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The Effects of Morning Versus Evening Stretching Exercises in Hamstrings Flexibility Gains

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Abstract

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Many human physiological functions, including muscle flexibility, exhibit a pattern over a 24-hour period, known as circadian rhythm. Muscle flexibility and its circadian rhythm have been researched, though much more information is needed, especially regarding the hamstring muscle group. The object of this study was to determine if stretching at different times of the day results in differences in hamstring flexibility. Since muscles and joints are most flexible at night, greater ranges of motion should be available, allowing for a greater degree of stretching to take place. We hypothesize that when utilizing the optimal type, duration, and frequency of stretch, subjects who stretch later in the day will have more significant increases in hip range of motion post intervention, as compared to subjects who stretch in the morning. The study was a randomized trial parallel-group research design; with hamstring flexibility being the outcome measure. Ten subjects between the ages of 21 to 40 years old were randomized into two intervention groups, one stretched between 0600 to 0900 the other between 1800 to 2100. Both intervention groups participated in active and passive knee extension stretches, performed for 5 days a week for 6 weeks. Pre and post intervention hamstring flexibility measurements were recorded, via manual goniometry of the hip angle while undergoing a passive straight leg raise. Data Desk Software was used to analyze the data, utilizing a 2-sample T test and one way

ANOVA, the results of this study were found to be insignificant for all variables. There is no significant difference in gains in hamstring flexibility with relation to Circadian Rhythm. Those who stretched in the evening did not have greater gains in ROM following a six week stretching protocol than those who stretched in the morning group.

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INTRODUCTION

Flexibility- What is it?

Flexibility is an intrinsic property of the body's tissues that determines the amount of motion available at a joint or group of joints without causing injury (Thacker, Gilchrist, Stroup, & Kimsey, 2004). It describes a joints ability to complete a full range of motion smoothly and easily (Kisner & Colby, 2007).

Flexibility is important for the performance of both simple activities of daily living and difficult athletic and professional feats. Multiple factors affect flexibility, including the viscoelasticity of muscles, ligaments, connective tissues, and joint mobility or hypomobility. Limitations in these structures can be caused by prolonged immobilization, trauma, muscle, tendon or fascial disorders, sedentary lifestyle, and postural malalignment. When these restrictions limit function, cause pain, or increase the risk of injury, stretching becomes a crucial component of the individual's health regimen. Stretching can be defined as "any therapeutic maneuver designed to increase the extensibility of soft tissues, thereby improving flexibility by elongating structures that have adaptively shortened and have become hypomobile over time" (Kisner & Colby, 2007, p. 66). In a clinical setting, stretching is indicated when range of motion (ROM) is limited functionally, due to adhesions, contractures, and/or scar tissue formation; when structural deformities arise due to restricted motion, and in its more common usage, before and after intense exercise to minimize soreness and to prevent musculoskeletal injuries (Kisner & Colby, 2007). Thacker et al. (2004) reported in a literature review on stretching, that since 1962, 27 articles have reported that stretching exercises improve the flexibility in the knee, hip, trunk, shoulder, and ankle joints.

Circadian Rhythm

Many human physiological functions, including muscle flexibility, exhibit a pattern over a 24-hour period. This cycle, known as a circadian rhythm, has high and low points of performance occurring at specific points throughout the day (Alter, 2004). In the human body, the circadian rhythm is regulated by the suprachiasmic nucleus, which is located in the anterior portion of hypothalamus, superior to the optic chiasm. This center receives information about the time of day from the retina and then coordinates daily biological rhythms (Weipeng, Newton, & McGuigan, 2011). Circadian rhythm has been well researched in multiple areas of physiology, including the circadian rhythm of muscle strength and performance. In 1983, Baxter and Reilly studied the time of day effects of eight females cycling at maximal exertion. The results of this study indicated that exercise tolerance time, total work done, and peak lactate production were highest at 2200 h when compared to 0630 h.

Deschenes et al, (1998) tested ten healthy males to determine whether muscle performance and the body's response to exercise were influenced by the time of day. Muscle performance using an isokinetic dynamometer with maximal effort was recorded at 0800 h, 1200 h, 1600 h, and 2000 h. The results of the study indicated significant time-of-day effects in measures of peak torque, power, total work per set, and maximal work in a single repetition. The study also found significant time-of-day effects of plasma levels of testosterone and cortisol, with testosterone to cortisol ratios highest at 2000 h.

Wyse, Mercer, and Gleeson (1994), looked at circadian rhythm with regard to isokinetic muscle strength in order to determine when peak lower extremity muscle performance takes place. Nine adult male sportsmen's isokinetic leg strength was tested for extension peak torque, flexion peak torque and peak torque ratio using a dynamometer between 0800-0900 h, 1300-1400 h and 1800-1930 h for three days. The results of the study using a one-way repeated measures ANOVA revealed that significantly higher scores were achieved between 1800-1930 h, showing that strength reaches its peak in the evening.

In 2007, Reilly et al. performed a study looking at the effect of circadian rhythm on different aspects of the body. The researchers looked at 8 male soccer players, focusing on body temperature, grip strength, reactions times, flexibility, juggling and dribbling, and wall-volley test. Measurements were taken on different days at 0800 h, 1200 h, 1600 h, and 2000 h. When ANOVA statistics were performed, the results showed significant influence of circadian rhythm on body temperature, reaction time, self-rated alertness, fatigue, forward (sit and reach) flexibility, and right hand grip strength, all peaking between 1600 h and 2000 h. However, they found that Circadian rhythm was insignificant for left-hand grip strength and whole body flexibility, measured by the stand and reach test (Reilly et al., 2007).

The circadian rhythm of muscle flexibility has been researched as well, though much more information is needed. Gifford (1987) took 25 subjects between the ages of 25 and 32 and tested lumbar flexion and extension, fingertip-to-floor distance, glenohumeral lateral rotation, and passive straight leg raising. Measurements were taken every two hours over a 24-hour period. Fingertip to floor values indicated maximum stiffness at 0600 h, increasing to maximum flexibility at midday to midnight. Similarly, lumbar flexion measurements showed the most stiffness in the morning with flexibility increasing to a peak in late afternoon and early evening followed by increased stiffness. Straight leg raising and glenohumeral lateral rotation values were less dramatic, however, both showed an overall rise in flexibility throughout the day, with straight leg raise values reaching their maximum between 0800 and 2200 h.

