The AAPS Journal, Vol. 19, No. 2, March 2017 (© 2016)
DOI: 10.1208/s12248-016-0024-x

Research Article

Theme: Recent Advances in Musculoskeletal Tissue Engineering
Guest Editor: Aliasger K. Salem

GDF11 Treatment Attenuates the Recovery of Skeletal Muscle Function After Injury in Older Rats

Yu Zhou,[1] Neel Sharma,[1] David Dukes,[1] Maria B. Myzithras,[2] Priyanka Gupta,[2] Ashraf Khalil,[2] Julius Kahn,[2] Jennifer S. Ahlberg,[2] David B. Hayes,[2] Michael Franti,[2] and Tracy Criswell[1,3]

Received 16 September 2016; accepted 24 November 2016; published online 6 December 2016

Abstract. Loss of skeletal muscle mass and function results in loss of mobility for elderly patients. Novel therapies that can protect and/or restore muscle function during aging would have profound effects on the quality of life for this population. Growth differentiation factor 11 (GDF11) has been proposed as a "youthful" circulating factor that can restore cardiac, neural, and skeletal muscle functions in aging animals. However, conflicting data has been recently published that casts doubt on these assertions. We used a complex rat model of skeletal muscle injury that physiologically mimics injuries seen in patients; to investigate the ability of GDF11 and to enhance skeletal muscle regeneration after injury in older rats. Our data showed that GDF11 treatment resulted in a significant increase in tissue fibrosis, accompanied by attenuated functional recovery, as compared to animals treated with vehicle alone. GDF11 impaired the recovery of skeletal muscle function in older rats after injury.

KEY WORDS: GDF11; regeneration; sarcopenia; skeletal muscle.

INTRODUCTION

According to the American College of Sports Medicine, the number of individuals over 65 years of age will reach 70 million by 2030, indicating an upcoming increase in aged-related disorders seen in the clinic (1). Sarcopenia refers to age-dependent skeletal muscle dysfunction that often leads to significant loss of mobility and attenuation of recovery from muscle injury (2). Any therapy that could maintain or improve muscle mass quality and function during aging would greatly increase the quality of life for this burgeoning elderly population.

Sarcopenia is thought to occur through age-dependent changes in the tissue microenvironment and intrinsic changes in the satellite cell/muscle progenitor cell population. Changes in the tissue microenvironment have been shown to include decreased perfusion, changes in innervation, and increased fibrosis and adiposity (3). Additionally, intrinsic changes in the satellite cell population affect cell proliferation, self-renewal, and differentiation after injury (3).

Many of these processes were shown to be mediated by members of the TGFβ signaling family (3,4), suggesting that the TGFβ pathway may be directly involved in the development of sarcopenia. Growth differentiation factor-11 (GDF11) is a TGFβ family member that is highly homologous to another TGFβ family member, myostatin (GDF8), a known inhibitor of skeletal muscle hypertrophy. Both myostatin and GDF11 act through the activin type II receptor (ActRIIB) and mediate downstream signaling through the activation of the Smad2/Smad3 complex (5,6). Loffredo *et al.* identified GDF11 as a circulating "rejuvenating factor" from an aptamer-based proteomics screen of sera from heterchronic parabiosed mice; the serum level of which was supposedly decreased in the aged animals (7). Further reports from high impact journals have suggested that this supposed age-dependent decrease in circulating GDF11, but not myostatin, can be rescued via exogenous administration of GDF11 and reverse age-related defects in the heart, brain, and skeletal muscle (7–9). More recently, several groups have published data that conflicts with the original GDF11 reports. Egerman *et al.* demonstrated that the GDF11 antibody used by Loffredo *et al.* was not specific for GDF11, but instead was able to bind to both GDF11 and myostatin (10). Furthermore, they demonstrated that circulating GDF11 increased with age and inhibited skeletal muscle regeneration (10) Data from Rodgers *et al.* demonstrated that the circulating molar concentration of myostatin is 500× higher than that of GDF11 (11). Since myostatin and GDF11 both compete for binding to the ActRIIB, the concentration discrepancy suggests

[1] Wake Forest Institute for Regenerative Medicine, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, North Carolina 27157, USA.
[2] Boehringer-Ingelheim Pharmaceuticals, Ridgefield, Connecticut, USA.
[3] To whom correspondence should be addressed. (e-mail: tcriswel@wakehealth.edu)

