Insulin-Like Growth Factor I Deficiency Prolongs Survival and Antagonizes Paraquat-Induced Cardiomyocyte Dysfunction: Role of Oxidative Stress

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ABSTRACT

Interruption of insulin-like growth factor I (IGF-1) signaling has been demonstrated to prolong life span although the underlying mechanism has not been elucidated. The aim of this study was to examine the influence of severe IGF-1 deficiency on survival rate, cardiomyocyte viability, contractile function, and intracellular Ca\textsuperscript{2+} property in response to challenge with the pro-oxidant paraquat. C57 negative and liver IGF-1 deficient (LID) transgenic mice were administrated paraquat (75 mg/kg) and survival was monitored. LID mice displayed a significantly improved survival than did C57 mice evaluated by the Kaplan-Meier curve. MTT assay revealed that in vitro IGF-1 treatment significantly sensitized paraquat-induced cell death in both C57 and LID groups, with significantly better cell viability in LID cardiomyocytes. Compared to C57 mouse cardiomyocytes, LID myocytes displayed reduced peak shortening (PS), decreased maximal velocity of shortening/relengthening (\(\pm\) dL/dt), prolonged time-to-90\% relengthening (TR\textsubscript{90}), and comparable tolerance to high stimulus frequency and intracellular Ca\textsuperscript{2+} homeostasis. Paraquat treatment for 48 hours reduced PS, \(\pm\) dL/dt, tolerance to high stimulus frequency, resting and rise in intracellular Ca\textsuperscript{2+}, and prolonged TR\textsubscript{90}, all of which were nullified or masked by IGF-1 deficiency. Paraquat increased reactive oxygen species and carbonyl production upregulated the Ca\textsuperscript{2+} regulating protein SERCA2a, and downregulated Na\textsuperscript{+}-Ca\textsuperscript{2+} exchanger, the effects of which were nullified or masked by IGF-1 deficiency. Although LID mice displayed reduced whole body glucose clearance, cardiomyocytes from LID mice exhibited dramatically enhanced insulin-stimulated phosphorylation of insulin receptor and Akt. These data demonstrated that IGF-1 deficiency may antagonize or mask the paraquat-induced decrease in survival, cardiomyocyte dysfunction, oxidative stress, and change in Ca\textsuperscript{2+} regulating proteins.

INTRODUCTION

GROWTH HORMONE (GH) and its major gene target insulin-like growth factor-1 (IGF-1) are essential for the maintenance of normal cardiac structure and function. GH/IGF-1 deficiency has been associated with altered body composition, cytokine and neuroendocrine activation, cardiac atrophy, and compromised cardiac function.\textsuperscript{1,2} 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.\textsuperscript{3} Recent evidence has suggested a controversial role of the GH and
IGF-1 in cardiac aging and life span. Clinical data revealed signs of early aging (wrinkled skin, obesity, insulin resistance, and osteopenia) in patients with isolated GH deficiency due to GH gene deletion, PROP-1 gene mutation-induced pituitary hormone deficiency, and isolated IGF-I deficiency due to deletion or mutation of the GH receptor gene (Laron syndrome) despite a long life span, reaching ages of 80–90 years. This is supported by an experimental finding of overt longevity in animal models of genetic GH deficiencies, such as Snell mice (Pit-1 gene mutation), the Ames Dwarf mice (PROP-1 gene mutation), and the Laron mice (GH receptor gene knockout). To the contrary, GH transgenic mice and acromegalic patients display premature death. Nonetheless, data from our lab as well as others have depicted compromised cardiac function in IGF-1 deficiency and hyper-contractile state of the heart with GH surplus. These findings led to the initiation of trials with GH or IGF-1 treatment in elderly subjects and the establishment of so-called “rejuvenation clinics.” Given the apparent contradiction between heart function and longevity at various GH/IGF-1 levels, the jury is still out in determining whether the seemingly compromised cardiac function under IGF-1 deficiency is indeed “detrimental” to cardiac health and ultimate longevity.

The aim of this investigation was to examine the effect of severe liver IGF-1 deficiency on resistance to paraquat toxicity, cardiomyocyte function, and oxidative stress. We took advantage of a murine model lacking the igf-1 gene specifically in liver (liver IGF-1 deficient [LID]) generated using the Cre/loxP system. The LID mice display a marked reduction (~75%) in circulating IGF-I and elevated GH levels. These mice exhibit extremely low levels of circulating IGF-1, hyperinsulinemia associated with muscle insulin resistance.

