Differential expression of 14-3-3ɛ during physiological, pathological cardiac hypertrophy and chronic heart failure in mice

Research Article

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Abbreviations: (CHF), chronic heart failure, (TAC), transverse aortic constriction, (LVIDd), inner dimension of diastolic LV, (LVIDs), inner dimension of systolic LV, (LVPWd), LV end-diastolic posterior wall thickness, (LVPWs), LV end-systolic posterior wall thickness, (LVAWd), LV end-diastolic anterior wall thickness, (LVAWs), LV end-systolic anterior wall thickness, (EDV), end-diastolic volume, (ESV), end-systolic volume, (FS), fractional shortening, (EF), eject fraction

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Summary

Physiological cardiac hypertrophy associated with regular exercise is usually beneficial, in marked contrast to pathological hypertrophy associated with disease. 14-3-3 proteins play a critical antiapoptotic function in cardiomyocytes. Whether it or other genes activated in the athlete’s heart might have an impact on cardiac function and survival in a setting of heart failure is unknown. To examine whether different changes of 14-3-3 proteins expression in physiological cardiac hypertrophy, pathological cardiac hypertrophy and chronic heart failure (CHF), we constructed mouse models of physiological cardiac hypertrophy to swim training, pathological cardiac hypertrophy to transverse aortic constriction (TAC) for 4 weeks and chronic heart failure to TAC for 16 weeks. In response to swimming training and TAC, mice showed significant increases in left ventricular diastolic posterior wall thickness (LVPWd), heart weight and normalized heart weight to body weight ratio. However, in CHF mice, LVPWd decreased, end-diastolic volume (EDV) increased and marked cardiac fibrosis was formed. Thus, pressure overload induced decompensate heart failure and eccentric hypertrophy. Moreover, 14-3-3ɛ protein expression of hearts was increased in response to swimming training but decreased in CHF mice. However, other isoforms (β, ɛζ) of 14-3-3 proteins were no obvious changes in these three models. Therefore, our results suggest that the expressions of 14-3-3ɛ are different in physiological and pathological hypertrophy, which may provide a potential gene strategy for the treatment of heart failure.

I. Introduction

Cardiovascular disease remains the number one cause of mortality in the world, with heart failure representing the fastest growing subclass over the past decade (Kannel et al, 2000; Levy et al, 2002; Hobbs et al, 2004). Cardiac hypertrophy is characteristics of most forms of heart failure, and is induced by pathological stimuli (e.g., pressure or volume overload) (Izumo et al, 1988; Izemitsu et al, 2001). When disease causes pressure or volume overload (e.g., hypertension, valvular disorders) of the heart, the failing cardiac hypertrophy is initially a compensatory response to the increased load. However, function in the hypertrophied heart eventually decompensates, leading to left ventricle (LV) dilatation, increased interstitial fibrosis and heart failure (Levy et al, 1990; Cohn et al, 1997). A paradoxical exception is the cardiac hypertrophy that occurs in the athlete (physiological hypertrophy), which is induced by physiological stimuli (e.g., developmental growth, exercise training) (Iemitsu et al, 2001). In contrast to pathological hypertrophy in disease states, which is associated with fibrosis, dysfunction, altered cardiac gene expression, and increased morbidity and mortality, physiological hypertrophy shows a normal organization of sarcomeres and fibers, normal or enhanced cardiac function, and a relatively normal pattern of cardiac gene expression (Heneke et al, 2006; McMullen et al, 2007).

