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Expression of Systemic and Localized MicroRNAs after a Tendon Loading Activity

Daniel Conde[1], Manuel Gomez[2], Raul Zubia[3], Ricardo Rangel[4] and Gabriel Ibarra-Mejia[5]

[1]Physical Therapy and Movement Sciences Department, The University of Texas at El Paso

[2]Interdisciplinary Health Sciences Ph.D. Program, The University of Texas at El Paso

[3]Department of Kinesiology, The University of Texas at El Paso

[3]Bio Eternal Science Lab, Mexico City, Mexico

[4]Department of Public Health Sciences, The University of Texas at El Paso

Corresponding author's Email: daconde@utep.edu

Author Note: Dr. Daniel Conde has a Ph.D. in Interdisciplinary Health Sciences from the University of Texas at El Paso. He is currently a postdoctoral researcher in the Physical Therapy and Movement Sciences Department at the University of Texas at El Paso. Manuel Gomez has a Master's in Kinesiology from the University of Texas at El Paso, and current Ph.D. student and Graduate Research Associate at The University of Texas at El Paso. Raul Zubia has a bachelor's in nursing and a Bachelor's and Master's in Kinesiology from The University of Texas at El Paso; he is currently a Registered Cardiology Nurse. Dr. Ricardo Rangel-Martinez holds a Ph.D. degree in biological sciences and is the scientific director for Bio Eternal Science Lab in Mexico City, Mexico. Dr. Gabriel Ibarra-Mejia has an MD and MS degree from Universidad Autonoma de Ciudad Juarez, a MS degree in Ergonomics from Lulea Tekniska Universitet in Sweden, and a Ph.D. in Environmental Science and Engineering from the University of Texas at El Paso. He is currently an Associate Professor in the Department of Public Health Sciences at the University of Texas at El Paso.

Abstract: This exploratory case study investigated the acute changes in mir-192-5p expressions after a hand gripping exercise with and without compression sleeves, to assess both localized and systemic responses. MicroRNAs (miRNAs) are crucial in regulating gene expression post-transcriptionally, including inhibiting translation. Specifically, mir-192-5p has been identified in the peripheral blood following tendon injuries, highlighting their potential role in tendon health. Compression sleeves are widely utilized for both preventing and treating tendon injuries, yet the impact of these sleeves on miRNAs related to tendon injuries remains unexplored. The purpose of this exploratory case study was to determine the acute localized and systemic changes in mir-192-5p after a hand gripping exercise performed with and without a blood flow restriction (BFR) cuff and graded compression sleeves. The study involved two apparently healthy 20-year-old males who underwent anthropometric measurements during their initial visit. Subsequent visits involved drawing a 10 mL venous blood sample from each hand, assessment of resting blood pressure to determine the pressure needed to achieve BFR, and maximal grip strength to calculate the 60% of the max used for the gripping task. Participants completed a 2-minute gripping exercise, alternating 3 seconds of gripping and 2 seconds of rest, under four different conditions: without sleeves and no cuff (NS + NC), with sleeves and no cuff (S + NC), without sleeves but with a cuff (NS + C), and with both sleeves and cuffs (S + C). Blood samples were collected immediately post-exercise for miRNA analysis using RTqPCR. The study found differences in mi-192-5p expression, indicating a localized response influenced by compression sleeves and cuffs. Also, the results show a difference in the expression on the active and resting hands across different conditions, except for the S + C condition. These findings suggest that there is a localized miRNA response to acute tendon load, leading to the potential use of miRNAs as early indicators of tendon damage due to mechanical overload. We conclude that further research into miRNA expression changes during repetitive handgrip motions will unveil early signs of tendon strain due to mechanical overload, supporting the development of more effective injury prevention strategies.

Keywords: miRNA, *tendon load, tendon injury, compression sleeves, gene expression*

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1. Introduction

Work-related musculoskeletal disorders (WMSDs), including tendon injuries, are among the most reported cases resulting in days away from work in the U.S. In 2022, WMSDs accounted for a total of 502,380, including 365,420 from injuries associated with muscles, tendons, ligaments, and joints (Da Costa & Vieira, 2010; Statistics, 2024). Tendons are the specialized tissues that connect muscles to bones, allowing mechanical forces to be transmitted to the musculoskeletal system (Gracey et al., 2020). Repetitive mechanical loading and degeneration of their collagen fibers may result in acute or chronic tendon injuries (Lin et al., 2004). Healthy tendons are predominantly made of collagen I and contain a small amount of collagen III. When tendons are continuously exposed to repeated mechanical load and become damaged, collagen III becomes the main component (Liu et al., 2021; Maffulli et al., 2000). When a tendon is injured, a series of molecular mechanisms are activated (Nichols et al., 2019). Some of these mechanisms can be regulated by micro RNAs (miRNAs), including inflammation, cell proliferation and differentiation, remodeling of the extracellular matrix, angiogenesis and cell death (Liu et al., 2021).