**MATERIALS AND METHODS**

*Experimental animals, genotyping, and serum IGF-1 measurement*

The experimental procedure was approved by the Institutional Animal Use and Care Committee at the University of Wyoming (Laramie, WY). All animal procedures were in accordance with the National Institute of Health animal care standards. LID mice on a mixed C57BL/6, FVB/N, and 129sv background were generated using the Cre/loxP system. To determine the presence of the IGF-1/loxP transgene, genomic DNA was isolated from tail clips using a Quick extraction and amplification kit (BioPioneer, San Diego, CA). To test the presence of Cre transgene (i.e., liver-specific IGF-1 gene knockout), primers Cre-5’ and Cre-3’ were used, which yielded a 0.6 kb band for the Cre transgene. Mice homozygous or heterozygous for IGF-1/loxP carrying the albumin-Cre transgene were crossed. The homozygous offspring along with negative controls were used for experiment. The mouse genotyping was executed using a double PCR strategy. To identify the genotype of IGF-1/loxP, primers of IA6, IA8, and ID3 were used in PCR reaction. Mice that yield one 0.4 kb band are negative for IGF-1/loxP whereas those with one 0.2 kb band are positive. The presence of both 0.4 and 0.2 kb bands indicates for heterozygous IGF-I/loxP. To determine the presence of the Cre transgene, primers Cre-5’ and Cre-3’ were used, which yield a 0.6 kb band. Mice positive for both IGF-1/loxP and Cre transgene were deemed the LID mice, while the IGF-I/loxP negative mice with or without Cre transgene were used as LID negative (C57) mice. Female positive transgenic mice and negative littermates were used for our current study at 6 months of age. Quantitative determination of mouse serum IGF-1 concentration was performed using a Quantikine ELISA kit from R&D System (Minneapolis, MN).

*Kaplan-Meier analysis of survival (paraquat tolerance test)*

Six-month-old LID mice and negative littermates were administered 75 mg/kg paraquat (methyl viologen i.p., Sigma, St. Louis, MO). Mice were monitored every 2 h for survival for up to 84 h. A total of 18 LID and 23 C57 wild-type mice were used in three independent Kaplan-Meier survival tests.

*Cell isolation procedures*

Mouse hearts were removed under anesthesia (ketamine/xylazine at 3:1, 1.32 mg/kg) and were perfused with Krebs-Henseleit bicarbonate...
ate buffer: 118 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO$_4$, 1.2 mM KH$_2$PO$_4$, 25 mM NaHCO$_3$, 10 mM HEPES, 11.1 mM glucose, and 10 mM butanedione with 5% CO$_2$/95% O$_2$. Hearts were subsequently digested with 223 U/mL collagenase D (Boehringer Mannheim, Indianapolis, IN) for 10 min at 37°C. After perfusion, left ventricles were removed and minced. Extracellular Ca$^{2+}$ was added back to 1.25 mM. Functional studies were conducted between 1 and 8 h of isolation. Myocytes with obvious sarcolemmal blebs or spontaneous contractions were not used for study.\(^{13}\)

**MTT assay for cell viability in response to paraquat with or without IGF-1 supplementation**

The MTT assay is based on transformation of the tetrazolium salt MTT by active mitochondria to an insoluble formazan salt.\(^{14}\) Fresh cardiomyocytes from control and LID mice were isolated using the above-mentioned procedure.\(^{13}\) Cells were plated in microtiter plate at a density of $3 \times 10^5$ cells/mL. Cardiomyocytes from control C57 and LID mice were treated with IGF-1 (15 nM) or vehicle for 2 h at 37°C before being exposed to paraquat (6 mM) for another 3 h at 37°C. The level of IGF-1 supplemented (15 nM or 114 ng/mL) was based on the physiological levels of the growth factor.\(^{15}\) The concentration of paraquat (6 mM) was determined based on the estimation of paraquat used in Kaplan-Meier in vivo survival study (75 mg/kg) and the average total blood volume of a mouse (77–80 mL/kg).\(^{16}\) Following paraquat incubation, MTT was added to each well with a final concentration of 0.5 mg/mL, and the plates were incubated for another 2 h at 37°C. The formazan crystals in each well were dissolved in dimethyl sulfoxide (150 µL/well). Formazan was quantified spectroscopically at 560 nm using a SpectraMax 190 spectrophotometer.