14-3-3 proteins were first found in fetal bovine brain
in 1967 and this class of proteins has been found in all eukaryotic organisms studied so far (van Hemert et al., 2001; Daniela et al., 2003). There are seven known mammalian 14-3-3 isotypes (β, γ, ε, η, ζ, σ and τ/θ) (Martin et al., 1993). 14-3-3 monomers have a molecular weight of approximately 30 kDa and an isoelectric point of about 5, but functional 14-3-3 exists as a dimer. 14-3-3 Proteins specifically recognize phosphoserine/threonine-containing sequence motifs on target proteins, such as RSXpSXP, RXSX (ST) XP or RX (Y/F) XpSXP. In addition, they can bind to unphosphorylated motifs: GHSL and WLDLE (Petosa et al., 1998; Hallberg, 2002; Berg et al., 2003). 14-3-3 proteins have been shown to interact with an array of partners, ranging from enzymes to structural proteins. Often, these proteins are important in vital cellular processes including cell cycle control and apoptosis. Through its interaction, 14-3-3 either regulates the catalytic activity of its bound enzymes, determine the subcellular localization of target proteins, or both (Fu et al., 2000; Tzivion et al., 2002). For example, 14-3-3 inhibits ASK1 (apoptosis signal regulating kinase-1) activity by binding to specific residues surrounding Ser967 (Zhang et al., 1999; Goldman et al., 2004). This interaction also controls the subcellular distribution of ASK1 (Subramanian et al., 2004; Zhang et al., 2003). The binding of 14-3-3 with phosphoinositide 3-kinase (PI3K), protein kinase C (PKC) and Raf can either inhibit or enhance the activities of these enzymes (Hekman et al., 2004; Light et al., 2002). 14-3-3 Proteins associate with cdc25c, FKHR1 (also called FOXO3a), histone deacetylase5/7(HDAC5/7), nuclear factor of activated T cells (NFATc), p27 and PKUa, preventing their entry into the nucleus (Timothy et al., 2001; Sekimoto et al., 2004). 14-3-3 Proteins can also modulate protein–protein interactions. For example, 14-3-3 interacts with the apoptosismodulating protein BAD, preventing BAD from binding to and inhibiting the antiapoptotic function of Bel-7 (Datta et al., 2000; Yang et al., 2001).

14-3-3 proteins have been found to be up- or down regulated in human disease, but their direct role in disease progression has not been clearly established. In neuronal system, 14-3-3γ gene has been implicated in neurological disorders (Zanusso et al., 2005); 14-3-3γ isoform in the cerebrospinal fluid (CSF) can be used as a marker for sporadic Creutzfeldt-Jakob disease (CJD) (Peoc’h et al., 2001; Van Everbroeck et al., 2005). Moreover, 14-3-3β, ε and η genes are also found in the CSF of CJD patients (Wiltfang et al., 1999); In addition to their possible role in neuronal function, 14-3-3 proteins have attracted much recent interest owing to their possible involvement in the pathophysiology of various cancer diseases. The 14-3-3σ gene expression is much lower in breast carcinoma cells than in normal breast epithelium resulting from high frequency of hypermethylation at the 14-3-3σ locus leads to gene silencing in breast cancer (Anne et al., 2000); however, 14-3-3β, γ and τ gene expression levels are increased in lung cancer as compared with the equivalent normal tissues (Qi et al., 2005). The mechanism underlying the development of pathological hypertrophy versus physiological hypertrophy in the adult is poorly understood and whether 14-3-3 proteins are involved in physiological, pathological cardiac hypertrophy and chronic heart failure (CHF) are little known.

In preliminary studies of our lab, using a gene micro assay, we found the gene of 14-3-3e mRNA was up-regulated in hypertrophic rat heart induced by continuous norepinephrine (NE) infusion (Li et al., 2003). Subsequently, we constructed a recombiant adenovirus which encoding R18, a general 14-3-3 peptide inhibitor, to disrupt 14-3-3 functions in cardiacmyocytes and cardiac fibroblasts. We found that 14-3-3 proteins inhibited cardiomyocyte hypertrophy induced by norepinephrine in rats and serum-induced proliferation of cardiac fibroblast (Liao et al., 2005; Du et al., 2005). These data suggested that 14-3-3 proteins were involved in cardiac hypertrophy. However, whether 14-3-3 proteins are different expressed in physiological cardiac hypertrophy, pathological cardiac hypertrophy and heart failure are still unclear. To examine the expression of 14-3-3 proteins in the hearts under physiological and pathological conditions, we carried out studies in mice which were subjected to chronic swimming training for 8 weeks induced to physiological cardiac hypertrophy, or pressure overload by transverse aortic constriction (TAC) for 4 weeks induced to pathological cardiac hypertrophy and 16 weeks induced to chronic heart failure.

