



Original Article

Insulin-like growth factor-1 overexpression in cardiomyocytes diminishes ex vivo heart functional recovery after acute ischemia

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Abstract

Background: Acute insulin-like growth factor-1 administration has been shown to have beneficial effects in cardiac pathological conditions. The aim of the present study was to assess the structural and ex vivo functional impacts of long-term cardiomyocyte-specific insulin-like growth factor-1 overexpression in hearts of transgenic α MHC-IGF-1 Ea mice. **Methods:** Performance of isolated transgenic α MHC-IGF-1 Ea and littermate wild-type control hearts was compared under baseline conditions and in response to 20-min ischemic insult. Cardiac desmin and laminin expression patterns were determined histologically, and myocardial hydroxyproline was measured to assess collagen content. **Results:** Overexpression of insulin-like growth factor-1 did not modify expression patterns of desmin or laminin but was associated with a pronounced increase ($\sim 30\%$) in cardiac collagen content (from ~ 3.7 to $4.8 \mu\text{g}/\text{mg}$). Baseline myocardial contractile function and coronary flow were unaltered by insulin-like growth factor-1 overexpression. In contrast to prior evidence of acute cardiac protection, insulin-like growth factor-1 overexpression was associated with significant impairment of acute functional response to ischemia–reperfusion. Insulin-like growth factor-1 overexpression did not modify ischemic contracture development, but postischemic diastolic dysfunction was aggravated (51 ± 5 vs. 22 ± 6 mmHg in nontransgenic littermates). Compared with wild-type control, recovery of pressure development and relaxation indices relative to baseline performance were significantly reduced in transgenic α MHC-IGF-1 Ea after 60-min reperfusion ($34 \pm 7\%$ vs. $62 \pm 7\%$ recovery of $+dP/dt$; $35 \pm 11\%$ vs. $57 \pm 8\%$ recovery of $-dP/dt$). **Conclusions:** Chronic insulin-like growth factor-1 overexpression is associated with reduced functional recovery after acute ischemic insult. Collagen deposition is elevated in transgenic α MHC-IGF-1 Ea hearts, but there is no change in expression of the myocardial structural proteins desmin and laminin. These findings suggest that sustained cardiac elevation of insulin-like growth factor-1 may not be beneficial in the setting of an acute ischemic insult. © 2010 Elsevier Inc. All rights reserved.

Keywords: Insulin-like growth factor-1; Fibrosis; Myocardial; Ischemia; Collagen; Langendorff

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1. Introduction

Insulin-like growth factor (IGF)-1 is critical in regulating cardiac function and structure. Insulin-like growth factor-1 promotes cardiac myocyte growth in vitro [1,2], and differentiation and survival of cardiomyocytes following ischemic injury [3–7]. Insulin-like growth factor-1 enhances cardiac function in vivo in experimental models of myocardial infarction or cardiac failure [8–11]. Administration of IGF or growth hormone also improves cardiac function in human heart failure [12,13], highlighting the potential therapeutic value of IGF-1. Moreover, correlations exist between low levels of acute IGF-1 and increased risk of heart failure [3,14–17], and a polymorphism in the IGF-1 promoter that reduces IGF-1 production [18–20] is linked to increased mortality postinfarction [19,20]. These observations collectively implicate a myocardial protective role for IGF-1 in humans.

While IGF-1 offers potential value in the setting of infarction, adverse systemic effects limit clinical utility [21]. To overcome problems associated with systemic delivery of IGF-1, targeted approaches have been tested, including intrapericardial administration [22], infusion (in an ex vivo ischemia–reperfusion model [23]), adenoviral transfection [24], and cardiac-specific transgenic expression in α MHC-IGF-1 Ea mice [25]. These all improve outcomes from the different insults assessed (both chronic and acute). A number of studies have identified IGF-1 as a cardiac myocyte survival factor. There are increased cardiac myocyte apoptosis after ischemia in IGF-1 knockout mice [26], reduced postischemic myocyte apoptosis and cardiac dilation with cardiac-specific IGF-1 overexpression [10,27], and restoration of postinfarct cardiac damage facilitated by modulation of the inflammatory response and increased antiapoptotic signaling function in α MHC-IGF-1 Ea mice [25].

The effects of IGF-1 are varied, and multiple isoforms exist which may play distinct physiological roles. These IGF-1 isoforms differ in amino terminal peptide sequences and carboxy-terminal E peptides [28]. Although the specific roles of the isoforms remains unclear, IGF-1 Ea (sometimes referred to as muscle (m)IGF-1 when expressed in myofibers) has wide extrahepatic tissue distribution, while IGF-1 Eb (sometimes referred to as mechano-growth factor) is produced by the liver and skeletal muscle in response to damage or increased workload [29,30]. Studies of mice overexpressing the circulating IGF-1 Eb isoform report increased myocyte number due to inhibition of apoptosis [4], and induction of cardiomyocyte hypertrophy [4–7,31–34]. Insulin-like growth factor-1 Ec, a third isoform of IGF-1 isolated from human liver, shares 73% homology with, and is believed to be the human homologue of, rat IGF-1 Eb [35].

