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Effects of Diet-Induced Obesity on Extracellular Matrix Remodeling During Skeletal Muscle Regeneration

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THE EFFECT OF DIET-INDUCED OBESITY ON EXTRACELLULAR MATRIX REMODELING DURING SKELETAL MUSCLE REGENERATION

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ABSTRACT

Skeletal muscle has the ability to regenerate from damage; however, recent studies have reported a negative effect of obesity on skeletal muscle regenerative capacity. The extracellular matrix (ECM) contributes to skeletal muscle structure acting as a scaffold for skeletal muscle. Additionally, skeletal muscle serves as a reservoir for proteins and growth factors that promote regeneration. Optimal skeletal muscle regeneration includes inflammation, ECM remodeling, and myofiber growth. Disruption to any of these processes can negatively affect skeletal muscle regeneration. **PURPOSE:** The purpose of this study was to determine how diet-induced obesity (DIO) affects ECM remodeling during skeletal muscle regeneration. **METHODS:** Fifty-six male C57BL/6J mice were randomly assigned to two groups; lean diet (10% fat) and high fat diet (HFD) (60% fat). Within those two groups, mice were randomly assigned to either a PBS (uninjured) group or a bupivacaine (injured) group. Bupivacaine is a myotoxin which induces injury to skeletal muscle. Both groups received injections into the tibialis anterior (TA). Three or 28 days post-bupivacaine injection, the TAs were extracted and PCR reaction was done to quantify ECM-related gene expression (i.e. Collagen-I, Collagen-III, Fibronectin, TGF-β, MMP-2, MMP-9, and TIMP-I). **RESULTS:** There was no difference in Collagen III:I gene expression 3 days post-injection in the lean group ($p>0.05$). However, there was a 3 fold increase ($p<0.05$) in Collagen III:I gene expression in the obese group 3 days post-injection. Twenty-eight days post-injection there was an increase in Collagen III:I ratio gene expression in the lean group due to injury as compared to the uninjured lean group ($p<0.05$). Three days post injection there was a main effect of injury to increase TGF-β gene expression ($p<0.05$). Twenty-eight days post injection there were no significant differences in TGF-β gene expression between groups ($p>0.05$). Three and 28 days post injection there was a main effect of injury to increase MMP-2 gene expression ($p<0.05$). Three days post injury there was a main effect of injury to increase MMP-9 gene expression ($p<0.05$). Twenty-eight days post injection there was a main effect of HFD to decrease MMP-9 gene expression ($p<0.05$). Three days post injection there was a main effect of injury to increase TIMP-I gene expression ($p<0.05$). Twenty-eight days post injection there was a main effect of HFD to decrease TIMP-I gene expression regardless of diet ($p<0.05$). **CONCLUSION:** Obesity altered ECM composition during skeletal muscle regeneration. This could negatively impact the ability of obese muscle to recovery form injury. These findings suggest that an altered composition could lead to a change in exercise prescription for this specific population.

INTRODUCTION

Obesity is an increasingly prevalent disease in the United States. Approximately 33% of American are obese (BMI > 30) (47). Obesity is associated with pathophysiological conditions such as cardiovascular disease, type II diabetes and high blood pressure (3, 61). Obesity is also associated with negative financial affects. It cost approximately $1000 more to care for an obese individual than an individual of normal weight (22). As the trend for obesity is increasing, two documented ways to combat this are to alter diet and increase physical activity (20, 30). We know that exercise with a large eccentric component will induce muscle damage (15). It has been demonstrated that obese muscle takes longer to regenerate following injury (21). Understanding the mechanisms related to impaired muscle function associated with obesity could lead to a change in exercise prescription for this specific population.

Optimal skeletal muscle regeneration includes satellite cell activation, inflammation responses, ECM remodeling, and myofiber growth (13). Disruption to any of these processes negatively affects skeletal muscle regeneration. At the onset of skeletal muscle regeneration, two to three days post-injury, there is a robust elevation of cellular activity (54). By three weeks muscle has been shown to be fully regenerated and back to full power (53). Obesity negatively effects skeletal muscle's regenerative capacity. It was demonstrated that obesity alters muscle regeneration by reducing MyoD and IL-6 mRNA abundance (8). Another study showed that muscle regeneration was delayed in obese mice (45). It has been shown that in mice fed a HFD PIP3 activity was reduced during skeletal muscle regeneration. This study also showed that in mice fed a HFD PTEN remained elevated during skeletal muscle regeneration (29). These two finding support that there is reduced signaling through Akt and thus reduced protein synthesis.

The extracellular matrix (ECM) acts as a scaffold for skeletal muscle structure and also serves as a reservoir for proteins and growth factors that promote regeneration (64). Collagen networks assist with force transmission which is another important function of the ECM (51). The ECM contains many collagen molecules, proteoglycans and glycoproteins (37). Collagen-I and III are the most abundant of the collagens that make up the musculoskeletal ECM (37). Type I and III collagen expression are centrally regulated by TGF-β pathway (59) It has been observed that skeletal muscle in obese mice has an impaired ability to regenerate possibly due to an increase in collagen deposition (29, 45, 55). Collagen synthesis is decreased within two days following hind limb suspension (1). Type I and III collagen gene expression increases with acute muscle loading within one day (25). It has been demonstrated that collagen content was elevated in DIO mice after damage (21).

