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Dehydration Reduces Posterior Leg and Trunk Flexibility and Increases Stiffness in Male Collegiate Age Runners

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ABSTRACT

Dehydration reduces flexibility and increases stiffness in male collegiate age runners.

Dehydration has been shown to negatively affect collagen in vitro; however the literature lacks works exploring the in vivo effects of dehydration on collagenous tissue. This study addresses this gap in the literature, by exploring the effects of dehydration on the muscles and connective tissues of the posterior leg. It was hypothesized that when dehydrated, the collagen within these tissues would become stiffer, decreasing flexibility and increasing stiffness.

A cross-over cohort design was conducted to evaluate nineteen male collegiate runners. Each subject attended three sessions: baseline, dehydration and euhydration. The order of testing was randomly assigned and the PI was blinded throughout. Mean sit and reach (MSnR), mean terminal straight leg raise (MTSLR) and mean posterior leg stiffness (MPLS) scores for each testing condition were analyzed using a repeated measures ANOVA.

Dehydrated, subjects demonstrated statistically significant decreases in MSnR scores, $p<0.001$, $d=0.469$ (MSnR dehydrated 26.83 ± 7.53 cm and MSnR euhydrated 30.36 ± 7.53 cm) and MTSLR, $p<0.001$, $d=1.068$ (MTSLR dehydrated 51.38 ± 9.39 and MTSLR euhydrated 60.58 ± 7.74), with a concurrent increase in MPLS, $p=0.005$, $d=1.023$ (MPLS dehydrated 0.899 ± 0.357 and MPLS euhydrated 0.508 ± 0.409), as compared to when they were euhydrated.

The large effect size for MPLS and MTSLR and moderate for MSnR indicates that when dehydrated subjects became stiffer and has less flexibility as compared to when they are euhydrated. These changes may impede performance and increase the risk of injury in dehydrated individuals.

Keywords: Euhydration, Straight leg raise, Sit and reach test, Lower extremity injury, Hamstring

INTRODUCTION

Optimal performance is the primary goal of every athlete regardless of age or skill level. Strength, power, endurance, flexibility, range of motion (ROM) and neural control are performance measures used to delineate stages of recovery by athletes, coaches and medical professionals. Previous studies have evaluated the effect of dehydration on strength, power and endurance in various populations, including skilled and unskilled athletes and individuals in uniform [1-6]. It is generally accepted that performance is impeded when individuals, and the connective tissue and muscles within their body, become dehydrated.

Laboratory studies have shown that human collagen fibers in the skin, vocal cords and intervertebral discs stiffen and become brittle when dehydrated, leading to mechanical change in the properties of the tissue [7-11]. Specifically

these studies have shown that when dehydrated, collagens' ability to tolerate deformation, elongation and compression, is compromised [7-11]. Despite the in vitro evidence that collagens' mechanical properties are altered when dehydrated, there is a lack of in vivo studies connecting this physiological induced phenomenon with an effect on performance measures commonly assessed in athletes such as posterior leg stiffness (PLS), hamstring flexibility and straight leg raise range of motion (SLRROM). These performance measures are important as they have been linked to both athletic performance [6] as well as risk of hamstring injury [12,13].

Research has shown that the structures responsible for PLS during the straight leg raise (SLR) maneuver include the hamstring musculotendinous unit and the connective tissues and fascia which surround it [14,15]. Adhesions in the surrounding structures and tissues of the posterior leg and hamstring as well as friction occurring within the hip joint itself are also factors associated with PLS [15].

During athletic movement and during the performance of clinical based measures, the connective tissues and fascia in the posterior leg surround the hamstring musculotendinous unit and restrict the movement of the muscles and the neurovascular structures located within the posterior leg [16,17]. These non-contractile connective tissues are comprised of collagen fibers and are influenced by changes in hydration levels and therefore, may be responsible for the development of stiffness in the posterior leg [7-9,18-20]. This study was designed to explore whether common performance measures for flexibility and stiffness would be affected by differing levels of hydration as has been reported in the laboratory setting. It was hypothesized that when dehydrated, subjects would exhibit decreased posterior leg flexibility as measured by the sit and reach test and SLRROM (MTSLR and MSnR measures) with a concurrent increase in PLS (MPLS) as compared to the euhydrated condition.