Guariglia et al. (2011) looked at hamstring length of 26 males who did not regularly exercise, taking measurements at 0800 h, 1300 h, and 1800 h, using the Sit and Reach Test

(SRT), as well as the the Angle of the Hip Joint (AHJ). An ANOVA analysis showed that flexibility increased significantly throughout the day and was greatest at 1800 h. Similarly, Dhariwal and Malik (2011) investigated flexibility in 25 males studying physical education using SRT, taking measurements at 0700, 1300, and 1900 h. Using an ANOVA analysis and Scheffe's post-hoc test, researchers found decreased flexibility at 0700 h rising through 1300 h and finally peaking at 1900 h. Pearson and Onambele (2005) measured the time-of-day variability of internal muscle structure, measuring knee extension torque, fiber pennation and infrapatellar tendon characteristics, with results showing that tendons are more compliant at night. The results of all of these studies strongly indicate the presence of increased muscle compliance at night, and greater flexibility as a result.

There are many explanations for the circadian changes seen in muscle flexibility. Weipeng, Newton, and McGuigan, (2011) explain that body temperature peaks in the early evening facilitating increased energy metabolism and muscle compliance. Deschenes et al. (1998) attribute changes in tendon stiffness to the pulsatile production of testosterone which peaks in the morning and declines in early evening. This study also points out that there is significant circadian impact on nerve conduction velocity, sensitivity, and neuromuscular efficiency that follow fluctuation of core temperature. Others attribute time-of-day changes in flexibility to anabolic steroid levels (Miles et al. 1992 cited by Pearson and Onambele, 2005).

The Hamstrings

The hamstring muscle group is one of the most commonly tight muscles, and as such, will be the muscle focused on in this study to further examine the effects of circadian rhythm. The hamstrings are responsible for extension at the hip and flexion at the knee (Moore, Dalley, & Agur, 2006), and eccentrically controlling the forward swing of the leg during the terminal swing phase of gait. The hamstrings also provide posterior support to the knee capsule during knee extension in stance phase, and will thus limit posterior translation of the femur on the tibia. If the hamstrings are not functioning properly, the knee may snap into hyperextension and/or genu recurvatum may occur as well, which could potentially lead to ligamentous, tendinous, muscular, or joint deformity and damage (Kisner & Colby, 2007). In addition, the hamstrings also affect pelvic tilt and rotation, sacral rotation, and rotation of the hip (Carlson, 2008).

When the hamstring muscles are tight, there is less stretch and force absorption, putting the muscle at risk when it needs to lengthen (Prior, Guerin, & Grimmer, 2009). Athletes with an increased tightness of the hamstring and/or quadriceps muscles have been found to have a statistically higher risk for a subsequent musculoskeletal lesion secondary to having a muscular/ biomechanical disadvantage (Witvrouw, Dannels, Asselman, D'Have, & Cambier, 2003). The hamstrings muscle group is commonly tight in individuals who do not perform a stretching routine on a regular basis; furthermore, Carlson (2008) showed that those individuals with shortened or tight hamstrings who run with a longer stride length can have a predisposition to a potential hamstring injury. Hamstring injury is most common in sports that involve sudden bursts of acceleration and deceleration, such as soccer, football, and track/field. Unfortunately for athletes and nonathletes alike, hamstring strains or injuries tend to have a relatively high recurrence rate. In a study performed by Witvrouw et al. (2003), researchers correlated a high percentage of football players who suffered a hamstring injury with significantly less hamstring flexibility. Tight hamstrings may also lead to restricted talocrural dorsiflexion, which in turn leads to a biomechanical disadvantage, and the potential promotion of subtalar pronation, as well as excessive knee flexion. This can lead to increased compression of the patella on the femur, which will cause patellofemoral pain syndrome (Green, 2005). Reduced hamstring flexibility is often a cause of lower extremity injuries and low back pain as it can cause a posterior pelvic tilt, leading to lumbar spine dysfunction. (Decoster, Scanlon, Horn, & Cleland, 2004). It is therefore important to include hamstring stretching in a prevention, as well as a rehabilitation, protocol in order to prevent initial hamstring injury, recurrence of hamstring injury, and other musculoskeletal complications (DePino, Webright, & Arnold, 2005).

Type, Frequency and Duration of Stretch

Due its widespread usage both clinically and athletically, much investigation has been conducted to determine the most effective type of stretch, as well as the appropriate frequency and duration. The research is not definitive on the ideal parameters for hamstrings stretches. Duration of stretch ranges from 5-60 seconds, with frequency ranging from 1 to 3 times per day and up to 5 days per week. The length of the stretching program ranges from 1 day to 8 weeks, however 6 weeks has consistently shown to be most effective.

Bandy and Irion (1994) compared four different combinations of duration and frequency of hamstring stretching in their study. They found that both a 30 and 60 second stretch of the hamstrings once per day, was more effective than a 15 second stretch at increasing range of motion. As a corollary to these findings, Bandy and Irion (1994) noted that the 60 second stretch was no more effective than the 30 second stretch. In a later study, Bandy, Irion, and Briggler (1997) further found that increasing the frequency of the stretch by either more repetitions, or more times per day, did not show increases in flexibility. Bandy, Irion, and Briggler (1998) whom fellow researchers have used as the gold standard, found that the most commonly used type of stretch is the static stretch.

Static stretching is a common technique used by specialists within the sports medicine world in order to increase muscle length without potentially over-traumatizing tissue. Static stretching takes a muscle to its end range, and then maintains this position for a specified duration until a “release” or decreased tissue tension is felt (Meroni et al, 2010; Band and Irion, 1997; O'Sullivan, K., Murray, E., & Sainsbury, D., 2009). The mechanism of action for static stretching is based on the facilitation of the Golgi Tendon Organ, which is a proprioceptive sensory receptor found at both the origins and insertions of muscle, and responsible for sensing changes in muscle tension. Multiple studies have shown that static tension that is placed on the musculotendinous unit leads to activation of the watch GTO, which in response to increased tension leads to autogenic inhibition of the muscle being placed on stretch, thus decreasing tissue resistance and improving ROM (Meroni et al. 2010; Deyne, 2001).

Active stretching, over the last 15 years, has been researched extensively in order to determine its efficacy and use within the rehabilitation world, as well as the world of sports medicine. Active stretching, unlike passive stretching, consists of performing an active contraction of the agonist muscle group through the full ROM in order to increase or improve the range of motion of the antagonist muscle group. The primary physiological response within the body related to active stretching is related to the use of the principle of reciprocal inhibition; essentially meaning that as one muscle is actively contracting (agonist), the body has a natural stretch reflex that is initiated, leading to the relaxation of the antagonist or opposing muscle group. Sahrmann and White have advocated for the use of an active stretching protocol to not only improve muscular flexibility, but concurrently improving function of the antagonist muscle group.