II. Materials and methods
A. Experiments protocols
The animal care and experimental protocols were in compliance with the Animal Management Rule of the People’s Republic of China (Ministry of Health, P. R. China, document no. 552001), and the study was approved by the Animal Care Committee of the Third Hospital, Peking University.

Protocol I
For aortic banding, mice were subjected to transverse aortic constriction or a sham operation by the same surgeon as described previously (Ding et al., 1999). Briefly, transverse aortic constriction was performed in 8–10-week-old male C57/BL6 mice (weight 15–20 g). Animals were anesthetized with intraperitoneal tribromethanol 0.2 ml/mg and atropine 1 μl/mg, and aortic constriction was created via a center thoracotomy by placing a ligature securely around the transverse aorta and a 26-gauge needle and then removing the needle. Animals with TAC were allowed access to food and water ad libitum for 4 weeks, and age-matched sham-operated animals served as controls (SHAM1). CHF was made by TAC for longer time to 16 weeks, also age-matched sham-operated animals served as controls (SHAM2).

Protocol II
Swimming training was performed as previously described (McMullen et al., 2003). Briefly, 8–10-week-old male C57/BL6 mice weighing 15–20 g were made to swim in water tanks with a surface area of 225 cm² and a depth of 15 cm for 90 min and water temperature of 30–32 °C. Animals were initially exercised for 20 min twice daily, and the duration of exercise was increased in 20-min increments daily, reaching 90 min, twice daily. This duration of exercise was maintained until the end of the study. Mice were trained 6 days a week and age-matched animals served as controls. All animals were allowed access to food and water ad libitum. Two of eight animals were unable to complete
this course of exercise, but all other animals were conditioned successfully.

B. Echocardiography
Before euthanasia, in vivo left ventricular (LV) function and LV hypertrophy were assessed by measuring fraction shortening (FS), and left ventricular diastolic posterior wall thickness (LVPWd), recorded by echocardiography using a Vevo 770 echocardiography system (Visual Sonics, Toronto, Canada) with a 30 MHz linear array transducer (McMullen et al, 2007). Briefly, Animals were anesthetized with inhaling isoflurane/oxygen, once the short-axis two-dimensional (2D) image of the left ventricle was obtained at the papillary muscle level, 2D guided M-mode images across the anterior and posterior walls were recorded. Parameters measured digitally on the M-mode trace were the LVPWd and inner dimension of diastolic or systolic left ventricles (LVIDd and LVIDs), and FS = (LVIDd−LVIDs)/LVIDd, EDV = ((7.0 / (2.4 + LVIDd))×LVIDd³, ESV = ((7.0 / (2.4 + LVIDs))×LVIDs³.

For aortic banding studies, to evaluate the degree of stenosis, the pressure gradient across the constriction was assessed using Doppler echocardiography (McMullen et al, 2004). A no imaging Doppler pencil transducer (continuous wave) was placed at the apex and orientated towards the proximal ascending aorta. The peak velocity (in meters per second) was measured, and the maximum instantaneous gradient (millimeters of Hg) was calculated using the following Bernoulli equation: pressure gradient = 4 × (velocity)².

C. Measurement of BP and HR
Swim-training mice along with their age-matched control siblings at 4 months were used for blood pressure (BP) and heart rate (HR) measurement. The systolicBP, diastolic BP and HR were measured by a programmable sphygmomanometer (BP-98A; Softron, Japan) using the tail-cuff method as described previously (Fukamizu et al, 1993). Anaesthetized mice were introduced into a small holder mounted on a thermostatically controlled warming plate and maintained at 37 °C during measurement.

D. HW assessment and histologic examination
At the completion of the experiment, animals were euthanized and their hearts were removed, the left ventricle was quickly separated from the atria and right ventricular free wall and their heart [left ventricle + right ventricle] weights (HW) and body weights (BW) were determined. Then, left ventricles were fixed overnight in 4% paraformaldehyde before embedding in paraffin. Sections of 7.5 µm were prepared, and stained with hematoxylin-eosin (HE) or Sirius red for evaluation of myocyte hypertrophy and collagen content, respectively.