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidases that degrade collagen fibrils within the ECM in order to allow for cell migration (37, 48). MMPs are produced in an inactive form and upon injury become active. Because of this, in healthy tissue active MMP expression is relatively low. MMP activity increases in response to inflammation associated with skeletal muscle regeneration (38). MMP-2 and 9 are the most abundant in skeletal muscle. There is conflicting research reporting MMP-2 and 9 levels as a result of obesity. MMP- 2 activity has been shown to increase with obesity (56). In a different study MMP-2 mRNA abundance was showed to have increased as a result of obesity but MMP-2 activity was no different as a result of obesity (4). Tissue inhibitor of metalloproteinases (TIMPs) are a class of proteins that regulate the activity of MMPs by binding to the active site. Four TIMPs have been identified and all have been found to be expressed in skeletal muscle (2,

7). It has been shown that TIMP-1 levels increased 1 and 2 days post-injury and then was not different from control 3 days post-injury (60).

The purpose of this study is to determine if obesity alters extracellular matrix remodeling during skeletal muscle regeneration. I hypothesize that there will be an increase in the collagen III:I ratio due to an increase in the collagen-III gene expression. I hypothesize that MMP-2 and 9 gene expression will be down at the onset of skeletal muscle regeneration and beyond due to obesity. It has been previously discussed that collagen deposition is increased with obesity and since MMP-2 and 9 are responsible for the breakdown of collagen, MMPs might be reduced. I hypothesize that TIMP-1 gene expression will be increased 3 and 28 days post injection as a result of obesity. If MMP-2 and 9 levels are lower than TIMP-1 which regulates these MMPs could be increased leading to this decrease in MMP gene expression.

METHODS

Animals and Housing

Forty-eight 3 week old C57BL/6 mice were purchased from Jackson Laboratories. Animals were housed in the University of Arkansas Central Laboratory Animal Facility. The overall study consisted of 2 separate animal experiments. Experiment 1 examined gene expression 3 days post-bupivacaine injection. Experiment 2 examined gene expression 28 days post-bupivacaine injection. For experiment 1 and 2 mice were randomly assigned to one of four groups: 1) lean uninjured (n = 5-9); 2) lean injured (n = 5); 3) obese uninjured (n = 4-6); 4) obese injured (n = 6-8). Mice were fed a lean diet (lean; 10% kcals fat Research Diets, New Brunswick, New Jersey) or a high fat diet (HFD; 60% kcals fat, Research Diets, New Brunswick, New Jersey) for 12 weeks. All procedures are approved by the University of Arkansas Institutional Animal Care and Use Committee (IACUC).

Bupivacaine Injection

At fifteen weeks of age, bupivacaine (Hospira, Lake Forest, IL) injections were performed as previously described (63). Mice will be anesthetized with a subcutaneous injection of a cocktail containing ketamine hydrochloride (45 mg/kg body weight), xylazine (3 mg/kg body weight), and acepromazine (1 mg/kg body weight). Muscle damage was induced by injecting 0.03ml of 0.75% bupivacaine (Marcaine) in the left and right tibialis anterior muscles (TA). A 25-gauge, 5/8 (0.5 x 16 mm) needle was inserted along the longitudinal axis of the muscle, and the bupivacaine was injected slowly as the needle was withdrawn. The control group was injected with 0.03 ml of phosphate buffered saline (PBS).

Muscle and Tibia Extraction

Muscle and tibia extractions were performed as previously described (63). Three and 28 days post-injection, the TA and tibias were extracted to examine the early and late regenerative response in skeletal muscle. Mice were anesthetized with a subcutaneous injection of a cocktail containing ketamine hydrochloride (90 mg/kg body weight), xylazine (3 mg/kg body weight), and acepromazine (1 mg/kg body weight). The left TA was snap frozen in liquid nitrogen and stored at -80°C for gene expression analysis. After the TA was dissected out, the tibia was removed and measured with a plastic caliper (VWR, Radnor, PA, USA). Tibia measurements were utilized to normalize muscle weights as an estimate of total body size.

RNA Isolation, cDNA synthesis, and quantitative RT-PCR

RNA isolation, cDNA synthesis, and Real-Time PCR were completed as previously described (63). RNA was extracted with Trizol reagent (Life Technologies, Grand Island, NY, USA). TA muscles were homogenized in Trizol. Total RNA was isolated, DNase was treated and concentration and purity was determined by fluorometry using the Qubit 2.0 (Life Technologies). cDNA was reverse transcribed from 1 µg of total RNA using the Superscript Vilo cDNA synthesis kit (Life Technologies, Carlsbad, CA, USA). Real-time PCR was performed, and results were analyzed by using the StepOne Real-Time PCR system (Life Technologies, Applied Biosystems, Grand Island, NY). cDNA was amplified in a 25 µL reaction containing appropriate primer pairs and TaqMan Universal Mastermix (Applied Biosystems). Samples were incubated at 95°C for 4 min, followed by 40 cycles of denaturation, annealing and extension at 95°C, 55°C and 72°C respectively. TaqMan fluorescence was measured at the end of the extension step each cycle.

