COMPARISON OF MUSCLE AND BONE CHARACTERISTICS AND STRETCH-SHORTENING CYCLE CAPABILITIES BETWEEN INDIVIDUALS WITH AND WTIHOUT CHRONIC ANKLE INSTABILITY

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Abstract

Chronic ankle instability (CAI) is the most common injury in athletics and can result in lasting deficits in strength, ability to perform everyday tasks and recurrent-sprains. There are many studies that have observed long term ligamentous or osteochondral damage about the ankle joint specifically, but no known research has studied the possible downstream effects such as differences in bone and muscle health in the lower limb and stretch-shortening cycle performance. Therefore, the purpose of this study was to compare muscle and bone characteristics as well as stretch-shortening cycle capabilities of female individuals with chronic ankle instability with an uninjured control group. Individuals with CAI (n=10; age= 21 yrs \pm 1.4; height= 165.6 cm \pm 9.5; body mass= 72.7 kg \pm 18.1) and controls with no history of ankle injury (n=10; age= 20.5 yrs \pm 1.0; height= 162.2 cm \pm 7.4; body mass= 59.8 kg \pm 7.3) were recruited to participate in this study. Subjects' lower limb morphology was tested using peripheral quantitative computed tomography (pQCT) and performance measures were obtained using a unilateral hopping protocol on a custom-made sled. The CAI group was found to have significantly lower tibia mass and density at the most distal scan when compared to controls ($p \leq p$ 0.05). There were also notable trends that suggested muscle density and force production in the CAI group was lower than controls. This investigation adds to existing evidence that there are long term deficits present in individuals with CAI muscle and bone health in the distal portion of the lower leg and these may be associated with possibly lower levels of force production capabilities especially under heavy eccentric loading such as drop hops.

INTRODUCTION

Ankle sprains are one of the most common injuries experienced both in the general population and across different athletic backgrounds. In a study conducted by Hiller et al. (2012), 61.1% of the general population surveyed was found to have a history of ankle injury and most commonly ankle sprains. Up to 50% of individuals that reported lateral ankle sprains continue to experience mechanical laxity, functional instability, pain and weakness years after the original injury and are said to fall into a category of people with Chronic Ankle Instability (CAI) (Hertel 2002, Hiller et al. 2011; Tanen et al. 2014). Mechanical laxity can arise from anatomic changes such as ligamentous damage after ankle sprain (Hertel 2002). Functional instability has been thought to arise at least in part due to proprioceptive deficits, but additions to this definition have been made (Freeman et al. 1965, Hertel 2002). Needle et al. assert that altered perception of proprioception may be the culprit for seemingly lowered joint position sensing in individuals with CAI (2017). CAI incidences and negative long term outcomes after ankle sprain are thought to be equally likely regardless of age, gender and type of sport; however one recent study found a higher incidence of CAI in female athletes than their male counterparts (Anandacoomarasamy and Barnsley 2005, Tanen et al. 2014). The current study will utilize the IdFAI questionnaire to identify subjects that qualify for the CAI group (Tanen et al. 2014).

Patients with CAI suffer from mechanical and functional deficits that limit their daily activities and there are some known anatomic changes that occur as a result of CAI that may impact biomechanical function (Donovan and Hertel 2012). For instance the talus has been seen to shift anteriorly under the tibia, which can lead to changes in the way joint loading occurs in impact or weight-bearing activities (Vicenzino et al. 2006; Ferkel and Chams 2007). These changes effect more than just the ankle joint as well, Simpson et al. 2018 found increased knee

and hip flexion to be present in the landing mechanics of individuals with CAI, as well as increased ankle inversion prior to landing. These adaptive mechanisms may result partially due to arthrogenic muscle inhibition (AMI) and arise to compensate for the inability to fully activate stabilizing muscles around the ankle joint in preparation for impact (McVey et al. 2005). This inhibition is designed as a protective measure to prevent further injury to the joint, but this process includes the inhibition of spinal reflexes in the surrounding musculature (Hopkins and Ingersoll 2000, Lepley et al. 2017). This in turn makes rehabilitation of the injury much harder because it prevents the effectiveness of strength training in the dynamic stabilizers of the ankle (McKeon and Wikstrom 2016, McVey et al. 2005). AMI is also associated with resulting muscle weakness and atrophy which can further compound the risk of subsequent injuries (Hopkins and Ingersoll 2000). McVey et al. 2005 found the peroneals and the tibialis anterior to be effected by AMI and suggested that the output of the soleus may also be decreased. A combination of decreased muscle pre-activity and alterations to landing mechanics also predispose the ankle to further instances of giving way (Simpson et al. 2019, Simpson et al. 2018).