**Cell shortening/relengthening**

Mechanical properties of ventricular myocytes were assessed using a SoftEdge MyoCam system (IonOptix, Milton, MA).\(^{15}\) In brief, left ventricular myocytes were placed in a chamber mounted on the stage of an inverted microscope (Model IX-70, Olympus, Tokyo, Japan) and superfused at 25°C with a buffer containing: 131 mM NaCl, 4 mM KCl, 1 mM CaCl$_2$, 1 mM MgCl$_2$, 10 mM glucose, and 10 mM HEPES, at pH 7.4. The cells were field stimulated with supra-threshold voltage at a frequency of 0.5 Hz (unless otherwise stated), 3 ms duration, using a pair of platinum wires placed on opposite sides of the chamber connected to a FHC stimulator (Brunswick, NE). The myocyte being studied was displayed on a computer monitor using an IonOptix MyoCam camera. The IonOptix SoftEdge software was used to capture changes in cell length during shortening and relengthening. In case of altering stimulus frequency, the steady-state contraction of myocytes was achieved (usually after the first five to six beats) before peak shortening was recorded.\(^{13}\)

**Intracellular Ca$^{2+}$ transient measurement**

Intracellular Ca$^{2+}$ was measured using a dual-excitation, single-emission photomultiplier system (IonOptix) in myocytes loaded with fura 2-AM (0.5 µM). Myocytes were placed on an inverted microscope and imaged through an Olympus (IX-70) Fluor 40 oil objective. Myocytes were exposed to light emitted by a 75 W halogen lamp through either a 360 or 380 nm filter while being stimulated to contract at 0.5 Hz. Fluorescence emissions were detected between 480 and 520 nm by a photomultiplier tube after initial illumination at 360 nm for 0.5 s and then at 380 nm for the duration of the recording protocol. The 360 nm excitation reading was repeated at the end of the protocol. Qualitative evaluation of intracellular Ca$^{2+}$ was inferred from fura fluorescence intensity (FFI) changes ($\Delta$FFI). A Chebyshev equation was used to evaluate the intracellular Ca$^{2+}$ decay constant. Myocyte shortening was also evaluated in a cohort of the fura two-loaded ventricular myocytes simultaneously to compare their temporal relationship with the fluorescence signal. However, their mechanical properties were not used for data summary due to the apparent Ca$^{2+}$ buffering effect of fura-2.\(^{17}\)

**Intracellular fluorescence measurement of reactive oxygen species**

Intracellular oxidant stress was monitored by changes in fluorescence intensity resulting from oxidation of the intracellular probe 5-(6)-
chloromethyl-2’, 7’-dichlorodihydrofluorescein diacetate (CM-H2DCFDA, Molecular Probes, Eugene, OR), which enters cells and produces a fluorescent signal following intracellular oxidation by reactive oxygen species (ROS) such as H2O2. Freshly isolated cardiomyocytes were loaded with 10 μM CM-H2DCFDA for 30 min at 37°C before being washed with PBS buffer. The fluorescence intensity was then measured with a fluorescence microplate reader at an excitation wavelength of 480 nm and an emission wavelength of 530 nm with SpectraMax 340PC Microplate Reader System (Molecular Device, Sunnyvale, CA). CM-DH2DCFDA untreated cells showed no fluorescence and were used to determine background fluorescence, which was subtracted from the treated samples. The fluorescence intensity was normalized with protein amount.18

Western blot analysis of SERCA2a, NCX, PLB, foxo3a, insulin receptor, Akt, and carbonyl

Cardiomyocytes from paraquat-treated (48 h at 75 mg/kg) mice were dispersed and sonicated in a lysis buffer containing 20 mM Tris (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton, 0.1% SDS, and protease inhibitor cocktail. To stimulate phosphorylation of insulin receptor and Akt, cardiomyocytes were treated with 100 nM insulin for 10 min prior to protein collection. After protein determination, equal amount of protein samples (50 μg) was loaded onto 7%/15 % SDS-polyacrylamide gels in a minigel apparatus and was transferred to nitrocellulose membranes. For protein carbonyl, samples were prepared following the instruction of S7150 OxyBlotTM Protein Oxidation Detection kit (Chemicon International, Temecula, CA) before being loaded onto the SDS-polyacrylamide gels. Membranes were probed with anti-rabbit phosphor-Akt (Ser473, 1:1000; Cell Signaling Technology, Beverly, MA), anti-rabbit IGF-1 receptor (tyr1131)/insulin receptor (tyr 1146, 1:1000; Cell Signaling), anti-rabbit SERCA2a (1:1000; Bethyl Laboratories, Montgomery, TX), anti-rabbit NCX polyclonal (1:1000; Swant, Bellinzona, Switzerland), anti-mouse PLB monoclonal (1:2000; Abcam, Cambridge, MA), anti-rabbit Foxo3a polyclonal (1:1000; Upstate, Lake Placid, NY), rabbit anti-DNP (dinitrophenyl for carbonyl determination, 1:150; Chemicon International, Temecula, CA) and anti-rabbit GAPDH (1:1000, as internal loading control; Cell Signaling), followed by incubation with horseradish peroxidase-coupled anti-mouse secondary antibody (Cell Signaling). After immunoblotting, the film was scanned and detected with a Bio-Rad Calibrated Densitometer (Hercules, CA) and the intensity of immunoblot bands was normalized to the loading control GAPDH.