METHODS

Nineteen male runners, with a mean age of 20 ± 2.1 years (range 18-23 years), participated in this study. Prior to participation each subject was informed of the benefits and risks of the investigation and signed an informed consent approved by the Institutional Review Board. The study protocol consisted of an intake day, to familiarize subjects with the testing protocols and data collection procedures. Subjects returned on two subsequent occasions for testing using the euhydration and dehydration protocols.

Repeated measures, cross-over design in which each subject underwent testing for each of the two testing conditions, dehydrated and euhydrated, was implemented.

Testing order was randomized and the primary investigator (PI) was blinded to testing condition. Subjects were tested at the same time of day to ensure that time of day would not affect results. Subjects underwent identical upper body exercise protocols in a heated room. Fluids were restricted for the dehydration condition and provided for the euhydrated condition. This is similar to designs used in previous research to induce a dehydrated and euhydrated state in subjects [4,21,22]. The sit and reach test is commonly performed to measure trunk and posterior leg flexibility and had been reported, but not analyzed, in previous studies discussing flexibility and hydration levels [1,23]. The straight leg maneuver is commonly used as a measure of posterior leg stiffness and has been correlated with injury risk in athletic populations [24].

PLS has been defined as the resistance to passive movement during the straight leg raise maneuver which cannot be accounted for by either limb weight or muscular activity [25-27]. PLS has been analyzed in previous studies by comparing the slopes between two testing conditions, as described by Wright [26] and Dugan et al. [25]. PLS was measured using an isokinetic device which measures the resistance to passive movement in Newton's throughout the arc of motion of the SLR maneuver.

Subject inclusion criteria included subjects who were male runners between the ages of 18 and 23 years of age and in good health. Runners were classified based on current participation in either indoor/outdoor track or cross-country at the club or collegiate level. Subject demographics and running history are listed in Table 1.

Table 1: Subject demographics.

	Mean	Standard Deviation
Age (years)	20.2	2.1
Height (cm)	175.9	4.6
Years Running (years)	5.3	1.9

BMI	8.3	3.6
Nude Weight (kg)	70.5	7.6
Miles ran during the week prior to experiment (miles)	42.1	17.2

Volunteers were excluded if they would be potentially intolerant of the testing procedures, based on a history of upper or lower extremity pain, including the neck or back or self-reported injury within the past month. Similarly, any volunteer with a history of neurological disorders (multiple sclerosis, Parkinson's disease, spasticity, hypertonicity) and other connective tissue disorders or who had a history of hypervolemia, smoking, hypertension, cardiac problems, syncope, obesity, anorexia, bulimia or who were currently taking diuretics were also excluded from participation in the study.

PROCEDURES

Prior to testing each subjects' urine specific gravity (USG) was measured using an Atago PAL-10S digital refractometer (Atago U.S.A., Inc., Bellevue, WA) and recorded along with height, body mass index (BMI), resting heart rate and blood pressure. Nude body weight was measured in a private bathroom using a digital scale (model BWB-800 A; Tanita Corporation, Tokyo, Japan) assigned to the subject, each subject was asked to disrobe and dry their body with a towel prior to stepping on the scale. Subjects were then asked to don a dry t-shirt, loose fitting shorts and remove their shoes and socks for the remainder of the study. Each subject then performed six trials of the sit and reach test using the Flex-Tester® (Novel Products, Inc., Rockton, IL) with each measurement rounded down to the smallest half centimeter (Figure 1).

Figure 1: Sit and reach testing position.

