Cardiac-specific overexpression of insulin-like growth factor 1 attenuates aging-associated cardiac diastolic contractile dysfunction and protein damage

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Li Q. Wu S, Li SY, Lopez FL, Du M, Kajstura J, Anversa P, Ren J. Cardiac-specific overexpression of insulin-like growth factor 1 attenuates aging-associated cardiac diastolic contractility dysfunction and protein damage. Am J Physiol Heart Circ Physiol 292: H1398–H1403, 2007. First published November 3, 2006; doi:10.1152/ajpheart.01036.2006.—Aging is associated with hepatic growth hormone resistance resulting in a fall in serum insulin-like growth factor 1 (IGF-1) level. However, whether loss of IGF-1 contributes to cardiac aging is unclear. This study was designed to examine the effect of cardiac overexpression of IGF-1 on cardiomyocyte contractile function in young (3 mo) and old (26–28 mo) mice. Cardiomyocyte contractile function was evaluated, including peak shortening (PS), time to 90% PS, time to 90% relengthening (TR90), and maximal velocity of shortening/relengthening (±dL/dt). Levels of β-glycolysis end product, protein, carboxyl, sarco(endo)plasmic reticulum Ca2+/ATPase (SERCA2a), phospholamban, and Na+/Ca2+ exchanger were assessed by Western blot analysis. SERCA activity was measured by 45Ca2+ uptake. Aging induced a decline in plasma IGF-1 levels. Aged cells exhibited depressed ±dL/dt, prolonged TR90, and a steeper PS decline in response to increasing stimulus frequency compared with those in young myocytes. IGF-1 transgene alleviated aging-induced loss in plasma IGF-1 and aging-induced mechanical defects with little effect in young mice. The beneficial effect of IGF-1 transgene on aging-associated cardiomyocyte contractile dysfunction was somewhat mimicked by short-term in vitro treatment of recombinant IGF-1 (500 nM). Advanced glycation end product, protein, carboxyl and voltage levels were higher in aged mice, which were not affected by IGF-1. Expression of SERCA2a (but not Na+/Ca2+ exchanger and phospholamban) and SERCA activity were reduced with aging, which was ablated by the IGF-1 transgene. Collectively, these data suggest a beneficial role of IGF-1 in aging-induced contractile dysfunction, possibly related to improved Ca2+ uptake.

cardiomyocytes; contractile function; calcium regulatory protein; senescence

CARDIAC AGING is a continuous and irreversible biological process contributing to the high morbidity and mortality in the elderly (4, 10, 11). Several theories have been made for cardiac aging, including prolonged action potential duration, myosin heavy chain isozyme switch, impaired intracellular Ca2+ homeostasis, altered membrane structure and permeability, and accumulation of reactive oxygen species, which may lead to abnormal cardiac contractile function (8–10). In addition, aging induces reduction in growth hormone (GH) and subsequent insulin-like growth factor-1 (IGF-1) deficiency, both of which are essential for the maintenance of normal cardiac structure and function. GH/IGF-1 deficiency has been reported to be associated with altered body composition, cytokine and neuroendocrine activation, cardiac atrophy, and impaired cardiac function. The risk of cardiovascular disease is increased in subjects with IGF-1 deficiency. IGF-1, the mediator of many of GH-associated effects in peripheral tissues, improves myocardial function in the setting of both healthy and failing hearts (22). The therapeutic potential of IGF-1 has been implicated in cardiac disorders related, but not limited, to heart failure, myocardial infarction, and diabetes (22). Nonetheless, the role of IGF-1 in cardiac aging remains largely obscure. Therefore, the present study was designed to determine whether cardiac-specific overexpression of IGF-1 alleviates aging-associated cardiomyocyte contractile function and affects expression/function of key Ca2+ regulatory proteins.

MATERIALS AND METHODS

Experimental animals. All animal procedures used in this study were approved by the Animal Care and Use Committees at the University of North Dakota (Grand Forks, ND) and the University of Wyoming (Laramie, WY). Male FVB and IGF-1 heterozygous transgenic mice at young (3 mo old) and old (26–28 mo old) ages were used. The pigmentation of fur color was used as a marker for heterozygous IGF-1 (light brown) or wild-type FVB (white) mouse identification as described (20). All animals were kept in our institutional animal facility at the University of Wyoming with free access to standard laboratory chow and tap water. At the time of death, blood glucose and plasma IGF-1 levels were measured using a glucose monitor (Accu-ChekII, model 792, Boehringer-Mannheim Diagnostics, Indianapolis, IN) and an ELISA commercial kit from Diagnostic System Laboratory (Webster, TX), respectively.

