THE EFFECTS OF DISUSE FROM LOWER LIMB IMMOBILIZATION ON PROPRIOCEPTION

By

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THE EFFECTS OF DISUSE FROM LOWER LIMB

IMMOBILIZATION ON PROPRIOCEPTION

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Title of Study: THE EFFECTS OF DISUSE FROM LOWER LIMB IMMOBILIZATION ON PROPRIOCEPTION

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Abstract: The purpose of this study was to examine the effects of two-weeks of left leg immobilization on proprioception using tendon taps (TT) and a reactive leg drop (RLD) test in young healthy adults. Twenty-seven subjects (26.1 ± 4.8 yrs) volunteered to participate in either a disuse or control group. Disuse subjects were required to not use their left leg for two consecutive weeks while wearing a knee brace set to 100° of flexion and were to use crutches. The control group did not change their living habits within the two-week period. Both groups were tested pre- and post- two weeks. During testing an electronic goniometer was attached to the participants leg at a 90° and 180° angle for tendon tap (TT) and the reactive leg drop (RLD) test, respectively. During both tests, participants performed the Jendrassik maneuver; hands clasped while pulling in opposite directions, eyes closed with the head back. During the TT, the patellar tendon was struck with a weighted hammer to induce a knee jerk reflex. During the RLD, an investigator supported the weight of the participants leg while it was fully relaxed, at random intervals the leg was dropped, the participant was instructed to kick their leg back to the previous position as quickly as possible when they felt their leg begin to fall. Reflex magnitude (°) was recorded during TT and drop angle (°) was recorded during RLD tests. Due to the difficulty of this study, a total of 18 subjects finished the protocol (disuse n=7, control n=11). A 3-way mixed factorial ANOVA was used to investigate the reflex magnitude of TT and the drop angle of the RLD in Time × Limb × Group. There was a significant interaction of Time and significant correlation between Limb × Group from RLD and a significant correlation between Time × Group from TT. Those in the disuse group had a significantly lower TT change than the controls, but they had a significantly greater RLD change than the controls. With modifications to these measures, these tests will be an effective way to measure proprioception in future research.

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CHAPTER I

INTRODUCTION

Proprioception, originally termed 'muscle sense' in the 19th century, is the body's ability to sense position, movement, and location of the limbs and trunk. Before the early 20th century, it was theorized that your 'muscle sense' was the brain constantly monitoring the body's position and movement. It wasn't until definitive evidence by Charles Sherrington proved that sensory afferents have influence on the muscle contraction through a peripheral source (Tuthill & Azim, 2018). With this evidence, more research was done by Goodwin in 1972 that demonstrated the key role muscle spindles play in proprioception (Winter, Allen, & Proske, 2005).

Muscle Spindles, as stated by Zhao et al. (2010), are stretch receptors that primarily detect changes in length of the muscle and convey the information to the central nervous system. Each muscle spindle has a primary sensory nerve ending (Macefield & Knellwolf, 2018) responsible for sensing the movement and positioning of our body without visual feedback (Winter, Allenn, & Proske, 2005). Muscle spindle research is predominantly done in animal models in order to extract and examine the spindles. Few studies have been done with human models, one by Winter, Allen, & Proske (2005) observed a strong correlation between muscle spindles and muscle position sense.

Disuse is used in research to induce decreases in physiological mechanisms (Victor, Bloomfield, & Greenleaf, 1997) in the body (e.g., muscular atrophy and strength loss). Limb immobilization and bed rest protocols are primary used to induce these physical changes in research. Both are used in clinical settings, limb immobilization targets specific muscle groups, while bedrest is used to see the physiological changes of the entire body during weightlessness (de Boer et al, 2008, Manganotti et al, 2021), and is used to simulate spaceflight. Disuse studies are used in both human and animal models. In human models, it's been observed that the quadriceps force, volume, and muscle cross sectional area (CSA) significantly decrease after 10 days of bedrest (Maganotti et al, 2021) and significant muscle thickness decrease of the vastus lateralis (-12.2%) and gastrocnemius medias (-8%) after five-weeks of bedrest (de Boer et al, 2008). Leukel et al (2014) investigated the changes in the excitability of the corticospinal pathways after eight-weeks of ankle immobilization, with no changes found pre- to post-disuse. Hind-limb immobilization of rats has shown to decrease muscle spindle discharge between 14- and 21-days post disuse (Zhao et al. 2010, Rosand et al. 2006). To this authors knowledge, no study has looked at all these measures in a human model disuse study.

While many studies have examined the motor deficits that arise from disuse, very little is known in regards to sensory deficits. One study showed a decrease in upper limb proprioception within the first 24-hours of immobilization (Moisello et al. 2008). However, the upper and lower limbs often have different responses to extended immobilization. Simple ways to measure sensory responses in the lower limb are through tendon taps (TT) and using a novel reactive leg drop (RLD) test. TT are used to elicit a response in a muscle group after a tendon is tapped or stimulated. Tham et al. (2012) observed that 45° and 60° of knee flexion elicits the best patellar tendon tap response. Disuse has been shown to hinder the Achilles tendon response after 72-hours of lower leg suspension (Seynnes et al. 2008). The RLD test is a novel way to assess voluntary sensory responses in the lower limb (Magrini et al. 2017). This tests the speed in which an individual can blindly correct their limb after displacement.

1.2 Purpose of the Study

The purpose of this study was to examine the effects of two-weeks of left leg immobilization on proprioception using tendon taps (TT) and a reactive leg drop (RLD).

1.3 Research Questions/Hypotheses

How will two weeks of lower limb immobilization affect the proprioceptors in the lower limb?

- Hypothesis: There will be a decrease in TT reflex magnitude.
- Hypothesis: The immobilized leg will have a slower reaction time, resulting in a greater loss in degrees from pre values during the RLD test.