For the purpose of this research study, it is imperative to understand how hamstring flexibility can be objectively measured with reliability and validity. The most common, as well as oldest method of measuring hamstring flexibility is the sit-and-reach test (SRT); first described by Wells and Dillion in 1952. As years have passed, additional research and knowledge about flexibility and muscular tightness has led to multiple variations of the SRT. One of the newest variations of the SRT, known as the Toe-Touch Test (TT), is the second most commonly selected tests used in order to determine hamstring flexibility. The major difference between the SRT and TT tests is patient positioning; long sitting versus standing, respectively (D Mayorga-Vega, 2014; Ayala et al, 2012).

The current study utilizes the passive straight leg raise test (pSLR) and active straight leg raise (aSLR) as a means of measuring hamstring flexibility. Lee and Munn (2000) determined that the pSLR has an overall reliability of .97 which is significantly higher than the reliability of both the SRT and the TT test. Similarly, a 1982 study by Ekstrand et. al determined there was a high reliability (>than .85) when performing objective measurements of hamstring flexibility with a Myrin goniometer. The researchers felt that this was due to pSLR test's ability to isolate the hip joint, as opposed to the multiple joints involved in measuring during an SRT, thus providing a more valid and reliable measure of hamstring flexibility. In support of this theory, Kendall et al. (1971) determined that the SRT does not isolate the joint at the time of the measurement, thus influencing the validity of this objective measure. Kendall et al. (1971) states that "the final result measurement of using a SRT test can be strongly influenced by the overall physiology of the person, neural tension, or by both contractile and non-contractile tissues in the posterior aspect of the knee, triceps surae complex, and back." In contrast to the potential limitations using the SRT test, a manual goniometric measurement of the hip flexion angle while performing a passive straight leg raise provide direct isolation of the hamstring muscle group (Kendall et al., 1971; Davis, Ashby, McCale, McQuain, & Wine, 2005).

In a more recent study from 2010, Bakirtzoglou et al. compared outcomes of hamstring flexibility using the SRT and the pSLR on athletes and nonathletes. The results of the study showed a statistically significant difference when using the pSLR; however no significant difference between the two populations was found with the SRT. The authors concluded that the two test are in fact not comparable, and recommended the use of the pSLR, as it is better at isolating the hip joint, thus providing a more valid measurement of hamstring flexibility.

Static stretching is a common technique used by specialists within the sports medicine world in order to increase muscle length without potentially over-traumatizing tissue. Static stretching takes a muscle to its end range, and then maintains this position for a specified duration until a “release” or decreased tissue tension is felt. The mechanism of action for static stretching is based on the facilitation of the Golgi Tendon Organ, which is a proprioceptive sensory receptor found at both the origins and insertions of muscle, and responsible for sensing changes in muscle tension. Multiple studies have shown that static tension that is placed on the musculotendinous unit leads to activation of the watch GTO, which in response to increased tension leads to autogenic inhibition of the muscle being placed on stretch, thus decreasing tissue resistance and improving ROM.

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With the knowledge that muscles exhibit the most flexibility at specific times of the day, the object of this study is to determine if variability exists in gains in flexibility, depending on the time of day that a stretching protocol is conducted. If muscles and joints have the most flexibility at specific times, then assumedly, greater ranges of motion will be available, allowing for a greater degree of stretching to take place. We therefore hypothesize that utilizing the optimal type, duration and frequency of stretch as noted above, subjects who stretch later in the day will have greater gains in flexibility than those who stretch in the morning.

METHODS

Trial Design

This was non-controlled, two group, randomized prospective study. Within this study there were two experimental groups with participants randomly assigned to one of two groups: stretching in the morning or at night. Both groups participated in the same hamstring stretching protocol, and thus there was no comparative control group.

Subjects

The participants in the study were recruited from the Hunter College Department of Physical Therapy, Brookdale Campus. Recruitment flyers were posted in the classrooms and hallways beginning a month prior to commencement of the study. As an adjunct to the flyers, information sessions were conducted in the student classrooms for each respective class. These meetings, as well as the aforementioned flyer, briefly discussed the purpose and length of the study, as well as the protocol to be used.

Approximately 80 people, both male and female, were given the opportunity to participate in this study. Although there were specific inclusion and exclusion criteria for participation of this study, initial recruiting was non-specific.

After the initial recruiting, a total of 18 subjects provided verbal consent to participate in the eligibility screening for the study. Prospective participants were then provided with an eligibility questionnaire consisting of six questions to further ensure that they met inclusion criteria, and that it was medically safe for them to participate in the study. Finally, if the prior questions implicated that the potential participant was eligible for the study, bilateral hamstring measurements were taken in order to determine a flexibility limitation; both passive and active SLR measurement were assessed by the researchers using the protocol described below. If participants displayed <80 degrees of active and passive SLR ROM, the participants hamstrings were considered tight, and they were eligible for inclusion in the study.

The study ultimately included 18 participants who met the following inclusion criteria: 1) Hunter College Doctorate of Physical Therapy student ages 21-40, 2) no previous muscle strain injury in their lower extremity or lower back that required medical attention, 3) no pain or discomfort in their lower back or lower extremities within the past 2 weeks, 4) currently medically cleared for full participation in all forms of exercise, 5) no medical, physical, or psychological impairments limiting participation, 6) had bilateral limited hamstring flexibility, <80* as measured with a 14” universal goniometer.

Once it was determined that participants were eligible, verbal consent was obtained to participate in the study. Prior to baseline measurements, one researcher demonstrated the two selected exercises from the provided protocol, answered questions that participants had, and then observed the participants performing the exercises while providing feedback. Each participant was then provided with a stretching checklist to keep a record of the days and times they performed the exercises, and finally randomized into one of two groups; morning (6:00am-9:00am) or evening (6:00pm – 9:00pm).

Baseline Measurement Protocol

Baseline goniometric measurements were performed on all participants within a 3 day window of time. Prior to the day of baseline measurements, participants were instructed to wear loose clothing in order to ensure that clothing would not provide a restriction while measuring hamstring flexibility. All measurements were performed on the same high-low physical therapy mat in order to further decrease a potential limitation of the study. Three researchers participated in the goniometric measurement of hamstring flexibility: one performed goniometric measurement with a 14” universal goniometer, one stabilized each participant’s bilateral anterior superior iliac spine (ASIS) in order to decrease and palpate for pelvic rotation, and one performed the PROM straight leg raise (SLR). Two researchers took turns taking goniometric measurements in order to increase reliability of baseline measurements.