Isolation of mouse ventricular myocytes. Hearts were rapidly removed from anesthetized mice and mounted onto a temperature-controlled (37°C) Langendorff system. After being perfused with a modified Tyrode solution (Ca2+ free) for 2 min, the heart was digested for ~10 min with 0.9 mg/ml collagenase D (Boehringer-Mannheim Biochemicals) in the modified Tyrode solution. The modified Tyrode solution (pH 7.4) contained the following: (in mM) 135 NaCl, 4.0 KCl, 1.0 MgCl2, 10 HEPES, 0.33 Na2HPO4, 10 glucose, and 10 butanedione monoxime, and the solution was gassed with 5% CO2–95% O2. The digested heart was then removed from the cannula, and the left ventricle was cut into small pieces in the modified Tyrode solution. Tissue pieces were gently agitated, and the pellet of cells was resuspended. Extracellular Ca2+ was added incrementally back to 1.20 mM over a period of 30 min. Isolated myocytes were used for experiments within 8 h of isolation. Only rod-shaped myocytes with clear edges were selected for mechanical and intracellular Ca2+ studies (3).

Cell shortening/relengthening. Mechanical properties of ventricular myocytes were assessed by using a SoftEdge MyoCam system to
shortening and relengthening were assessed by using the following first 5–6 beats) before peak shortening (PS) was recorded. Cell steady-state contraction of myocyte was achieved (usually after the first 5–6 beats) before peak shortening (PS) was recorded. Cell shortening and relengthening were assessed by using the following indexes: PS, time to 90% FS (TPS90), time to 90% relengthening (TR90), and half-width duration (HWD), indicating late cell shortening and early relengthening, as well as maximal velocity of shortening (+dL/dt) and relengthening (−dL/dt). To examine the acute effect of IGF-1 on cardiomyocyte contractile function, cohorts of cardiomyocytes from young and aged FVB mice were treated with recombinant IGF-1 (500 nM) for 2 h at 37°C with 100% humidity and 5% CO2 as described previously (14).

**Western blot analysis.** Protein levels of sarco(endo)plasmic reticulum Ca2+-ATPase (SERCA2a), phospholamban (PLB), Na+/Ca2+ exchanger (NCX), advanced glycation end product (AGE), and protein carbonyl were examined by standard Western blot analysis. Left ventricular tissues were homogenized and centrifuged at 70,000 g for 20 min at 4°C. The supernatants were used for immunoblotting of SERCA2a, PLB, NCX, AGE, and carbonyl. The extracted proteins were separated on 10–15% SDS-polyacrylamide gels and transferred to polyvinylidene difluoride membranes. After being blocked, the membrane was incubated with mouse anti-AGE monoclonal (1:1,000, TransGenic, Kumamoto, Japan), rabbit anti-NCX polyclonal (1:1,000, Swant, Bellinzona, Switzerland), mouse anti-PLB monoclonal antibody (1:2,000, Abcam, Cambridge, MA), rabbit anti-SERCA2a (1:1,000, Affinity BioReagents, Golden, CO), and rabbit anti-dinitrophenvyl (1:150, Chemicon International, Temecula, CA) overnight at 4°C, followed by incubation with secondary antibodies. The antigens were detected by the luminomodification method. Quantification of band density was determined by using Quantity One software (version 4.4.0, build 36, Bio-Rad) and reported in optical density per square millimeter (16).

**SERCA activity measured by 45Ca2+ uptake.** Cardiomyocytes were sonicated and solubilized in a Tris-sucrose homogenization buffer consisting of 30 mM Tris-HCl, 8% sucrose, 1 mM PMSF, and 2 mM dithiothreitol (pH 7.1). To determine SERCA-dependent Ca2+ uptake, samples were treated with and without the SERCA inhibitor thapsigargin (10 μM) for 15 min. The difference between the two readings was deemed the thapsigargin-sensitive uptake through SERCA. Uptake was initiated by the addition of an aliquot of supernatant to a solution consisting of (in mM) 100 KCl, 5 Na2S, 6 MgCl2, 0.15 EGTA, 0.12 CaCl2, 30 Tris/HCl (pH 7.0), 10 oxalate, 2 ATP, and 1 μCi 45CaCl2 at 37°C. Aliquots of samples were injected onto glass filters on a suction manifold and washed three times. Filters were then removed from the manifold, placed in scintillation fluid, and counted. SERCA activity was expressed as counts per million per milligram protein (15).