1.4 Significance of the Study

This study has the potential to increase our understanding on the effect that bedrest and limb immobilization has on proprioception. The findings could be in not only an athletic population, but a clinical one, using this information for both the young and old. Proprioception of the lower limb is still something that needs to be studied, current literature focuses on upper limb proprioception rather than lower limb. These measures have not been used to test proprioception after disuse.

1.5 Delimitations

1. Approximately 10-15 disuse subjects and 10 control subjects were needed for this study.

2. Participants were between 18-39 years of age

3. All participants were able to walk independently without aid, and no physical limitations, neuromuscular or muscular disease or illness.

1.6 Limitations

1. Not all the disuse participants complied to the protocol.

2. Approximately half of the disuse subjects dropped midway through protocol due to difficulties with the study.

3. The TT and RLD tests were not done by the same investigator throughout the study

4. It was difficult to find a TT reflex in multiple participants, causing one disuse and three control subjects TT data to be excluded.

5. The study was underpowered.

1.7 Assumptions

1. It was assumed the participants would fully adhere to the disuse protocol.

2. Participants would follow directions during the TT and RLD tests.

CHAPTER II

LITERATURE REVIEW

2.1 Proprioception

Avanzino et al. (2013)

The aim of this study was to assess the role of proprioception in preventing interhemispheric imbalance induced by short-term limb immobilization.

Twenty-eight subjects were divided into three groups, no-vibration (10), proprioceptive vibration (10), and tactile vibration (8). The two vibration groups received a high-frequency (80Hz) and low-frequency vibration (30Hz) during the immobilization period. All participants were right-handed, had no neurological disorders, no orthopedic problems, and were unaware of the purpose of the experiment. All subjects wore an arm sling that stabilized the hand, wrist, fingers and forearm for a 10-hour period. Subjects were instructed not to move their right hand for the entirety of the period. The left arm was monitored via an accelerometer, though subjects were free to move the left arm. The accelerometer was worn the day before and during the experiment period for the same amount of time (10 hours, 8am-6pm) both days. The energy expended was measured in METs.

EMG electrodes were placed on the FDI and first metacarpophalangeal joint bilaterally, with the ground electrode placed on the elbow. Cortical excitability was tested in the left and right primary motor cortex using input/output recruitment curve and interhemispheric inhibition via TMS. This was conducted the day before (pre) and immediately after the 10-hour immobilization (post).

For both vibration groups, the right hand was vibrated through a small hole in the sling perpendicular to the FDI. The proprioceptive vibration group received a vibration of 80 Hz, an amplitude of 80% of the maximal amplitude, while the tactile vibration group received a vibration of 30 Hz, an amplitude of 15% of the maximal amplitude. Subjects were blindfolded during the first two vibration sessions. Vibration sessions occurred twice every hour between 9am and 5pm. The sessions lasted 7.5 minutes, with 1-minute of vibration followed by 30s of no vibration, this was repeated 5 times.

RTM of left M1, RM-ANOVA showed significant interaction TIME*GROUP. RMT increased significantly in the no-vibration and tactile vibration group, but not the proprioceptive vibration group. The excitability of the left hemisphere was significantly reduced by the immobilization in all groups. A positive linear correlation was found between the MEP amplitude and the TMS intensity for both the left and right M1s. No differences in the slope was observed between the two vibration groups before immobilization. The slope of the left M1 in all groups was reduced due to immobilization. The RMT of the right M1 did not change after immobilization in any group. Excitability of the right M1 increased significantly in the no-vibration and tactile vibration groups, but not the proprioceptive vibration group. Left-Right IHI was reduced in the no-vibration and tactile vibration groups. Right-left IHI was shown to increase in the no-vibration and tactile vibration groups after immobilization. The proprioceptive vibration group showed no change in either of these trials.

Balslev et al. (2008)

Ten right-handed subjects participated in this study. All were healthy, had normal vision, and used a computer mouse in their daily life. The participants were sat in front of a computer screen, they used their right hand to control the computer mouse, and their right hand was hidden from view. Each trial began with the starting position in the upper right corner of the screen. A red square would appear 45 degrees from the target with a visual line connecting the start position with the target. Subjects were instructed to move the cursor from starting position to the target as fast and accurately as possible. At the end of the trail, the target would disappear, and the next trial wouldn't start until the cursor was at the starting position again. Each trial lasted 1 second. During the trials, the target would move either 20 degrees clockwise or counterclockwise of the cursor trajectory or jump 20 degrees away from the cursor trajectory. The subjects were told what to expect before each trial began. Subjects participated in two sessions consisting of 4 blocks of 50 trials. rTMS was conducted between the sessions.

Before rTMS began, each subject's "hot spot" was determined on the left hemisphere, 3 cm posterior to the hot spot was the stimulation site, and the stimulation was 110% of the individual's resting motor threshold. The resting motor threshold was determined by the lowest stimulation intensity the FDI muscle of the right hand would react to. Subjects went through two sessions, real and sham.

There was no difference found between the rTMS and the sham rTMS in either cursor rotation or the target jump trial. There was a significant interaction between trial type and intervention type. There was a significance between reaction time and cursor distance (+0.41), between reaction time and visual error (-0.28), start value for both were 0.

Alghadir et al. (2020)

The aim of this study was to observe the effect of chronic ankle sprains on pain, range of motion, proprioception, and static/dynamic balance among athletes.

