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GSK3β inhibition and LEF1 upregulation in skeletal muscle following a bout of downhill running

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Abstract Canonical Wnt signaling is important in skeletal muscle repair but has not been well characterized in response to physiological stimuli. The objective of this study was to assess the effect of downhill running (DHR) on components of Wnt signaling. Young, male C57BL/J6 mice were exposed to DHR. Muscle injury and repair (MCadherin) were measured in soleus. Gene and protein expression of Wnt3a, active β-catenin, GSK3β, and LEF1 were measured in gastrocnemius. Muscle injury increased 6 days post-DHR and MCadherin protein increased 5 days post-DHR. Total and active GSK3β protein decreased 3 days (9-fold and 3.6-fold, respectively) post-DHR. LEF1 protein increased 6 days (5-fold) post-DHR. DHR decreased GSK3β and increased LEF1 protein expression, but did not affect other components of Wnt signaling. Due to their applicability, using models of physiological stimuli such as DHR will provide significant insight into cellular mechanisms within muscle.

Keywords DHR · Skeletal muscle repair · Wnt · Exercise

Introduction

When skeletal muscle is injured (e.g., novel bout of exercise, mechanical overload, freeze injury, chemical injury),

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complex molecular and cellular mechanisms are activated to initiate repair of injured myofibers. These responses include inflammation, structural alterations, and myogenesis [1]. The ability of adult skeletal muscle satellite cells to initiate the myogenic pathway is critical for recovery from skeletal muscle injury, and involves signals released from a variety of sites including the injured muscle itself, neighboring muscle fibers, or infiltrating inflammatory cells. In response to these signaling events, quiescent satellite cells are activated and develop into proliferating myoblasts that express myogenic markers such as MCadherin and MyoD. During differentiation, the proliferating myoblasts express myogenic factors such as myogenin and myosin heavy chain (MHC), which direct differentiation and promote the formation and fusion of myotubes that ultimately repair injured myofibers [2].

Wnt signaling is important for skeletal muscle repair following injury [3–6]. The canonical Wnt signaling pathway is regulated by Frizzled and low-density lipoprotein receptor-related protein receptor (LRP) binding with soluble Wnt ligands. Frizzled and LRP activation leads to inhibition of phosphorylation of β-catenin by the Wnt signaling inhibitor, glycogen synthase kinase β (GSK3β). With the assistance of Axin, β-catenin is not degraded and therefore translocates to the nucleus and binds to transcription factors such as T cell factor-1 (TCF1) and lymphoid-enhancing factor-1 (LEF1). TCF/LEF may increase expression of myogenic regulatory factors (MRF), such as Myf5 of MyoD, or increase expression of MRF co-activators, ultimately resulting in skeletal muscle repair [6–10].

The components of Wnt signaling were first characterized during skeletal muscle repair using artificial muscle injury models, including freeze injury, myofiber explantation, and chemical injury [3–5, 11, 12]. Wnt3a is one of the

19 Wnt ligands that are known to increase the Wnt downstream target, LEF1, and is critical for skeletal muscle formation in mouse embryos [13, 14]. Evidence suggests that Wnt3a may be important for adult skeletal muscle repair, since there is increased Wnt3a protein expression in cultured myofibers [4, 12, 15], and it is the Wnt of choice in force activation of Wnt signaling using in vitro models of muscle regeneration [4]. More work is needed to further characterize Wnt3a and determine its importance to adult skeletal muscle repair.

The expression pattern and role of GSK3β in regenerating skeletal muscle is not fully understood [16]. GSK3β activity was decreased in skeletal muscle growth models (reloading of atrophic muscle), and inhibiting GSK3β in C2C12 cells or single muscle fibers increased MRF expression, while enhancing GSK3β activity reduced satellite cell activation and skeletal muscle hypertrophy [4, 11, 17]. Since Wnt3a has been reported as responsible for GSK-3β activity depression in primary progenitor cells [4], it is possible that Wnt3a-induced depression of GSK3β is a critical step during skeletal muscle repair, but this hypothesis requires confirmation.

The majority of muscle repair models used in characterizing Wnt3a, active β-catenin, GSK3β, and LEF1, have hitherto involved in vitro or artificial models of muscle injury [3–5, 12, 18]. Although freeze injury and chemical injury induce extensive muscle damage, these methods are focal in nature and may be limited in their inclusiveness of other biological systems (neurological, circulatory, immunological) that play a role during skeletal muscle repair. Using physiological stimuli such as downhill running (DHR) is more relevant to common muscle function scenarios. If used as a model of contraction-induced muscle injury, physiological stimuli may help elucidate intracellular responses such as Wnt signaling that are important for skeletal muscle repair. Since it is possible that myogenic processes involved in muscle repair following freeze- or chemically-induced injury differ from contraction-induced injury, physiological stimuli could be critical for accurately describing skeletal muscle repair [6]. Eccentric contractions during DHR induce a physiological stimulus that is known to induce a myogenic response [19–22], and therefore DHR is a useful tool for investigating signaling mechanisms within skeletal muscle. Furthermore, if used as an exercise model, DHR can be used as a model that examines the effects of the orchestration of multiple biological systems on signaling pathways within skeletal muscle. The overall importance of this knowledge is that understanding the potential for skeletal muscle repair from contraction-induced injury is a significant public health concern (e.g., sarcopenia).