The skin over the semimembranosus and rectus femoris muscle bellies, where the EMG, Patheway™ MR-20 portable EMG (The Prometheus Group, Dover, NH), leads were placed, was scrubbed with alcohol prep pads and shaved prior to placement of self-adhering surface EMG leads. Subjects were placed in supine on a Triton Treatment TRT-300 (Chattanooga Group, Hixsen, TN) high low table, with the posterior thigh and lower leg located over the table's adjustable face cradle and greater trochanter centered with the Kincom®'s axis of rotation (Kinetic Communicator, Chattex Corp., Chattanooga, TN). The subject's leg was then placed on top of the Kincom®'s force transducer which was attached to a steel bar connected to the lever arm. Careful attention was used to position the force transducer approximately 2-4 cm proximal to the medial malleolus to minimize compression of the gastrocsoleus complex and maintain subject comfort. An "L" shaped PVC bar was lowered and locked into position to ensure that the knee was fixed in 0 of extension, while maintaining appropriate EMG electrode placement. A strap was then attached to the vertical PVC bar and the subject's foot to fix the foot in a neutral position (Figure 2). The table's face cradle was lowered allowing the leg to be fully supported by the Kincom. Lever arm length, the distance between the axis of rotation on the Kincom® and the set point on the force transducer, was recorded and entered into the Kincom's® software in order to accurately calculate torque, and to allow for easy replication during the next two testing days.

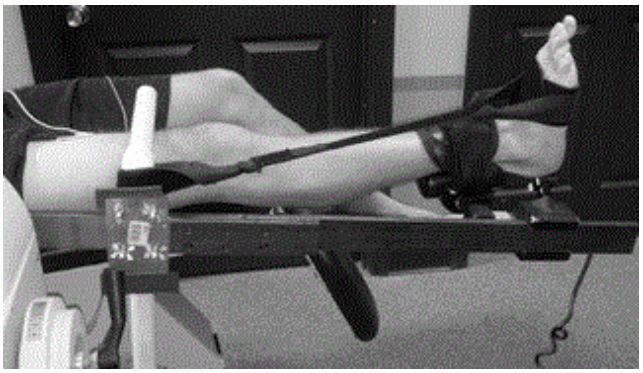

Figure 2: Leg positioned on Kincom. Note "L" shaped PVC bar used to stabilize the knee with foot strap in place to lock the ankle in neutral position.

To ensure that the EMG would detect muscular activity the subjects were asked to perform a maximal isometric contraction of the quadriceps and hamstring muscles. This procedure ensured that the electrodes were located properly and were able to record muscular activity while the subject was positioned on the Kincom. Once electrode placement was confirmed, a permanent marker was used to indicate the corners of the electrodes, on the subject's skin, which maintained consistency in electrode placement for each testing condition.

The Kincom's gravity correction subroutine was used to correct for force against the lever arm created by the weight of the subjects' limb as well as the weight of the cradle itself. Prior to each trial, the PI passively raised the limb to each subject's maximally tolerated SLR position. This subject-selected endpoint was used as the Kincom's stop position and was automatically recorded by the device and later used to calculate MTSLR for each condition. The limb was then lowered to the horizontal position and the subject allowed resting for 30 s prior to the commencement of testing. The PI then activated the Kincom® which passively elevated the subject's leg at a rate of 5/s until the device reached the set end range. The limb was then lowered to the horizontal starting position at 5/s, ensuring that the limb was never in a maximally stretched position for more than 2 s at a time. A rate of 5/s has been used in previous studies and was chosen in order to prevent reflexive motor activation due to stimulation of the stretch reflex, and allow for accurate measurement of stiffness and flexibility [16,25,27].

Prior to departure, subjects were given the necessary quantity of HydraTrend™ (UriDynamics, Inc., Indianapolis, IN) USG test strips for the number of days between intake and final testing, so that they could monitor their USG between trials. Subjects were provided written and verbal instructions on the proper use of the USG test strips, and instructed to record their urine pH and USG using each morning's first void. Subjects were also given instruction on the use of a digital scale in order to record daily nude body weight at the same time each day, over the course of the experiment.