Hamstring flexibility was measured using both a passive and active SLR. The SLR was performed with the subject positioned supine on a mat table, with their upper extremity and spine in neutral position, and bilateral lower extremities extended. AROM measurements were taken first for all participants to standardize baseline measurements. The researcher positioned the pivot of goniometer at the greater trochanter (found via palpation), the static arm in line with the mid-axillary line, and the moving arm in line with fibular head of the test leg (Hazel, 156). For AROM measurements, participants were instructed to maintain full knee extension, and a neutral ankle position while performing the SLR, stop at the first point of resistance (if pelvic movement had not begun prior to resistance), and then maintain that position for approximately 3 seconds in order to obtain an accurate measurement. Following AROM, PROM measurements were then performed on the same leg. Goniometric placement for the PROM measurement was the same as for AROM. The researcher who provided the passive movement maintained the knee in an extended position, instructed the participant to again maintain a neutral ankle position, and then perform the SLR. The researcher was instructed to stop at the first point of resistance, if the researcher providing the stabilization had not reported pelvic movement. Goniometric measurement was again taken while the test leg was maintained at the end position for approximately 3 seconds (Davis, Ashby, McCale, McQuain, & Wine, 2008; Fasen et al, 2009). After AROM and PROM measurements were assessed for the same leg, the same procedure was performed for the contralateral leg. After baseline measurements were taken, the participants were again reminded of the length of the study, the participation checklist, and to perform both stretches provided on the protocol handout.

Exercise Program and Selection

Each group in the study was expected to participate in the stretching protocol provided 5 days a week for 6 weeks from their start date. Regardless if a participant was in the morning or night group, the stretching protocol consisted of the same two exercises. The equipment necessary to perform the stretching exercises were 1 towel roll, 1 pillowcase/towel, 1 timer, and a mat or firm surface. In the appendix section, there is a copy of the stretching exercise protocol handout provided by the researchers to each participant in the study. The handout includes inclusive instructions for the participants, visuals of the two stretching techniques chosen (one passive stretch, and one active stretch), detailed instructions on how to perform each stretch, duration, frequency, and an additional disclaimer for participants (Bandy & Irion, 1994; Meroni et al, 2010; Decoster et al, 2005; Davis et al, 2005; Gajdosik & Lusin, 1983).

Outcome Measure

The only outcome measure chosen by the researchers for this study was bilateral active and passive straight leg raise measurements. An initial baseline was taken at Day 0, and the final measurement was taken 6 weeks post stretching protocol for both groups.

Statistical Analysis

Data Desk Software was used to analyze the collected data. Descriptive analysis was used to analyze the demographic data of all the participants as shown in Table 1. Table 2 shows all the variables for which statistical analysis was performed with emphasis on variables number 2-10 as closely related to our hypothesis. Means and standard deviations were first determined as shown in Table 3, as well as 2-Sample T-test to check for a difference in range of motion between pre and post measurements and one-way ANOVA to analyze if there is a statistical significance as shown in Table 4. In addition, age regression analysis was performed to see if there is a pattern between age and change in range of motion.

Table 1. Description of the sample (n=12)

VARIABLE	**Number of Subjects (%) or Mean+/-Standard Deviation (Range)**
Gender	
Female	**8 (67%)**
Male	**4 (33%)**
Physically Active	
Athletes	**8 (67%)**
Non-Athletes	**4 (33%)**
Race	
White	**10 (83%)**
Asian	**2 (17%)**
Age	**27+/-3.2 (21-40yo)**

**The above table format was borrowed from a research article by Prather et al., 2010.*

RESULTS

Eighteen subjects were recruited, but only 12 subjects completed the research study. The sample consisted of 4 men and 8 women ages 21-40 year old healthy physical therapy student volunteers from Hunter College, NY. Out of 12 subjects, 83% were white and 17% Asian and 67% were athletes, and 33% non-athletes. The average age of the sample population was 27 years old. Mean and standard deviation was calculated for baseline and final measurements, as listed in Table 3. 2-Sample t-test and ANOVA were used to analyze and compare pre measurements at week 0 and post measurements at week 6 for all the variables listed in Table 4. Figures 1-20 are a graphic representation of the statistically analyzed results via box-plots and age regression line.

Results showed that there was slight improvement in hamstrings flexibility in both morning and evening groups from pre to post measurements, but there was no statistical significance for any of the variables listed in Table 2. The hamstring flexibility for the two intervention groups together improved an average of 8.28 degrees, the morning group improved hamstring flexibility an average of 8.83 degrees, the morning group AROM improved hamstring flexibility an average of 8.42 degrees, the morning group PROM improved hamstring flexibility an average of 9.25 degrees, the evening group improved hamstring flexibility an average of 7.73 degrees, the evening group AROM improved hamstring flexibility an average of 8.96 degrees, the evening group PROM improved hamstring flexibility an average of 6.5 degrees. The morning group improved hamstring flexibility an average of 1.1 degrees more than the evening group ($p=0.52$), the evening group AROM improved hamstring flexibility an average of 0.54 degrees more than the morning group AROM ($p=0.83$), the morning group PROM improved hamstring flexibility an average of 2.75 degrees more than the evening group PROM ($p=0.28$). In addition, there were no statistically significant differences in hamstring flexibility between AROM and PROM, athletes and non-athletes, males and females, Asian and white subjects, and right lower extremity and left lower extremity as listed in Table 4. Age regression analysis showed slight trend of improvements in hamstrings flexibility for PROM and decrease in AROM as we age, but again the results were not statistically significant.

Table 2. Means+/- Standard Deviations for baseline and final measurements

		Mean +/- Standard Deviation	**Box-Plot**		
		BASELINE	FINAL	CHANGE	
1	Morning and Evening Groups Together	49.3+/-9.0	57.5+/-8.7	8.3+/-5.9	Figure 1
2	Morning Group	49.5+/-8.5	58.4+/-7.7	8.8+/-6.1	Figure 2
3	Morning AROM	51.4+/-9.8	59.8+/-7.3	8.4+/-6.6	Figure 3
4	Morning PROM	47.7+/-6.7	56.9+/-8.2	9.3+/-5.9	Figure 4
5	Evening Group	49.0+/-9.7	56.7+/-9.7	7.7+/-5.8	Figure 5
6	Evening AROM	50.9+/-10.2	59.9+/-8.5	9.0+/-5.2	Figure 6
7	Evening PROM	47.0+/-9.2	53.5+/-10.1	6.5+/-6.3	Figure 7

DISCUSSION

The results of this study show that there are no statistically significant gains in hamstrings flexibility in relation to circadian rhythm after a 6-week stretching protocol. As there were no statistically significant gains in hamstrings flexibility between the two intervention groups, we fail to accept our research hypothesis, namely, that the evening group would have greater improvements in hamstrings flexibility in comparison to the morning group. Furthermore, the statistical analysis for variables such as age, race, gender, and physical activity also showed no statistical significance, and we therefore cannot correlate how these variables may affect muscle flexibility.