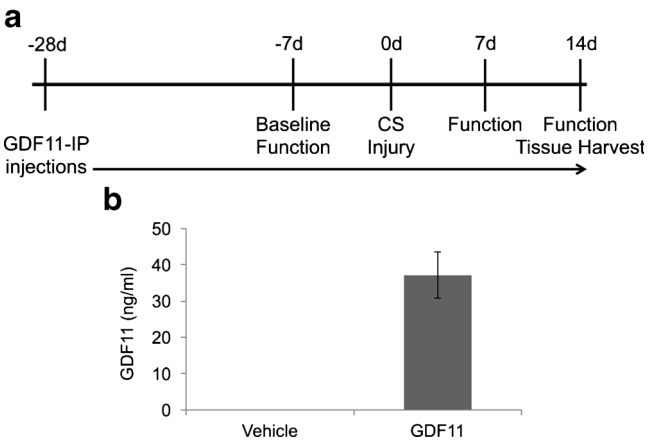

Fig. 1. Experimental design. **a** GDF11 injections (0.1 mg/kg/day) were started 28 days prior to injury and continued until the end of the experiment (14 days after injury). Baseline isometric torque was determined 7 days prior to injury and repeated at 7 and 14 days after injury. **b** Quantitative measurement of circulating GDF11 (ng/ml) in treated animals at time of euthanasia. No circulating GDF11 was detected in vehicle only-treated animals. Eight rats were used per group

that circulating GDF11 may not be physiologically relevant and may only be active as an autocrine/paracrine factor at the tissue level (11). Subsequent data from independent groups further cast doubt on the ability of GDF11 to affect age-dependent changes in the heart and brain (10–13).

Our group has developed and characterized a rat model of compartment syndrome (CS) skeletal muscle injury that mimics the complex damage to the muscle, neural, and vascular tissue compartments found in patients after crush injury (14). Furthermore, we have shown that the regenerative capacity of skeletal muscle in older rats after CS injury is diminished as compared to young animals, similar to the regenerative ability of older people (15). Therefore, this model provides a unique opportunity to test the effect of GDF11 treatment on the recovery of skeletal muscle function in older animals in a physiologically relevant model of injury. Herein, we provide data showing that GDF11 treatment resulted in increased fibrosis in injured muscle and decreased recovery of function after CS injury.

MATERIALS AND METHODS

Animals

All animal studies were performed in accordance with the Wake Forest University Institutional Animal Care and Use Committee and NIH standards. Male, 10–12-month-old Lewis rats were used in these studies (Envigo, Cambridgeshire, UK).

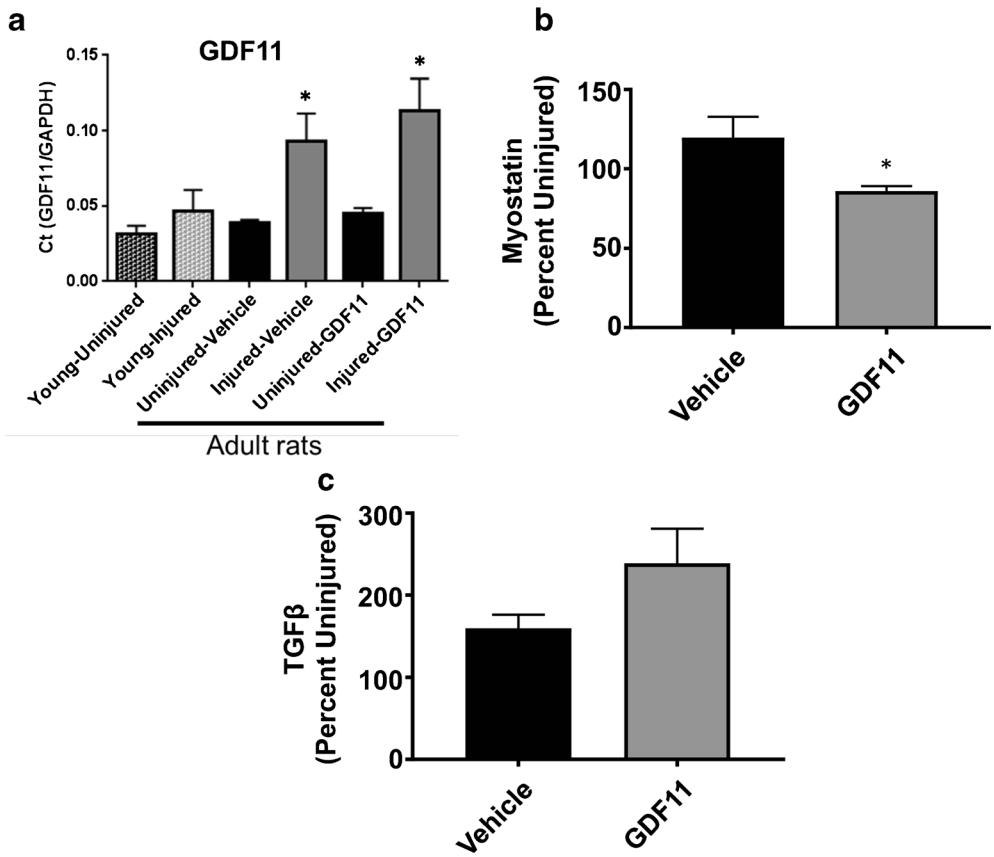

Fig. 2. Quantitative (qPCR) analysis of TGFβ family transcripts. qPCR analysis of **a** GDF11, **b** myostatin, and **c** TGFβ transcripts 14 days after injury. Young rats were 3–4 months of age, and adult rats were 11–12 months of age. mRNA was harvested from at least four uninjured and eight injured EDL muscles for each analysis. Significance: GDF11 (*asterisk*) $p < 0.05$ between injured and uninjured samples, myostatin (*asterisk*) $p < 0.03$ and TGFβ (*asterisk*) $p < 0.06$ between vehicle and GDF11 samples. *GAPDH* glyceraldehyde-3-phosphate dehydrogenase