Euhydration and dehydration testing days were scheduled a minimum of two days and a maximum of seven days apart. The subjects were instructed to create and record a Standardized Exercise Routine (SER) to be used the day prior to undergoing each testing condition. The SER consisted of a running route of moderate intensity that could be exactly duplicated (distance, time, intensity and route) the day prior to each testing condition. This allowed each subject to continue training, while preventing variations in training routine from confounding the results. Subjects were asked not to exercise the morning of testing but could continue with regular training on off days. Care was taken to ensure that subjects were scheduled to return to the clinic at the same time of the day for both the dehydration and euhydration testing procedures

Dehydration testing protocol (DTP)

Subjects were given verbal and written instructions to abstain from fluids from 6 PM the evening prior to testing until the completion of the DTP the next day. Subjects were allowed to consume no more than 8 oz. of fluids with dinner, if needed. Subjects were educated and provided written guidelines outlining the warning signs of severe dehydration. All subjects had 100% compliance completing the SER, prior to their arrival for testing, as instructed.

Prior to testing, each subject's BMI, resting heart rate and blood pressure were recorded. Subjects next provide a urine sample for USG measurement. Lastly subjects, removed all clothing, wiped their bodies with a clean, dry towel, to absorb sweat prior to weighing. Baseline core temperature was measured using a digital rectal thermometer

(Barrington Diagnostics, Barrington, IL). To ensure cleanliness and comfort, subjects used a clean plastic cover designed specifically by the manufacturer for the rectal thermometer and personal lubricant jelly (CVS Corporation, Woonsocket, RI) for each measurement. Subjects were prohibited from drinking and required to perform a heated exercise protocol, described below. This protocol has been used in other studies to ensure that each subject became adequately dehydrated and ensured that the subjects did not utilize their lower extremities prior to testing.

Heated exercise protocol. Subjects were placed in a hot (26.6C), dry (<20% humidity) room and utilized the upper body portion of the SciFit Pro II Total Body Ergometer (SCIFIT, Tulsa, OK) for eight minute bouts at a constant work load of 30 W, while maintaining a heart rate below 80% maximal heart rate. Following each exercise bout, subjects were required to rest for eight minutes in the heated room, while heart rate and blood pressure was monitored. Subjects continued to alternate upper body ergometry and rest for 4 complete cycles, lasting approximately 64 min.

Once the heated exercise protocol was completed, subjects were placed in an air conditioned (20°C) room and allowed to rest for 60 min. This allowed core temperature to return to baseline, preventing internal temperature from affecting muscle and connective tissue flexibility and stiffness. Once a subject's rectal temperature had returned to baseline, a final USG measurement was obtained and recorded.

Subjects were asked to wear a t-shirt and loose fitting shorts for the remainder of the study, but had to remove socks and shoes and were not allowed to consume fluids until testing had concluded. MSnR, MTSLR and MPLS were calculated for each condition using procedures previously described. Data was only included for statistical analysis, if the subject's final USG measurement was ≥ 1.020 µg. Following measurements, subjects were given water as well as an electrolyte drink, Gatorade G-Series (PepsiCo, Inc., Purchase, NY), and monitored until such time that baseline weight was achieved. Subjects were instructed to continue drinking fluids and to report any adverse symptoms or reactions to the PI.

Euhydration testing protocol

The day prior to euhydrated testing, subjects were instructed to measure and record USG and nude body weight at 6 PM. Subjects were given verbal and written instructions to consume eight 10 oz. glasses of water, juice or sports drink between 6 PM and before bed. Upon waking, subjects measured and recorded USG and nude body weight prior to consuming an additional three 10 oz. glasses of water or juice.

USG, body weight, BMI, resting heart rate, blood pressure, and core temperature measurements were performed as previously described.

Subjects were then placed in a hot (26.6°C), dry (<20% humidity) room and performed the heated exercise protocol as previously described. Unlike the dehydration protocol, subjects were offered water as well as Gatorade G Series® and drank liberally throughout the heated exercise protocol. During the eight minute resting period between exercise bouts, subjects were encouraged to drink a minimum of 200 ml of water or Gatorade G Series® to ensure maintenance of hydration [28]. In the event that there was a reduction in body weight following the heated exercise protocol, subjects consumed water equal to the number of grams of body weight lost [28].