Cardiomyocytes from LV cross sections were stained with hematoxylin-eosin, and mean values from each mouse were calculated by use of the measurements from 60 to 80 cells from an individual mouse using light microscopy at × 400 magnification. Sirius-stained sections were quantitatively analyzed using light microscopy at × 40 magnification to evaluate myocardial fibrosis using the difference in color (red fibrotic area as opposed to yellow myocardium). Digital photographs were obtained by using a color image analyzer (QWin Colour Binary 1, LEICA).

E. Citrate Synthase Activity
Citrate synthase (CS) analysis was performed as described before (Srere et al, 1969; McMullen et al, 2003). Briefly, the tibialis anterior and gastrocnemius were dissected, weighed, and rapidly frozen in liquid nitrogen. Frozen tissue was then homogenized with a glass homogenizer on ice in 100 mM Tris-HCl. Muscle homogenate protein concentration was determined using the Bio-Rad protein assay (Bio-Rad Laboratories, Hercules, CA). Sample homogenate was then added to a reaction mix of 100mM Tris-HCl, 1.0mM diithio-bis (2-nitrobenzoic acid), and 3.9mM acetyl coenzyme A. After an addition of 1.0mM oxaloacetate, absorbance at 412 nm was recorded for a 2-min period. Mean absorbance change per minute was recorded for each sample, and CS activity (in µmol/mg protein/min) was then calculated by using the mercaptoide ion extinction coefficient of 13.6.

F. Western blot analysis
Western blot was performed as previously described (Yin et al, 2003). Briefly, cell samples were lysed in 100 µl buffer containing 20 mM Tris-HCl (pH 7.4), 100 mM NaCl, 10 mM sodium pyrophosphate, 5 mM EDTA, 50 mM NaF, 1 mM sodium vanadate, 0.1% SDS, 10% glycerol, 1% Triton X-100, 1% sodium deoxycholate, 1 mM leupeptin, 0.1 mM aprotinin and 1 mM PMSF. Protein concentration was determined with a BCA protein assay kit (Pierce Biotechnology, Inc, Rockford, IL, USA), and proteins were separated on a 10% SDS-polyacrylamide gel and then electrophotographically transferred to nitrocellulose membranes (Pall Corporation, East Hill, NY, USA). eIF5 was used as a loading control (Du et al, 2005). Results are expressed as the changed fold over the control (Con) or sham (SHAM1 or SHAM2 of CHF group). Anti-14-3-3ε, β, γ, ζ, and anti-eIF-5 were purchased from Santa Cruz (Delaware, CA, USA).The sheets were analyzed with antibodies according to the supplier’s protocol, and visualized with peroxidase and an enhanced-chemiluminescence system (ECL kit, Pierce Biotechnology, Inc.). Bands were visualized by use of a super western sensitivity chemiluminescence detection system (Pierce, IL). Autoradiographs were quantitated by densitometry (Science Imaging system, Bio-Rad, Hercules, CA).

G. Statistical analysis
Data are presented as mean ± S.D. Statistical analysis was performed by one-way analysis of variance followed by Tukey’s method or unpaired two-tailed Student’s t-tests. Correlations between groups of values were evaluated, calculating the best fit based on least squares regression analysis, and the coefficient of correlation (r²) was estimated. Results were considered statistically significant at p < 0.05.

III. Results
A. Cardiac hypertrophy and no fibrosis in mice response to swimming training
To analyze the role of 14-3-3ε proteins in physiological cardiac hypertrophy, we copied the model by swimming training for 8 weeks in mice. The mice were adept at swimming for increasingly longer intervals after a few days of acclimatization. Body weight and cardiac wall thickness determinations were made throughout the course of study to assess the development of cardiac hypertrophy. The swimming mice failed to gain weight relative to the controls since 2wk whereas increased LVPWd at 6-8wk (Figure 2D, 2F). Animals were randomized by body
weight at the outset, at the end of 8 wk of exercise, the swimmers manifested a significant (12%) increase in heart weight whereas failed to gain weight significantly at the same time, resulting in a 32% increase in heart weight-to-body weight ratio (P < 0.001, Table 1). The swimming mice showed cardiac hypertrophy and no fibrosis (Figure 1A-C). Furthermore, we measured FS to detect LV systolic function. As shown in Figure 2E, there was no significant change in FS in swimming mice compared with control, which meant swimming training did not reduce cardiac systolic function.