Sixty track and field athletes (18-25yrs) were used in this study. They were split into two groups, group A consisted of athletes with a history of grade 1 or 2 ankle sprain requiring medical care who reported ankle giving way at least three times in the last 12 months. Group B consisted of athletes without a history of lower limb sprain in the past year, acted as control. Athletes with a grade 3 sprain were excluded from the study. Pain was measured using the Visual Analog Scale (VAS), active dorsiflexion, plantarflexion, inversion, and eversion ROM was measured using a goniometer while the athlete lay in a supine position. With the athletes' eyes closed, proprioception was measured by positioning the non-affected ankle joint in some degree of dorsi- or plantarflexion and asking the athlete to match the position with their affected ankle. Static balance was measured using a single leg stance time test. Athletes were to stand on one leg with their eyes open and closed and were timed (in sec) to see how long the athlete could maintain their stance with minimal movement. Dynamic balance was assessed using the Y-balance test (YBT), the investigators were able to measure the athletes' reach distance in anterior, posteromedial, and posterolateral directions. Group A reported a significantly higher pain score at assessment compared to group B. There was a significantly higher mean difference in position sense in group A compared to group B. Single leg stance was significantly higher in group B than in group A during both eyes open and closed. Group B reached distance was significantly higher in all three-direction compared to group A.

2.2 Muscle spindles

Rosant et al. (2006)

The aim of this study was to see if muscle spindle discharge would differ after 21 days of hindlimb unloading in rats. Twenty-four male Wistar rats were randomly assigned to control (12) or hindlimb unloading (12) groups. The rats were suspended by the tail and connected to the top of the cage to a swivel that allowed 360º rotation. At the end of the suspension period, the rats were anesthetized, and the right or left soleus was randomly selected for either the neuromechanical or immunohistochemistry study.

Neuromechanical: To measure the soleus, the knee and ankle were flexed to 90°. The soleus nerve was severed and dissected; this was to remove the muscle spindles motor control. The Achilles tendon was severed and attached to a device that measured passive force and muscle length. The muscle was stretched to two different lengths, a slack length and to the length that corresponded to the ENG threshold. The muscle was first stretched to slack length, ramp and hold stretches were randomly inflicted at 0,5, 1, and 2mm at velocities of 1,2,5,10,30,50, and 80 mm/s. This was done for three trials, then a second series began using the second stretch length.

Immunohistochemical: This experiment used 8 control and 8 unloading rats. The soleus muscle was excited, stretched, and frozen in its resting length. The muscle was cut in transverse sections, 5 sections were cut every 200-mu m. The first was used as control, the next portion was processed for ATPase, the final three sections were labeled with antibodies. All sections were then incubated at 25°C for 90 minutes. The slides were washed in three different solutions for a total of 20 minutes, put back in the incubator for 10 minutes, and washed again for 8 minutes before being dehydrated. The muscle spindles were stained and examined under a microscope.

The muscles in situ were significantly shorter in the unloading group than in the control group. The slack length and neurogram length in the control group was considered identical but was significantly different in the unloading group (P=0.0024). There was a significant decrease in muscle CSA, the unloading groups peak and steady stress values were significantly higher at slack length. Static and dynamic conditions were significantly higher in the unloading groups than the control group during slack length. A significant difference was found in the spindle efficacy index (SEI) values of the unloading group. As the values decreased the stretch amplitude increased, this difference was significant at the slack length. During slack length, the SEIs were significantly lower whatever the stretch amplitude was.

Zhao et al. (2010)

The aim of this study was to see the changes of muscle spindle discharge activity in rats after 14 days hindlimb unloading (HU). Fourty-one Female Sprague-Dawley rats were randomly split into two groups, control (CON) (17) and HU (24) for this study. The rats' muscles were dissected and measured during flexion and extension to measure the muscle lengths from knee to ankle joint. Muscle spindles and sensory axons were isolated, approximately 4-8 spindles were isolated per rat.

The isolated muscle spindles were placed in a salt solution, one side was fixed rigidly, while the other was attached to a mechanical stimulator to provide stretching. The soleus was placed in its shortened position and was held for 30-40 minutes. During this time, the afferent discharges of the spindle were recorded. The spindles were placed in a solution and soaked overnight. The following day they were then repeatedly rinsed in a different solution. The tissues were sliced and examined under a light microscope. Selected sections were stained and observed under an electron microscope.

A total of 238 muscle spindles were isolated: 73 from the CON group and 165 from the HU group. Spontaneous discharges appeared in all CON receptors and only 37 spindles in the HU group during the shortened position. The most typical discharge characteristics observed were high-frequency spike clusters. 76% of discharges were organized in clusters after 14 days of HU, this was a significant change. After HU, discharge rates significantly decreased in the extended position.

Winter, Allen, & Proske (2005)

The aim of this study was to examine if muscle spindle signals and sense of effort contribute to position sense (proprioception) of the forearm.

Fifteen subjects were used in four different experiments, not all subjects participated in all the experiments. They attended control trials to familiarize themselves with the procedure, they were also required to achieve certain accuracies to participate in the experiments. During all experiments, participants had their arms strapped to paddles to keep their forearms in position.

Experiment 1: Subjects were blindfolded with both arms resting in a horizontal position (0°). When instructed, the subjects would flex their reference arm into an approximate 45° position, cued when to stop flexion. Using their other (indicator) arm, they were asked to match the position of the reference arm. They did this for a total of 10 trials. The following 10 trials, the subject was asked to keep their reference arm totally relaxed as an experimenter moved the arm to a 45° position, the arm was then placed on a support so the subject could remain relaxed. Audio feedback was provided from electromyographic (EMG) activity recordings from the biceps brachii to ensure subjects adhered. Again, they were asked to match the position of the reference arm with their indicator arm.

Experiment 2: The supported portion of experiment one was repeated, but the muscles were conditioned before the experiment began. Two forms of conditioning were applied, elbow flexion and elbow extension.

Experiment 3: Had the same arrangement as experiment one. The position matching done previously was used with and without a 2kg weight. The weight was attached to the reference arm so it induced 6 Nm of torque when the elbow was flexed to 45°. Experiment 4: Experiment three was repeated, but with muscle conditioning.

There was a significant difference between the degrees of error in the supported and unsupported arm, the supported arm had a significantly higher rate of error. After flexion conditioning, the indicator arm acquired a more extended position when matching the reference arm. Errors of position between weighted and unweighted arm were significantly different in experiment three. There was a significant difference of position errors after flexion conditioning compared to extension conditioning.