Subjects were then placed in an air conditioned (20°C) room and allowed to rest for 60 min to allow core temperature to return to baseline. Subjects consumed 200 ml of Gatorade G Series® every 20 min throughout the cooling phase to enhance hydration [29]. A final rectal temperature was obtained and recorded. At the conclusion of the cool down phase, USG was measured and recorded. All subjects were able to demonstrate a USG<1.010 µg (Table 2).

Table 2: Descriptive statistics for urine specific gravity at time of testing

	Mean (µgm)	Standard Error	Lower Bound	Upper Bound
Intake	1.022	0.002	1.018	1.025
Dehydrated	1.025	0.001	1.023	1.027
Euhydrated	1.004	0.001	1.002	1.005

MSnR, MTSLR and MPLS were calculated for the euhydrated condition as previously described. Subjects were given salty snacks and monitored for signs and symptoms of hypervolemia for thirty minutes at the conclusion of testing prior to being allowed to leave the facility.

Statistical Analyses

Using Atha and Wheatley's [30] work as a template for data analysis, the last three measurements was used when calculating MSnR and MTSLR for each testing condition. All analyses were performed using SPSS, version 23 (IBM Corporation, Armonk, NY) with a statistical significance level of $p < 0.05$ and a power of 0.80. A repeated measures ANOVA was used to determine if the two testing condition's USG measurements were statistically different from each other. A statistically significant difference between testing conditions was followed by a repeated measures ANOVA for variables MSnR, MTSLR and MPLS and Cohen's d was calculated to determine effect size.

The study design created two statistically significant different testing conditions, $p<0.001$, based upon USG. The euhydrated mean USG=1.004 µg (range of 1.00-1.005), dehydrated mean USG=1.025 µg (range 1.023-1.027) (Table 3).

Table 3: Urine specific gravity statistical analysis

	Mean Difference	Std. Error	Significance
Dehydrated–Euhydrated	0.022	0.001	<0.001

Statistical analysis of average urine specific gravity measurements at time of testing for both conditions, n=19.

Descriptive statistics for MSnR, MTSLR and MPLS are provided in Table 4 for each testing condition.

Table 4: Descriptive statistics; Abbreviation: TSLR: Terminal Straight Leg Raise

	Mean	Standard Deviation
Sit and reach (Dehydrated Condition)	26.833 cm	7.529
Sit and reach (Euhydrated Condition)	30.364 cm	7.526
TSLR (Dehydrated Condition)	51.384°	9.388
TSLR (Euhydrated Condition)	60.578°	7.744
Dehydrated Slope	0.899	0.357
Euhydrated slope	0.508	0.409

Statistical analysis reveals a statistically significant difference between the dehydrated and euhydrated conditions for MTSLR and PLS scores with a large effect size. MSnR scores were also significant with a moderate effect size between the two conditions (Table 5).

Table 5: Statistical analysis of data

Test	Type III sum of squares	Mean square	F	Significance	Cohoen's d
Mean Sit and Reach	59.941	59.941	24.764	<0.001	0.469
Mean Terminal SLR	403.433	403.433	66.181	<0.001	1.068
Mean PLS	0.649	0.649	10.021	0.005	1.023
Abbreviation: SLR: Straight Leg Raise					

DISCUSSION

The purpose of this study was to determine if posterior leg flexibility and stiffness differs when collegiate age male runners are dehydrated as compared to when they are euhydrated. USG measures define both the dehydration and euhydration conditions. Researchers have previously defined USG values for both dehydration, USG 1.020 µg [29,31,32] and euhydration, USG<1.010 µg [29,31]. These two values were therefore used as threshold indicators to classify subjects as either dehydrated or euhydrated. The study design was able to create two statistically different testing conditions using the procedures described above.

The sit and reach is a global test of flexibility and is commonly used to assess flexibility in many populations. The observed moderate reduction in MSnR measurements, when the subjects were dehydrated as compared to the euhydrated indicates that there is a global reduction in flexibility which is both measurable and statistically significant (Figure 3). These finding are consistent with observations reported elsewhere in the literature [1,33].