The purpose of this study was to characterize gene and protein expression of Wnt signaling components following exposure to an acute bout of DHR. We hypothesized that DHR would increase Wnt3a, active β-catenin, and LEF1 and decrease expression of total GSK3β and active GSK3β ($GSK3\beta^{pY216}$). Since physiological stimuli induce systemic effects [23], the DHR model may influence mechanisms of skeletal muscle processes (such as muscle repair) differently from localized muscle injury models. Therefore, the results from this DHR study will aid in deciphering factors important for skeletal muscle repair which could be applied to therapeutic approaches towards muscle-associated ailments such as aging and disease.

Methods

Animals

Twelve-week-old C57BL/J6 male mice (body mass ∼27 g, $n = 44$) (Jackson Laboratory, Bar Harbor, ME, USA) were housed at the University of North Carolina at Charlotte Vivarium under standard conditions (18–22 °C; 20–40 % humidity; 12:12 h light:dark cycle) and provided with standard chow and water ad libitum. All procedures were approved and performed in adherence to the Institutional Animal Care and Use Committee at the University of North Carolina at Charlotte and the American Physiological Society animal care guidelines.

Downhill running (DHR)

Mice were randomly divided into two groups; control (normal cage activity) and experimental (DHR protocol). In the experimental group, mice were familiarized with the treadmill (5.08 × 38 cm; Columbus Instruments, Columbus, OH, USA) and completed an acute bout of treadmill exercise which comprised of running (22 m/min) downhill (−15 % grade) for ∼50 min (modified from [24]). An electrical grid shock (1.0 mA at 150 V) was used to encourage running.

Tissue collection and preparation

Randomly selected mice from the experimental group were euthanized by asphyxiation (CO_2) at 24-h intervals for 6 days post-DHR (five muscles per time point) and control (five muscles). Different cellular techniques were performed on the soleus [Hematoxylin and Eosin staining (H&E) and immunofluorescence] or gastrocnemius (western blot, and rT-PCR) of the same mouse since different muscle preparations were required for the histology/immunofluorescence relative to western blot/rT-PCR techniques. We acknowledge that gastrocnemius and soleus muscles have different fiber-type characteristics, but

it was necessary to use both muscles to generate enough tissue samples for our analyses. Upon harvesting, gastrocnemius muscles were immediately frozen in liquid nitrogen and stored in −80 °C until processed for western blot analysis or RNA analysis. Prior to analysis, the gastrocnemius muscles were divided equally in the transverse plane at the mid-belly. One-half of the muscle was used for RNA isolation the other half for protein expression analysis. The soleus muscle was also excised, coated with optimal cutting temperature gel, and frozen in isopentane cooled in liquid nitrogen and stored in −80 °C until processed for histology or immunofluorescence.

Histology

Transverse sections (10 μm) from the mid-belly of soleus muscles were cut using a microtome cryostat (HM 505E; Microm, Germany). Single sections, every 15 cuts, were mounted on gelatin-coated slides and stored in −80 °C until processed for histology or immunofluorescence. To confirm muscle injury induced by DHR, Mayer's H&E (Sigma, Minneapolis, MN, USA) was performed on cross-sections. The number of injured fibers on the entire soleus cross-section were counted using muscle injury criteria (pale cytoplasm, centrally-located nuclei, and infiltrated muscle fibers) and results were expressed as a percentage of the total number of fibers within each cross-section [19, 25].

Immunofluorescence

Muscle regeneration was quantified using immunofluorescence on soleus cross-sections using antibodies directed to myogenic marker, M-Cadherin. Soleus cross-sections were fixed with 4 % paraformaldehyde (PFA) for 10 min at room temperature, washed with a standard washing buffer (1 % normal goat serum (NGS) in PBS + 0.1 % Triton-X 100 detergent; Sigma-Aldrich) and nonspecific sites were subsequently blocked for 1 h with 5 % NGS for 40 min. Either the primary antibody of mouse anti-M Cadherin antibody (1:75; Cat #: 611100; BD Biosciences, San Diego, CA, USA), or an isotype negative control, mouse IgG (BD Biosciences) [diluted to the same concentration (μg/μl) as anti-MCadherin antibody] were made in washing buffer, applied to the section and incubated overnight at 4 °C. Sections were then incubated with rabbit anti-mouse Alexa 546 (1:1,000; Invitrogen; Carlsbad, CA, USA) and nuclei staining 4′-6-Diamidino-2-phenylindole (DAPI; 1:10,000; Sigma) made in washing buffer for 1 h at room temperature. Immunofluorescence images were visualized using a fluorescent microscope (Olympus IX-71; Parkway Valley, PA, USA). The percentage of MCadherin-expressing cells was expressed relative to DAPI-positive cells within an entire section.

Western blot analysis

Total tissue lysates were prepared in RIPA buffer and protease inhibitor cocktail. Protein samples were electrophoresed on a 4–12 % (w/v) NuPAGE®Bis–Tris gel prior to transfer onto a polyvinylidene fluoride membrane. Antibodies [Wnt 3a (R&D), total GSK3β and GSK3β^{pY216} (BD Transduction), LEF 1 (Cell Signaling), β-catenin (Millipore), GAPDH (Millipore)] directed against proteins of interest were added to the blots. An HRP-conjugated secondary antibody was used and bound enzymes were detected with enhanced chemiluminescent solution. Densitometric quantification was performed using a densitometric analysis program (Multiguage; GE Healthcare). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an invariant control and has been demonstrated to have consistent mRNA expression in the gastrocnemius muscle using quantitative RT-PCR and SYBR Green detection [26].