2.3 Disuse

de Boer et al. (2008)

The aim of the study was to assess the adaptations of muscle size and architecture in the gastrocnemius medialis (GM), tibialis anterior (TA), vastus lateralis (VL), and biceps brachii (BB) muscles following 5 wks of horizontal bed rest. Ten healthy men were used. A week prior to the beginning of bed rest, the participants visited the laboratory to familiarize themselves with the testing procedures, the baseline (pre) data was collected during this time. Ultrasound images of the GM, TA, VL, and BB were taken for pentation angle, fascicle length, and muscle thickness. The GM and VL thicknesses decreased significantly, 12.2% and 8.0%, respectively. The fascicle length in the VL decreased by 5.9% and the pentation angle decreased by

13.5%. The GM fascicle length decreased by 4.8% and the pentation angle by 14.3%. There were no changes to the TA and BB. The greater atrophy in the VL and GM may be related to the amount of loading both muscles undergo daily.

Leukel et al. (2014)

Aim of the study was to investigate the changes in the excitability of the corticospinal pathways after 8-week of ankle immobilization.

Nine healthy males were subjects in this study and went through 8 weeks of ankle immobilization. The immobilized leg (left or right) was randomly chosen before the study began. This was part of a multidisciplinary study looking at the physiological consequences of immobilization, seven control subjects were recruited for this portion of the study. 48 hours before the beginning of the intervention (pre) and immediately following the removal of the cast (post), electrophysiological measurements were taken. The measurements and order are as follows: stretch reflex (sitting and standing),, H-reflex recruitment curves, MEP recruitment curves, Hcond. Stretch reflex and H-reflex were not measured in the control group. EMG was obtained from the soleus (SOL) and tibialis anterior (TA) muscles using bipolar surface electrodes of the immobilized leg. A custom ankle ergometer was used to measure the fast and slow stretch reflex of the SOL post immobilization. The fast and slow stretches (300°/s and 150°/s, respectively) were performed with either sitting with the knee and ankle at 90° or standing with the knee at 180° and the ankle at 90°. There were four conditions randomly administered with a total of 10 consecutive stretches per condition. H-reflex was measured, sitting or standing, to detect strength changes in Ia sensory fibers in spinal motoneurons. This was done using electrical stimulation on the posterior tibial nerve. Participants sat during the TMS portion. Using a figure 8 coil, the hotspot was identified for the optimal MEPs in the SOL. Two different conditions were tested, MEP recruitment curves and Single-pulse magnetic stimuli. Peak-to-peak-amplitude of 10 conditioned and 10 unconditioned control H-reflexes were calculated from the SOL EMG. These H-reflexes were taken at each interstimulus interval (ISI) and averaged in each subject.

There was no significant difference in stretch reflex between pre to post in the immobilized group. A significant difference was found between control group and immobilized group pre vs post MEP, and a significant reduction in Mmax values in the immobilized group, but no change in the control group. There was a significant stimulation intensity x time interaction found in the MEP recruitment curve. There was a significant difference in the ISI in both groups. There was a significant increase of Hcond in the immobilized group, ISIs -1, 0, and 4ms.

It is believed that the stretch reflex was not affected by the immobilization, however the immobilization caused morphological alterations to the muscles due to the significant reduction in Mmax in the immobilized group but not the control group.

Lambertz et al. (2002)

Aim of the study was to see if reflex excitability is altered by long-term spaceflight, and if these changes are due to central or peripheral mechanisms.

Twelve male cosmonauts that spent approximately 180 in space were used for this study, data was collected 28-42 days before flight, soon after spaceflight, and 5-6 days after return. The tests consisted of tendon taps and electronic stimulations to the Achilles tendon to measure the neuromechanical properties of the triceps surae" via EMG.

The astronauts laid prone on a table to find submaximal H and T reflexes and maximal M waves of the Soleus (Sol). Surface EMGs were placed on the triceps surae muscle group (TS). Additional EMG signals were recorded for the tibialis anterior (TA). Test sessions were done in the following order: 1. Electrical stimulation to record H reflex and maximal M waves, 2. Achilles tendon taps to elicit T reflex at rest, 3. MVCs under isometric conditions, 4. Quick-release movements to determine musculotendinous stiffness, 5. Sinusoidal perturbation during voluntary contractions to recorded stretch reflex, 6. Sinusoidal perturbation at rest to determine musculoarticular stiffness. The total duration of this was 90 minutes.

ANOVA indicated a significant variability between the cosmonauts. A significant main effect of spaceflight was found for differences in T/Mmax, the mean significantly increased between BDC and R+2/+3 and decreased between R+2/+3 and R+5/+6. MVC decreased significantly (17%) when tested on R+2/+3.

Manganotti et al. (2021)

"The aim of this study was to measure the electroneurographic and morphological characteristics of the peripheral nerve stem in both the upper and lower limbs in healthy adults pre- and post 10-day bed rest protocol."

Ten healthy adult males participated in this study, passed all physical exams, and had no exclusion criteria. This was a part of a larger study. The protocol consisted of 10 days horizontal bedrest (BR) in a hospital with 24-hour video surveillance and medical supervision. MRIs of the thighs were done after 8 hours of BR on the first (BR0) and last (BR9) day of the protocol. The median, deep peroneal (DPN), and fibular nerve were identified using ultrasound on days BR0 and BR9, and the cross-sectional area (CSA) of each nerve was measured. Nerve conduction velocity (NCS) was done bilaterally on all participants. F and H responses were recorded from the extensor brevis digitorum and flexor digitorum by stimulating the median and peroneal nerves. One day before BR and BR9, participants tested the MVC of their quadriceps femoris, MVCs consisted of three isometric contractions lasting 4s with a 60s rest between.