Bone tissue is highly dynamic and remodeling occurs constantly in response to changes in the mechanical environment such as strain (Zhang et al. 2007; Schipilow et al. 2013). Stronger bone structures have been noted in individuals that participate in impact training as a result of this remodeling (Nikander et al. 2006). Proper impact loading is also positively associated with higher bone quality, and reported lower activity levels in individuals with CAI may negatively impact bone mass near the ankle (Schipilow et al. 2013, Hiller et al. 2012). Bone measures such as density and cortical thickness have also been shown to improve with training, indicating that physical activity and impact loading do provide positive bone remodeling

adaptations (Nikander et al. 2006; Rantalainen et al. 2008). Decreased lower limb bone density and mass are also risk factors for fractures (Erlandson et al. 2016, Schipilow et al 2013).

Individuals with CAI are also at risk for early-onset osteoarthritis and osteochondral lesions stemming from the damage caused by the initial injury (Golditz et al. 2014 and Takao et al. 2005). Ferkel and Chams (2007) found osteophytes and osteochondral lesions of the talus in individuals with CAI. In a study done with subjects that had anterior cruciate ligament damage and subsequent reconstruction, it was found that the bone density in the distal femur and proximal tibia was lower than in controls which was identified as a step towards developing osteoarthritis (Kroker et al. 2018). This study also observed differences in joint biomechanics after ligamentous injury and the findings suggested that modified joint loading may impact the underlying bone density surrounding the joint (Kroker et al. 2018).

The potential decrease in bone density and mass of the tibia as a result of CAI, along with the known increased risk for osteoarthritis leads to questions about whether downstream effects of injury to the joint could be seen in bone strength measures such as ultimate fracture load and stress-strain index. Ultimate fracture load (UFL) of a bone is a measure that reflects the force (N) that would be required to break a bone laterally in either a medial-lateral or anterior-posterior direction, known as the x and y plane respectively (Rice et al. 2018). Stress-strain index is another measure of the strength of a bone, but this measure refers specifically to torsional strength (Rice et al. 2018). It has been found that bone cross-sectional area is associated with bone strength measures and training can improve this variable (Nikander et al. 2006).

Higher bone mineral density is associated with higher muscle mass, and muscle crosssectional area (CSA) is related to higher force production and overall strength (Rantalainen et al. 2008; Rice et al. 2018). In individuals with CAI, it is possible that muscle CSA may be

negatively affected by atrophy related to AMI (Hopkins and Ingersoll 2000) and kinesiophobia, or the fear of movement causing pain, may have compounded this loss. As discussed above, studies have been able to find weakness in the peroneals and tibialis anterior muscles that can be attributed to CAI. As the prime-mover of the joint, it would stand to reason that the gastrocnemius may experience similar atrophy and resultant decreases in force production in individuals with CAI. Peripheral Quantitative Computed Tomography (pQCT) scans were used to measure bone health variables as well as CSA of the tissues in the lower leg due to the non-invasive nature of the procedure (Erlandson et al. 2016). To this author's knowledge, there are no studies that have utilized pQCT to compare these variables between individuals with CAI and uninjured controls.

To test performance measures such as peak force and power, this investigation focused on the stretch-shortening cycle (SSC) as a mechanism that demonstrates strength and neuromuscular control. The SSC is characterized by a lengthening of the muscle-tendon unit (MTU) in an eccentric contraction followed by a transition to concentric shortening (Rice et al. 2018). The stretch that occurs in the lengthening phase of the cycle stores elastic potential energy that is used to generate force upon the completion of the shortening phase (Komi 2000, Kubo et al. 2007, Rice et al. 2018). This mechanism is highly studied in relation to the effects of different types of training on performance levels, however it is also highly relevant in the execution of everyday movements that utilize combinations of eccentric, concentric and isometric contractions (Rice et al. 2016, Sheppard et al. 2007, Malisoux et al. 2006, Cormie et al. 2009, Komi 200). It has been observed that strength and power athletes demonstrate higher peak force and power production and that the method of training is important to performance levels as well (Cormie et al. 2009, Rice et al. 2018). Based on the knowledge of the effects of CAI

limitations on neuromuscular function, postural control and motor neuron recruitment, it would make sense that these individuals may demonstrate different performance levels than the control group in countermovement and drop hops. Tibia bone strength measures have been correlated with higher force and power in trained athletes (Nikander et al. 2006). Residual damage in the musculature surrounding the ankle joint after subsequent strains and reduced firing or motor neuron recruitment as noted by Lepley et al. 2017, may also contribute to lower peak force.