RNA isolation and real-time polymerase chain reaction (qRT-PCR) analysis

Total RNA was isolated from gastrocnemius muscle tissues (miRNeasy Mini Kit) and quality was confirmed Agilent 2100 Bioanalyzer profiles. First-strand complementary DNA was synthesized using an iScript™ cDNAsysthesis kit. The reverse transcription reaction was incubated at 42 °C for 30 min and stopped by heating to 85 °C for 5 min. Then, 50 ng of final product was used as a template for PCR. qRT-PCR was performed using TaqMan® Probe-Based Detection with an ABI Prism 7500 Fast Real-Time PCR System using Taqman® gene expression assays and Taqman® Gene expression master mix. The template was amplified by 40 cycles of denaturation at 95 °C for 15 s, annealing of primers and probe, together with extension at 60 °C for 1 min in triplicate reactions. Fluorescence data were acquired during a combined anneal/extension step. RT negative reactions were run on each plate to confirm the absence of DNA contamination. Fold change values were calculated using comparative Ct analysis and normalized to those of GAPDH, which was an invariant control.

Statistical analyses

A one-way analysis of variance was used to assess the effects of DHR on muscle injury, muscle regeneration, or the signaling components assessed. Means were considered different when the observed F ratio was statistically significant ($P < 0.05$). Newman–Keuls post hoc was used for pairwise comparisons. Data are reported as mean ± SEM.

Results

Downhill running induces injury in soleus muscle

DHR protocol induced significant injury in the soleus muscle at 1–6 days post-exercise (Fig. 1). Specifically,

there was a significant increase in muscle injury at 3 days ($P = 0.038$; 3.7-fold), 5 days ($P = 0.040$; 3.7-fold) and 6 days ($P = 0.002$; 5.7-fold) post-DHR relative to control in the soleus. Also, the degree of soleus injury was significantly higher at 3 days ($P = 0.031$), 5 days ($P = 0.024$), and 6 days ($P = 0.001$) post-exercise relative
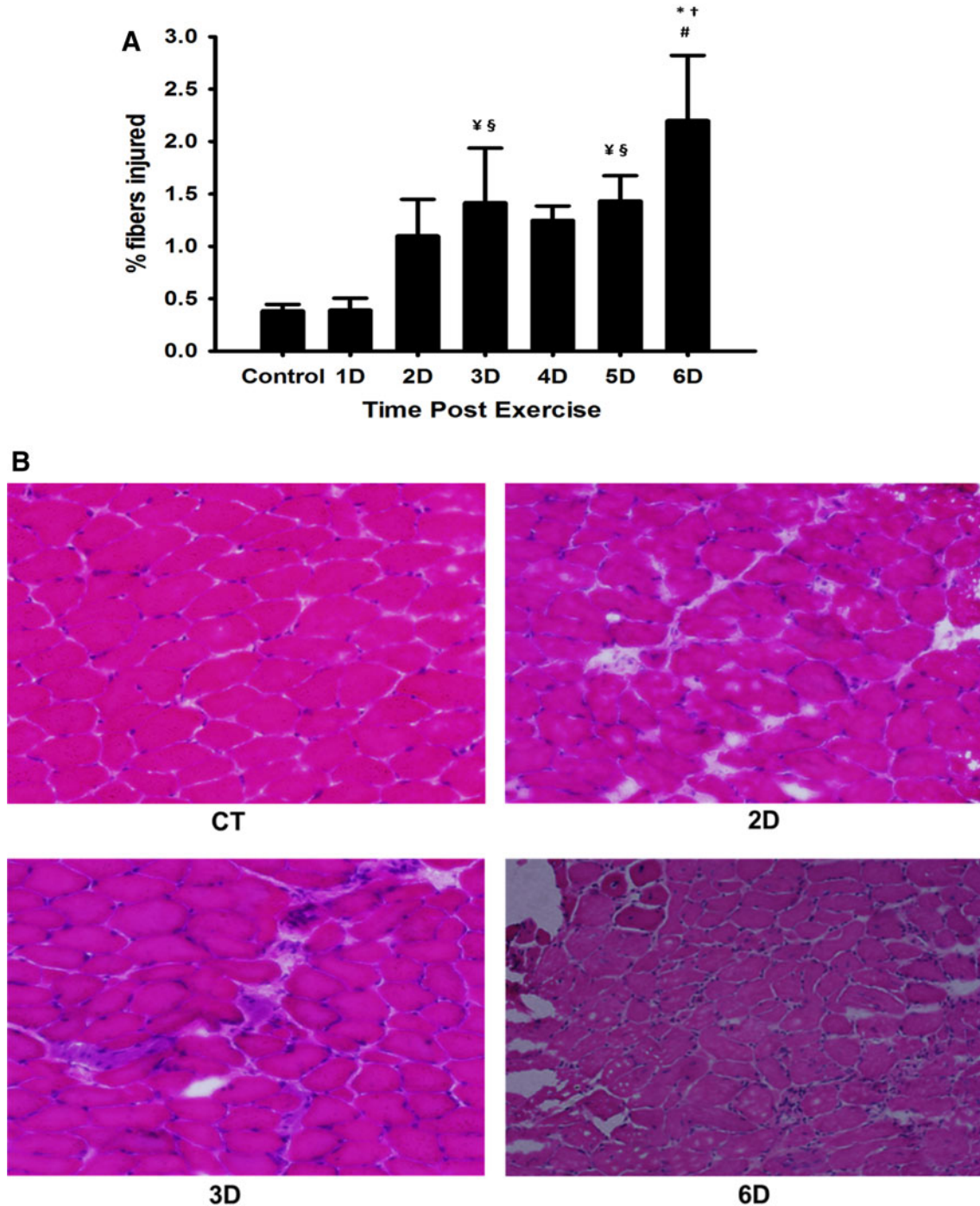