In the current study, we assessed the effects of cardiac overexpression of the IGF-1 Ea peptide on myocardial structural elements and on intrinsic resistance to injury during

ischemia–reperfusion. Based on evidence of cardiac protection by IGF-1 from previous studies [4,5,8,10,23,24,36] and of the regenerative action of the IGF-1 Ea transgene in vivo [25], we reasoned that cardiac-specific overexpression of this form of IGF-1 would improve functional recovery after acute ischemia. Fibroblast collagen production is known to be stimulated by IGF-1 exposure. We sought evidence of increased collagen deposition and altered chamber compliance in IGF-1-Ea-overexpressing hearts. Desmin and laminin are important myocardial structural proteins which are key to maintaining myocyte and tissue integrity under stress conditions. Acute IGF-1 exposure has been reported to stimulate desmin and laminin production in cardiac and noncardiac tissues [37,38]. We hypothesized that chronic IGF-1 Ea overexpression would increase desmin and laminin levels and confer increased resilience to ischemic mechanical stress.

Surprisingly, our findings reveal that chronic IGF-1 expression is associated with reduced functional recovery after acute ischemic insult and that there are no changes in myocardial desmin and laminin components.

2. Methods

All animal experiments were approved by and conducted in accordance with the guidelines set out by the University of Western Australia and Griffith University Animal Ethics Committees and by the National Health and Medical Research Council, Australia.

2.1. α MHC-IGF-1 Ea transgenic mice

The establishment of the α MHC-IGF-1 Ea transgenic mouse model has been previously described, and the presence of chronic IGF-1 overexpression has been confirmed to have no significant effect on systolic arterial pressure or heart rate in vivo [25]. Transgenic mouse colonies were maintained by crossing the IGF-1 Ea transgenic mice with background strain FVB/N mice. Transgenic animals were maintained as heterozygotes in a colony established at the University of Western Australia, and transgenic mice were identified by standard polymerase chain reaction (PCR) amplification using genomic DNA isolated from tail tip digests. The primers used for PCR were IGF-1 sense 5'-TTCCTGTCTACAGTGTCTGTG-3 and IGF-1 antisense 5'-GAGCTGACTTTGTAGGCTTCA-3'. As previously reported [25], the IGF-1 Ea transcript level is 35-fold elevated in transgenic compared with wild-type hearts. Nontransgenics and transgenics are designated as IGF-WT and IGF-TG. All analyses were performed on male mice.

2.2. RNA preparation and Northern blot analysis

Total RNA was isolated from IGF-TG and IGF-WT hearts using TRIZOL RNA extraction protocol (Invitrogen Life

Technologies, Mount Waverly, Australia). Total RNA (10 µg) was resolved on a 1% agarose gel and transferred to Hybond-N⁺ membrane (Amersham Biosciences, Sydney, Australia). The IGF-1-Ea-specific cDNA probe was labeled with [α -³²P] dCTP using the Rediprime labeling system kit (Amersham Biosciences). After overnight hybridization with a ³²P-labeled probe at 42°C prepared in hybridization solution (50 % deionized formamide, 5× SSPE, 2× Denhardt's, 0.1% SDS), the membrane was washed twice in stringency wash solution I (2× SSC, 0.1% SDS) for 5 min at RT, followed by two washes in stringency solution II (0.1× SSC, 0.1% SDS) at 42°C for 15 min. The membrane was exposed on a phosphorimager intensity screens (Fuji) using the MacBas 2500 phosphorimaging system (FujiFilm) and Image Reader Version 1.5E/Image Gauge V3.0 software.

2.3. Antibodies

Anti-desmin and anti-pan-laminin antibodies (Dako, Carpinteria, CA, USA), Rhodamine-conjugated phalloidin, and Alexa goat anti-rabbit 488 IgG (Molecular Probes, Eugene, OR, USA) were all used according to manufacturers' guidelines.

2.4. Preparation of paraffin sections of heart tissue

Hearts isolated from transgenic IGF-TG mice or IGF-WT littermates were fixed in 4% paraformaldehyde (pH 7.4) overnight at 4°C and embedded in paraffin. Longitudinal and transverse sections of heart muscle from nontransgenic and transgenic mice were collected onto silanated slides and stained with hematoxylin and eosin (H&E) for histological analysis.

2.5. Immunohistochemical staining and confocal laser scanning microscopy

Following antigen retrieval by heating in 10 mM citrate buffer, pH 6, sections were blocked for 1 h in 10% horse serum in phosphate-buffered saline (PBS) and incubated with the appropriate concentration of primary antibody overnight at 4°C. After washing with PBS, tissue sections were incubated with Alexa 488 goat anti-rabbit secondary antibody. Sections were mounted in Vectashield mounting medium (Vector Laboratories, Sydney, Australia) with DAPI and analyzed using confocal laser scanning microscopy. All fluorescent analyses of tissue sections were conducted on a Laser Scanning Confocal Microscope (BioRad 1000/1024 UV).

2.6. Ex vivo perfused heart model

Hearts were prepared and instrumented as outlined in detail previously [39–42]. Briefly, mice (2–4 months of age) were anesthetized, a thoracotomy was performed, and hearts were rapidly excised into ice-cold Krebs bicarbonate buffer.