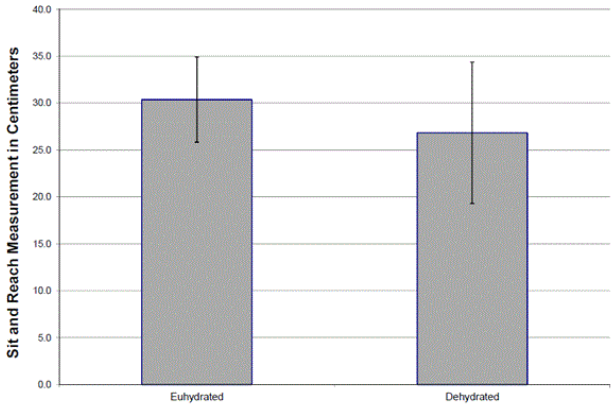

Figure 3: Mean sit and reach for both conditions

This study found a statistically significant difference when comparing MTSLR measures between the dehydrated and euhydrated conditions (Figure 4). The large effect size, d=1.068, indicates that when the subjects where dehydrated they exhibited a significant drop in SLRROM. A reduction in SLRROM has been linked to both a reduction in athletic performance [6] and an increase in hamstrings injury risk [12,13].

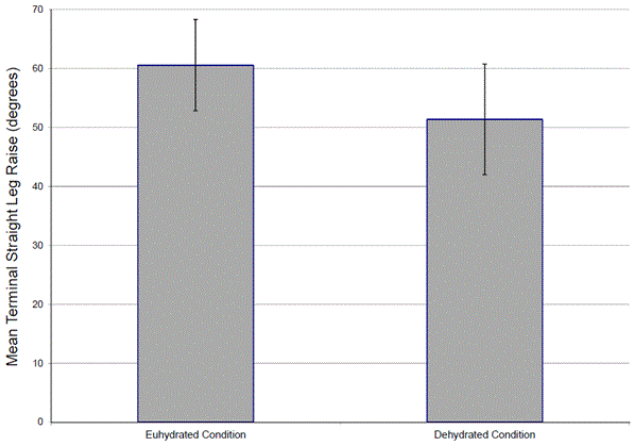

Figure 4: Terminal straight leg raise measures for both conditions

Studies measuring lower extremity stiffness have used the slope of the line created when plotting the measured passive resistance to movement on the X-axis and degrees of movement on the Y axis. Accordingly, a smaller slope indicates passive resistance to movement increased at a slower rate per degree of SLR range of motion compared to a larger slope. MPLS represents the inherent resistance to lengthening throughout the entire SLRROM for the tissues within the posterior leg, rather than a specific end point such as the MSnR or MTSLR. The MPLS may, therefore, be a better measure than SLRROM alone [12,13]. When subjects were dehydrated, the MPLS slope was statistically greater, with a large effect size, as compared to the slope obtained during the euhydrated condition. This would indicate a greater increase in PLS per degree of SLR range of motion during the dehydrated condition as compare to the euhydrated condition and that there was a measurable increase in passive resistance to movement of the lower extremity during the SLR maneuver (Figure 5). As motor activity within the quadriceps and hamstrings was absent throughout SLR maneuver on the KinCom this resistance can be attributable to tension within the skin, connective tissues, ligaments and tendons located within the posterior leg.