In the research literature, there are many studies conducted on hamstrings flexibility with similar stretching protocols. Unlike our study, many of these studies found significant increases in ROM from the time of their baseline measurements until the completion of their intervention. For example, Bandy et al (1994) found increases in three treatment groups when stretching was performed five times a week for six weeks. While our protocol was largely similar to theirs, Bandy's study utilized only a static stretch, while our study included a passive one in addition to a static stretch. Additionally, we utilized a popliteal angle stretch as an intervention and a SLR as our pre/post test measures, while Bandy's study did the opposite. Bandy's study also had 56 subjects while ours had only 12. In another study by Bandy et. al (1997) they found that a 30 second stretch 1 time per day is just as a effective as stretching for longer durations multiple times per day. However their study differed from ours in that they had 93 subjects while we only had 12. Additionally, they measured with the popliteal angle to determine hamstring length and utilized a standing hamstring stretch for the intervention. The protocols of these studies are not dramatically different from ours, however their sample sizes were drastically larger which is likely to account for the presence of statistical significance in those studies.

It is difficult to compare our study to other studies on circadian rhythm that exist in the literature. All prior studies measured the change in each subject's flexibility over the course of the day. Our study is the first to divide subjects into morning and evening groups for comparison, based on the assumption that there is enough variability between morning and evening flexibility to affect overall gains. This assumption may have been too big leading to the lack of consistent results in our study.

It is important to address the several limitations of our research study which more or less contributed to the lack of statistical significance in our results. Our sample size was small and homogenous, consisting of 12 healthy physical therapy students from Hunter College. The reasons for the small sample size were subject drop-outs, non-compliance with the stretching protocol, as well as the limited time-frame the researchers had for recruiting subjects.

Another limitation in this study was the fact that we utilized both active and passive stretching. Both were used as there is much literature to support the effectiveness of both these stretches, which was our rationale for using both. We also hoped to compare which specific type of flexibility improved more with a stretching protocol that consisted of both active and passive stretching techniques. The mechanism of action for increased flexibility and improved muscular length within the human body varies depending on whether the muscle is actively or passively stretched.

Bandy et al. (1998) performed a study comparing the effects of static and active (dynamic) stretching on hamstring flexibility; prior to this study, there had not been an objective study comparing these two techniques. Although there had not been a study which analyzed the effects of active stretching, it was a common subjective belief that active stretching improved hamstring flexibility to a greater degree than static stretching. The results of this study determined that a 30 second, static stretch was more effective than the active stretch in improving hamstring flexibility, although both were found to improve hamstring flexibility. Contrary to this study, Meroni et. Al performed a study in 2010 that also compared active and static passive stretching techniques on hamstring flexibility. Similar to the study by Bandy, as well as within this study, active knee extension ROM were the objective measures used in the study. Meroni et. Al determined that active knee extension ROM improved more in the active stretching group and not the passive stretching group. Another important thing to note within this study is that the gain in flexibility made by the active stretching group was maintained 4 weeks after cessation of the study, while comparatively the hamstring flexibility of the passive group returned essentially to baseline. Fasen et. al (2008) performed a randomized controlled trial of hamstring stretching, in which the researchers compared 4 different stretching techniques. One of the main stated objective of this previously stated study, was to determine whether active stretches were more effective than passive stretches. Although this study did not have the same purpose as our study or the same objective outcome measure (SLR vs. AKE), the rationale of using both passive and active stretching is addressed within this study. The researchers determined that after 4 weeks of stretching, there was a statistically significant improvement in hamstring length in the active stretching group but not the passive after only 4 weeks. By the final measurements at eight weeks, they discovered that hamstring flexibility actually had decreased from the 4 week measurement in the active stretching group. On the other hand, the passive stretching group kept increasing their flexibility for the entire duration of the study, and thus the researchers were able to conclude that the greatest improvement in hamstring flexibility occurred for the static stretching group. The major implication of this study is that an active stretching protocol lasting longer than 6 weeks, could decrease or have an inverse effect on improving hamstring flexibility. It is possible that one reason our study did not show statistical significance was that we utilized both active and passive stretching in our intervention for a duration of 6 weeks. Future research could take measurements at the halfway mark of the intervention time to determine if there might have been an increase in flexibility followed by a decrease as occurred in Fasen's study. It is the belief of this research team that even though an active stretch was utilized for greater than 4 weeks, both the morning and the evening groups both had the same stretching protocol, thus essentially negating the potentially inverse effects. Simply speaking, because both groups used the same protocol, the potential decrease in hamstring flexibility would have been seen in both groups and not one, thus not affecting the outcome of the study.

Tight hamstrings may not be a result of shortened muscle length, but rather altered neurodynamics. Mhatre, Singh, Tembhekar, and Mehta (2013) studied 56 female physiotherapy students with tight hamstrings. The students were divided into two groups, the first doing Mulligan's Bent Leg Raise stretch and then Two Leg Rotation technique to improve neurodynamics. The second group received a passive hamstring stretch. Both groups were found to have statistically significant hamstring lengthening, but greater gains were made in the first group and thus researchers drew the conclusion that neural tension stretches are more beneficial for patients with tight hamstrings.

Another reason for the lack of statistical significance in our study may have been due to unreliable goniometric measurements. Goniometric tools for measuring range of motion of joints are commonly used in physical therapy settings (Brosseau et al., 2001). The reliability and validity of goniometric measurements has been extensively researched in the evidence-based literature and show variable results. Studies by Brosseau et al. (2001) and Bierma-Zeinstra et al. (1998) examined the intra and inter-tester reliability and validity of various tools for measuring range of motion of joints including a universal goniometer. Results of a study by Brosseau et al. (2001) yielded high intra and inter-tester reliability and validity for a parallelogram and a universal goniometer with intra-tester reliability being slightly higher than inter-tester reliability for both measuring tools. Results of a study by Bierma-Zeinstra et al. (1998) found high intra and inter-tester reliability for both a universal goniometer and inclinometer with higher inter-tester reliability for the inclinometer. This study suggests that the inclinometer is a more reliable tool when measurements are taken by different examiners, but there is no statistically significant difference between the inclinometer and universal goniometer when measurements are taken by the same examiner except for passive range of motion which favors the inclinometer (Bierma-Zeinstra et al., 1998). As cited in Gajdosik and Bohannon (1978), a research study by Amis and Miller (1982) found that passive range of motion measurements are difficult to reproduce because the end range is affected by the amount of manual force applied by the examiner. Results from these research studies suggest that one examiner can effectively utilize a universal goniometer to reliably measure range of motion, but two examiners or more should rather utilize inclinometer to yield more accurate and precise results. In relation to validity, a study by Gajdosik and Bohannon (1987) suggests that the best way to confirm validity of goniometric measurements is by using still photography, cinematography, motion analysis, and radiography as the most common method. For the purposes of our research study, two examiners utilized a universal goniometer for measuring range of motion and radiography was not utilized to ensure high validity of goniometric measurements.