The aorta was cannulated, and hearts were perfused in a Langendorff mode at a pressure of 80 mmHg with a modified Krebs bicarbonate buffer: 119 mM NaCl, 22 mM NaHCO₃, 4.7 mM KCl, 1.2 mM KH₂PO₄, 2.5 mM CaCl₂, 1.2 mM MgSO₄, 0.5 mM EDTA, and 2 mM pyruvate. The perfusion fluid was saturated with 95% O₂ and 5% CO₂ at 37°C (yielding a pH of 7.4 and a PO₂>550 mmHg at the aortic cannula) and filtered via a 0.45-µm in-line filter. The left ventricle was vented with a polyethylene tube to prevent fluid accumulation, and an intraventricular balloon was introduced for assessment of contractile function [39]. Hearts were introduced into a water-jacketed chamber superfused with warmed buffer at 37°C, ensuring stable temperature throughout the protocols. Coronary flow was monitored via an ultrasonic flow-probe in the aortic perfusion line connected to a T106 flowmeter (Transonic Systems Inc., Ithaca, NY, USA). Functional data were recorded at 1 KHz on a four-channel MacLab data acquisition system (ADInstruments, Castle Hill, Australia). Ventricular pressure was digitally processed to yield systolic and diastolic pressures, +dP/dt (reflecting myocardial contractility or inotropic state), -dP/dt (reflecting the efficacy of myocardial relaxation or lusitropic state), and heart rate. Following a 15-min stabilization period at intrinsic heart rate (IGF-WT and IGF-TG not different), hearts were switched to ventricular pacing at 420 beats per min (bpm) and stabilized for a further 15 min.

Baseline functional measurements were made before subjecting hearts to a 20-min period of global zero-flow normothermic ischemia. Hearts were then aerobically reperfused. Ventricular pacing was discontinued on induction of ischemia and resumed after 2 min of reperfusion [43]. Pacing at baseline and during reperfusion allows for direct comparison of mechanical performance under relatively standard conditions.

2.7. Collagen measurement

Heart collagen content was assessed by measuring hydroxyproline content by a standardized high-pressure liquid chromatography (HPLC) method [44]. The total amount of collagen in each heart was calculated, assuming that collagen contains 12.2% wt/wt hydroxyproline, and expressed both as µg collagen per mg heart tissue and µg collagen per heart.

2.8. Statistical analysis

Data are presented as mean±S.E.M. Comparisons of individual parameters between wild-type and transgenic mice were performed by means of a Student's *t* test. For analysis of functional responses to ischemia–reperfusion, repeated-measures analysis of variance was employed followed by a Tukey's post hoc analysis for individual comparisons where significance was detected. A value of *P*<.05 was considered statistically significant.

3. Results

3.1. IGF-1 Ea gene expression in control IGF-WT and IGF-TG mice

Transgenic α MHC-IGF-1 Ea mice, where the rat IGF-1 Ea isoform was specifically overexpressed in the heart under the α MHC promoter (IGF-TG), were compared with nontransgenic littermate controls (IGF-WT) and background strain FVB/N mice. Northern blot analysis of total RNA isolated from brain, liver, kidney, lung, heart, and skeletal muscle of 12-week FVB/N and IGF-TG confirmed

that rat IGF-1 Ea expression was restricted to the hearts of IGF-TG mice (Fig. 1A). Analysis of 12-week IGF-TG mice and nontransgenic littermates (IGF-WT) revealed no significant difference in heart weight (mg), heart to body weight ratio (mg/g), or heart weight to tibial length ratio (mg/mm) (Fig. 1B). Histological analysis of heart tissue (Fig. 1C) showed no overt difference in the heart size of IGF-TG mice when compared to their nontransgenic IGF-WT littermates. Higher-magnification images taken in transverse and longitudinal section found no apparent difference in the size of the cardiac myocytes (Fig. 1C), indicating that in this cohort, IGF-1 overexpression is not