Fluorescence labeled probes for TGF-β (FAM dye), Collagen-I (FAM dye), Collagen-III (FAM dye), and MMP-2 (FAM dye), MMP-9 (FAM dye), Fibronectin-I (FAM dye), TIMP-1 (FAM-dye), and 18S (VIC dye) were purchased from Applied Biosystems and quantified with TaqMan Universal mastermix. Cycle threshold (Ct) was determined, and the ΔCt value was calculated as the difference between the Ct value and the 18s Ct value. Final quantification of gene expression was calculated using the ΔΔCT method Ct = [ΔCt(calibrator) – ΔCt(sample)]. Relative quantification were then calculated as $20^{-\Delta\Delta Ct}$.

Statistical Analysis

All data was analyzed using Statistical Package for the Social Sciences (SPSS version 22.0, Armonk, NY). Results were reported as mean ± SEM. A two-way ANOVA was performed to analyze main effects of treatment and diet and to determine if there are any interactions between the dependent variables at 3 and 28 days post-bupivacaine injection. When a significant interaction was detected, differences among individual means was assessed with Fisher's LSD post-hoc analysis. Statistical significance was set at $P \leq 0.05$.

LITERATURE REVIEW

This chapter includes literature relevant to the research purposes of this thesis. It is organized into 3 sections: (1) the extracellular matrix, (2) obesity, (3) and skeletal muscle regeneration. At the end of each section, the relevance of the literature to the research reported in this thesis is discussed.

I. Extracellular Matrix

ECM Basic Structure

The extracellular matrix (ECM) is a dynamic tissue that serves many roles within skeletal muscle. These roles include force transmission, protecting other tissues and muscle regeneration after injury (17). The ECM contains many collagen molecules, proteoglycans and glycoproteins (37). Some other factors that are included in the specialization of the ECM structure are the basement membrane, laminin, enactin, collagen IV, fibronectin and a plethora of growth factors and proteases (52). The essential building block of the ECM is collagen, a triple helical protein (17, 37). The most numerous collagen in the ECM of muscle is type I and III (37). Collagen-I is known to create strong and rigid parallel fibers. Collagen-III on the other hand is known to form a loose framework of fibers (37). There are numerous different members of the collagen protein family which allows the ECM to be specialized (17). The specific molecules that make up the ECM vary based upon its mechanical demands and specialized location to promote optimal functioning (2, 24, 36, 39). The major cells that works to maintain muscle and modify the ECM are fibroblasts (35). Fibroblasts function to repair in congruence with satellite cells. The homeostatic composition of the ECM is very important for satellite cells, fibroblasts, and other cells migrate through the ECM via the breakdown via MMPs (48).

ECM in skeletal muscle form the divisions of endo-, peri-, and epimysium surrounding muscle fibers, bundles of muscle fibers, and then the whole muscle. The endomysium is anchored to the basement membrane and changes as the muscle is stretched. The perimysium is composed of crossing bundles of collagen fibers (37).

Collagen Synthesis

Collagen synthesis is an important aspect of the ECM. Synthesis by modified polypeptide chains takes place in two places: ribosomes of the rough endoplasmic reticulum and inside the ECM (35). Translation of preprocollagen mRNA takes place inside ribosomes (41, 42, 62). Specific genes for the different collagen types combine to create the triple-helical procollagen molecule. For example, Col1α1 and Col1α2, typically two α1 and one α2, combine to assemble the triple-helical collagen-I (35). This procollagen assembly takes place in the endoplasmic reticulum (42). The golgi apparatus then moves the procollagen molecule from the endoplasmic reticulum to the extracellular space where it is cleaved at the amino- and carboxyterminal ends and the final product is collagen (26, 35). The collagens then self-assemble into fibrils (50). Elevated levels of TGF-β has been shown to be correlated with collagen levels (33).

Collagen Degradation

ECM can also be regulated by regulating Collagen-I and III. This can be done by the TGF-β pathway (59). Collagen degradation is the other important aspect of ECM remodeling. Collagen is degraded by matrix metalloproteinases (MMPs), zinc-dependent endopeptidases (19). MMP expression in healthy tissue is relatively low (37). Disruption to MMP activity can lead to an altered ECM composition and result in injury, disease and/or altered force transmission (2, 44).

MMP-2 and 9 are the most abundant in skeletal muscle. MMPs are secreted by inflammatory cells and regulated by tissue inhibitors of matrix metalloproteinases (TIMPS) (10, 14). TIMP-I acts on inhibiting active MMPs while TIMP-2 inhibits the inactive MMPs (10). There are only four TIMPs that have been identified so far (57). ECM structure is important to this study because it is looking at how skeletal muscle in obese mice is regenerating. The ECM is a main factor in skeletal muscle generation and so looking at how obesity might be affecting this structure could help to explain the differences that have been previously found.

II. Obesity

Obesity is now classified and a health related disease and has become an increasingly prevalent disease in the United States. Over one third of American are obese (BMI > 30) and approximately two thirds are overweight (BMI >25) and obese (47) . Obesity is associated with pathophysiological conditions such as cardiovascular disease, type II diabetes and high blood pressure (3, 61). Obesity was shown to increase mortality rates in a J shaped curve which was adjusted for age (40). After eliminating smoking and subclinical diseases, women with a BMI of less than nineteen displayed the lowest mortality rates (40). Obesity increases the number of risk factors associated with cardiovascular disease such as: high bold pressure, dyslipidemia, and impaired blood glucose levels (31). Obesity greatly affects total cholesterol levels, which can be characterized by high levels of triglycerides and LDL-cholesterol and low levels of HDL-cholesterol (31). However, studies have shown that these negative effects of obesity on cholesterol can be reversed. A meta-analysis has concluded that weight loss of 1kg can reduce total cholesterol and LDL levels by 1%, increase HDL levels by 1% and reduce triglyceride levels by 3% (16). One study examined obesity and coronary heart disease and reported that