The documented neurological and physiological differences in individuals with CAI compared to uninjured individuals encourage further investigation and comparison of musculotendinous and bone health characteristics of the lower limb well as performance measures between these populations. The current study aims to bring to light possible differences in morphological characteristics in the lower limb such as bone density and muscle cross-sectional area between the CAI group and the controls that may influence performance measures like peak force, velocity and power. In the current study, it is hypothesized that lower bone strength measures such as UFL and stress-strain index will be observed in the CAI group. It is also expected the CAI group will demonstrate lower bone mass and muscle cross-sectional area when compared to controls. In the hopping trials, it is expected the controls will outperform the CAI group in measures of peak force, velocity and power.

METHODS

Participants

Twenty healthy female subjects (CAI n=10, Control n=10) aged 18-25 years volunteered for the study. All subjects completed informed consent forms as well as the American College of Sports Medicine (ACSM) questionnaire (Balady et. al. 1998) to determine their general healthy condition before participation. Subjects that qualified for the CAI group completed an additional questionnaire (IdFAI) with a score of an 11 or above (Simon et al. 2012). All subjects reported no musculoskeletal injury, neuromuscular disease or lower limb injury in the 6 months prior to the testing session. Subjects were recruited from the Appalachian State University community by means of email and flyers to gauge interest. Exclusion criteria included pregnancy and musculoskeletal injury, neuromuscular disease or lower limb injury less than 6 months before testing.

Questionnaires

Two questionnaires were used to ensure participants met the criteria of the study. The ACSM questionnaire was used to assess the subjects' ability to participate in research involving exercise based on cardiovascular fitness measures. The IdFAI questionnaire was used as a reliable tool to identify subjects in the CAI group based on the frequency and severity of injuries and related symptoms. This questionnaire was scored by an investigator and had to exceed 11 for the participant to qualify for CAI.

Study Design

The Appalachian State University Institutional Review Board approved all experimental procedures. Data collection consisted of one testing session, lasting no more than 60 minutes. Upon arrival, participants signed an informed consent form and completed the ACSM questionnaire. Subjects in the CAI group then completed the IdFAI questionnaire. Subjects were thoroughly debriefed on experimental procedures and protocols prior to testing. The subject's anthropometric measurements were measured and recorded including height, body mass and lower leg length. Each subject then participated in experimental procedures to measure

muscle and bone architectural elements and hopping tasks to yield different measures of performance. Subjects were instructed to 1) wear appropriate exercise clothes and shoes for testing; 2) be well nourished and hydrated prior to testing; 3) avoid alcohol within 12 hours of testing and caffeine and tobacco within 3 hours of testing; and 4) be rested and avoid significant exercise the day of testing.

Anthropometric Measurements

Body mass was recorded using a Biomechanics Lab scale. Height was measured using a stadiometer. The lower limb length was determined by palpating the head of the fibula and measuring to the distal end of the lateral malleolus.

pQCT Scans

A Peripheral Quantitative Computed Tomography (pQCT) (Stratec Medizintechnik, Pforzheim, Germany) scanner was used to obtain bone density, muscle mass and cross-sectional area measures to assess the overall health of the lower limb. The pQCT scans were administered to either the unstable ankle (CAI group) or the preferred leg (uninjured control group). The subject placed their lower limb (unstable ankle or preferred leg) into the device and were instructed to sit motionless with their lower limb in the scanner for the 8-12 minute duration of the entire scan. The pQCT conducted scans at 4%, 14%, 38% and 66% of the lower limb with 0% representing the lateral malleolus. Variables of interest were extracted from the pQCT computer program and values were expressed relative to body mass and used for comparison between groups.

Countermovement and Drop Hops

Subjects were asked to perform a series of unilateral countermovement and drop hops on the testing limb used for the pQCT scan. A custom-made sled at an inclination of 10° with dual force plates (Bertec, Columbus, OH, USA) was used for hopping trials. Subjects laid flat on the sled with the test limb fully extended and a strap tethered just proximal of the patella to insure that no movement could be attributed to the knee joint. The limb not being used for experimental purposes was secured to eliminate its contribution in a hip, knee and ankle flexed position with foot resting on the pad of the sled. Subjects were instructed to rise onto their toes, remain still for a three second countdown and then flex the ankle as quickly and with as much force as they could generate to complete a countermovement hop. Two-minute rest periods were provided between trials, with three trials completed. The subjects then performed drop hops at 30 cm above their standing position. One investigator lifted the sled to 30 cm while another investigator counted down from three to signal the release of the sled. Subjects were instructed to push back up as quickly and with as much force as they could to hop off the force plate upon contact. Two-minute rest periods were provided between trials, with 3 trials completed at the same height.