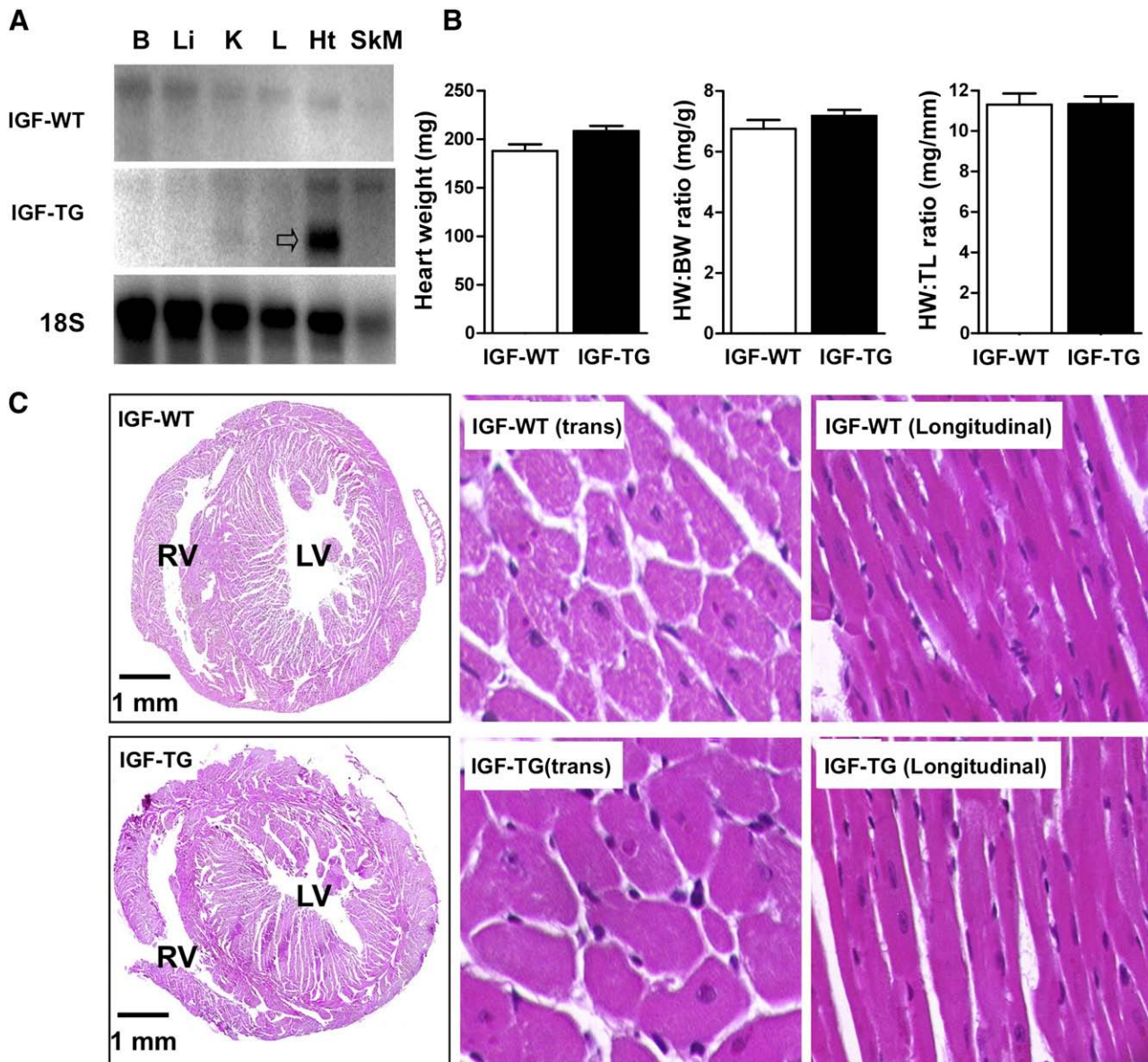


Fig. 1. Characterization of IGF-TG mice. (A) Northern blot analysis of total RNA (10 μ g) isolated from brain (B), liver (Li), kidney (K), lung (L), heart (Ht), and skeletal muscle (SkM) of 12-week IGF-WT and IGF-TG mice using a rat IGF-1 Ea ^{32} P-labeled probe. The 18S probe was used to determine RNA loading and RNA integrity. (B) Heart weight of 12-week IGF-WT and IGF-TG mice expressed as mg or mg/g normalized as mg/g body weight (IGF-WT $n=23$ and IGF-TG $n=18$), or as mg/mm of tibial length (IGF-WT $n=13$ and IGF-TG $n=10$). (C) The H&E-stained paraffin sections of heart muscle tissue isolated from IGF-WT and IGF-TG mice. Low magnification demonstrates no gross morphological changes, and higher magnification of heart muscle in transverse and longitudinal section (TS and LS) confirms that IGF-1 overexpression does not alter cellular and gross cardiac morphology.

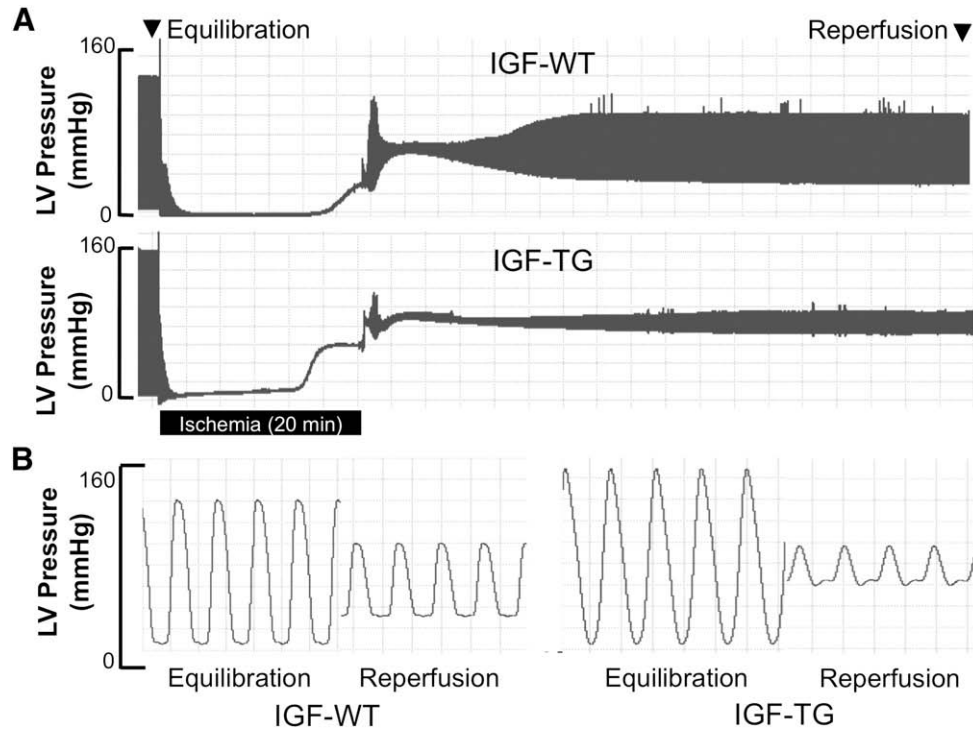


Fig. 2. Records of representative ex vivo Langendorff experiment time course for IGF-WT and IGF-TG hearts. (A) Compressed time-scale depiction showing diastolic/systolic pressure ‘envelopes,’ including time point of baseline measurement at end of equilibration, indicating ischemia and reperfusion periods. During an initial stabilization period (not shown), intrinsic heart rates were measured and found to be similar for IGF-WT (384 ± 24 bpm) and IGF-TG hearts (399 ± 22 bpm). (B) Expanded time records of representative pressure waveforms.

associated with altered cardiomyocyte size or macroscopic cardiac morphology.