**Statistical analysis.** Data were presented as means ± SE. Statistical significance (P < 0.05) for each variable was determined by ANOVA or t-test, where appropriate.

**RESULTS**

**General feature of young and old experimental animals.** General features of young and aged FVB and IGF-1 transgene mice are shown in Table 1. At young age, transgenic IGF-1 overexpression did not elicit any notable effect on body, liver, and kidney weights compared with those in age-matched FVB mice. However, IGF-1 significantly increased heart weight and heart size (heart-to-body weight ratio) in young mice. The enlarged heart weight and size seen in young IGF-1 mice prevailed through the older age. Aged mice had heavier body and organ weights (although not organ size) compared with those of young counterparts, with the exception of a large kidney size. Aging significantly reduced plasma IGF-1 levels. IGF-1 overexpression significantly elevated plasma IGF-1 levels in young mice and nullified aging-induced decline in plasma IGF-1 levels. Fasting blood glucose levels were not affected by either IGF-1 transgene or age.

**Mechanical properties of cardiomyocytes from young or aged FVB and IGF-1 mice.** Mechanical properties obtained at a pace frequency of 0.5 Hz revealed that resting cell length and PS amplitude were similar in ventricular myocytes among young and aged FVB and IGF-1 mice, with the exception of a reduced resting cell length in aged IGF-1 mice. However, myocytes from aged FVB mice displayed significantly reduced maximal velocity of shortening/relengthening (+dL/dt) compared with that in myocytes from young FVB mice. The reduced ±dL/dt was associated with normal shortening duration (TPS90), prolonged relengthening duration (TR90), and HWD in aged FVB myocytes. Interestingly, these aging-induced mechanical dysfunctions were not observed in aged IGF-1 transgenic mice, suggesting a beneficial role of IGF-1 against aging-associated cardiac dysfunction. IGF-1 itself did not affect mechanical contractile properties in young mice. To explore whether IGF-1 transgene-elicited beneficial effect against cardiac aging was due to IGF-1-induced cardiac hypertrophy or positive inotropy as described previously (20–22), cohorts of cardiomyocytes from young and aged FVB mice were treated in vitro with recombinant IGF-1 (500 nM) for 2 h before mechanical property was recorded. Our data revealed that IGF-1 treatment alleviated aging-induced decrease in ±dL/dt, prolongation in TR90, and HWD, in a manner similar to endogenous IGF-1 overexpression. In vitro IGF-1 treatment did not significantly affect cardiomyocyte mechanical function in young mice with the exception of significantly shortened HWD (Fig. 1).

### Table 1. General features of young and old FVB and IGF-1 mice

<table>
<thead>
<tr>
<th></th>
<th>FVB Young</th>
<th>FVB Old</th>
<th>IGF-1 Young</th>
<th>IGF-1 Old</th>
</tr>
</thead>
<tbody>
<tr>
<td>BW, g</td>
<td>19.1 ± 0.5</td>
<td>28.7 ± 0.8*</td>
<td>20.7 ± 0.9</td>
<td>30.2 ± 0.6*</td>
</tr>
<tr>
<td>HW, mg</td>
<td>98 ± 2.4</td>
<td>156 ± 8*</td>
<td>126 ± 6*</td>
<td>185 ± 10†</td>
</tr>
<tr>
<td>HW/BW, mg/g</td>
<td>5.12 ± 0.18</td>
<td>5.31 ± 0.22</td>
<td>6.12 ± 0.17†</td>
<td>6.19 ± 0.19</td>
</tr>
<tr>
<td>LW, g</td>
<td>0.89 ± 0.03</td>
<td>1.51 ± 0.07*</td>
<td>0.98 ± 0.05</td>
<td>1.40 ± 0.06*</td>
</tr>
<tr>
<td>LW/BW, mg/g</td>
<td>47.6 ± 1.4</td>
<td>51.3 ± 1.2</td>
<td>47.6 ± 1.3</td>
<td>46.1 ± 1.4</td>
</tr>
<tr>
<td>KW, g</td>
<td>0.23 ± 0.01</td>
<td>0.42 ± 0.02*</td>
<td>0.25 ± 0.02</td>
<td>0.45 ± 0.02*</td>
</tr>
<tr>
<td>KW/BW, mg/g</td>
<td>12.2 ± 0.4</td>
<td>14.6 ± 0.6*</td>
<td>12.1 ± 0.4</td>
<td>15.0 ± 0.5*</td>
</tr>
<tr>
<td>Blood glucose, mg/dl</td>
<td>97.0 ± 5.6</td>
<td>99.1 ± 6.4</td>
<td>95.2 ± 6.4</td>
<td>100.8 ± 7.0</td>
</tr>
<tr>
<td>Plasma IGF-1, ng/ml</td>
<td>101.8 ± 6.0</td>
<td>48.6 ± 4.2*</td>
<td>154.4 ± 5.3†</td>
<td>87.3 ± 5.7†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 17–23 mice/group. BW, body weight; HW, heart weight; LV, liver weight; KW, kidney weight. *P < 0.05 vs. corresponding young group; †P < 0.05 vs. corresponding FVB group.
Effect of increasing stimulation frequency on myocyte shortening. To evaluate the impact of aging on cardiac contractile function under various frequencies, stimulus frequency was raised from 0.1 to 5.0 Hz, and the steady-state PS was recorded. Cells were initially stimulated to contract at 0.5 Hz for 5 min to ensure steady state before commencing the frequency response study. All recordings were normalized to PS at 0.1 Hz of the same myocyte. Fig. 2 shows a steeper negative staircase in PS in aged FVB myocytes with increased stimulus frequency (at 0.5 Hz or higher) compared with young FVB myocytes, suggesting reduced stress intolerance under advanced aging. Interestingly, IGF-1 transgene ablated aging-induced stepper reduction in PS amplitude at high-stimulating frequencies.