women with a BMI of greater than twenty-nine had almost a 4 fold increased risk for coronary disease (66). Obesity is also associated with negative financial affects. It cost approximately $1000 more to care for an obese individual than an individual of normal weight (22). As the trend for obesity is increasing, two documented ways to combat this are to alter diet and increase physical activity (20, 30). We know that exercise with a large eccentric component will induce muscle damage (15). It has been demonstrated that obese muscle takes longer to regenerate following injury (21). Understanding the mechanisms related to impaired muscle function associated with obesity could lead to a change in exercise prescription for this specific population.

III. Skeletal muscle regeneration

Skeletal muscle is another dynamic structure which contains the ability to regenerate after trauma or damage. Acute damage includes injury to muscle fibers, plasma membrane, basal lamina and increased calcium levels (12). Muscle has been shown to be regenerated and back to peak power by three weeks post injury (53). Obesity has been shown, in a study using mice on a high fat diet, to delay the process of skeletal muscle regenerate as compared to the lean control group (29). Skeletal muscle regeneration takes place in three steps: inflammation response, ECM remodeling and myofiber growth.

Inflammation Response

Part of the inflammation response includes leukocytes, macrophages and satellite cells arriving at the site of injury (12). Macrophages assist with skeletal muscle repair by secreting pro- and anti-inflammatory cytokines which helps with repair and removal of dead tissue (18). One study

shows that inflammatory responses, after cardiotoxic injury in obese mice, such as macrophages were approximately reduced by fifty percent in the injured group. This disruption could lead to impaired skeletal muscle regeneration (45). Obesity leads to chronic low-grade inflammation (23, 46). This constant inflammation affects in skeletal muscle regeneration by leading to a reduction in phagocytes (45).

Extracellular Matrix Remodeling

ECM remodeling required both degradation and synthesis (57) and as previously stated ECM degradation is regulated by MMPs which degrade the collagen fibrils that make up the ECM. Disruption to ECM remodeling can lead to pathophysiological diseases such as muscular dystrophy (44). MMP activity and expression can be triggered most often by inflammation (11). It has been demonstrated in a study using rats that isometric, concentric and eccentric exercise led to an increase in collagen-I, collagen-III, MMP-2, TIMP-I and TIMP-2 (28). MMP-2 and MMP-9 levels were seen to be increased after cardiotoxic injury and then 7 days later they were no different than the control groups (34).One study performed with mice fed a high fat diet to induce obesity found that the ECM structure was compromised by an increase in collagen deposition at the onset of skeletal muscle damage and throughout the regeneration process (29). This altered composition of the ECM in obese mice could help to augment explanation and understanding of why skeletal muscle in obese individuals takes longer to regenerate (29).

Myofiber growth

Quiescent satellite cells, located in the basal lamina, awaken after injury, migrate to site of injury and fuse to damaged muscle fibers to promote regeneration (27). Fusion of satellite cells to

damaged muscle keep the process of regeneration focused on the site of injury (5). New fibers that are formed are basophilic (65). Since these fibers are regenerating within the same basal lamina this leads to some fibers splitting (5, 6). After fusion, myofiber size is increased and the new muscle is ready for full functional use (32). One study looked at the difference in satellite cell activation in lean and obese zucker rats. It was found that MyoD and Myogenin levels were reduced in the obese control group as compared to the lean control group (49). Obesity has also been found to delay the regeneration process. Mice fed a high fat diet compared to those fed a normal diet displayed at 6, 12, and 21 days to have more interstitial space of muscle and smaller regenerating myofibers (29). It is important to look at the process of skeletal muscle regeneration because in this study we are looking at how obesity is affecting this process of skeletal muscle regeneration. This study focuses on the specific process of ECM remodeling.

Results

ECM Gene Expression

Collagen I and III

Three days post injection there was a main effect of injury to increases Collagen-I gene expression ($p<0.05$). Twenty-eight days post injection there was a main effect of injury to increases Collagen-I gene expression ($p<0.05$). Three days post injection there was a main effect of injury to increase Collagen-III gene expression ($p<0.05$). Twenty-eight days post injection there were no significant differences in Collagen-III gene expression between groups ($p>0.05$). There was no difference in Collagen III:I gene expression 3 days post-injection in the lean group ($p>0.05$). However, there was a 3 fold increase ($p<0.05$) in Collagen III:I gene expression in the obese group 3 days post-injection. Twenty-eight days post-injection there was an increase in Collagen III:I ratio gene expression in the lean group due to injury as compared to the uninjured lean group ($p<0.05$).

Fibronectin

Three days post-injection there was a main effect of injury to increase Fibronectin-I gene expression ($p<0.05$). Twenty-eight days post-injection there was a main effect of injury to increase Fibronectin-I gene expression ($p<0.05$).

Regulation of ECM Remodeling

TGF-β

Three days post injection there was a main effect of injury to increase TGF-β gene expression ($p<0.05$). Twenty-eight days post injection there were no significant differences in TGF-β gene expression between groups ($p>0.05$).