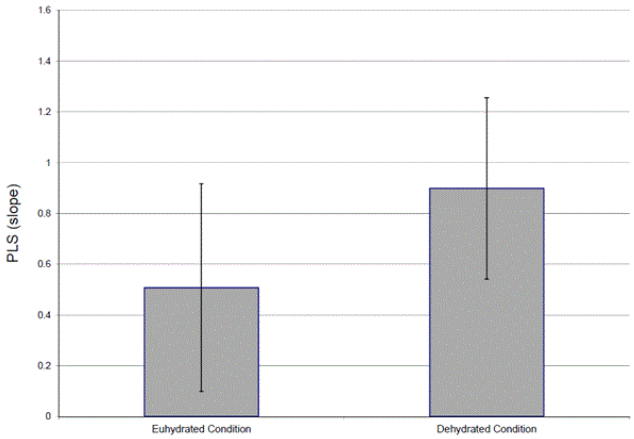

Figure 5: Slope comparison between both conditions

Previous in vitro studies demonstrated that human tissue is affected by variations in hydration levels [7-11]. This was the first in vivo study to show that posterior leg stiffness and flexibility are different when subjects are dehydrated versus euhydrated. Motor activity, of the quadriceps and hamstrings was monitored and if detected on EMG, the trial was thrown out. Subject positioning was consistent between both testing conditions. The protocol ensured that subject activity level prior to testing, time of testing, and motor activity of the quadriceps and hamstrings did not confound the test results. This ensured that the changes in MSnR, MTSLR and MPLS found between both testing conditions could only be attributable to the non-contractile structures located within the posterior leg.

The anatomical structures located within the posterior leg which were affected by the dehydration and euhydration protocols included the musculotendinous unit of the hamstrings as well as the connective tissues within and surrounding the muscles of the posterior leg, including the skin and fascia, extending from the hip joint to the foot, as well as friction from within the hip joint itself [15,26,34,35]. Collagen is a component of all these anatomical structures as well as the extracellular matrix (ECM) within and surrounding them [17,36]. Authors have described the ECM as critical in the absorption and transmission of tensile forces in tendons and muscles [17,36]. Collagen cross linking within the ECM allows for transmission of tensile loads while simultaneously limiting mobility of the structures based upon their alignment [17,36]. Water contained within the ECM allows the entire structure to absorb and dissipate tensile forces while simultaneously separating and lubricating the individual fibers and adjacent structures [17]. Previous studies have shown that collagen fibers become stiffer and more brittle when dehydrated [8,37]. A decrease in the water content within the ECM, muscles, tendons and connective tissue of the posterior leg would therefore effect stiffness while simultaneously decreasing the separation and lubrication between fibers and adjacent structures within the ECM. These cumulative factor, all lead to enhanced stiffness and limited extensibility [9,17].

The improvements in flexibility and stiffness seen in the euhydrated condition can be explained, by previous laboratory studies. These studies found that increasing hydration levels within collagen will act to increase lubrication and separation within the ECM resulting in a decrease in collagen stiffness/brittleness [38]. It is conceivable that when subjects became euhydrated the collagenous tissues located within their posterior legs exhibited increased mobility and spacing and decreased friction which leads to increased flexibility as measured by the sit and reach test and SLR maneuver while, it also allows for a concurrent drop in passive stiffness [17,38].

CONCLUSION

This study is the first to demonstrate in vivo changes in posterior leg flexibility and stiffness based on hydration level. It is conceivable to attribute the observed changes in flexibility and stiffness to changes in the mechanical properties of collagen located within and surrounding the musculotendinous unit of the hamstring muscles, the connective tissues, skin and fascia of the posterior leg. When dehydrated, subjects demonstrated an increase in resistance to passive movement earlier in the ROM and at greater amplitudes throughout the SLR maneuver as compared to euhydrated subjects. Conversely, subjects demonstrated increased flexibility with a subsequent decrease in PLS with euhydrated condition compared to the dehydrated condition.

While controversial others have linked high levels of stiffness and lack of flexibility to increased risk of injury within the posterior leg as well as impeded athletic performance. Studies exploring injury risk as well as athletic performance do not routinely account for or monitor hydration level as part of their reported variables. This may pose a threat to their validity because, as this study has shown, flexibility and stiffness are affected by hydration level. Future research in this area should therefore monitor and report on hydration levels to ensure that this possible mechanism of injury is confirmed and addressed. Similarly, future studies must prevent variations in hydration level from confounding their results when investigating the efficacy of therapeutic interventions involving flexibility or stiffness.

Limitations

This study utilized a sample size of nineteen male collegiate age runners who lived or attended school near the PIs clinic. This may reduce its applicability to other populations with differing genders and/or ages. Additionally, EMG activity was not monitored in the gluteal muscles, iliopsoas or tensor fascia lata due to equipment limitations. Without monitoring of these groups it is impossible to say if they hold a contributory influence.

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