Fig. 1 **a** Quantification of H&E staining as a marker of muscle injury. Two sections counted for every muscle (6–7 muscles/time point). Percentage of injured fibers relative to total fibers of cross section. Values are mean ± SE. *$P < 0.001$ vs. control, †$P < 0.001$ vs. 1D, #$P < 0.05$ vs. 2D, ¥$P < 0.05$ vs. control, §$P < 0.05$ vs. 1D. **b** H&E representation of control muscle and 6 days post-DHR soleus muscle. *D* days
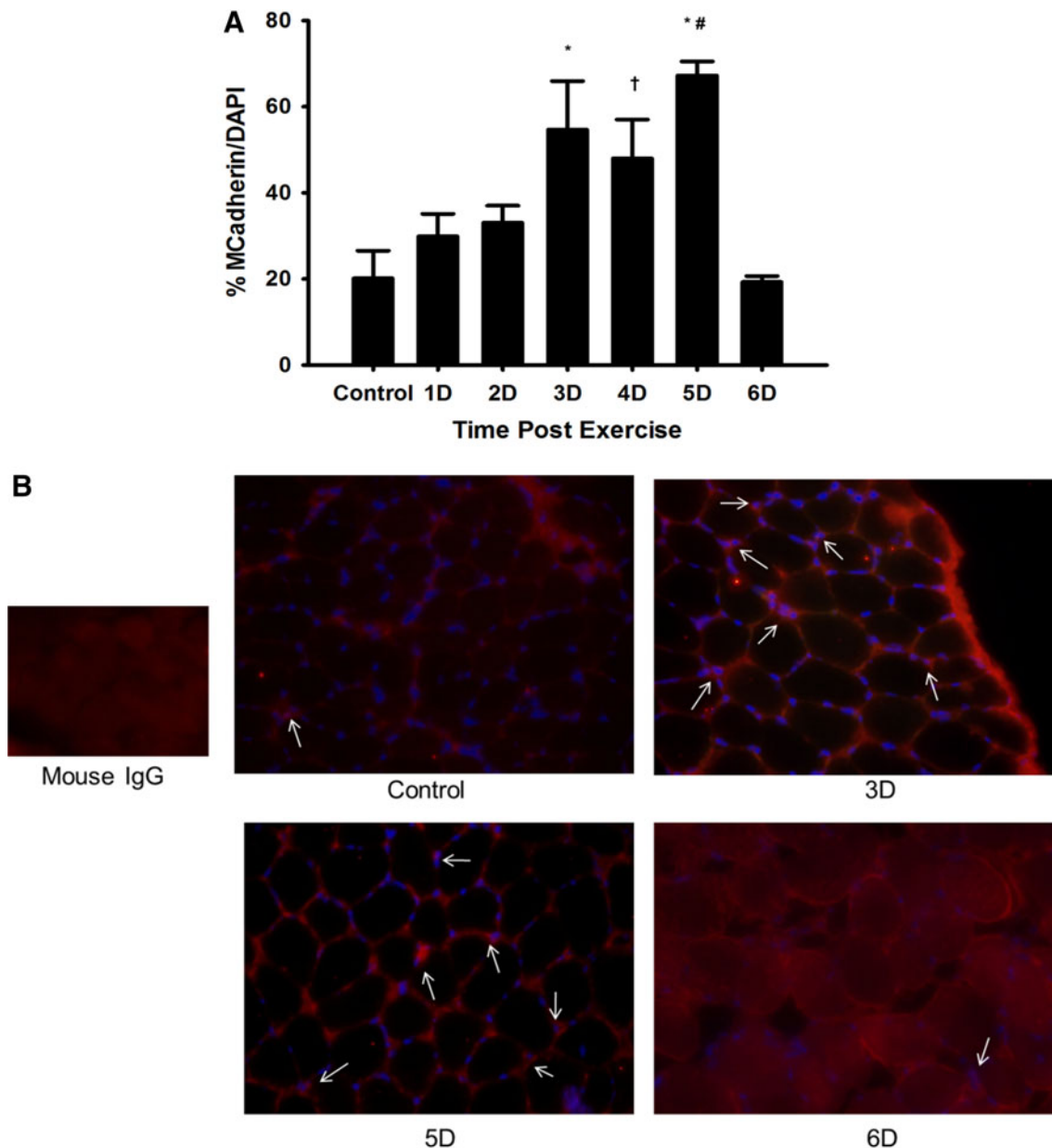
Fig. 2 a Immunofluorescence quantification of % MCadherin-positive cells from total cells (DAPI-positive) on muscle cross-sections. Two sections counted for every muscle (6–7 muscles/time point). Values are mean ± SE. *$P < 0.01$ vs. baseline and $6D$, [†] to 1D. Furthermore, soleus injury was significantly higher $P < 0.05$ vs. baseline and $6D$, [#]$P < 0.01$ vs. $1D$ and $2D$. **b** Immuno-fluorescence representation of mouse IgG isotype control with Alexa 546, with representative time points probed for MCadherin (*red*) and DAPI (*blue*). *D* days

to 1D. Furthermore, soleus injury was significantly higher at 6 days relative to 2 days post-exercise ($P = 0.047$).