MMPs

Three days post injection there was a main effect of injury to increase MMP-2 gene expression ($p<0.05$). Twenty-eight days post injection there was a main effect of injury to increase MMP-2 gene expression ($p<0.05$). Three days post injury there was a main effect of injury to increase MMP-9 gene expression ($p<0.05$). Twenty-eight days post injection there was a main effect of HFD to decrease MMP-9 gene expression ($p<0.05$).

TIMP-1

Three days post injection there was a main effect of injury to increase TIMP-I gene expression ($p<0.05$). Twenty-eight days post injection there was a main effect of HFD to decrease TIMP-I gene expression regardless of diet ($p<0.05$).

DISCUSSION

The purpose of this study was to determine if diet-induced obesity had an effect on ECM remodeling during skeletal muscle regeneration. To the best of our knowledge we are the first to demonstrate that obesity alters collagen III:I response during skeletal muscle regeneration. The ratio was altered as compared to the lean group at both early and late time points. This suggests that obesity could have a negative effect on ECM remodeling during skeletal muscle regeneration. This novel discovery could lead to changes in exercise prescription for obese individuals.

The primary component of the ECM is collagen, specifically Collagen I and III (37). One study showed that hindlimb unloading altered the Collagen III:I ratio leading to possible effects of decreased muscle stiffness and alter functional ability (43). Therefore an altered Collagen III:I ratio is strongly correlated with alterations to ECM composition. For the purpose of this study we are letting the Collagen III:I ratio imply ECM composition. With an altered collagen III:I ratio, satellite cells and other cells that must migrate through the ECM to assist with regeneration, may have a more difficult time moving through the ECM. This could slow the skeletal muscle regeneration process. It has been shown that obese mice during regeneration displayed increased collagen deposition in skeletal muscle (29). We hypothesized that there would be an altered ECM composition during regeneration due to dysregulated collagen isoform gene expression in the obese group. Our data did support this hypothesis and showed in increased ratio after injury in the obese group three days post-injection. It has been observed in previous studies that both Collagen III and I gene expression decreased after 5 weeks of being on a HFD (58). This implies that obesity itself can alter Collagen gene expression. Another study showed that Collagen I gene expression is elevated as a result of skeletal muscle hypertrophy (67). Collagen III and I gene expression were shown to have increased after concentric, eccentric and isometric exercise

in another study (28). These studies demonstrate the many physiological stimuli that could affect Collagen gene expression and therefore ECM composition. Our data shows an altered Collagen III:I ratio in obese mice during skeletal muscle regeneration. This suggest that obese mice could take longer to recover due to an altered ECM composition. Collagen I is known to create strong and rigid fibers while Collagen III is known create more of a loose framework (37). An altered ratio could change the entire structure and integrity of the ECM leading to disruption of skeletal muscle regeneration or even force transmission. An altered ECM composition could lead to a more difficult area for cells to migrate through to get to the damaged tissue.

Collagen is degraded by MMPs therefore helping to regulate the ECM (17). There are many different MMPs including different classes however, in skeletal muscle MMP-2 and MMP-9 are the most abundant. It was shown in a previous study that MMP-2 gene expression after eccentric, concentric and isometric exercise, was increased (28). In another study that induced hypertrophy by ablation, 2 days post hypertrophy, MMP-2 gene expression was decreased but MMP-9 increased. Then at 7 and 14 days post overload MMP-2 gene expression increased and MMP-9 gene expression decreased (9). After cardiotoxic injury MMP-2 and MMP-9 levels were increased but returned to levels with no difference from control after 7 days (34). We hypothesized that MMP-2 and MMP-9 gene expression would be lowered during regeneration in the obese group. Our results did not support this hypothesis. Our data supports the increase of MMP-2 gene expression due to injury at the onset of regeneration. However, our data showed no difference between diet. At the 28 day time point MMP-2 levels were still elevated as a result of injury not back to control group. Our data suggests that MMP-2 and MMP-9 may not be involved in regulating the altered ECM composition in obese mice at the onset of regeneration MMPs are enzymes therefore gene expression in not necessarily an

accurate representation of activity. MMP activity could be changed due to damage to the obese group while expression remains the same. This mismatch of gene expression and protein content has been seen in other studies. For example, collagen gene expression was shown to be lowered after being on a HFD, however these differences were not observed by protein analysis (58).

TIMPs are regulators of MMPs (10). In one study that induced hypertrophy by ablation, 2 days post hypertrophy, TIMP-I gene expression increased. Then at 7 and 14 days post overload TIMP-I levels were still increased as compared to control (9). Our hypothesis was that TIMP-I gene expression would be increased in the obese group at the onset of regeneration. However, our results showed no significant differences between diet post-injection during regeneration. Future studies could look at protein content and activity instead of gene expression. They could also look at other MMPs or TIMPs. Studies could also preform comparisons between lean and obese uninjured mice.

In conclusion, our novel finding of an altered Collagen III:I gene expression, implying ECM composition, could have a drastic impact of skeletal muscle regeneration in obese individuals. This could lead to skeletal muscle regeneration taking longer in these individuals or even an inability to fully recover. These findings could lead to changes in exercise prescription for the obese population to compensate this altered ratio during skeletal muscle regeneration. Further research is needed to pinpoint exactly what is causing this altered ratio.

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Figure Legend

Figure 1: The effect of obesity on Collagen-I gene expression during skeletal muscle regeneration. A) Collagen-I gene expression 3 days post-injection. B) Collagen-I gene expression 28 days post-injection. Values are means ± SE. ME denotes a main effect of injury, $p \leq 0.05$.

