tr>
</tbody>
</table>

LV, left ventricular; LVPW, LV posterior wall thickness; IVS, interventricular septum thickness; LVEDD, LV end-diastolic dimension; LVESD, LV end-systolic dimension; FS, fractional shortening; AoPg, aortic pressure gradient. No statistically significant differences were detected for any variable by repeated measures ANOVA, one-way ANOVA or unpaired t-tests.
**Supplementary Table VI.** Echocardiography data of male C57BL/6 mice at baseline and 8 weeks after an injection of $2 \times 10^{11}$ vector genomes of rAAV6-caPI3K or rAAV6-null (control).

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>8 weeks post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>rAAV6-caPI3K</td>
</tr>
<tr>
<td>No. of animals</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>547 ± 24</td>
<td>541 ± 18</td>
</tr>
<tr>
<td>LVPW, mm</td>
<td>0.8 ± 0.0</td>
<td>0.8 ± 0.0</td>
</tr>
<tr>
<td>IVS, mm</td>
<td>0.9 ± 0.0</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>4.1 ± 0.1</td>
<td>4.1 ± 0.1</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>2.6 ± 0.1</td>
<td>2.6 ± 0.1</td>
</tr>
<tr>
<td>FS, %</td>
<td>36 ± 2</td>
<td>36 ± 2</td>
</tr>
</tbody>
</table>

LV, left ventricular; LVPW, LV posterior wall thickness; IVS, interventricular septum thickness; LVEDD, LV end-diastolic dimension; LVESD, LV end-systolic dimension; FS, fractional shortening. As there was a significant difference in heart rate between time points by repeated measures ANOVA ($P<0.0001$), unpaired t-tests were performed to compare the groups at each time point. No statistically significant differences were detected.
**Supplementary Table VII.** Echocardiography data of male FVBN mice subjected to transverse aortic constriction (TAC) for 4 weeks prior to randomization to control group or PI3K-treatment group.

<table>
<thead>
<tr>
<th></th>
<th>Pre-surgery</th>
<th>4 weeks post-TAC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>rAAV6-caPI3K</td>
</tr>
<tr>
<td>No. of animals</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>615 ± 13</td>
<td>575 ± 24</td>
</tr>
<tr>
<td>LVPW, mm</td>
<td>0.9 ± 0.0</td>
<td>0.9 ± 0.0</td>
</tr>
<tr>
<td>IVS, mm</td>
<td>1.1 ± 0.0</td>
<td>1.0 ± 0.1</td>
</tr>
<tr>
<td>LVEDD, mm</td>
<td>3.8 ± 0.1</td>
<td>3.8 ± 0.1</td>
</tr>
<tr>
<td>LVESD, mm</td>
<td>2.2 ± 0.1</td>
<td>2.2 ± 0.1</td>
</tr>
<tr>
<td>FS, %</td>
<td>42 ± 1</td>
<td>42 ± 1</td>
</tr>
</tbody>
</table>

LV, left ventricular; LVPW, LV posterior wall thickness; IVS, interventricular septum thickness; LVEDD, LV end-diastolic dimension; LVESD, LV end-systolic dimension; FS, fractional shortening. Data are shown as mean ± SEM. Repeated measures ANOVA (HR: NS, LVPW: \( P<0.0001 \), IVS: \( P<0.0001 \), LVEDD: NS, LVESD: \( P=0.03 \), FS: \( P=0.001 \)) followed by Fisher’s least significant difference post-hoc pairwise t-tests. *\( P<0.05 \) compared with baseline/pre-surgery, † \( P=0.08 \) compared with baseline/pre-surgery.
Supplementary Figure 1

[Diagram showing a bar chart with two groups: Ntg and caPI3K. The chart compares Citrate synthase activity (μmol/mg protein/min) between Non-swim and Swim conditions. There are asterisks (*) indicating statistical significance, and "ns" indicating no significant difference.]
Supplementary Figure 2
Supplementary Figure 3
Supplementary Figure 4
Figure legends

Supplementary Figure 1
Citrate synthase activity in mixed gastrocnemius of Ntg and caPI3K mice following 4 weeks of chronic swim training. Mice were trained for up to 90 minutes, twice daily for 28 days. There was no significant difference (ns) in citrate synthase activity between Ntg and caPI3K swimmers, indicating that mice exercised to a similar degree despite initial differences in heart size. n=3-5 per group. *P<0.01 vs non-swim of the same genotype.

Supplementary Figure 2
Western blot and quantitation of phosphorylation of Akt (S473), in heart tissue from Ntg, caPI3K (caP; caPI3K-wildtype or het; comparable Hsp70 protein expression), and caPI3K-Hsp70 KO mice. Banding had no effect on pAkt/total Akt in caPI3K or caPI3K-Hsp70 KO, thus groups were combined due to limited samples. *P<0.05, n=3/group. Values are expressed relative to a control Ntg mouse normalized to 1.

Supplementary Figure 3
A) Gene expression of C/EBPβ and CITED4 in hearts of adult mice (basal conditions) administered rAAV6-caPI3K (PI3K) or a control vector (CON). Representative Northern blots and quantitation of gene expression. n=5/group. B) C/EBPβ and CITED4 in hearts of banded mice administered rAAV6-caPI3K or a control vector (CON). n=5-6/group.

Supplementary Figure 4
Apoptosis in ventricular sections from banded mice administered rAAV6-caPI3K or a control vector (CON) compared with a control unoperated mouse (control). Representative LV sections labeled by TUNEL staining. Positively stained apoptotic nuclei appear dark blue (indicated by
arrow); magnification 200x; scale bar=20μM. Quantitation of positively-stained nuclei/total number of nuclei versus control unoperated mouse. $n=3$/group. Apoptosis was relatively low ($\leq 0.04\%$) in the pressure overload model as previously reported.$^{9,10}$
References