In addition to the development of cardiac hypertrophy, two other measures of exercise conditioning were assessed: heart rate response to a work load and skeletal muscle C5 activity (Kaplan et al, 1994). Figure 2A shows the mean heart rate response to swimming training in rest was significantly decreased (449 beats/min) compared with the control (599 beats/min) at 8wk (P < 0.01), whereas the systolic blood pressure (SBP) and diastolic blood pressure (DBP) did not decrease significantly (Figure 2B). Citrate synthase activity, an index of muscle oxidative capacity and hence physical training, was measured in mixed gastrocnemius muscle of mice that underwent swimming training. After 8 weeks of exercise, citrate synthase activity was significantly elevated in skeletal muscle of swimming mice compared with control (P < 0.001, Figure 2C). Both measures indicated that the animals had become conditioned during the course of training.

B. Pathological hypertrophy after TAC and cardiac dysfunction in CHF mice

At the same time, we copied the models of TAC and CHF respectively. There was no difference in body weight among the groups. To determine that the magnitude of systolic load was comparable in both TAC groups, in vivo aortic pressure gradient (AoPg) measurements were performed. AoPg was elevated significantly both in TAC and CHF group compared with SHAM1 and SHAM2 (p<0.01, Figure 3A). At the end of 4wk in TAC mice, detected by echocardiography, LVPWd was increased whereas FS was normal compared with SHAM1 (Figure 4B, Table 1), and there is a positive linear correlation between the weights of echocardiographic left ventricular myocardium (Echo LVM) and anatomy LVM in TAC and CHF mice (Figure 3B). Moreover, hearts removed from TAC mice displayed mild fibrosis (Figure 1A).

To copy the model of chronic heart failure (CHF), we performed the mice of TAC for a long time to 16 weeks. In CHF mice, there was no obvious change of cardiac systolic function in the compensatory stage of TAC from 0wk to 15wk (Figure 3C). When pressure was persistent, the cardiac systolic function of mice depressed, fraction shortening (FS) decreased to 21.2±1±2.61 %, compared with 31.5±1±3.17% in sham2 mice at 16wk(P < 0.05; Figure 3C and Table 1). In addition, This LV dysfunction was associated with pulmonary edema, with lung weight in CHF mice increasing to 170 ± 16.33 mg compared with 142.5 ± 20.62mg in sham2 mice (P<0.05; Table 1). Also, LVPWd was increased most significantly in the first 4 weeks, with time passing by, LVPWd decreased mildly, till there was no significant change when FS was decreased at 16wk (Figure 3D). Thus, pressure overload induced decompensated heart failure in CHF mice.

C. Different changes in comparison with three cardiac hypertrophy models

To investigate the different changes in three models of cardiac hypertrophy, we compared the data of echocardiography and anatomy in swimming training, TAC and CHF mice (Table. 1). As we showed in Figure 4, there were no obvious changes of FS in swimming training and TAC mice whereas FS decreased in CHF mice. LVPWd increased mild in response to swimming training and marked increased in response to TAC, whereas decreased in CHF mice. These revealed that mice compensated to the pressure load of swimming training and TAC, whereas decompensated in CHF mice. Furthermore, there was obviously increased magnitude of hypertrophy in CHF mice compared with TAC mice, and also the functional response was dramatically different between these groups. TAC mice compensated to the pressure load without change in LV function, LV diameter, or lung weight (Table 1). In contrast, CHF mice developed severe LV dysfunction, as measured by FS and inner dimension of diastolic LV (LVIDd). At the end of 16wk of CHF mice, the heart became dilated, with LVIDd of 4.25±0.17mm, compared with 4.00±0.1 mm in SHAM2 mice (P<0.05; Figure 4D), and EDV (end-diastolic volume) of 80.86±7.78 µl, compared with 70.12±4.13 µl in SHAM2 mice (P<0.05; Table 1). CHF mice developed severe LV systolic dysfunction, as measured by FS and eject fraction (EF) in response to pressure overload (Figure 4A, Table 1). When analyzed cardiac morphology, we found that there were no fibrosis in swim-training mice, mild fibrosis in TAC mice and marked fibrosis in CHF mice (Figure 1A, 1D). Therefore, these results reflected the changes from adaptive cardiac hypertrophy to compensate and decompensate maladaptive hypertrophy.