The switch from collagen I to collagen III happens in the tendons' microenvironment, where different biomolecules interact, including miRNAs (Dubin et al., 2018). The miRNAs are small non-coding RNAs formed by 19 to 24 nucleotides. Their primary function is the inhibition of protein synthesis by binding to the three prime untranslated region (3'-UTR) of target messenger RNA (mRNA) to prevent its translation to proteins (Castell-Auvi et al., 2013; Ye et al., 2019). Some studies suggest miRNA can regulate gene expression by interacting directly with proteins (Qin & Xu, 2013). Most of the miRNAs are within cells, but some are extracellular miRNAs, commonly found in different biological fluids, including circulating blood (Ohshima et al., 2010).

In tendons, miRNAs are associated with tenocyte differentiation and collagen type modification (Greene, 2015). One of the miRNAs associated with tendon injury includes miR-192-5p. This miRNA has been found to have a role in rotator cuff tendinopathy having a downregulation in degenerative, tears, and chronic tendinopathies. However, the acute expression of this miRNA after mechanically loading the tendon is unknown. Therefore, the purpose of this research was to assess localized and systemic differences in the acute expression of miR-192-5p after completing a hand-gripping task with the dominant hand.

2. Methods

2.1 Participants

Two apparently healthy, 20-year-old males from the University of Texas at El Paso agreed to participate in the study. Only male participants were included to minimize the possible effects of estradiol and progesterone on miRNA expression (Schisterman et al., 2014). The participants did not have known musculoskeletal injuries, did not have surgeries, were not engaging in strenuous or repetitive physical activities (i.e., CrossFit, powerlifting, construction/manufacturing work), and were not taking any anti-inflammatory medications for the past year. Both participants were asked to refrain from strenuous physical activity involving the upper limbs while participating in the research. Participants were asked to continue their normal routine if they engaged in regular physical activity.

2.2. Anthropometric Measurements

Body weight (kg) and height (m) were measured using a Detecto™ scale with a stadiometer. Body mass index (BMI) was calculated by dividing the weight in kilograms by the square of the height in meters (BMI = weight (kg) / height (m)^2). Resting blood pressure (mmHg) and heart rate (BPM) were measured using an Omron™ digital blood pressure monitor after the participants rested for at least 10 minutes after arriving at the laboratory.

2.3 Surface Electromyography and Maximal Grip Strength

During each visit, before the start of the hand-gripping task, the research assistant attached dual surface electromyography (sEMG) electrodes in the extensor digitorum longus and the flexor digitorum superficialis muscles of the participant's dominant hand. After the electrodes were attached, the participants were asked to hold a wireless hand dynamometer with their dominant hand, grip it as hard as possible, and maintain a 90° at the elbow (Figure 1a, 1b, and 1c).

The maximal amount of force (N) was recorded during the grip strength assessment and used to calculate 60% of the participant's maximal grip force for the hand-gripping task.

Figure 1. a) Participant showing having a sEMG electrode placed on the extensor digitorum muscle. b) Participant showing a sEMG electrode placed on the flexor digitorum superficialis muscle. c) Participant showing gripping the hand dynamometer while maintaining a 90° at the elbow.

2.4 Blood Sampling, miRNA Extraction and Lactate

After measuring the maximal grip strength, a registered nurse (RN) inserted an intravenous catheter with an extension line into one of the dorsal veins of the dominant and non-dominant hands. The catheter was secured using Tegaderm to prevent it from moving during the hand-gripping task (Figure 2a). A blood sample of approximately 20 cc was obtained from the dominant hand (localized) and the non-dominant hand (systemic) before and after the hand-gripping task. To ensure a reliable sample was obtained, the RN washed the line with a saline solution of approximately 10 cc before obtaining a small "waste" sample; once the sample was discarded, a 20-cc sample was obtained and immediately centrifuged to separate the plasma from red and white blood cells (Figure 2b). Total miRNAs were immediately extracted and stored at -80°. The remaining plasma samples were also stored at -80°C.

Circulating blood lactate was measured using the Nova Biomedical lactate meter from a drop of blood of approximately 100 µL obtained from the 20-cc sample. A pipette was used to obtain and load the sample into the lactate meter strip.

2.5 Blood Flow Restriction and Graded Compression Sleeves

A blood pressure cuff (sphygmomanometer) was placed on the dominant arm's biceps and inflated to 50 mmHg above the resting diastolic pressure to obtain a localized blood sample. The cuff created venous occlusion while maintaining arterial blood flow to the arm. The correct sleeve size was determined by measuring the wrist circumference following the manufacturer's instructions. The compression sleeves created a graded compression in the forearm equal to 12 mmHg at the wrist and 8 mmHg just below the elbow (Figure 3).