To determine if regeneration processes were activated in soleus muscle exposed to DHR, myoblast proliferation marker MCadherin protein was quantified on soleus cross-sections (Fig. 2). There was a significant increase in MCadherin protein expression on soleus muscle at 3 days ($P = 0.005$; 2.7-fold), 4 days ($P = 0.023$; 2.4-fold), and 5 days ($P < 0.001$; 3.3-fold) (overall $P < 0.001$) post-DHR relative to control. Also, there was a significant increase in MCadherin expression at 3 days ($P = 0.006$),

4 days ($P = 0.028$), and 5 days ($P < 0.001$) relative to 6 days post-DHR. Moreover, MCadherin expression was significantly higher at 5 days relative to 1 day ($P = 0.002$) and 2 days ($P = 0.002$) post-DHR.

Downhill running decreases GSK3β and increases LEF1 expression in gastrocnemius muscle

To test the hypothesis that DHR would influence mRNA and protein expression of signaling components associated with muscle regeneration, Wnt3a, total and active GSK3β,

Fig. 3 a qRT-PCR analysis of Wnt3a expression. Fold change relative to control.
b Quantification of immunoblot probed for Wnt3a protein on lysates of gastrocnemius muscle that were exposed to DHR versus control. c Western blot representation of gastrocnemius lysates probed for Wnt3a antibody and striped and reprobed with GAPDH antibody
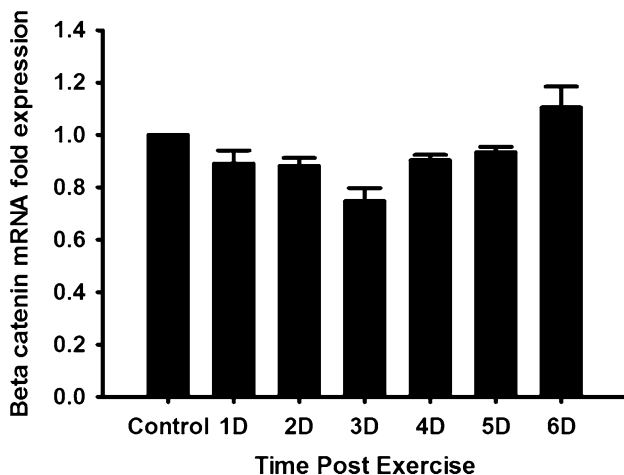
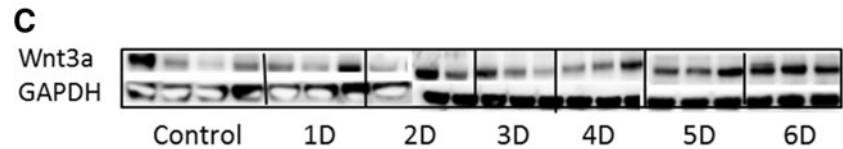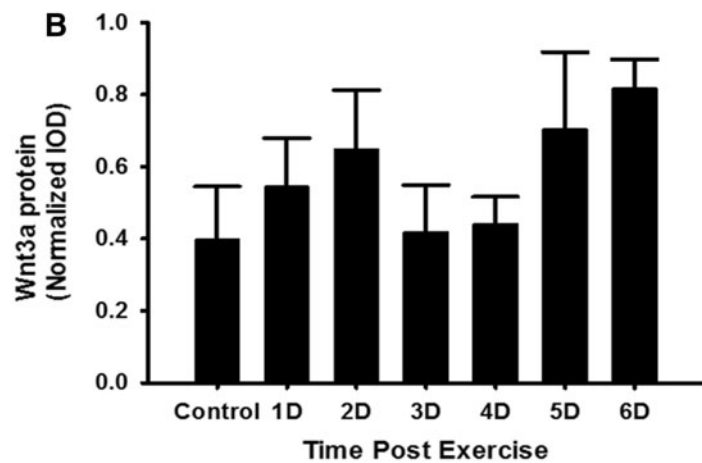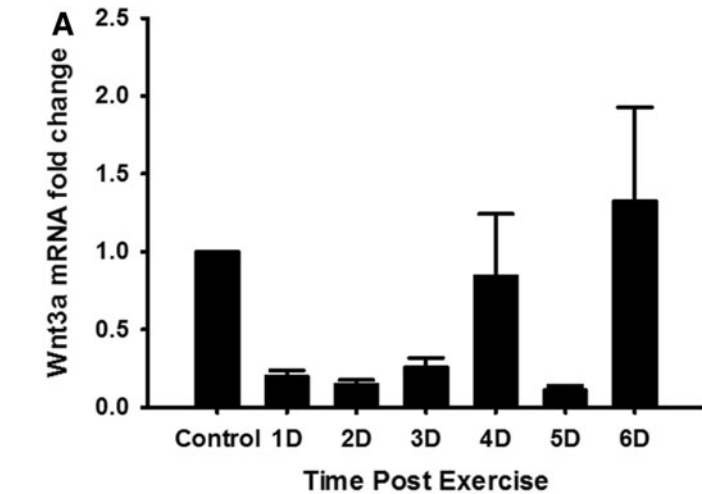Fig. 4 qRT-PCR analysis of total β-catenin expression. Fold change relative to control