Quadriceps force, volume, and muscle CSA decreased significantly due to BR. No significant changes to the nerve CSA were observed, however latency of the median nerve significantly increased. There was a significant decrease in F-wave amplitude in both lower limbs in the peroneal nerve. Altered H-reflex was found in only the dominant limb after BR. A repeated measures ANOVA showed significant TIMExEFFECT in the lower limbs, not significance was found in the upper limb.

Moisello et al. (2008)

Forty-six right-handed males participated in the study. For the first experiment (A), 21 participants were split into three groups. 7 were assigned to 12 hours of arm immobilization, 7 to 6 hours of immobilization, and 7 were not immobilized and acted as control. For experiment B, 18 participants were immobilized for 12 hours and 7 acted as controls. All splints were attached to the upper left arm via Velcro straps. The elbow joint was kept at 90°, preventing the joint from moving, and the arm was placed in a shoulder sling.

Prior to being immobilized, participants were seated at a computer screen with their elbows and wrists at 90° and 45°, respectively. Targets were displayed on the screen 45, 90, and 135° from the cursor's starting point, participants were instructed to make out and back movements from the starting point to each target. They were told to make the lines as straight as possible, not to correct any mistakes, and do it as quickly as possible. The participants did this with and without visual feedback (visual baseline and baseline). Participants were then immobilized and were allowed to perform their daily tasks. Once back in the lab after 6 or 12 hours, the splint was removed and the groups from experiment A. did the tests again without visual feedback. The group from experiment B did the tests with visual feedback. All the control subjects from both experimental groups returned to the lab after 12 hours and did the same tests as their respective groups.

Significant differences between pre and post in the 12 hours immobilization group was found. Distribution of movement onsets, Group x Block interaction (p=.004), variability of IJT increased significantly, and the mean and variability of normalized hand-path area increased significantly. No significance was in pre to post in the 6 hour or control group.

2.4 Reactive Leg Drop Test

Magrini et al. (2017)

The aim of this study was to assess the sensory-motor function in older adults using a novel test, the reactive leg drop. Two groups of participants, older and younger females, visited the lab once for the tests administered. A balance test was done to measure the participants proprioception using a sway index, the participants center of gravity relative to the center of the platform. Four different conditions were done for 30s each, with 30s rest in between conditions. Patellar tendon taps were performed while the participants were sitting with their right leg at 90°. The participants closed their eyes and performed the Jendrassik maneuver, while the knee was tapped to get a stretch reflex response. A reactive leg drop test was performed to measure the participants reaction time. Participants were seated with their leg extended to 180°, supported by an investigator. Subjects were instructed to kick out as quickly as possible as soon as they felt their leg fall. The investigator then dropped the leg in random intervals during multiple trials: eyes open and watching, eyes closed, eyes closed while performing the Jendrassik maneuver, and eyes open with lower limb blocked.

There was a significant difference between the age groups, younger women did significantly better in the eyes closed and blocked view leg drop tests than older women. There was a significant relationship between age and these two leg drop tests. There was a significant relationship between blocked view and eyes closed diverted to ECFS. When groups were combined, there was a significant relationship between eyes closed and reflex latency.

2.5 Tendon Taps

Seynnes et al. (2008)

The aim of this study was to induce tendinous and synaptic changes using unilateral lower limb suspension on tendon tap reflex

Sixteen healthy males between 18-29 years were subjects for this study. All participants were examined by a doctor at baseline and screened for DVT every 72 hours during the suspension period. Subjects were randomly assigned to the suspension (8) and control (8) group. The unilateral lower leg suspension (ULLS) protocol lasted 23 days, the participants were tested on days 0, 14, and 23 of suspension, and six subjects were tested one week as a follow-up. "The control group was tested at baseline and after 3 weeks". Testing measures were performed in the following order: muscle cross sectional area (CSA), T reflex recruitment curve, maximal H reflex (Hmax), maximal M-wave (Mmax), maximal voluntary contraction (MVC), and tendon aponeurosis rate of load-elongation. Testing familiarization occurred one week before baseline.

The right leg was suspended via shoulder strap in a flexed position. Participants were instructed to not load the right leg during the entire study, either by touching the ground or using the muscles in the leg. Compliance to the protocol was monitored by communication with the subjects, who were encouraged to report any failure. EMG of the right plantar flexors were taken prior to leg suspension. The activity of the soleus (SOL) and gastrocnemius lateralis (GL) during regular walking was 16% and 21%, respectively. Maximal CSA was taken of the triceps surae muscles and plantar flexor MVC was assessed before leg suspension. Mmax, H, and T reflexes were measured on the SOL and GL using electrical stimulation while the subjects were lying prone on a table. The posterior face of the Achilles tendon was tapped to elicit a T reflex, this reflex was measured at four different intensities. T1 was defined as the lowest intensity to evoke a response while T4 was defined as the intensity to evoke a maximal reflex.

Mmax amplitude increased in the SOL (5.6 to 6.7mV), but the GL remained unchanged after ULLS. MVC in the SOL decreased significantly, and the MVC in the GL decreased, however, this was not significant. The CSA of the SOL, GL, and GM decreased by 7.1%, 8.8%, and 9.3% respectively. Mean Hmax and Mmax increased significantly (0.60 to 0.74), normalized T and Mmax ratios had no significant change. For the T reflex, the slope of the recruitment curve became significantly steeper 14 days after ULLS. No significance was found in the control group.

Tham et al. (2012)

The aim of this study is to determine the greatest tendon tap response depending on the angle of the leg using motion analysis.

One hundred healthy individuals (50 male, 50 female) between the ages of 21-32 years were recruited to participate in this study. Each participant sat on a high table that allowed their lower leg to dangle freely. The knee was angled at 15º, 30º, 45º, 60º, 75º, and 90º, and tapped five times at each angle. 16 reflective markers were attached to the lower leg of each subject, and motion capture software was used to determine the displacement of the leg after each tendon tap.