A potentiometer (Celesco, Chatsworth, CA, USA) attached to the top of the custom-made sled was used to calculate displacement and provided the maximum hop height of each trial. A custom-made LabView program (National Instruments-Version , Austin, TX, USA) was used to analyze data files to generate peak force, impulse, peak velocity, maximum hop height and peak power for both the countermovement and drop hop trials. All values were expressed relative to body mass and used for comparison between groups.

Statistical Analysis

SPSS version 12.0 (SPSS Inc., Chicago, IL, USA) was used to perform all statistical analyses and a priori value (p) \leq 0.05 was defined as statistically significant. A multivariate ANOVA was performed to compare the CAI group with the controls for each dependent variable.

RESULTS

Individuals qualifying for CAI (n=10; age= 21 yrs \pm 1.4; height= 165.6 cm \pm 9.5; body mass= 72.7 kg \pm 18.1) and controls with no history of ankle injury (n=10; age= 20.5 yrs \pm 1.0; height= 162.2 cm \pm 7.4; body mass= 59.8 kg \pm 7.3) were tested for the current study. There were no significant differences ($p \le 0.05$) between groups in anthropometric measures, however a notable trend existed in body mass with the CAI group tending to be larger than the controls (p =0.054) as shown in Table 1. This trend was accounted for in all variables by normalizing the values to body mass to allow for further comparison.

Table 1. Anthropometric Measures ($M \pm SD$)

Group	Age (yrs)	Height (cm)	Mass (kg)	Lower Leg Length (mm)
Control	20.5 ± 1.0	162.2 ± 7.4	59.8 ± 7.3	348.3 ± 20.2
CAI	21 ± 1.4	$165.6\pm~9.5$	72.7 ± 18.1•	360.4 ± 27.9

• Trend with (0.05 < p < 0.09) compared to controls

CAI individuals had significantly less tibia mass at 4% of the lower leg when compared to the controls as shown in Table 2. The CAI group also had significantly less trabecular density according to their 4% scans than the control group (Table 2). While not statistically significant, there is also a notable trend present (0.05 in cortical density across the 14, 38 and 66percentage scans indicating the CAI group tended to have less cortical density than controls(Table 2, Figure 3). Another trend was evident in the muscle density at 66% of the lower leg.The CAI group tended to have less muscle density than the controls (p=0.054) as shown in Table2. There was no statistical significance found in measures of bone or muscle area (Table 3),stress-strain index or ultimate fracture load between groups (Table 4).

Lower Leg	Group	Rel Trb Density (mg/cm^3)	Rel Tibia Mass (mg/cm)	Rel CrtDen (mg/cm^3)	Rel Muscle Den (mg/cm^3)
4%	Control	4.2 ± 0.7	0.054 ± 0.005		
	CAI	3.5 ± 0.6 *	$0.047 \pm 0.008*$		
14%	Control		0.039 ± 0.005	18.8 ± 3.1	
	CAI		0.036 ± 0.006	16.1 ± 3.5•	
38%	Control		0.056 ± 0.007	19.8 ± 2.9	
	CAI		0.050 ± 0.010	17.1 ± 3.9•	
66%	Control		0.075 ± 0.008	19.1 ± 2.9	1.3 ± 0.2
	CAI		0.071 ± 0.013	$16.4 \pm 3.9 \bullet$	1.1±0.3•

Table 2. *Relative Bone Health Measures and Muscle Density* $(M \pm SD)$

Note. Rel Trb Density = Relative trabecular density; Rel Tibia Mass = Relative tibia mass; Rel

CrtDen = Relative cortical density; Rel Muscle Den = Relative muscle density.