Figure 2: The effect of obesity on Collagen-III gene expression during skeletal muscle regeneration. A) Collagen-III gene expression 3 days post-injection. B) Collagen-III gene expression 28 days post-injection. Values are means ± SE. ME denotes a main effect of injury, $p \leq 0.05$.

Figure 3: The effect of obesity on Collagen III:I ratio gene expression during skeletal muscle regeneration. A) Collagen III:I gene expression 3 days post-injection. B) Collagen III:I gene expression 28 days post-injection. Values are means ± SE. $p \leq 0.05$. Significant difference denoted by when letters between groups are different.

Figure 4: The effect of obesity on Fibronectin gene expression during skeletal muscle regeneration. A) Fibronectin gene expression 3 days post-injection. B) Fibronectin gene expression 28 days post-injection. Values are means ± SE. ME denotes a main effect of injury, $p \leq 0.05$.

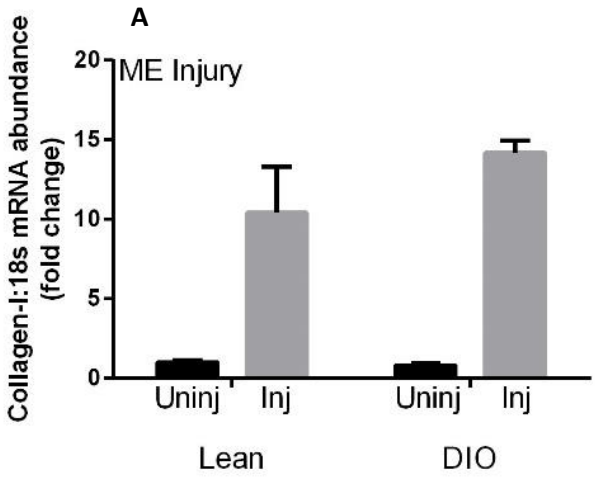
Figure 5: The effect of obesity on TGF-β gene expression during skeletal muscle regeneration. A) TGF-β gene expression 3 days post-injection. B) TGF-β gene expression 28 days post-injection. Values are means ± SE. ME denotes a main effect of injury, $p \leq 0.05$.

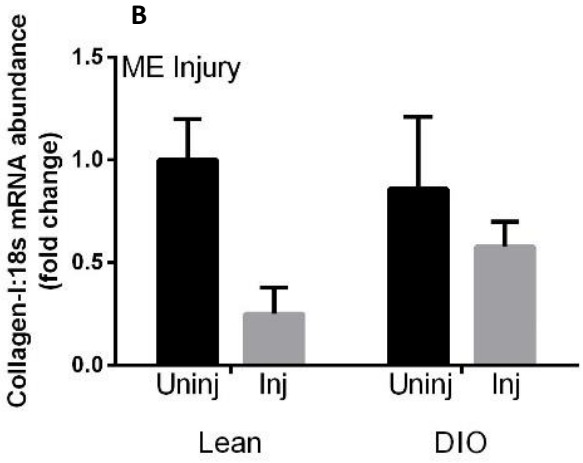
Figure 6: The effect of obesity on MMP-2 gene expression during skeletal muscle regeneration. A) MMP-2 gene expression 3 days post-injection. B) MMP-2 gene expression 28 days post-injection. Values are means ± SE. ME denotes a main effect of injury, $p \leq 0.05$.

Figure 7: The effect of obesity on MMP-9 gene expression during skeletal muscle regeneration. A) MMP-9 gene expression 3 days post-injection. ME denotes a main effect of injury B) MMP-9 gene expression 28 days post-injection. ME denotes a main effect of diet. Values are means ± SE. $p \leq 0.05$.

Figure 8: The effect of obesity on TIMP-1 gene expression during skeletal muscle regeneration. A) TIMP-1 gene expression 3 days post-injection. ME denotes a main effect of injury B) TIMP-1 gene expression 28 days post-injection. ME denotes a main effect of diet. Values are means ± SE. $p \leq 0.05$.

Figure. 1





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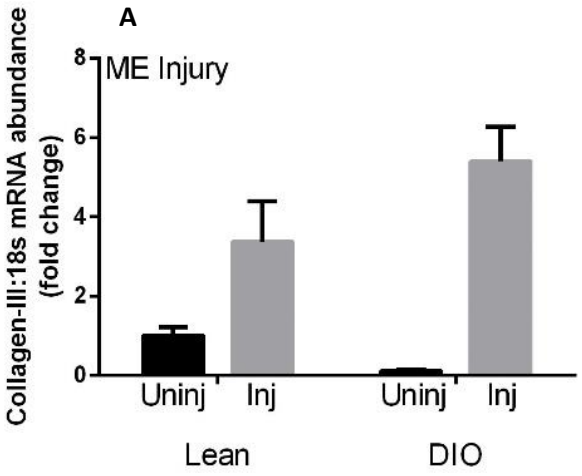