D. 14-3-3ε is increased in hearts of swimming-training mice, whereas decreased in CHF mice

Subsequently, we detected protein expression of 14-3-3 isoforms (β, ζ and ε) in hearts of mice from physiological, pathological hypertrophy and heart failure respectively. As we showed in Figure 5A-D, it manifested that 14-3-3β protein expression increased in swimming mice and TAC mice, and the same tendency as 14-3-3ζ in TAC mice, but there were no obvious changes in statistics. Interestingly, the expression of 14-3-3ε was increased 2-fold above control in swimming-training mice (p<0.01), and no obvious changes in TAC mice, whereas decreased 2.5-fold compared with sham2 group in CHF mice (p<0.05, Figure 5E, F). Therefore, these results suggest that differential expression of 14-3-3ε may play an important role in cardiac hypertrophy.
Table 1: Anatomical and echocardiographic data of three models (SWIM, TAC and CHF mice). TAC, transverse aortic constriction; CHF, chronic heart failure; BW, body weight; HW, heart weight; LW, lung weight; TL, tibial length; LVIDd, inner dimension of diastolic LV; LVIDs, inner dimension of systolic LV; LVPWd, LV end-diastolic posterior wall thickness; LVPWs, LV end-systolic posterior wall thickness; LVAWd, LV end-diastolic anterior wall thickness; LVAWS, LV end-systolic anterior wall thickness; EF, ejection fraction; FS, fractional shortening; EDV, end-diastolic volume; ESV, end-systolic volume.*p<0.05, **p<0.005, *p<0.001 compared to control or SHAM1 or SHAM2 from the same group.

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<th>Anatomical data</th>
<th>CON(n=8)</th>
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<th>TAC(n=7)</th>
<th>CHF(n=7)</th>
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<td>LW (g)</td>
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<td>TL (mm)</td>
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<td>LVPWd (mm)</td>
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Figure 1: Histological sections and echocardiograms from the left ventricular (LV) wall of three models (SWIM, TAC and CHF mice).
(A) The LV cross sections of the three models stained with hematoxylin-eosin (×400 magnification, Scale bar, 200 μm) and Sirius red (red staining, × 40 magnifications, Scale bar, 200 μm). (B) Representative echocardiograms used to measure the LV wall thickness in three models (swim training, TAC and CHF mice) during cardiac cycles. (C) Mean cross-sectional area of cardiomyocytes in three models was measured. **p<0.01, ***p<0.001. (D) The area fraction of fibrotic tissue of hearts was compared among three models (SWIM, TAC and CHF mice). ***p<0.001.
Figure 2: The stress of swim training in mice results in physiological cardiac hypertrophy. (A) Heart rate (HR), (B) Blood pressure (BP, including systolic blood pressure, SBP and diastolic blood pressure, DBP) and (C) Citrate synthase (CS) was assayed at the end of 8wk in control (CON) and swim-training (SWIM) mice. *p<0.05, **p<0.01 compared to control from the same group. (D) Body weight (BW), (E) Fraction shortening (FS), (F) Left ventricular end-diastolic posterior wall thickness (LVPWd) were observed dynamically from 0wk, 2wk, 4wk, 6wk, 8wk. *p<0.05, **p<0.01, ***p<0.001 compared to control at the same time.

Figure 3: The changes of AoPg and LVM in three models and dynamic changes of LVPWd and FS observed by echocardiography in CHF mice. (A) Aortic pressure gradient (AoPg) was detected in three models (SWIM, TAC and CHF mice). **p<0.01, ***p<0.001 compared to SHAM1 or SHAM2 from the same group. (B) Positive linear correlation between the weights of echocardiographic left ventricular myocardium (Echo LVM) and Anatomy LVM in three models (SWIM, TAC and CHF mice). (C) FS (D) LVPWd were observed dynamically for every 2 weeks over the 16-week study period in CHF mice. *p<0.05, **p<0.01, ***p<0.001 compared to SHAM2 at the same time.
Figure 4: Different changes in comparison with three models (SWIM, TAC and CHF mice). (A) FS, (B) LVPWd, (C) LVIDs, (D) LVIDd were compared among SWIM, TAC and CHF mice. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ compared to control (CON) or SHAM1 or SHAM2 from the same group.