There was a linear relation between all the different angles. There was a significantly lower reflex response for smaller tapping angles, 15°, 30°, and 45°. It is suggested that 45º and 60º is the best angle to elicit an adequate response for tendon taps.

CHAPTER III

METHODOLOGY

3.1 Subjects

Twenty-seven subjects (19-37yrs) who could walk independently without aid, had no physical limitations, neuromuscular or muscular disease or illnesses were recruited for this study. Subjects volunteered to participate as a disuse subject or a control subject. Individuals participating in the disuse group were excluded if they had a BMI ≥30 and had an increased risk of blood clotting. The subjects were Oklahoma State University students, faculty, and Stillwater community members. Recruitment was done via Oklahoma State mass emails, flyers hung up in busy campus buildings, as well as presentations in classrooms to undergraduate students in the school of Kinesiology, Applied Health, and Recreation. All subjects filled out an informed consent, health history questionnaire, and a COVID-19 symptom questionnaire.

3.2 Immobilization

Participants in the disuse group were fitted for a Breg TScope knee brace set to approximately 100º of knee flexion. A pair of Sport Swings crutches were fixed to their height. Investigators demonstrated how to properly use the crutches and allowed participants to practice and adjust the crutches to make it the most comfortable as possible. Compression socks were measured to the length of the left leg (upper thigh to the lateral malleolus) and was used to help with blood circulation while the brace was on. Participants were instructed to not use their left leg for the duration of two consecutive weeks, and to keep the brace on at all times excluding bathing and sleeping. They were also given mobility exercises to do twice a day to ensure blood circulation during protocol.

3.3 Tendon Taps

The participants sat comfortably in a Biodex 4 chair with their legs freely dangling. An electronic goniometer was attached to the subject's leg using tape and the knee was bent to approximately 90°. The participant was asked to perform the Jendrassik maneuver (interlocked hands, pulling in opposite directions), with their heads back and eyes closed. The patellar tendon was tapped manually by a researcher using a weighted hammer 10-15 times per leg.

3.4 Reactive Leg Drop Test

The participant was seated in a Biodex 4 chair with their left leg passively extended to their maximal range of motion and supported by a towel held by the researcher. An electronic goniometer was attached to the leg and the knee was extended to approximately 180°. The participant was instructed to relax their leg, so the leg was fully supported by the researcher. The participant was again asked to perform the Jendrassik maneuver with their heads back and eyes closed and was instructed to fully relax their quadriceps, so the leg was fully supported by the researcher. Once the muscles were fully relaxed, the towel was dropped at random intervals, the participants were instructed to fully extend their leg as rapidly as possible as soon as they felt their leg free fall.

3.5 Statistical Analysis

To run statistics, SPSS software version 24 (SPSS Inc., Chicago Illinois, USA) was used. For all statistical tests, $\alpha = .05$. A 3-way mixed factorial ANOVA (group [disuse vs. Control] x limb [left vs right] x time [pre vs post]) was run for both TT and RLD data. A Cohen's d was run to find effect size (ES) and percent change (%Δ) was found for both legs pre and post two weeks.

CHAPTER IV

RESULTS

4.1 Descriptive

Twenty-Seven subjects participated in this study. Eighteen subjects, composing of 8 females, finished the two-week protocol (age = 25.2 ± 4.2 yrs, height = 172 ± 9.5 cm, mass = 82 ± 22.5kg). Seven subjects (age = 22 ± 3.8 yrs, height = 168.8 ± 11 cm, mass = 67.68 ± 11 kg) completed the disuse protocol, while eleven subjects (age = 27.5 ± 3.2 yrs, height = 174.3 ± 7.8 cm, mass = 92.04 ± 23.4 kg) acted as controls. This is represented in Table 1 below.

Table 1. Demographic for Disuse and Control Groups

	Disuse (n=7) Males (n=3) Mean ± SD	Control (n=11) Males (n=7) Mean ± SD	p-value
Age (yrs)	22 ± 3.8	27.5 ± 3.2	0.007*
Height (cm)	168.8 ±11	174.3 ±7.8	0.01*
Weight (kg)	67.68 ± 11.1	92.04 ± 23.4	0.12

* = Significance

4.2 RLD

A 3-way Mixed Factorial ANOVA (Group × Limb × Time) revealed a Limb × Group interaction, $F(1,16) = 7.432$, $p = 0.015$, with the right limb of the control group dropping on average 10.003° (42.977%) more than the disuse group, as shown in Figure 1. A 17.4% and 25% improvement was found in the disuse groups left and right lim b, and a 6.8% and 3.5% improvement in the control groups left and right limbs drop angle, respectively. This is represented in Table 2 below. There was a significant main effect of Time (p=0.033).

Table 2. Summary of Statistical Description of Reactive Leg Drop (°)

	Pre	Post	%Δ	p-values	ES
Disuse Group					
Left	-34.8 ± 18.7	-25.4 ± 8.9	17.4	0.08	0.63
Right	-26.6 ± 12.3	-19.9 ± 9.8	25	0.05*	0.59
Control Group					
Left	-32.2 ± 13.3	-29.8 ± 8.2	6.8	0.37	0.22
Right	-34.8 ±17.7	-32.7 ± 15.4	3.5	0.77	0.13

* = Significance
Pre/Post values are in Mean ± SD
ES = Effect Size, measures in Cohen's *d*

4.3 TT

A 3-way Mixed Factorial ANOVA between Group×Limb×Time revealed a significant interaction between Group×Time, F (1,12) = 10.504, p=0.007, with the left limb of the control group extending on average 8.402° more than that of the disuse group, as shown in Figure 2. A -23.1% and -15.5% decrease in reflex magnitude was found in the left and right limb of the disuse group, and a 44.8% and 36.3% improvement in reflex magnitude was found in the left and right limb of the control group, respectively. This is represented in Table 3 below.