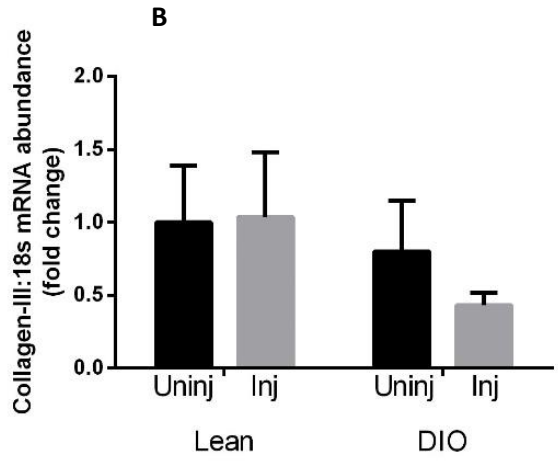

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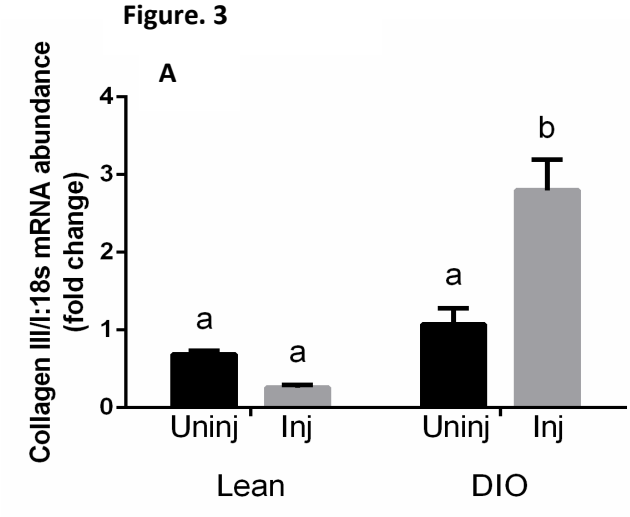

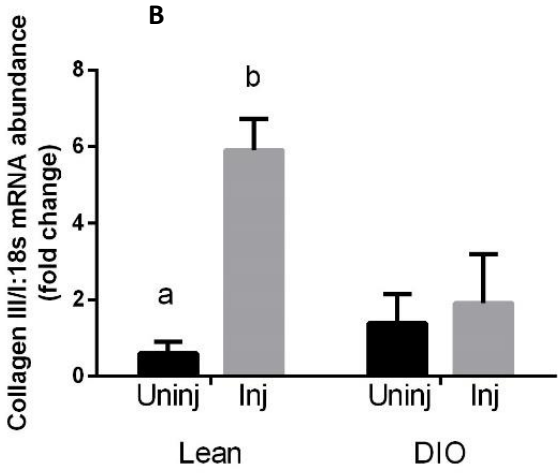

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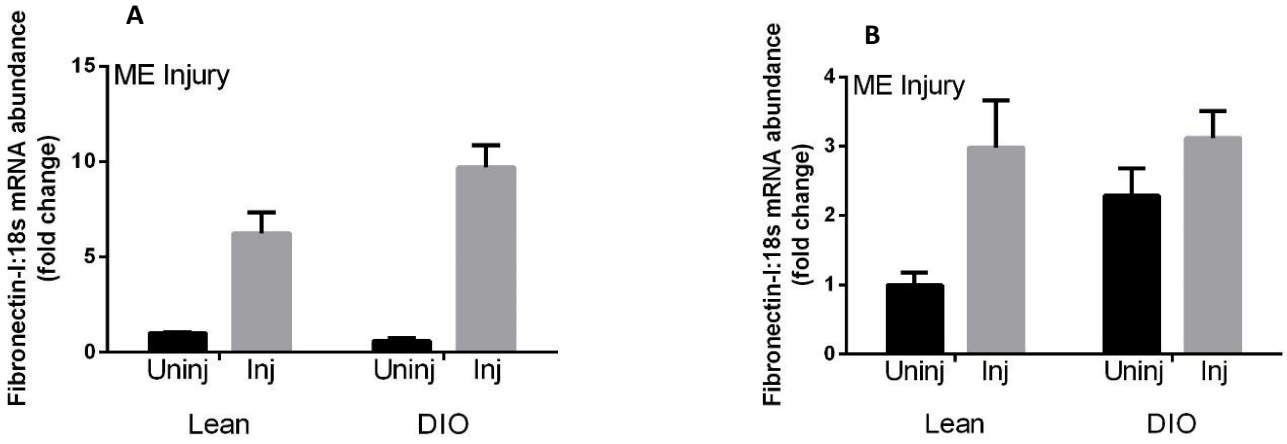