active β-catenin, and LEF1 were measured in gastrocnemius tissue from the same mice for which soleus injury was assessed, following DHR. Within 6 days post-DHR no changes in either mRNA or protein levels of Wnt3a expression were found (Fig. 3). Moreover, there were no changes in mRNA levels of total β-catenin (Fig. 4), nor were there detectable levels of active β-catenin protein with the antibody used (clone 8E7; Millipore). There were no changes in mRNA of total GSK3β from control to 6 days post-DHR (Fig. 5a). There was a significant decrease in total GSK3β at 2 days ($P = 0.010$; 2.4-fold), 3 days ($P < 0.001$; 9-fold), 4 days ($P = 0.002$; 3.79fold), and 6 days ($P = 0.007$; 2.49fold) post-DHR relative to control (Fig. 5b). Also, there was a significant decrease in total GSK3β protein levels at 3 days ($P = 0.003$) and 4 days ($P = 0.016$) relative to 5 days post-DHR. Furthermore, total GSK3β protein expression was significantly
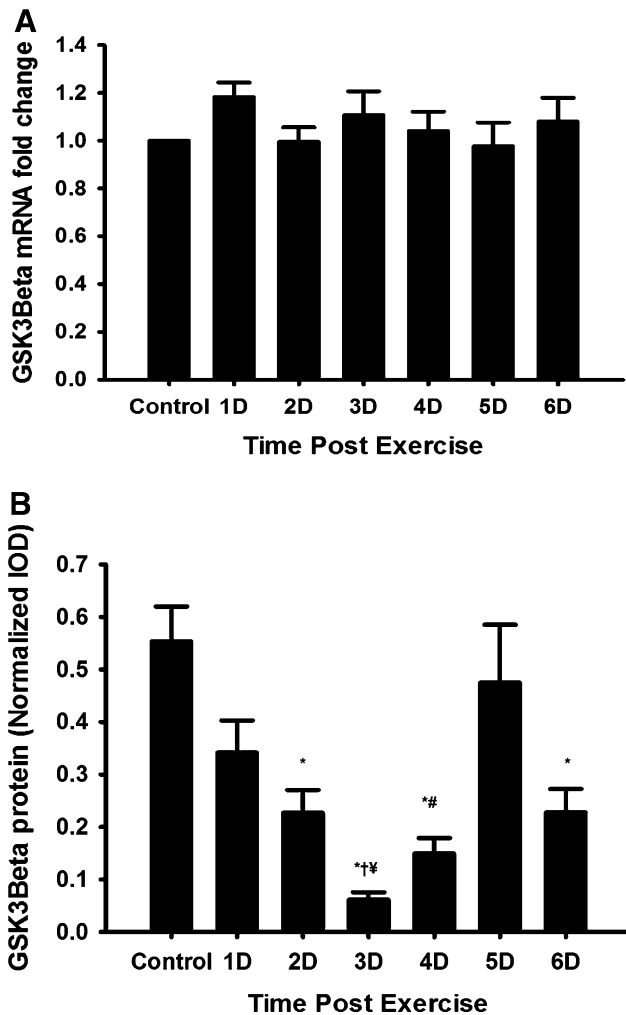
Fig. 5 a qRT-PCR analysis of total GSK3β expression. Fold change relative to control. **b** Quantification of immunoblot probed for total GSK3β protein on lysates of gastrocnemius muscle that were exposed to DHR vs. control. Values are mean ± SE. *$P < 0.01$ vs. control, †$P < 0.01$ vs. *5D*, #$P < 0.05$ vs. *5D*, ¥$P < 0.05$ vs. *1D*. *D* days

lower at 3 days ($P = 0.044$) relative to 1 day post-DHR. GSK3β^{pY216} protein decreased at 2 days ($P = 0.028$; 2.3-fold), 3 days ($P = 0.005$; 3.6-fold), 4 days ($P = 0.042$; 2-fold) and 6 days ($P = 0.039$; 3.4-fold) post-DHR relative to control (Fig. 6). No significant changes in LEF1 mRNA levels after DHR were found (Fig. 7a), but LEF1 protein expression was significantly elevated at 5 days ($P < 0.001$; 4.3-fold) and 6 days ($P < 0.00$; 5-fold) compared to control through 4 days post-DHR (Fig. 7b).

Discussion

The purpose of this study was to determine if DHR influences the expression of canonical Wnt signaling components. Although an acute bout of DHR did not influence Wnt3a or active β-catenin expression, the novel finding of

this study was that DHR did affect LEF1 and total and active GSK3β expression in the gastrocnemius within 6 days post-exercise. Accumulating evidence suggests that Wnt3a up-regulates LEF1 and is an important component in Wnt signaling activation during skeletal muscle repair [4, 6–8, 13–15]. Previous studies characterizing Wnt3a used artificial muscle injury models. Therefore, Wnt3a responses to an exercise model, such as DHR, may provide further insight into intracellular responses to physiological stimuli that are more relevant to common muscle function scenarios including skeletal muscle injury and repair.

Although the DHR model used in this study induced muscle injury and possibly regeneration in the soleus, injury nor repair were quantified in the gastrocnemius, which was the muscle of choice in characterizing Wnt3a and the other Wnt signaling components. Therefore, it is not known if DHR induced muscle injury in the gastrocnemius, and hence the responses observed may not have been associated with muscle repair. In this study, a single bout of DHR did not up-regulate Wnt3a mRNA or protein expression which was consistent with previous studies that included a variety of skeletal muscle injury models [3, 12]. While it is possible that other Wnt proteins may have responded to DHR in this study (e.g., Wnts 5a, 5b, 7a, and 7b; [3, 4, 27], we were primarily interested in Wnt3a signaling responses to DHR because of its suggested importance in skeletal muscle repair [4, 6–8, 13–15].