CS injury was performed as previously described (14). Briefly, neonatal blood pressure cuffs were wrapped around the upper hind limb, proximal to the anterior muscle compartment, of isofluorane-anesthetized rats and held at a pressure of 140 mmHg for 3 h. All rats were monitored daily for lameness and weighed weekly throughout the course of the experiment.

Lyophilized human, *E. coli* derived, GDF11 was purchased from the R&D Systems. GDF11 was reconstituted with 30 mM acetate, pH 5.0 buffer, and total protein concentration was confirmed using a Pierce BCA Protein assay kit (Thermo Scientific, Waltham, MA). The GDF11 stock solution was diluted to 0.1 mg/ml with 30 mM acetate buffer for intraperitoneal (IP) injections. IP injections of GDF11 (0.1 mg/kg/day) or vehicle only (30 mM acetate buffer) were given 28 days prior to injury and continued until the end of the experiment (14 days after injury) (Fig. 1a). Blood samples were taken at the time of euthanasia via cardiac puncture and processed for serum. Circulating levels of GDF11, in both vehicle and GDF11-treated animals, were determined by MSD assays of flash frozen serum samples as previously described (16).

Contractile function of the left anterior crural muscles was assessed *in vivo* 7 and 14 days after injury by measuring maximal isometric torque as a function of stimulation frequency after direct stimulation of the peroneal nerve and compared to baseline function measured prior to injury as previously described (14). Functional isometric and isokinetic measurements were assessed and analyzed using a custom-designed LabView-based software program (provided by the US Army Institute of Surgical Research).

Histology

Animals were euthanized 14 days after injury and the injured tibialis anterior (TA), extensor digitalis longus (EDL), and soleus (Sol) muscles, along with the contralateral uninjured muscles, were dissected and weighed prior to being flash frozen in liquid nitrogen or fixed in 10% neutral buffered formalin and embedded in paraffin for further analyses. Sections (8 μm) were stained with either Masson's trichrome or hematoxylin and eosin (H+E). Fibrosis was quantified from Picro-Sirius Red (Abcam, Cambridge, MA) stained slides using the ImageJ software (NIH, Bethesda, MD) as previously described (14,15). At least 10 high-powered fields (HPFs) were analyzed per muscle.

Quantitative PCR

The PerfectPure RNA Fibrous tissue kit (5 Prime Inc., Gaithersburg, MD) was used to isolate the total RNA from the extensor digitalis muscles, according to the manufacturer's protocol. cDNA was prepared using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems, Foster City, CA). Quantitative PCR (qPCR) was performed in 20-mL reactions in 96-well plates using cDNA samples generated from 12.5 ng of total RNA as previously described (15). Taqman probes for GAPDH (Rn01775763_g1), rat GDF8 (myostatin) (Rn00569683_m1), rat GDF11 (Rn01756258_m1), Pax7 (Rn01518732_m1), and rat TGFβ1 (Rn01475963_m1) were ordered from Thermo-Fisher Scientific (Waltham, MA).

Statistics

Each functional and morphological measure was compared among groups using either a one-way or two-way analysis of variation (ANOVA). In the event of a significant ANOVA, post hoc means comparison testing was performed with uncorrected Fisher's LSD or with Tukey's HSD tests. Statistical significance was achieved at $p < 0.05$. All statistical analyses were performed using the GraphPad Prism Software (GraphPad Software, San Diego, CA) or Microsoft Excel (Redmond, WA).

RESULTS

Adult male Lewis rats were injected IP with 0.1 mg/kg/day GDF11 or vehicle alone for 28 days prior to CS injury using a protocol similar to what was described by Sinha *et al.*, in the original *Science* paper demonstrating GDF11 rescue of age-dependent dysfunction in skeletal muscle (8). IP injections were continued for 14 days after injury, at which time the animals were euthanized and muscle tissues were harvested for analyses (Fig. 1a). Blood, taken via cardiac puncture at the time of euthanasia, was processed for serum which was used to determine the amount of circulating GDF11 present in each animal. All treated animals had