Figure 5: 14-3-3ε is increased in hearts of swim-training mice whereas decreased in CHF mice. (A) 14-3-3β, (C) 14-3-3ζ and (E) 14-3-3ε were detected by Western blot in the myocardium of swim training, TAC and CHF mice respectively; eIF5 was used as a loading control. A representative western blot for each treatment from three independent experiments is shown. Quantitative analysis of 14-3-3β (B), 14-3-3ζ (D) and 14-3-3ε (F), Results are represented as mean ± S.D. (n = 3). *$p < 0.05$, **$p < 0.01$ compared to control or SHAM2 from the same group.

V. Discussion

Examples of stimulus-dependent hypertrophic responses can be found in both physiological and pathological hypertrophy. Exercise-induced cardiac hypertrophy is a good example of physiological hypertrophy, which is a favorable adaptive response of the heart to increases in bodily demand. In comparison, pathological hypertrophy is a maladaptive response to pathological stimuli, such as pressure or volume overload. These differences are particularly evident clinically, for pathological hypertrophy often progresses to heart failure, especially when pathological stimuli are persistent, whereas physiological hypertrophy usually does not. Regular aerobic exercise has many effects that are important in the prevention and treatment of cardiovascular disease (Belardinelli et al, 1999; Giannuzzi et al, 2003). To develop more effective gene therapies to treat cardiac hypertrophy and failure it is critical to
understand the genes and proteins involved in the development of pathological versus physiological cardiac hypertrophy. Although previous studies have shown that physiologic and pathologic hypertrophies have distinct functional characteristics, it was not clear whether these two forms of hypertrophy were mediated by common or distinct proteins. This work suggests that 14-3-3ε is involved in the induction of cardiac hypertrophy including physiological (swimming induced), pathological and heart failure (pressure overload induced). Of clinical relevance with regard to the transition from hypertrophy to heart failure, mice displayed marked cardiac dysfunction in response to pressure overload, also we show that 14-3-3ε is up-regulated in swimming-training mice in contrast to down-regulated in mice of chronic heart failure.

Homodynamic overload initially results in an adaptive concentric hypertrophy in the heart, normalizing wall tension. Sustained pressure overload will override these adaptive mechanisms, leading to eccentric hypertrophy, decreased contractile function, and the development of heart failure (Chien et al, 1999). Molecular pathways involved in cardiac hypertrophy can be specifically studied in vivo by exposing mice to either biomechanical stress through TAC (Rockman et al,1991) or hormonally induced hypertrophy (Brancaccio et al, 2003; Oudit et al, 2003). This approach led to the identification of many signal transduction pathways involved in these processes (Frey and Olson 2003; Olson and Schneider 2003). As we showed in this study, swimming-training stress result in physiological cardiac hypertrophy, which shows normal cardiac function, hypertrophic myocytes and no fibrosis. In contrast, TAC firstly results in concentric hypertrophy with hearts weight and LVPWd increased mild fibrosis. When pressure overload are persistent, LVIDs, LVIDd, EDS and EDV increased, FS decreased, marked fibrosis formed, leading to decompensate cardiac hypertrophy and heart failure (Figure 1).