Interestingly, DHR did not alter either total or active β-catenin in gasctrocnemius, which is a component of Wnt signaling [8, 10], although this may have been related to the lack of Wnt3a responses to DHR in this study. Our findings contrast the results of previous studies that reported up-regulation of β-catenin in skeletal muscle following mechanical overload [12, 28–30]. The absence of changes in Wnt3a, total β-catenin, and active β-catenin expression suggests that a single bout of DHR may not induce a profound enough stimulus to activate canonical Wnt signaling or that other systemic factors attenuated β-catenin activation. This has important implications for accurately describing interactions among signaling pathways following exposure to physiological stimuli.

In this study, total GSK3β and active GSK3β (GSK3β^{pY216}) decreased after a single bout of DHR at 3 days post-exercise resulting in the greatest magnitude of change of sixfold and fourfold for total and active GSK3β, respectively. GSK3β is an isoform of serine/threonine protein kinases and regulates Wnt signaling [31]. When GSK3β is activated after phosphorylated at tyrosine 216 (Tyr 216; GSK3β^{pY216}), subsequently phosphorylating β-catenin, promoting its degradation, then canonical Wnt signaling is inhibited [4, 32]. When GSK3β is inactive, β-catenin is translocated to the nucleus and interacts with TCF/LEF transcription factors, which may induce the

Fig. 6 **a** Quantification of immunoblot probed for GSK3β (pY216) protein on lysates of gastrocnemius muscle that were exposed to DHR vs. control. Values are mean ± SE. *$P < 0.01$ vs. control, †$P < 0.05$ vs. control. **b** Western blot representation of gastrocnemius lysates probed for GSK3β (pY216 and total) and striped and reprobed with GAPDH antibody
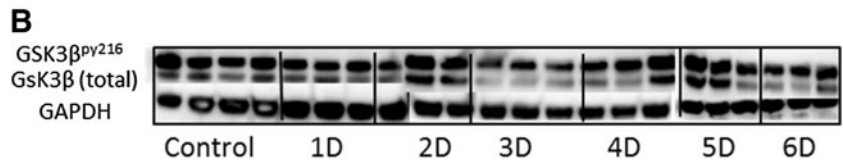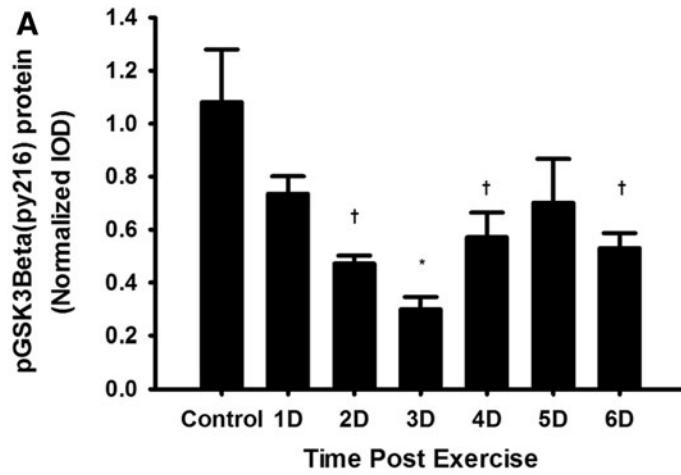

**Fig. 7** **a** qRT-PCR analysis of LEF1 expression. Fold change relative to control. **b** Quantification of immunoblot probed for LEF1 protein on lysates of gastrocnemius muscle that were exposed to DHR versus control. Values are mean ± SE. *$P < 0.01$ relative to baseline. **c** Western blot representation of gastrocnemius lysates probed for LEF1 antibody and striped and reprobed with GAPDH antibody
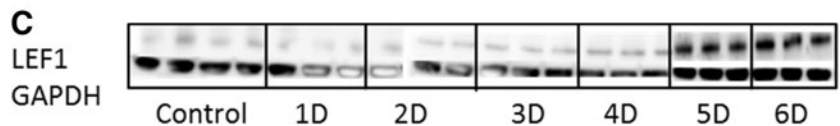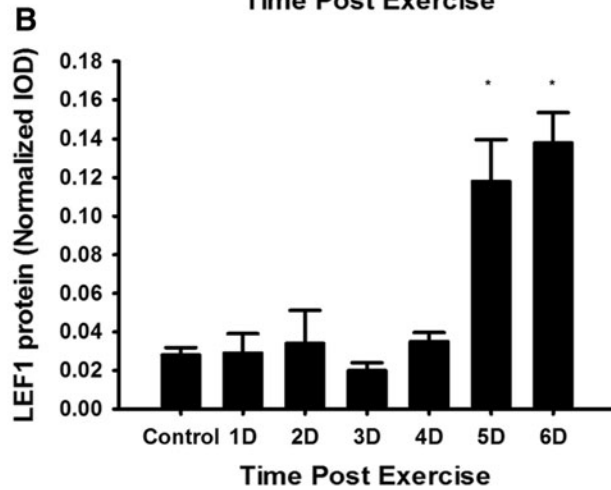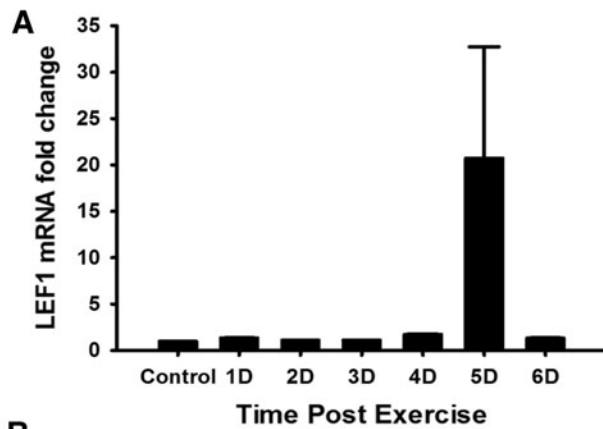
expression of MRFs or MRF co-activators [6–10]. Our results are consistent with previous studies that reported deactivation of GSK3β in response to physiological stimuli. For example, in rat gastrocnemius exposed to treadmill exercise, expression of inactive GSK3β (GSK3β Ser^9 phosphorylation) was up-regulated with a concomitant increase in the interaction between the Wnt protein Dishevelled and GSK3β (an indicator of GSK3β inactivation) [33]. The changes in GSK3β and GSK3β^{pY216} expression, independent of Wnt3a and β-catenin, suggests that GSK3β and GSK3β^{pY216} expression responses may have been associated with a different Wnt ligand associated with skeletal muscle repair, including Wnt5a, 5b, 7a, or 7b [3, 4, 27]. In fibroblast cultures, administration of Wnt5a has been demonstrated to inactivate GSK3β [34], suggesting that other Wnt ligands, not measured in this study, could be responsible for decreasing GSK3β. Therefore, more work is needed to determine the contribution of other Wnt ligands to the inactivation of GSK3β,