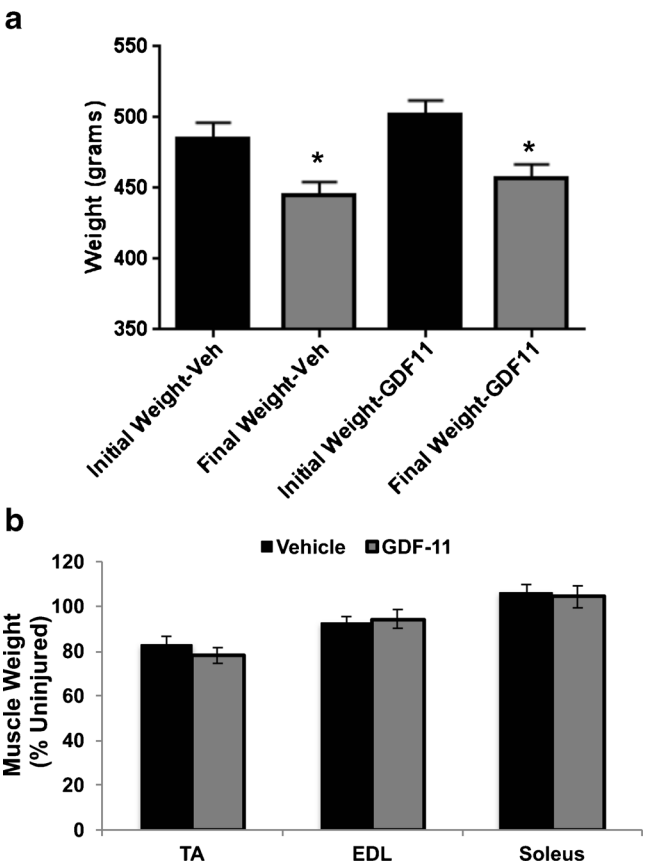

Fig. 3. The effect of GDF11 treatment on final animal body and muscle weight. **a** Initial and final body weights (grams) of rats. **b** Wet weights of vehicle- and GDF11-treated tibialis anterior (*TA*), extensor digitalis longus (*EDL*), and soleus muscles. Significance: (*asterisk*) $p < 0.05$ between initial and final body weights

detectable levels of circulating GDF11 ranging from approximately 20–75 ng/ml (Fig. 1b), whereas no circulating GDF11 was detected in the vehicle-treated animals. Quantitative PCR (qPCR) was performed on RNA isolated from injured and contralateral uninjured EDL muscles from vehicle- and GDF11-treated rats using probes specific to GDF11, myostatin and TGFβ (Fig. 2). Significant increases in GDF11 mRNA after injury in both vehicle- and GDF11-treated rats were detected (Fig. 2a). Sinha *et al.* suggested that GDF11 decreased in response to aging; therefore, we compared our GDF11 data in adult rats (11–12 months of age) to similarly treated uninjured and injured muscles from young rats (3–4 months of age) (Fig. 2a). Our data showed a more robust induction of GDF11 message after injury in the older rats at 14 days after injury than the younger rats in contrast to previous reports ($p < 0.05$) (Fig. 2a), although there was no difference in basal GDF11 levels from uninjured muscles in either the young or adult rats. It must be cautioned that this data compared GDF11 mRNA changes and not circulating GDF11 changes in these animals and thus may not be representative of changes in GDF11 protein levels between the different aged animals. Furthermore, myostatin mRNA was significantly decreased in muscles treated with GDF11 compared to vehicle-treated muscles ($p < 0.03$) (Fig. 2b) and a trend for increased TGFβ in GDF11-treated muscles was noted ($p < 0.06$) (Fig. 2c).

All animals, regardless of treatment, lost 10–15% body weight over the course of the experiment (Fig. 3a). The TA, EDL, and Sol muscles were weighed immediately after dissection. The TA and EDL muscles are both in the anterior muscle compartment and tend to be similarly affected by the CS injury, whereas the Sol muscle is from a posterior muscle compartment that is not significantly damaged in our model and thus serves as a control. We found no difference in the wet weights of the muscles between the vehicle- and GDF11-treated groups, with the TA and EDL muscles recovering 80–95% of their weight, as compared to uninjured controls, respectively (Fig. 3b).

After injury, skeletal muscle undergoes a well-orchestrated series of transcription factor activation and deactivation that results in degeneration of the damaged fibers followed by regeneration and maturation of immature fibers into mature myofibers (17). This process is mediated by local Pax7+ skeletal muscle stem cells (satellite cells). We have previously characterized and compared the timing of these events in young and old rats after CS injury and demonstrated that young rats efficiently recover muscle mass and function, while older animals have a delayed or deficient recovery (15). H+E staining of TA muscles from vehicle- and GDF11-treated groups similarly showed small regenerating myofibers with centrally located nuclei (Fig. 4a, black arrows), adipose deposits (Fig. 4a, black arrowheads), and small foci of infiltrating immune cells (Fig. 4a, white arrows). Myofiber cross-sectional area (CSA) is an indicator of myofiber maturity, with more mature myofibers having a larger CSA as compared to newly regenerating/regenerated