Protein expression of intracellular Ca²⁺ regulatory proteins, AGE, and carbonyl formation. As depicted in Fig. 3, Western blot analysis demonstrated a significantly reduced expression of SERCA2a in the aged FVB group, which was nullified by IGF-1. IGF-1 itself did not affect the expression of SERCA2a. Neither aging nor IGF-1 affected expression of NCX and PLB (monomer). IGF-1 but not aging significantly reduced the expression of pentamer PLB. Our data further revealed a significantly elevated formation of AGE and protein carbonyl in aged FVB mouse hearts. IGF-1 attenuated aging-elicited elevation of protein carbonyl but not AGE. Interestingly, IGF-1 itself significantly reduced the levels of AGE and protein carbonyl in young mouse hearts (Fig. 4).

Effect of IGF-1 on SERCA activity in young and aged mice. Consistent with reduced SERCA2a protein expression in aged FVB mouse hearts shown in Fig. 3, direct measurement of SERCA activity using ⁴⁵Ca²⁺ uptake technique revealed that aging significantly dampened SERCA activity, which may be rescued by IGF-1. Similar to its effect on SERCA2a protein expression, IGF-1 transgene itself did not affect SERCA activity in young FVB mouse hearts (Fig. 5).
DISCUSSION

Our study revealed that cardiac overexpression of IGF-1 rescued aging-induced cardiac contractile abnormalities. The IGF-1-induced cardiac protection against aging-induced cardiac defects may be underscored by alteration in SERCA protein expression and function as well as protein carbonyl formation. Furthermore, cardiac-specific overexpression of IGF-1 helped to alleviate aging-induced decline in plasma IGF-1 levels. Since IGF-1 itself did not significantly affect cardiac contractile function in young mice, its protective role against aging-induced cardiac dysfunction may implicate certain clinical potential of this growth factor in delaying cardiac aging process and minimize senescence-associated high cardiovascular mortality.

Ample evidence has confirmed dysregulated cardiac function in senescence (4, 9, 13). Our results revealed reduced maximal velocity of contraction and relaxation, prolongation of relaxation, and HWD in aged FVB cardiomyocytes. We found that IGF-1 attenuated aging-induced cardiac contractile dysfunction, SERCA expression/function, and protein carbonyl formation, indicating a possible contribution of facilitated intracellular Ca\(^{2+}\) resequestration and reduced protein damage from IGF-1 transgene. The fact that IGF-1 itself did not affect myocyte function, SERCA expression and function in young mouse hearts indicate that the excessive amount of this growth factor is not innately harmful to cardiac function. It was demonstrated previously that IGF-1-overexpressing transgenic mice exhibit protection against diabetes-induced cardiac pathology, oxidative damage, activation of oncogene p53, and cardiac contractile dysfunction (7, 18). IGF-1 is known to trigger cardiac hypertrophy (20, 22), as evidenced by significantly increased cardiac mass and heart-to-body weight ratio (Table 1). It is possible that the IGF-1 overexpression-associated changes in cardiomyocyte function and protein expression under aging could be consequences of cardiac hypertrophy. Nonetheless, data from our short-term in vitro treatment of recombinant IGF-1 indicated that IGF-1-induced cardiac hypertrophy is less likely responsible for the IGF-1-elicited protective effect against cardiac aging. The observation that short-
term IGF-1 incubation did not significantly affect young cardiomyocyte contractile function (with the exception of shortened HWD) is consistent with our previous report (14), indicating a possibly sufficient IGF-1 levels in young cardiomyocytes. The fact that recombinant IGF-1 short-term treatment shortened HWD without affecting TPS90 and TR90 in young cardiomyocytes indicates that IGF-1 may predominantly facilitate late-phase contraction and early-phase relaxation (which comprises HWD). However, such response possibly elicited by acute IGF-1 treatment may be adapted with sustained high IGF-1 levels in IGF-1 overexpression condition.