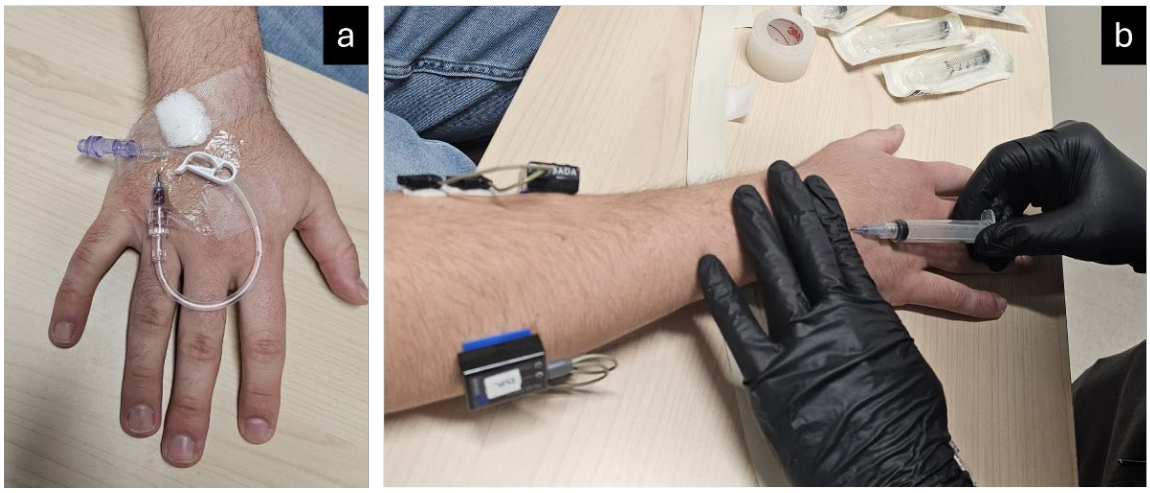


Figure 2. Venous blood samples. a) An intravenous catheter attached to an extension line was inserted into one of the dorsal veins of the hand to procure a blood sample before and after the hand-gripping task. b) The blood sample was taken after washing the line with 10cc of a sterile saline solution.

Figure 3. The participant is seen wearing the graded compression sleeve. The gray part of the sleeve creates a graded compression equal to 12 mmHg distal to 8 mmHg proximal.

2.6 Hand Gripping Task

The participant was asked to grip the hand dynamometer, producing and maintaining a force equal to 60% of the maximal grip for two seconds and resting for three seconds. The participants repeated this cycle until they completed two minutes. The participants completed the same task under four conditions randomly assigned (Figure 4a, 4b, 4c, 4d).

1. With no sleeves and no blood pressure cuff (NS, NC)
2. With no sleeves and the blood pressure cuff (NS, C)
3. With sleeves and no blood pressure cuff (S, NC)
4. With sleeves and the blood pressure cuff (S, C)

Figure 4. Participant showing the different hand-gripping task conditions. a) no sleeves, no cuff. b) no sleeves, cuff. c) sleeves, no cuff. d) sleeves, cuff