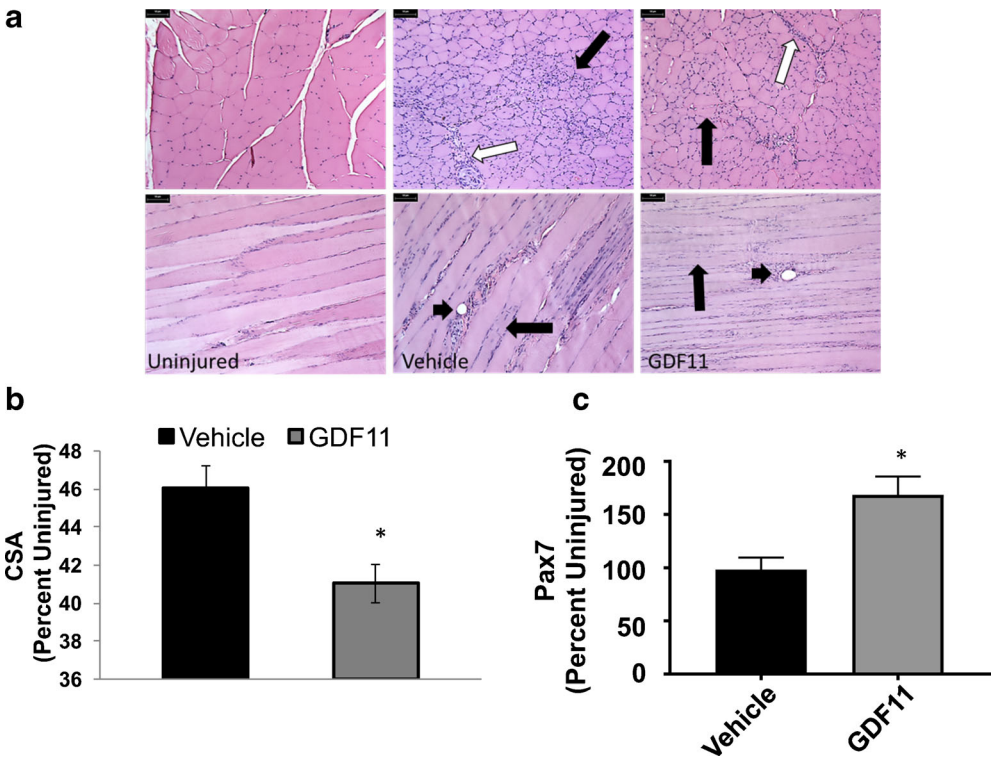

Fig. 4. Morphology of TA muscles from GDF11- or vehicle-treated rats. **a** H+E stained TA muscle showed foci of regenerating fibers (*black arrows*), infiltration of immune cells (*white arrows*), and adipose tissue deposition (*black arrowheads*). **b** Myofiber cross-sectional area (*CSA*) was measured from H+E stained sections and at least 10 HPFs were measured from each muscle. **c** qPCR analysis of Pax7 expression in EDL muscles from vehicle- and GDF11-treated rats. mRNA was harvested from at least four uninjured and eight injured EDL muscles for each analysis. Significance: CSA $p < 0.001$, Pax7 $p < 0.01$

myofibers. The CSA of myofibers in muscle from GDF11-treated animals were significantly smaller than the CSA from vehicle-treated rats ($p < 0.001$) (Fig. 2b). Moreover, the satellite cell marker Pax7 is significantly increased in muscles from GDF11-treated animals as compared to vehicle alone ($p < 0.01$) (Fig. 4c), suggesting that these muscles are undergoing delayed regeneration as compared to their counterparts that were treated with vehicle alone.

Fibrotic lesions that are not resolved after injury are a major hindrance to recovery of full muscle function. This is particularly true for older individuals where the regenerative process is inefficient and often results in adipose deposition and unresolved pathologic fibrosis. As expected, increased adipose deposits were detected in all of the injured muscles (Fig. 5a, arrows). Quantification of tissue fibrosis showed that the area of tissue containing fibrotic lesions was significantly higher in muscles of GDF11-treated animals as opposed to vehicle alone ($p < 0.001$) (Fig. 5b).

Functional recovery of the anterior muscle compartment was determined by measuring isometric torque output after electrical stimulation of the peroneal nerve. At day 7 after injury, vehicle- and GDF11-treated muscles showed 10% maximum isometric torque as compared to baseline function measurements taken prior to injury (data not shown). Muscles from vehicle-treated rats showed a significant improvement of force output at day 14 normalized to day 7, at all stimulation frequencies except 50 Hz ($p < 0.06$), as compared to GDF11-treated rats ($p < 0.05$) (Fig. 6a). Furthermore, there was a strong trend towards significance in maximal (Max) isometric torque production from muscles treated with vehicle as compared to those treated with GDF11 ($p < 0.06$) (Fig. 6b).