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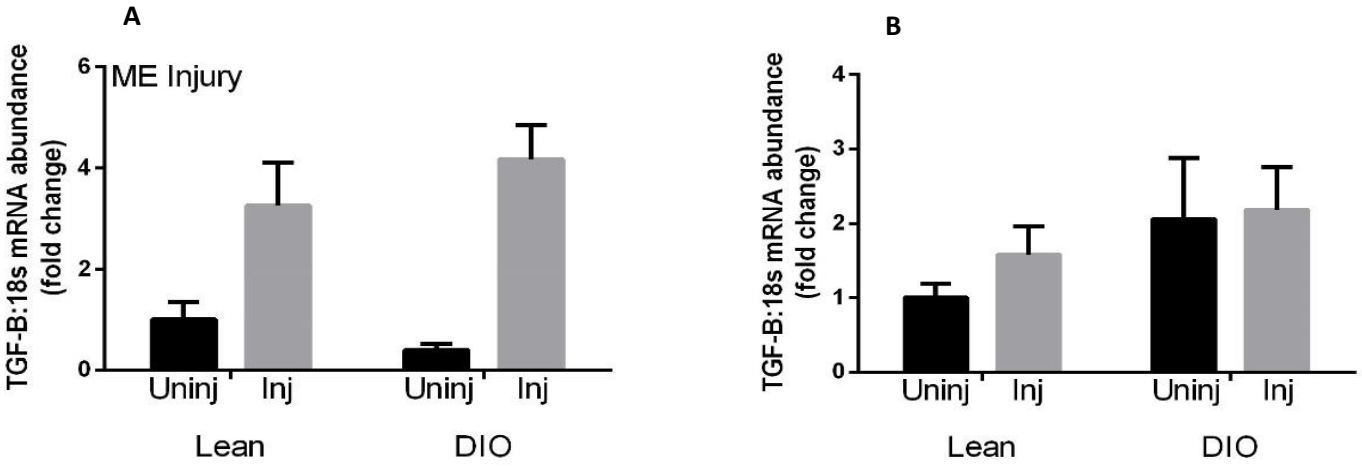

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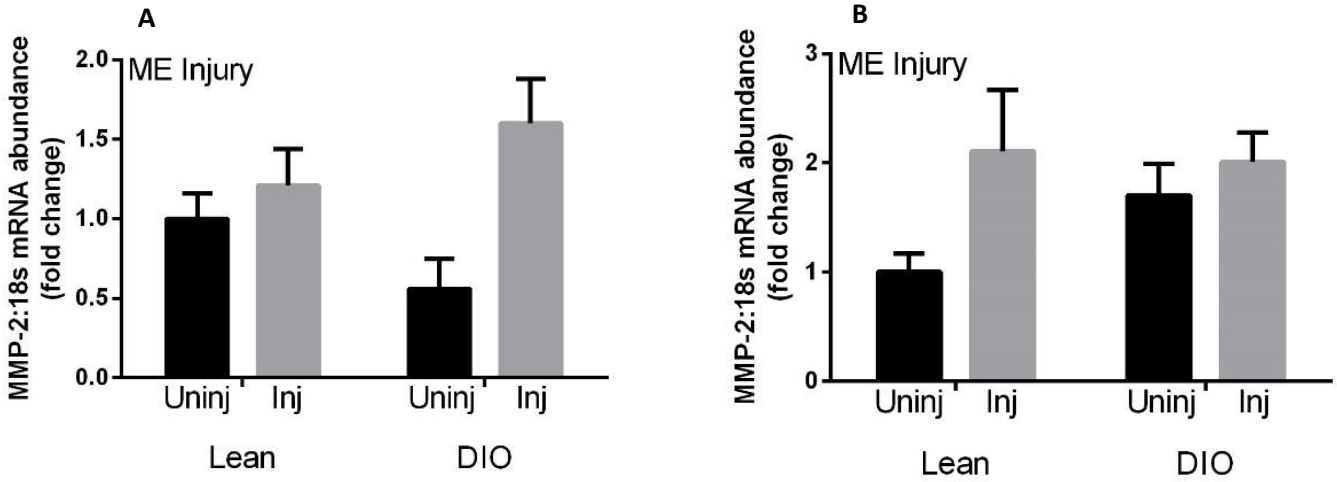

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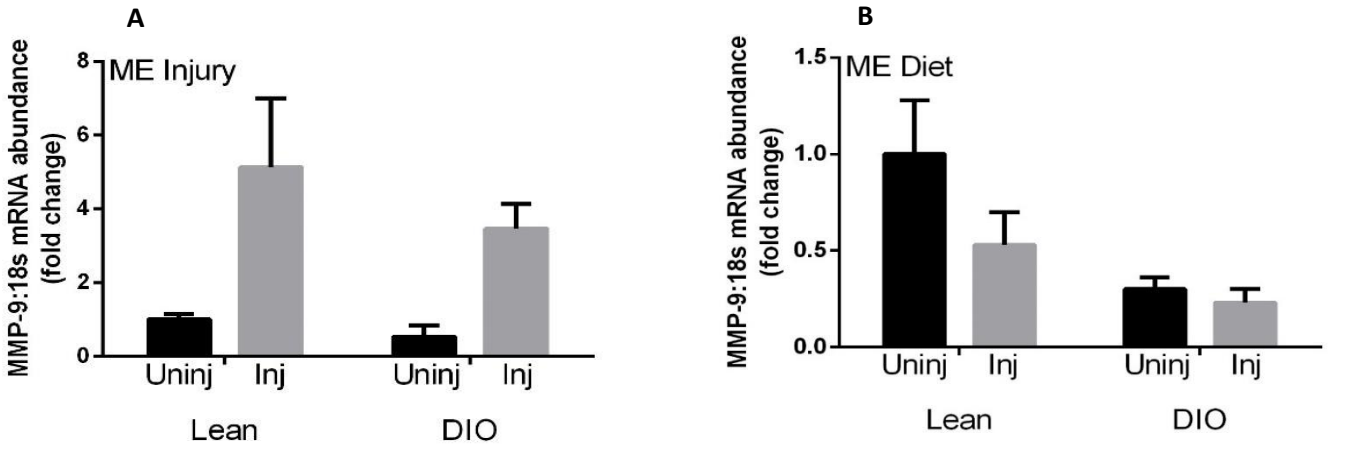

Figure. 8