Alternatively, a different signaling pathway, serine-threonine protein kinase Akt (Akt)/phosphoinositide 3-kinase (PI3K)/mammalian target of rapamycin (mTOR), may have interacted with GSK3β and GSK3β^{pY216} following DHR [27, 35, 36]. Akt/PI3 K/mTOR are known to be up-regulated following DHR [37–40]. Upon activation during skeletal muscle repair, Akt inhibits GSK3β [27, 35, 36], and therefore it is possible that alternative pathways such as Akt/PI3K/mTOR inhibited GSK3β and GSK3β^{pY216} in this study.

DHR induced a 4.2- and 5.0-fold increase in LEF1 mRNA at 5 and 6 days post-DHR, respectively. LEF1 is a sequence-specific DNA-binding transcription factor which is an important downstream regulator of canonical Wnt signaling that may induce the expression of MRFs or MRF co-activators [6–8, 10]. Our findings are in agreement with previous studies that reported LEF1 mRNA expression increases in skeletal muscle exposed to physiological stimuli such as mechanical overload and strength/power training programs [28, 41]. Although DHR increased LEF1, the acute bout of exercise did not effect Wnt3a or β-catenin, suggesting that other factors may be responsible for LEF1 regulation. There is evidence for increased LEF1 with no changes in β-catenin in skeletal muscle exposed to physiological stimuli [41]. Leal et al. [41] demonstrated increases in LEF1 gene expression in vastus lateralis muscles from subjects who performed a strength or power training program, but total β-catenin gene expression increased only in the power-trained group and not in the strength-trained group.

Moreover, it is possible that other factors, independent of β-catenin, could have induced the observed up-regulation of LEF1 expression [42–45]. The integrin-linked kinase pathway has been demonstrated to inhibit GSK3β

activity and to increase LEF1 expression [42]. In addition, transforming growth factor β signaling pathway can also activate LEF1 independent of β-catenin [43]. Furthermore, an interaction between vitamin D receptor and LEF1 that was independent of β-catenin has been reported [44]. Immunoprecipitation experiments in mammalian COS (CV-1 origination with SV40 genetic material) cells showed that vitamin D receptor and LEF1 interact while β-catenin did not, suggesting that β-catenin is not the sole activator of LEF1 expression [44]. Lastly, co-immunoprecipitation and binding assays have demonstrated that myogenic regulator Pax3 binds to LEF1 and increases LEF1 activity, independent of β-catenin [45]. This demonstrates that there are a variety of potential mechanisms for increased LEF1 expression and activity that support our findings of increased LEF1 expression independent of β-catenin activation following DHR.

Conclusion

The results of this study indicate that an acute bout of DHR induces significant injury to the soleus muscle at 3, 5, and 6 days post-exercise as well as increases in myogenic protein levels of M-Cadherin at 3–5 days post-exercise in the soleus muscle. Although skeletal muscle injury and repair was not measured in the gastrocnemius muscle, DHR resulted in decreased total and active GSK3β in the days preceding increased expression of LEF1, suggesting that physiological stimuli affects these proteins.

We consider the DHR model to be ideal in investigating the cellular mechanisms within skeletal muscle because DHR uses a systems approach, may include the influence of other biological systems, and are applicable to the 'real world' muscle function, including the injury and regeneration cycle. Findings from this study provide further insight into the complex role of signaling pathways in skeletal muscle exposed to exercise, which may contribute to the development of therapeutic approaches for regenerative medicine as well as skeletal muscle dysfunctions associated with aging and disease. Importantly, this study suggests that skeletal muscle cellular responses to the muscle model used and physiological stimuli may provide an accurate description of cellular events involved within a contracting skeletal muscle and during skeletal muscle repair. This is a critical component in the translation of the results from animal studies to human populations.

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