* Significantly less ($p \le 0.05$) than Controls

• Trend with (0.05<*p*<0.09) compared to controls

Lower Leg	Group	Rel Tibia Area (mm^2)	Rel CrtArea (mm^2)	Rel Muscle Area (mm^2)
4%	Control	18.0 ± 1.5		
	CAI	16.0 ± 4.0		
14%	Control	7.3 ± 0.9	2.5 ± 0.4	
	CAI	7.0 ± 1.3	2.3 ± 0.4	
38%	Control	6.2 ± 0.7	4.3 ± 0.6	
	CAI	5.6 ± 1.0	3.9 ± 0.8	
66%	Control	10.2 ± 1.1	5.7 ± 0.7	0.621 ± 0.05
	CAI	10.3 ± 1.7	5.3 ± 1.1	0.628 ± 0.09

Table 3. Relative Bone and Muscle Cross Sectional Area $(M \pm SD)$

Note. Rel Tibia Area = Relative tibia area; Rel CrtArea = Relative cortical area; Rel Muscle Area = Relative muscle area.

Table 4. Relative Bone Strength Variables ($M \pm SD$)

Lower Leg	Group	Rel UFL X (N)	Rel UFL Y (N)	Rel SSI (mm^3)
4%	Control			
	CAI			
14%	Control	55.4 ± 10.4	47.6 ± 7.2	22.4 ± 3.5
	CAI	50.8 ± 12.5	43.8 ± 7.9	22.0 ± 3.8
38%	Control			24.5 ± 3.9
	CAI			22.3 ± 4.8
66%	Control			
	CAI			

Note. Rel UFL X = Relative ultimate fracture load x-plane; Rel UFL Y = Relative ultimate fracture load y-plane; Rel SSI = Relative stress-strain index.

Two other measures related to the 4% scan were found to be significantly different between groups. The CAI group was found to have less total tibia density than the controls as shown in Figure 1. A ratio was calculated to compare the mass of the tibia at 4% to its mass at 38% of the lower leg. This ratio was found to be statistically smaller in the CAI group compared with the controls as shown in Figure 2.



Figure 1. Bone density measures between groups.

* Significantly less ($p \le 0.05$) than Controls.



Figure 2. Bone mass measures between groups

* Significantly less ($p \le 0.05$) than Controls.



Figure 3. Cortical density across lower leg between groups

No significant differences existed in the stretch-shortening cycle performance measures shown in Table 5. While not significant, there was a trend noted that the CAI group tended to have a lower mean relative peak force in the drop hop trials than the controls.

Нор Туре	Group	Rel PF (N)	Rel Imp (N*s)	PV(m/s)	Rel PP (W)
СМН	Control	12.5 ± 2.0	4.8 ± 0.3	0.573 ± 0.1	4.4 ± 1.3
	CAI	11.5 ± 2.4	5.2 ± 0.9	0.608 ± 0.1	4.6 ± 1.3
DH	Control	25.5 ± 3.2	4.9 ± 0.4	0.800 ± 0.1	7.3 ± 1.9
	CAI	22.0±4.3•	4.9 ± 0.4	0.786 ± 0.1	6.5 ± 1.3

Table 5. *Relative Stretch-Shortening Cycle Performance Measures* $(M \pm SD)$

Note. CMH = Countermovement hop; DH = Drop hop; Rel PF = Relative peak force; PV = Peak velocity; Rel PP = Relative peak power.

• Trend with $(0.05 \le p \le 0.09)$ compared to controls

DISCUSSION

The purpose of the present study was to determine any significant differences in bone or muscle morphological characteristics of the lower leg in individuals with CAI when compared to controls with no history of ankle injury. The main findings in this investigation provide evidence of detrimental effects in general bone health in the CAI group when compared with controls. These effects were most evident in the 4% scan of the distal tibia, however notable trends existed across the scans at different distances. The 4% cross-sectional scan provided by the pQCT revealed tibia mass and density measures to be significantly lower in the CAI group than the controls. The overall tibia mass at 4% of the lower leg was smaller in the CAI group along with a ratio comparing the 4% tibia mass to the mass at 38%. Not only was overall mass of the tibia lower in the CAI group, but a significantly lower tibia density and more specifically trabecular density were noted as well. Lower mass of the tibia at 4% could be attributed to lower levels of impact training activities in the CAI group (Nikander et al. 2006). Given the increased risk for osteoarthritis and cartilage degeneration in individuals with CAI, the detriments in tibia bone health found in this study reveal yet another deficit and potential risk factor for future injury in this population (Golditz et al. 2014). Cortical density was also tested in this study and was obtained at the 14, 38, and 66% scans of the lower leg. This measure was not found to be statistically significant between groups, but a clear trend was established that suggested that cortical density was lower in the CAI group than in the controls. The significant decreases in overall tibia and trabecular density in the 4% scan of the CAI group show evidence that decreased cortical density could be another plausible morphological effect. The lack of significance found in this variable could have been a result of a small sample size and may have become apparent had there been more subjects and a lower standard deviation. This trend may also indicate that the downstream effects of CAI may not be visible in the more proximal anatomic regions of the lower leg.