Statistical analysis

Data are presented as mean ± SEM. Log rank test was used for the Kaplan-Meier survival curve. Statistical significance (p < 0.05) for all other variables was determined by analysis of variance (ANOVA) followed by a Dunnett’s post-hoc analysis.

RESULTS

Kaplan-Meier analysis of survival (paraquat tolerance test) and glucose tolerance test

The serum IGF-1 levels were 623 ± 60 ng/mL and 263 ± 37 ng/mL in wild-type and LID mice (n = 9 mice per group, p < 0.05 between the two groups), respectively. These data are consistent with the previously reported serum IGF-1 levels using the same mice11 and confirm the state of severe IGF-1 deficiency in LID mice. Six-month-old LID mice and wild-type littermates were intraperitoneally administered 75 mg/kg paraquat (methyl viologen). Survival rate of mice were monitored every 2 h for 84 h. Figure 1A indicates that the LID group displayed a marked longer survival rate than their C57 littermates. The survival rate at 84 h after paraquat injection was 61.1% and 17.4% in LID and C57 mice, respectively. Figure 1B displays the glucose clearance ability elicited by LID and C57 mice. Following acute intraperitoneal glucose challenge, blood glucose levels in C57 mice started to drop after peaking at 15 min, and returned to nearly baseline after 120 min. However, the post-challenge glucose levels maintained at high levels from 15 through 60 min in LID mice before returning to normal levels after 120 min.
Cell viability in response to paraquat with or without IGF-1 supplementation

To further examine the effect of IGF-1 on paraquat-induced mortality, an in vitro experiment was performed to examine the effect of IGF-1 incubation (15 nM for 2 h) on paraquat (6 mM, 3 h treatment) elicited cell death in C57 and LID mouse cardiomyocytes. As indicated before, the levels of IGF-1 and paraquat were chosen based on the physiological IGF-1 concentration and the estimated in vivo level of paraquat used in in vivo survival study (75 mg/kg), respectively. Consistent with the Kaplan-Meier data, cardiomyocytes from LID mice are more tolerant to paraquat-induced cell death compared with the C57 group, both in the absence or presence of IGF-1 treatment. Interestingly, IGF-1 supplementation significantly reduced the cell survival rate in both C57 and LID groups in response to paraquat exposure. There was no difference in cell viability between C57 and LID cardiomyocytes in the absence of the paraquat insult (Fig. 1C). These data suggest a seemingly sensitizing effect of IGF-1 supplementation on paraquat-induced cell death.

Baseline mechanical and intracellular Ca\(^{2+}\) properties of left ventricular myocytes

Data shown in Figure 2 indicate that peak shortening (PS) amplitude and maximal velocity of shortening/relengthening (± dL/dt) were significantly reduced in LID mouse cardiomyocytes associated with unchanged time-to-PS (TPS) and prolonged time-to-90% relengthening (TR\(_{90}\)). While paraquat treatment (48 h) significantly reduced PS, ± dL/dt, and prolonged TR\(_{90}\) without affecting TPS in C57 group, the paraquat-induced mechanical effects were masked by IGF-1 deficiency. To explore the possible role of intracellular Ca\(^{2+}\) homeostasis in IGF-1 deficiency and paraquat-induced mechanical responses, we evaluated intracellular Ca\(^{2+}\) transients using the fura-2 fluorescence technique. Our result indicated that paraquat reduced resting and rise of (peak to resting) intracellular Ca\(^{2+}\) levels, as well as slowed down intracellular Ca\(^{2+}\) clearing rate (single and bi-exponential decay), all of which were nullified or masked by IGF-1 deficiency. IGF-1 deficiency itself did not affect the resting and peak intracellular Ca\(^{2+}\) levels as well as bi-exponential intracellular Ca\(^{2+}\) decay rate although it prolonged single exponential intracellular Ca\(^{2+}\) decay rate (Fig. 3).
Effect of increasing stimulation frequency on myocyte shortening