3.2. Reduced postischemic functional recovery in IGF-TG hearts

Fig. 2A shows compressed time-scale records of representative ex vivo Langendorff experiments for both IGF-WT and IGF-TG hearts. During an initial stabilization period, intrinsic heart rates were measured and found to be similar for IGF-WT and IGF-TG hearts (384 ± 24 vs. 399 ± 22 bpm, $P = \text{not significant}$). Under paced conditions, at the end of the equilibration period, baseline (normoxic) diastolic and systolic functional performance of both groups was also similar (Fig. 2B, Table 1). There was no significant difference in coronary flow between IGF-WT and IGF-TG hearts. Contracture development during the 20-min period of global ischemia was unaltered by IGF-1 overexpression, with almost identical elevations in diastolic tone in both groups (data not shown). However, a pronounced difference in functional recovery during reperfusion was evident between transgenic and nontransgenic control hearts (Figs. 2, 3, and 4). Although recovery of systolic pressure was comparable in both groups (data not shown), postischemic diastolic pressure was markedly elevated in IGF-TG hearts (Fig. 3A), associated with an impaired recovery of left ventricular pressure development (Fig. 3B). Recovery of coronary flow was equivalent in both groups (Fig. 3C),

suggesting that functional differences may not be attributed to vascular disturbance. Recoveries for both maximum rate of pressure development ($+dP/dt$) (Fig. 3A) and maximum rate of pressure decline ($-dP/dt$) were significantly reduced in hearts overexpressing IGF-1 compared with hearts of nontransgenic littermates (Fig. 4A and B).

3.3. Desmin, laminin, and collagen content in IGF-WT and IGF-TG hearts

Desmin is a cytoskeletal intermediate filament, and laminin is an extracellular matrix glycoprotein. Both are

Table 1

Baseline (preischemic) functional parameters in perfused isolated hearts of IGF-TG mice and IGF-WT littermates paced at 420 bpm

	IGF-WT ($n=9$)	IGF-TG ($n=8$)
EDP (mmHg)	3 ± 1	2 ± 1
LVDP (mmHg)	159 ± 7	168 ± 6
$+dP/dt$ (mmHg/s)	6115 ± 326	6445 ± 648
$-dP/dt$ (mmHg/s)	-4016 ± 227	-4695 ± 374
Flow (ml/min/g)	18 ± 1	16 ± 1

All values represent mean $\pm$ S.E.M. Functional parameters were measured following a 30-min stabilization period immediately prior to ischemia. Parameters measured: $+dP/dt$, end developed pressure (EDP), and left ventricular end-diastolic pressure (LVDP). Hearts paced at 420 bpm. Data presented are mean $\pm$ S.E.M. for IGF-TG ($n=8$) and IGF-WT littermates ($n=9$). No significant differences were observed ($P > .05$; IGF-TG vs. IGF-WT).

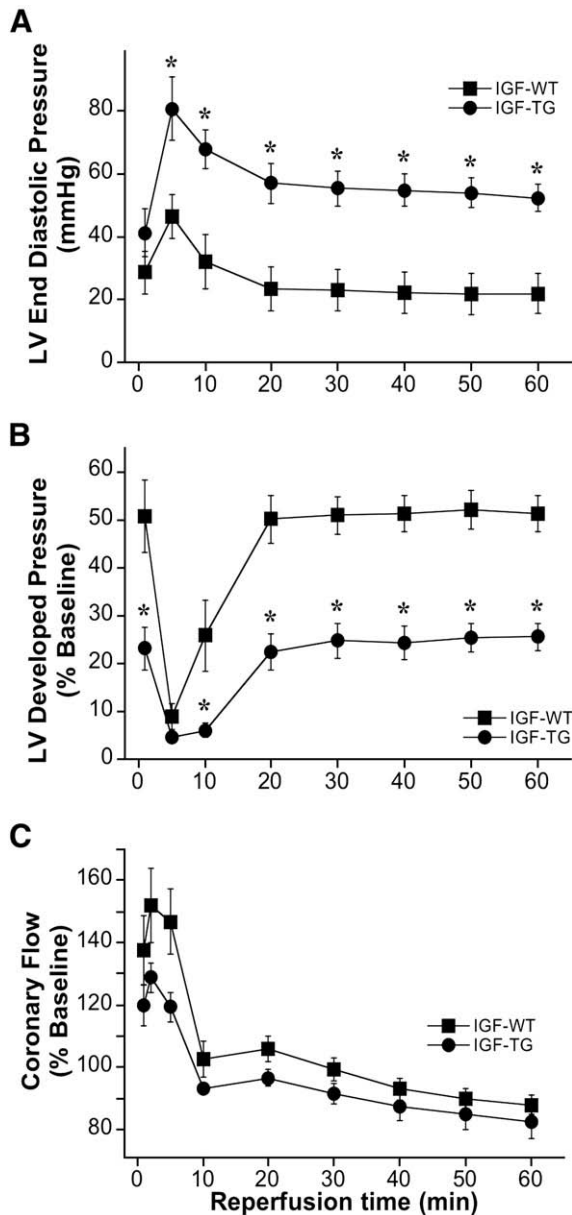


Fig. 3. Effects of IGF-1 overexpression on postischemic functional outcomes. (A) Left ventricular diastolic pressure, (B) left ventricular developed pressure, and (C) coronary flow rate. Data are shown for recovery in hearts from IGF-TG mice ($n=8$, circles) and IGF-WT ($n=9$, squares). Values are mean $\pm$ S.E.M.; * $P<.05$.

important structural components which are key to maintaining myocyte and tissue integrity under stress conditions, such as ischemic contracture. Acute IGF-1 exposure has been reported to stimulate desmin and laminin production in cardiac and noncardiac tissues [37,38]. Distributions of the cytoskeletal protein desmin and extracellular matrix protein laminin were determined immunohistochemically in transverse and longitudinal sections (Fig. 5). No differences in desmin staining at myocyte Z-lines and intercalated discs, or of laminin at the cardiomyocyte surface, could be detected between IGF-WT and IGF-TG hearts.