Cross-sectional bone area is also correlated with overall bone strength (Nikander et al. 2006). This study found no statistical difference in tibia area across all pQCT scans of the lower leg. Ultimate fracture load and stress-strain index were also measured at the 14 and 38% scans where there were no significant differences found between the CAI group and controls. Based on the results of the current study, it appears that CAI does not affect tibia cross-sectional area or these measures of bone strength.

Arthrogenic muscle inhibition has also been shown to negatively affect individuals with CAI in regards to rehabilitation, overall activity and strength (Hopkins and Ingersoll 2000). This along with peripheral deafferentation correlate with immobilizations that effect performance of daily activities and contribute to muscle weakness and atrophy in the stabilizers of the joint

(Needle et al. 2017; Hopkins and Ingersoll 2000). It is also recognized that individuals with CAI report lower levels of physical activity and that physical activity or training is correlated positively with measures such as muscle cross-sectional area (Hiller et al. 2012; Wernborn et al. 2007). It was hypothesized due to the documented differences in physical activity and heightened kinesiophobia associated with CAI, that this study would find a resultant decrease in muscle CSA in the CAI group when compared to the controls. This was in fact not the case as there was no significant difference between groups in the muscle CSA measure at 66% of the lower leg. While not significant, there was a trend noted in the muscle density measure at this location. A lower muscle density was reported in the CAI group compared with controls. When considering the long term implications of muscle CSA and density in relation to overall quality of life, it has been found that lower muscle density is associated with greater hospitalizations in older adults while there is no correlation in muscle size or CSA (Cawthon et al. 2012). This leads to the notion that muscle density that is detrimentally effected by pathologies such as CAI, but further investigations would need to find significance that supports this idea.

It is clear that individuals with CAI demonstrate deficits in functional performance tests that require lateral movement and extensive postural control (Madsen et al. 2018). The current study aimed to uncover differences in vertical hopping tasks about the ankle joint that may be influenced by lower limb morphologies of each group. Previous studies have noted a relationship between lower extremity bone health measures and performance variables like force and power (Nikander et al. 2006). Muscle CSA is also known to impact performance and strength measures (Jakobsen et al. 2012; Jones et al. 2008). This study employed the use of unilateral hopping tasks that isolated the ankle joint to obtain measures of force, velocity and power to compare between groups. The results indicated no statistical significance between

groups in any of the performance measures for either countermovement or drop hops. While there was again no significance noted in these variables, a trend in relative peak force suggested that the CAI group produced less force than controls in the drop hop condition. Since it was hypothesized that the CAI group would demonstrate lower muscle cross-sectional area, it was expected that there would be significant differences between groups in these performance measures. It seems that even though there were significant differences in bone health measures between groups, that muscle CSA is likely a stronger indicator of differences in performance measures.

The current study is not without limitations. One of these was that subjects were not screened for their pre-existing fitness level or exercise habits. Without this information it is impossible to determine if performance measures were influenced by previous training adaptations or even lack of experience. Something else to consider are the different proposed subgroups of CAI (Hertel 2002; Hiller et al. 2011). These subgroups take into account the different range of persisting symptoms that fall into the category of CAI. With the IdFAI questionnaire, it is possible that subjects could have qualified for CAI with only functional instability symptoms. While this is completely normal based on the range of subgroups proposed, it could have played a role in the performance variables in individuals within the CAI group. These limitations could in part have been addressed by a larger sample size that may have allowed for more groups to emerge based on the subjects' fitness levels, type of persisting symptoms and even whether or not they sought professional diagnosis or treatment after their injuries.

CONCLUSION

In conclusion, this investigation has shown that chronic ankle instability could be associated with deficits in various bone health measures in the distal tibia such as mass and density. The lack of downstream effects in other lower leg scans indicates that these differences are concentrated close to the ankle joint but further investigations should be done to corroborate this idea. There were also trends present such as decreased muscle density and force production during drop hops in the CAI group showing that mechanical damage after injury and resultant lower activity levels may influence measures of strength and performance. Further investigations could explore the differences between individuals in the CAI group with different persisting symptoms. Another interesting direction would be to examine the morphological differences between individuals who followed a rehabilitation regime after their injury and those who did not seek treatment from a professional. Overall, the damage associated with mechanical laxity and functional instability at the ankle joint have various downstream and long term effects in bone and muscle characteristics years after the initial injury.

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