Murine hearts contract at high frequencies. We examined the steady-state peak shortening amplitude under gradually increased stimulating frequency (0.1 to 5.0 Hz). All recordings were normalized to peak shortening at 0.1 Hz of the same cardiomyocyte. Results shown in Figure 4 revealed that PS amplitude decreases dramatically with the increased stimulus frequency from 0.1 to 5.0 Hz. The degree of decline in PS amplitude was significantly greater in paraquat-treated C57 mice, indicating decreased cardiac contractile reserve capacity at higher stress level. Interestingly, IGF-1 deficiency blunted paraquat-induced decline in peak shortening at high
frequencies without eliciting any effects on peak shortening itself.

Impact of IGF-1 deficiency on paraquat-induced oxidative stress, expression of protein carbonyl, insulin receptor, Akt, and Ca\(^{2+}\) regulating proteins

Paraquat is a known oxidant.\(^{19}\) To determine the potential mechanism(s) involved in the IGF-1 deficiency-elicited antagonism against paraquat-induced mortality, we evaluated ROS production and carbonyl content using DCF fluorescence and Western blot, respectively. Our data suggested that while there was no difference in ROS or carbonyl content between C57 and LID mouse hearts in the absence of paraquat treatment, IGF-1 deficiency significantly alleviated paraquat-elicited increase in ROS produc-
tion and carbonyl content (Fig. 5A and B). Although basal levels of phosphorylated insulin receptor and post-insulin receptor signaling molecule Akt were not affected by paraquat or IGF-1 deficiency, IGF-1 deficiency was capable of dramatically enhancing insulin-stimulated increase in phosphorylation of insulin receptor and Akt (Fig. 5C–E). Neither paraquat nor IGF-1 deficiency affected the expression of proapoptotic forkhead transcription factor Foxo3a and the Ca\(^{2+}\) regulating protein phospholamban. Nonetheless, expression of the Ca\(^{2+}\) regulating protein SERCA2a and NCX was up- and downregulated, respectively, by paraquat challenge. IGF-1 deficiency masked paraquat-induced increase in SERCA2a and decrease in NCX expression although IGF-1 deficiency itself upregulated expression of both SERCA2a and NCX (Fig. 6).

**DISCUSSION**

The major findings of our study are that severe IGF-1 deficiency nullified or masked the
oxidative stress inducer paraquat-elicited cardiac contractile and intracellular Ca\textsuperscript{2+} dysfunction. More importantly, IGF-1 deficiency enhanced survival rate against paraquat-induced mortality. These data are in line with the \textit{in vitro} observation where IGF-1 treatment significantly sensitized the paraquat-induced cell death in both C57 and LID groups, with LID cardiomyocytes being more resistant to IGF-1-induced decrease in cell survival in response to paraquat exposure. The IGF-1 deficiency-beneficial effects against paraquat on mortality, cardiac contractile, and intracellular Ca\textsuperscript{2+} function were causally associated with antagonism on paraquat-elicited production of ROS and carbonyl as well as alteration in the Ca\textsuperscript{2+} regulating proteins SERCA2a and Na\textsuperscript{+}-Ca\textsuperscript{2+} exchanger. Although LID mice displayed reduced whole body glucose clearance, cardiomyocytes from LID mice exhibited dramatically enhanced insulin-stimulated phosphorylation of insulin receptor and Akt. These results indicate that although IGF-1 deficiency itself appears to dampen the cardiac contractile function and intracellular Ca\textsuperscript{2+} homeostasis, it rather effectively enhanced resistance against oxidative stress-induced cardiac dysfunction, tolerance to stress, production of ROS and carbonyl, as well as alteration in Ca\textsuperscript{2+} regulating proteins, which may contribute to the overall improved survival rate.