SERCA and NCX are considered as the main machineries to remove Ca$^{2+}$ from cytosolic space for cardiac relaxation to occur. However, the relative contribution for intracellular Ca$^{2+}$ extrusion by SERCA and NCX are still debatable and may vary among different species (1, 5, 12). Bers (1) indicated that SERCA is responsible for 92% Ca$^{2+}$ removal, whereas NCX only contributes to <10% in mouse hearts. Our data revealed downregulation of SERCA but not NCX or PLB, an endogenous inhibitor of SERCA, in aged FVB hearts. The reduced SERCA2a protein expression is supported by reduced SERCA activity in aged FVB hearts, which may account for, at least in part, diastolic dysfunction (prolonged relaxation and HWD) in aged myocytes. One rather surprising finding from our study was that IGF-1 transgene reduced PLB pentamer expression without affecting that of PLB monomer. Although no precise mechanism regarding IGF-1-elicited response on PLB subunit can be offered at this time, two scenarios may be considered. First, the phosphorylation status of PLB may affect the affinity of PLB antibody (6). Although our earlier study did not reveal phosphorylation of PLB with short-term treatment of IGF-1 (14), the potential that sustained IGF-1 overexpression alters PLB phosphorylation and thus antibody affinity for unphosphorylated PLB cannot be ruled out. Secondly, it was depicted that PLB monomer rather than pentamer is deemed the “active” SERCA inhibitor (23). Therefore, the IGF-1-elicited down-regulation on PLB pentamer may be nonspecific and trivial to the overall effect of IGF-1 on SERCA function, given that PLB monomer is unaffected by IGF-1.

IGF-1 is a peptide growth factor structurally and functionally similar to insulin. It is synthesized by various cell types, including cardiomyocytes, and acts as an autocrine/paracrine factor (20, 22). IGF-1 regulates myocardial growth and function under both physiological and pathophysiological conditions. It improves myocardial function in postinfarction rat heart, in patients with chronic heart failure, and in healthy humans and experimental animals (2, 17, 22). Advanced age is associated with insufficient production of GH levels and hepatic GH resistance. This reduction in GH secretion is sufficient to cause a fall in the serum IGF-1 level, abnormal body composition, and metabolism (19). These changes are distinct from those associated with the hyposomatotropism of the elderly but are less severe than those seen in younger adults.
with organic GH deficiency. Evidence has indicated that GH and/or IGF-1 are involved in the regulation of cardiovascular function. Patients with GH/IGF-1 deficiency displayed comparable cardiac dysfunctions to aging, manifested primarily as reduced left ventricular mass, ejection fraction, and diastolic filling. IGF-1 is believed to mediate, to a large extent, the action of GH in cardiac growth and contraction. It improves cardiac contractility, tissue remodeling, glucose metabolism, insulin sensitivity, and lipid profile. Data from our present study suggest that cardiac-specific overexpression of IGF-1, which may be secreted from cardiomyocytes into circulation, may compensate for the reduced circulating IGF-1 levels and subsequently impaired SERCA expression/function under aging.

In conclusion, our study revealed that cardiac-specific overexpression of IGF-1 rescues aging-induced cardiomyocyte mechanical dysfunction, possibly through improvement in SERCA expression and function, in addition to its anti-protein damage capacity. It should be stated that other known beneficial effects of IGF-1, including antioxidant property, should not be ruled out at this time. These data have convincingly demonstrated the clinical potential of IGF-1 in the prevention and treatment of aging-associated cardiac dysfunction.

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GRANTS

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