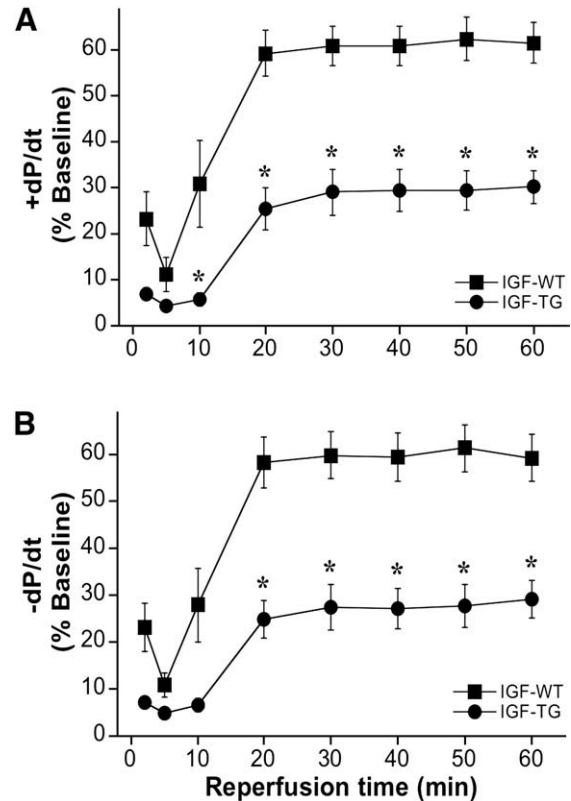


Fig. 4. Effects of IGF overexpression on postischemic recovery of +dP/dt and -dP/dt. (A) Left ventricular +dP/dt and (B) left ventricular -dP/dt. Data are shown for recovery in hearts from IGF-TG mice ($n=8$, circles) and IGF-WT ($n=9$, squares). Values are mean $\pm$ S.E.M.; * $P<.05$.

Insulin-like growth factor-1 is known to stimulate fibroblast production of extracellular matrix materials. To gain a quantitative measure of cardiac extracellular matrix composition — specifically of collagen content — the hydroxyproline content of IGF-TG hearts and IGF-WT littermate hearts was quantified by HPLC analysis (Fig. 6). A significant increase in both the relative collagen content (μg collagen/mg of heart tissue, Fig. 6A) and total amount of collagen per heart (Fig. 6B) was observed in hearts of IGF-TG mice.

4. Discussion

The aim of the present study was to determine the impact of long-term cardiomyocyte overexpression of IGF-1 Ea (in $\alpha\text{MHC-IGF-1}$ Ea mice) on cardiac structure and function, and on the acute functional response to ischemic insult.

4.1. Effects of IGF-1 overexpression on cardiac structure and basal function

Overexpression of the class 1: IGF-1 Ea isoform driven by the αMHC promoter was shown to be cardiac specific, as has been previously demonstrated with this promoter [4,45,46]. In this study, no significant differences in cardiac weight and cardiomyocyte size indices between IGF-WT and