Table 3. Summary of Statistic Description of Tendon Taps (º)

	Pre	Post	%Δ	p-values	ES
Disuse Group					
Left	18.6 ± 4.7	14.3 ± 5.5	-23.1	0.07	-0.79
Right	16.8 ± 4.9	14.3 ± 3.8	-15.5	0.03*	-0.59
Control Group					
Left	9.5 ± 4.7.	13.8 ± 6.5	44.8	0.71	0.1
Right	9.1 ± 7.7.	12.4 ± 6.3	36.3	0.47	0.06

* = Significance
Pre/Post values are in Mean ± SD
ES = Effect Size, measures in Cohen's *d*

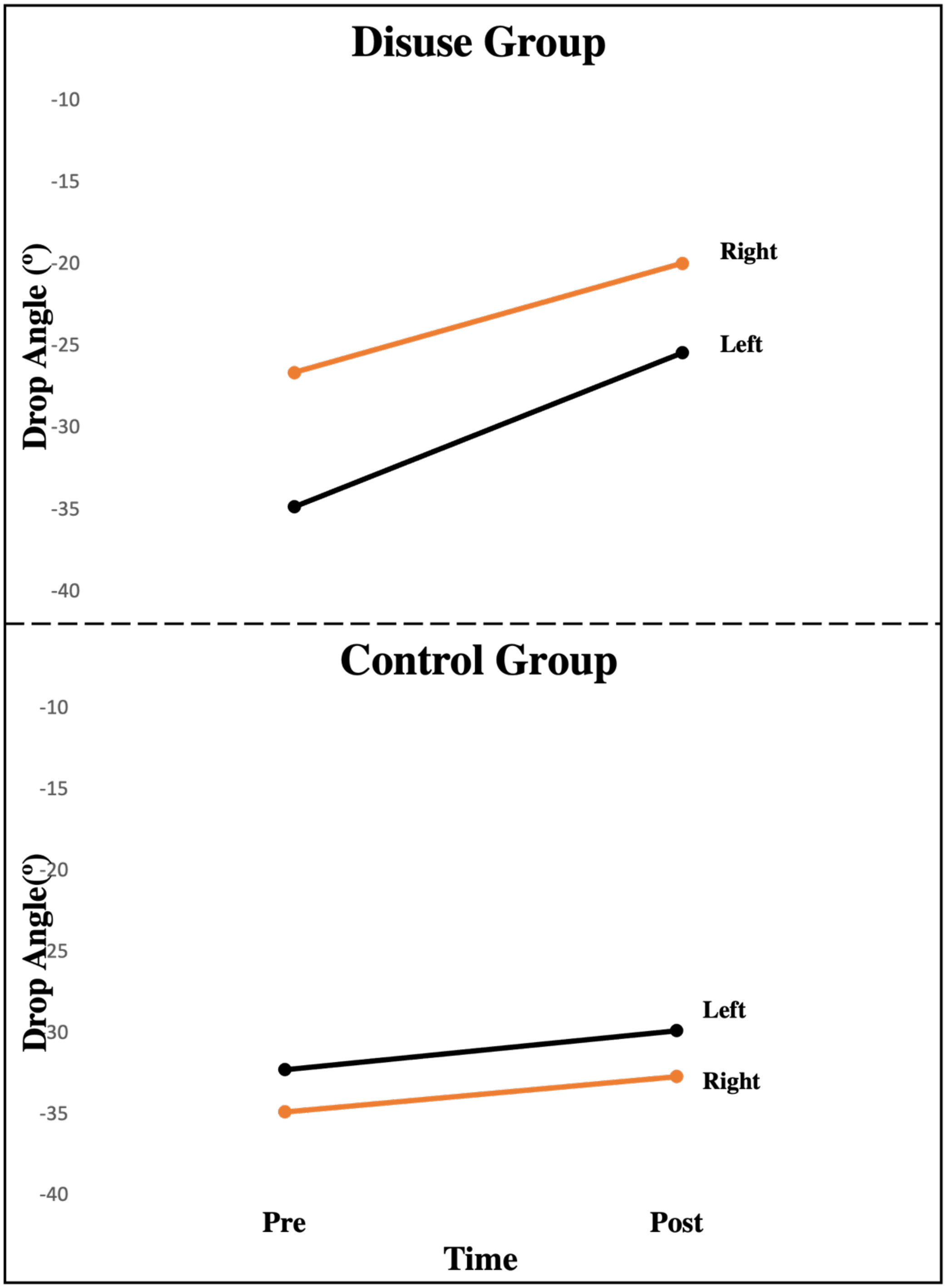

Figure 1. Pooled data (all subjects in their respective groups) and the percent change of the right and left leg pre- and post-two weeks in the RLD.