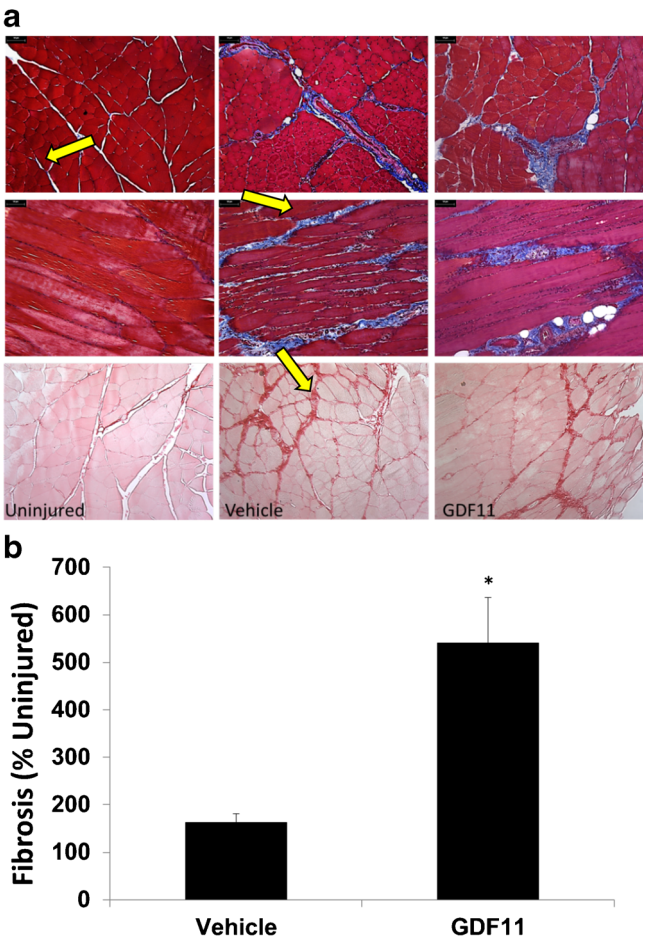

Fig. 5. The effect of GDF11 treatment on fibrosis after injury. **a** Masson's trichrome stain (*top 2 rows*) and Picro-Sirius red (*bottom row*) were used to analyze and quantitate TA muscle fibrosis. Adipose deposits were detected in all injured muscle, regardless of treatment (*yellow arrows*). **b** Fibrosis was quantitated from Picro-Sirius red stained slides using the ImageJ software. At least 10 HPFs were counted from each muscle. Significance: fibrosis $p < 0.001$

DISCUSSION

Skeletal muscle regeneration is mediated by a well-orchestrated series of events that begin with activation, expansion and differentiation of local satellite cells, fusion of differentiated myoblasts into myofibers, and ends with myofiber maturation (18). These events are mediated by a succession of transcription factor activation and deactivation that are controlled, at least in part, by Wnt/Notch and TGFβ signaling (4,19–21). GDF11 and myostatin (GDF8) are highly homologous members of the TGFβ family that both signal through the activin type II (ActRII) receptor pathway and are both antagonized by follistatin (5). Historical studies demonstrated that GDF11 signaling regulates axial vertebral patterning during embryogenesis and, similar to myostatin, was an inhibitor of muscle growth in the adult (22,23). Moreover, GDF11 was shown to be an inhibitor of neuronal and pancreatic islet precursor cells (24,25) and to have no effect on human umbilical vein endothelial cells (12).

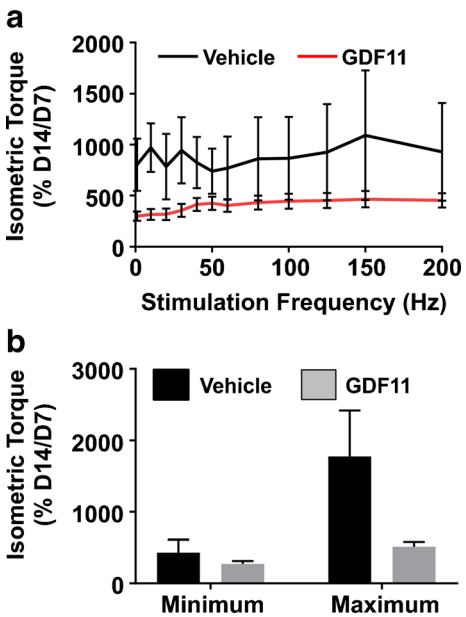

Fig. 6. The effect of GDF11 on the recovery of isometric torque after injury. Isometric torque force curves were measured as described in "Materials and Methods" at 7 and 14 days after injury. **a** Percent difference isometric torque at day 14 *versus* day 7 after injury. **b** Percent difference minimum (min) and maximum (max) isometric torque at day 14 *versus* day 7 after injury. Significance: force curve $p < 0.05$ at all stimulation frequencies except 50 Hz ($p < 0.06$), min/max $p < 0.06$ between vehicle, and GDF11 maximum isometric torque