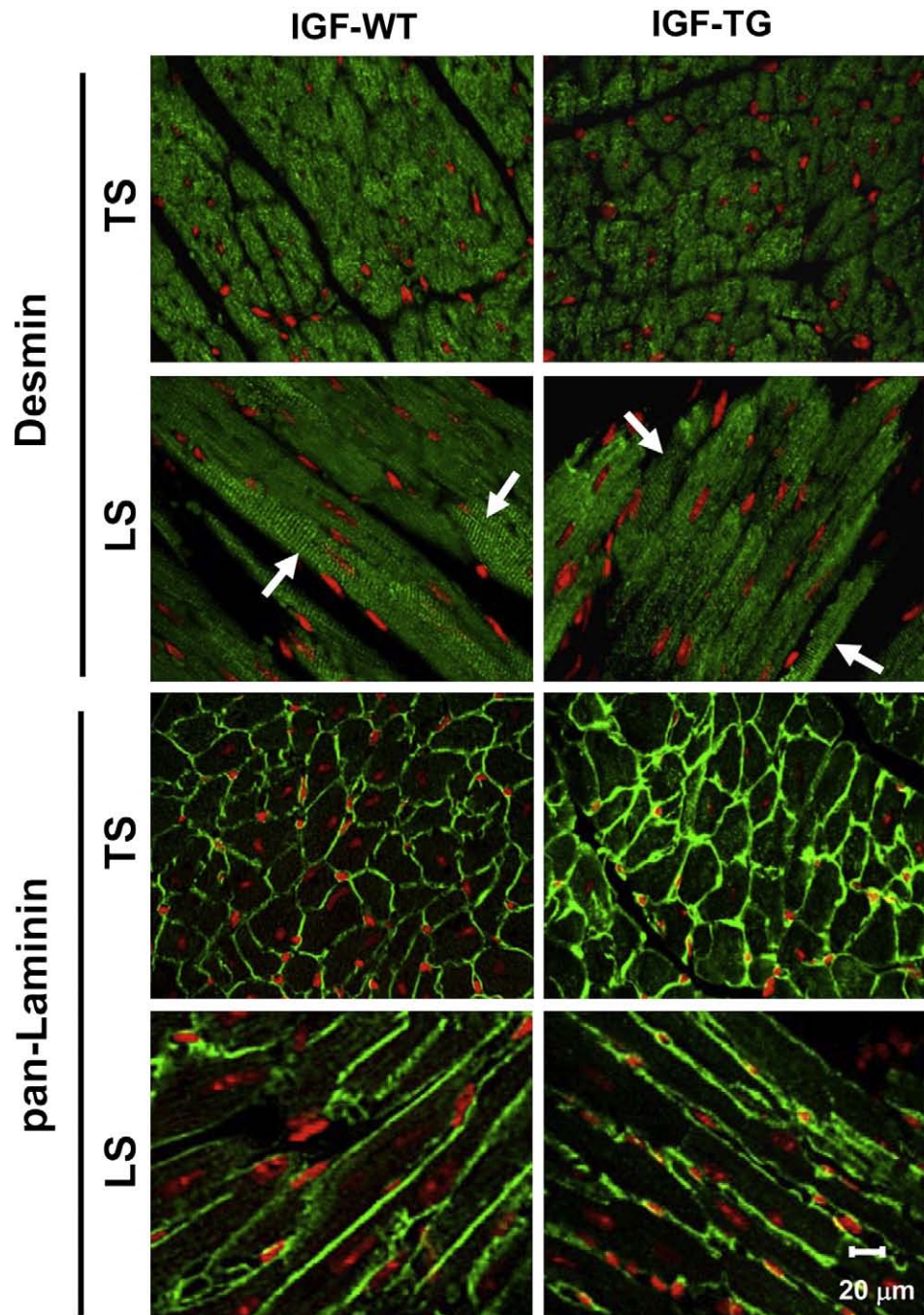


Fig. 5. Immunohistochemical analysis of desmin and laminin in hearts of transgenic and control mice. Confocal laser scanning microscopy of 5- μ m paraffin sections revealed no difference in the distribution of desmin or pan-laminin (green) in the hearts between IGF-TG and IGF-WT mice. Desmin localizes to the Z-lines of cardiomyocytes in vivo (arrow). Laminin localizes to the basement membrane surrounding the periphery of cardiomyocytes. Nuclei are shown in red. Images are displayed in longitudinal section (LS) and transverse section (TS).

IGF-TG were identified. Earlier in vivo echocardiographic investigations [25] have indicated occurrence of a minor hypertrophy in the IGF-TG which becomes less evident as the animals mature. In the present study, with more direct postmortem evaluation of tissues, a differential cardiac growth could not be statistically demonstrated, although some tendency for increased heart weight and heart weight/body weight ratio can be noted. Chronic cardiac-specific

IGF-1 overexpression is observed to have minimal impact on myocardial growth and morphology.

No impact of IGF-1 overexpression was detected on expression patterns for the major intermediate filament protein desmin or the basement membrane protein laminin. There is some evidence in cardiac and noncardiac tissues that short-term IGF-1 exposure increases the expression of these structural components [37,38]. Our hypothesis that, in the

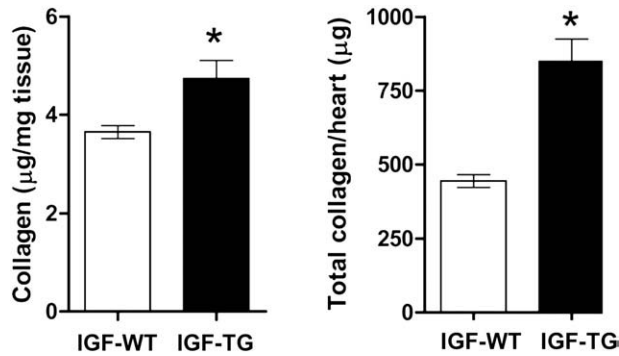


Fig. 6. The HPLC analysis of collagen content of IGF-TG and IGF-WT hearts. The collagen content of IGF-TG and IGF-WT hearts was quantified by measuring hydroxyproline levels by HPLC. A significant increase in both the relative collagen content (μg collagen/mg of heart tissue) and total amount of collagen per heart (μg) was observed in the hearts of IGF-TG mice ($n=4$) compared to IGF-WT littermates ($n=4$); $*P<.05$.

IGF-TG heart, increased desmin and laminin levels would be associated with increased resilience to ischemic mechanical stress has been refuted by both the structural and functional findings. In light of our surprising finding of reduced ischemic tolerance in IGF-TG mice, an alternative and valid interpretation is that the reduced postischemic contractile recovery cannot be attributed to reduction in the levels of desmin and laminin structural components. These findings also indicate that the acute and chronic effects of IGF-1 elevation may be dissimilar.

Insulin-like growth factor-1 overexpression was associated with significant elevation in cardiac collagen content, almost doubled in IGF-TG hearts compared with IGF-WT littermates (Fig. 6). Locally produced IGF-1 enhances collagen production by cardiac fibroblasts [47], and persistent IGF-1 expression in SIS2 transgenic mice also increases interstitial cardiac fibrosis [31]. This observation supports the conclusion that, in the IGF-TG heart, a fibroblast paracrine response to IGF-1 elevation mediates the increased production of interstitial collagen. Very recently, in a transgenic model of cardiac-specific IGF-1 receptor overexpression, collagen deposition has been found not to be increased [48].