As cited in Gajdosik and Bohannon (1987), a study by Moore (1949) discussed the importance of applying standardized procedures for measuring range of motion of joints with a universal goniometer. Reliability and validity of goniometric measurements are affected by many factors such as the complexity of the measured movement, variations among the measured body regions, active versus passive measurements, and intra-tester and inter-tester reliability. As cited in Gajdosik and Bohannon (1987) studies by Salter (1955) and Fish and Wingate (1985) concluded that inaccurate range of motion measurements mainly happen due to faulty use of the universal goniometer such as misidentification of bony landmarks and variations in manual force among the examiners. A study by Boone et al. (1978) found that inter-tester reliability of goniometric measurements is higher for the upper extremity motions rather than the lower extremity motions due to reasons such as difficulty in locating and palpating bony landmarks, difficulty in aligning the goniometer, and the size and weight of the lower extremities. Gajdosik and Bohannon (1987) emphasized that goniometric measurements are affected by many factors, but a strict standardization of the goniometric measurement procedure will greatly decrease sources of error. In our research study, we measured lower extremity range of motion of the hip joint and we didn't follow a strictly standardized measuring protocol as described in the research studies by Moore (1949).

In our research study, we utilized the straight leg raise test, but studies have challenged its validity in measuring hamstrings flexibility as during straight leg raise the pelvis moves together with the lower extremity (Gajdosik & Bohannon, 1987). A study by Bohannon et al. (1985), studied the contribution of pelvic and lower limb motions in the measurement of the passive straight leg raise. Results showed that the contribution of pelvic rotation to the passive straight leg raise angle measurements is substantial and starts in the first third of the motion and continues to increase until the end range of passive straight leg raise is achieved (Bohannon et al., 1985). A study by Sprigle et al. (2003) found that pelvic goniometer can be effectively utilized to measure both pelvic tilt and hip angle and thus it is more practical to use than universal goniometer. During the goniometric procedures of our research study, the examiners stabilized subjects' pelvis, but pelvic motion as a contribution to hamstring flexibility cannot be excluded from our study data since we didn't utilize a pelvic goniometer to measure if indeed a pelvic motion had occurred.

The position of the ankle during active and passive straight leg raise test may also affect its validity in measuring hamstrings flexibility. A study by Gajdosik et al. (1985) found that both active and passive straight leg raise measurements were decreased with the ankle held in dorsiflexion as opposed to the ankle held in plantarflexion. When utilizing straight leg raise test to measure hamstrings flexibility, the ankle is kept relaxed in plantarflexion because dorsiflexion of the ankle increases tension on the sciatic nerve and related neurological structures and limits range of motion. This study further emphasized the importance of standardizing testing procedures and documenting the position of the ankle while measuring hamstrings flexibility via a straight leg raise test (Gajdosik et al., 1985). Unfortunately, in our research study we didn’t follow a strictly standardized measuring protocol and we didn’t observe and document the position of the ankle while taking goniometric measurements.

A study by Atha and Wheatley (1976) found that the act of measuring joint range of motion and repeated measurements of the same increase tissue extensibility contributing to increased range of motion values. This study suggests that before measuring joint range of motion it is important that subjects perform warm up activities (Atha & Wheatley, 1976). In our research study, our subjects didn’t perform warm up activities of straight leg raises which may have contributed to variations and increase in range of motion each time the goniometric measurement was repeated due to human error in the measuring procedure.

The last issue we would like to raise is that of compliance, an issue that arises in any research studies that utilizes self reporting. The researchers must rely on the honesty of the participants when filling out logs. It is possible that our subjects wrote that they had stretched when in fact, they did not, and thus, we did not see the mean increase in flexibility in any group that we had anticipated.

Another factor to consider was whether the stretches given were performed properly. Every effort was made by the researchers to ensure proper performance of stretches, both with demonstration as well as written instructions with pictures. However, the possibility remains that the appropriate type frequency and duration of stretch did not occur, contributing to lack of significant findings. Additionally, our instructions included two different stretches, and perhaps a single stretch may have been easier to perform which could have increased compliance.

Interestingly, there may be a personality component that contributes to exercise compliance. Newcombe and Boyle (1995) reported that individuals who participated in sports exhibited significantly different personality profiles from non-participants. Univariate tests showed that the participants were more extraverted and vigorous, and less anxious, neurotic, depressed and confused. Similarly, Hoffman (2013) writes that exercisers are more confident in their physical abilities, more self-motivated, and more likely to begin and continue exercise programs, while less motivated individuals drop out or never start at all. When examining the demographics of our· morning and evening groups, we noticed that 100% of the morning group were exercisers, while in the evening group only two of the participants exercised regularly; the other participants were not involved in any form of exercise. Though participants were required to keep a log of days they completed protocol, as was mentioned prior, reliability of self- report measures is always questionable. These studies point to the fact that the morning (exercising) group may have been more compliant, resulting in greater than expected gains.

Clinical Implications

The results of our study cannot be generalized to the general population or in the clinical setting mainly due to the statistically insignificant results and the numerous limitations we discussed above. It is important to emphasize that this research was conducted with healthy physical therapy students while the population in the clinic presents with various diseased states.

Future Research

In a future study, our measuring protocol will be fully standardized to ensure the measurements are precise, reliable and valid. If we utilize a universal goniometer again, we will follow standardized goniometric procedures as described in articles by Moore (1949) and most recent publications by Norkin and White (2009). In our study, we utilized a straight leg raise test to measure hamstrings flexibility with a universal goniometer. In the research literature, aside from straight leg raise test researchers have utilized other tests for measuring hamstrings flexibility such as: popliteal angle, toe-touch, and sit and reach tests (Gajdosik & Lusin, 1983; Ayala et al., 2012). In addition, other tools for measuring range of motion such as: electrogoniometer, inclinometer, and pelvic goniometer have also been utilized (Christensen, 1999; Bierma-Zeinstra et al., 1998; Sprigle et al., 2003). For a future study, we will continue to extensively research the evidence-based literature and utilize the most reliable and valid tests for measuring hamstring flexibility and the most reliable and valid tools for measuring range of motion. We will also collect additional pertinent data for participating subjects such as dominant versus non-dominant extremity, as a study by Macedo and Magee (2008) compared ranges of motion of joints in dominant and non-dominant extremities in ninety healthy subjects and found statistically significant differences between the dominant and non-dominant side.

CONCLUSION

Results of this study showed that after six weeks of performing the stretching protocol there were no statistically significant differences in hamstrings flexibility for participants in the morning versus the evening group.