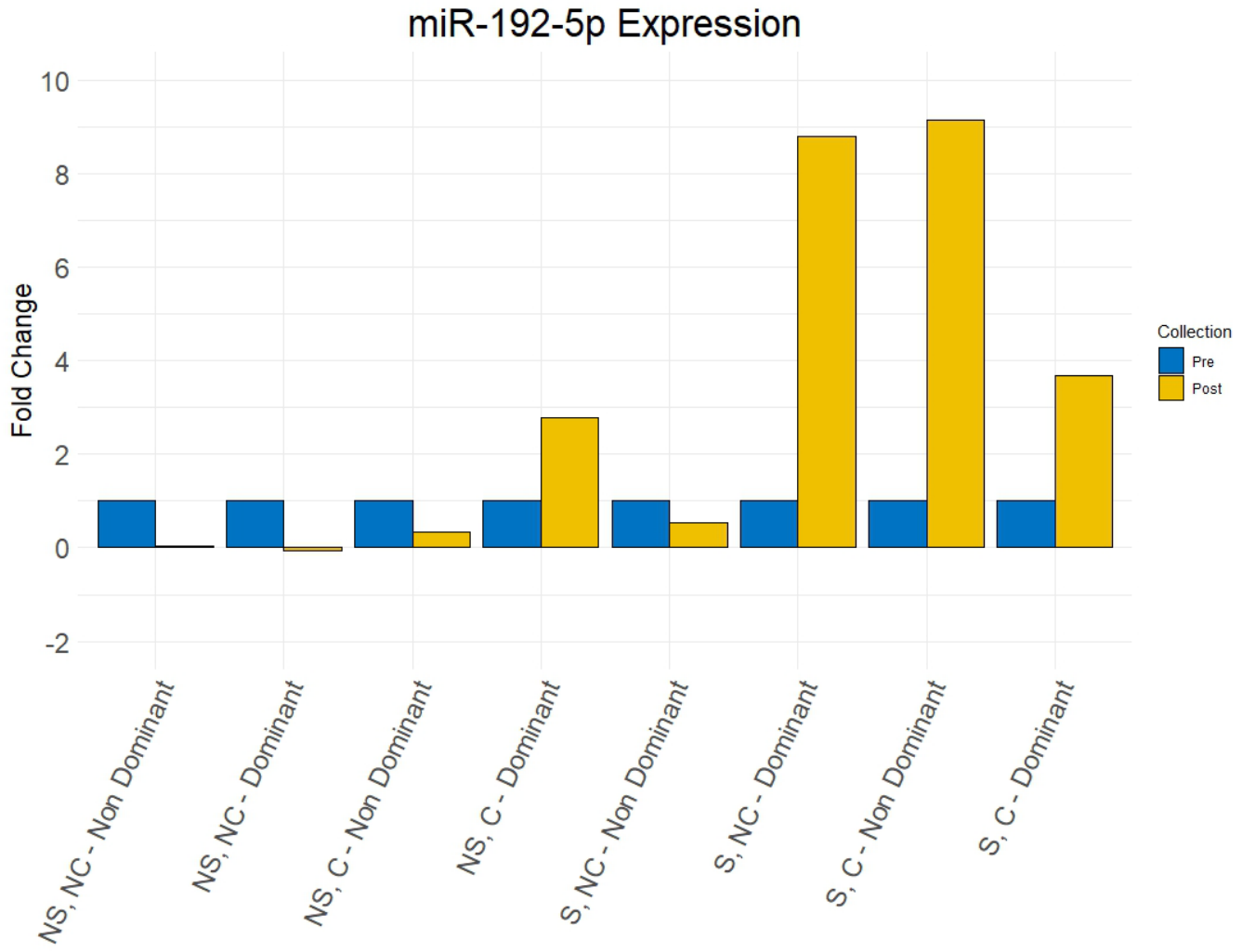
2.7 miRNA Expression

Total circulating miRNA was extracted using the Qiagen miRNeasy kit, following the manufacturer's instructions, and stored at -80°C until all samples were extracted and ready for analysis. Once all the miRNA was extracted from the samples, it was reverse transcribed into complimentary DNA (cDNA) using the Taqman Advanced miRNA cDNA synthesis kit. The target miRNA 192-5p were amplified using the Taqman Fast Advanced Master Mix, and their expression was assessed using the StepOne PCR system. The relative expression of miRNAs was determined using the ΔΔCt method, with small nuclear

RNA U6 (snU6) serving as the internal loading control for normalization. All results are presented as fold changes relative to snU6.

3. Results

The study revealed significant differences in miR-192-5p expression post-hand gripping task, with distinct patterns between dominant and non-dominant hands under various conditions, suggesting a localized response influenced by compression sleeves and cuffs. The relative fold changes presented on figure 5 show a difference in the expression of miR-192-5p before and after completing the hand-gripping task (Figure 5). Also, the results show a difference its expression in the dominant and non-dominant hands. There was an increase in the non-dominant hand with the sleeve and the cuff and decreases in the non-dominant with no sleeve and no cuff, with no sleeve and cuff, and with the sleeve and no cuff. Furthermore, there were increases in the dominant hand with no sleeves and the cuff, with sleeves and no cuff, and with sleeves and cuff, and a decrease with no sleeves and no cuff.



4. Concluding Remarks and Future Directions

The results of this experiment suggest a localized response in the expression of miR-192-5p after mechanically loading the tendons of the forearm. This response seems to be associated with the use of graded compression sleeves, and the cuff, rather than to the mechanical loading of the tendons. This response is shown by the crease in miR192-5p in the dominant arm with no sleeves and no cuff, and an increase when the sleeves, the cuff, or both are being used.

Even though the results do not seem to completely follow those found in the literature, where there is a downregulation after injury, it is important to note that when no compression is applied, there is a downregulation of the miRNA. These preliminary results will help us modify the methods to evaluate the expression of miRNAs associated with tendon damage and their possible use as markers of tendon mechanical load. Also, it will allow us to evaluate the effects of adding compression to mechanically loaded tendons.

Future studies by our research group will focus on gaining a deeper understanding of the impact of mechanical load on the tendon microenvironment and further exploring the role of miRNAs in tendon health and injury. We will study other miRNAs involved in tendon damage and healing, such as miR-210-3p and miR-145-5p. This understanding is essential for designing better work-to-rest ratios and may provide insights into the therapeutic use of miRNAs for the treatment of WMSDs

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