The elevation in collagen content in the IGF-TG was not associated with alteration in basal intact heart performance (Table 1). Extracellular collagen content can impact on myocardial stiffness or compliance and thus whole heart function. Increases in collagen content, and particularly in degree of collagen cross-linking, can increase stiffness and diastolic pressure [49–51]. However, despite a $\sim 30\%$ increase in collagen content normalized to heart mass with IGF-1 expression (Fig. 6), neither rate of force development ($+dP/dt$) nor decline ($-dP/dt$) was altered at baseline. Collagen content may be increased without necessarily impacting mechanical performance: in normal aged mice, basal function can be retained even when collagen level is doubled [52]. Future work might more specifically assay collagen subtype expression and extent of cross-linking. It

will also be informative to assess pressure–volume relationships in these hearts to determine whether detectable shifts in compliance and contractility are evident under conditions of altered ventricular loading.

4.2. Effects of IGF-1 overexpression on myocardial ischemic resilience

The functional recovery from ischemia was substantially impaired in IGF-TG hearts (Figs. 3, 4, and 5). This change cannot be attributed to alterations in coronary reflow since recoveries for tissue perfusion were almost identical in both groups (with the exception of an insignificant trend to reduced reflow at ~ 5 min of reperfusion in transgenic hearts, paralleling the peak in diastolic pressure and thus compression at this time; Fig. 3).

In the IGF-TG, there was a doubling of postischemic diastolic dysfunction and 40%–45% reductions in recovery of $+dP/dt$ and $-dP/dt$ (Figs. 2 and 3). Interestingly, these changes were not associated with any shift in peak systolic pressure generation. Thus, the impact of IGF-1 overexpression seems to be specific, modifying factors that dictate diastolic force and influence contractile kinetics in situ. It is possible that the substantially elevated collagen content with IGF-1 overexpression might impact more markedly on diastolic state and that a different compliance level in the IGF-TG due to increased interstitial collagen may exert more functional impact under conditions of stress and systolic dysfunction. Further studies are also required to examine the role of cardiomyocyte Ca^{2+} disturbances (and related electromechanical effects) which could be more prominent in the IGF-TG. From Fig. 3A, it may be inferred that diastolic Ca^{2+} levels could be elevated in the IGF-TG heart postischemia, contributing to the sustained elevation in left ventricular end-diastolic pressure.

Our findings contrast with a previously published study which examined the effects of acute IGF-1 infusion at the time of reperfusion. Davani et al. [23] investigated acute IGF-1 treatment in ischemic-reperfused isolated murine hearts, concluding that IGF-1 improved functional recovery. The experimental recording mode used in the acute treatment study did not allow for direct measurement of systolic and diastolic contractile function independent of perfusion pressure. Thus, direct benchmarking of data between the two studies is not possible, but the key finding is of opposite functional outcomes in the present study and the study reported by Davani et al. [23]. These discrepant outcomes may indicate that the consequences of an ischemic event in the setting of chronic and acute IGF-1 exposure are different. Response to acute IGF-1 treatment during ischemia may also be different in the context of chronic IGF-1 exposure. Further work is required to evaluate this possibility.

Differences in the response to ischemic insult in settings of acute and chronically elevated IGF-1 could

be attributed to altered status of the IGF-1 initiated signaling pathways. One explanation for the reduced performance of the IGF-TG hearts in reperfusion may be that chronic exposure to elevated IGF-1 limits the capacity of these hearts to use the PI3K/Akt pathway to provide acute protection in response to ischemic insult. Previous work in the IGF-TG has shown that signaling through the canonical PI3K/Akt pathway is not upregulated, but there is activation of the PDK1/SGK1 signaling axis [25]. Work in ischemic preconditioning has shown this latter signaling pathway to mediate protection [53]. Thus, an alternative interpretation of the reduced postischemic functional performance of IGF-TG hearts is that it may actually represent an adaptive state, where these hearts exhibit a low-functioning ‘preservation’ mechanical phenotype after ischemic insult. Reduced reperfusion recovery immediately postischemia may not be deleterious per se unless in vivo hemodynamics are severely compromised by inadequate pump function.

With chronic IGF-1 elevation, there may be alterations in the predominant PI3K isoforms activated. It is relevant that that PI3K γ signaling in particular has been shown to be important in mediating improved recovery after ischemic preconditioning [54]. Further work is required to characterize the signaling cascades initiated in the IGF-TG heart through ischemia–reperfusion and to determine the efficacy of preconditioning regimens in this model. It is also necessary to examine a longer time window postischemia. Although the immediate functional response may be diminished in the IGF-TG, longer-term tissue viability may be improved. In the present study, measures of cell death were not undertaken, and this will be an important avenue for future investigation.

In summary, the current study reveals that chronic elevation of cardiac IGF-1 in the hearts of IGF-TG mice is associated with reduced short-term functional recovery after an acute ex vivo ischemic insult, characterized by sustained elevation in left ventricular end-diastolic function and a decrease in contraction kinetic parameters. The IGF-TG hearts exhibited a significant increase in collagen content but no difference in the levels of the key structural proteins: desmin and laminin. Although reports of improved functional recovery from injurious stimuli following acute administration of IGF-1 highlight the potential cardioprotective effects of IGF-1 and encourage IGF-1-mediated therapeutic approaches, more sustained IGF-1 exposure may not be functionally advantageous in the context of an acute ischemic insult. Further work is required to consider the underlying signaling mechanisms involved and to determine how short-term functional outcomes and longer-term myocardial viability may be linked.

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