14-3-3ε is a member of the 14-3-3 protein family, which binds diverse proteins and functions as a scaffold to facilitate or attenuate the activities of the binding proteins (Tzivion et al, 2002). Recent studies demonstrated that brain 14-3-3ε was highly up-regulated in rats treated with rosiglitazone, 14-3-3ε bind to ligand-activated peroxisome proliferator-activated receptor-γ, and enhance sequestration of phosphorylated Bad and thereby suppress apoptosis to protect against ischemic cerebral infarction and neuronal apoptosis. Different signaling cascades may be important for the induction of 14-3-3 proteins in these two forms of hypertrophy is supported by other reports in the literature. Cardiac-specific over-expression of Gq, which is activated by a number of ligands, including angiotensin II (Ang II), NE and endothelin-1 (ET-1), induced cardiac hypertrophy akin to pathological hypertrophy (D’Angelo et al, 1997). Furthermore, pressure overload-induced hypertrophy but not swimming-induced hypertrophy was inhibited by Ang II receptor blockade (Geenen et al, 1996; Devereux et al, 2000). Downstream of Gq, p38, c-Jun NH (2)-terminal kinase (JNK), and PKCβ all have been implicated in mediating pathological hypertrophy (Bowman et al, 1997; Cook et al, 1999; Choukroun et al,1999). Calcineurin, another important hypertrophic mediator, has been implicated for the induction of pathological hypertrophy, but not resulted in physiological cardiac hypertrophy (Wilkins et al, 2004). Recent studies suggest that p38 plays a critical role in the development of fibrosis in response to pathological stimuli but not to cardiac growth itself (Zhang et al, 2003;Braz et al, 2003). Furthermore, using mice with cardiac-specific expression of dominant-negative (DN) mutants of 14-3-3 (DN 14-3-3/TG) and p38alpha/beta MAPK (DNp38alpha and DNs3β) mice, it was shown that 14-3-3 protein along with p38 MAPK mediated in left ventricular remodeling associated with swimming stress (Watanabe K et al, 2007).

The most likely candidate responsible for mediating exercise-induced cardiac hypertrophy is insulin-like growth factor-I (IGF-I), which activates PI3K (p110α) coupled to receptor tyrosine kinases. Cardiac-specific over-expression of the IGF-I receptor in transgenic mice resulted in cardiac hypertrophy that was characteristic of physiological hypertrophy, and the increase in heart weight was suppressed when these mice were crossed with dnPI3K transgenic mice. By contrast, cardiac formation of Ang II was increased in hypothyroided hearts of patients with heart failure (Serneri et al, 2001). It has been suggested that glycogen synthase kinase-3β (GSK-3β) provides a mechanism for cross talk between the Akt and calcineurin pathways (Antos et al, 2002). Akt directly phosphorylates GSK-3β, resulting in its inactivation. It was reported that mice expressing a constitutively-active form of GSK-3β did not develop exercise-induced hypertrophy, thereby, indicating the importance of the PI3K/Akt/GSK-3β pathway in physiological hypertrophy. Our laboratory previously reported that constructing AdR18, a recombinant adenovirus which encoding R18, a general 14-3-3 peptide inhibitor, to disrupt 14-3-3 functions. We found that 14-3-3 proteins inhibits norepinephrine-induced cardiomyocytes hypertrophy through regulation of the PI3K/PKB/GSK3β and Nfat pathway. Further, 14-3-3 proteins inhibit proliferation of cardiac fibroblasts, via p70 S6 kinase (p70S6K) phosphorylation and PI3 kinase activation, not through extra-cellular signal-regulated kinase (ERK) phosphorylation. Therefore, 14-3-3ε may be promote physiological cardiac hypertrophy via IGF-I/PI3K/AKT pathway and inhibit pathological cardiac hypertrophy via signaling molecules downstream of GPCRs activated by pathological stimuli (eg. NE, Ang II, ET-1, etc).

The cardiac function in mice are based on echocardiography in vivo, further evaluated can be performed by invasive hemodynamic measurements. Combined with anatomy data in mice, it is possible to analyze objectively. The use of isoform-specific inhibitors of 14-3-3 isoforms has generated great interest in oncology. Further studies are required to elucidate the mechanisms responsible for the depressed function in 14-3-3ε transgenics in response to TAC (e.g., increased.
fibrosis, necrosis, depressed excitation-contraction coupling). However, 14-3-3 proteins are ubiquitous proteins that are highly preserved from bacteria to humans and plants. Mice did not survive embryonic development when knockout ε isoform of 14-3-3 proteins. In consequent study, we may concentrate on knocking down the function of 14-3-3ε by using siRNA in vitro.

In summary, our findings suggest that exercise training and increased 14-3-3ε expressions have a favorable impact on physiological cardiac hypertrophy. The cardio-protective role of 14-3-3ε may be caused, at least in part, by promotion of physiological signaling cascades and inhibition of pathological signaling cascades. It may be of benefit to selectively target 14-3-3ε as a potential gene strategy for the treatment of heart failure.

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