APPENDIX

Stretching Exercises Protocol Handout

Inclusive Instructions for research study participants

- Never stretch a muscle past the point of resistance. Remember a stretch is a comfortable lengthening of a muscle; it should not be uncomfortable or painful.
- Always maintain appropriate positioning of the lower extremity that is being stretched, opposite lower extremity, and lower back as described below under participant position section.
- If you are feeling pain or compensating and cannot maintain the appropriate position that means you are stretching the muscle past resistance, and need to decrease the stretch length.
- Never “bounce” a muscle being stretched.
- Do not hold your breath while stretching.
- **Time to perform this exercise protocol:**
  - **AM Group** = anytime between 6:00am-09:00am
  - **PM Group** = anytime between 6:00pm-09:00pm
- **Equipment**: 1 towel roll, 1 pillowcase/towel, 1 timer, mat or firm surface

PLEASE NOTE:

- Performing this exercise protocol carries minimal risk.
- Participants may strain the hamstrings muscles if they do not adhere to the instructions given for the exercise protocol.
- Symptoms of a strain may include: localized stiffness, bruising/discoloration, swelling and soreness at the area of the strained muscle (Wikipedia; Drugs, n.d.).
  - http://www.drugs.com/cg/muscle-strain.html
- If participants feel that they've strained a muscle while performing the exercise protocol, they should STOP the exercise protocol and contact the investigators of this research study immediately.
- Further medical attention will be suggested as appropriate.
- Please feel free to contact the investigators of this research study if you have any questions or concerns at any time.

Table 3. Variables for which statistical tests were performed:

1	Morning and Evening Groups Together
2	**Morning Group**
3	**Morning AROM**
4	**Morning PROM**
5	**Evening Group**
6	**Evening AROM**
7	**Evening PROM**
8	**Morning versus Evening Group**
9	**AROM Morning versus AROM Evening**
10	**PROM Morning versus PROM Evening**
11	AROM versus PROM
12	Athletes versus Non-Athletes AROM
13	Athletes versus Non-Athletes PROM
14	Males versus Females AROM
15	Males versus Females PROM
16	Asian versus White AROM
17	Asian versus White PROM
18	Left versus Right Side
19	Age Regression Analysis

Figure 1. Morning and Evening Groups Change (Baseline to Final)

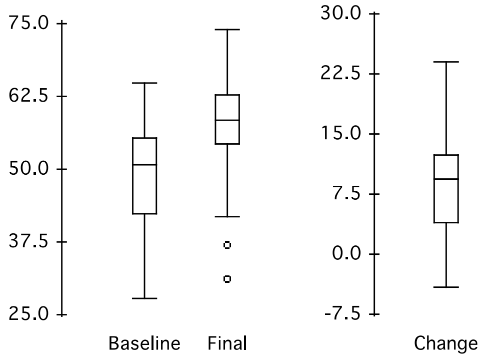


Figure 2. Morning Group Change (Baseline to Final)

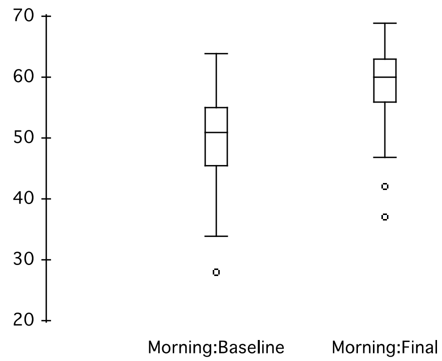


Figure 3. Morning Group Change AROM (Baseline to Final)

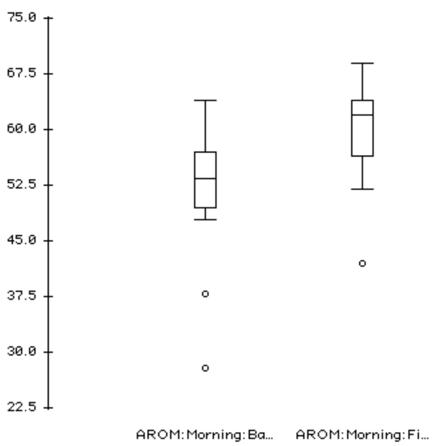


Figure 4. Morning Group Change PROM (Baseline to Final)

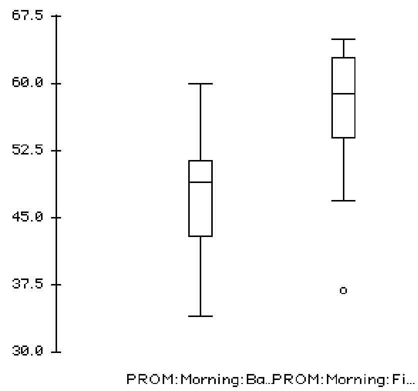


Figure 5. Evening Group Change (Baseline to Final)

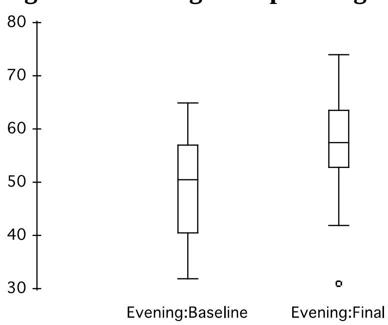


Figure 6. Evening Group Change AROM (Baseline to Final)

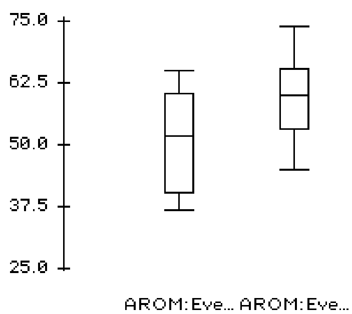


Figure 7. Evening Group Change PROM (Baseline to Final)

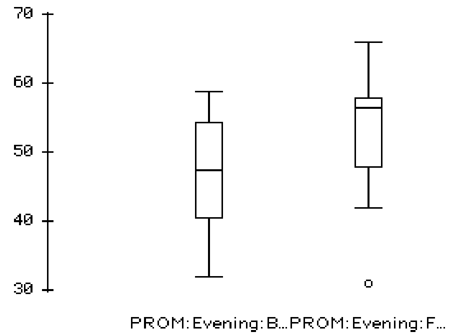


Table 4. 2-Sample T-test and ANOVA comparisons

	CHANGE IN:	2-Sample t-Test	ANOVA	Ho: Means are equal	Box-Plot
1	Morning versus Evening Group	p=0.5243	p=0.5243	Fail to reject	Figure 8
2	AROM Morning versus AROM Evening	p=0.8264	p=0.8263	Fail to reject	Figure 9
3	PROM Morning versus PROM Evening	p=0.2788	p=0.2787	Fail to reject	Figure 10
4	AROM versus PROM	p=0.6398	p=0.6398	Fail to reject	Figure 11
5	Athletes versus Non-Athletes AROM	p=0.4724	p=0.4941	Fail to reject	Figure 12
6	Athletes versus Non-Athletes PROM	P=0.5970	p=0.5813	Fail to reject	Figure 13
7	Males versus Females AROM	p=0.4332	p=0.5411	Fail to reject	Figure 14
8	Males versus Females PROM	p=0.5586	p=0.5813	Fail to reject	Figure 15
9	Asian versus White AROM	p=0.3014	p=0.6015	Fail to reject	Figure 16
10	Asian versus White PROM	p=0.4845	p=0.9653	Fail to reject	Figure 17
11	*Right LE versus Left LE	p=0.4192	p=0.8385	Fail to reject	Figure 18