With the known background of GDF11 and myostatin, it was surprising to find several recent high profile studies that have put forth GDF11 as a circulating factor that has the ability to "rejuvenate" old tissue. Using heterochronic parabiosis experiments, Conboy *et al.* demonstrated that systemic circulating factors from young mice could improve the proliferation and regenerative capacity of skeletal muscle progenitor cells (satellite cells) in older mice (26). Loffredo *et al.* proceeded to identify GDF11 as a circulating factor that declined with age and could reverse cardiac hypertrophy when injected into older animals (7). In the study by Sinha *et al.*, GDF11 was shown to exert its effect on the aged skeletal muscle satellite cell population by reducing DNA damage and caspase activation, by increasing myogenic differentiation, and by increasing the integration capacity of transplanted satellite cells (8). Further studies indicated that injection of exogenous rGDF11 into older animals could promote neurogenesis and cerebrovascular angiogenesis (7,8,27–29).

Several groups have now published data that is in direct conflict with that published by Loffredo *et al.* and Sinha *et al.* More specifically, the paper by Egerman *et al.* suggests that the reagents used in the earlier studies were not specific for GDF11, but also detected myostatin. This group then goes on to demonstrate inhibition of myoblast fusion *in vitro* and muscle regeneration *in vivo* after cardiotoxin injury in GDF11-treated mice, (10) and, in direct contrast to the Sinha *et al.* study, they demonstrated that circulating GDF11 increased, rather than decreased, in old mice. Furthermore, Rodgers *et al.* showed that circulating myostatin levels are approximately 500× higher than those of circulating GDF11 (11). Since myostatin and GDF11 compete for the same receptor, circulating GDF11 may not have any physiological relevance (11,30).

Due to the controversy over the ability of GDF11 to influence skeletal muscle regeneration, we examined the effect of GDF11 treatment on skeletal muscle regeneration in older rats after CS injury. Unlike commonly used models of skeletal muscle injury (myotoxin, vessel ligation), our model physiologically mimics injuries found in the clinic (14). The complex injury to the muscle, vascular, and neural compartments provides a unique model for testing therapies to aid regeneration and recovery of function after injury. Furthermore, we have characterized this model in young and old rats and have shown that older animals recover from injury slower than young animals (15), similar to the regenerative capacity of older people.

We found that systemic GDF11 treatment of rats, using the same protocol of Sinha *et al.*, had significant effects on myofiber CSA, pathologic fibrosis, and restoration of function, 14 days after injury. The decreased myofiber CSA (Fig. 4a) and increased Pax7 expression (Fig. 4b) found in muscles from GDF11-treated rats indicate attenuation of tissue regeneration after injury. Increased fibrosis in muscles from GDF11-treated animals (Fig. 5b) may be due to increased TGFβ expression (Fig. 2c). The detrimental effects of GDF11 treatment on skeletal muscle regeneration in our model of injury is confirmed by the decreased recovery in muscle function (Fig. 6). These data are consistent with recent reports showing no change, or an increase, in circulating GDF11 during aging and no effect, or a detrimental effect, of GDF11 treatment on tissue healing and regeneration (10,11,13).