Reduced IGF-1 levels in conjunction with enhanced oxidative stress are commonly found with advanced age.\textsuperscript{4,5,17} It was recently shown that overexpression of the antioxidant catalase and metallothionein reduces accumulation of reactive oxygen species and subsequently oxidative stress, and improves cardiac contractile function in advanced age and extends murine life span.\textsuperscript{17,20,21} Recent study from our laboratory revealed that overexpression of IGF-1 exerts similar effects against cardiac aging mani-
fested as prolonged diastolic duration, altered intracellular Ca\textsuperscript{2+} regulating proteins, and enhanced cardiac protein damage.\textsuperscript{15} This seems to be supported by evidence from our current study of reduced cardiac contractile function (PS, ± dL/dt) and prolonged relaxation (TR\textsubscript{90}) in the severely IGF-1 deficient LID mice, indicating a key role of IGF-1 in the maintenance of physiological cardiac function.\textsuperscript{3} Interestingly, while IGF-1 deficiency itself reduced cardiomyocyte contractile capacity and prolonged diastolic phase, it significantly antagonized paraquat-induced cardiomyocyte contractile and intracellular Ca\textsuperscript{2+} abnormalities as well as reduced tolerance to high stimulus frequency. Paraquat, a quaternary nitrogen herbicide, is highly toxic for humans and animals. It has been shown that generation of superoxide anion and formation of more toxic reactive oxygen species, including hydrogen peroxide and hydroxyl radical, may underscore the paraquat-induced toxicity.\textsuperscript{19} As a result of the abruptly enhanced oxidative stress, cellular NADPH, the major source of reducing agent, becomes oxidized and depleted, leading to the disruption of many important NADPH-mediated biochemical processes. This is supported by our data of enhanced production of ROS and carbonyl in paraquat-treated mouse hearts. Nonetheless, it should be pointed out that respiratory failure due to an oxidative insult andobliterating fibrosis is usually considered as the major cause of death in paraquat poisoning.\textsuperscript{19} Although paraquat treatment directly and significantly compromises cardiomyocyte contractile and intracellular Ca\textsuperscript{2+} properties, as well as stress tolerance to high stimulus frequency, to what extent such heart dysfunction and heart failure may contribute to paraquat-induced death is essentially unknown.

Like insulin, IGF-1 belongs to the most biologically characterized peptide involved in metabolism, growth, and development. Upon binding to its membrane IGF-1 receptor, the intrinsic tyrosine kinase domain of the receptor is activated. Data from animal models of both IGF-1 deficiency and surplus have implicated the importance of IGF-1 in carbohydrate and lipid metabolism, in a coordinate and interdependent manner with GH.\textsuperscript{2} Although LID mice displayed reduced glucose clearance capacity, consistent with the previous report,\textsuperscript{2,11} the fact that insulin-stimulated phosphorylation of insulin receptor and Akt was not dampened but rather enhanced in LID mouse cardiomyocytes indicates a possibly intact insulin sensitivity in the hearts. IGF-1 deficiency offered protection against paraquat-induced intracellular Ca\textsuperscript{2+} mishandling, similar to its effect on paraquat-induced cell death and cardiomyocyte contractile dysfunction. These data suggested the potential role of intracellular Ca\textsuperscript{2+} handling in IGF-1 deficiency-elicited protection against paraquat-induced effects on hearts. This notion is further supported by IGF-1 deficiency-elicited attenuating or masking effects on paraquat-induced changes of SERCA2a and Na\textsuperscript{+}-Ca\textsuperscript{2+} exchanger. The lack of changes in intracellular Ca\textsuperscript{2+} transients in conjunction with dampened cell shortening in LID mouse cardiomyocytes seems to indicate a likelihood of enhanced myofilament Ca\textsuperscript{2+} sensitivity under IGF-1 deficiency. This seems to be consistent with the upregulation of SERCA2a and Na\textsuperscript{+}-Ca\textsuperscript{2+} exchanger under IGF-1 deficiency. Further study is warranted to elucidate the precise role of Ca\textsuperscript{2+} regulating protein and oxidative stress in dampened cardiac function and longevity under IGF-1 deficiency.

Although IGF-1 improves cardiac contractility, tissue remodeling, glucose metabolism, insulin sensitivity and lipid profile,\textsuperscript{3} data from our present study suggested IGF-1 deficiency enhanced resistance of cardiomyocytes to the paraquat toxicity. This notion received further support from the observation that in vitro IGF-1 supplementation reverses the IGF-1 deficiency-elicited protective effect against paraquat. Reactive oxygen species and carbonyl production, as well as Ca\textsuperscript{2+} regulating proteins, may play a role in IGF-1 deficiency-induced beneficial effects. The controversial paradox of IGF-1 levels and life span continues to be an issue for a fine balance between cardiac function and longevity.

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