**Results are statistically significant if p<0.05*
**LE (Lower Extremity)*

Figure 8. Morning versus Evening Group Change (Baseline to Final)

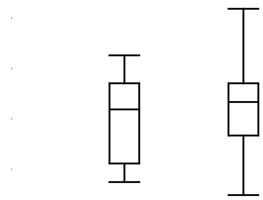


Figure 9. AROM Morning versus AROM Evening (Baseline to Final)

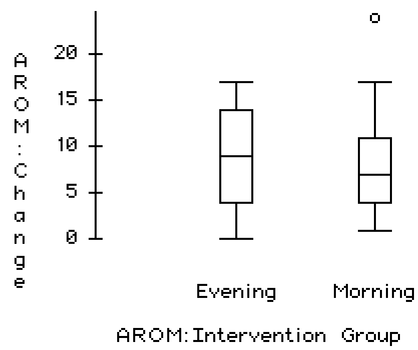


Figure 10. PROM Morning versus PROM Evening (Baseline to Final)

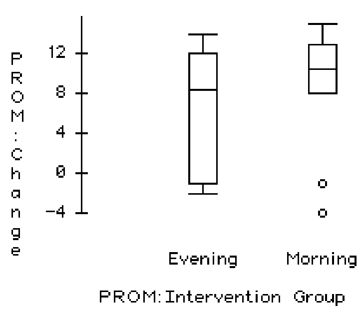


Figure 11. Change in AROM versus change in PROM (Baseline to Final)

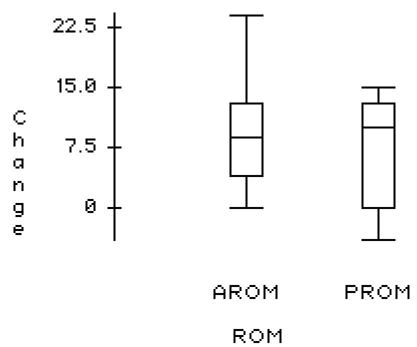


Figure 12. Athletes versus Non-Athletes AROM (Baseline to Final)

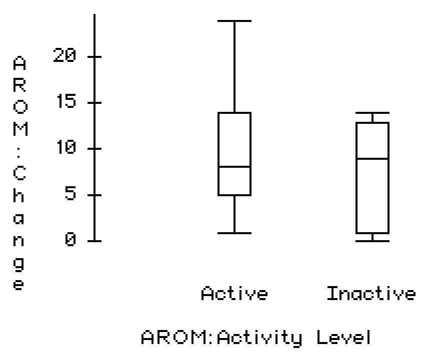


Figure 13. Athletes versus Non-Athletes PROM (Baseline to Final)

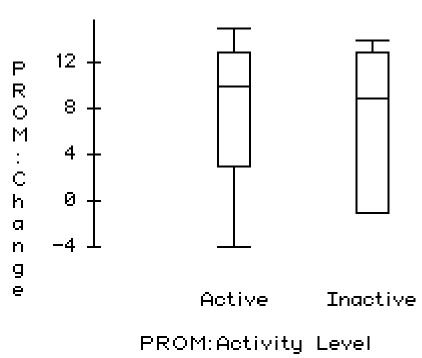


Figure 14. Males versus Females AROM (Baseline to Final)

Figure 15. Males versus Females PROM (Baseline to Final)

Figure 16. Asian versus White AROM (Baseline to Final)

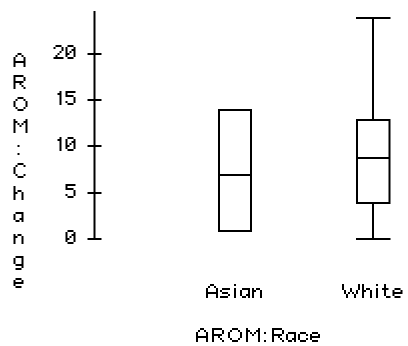


Figure 17. Asian versus White PROM (Baseline to Final)

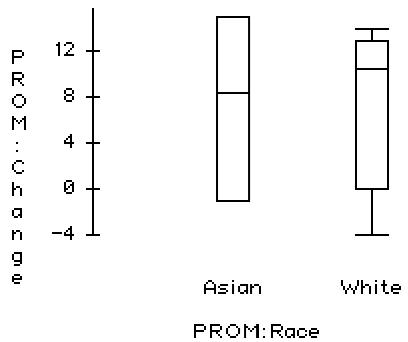
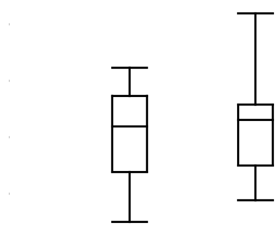


Figure 18. Right LE versus Left LE (Baseline to Final)



***Left versus Right Lower Extremity**

Figure 19. Age Regression Analysis Line

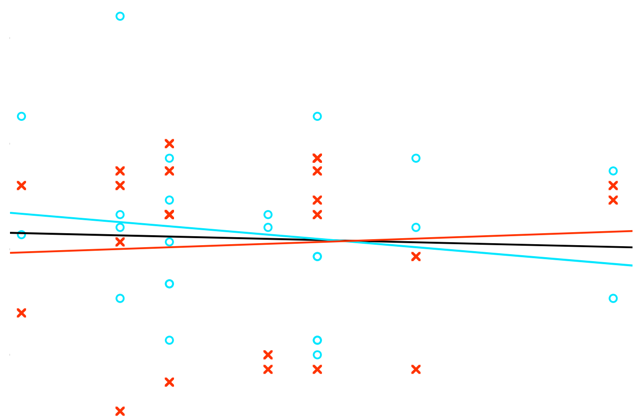
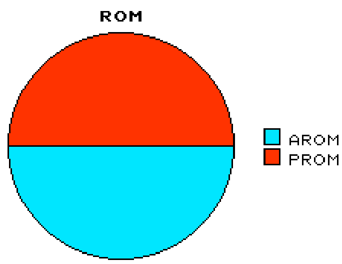


Figure 20. Pie Chart Explaining Variables for Age Regression Line



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