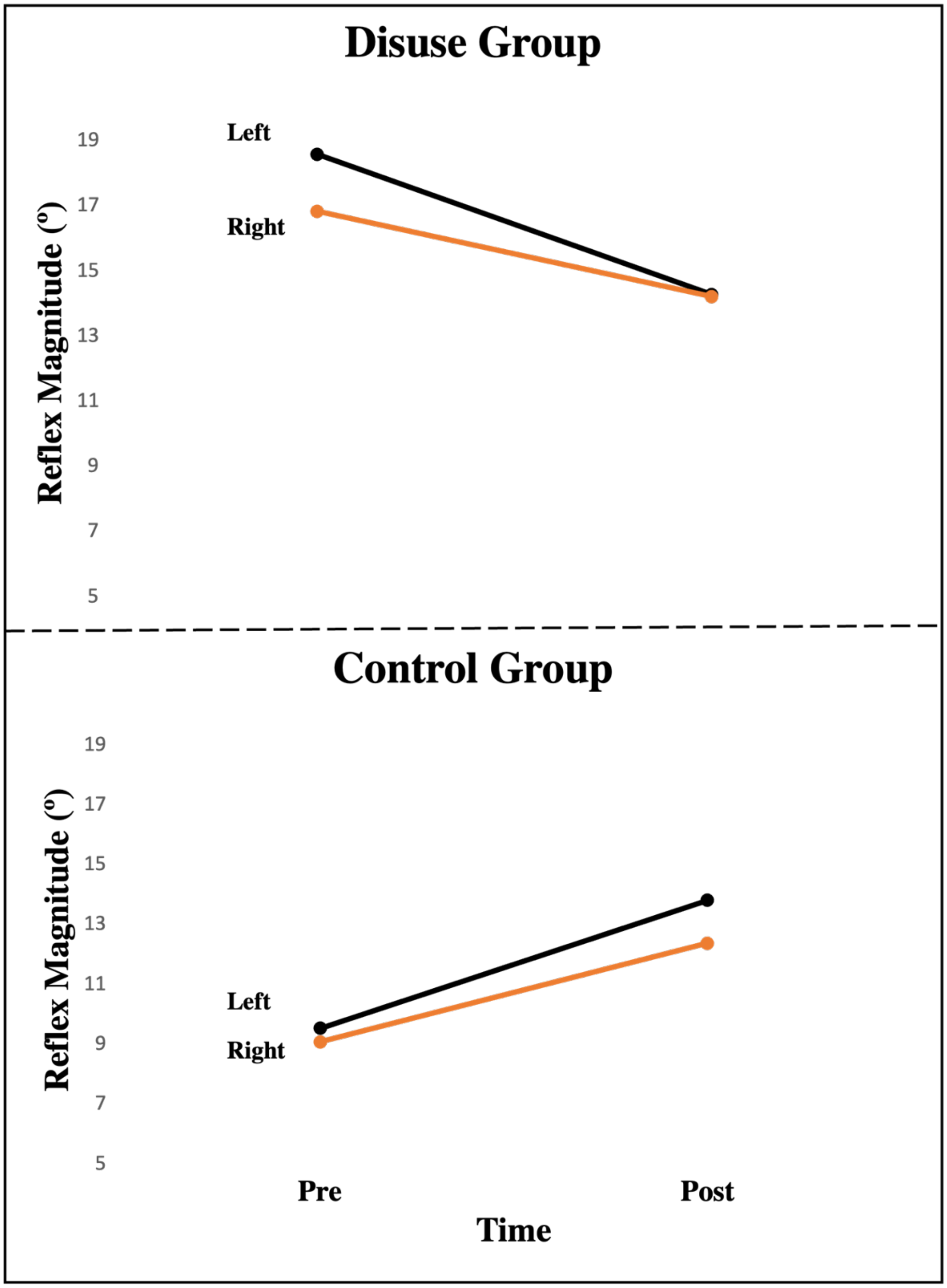

Figure 2. Pooled data (all subjects in their respective groups) and the percent change of the right and left leg pre- and post-two weeks in TT.

CHAPTER V

DISCUSSION

5.1 Discussion

The purpose of the investigation was to examine the effects of two-weeks of left leg immobilization on proprioception. The hope was to increase our understanding of proprioception after disuse and potentially use these findings for younger and older athletic and clinical population.

As hypothesized, the disuse group TT reflex magnitude decreased compared to the control group, which did not change significantly pre- to post-two weeks. A smaller sample size was used to calculate the changes due to the inability to produce a reflex from one disuse (n=6) subject and three control (n=8) subjects. While this was hypothesized for this study, a similar study done by Seynnes et al (2008) found that after 14-day unilateral lower limb suspension, the T reflex recruitment curve became significantly steeper. In Zhu et al (2008) hindlimb unloading study, muscle spindle responses in the rats soleus began decreasing at day 7 of unloading, and significantly decreased at day 14, suggesting that the exposure to hindlimb unloading gradually influences the function and structure of muscle spindles. Rosant et al (2006) also found a decrease in muscle spindle efficacy after 21-day hindlimb immobilization in rats. Both studies support our finding of the decrease in reflex magnitude post two weeks.

Primary data from the disuse group showed an increase in the RLD reaction time, showing a possible familiarization effect from pre- to post-disuse. A control group was recruited to test this theory, but the control group did not change as the disuse group did. There was a

significant difference between groups, with the disuse group having a significantly greater RLD change than the control group, who stayed almost the same from pre to post.

The improvement of RLD reaction time could be caused by tendon and muscle stiffness during the disuse period. This claim is supported by Kaneguchi et al. in their 2020 study where they found a 70° decrease in range of motion after three-weeks hind-limb immobilization in rats. This finding would help explain why there was an increased reaction time in the left leg, however, it doesn't explain why there was also an increase in the right leg.

There is a possible correlation between trained and untrained individuals and the RLD test. After reviewing the health history questionnaires all subjects filled out, the majority of disuse subjects were untrained (n=6) compared to our control group (n=2). This is just a speculation, and more testing will need to be done specifically comparing trained and untrained individuals in the RLD test.

Although the RLD test was a new novel test and the TT were an easy to complete and the data supported the hypothesis, some issues arose from this study. Not all the tests were done by the same investigator for the entire duration of the study. It was difficult to find a knee jerk reflex during the TT protocol on multiple participants, causing a total of four participants to be excluded. Due to the difficulty of the disuse protocol, approximately half of the disuse subjects dropped midway through the two-weeks or did not fully comply with the protocol.

5.2 Conclusion

Unfortunately, we don't fully know or understand why the increase in RLD reaction time post-disuse happened, and more research will need to be done. For future research the subjects should have a familiarization day for the RLD test. The Jendrassik maneuver should be altered slightly, with the participants eyes open and looking up instead of closed. It was observed in multiple disuse and control subjects that it was more difficult to fully relax the leg while the eyes were closed, and the subjects would tense up prematurely while performing the RLD. EMG sensors should also be placed on the subjects Rectus Femoris and Vastus Lateralis to ensure muscle relaxation before the leg is dropped.

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