REFERENCES

1. American College of Sports M, Chodzko-Zajko WJ, Proctor DN, Fiatarone Singh MA, Minson CT, Nigg CR, *et al*. American College of Sports Medicine position stand. Exercise and physical activity for older adults. Med Sci Sports Exerc. 2009;41(7):1510–30.
2. Narici MV, Maffulli N. Sarcopenia: characteristics, mechanisms and functional significance. Br Med Bull. 2010;95:139–59.
3. Dumont NA, Wang YX, Rudnicki MA. Intrinsic and extrinsic mechanisms regulating satellite cell function. Development. 2015;142(9):1572–81.
4. Carlson ME, Conboy MJ, Hsu M, Barchas L, Jeong J, Agrawal A, *et al*. Relative roles of TGF-beta1 and Wnt in the systemic regulation and aging of satellite cell responses. Aging Cell. 2009;8(6):676–89.
5. McPherron AC, Huynh TV, Lee SJ. Redundancy of myostatin and growth/differentiation factor 11 function. BMC Dev Biol. 2009;9:24.
6. Oh SP, Yeo CY, Lee Y, Schrewe H, Whitman M, Li E. Activin type IIA and IIB receptors mediate Gdf11 signaling in axial vertebral patterning. Genes Dev. 2002;16(21):2749–54.
7. Loffredo FS, Steinhauser ML, Jay SM, Gannon J, Pancoast JR, Yalamanchi P, *et al*. Growth differentiation factor 11 is a circulating factor that reverses age-related cardiac hypertrophy. Cell. 2013;153(4):828–39.
8. Sinha M, Jang YC, Oh J, Khong D, Wu EY, Manohar R, *et al*. Restoring systemic GDF11 levels reverses age-related dysfunction in mouse skeletal muscle. Science. 2014;344(6184):649–52.
9. Mendelsohn AR, Larrick JW. Systemic factors mediate reversible age-associated brain dysfunction. Rejuvenation Res. 2014;17(6):525–8.
10. Egerman MA, Cadena SM, Gilbert JA, Meyer A, Nelson HN, Swalley SE, *et al*. GDF11 Increases with Age and Inhibits Skeletal Muscle Regeneration. Cell Metab. 2015;22(1):164–74.
11. Rodgers BD, Eldridge JA. Reduced Circulating GDF11 Is Unlikely Responsible for Age-Dependent Changes in Mouse Heart, Muscle, and Brain. Endocrinology. 2015;156(11):3885–8.
12. Zhang YH, Cheng F, Du XT, Gao JL, Xiao XL, Li N, et al. GDF11/BMP11 activates both smad1/5/8 and smad2/3 signals but shows no significant effect on proliferation and migration of human umbilical vein endothelial cells. Oncotarget. 2016.
13. Smith SC, Zhang X, Zhang X, Gross P, Starosta T, Mohsin S, *et al*. GDF11 does not rescue aging-related pathological hypertrophy. Circ Res. 2015;117(11):926–32.
14. Criswell TL, Corona BT, Ward CL, Miller M, Patel M, Wang Z, et al. Compression-induced muscle injury in rats that mimics compartment syndrome in humans. Am J Pathol. 2011.
15. Zhou Y, Lovell D, Bethea M, Wang Z, Christ GJ, Soker S, *et al*. Age-dependent changes cooperatively impact skeletal muscle regeneration after compartment syndrome injury. Am J Pathol. 2014;184(8):2225–36.
16. Myzithras M, Li H, Bigwarfe T, Waltz E, Gupta P, Low S, et al. Development of an ultra-sensitive Simoa assay to enable GDF11 detection: a comparison across bioanalytical platforms. Bioanalysis. 2016.
17. Bentzinger CF, Wang YX, Rudnicki MA. Building muscle: molecular regulation of myogenesis. Cold Spring Harb Perspect Biol. 2012;4(2).
18. Dumont NA, Bentzinger CF, Sincennes MC, Rudnicki MA. Satellite Cells and Skeletal Muscle Regeneration. Compr Physiol. 2015;5(3):1027–59.
19. Conboy IM, Rando TA. The regulation of Notch signaling controls satellite cell activation and cell fate determination in postnatal myogenesis. Dev Cell. 2002;3(3):397–409.
20. Brack AS, Conboy MJ, Roy S, Lee M, Kuo CJ, Keller C, *et al*. Increased Wnt signaling during aging alters muscle stem cell fate and increases fibrosis. Science. 2007;317(5839):807–10.

21. Brack AS, Conboy IM, Conboy MJ, Shen J, Rando TA. A temporal switch from notch to Wnt signaling in muscle stem cells is necessary for normal adult myogenesis. Cell Stem Cell. 2008;2(1):50–9.
22. Gamer LW, Cox KA, Small C, Rosen V. Gdf11 is a negative regulator of chondrogenesis and myogenesis in the developing chick limb. Dev Biol. 2001;229(2):407–20.
23. McPherron AC, Lawler AM, Lee SJ. Regulation of anterior/posterior patterning of the axial skeleton by growth/differentiation factor 11. Nat Genet. 1999;22(3):260–4.
24. Gokoffski KK, Wu HH, Beites CL, Kim J, Kim EJ, Matzuk MM, *et al*. Activin and GDF11 collaborate in feedback control of neuroepithelial stem cell proliferation and fate. Development. 2011;138(19):4131–42.
25. Harmon EB, Apelqvist AA, Smart NG, Gu X, Osborne DH, Kim SK. GDF11 modulates NGN3+ islet progenitor cell number and promotes beta-cell differentiation in pancreas development. Development. 2004;131(24):6163–74.
26. Conboy IM, Conboy MJ, Wagers AJ, Girma ER, Weissman IL, Rando TA. Rejuvenation of aged progenitor cells by exposure to a young systemic environment. Nature. 2005;433(7027):760–4.
27. Vanbekbergen N, Hendrickx M, Leyns L. Growth differentiation factor 11 is an encephalic regionalizing factor in neural differentiated mouse embryonic stem cells. BMC Res Notes. 2014;7:766.
28. Katsimpardi L, Litterman NK, Schein PA, Miller CM, Loffredo FS, Wojtkiewicz GR, *et al*. Vascular and neurogenic rejuvenation of the aging mouse brain by young systemic factors. Science. 2014;344(6184):630–4.
29. Shi Y, Liu JP. Gdf11 facilitates temporal progression of neurogenesis in the developing spinal cord. J Neurosci. 2011;31(3):883–93.
30. Rodgers BD. The Immateriality of Circulating GDF11. Circ Res. 2016;118(10):1472–4.